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Aluminium Chloride-Induced Cerebral Toxicity in Wistar Rats: Anticholinesterase and Antioxidant Effects of Ascorbic Acid

Ebhodaghe, Clinton Iria[1], Enogieru, Adaze Bijou [1]*
[1]Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

ABSTRACT

Numerous literature evidence suggests that aluminium is a toxic metal that induces direct damage to primary human neural cells, leading to genotoxic effects while promoting neurodegeneration in different regions of the brain. However, the use of exogenous antioxidants to counteract neurodegeneration has been widely reported. Ascorbic acid (AA), also known as Vitamin C, is a water-soluble vitamin reported to exert antioxidant, anti-inflammatory and anti-apoptotic effects. Accordingly, this study investigated its protective activity against aluminium chloride ($AlCl_3$)-induced neurotoxicity in Wistar rats. Forty-eight Wistar rats were randomly divided into six groups (n=8) and treated as follows: A (control); B (100 mg/kg bw $AlCl_3$); C (100 mg/Kg bw AA + 100 mg/kg bw $AlCl_3$); D (200 mg/Kg bw AA + 100 mg/kg bw $AlCl_3$); E (100 mg/Kg bw AA); F (100 mg/Kg bw AA). The administration, via an oral gavage, lasted for 28 days. Thereafter, the weights, neurobehavioral, antioxidant enzymes, lipid peroxidation, acetylcholinesterase and histological assessments were carried out. Findings showed significantly ($p < 0.05$) impaired weights, neurobehavioural, and antioxidant enzymes as well as elevated lipid peroxidation and acetylcholinesterase in the $AlCl_3$-exposed rats when compared to control. Also, the cerebral cortex of $AlCl_3$-exposed rats displayed severe cytoplasmic vacuolations and degenerating cells, indicating cerebral dysfunction. However, pretreatment of $AlCl_3$-exposed rats with AA significantly ($p < 0.05$) attenuated these adverse effects. Altogether, AA exerted neuroprotective effects against $AlCl_3$-induced cerebral toxicity, possibly through its potent antioxidant and anticholinesterase activity.

Keywords: Ascorbic Acid; Vitamin C; Neurodegeneration; Neurobehavioural; Antioxidant; Anticholinesterase

***Corresponding author**: Adaze Bijou Enogieru
Email: adaze.enogieru@uniben.edu +234 7016780198

INTRODUCTION

Aluminium and its compounds constitute a substantial portion of the Earth's crust, accounting for approximately 8% [1]. This element is the third most abundant and naturally occurs in combination with oxygen, fluorine, silicon, sulfur, and other elements, rather than in its elemental form [1]. Although aluminium's essential biological function remains elusive, its detrimental effects on living organisms particularly concerning various cellular and metabolic processes within the nervous system have become increasingly apparent. For instance, aluminium is now widely acknowledged for its potential involvement in various neurological disorders, with Alzheimer's disease and cognitive impairments being prominent examples [2-4]. Literature evidence suggests that it accumulates in the brain, with a particular preference for regions such as the cerebellum, cerebrum and hippocampus, consequently leading to an array of detrimental effects encompassing oxidative stress, neuroinflammation, and cholinesterase dysfunction [5, 6]. Humans encounter aluminium through diverse environmental sources, including using aluminium sulfate (alum) in water purification, resulting in elevated levels of aluminium in drinking water [7]. Furthermore, aluminium is prevalent in containers, cooking utensils, and certain medications [8, 9].

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The prevalence of aluminium exposure in Nigeria has emerged as a pressing public health concern, with studies highlighting elevated environmental and food-related aluminium levels in the country [10]. Aluminium exposure poses a substantial global public health challenge, impacting the population's well-being, particularly concerning neurological health. Consequently, there has been a surge in the search for therapeutic agents capable of mitigating the toxicity of aluminium.

Ascorbic acid, also known as vitamin C, is a water-soluble organic compound with antioxidant properties [11]. It is an essential nutrient for humans and some other animals, meaning it must be obtained through diet or supplements since the body cannot produce it on its own. Ascorbic acid plays a crucial role in various biological processes in the body [12], and is reported to modulate and mitigate neuroinflammation and neuronal damage [13]. It can scavenge free radicals, reduce oxidative stress, and control various cellular processes involved in neuronal function and survival through its involvement in various neurotrophic pathways [14]. Ascorbic acid is involved in the synthesis of neurotransmitters and plays a role in the regulation of gene expression related to neuronal survival [11]. Although mitochondrial dysfunction is implicated in the pathogenesis of neurodegenerative diseases, ascorbic acid has been shown to protect the mitochondria from oxidative damage and improve its function [15], thus helping to maintain energy production and reduce neuronal stress. Overall, several reports show that ascorbic acid offers the potential for the enhancement of antioxidant activity, attenuation of oxidative stress, and modulation of neuroinflammation [16, 17]. However, there are few reports demonstrating the antioxidant and anticholinesterase activity of ascorbic acid in aluminium chloride-induced neurotoxicity. Accordingly, this study investigated such activity in the cerebrum of adult Wistar rats.

MATERIALS AND METHODS

Chemical and reagents

Aluminium chloride (98%) and L-ascorbic acid (99%) were manufactured by Molychem, Mumbai, India, and Normal saline by Unique Pharmaceuticals, Sango-Otta, Nigeria. Other reagents were all of the analytical grades.

Care and Management of Experimental Animals

A total of Forty-eight Wistar rats, weighing between 120g-140g were purchased and housed in the Department of Anatomy animal holdings. The rats, fed with standard rat chow (Bendel livestock feed, Edo state, Nigeria) and water liberally, acclimatized for two weeks before the beginning of the experiment. The experimental procedures performed on the animals were according to the guidelines of the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria with approval number CMS/REC/2023/446.

Experimental design

The rats were randomly assigned into six (6) different groups (n=8). The experimental design was as follows:

Group A (control) - received 1 ml of normal saline

Group B (Al) - received 100 mg/kg body weight (BW) of Aluminium chloride ($AlCl_3$) only.

Group C (AA1 + $AlCl_3$) - received 100 mg/kg BW/day of ascorbic acid (AA) and 100 mg/kg BW of $AlCl_3$.

Group D (AA2 + $AlCl_3$) - received 200 mg/kg BW/day of AA and 100 mg/kg BW of $AlCl_3$.

Group E (AA1) - received 100 mg/kg BW/day of AA.

Group F (AA2) - received 200 mg/kg BW/day of AA.

Rats were pretreated with AA one hour before the administration of $AlCl_3$. After 28 days, the rats were subjected to the neurobehavioural evaluation.

Evaluation of neurobehavioural activity

The novel object recognition test was carried out in a wooden open box device ($80 \times 60 \times 40$ cm) as previously described [18, 19]. Here, rats explored the device for a 2-minute session of familiarization on the 27th day of the

experiment. On the 28th day, a first 5-minute sample trial test (T1) was carried out, with two similar objects (named familiar objects FO1 and FO2) placed at the corners of the box. In the second 5-minute test (T2), FO2 presented in T1 was substituted with a novel object (NO), and the exploration times for FO1 and NO were recorded. The discrimination between FO1 and NO during T2 was determined by equating the time spent exploring FO1 and NO. For quality control, a discrimination index (DI) was calculated as follows: DI = NO−FO1/NO + FO1.

Determination of relative brain weight

Following the end of the novel object recognition test, rats were sacrificed and the brains of experimental rats were removed and weighed. The relative brain weight was expressed as a percentage of the final body weight at sacrifice, and the cerebrum was dissected out and processed for biochemical and histological assessment.

Evaluation of biochemical parameters

The cerebrum was homogenized in ice-cold 20 Mm Tris-HCl buffer (pH 7.4), and the homogenate was then centrifuged at 10,000g for 10 min at 4°C [20, 21]. The supernatant was collected and evaluated for Catalase – CAT [22], Superoxide dismutase – SOD [23], Glutathione peroxidase – GPx [24], Glutathione - GSH [25], Malondialdehyde – MDA [26], and Acetylcholinesterase - AChE activities [27].

Histological evaluation

After proper fixation of the cerebrum in 10% buffered formal saline for 72 h, processing through the paraffin wax embedding and the Hematoxylin and Eosin staining method was done as previously described [28].

Statistical analysis

Analysis of data was carried out using the GraphPad Prism Software V9. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test was utilized to determine statistical significance ($p < 0.05$). Values are presented as Mean ± Standard Error of Mean (SEM).

Results

Weight Findings

Table 1 shows weight parameters across the experimental groups. For weight change, whole brain, and relative brain weights, a significant decrease ($p < 0.05$) was observed in the $AlCl_3$-exposed rats when compared to the control. However, a significant increase ($p < 0.05$) was observed in the AA-pretreated rats, for whole brain and relative brain weights, when compared to the $AlCl_3$-exposed rats. No significant difference ($p > 0.05$) was observed between the AA-treated rats and control.

Table 1: Weight parameters across experimental groups.

Groups	Control	$AlCl_3$	AA1 + $AlCl_3$	AA2 + $AlCl_3$	AA1	AA2
Initial BW (g)	126.7 ± 4.22	131.4 ± 3.21	126.2 ± 4.41	128.7 ± 5.23	127.6 ± 3.24	131.6 ± 5.15
Final BW (g)	174.3 ± 3.10	162.0 ± 2.92	167.8 ± 7.60	166.7 ± 3.65	169.9 ± 4.25	175.1 ± 5.51
Weight Change (g)	47.6 ± 5.22	30.6 ± 2.39 #	41.6 ± 7.65	38.00 ± 2.38	42.71 ± 1.58	43.5 ± 2.07
Whole brain weight (g)	1.61 ± 0.03	1.31 ± 0.07 #	1.57 ± 0.07 *	1.58 ± 0.06 *	1.69 ± 0.06	1.70 ± 0.031
Relative brain weight (%)	0.93 ± 0.01	0.81 ± 0.04 #	0.94 ± 0.01 *	0.95 ± 0.02 *	0.99 ± 0.02	0.97 ± 0.02

# p<0.05 compared with the control group; * p<0.05 compared with $AlCl_3$ group

Neurobehavioural Findings

Table 2 shows the neurobehavioural findings from the novel object recognition test across the experimental groups. Here, no significant difference ($p > 0.05$) was observed in the mean exploration times for FO1, FO2, and NO. A significant decrease ($p < 0.05$) was observed in the total exploration time for T1 and DI in the $AlCl_3$-exposed rats when compared to the control. However, a significant increase ($p < 0.05$) was observed in the AA-pretreated rats, for DI, when compared to the $AlCl_3$-exposed rats. No significant difference ($p > 0.05$) was observed between the AA-treated rats and control.

Table 2: Neurobehavioural findings across experimental groups.

Groups	Control	$AlCl_3$	AA1 + $AlCl_3$	AA2 + $AlCl_3$	AA1	AA2
FO1 – 1st Test (s)	29.83 ± 2.04	13.00 ± 3.46	11.50 ± 7.59	19.75 ± 6.86	15.80 ± 5.41	17.80 ± 7.30
FO2 – 1st Test (s)	15.33 ± 2.12	06.67 ± 2.59	12.50 ± 4.41	13.75 ± 1.75	11.86 ± 2.60	17.43 ± 3.44
FO1 - 2nd Test (s)	04.17 ± 2.71	09.33 ± 1.48	06.60 ± 1.89	07.20 ± 2.13	05.80 ± 3.56	09.25 ± 5.96
NO	24.17 ± 3.44	11.50 ± 1.88	27.40 ± 3.23	26.00 ± 5.26	20.00 ± 3.86	29.00 ± 6.87
T1 (s)	45.17 ± 3.38	19.67 ± 5.45 #	24.00 ± 8.48	33.50 ± 5.84	25.80 ± 7.83	36.50 ± 8.29
T2 (s)	28.33 ± 6.02	20.83 ± 3.34	34.00 ± 4.14	33.20 ± 7.14	25.80 ± 7.33	38.25 ± 11.70
DI	0.82 ± 0.11	0.10 ± 0.02 #	0.63 ± 0.11 *	0.63 ± 0.10 *	0.73 ± 0.16	0.67 ± 0.20

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_3$ group. FO1 – Familiar object 1; FO2 – Familiar object 2; NO – Novel object; T1 – Total exploration time 1; T2 – Total exploration time 2; DI – Discrimination index

Biochemical Findings

Table 3 illustrates the activity of antioxidants, lipid peroxidation, and AChE in the cerebrum across experimental groups. Here, a significant decrease ($p< 0.05$) in SOD, CAT, GSH, and GPx as well as a significant increase in MDA and AChE was observed in the $AlCl_3$-exposed rats when compared to the control. However, a significant difference ($p < 0.05$) was observed in these parameters in the AA-pretreated rats following comparison to the $AlCl_3$-exposed rats. No significant difference ($p > 0.05$) was observed between the AA-treated rats and control.

Table 3: Biochemical findings across experimental groups

Groups	Control	$AlCl_3$	AA1 + $AlCl_3$	AA2 + $AlCl_3$	AA1	AA2
CAT (U/mg)	31.65 ± 3.37	08.62 ± 1.61 #	18.62 ± 1.33	20.65 ± 2.74 *	24.61 ± 2.37	25.49 ± 3.07
SOD (U/mg)	0.91 ± 0.05	0.72 ± 0.04 #	0.86 ± 0.04*	0.93 ± 0.04 *	0.90 ± 0.15	0.91 ± 0.03
GPx (U/mg)	57.40 ± 8.25	18.74 ± 3.03 #	43.71 ± 2.60 *	43.57 ± 3.02 *	52.54 ± 7.21	52.84 ± 4.08
GSH (µM)	25.32 ± 2.24	07.53 ± 2.83 #	20.09 ± 2.24 *	19.66 ± 1.62 *	22.17 ± 3.34	22.90 ± 2.07
MDA (mmol/mg)	01.76 ± 0.77	11.41 ± 2.45 #	03.55 ± 0.85 *	04.39 ± 0.82 *	04.62 ± 0.98	04.46 ± 0.39
AChE (ìmol/mg)	29.35 ± 15.72	508.2 ± 186.0 #	118.2 ± 26.98 *	91.27 ± 22.13 *	47.72 ± 10.56	36.93 ± 7.01

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $AlCl_3$ group.

Histological Findings

Figure 1 shows the histology of the inner granular layer of the cerebral cortex across experimental groups. Appearing in Figure 1A (control) are normal granular cells and typical features of the cerebral cortex. In Figure 1B, empty vacuoles, cytoplasmic vacuolization, and degenerating cell nuclei can be noticed in the cortex of the $AlCl_3$-exposed rats. Figures 1C-D show very sparse degenerating cell nuclei in the cortex of $AlCl_3$-exposed rats pretreated with AA. Figures 1E-F show normal features of the cortex of AA-treated rats comparable to the control.

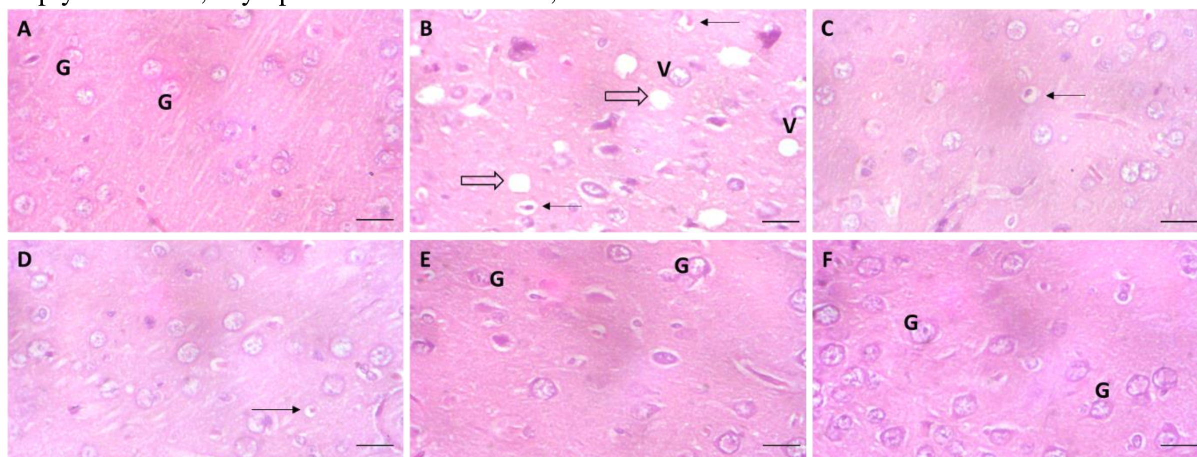

Figure 1: Representative histology of the cerebral cortex (internal granular layer, IV) across experimental groups. (A) Control group showing normal granular (G) cells. (B) $AlCl_3$ rats displaying empty vacuoles (double arrows) and cytoplasmic vacuolization (V); Degenerating cell nuclei can be noticed in the vacuolated neuropil (single arrow). (C&D) AA-Pretreated rats showing relatively normal features with few degenerating cell nuclei (single arrow). (E&F) AA-Treated rats showing normal features of the cortex. (H&E 400x; Scale bar: 25µm).

DISCUSSION

Aluminium is a well-known neurotoxic metal implicated in the development and progression of neurodegenerative disorders, including Alzheimer's disease [29]. Accordingly, this study investigated the protective activity of ascorbic acid against $AlCl_3$-induced neurotoxicity in Wistar rats. Findings from this study showed that the $AlCl_3$-exposed rats exhibited a significant decrease in body weight, whole brain weight, and relative brain weight when compared to control. This weight loss suggests that aluminium chloride exposure had an adverse effect on the overall health and well-being of the rats. Literature reports indicate that aluminium's effect on weight loss is a rather indirect process. For instance, aluminium exposure may lead to a loss of appetite, a reduction in food consumption, and a subsequent decrease in body weight. In addition, aluminium can interfere with various metabolic processes in the body, thus affecting the absorption and utilization of essential minerals and nutrients, leading to imbalances in the body's energy metabolism and ultimately weight loss [30, 31]. The findings from this study align with previous reports demonstrating that $AlCl_3$ exposure causes weight loss in experimental animals [4, 32, 33]. However, pretreatment with ascorbic acid mitigated the $AlCl_3$-induced weight loss in the rats, thus suggesting that ascorbic acid exerted a protective effect.

The Novel Object Recognition test is a valuable tool for assessing cognitive function and memory [34]. This test measures a rodent's ability to recognize and remember new or novel objects. Various cerebral regions are involved in memory [35], and the proper functioning of these cerebral areas is crucial for the encoding and retrieval of recognition memory. In this study, the total exploration times and the discrimination index showed a significant decrease in the $AlCl_3$-exposed rats when compared to control, thus indicating impaired cognitive function. The discrimination index serves as a valuable indicator of cognition, gauging proficiency in distinguishing between diverse stimuli, either objects or signals [36]. This metric quantifies the aptitude for precise

discrimination and categorization, particularly in contexts intertwined with memory and the processes of learning. The findings are consistent with earlier studies that have reported lowered discrimination index and cognitive deficits in aluminium-exposed animals [5, 37]. Conversely, ascorbic acid-pretreated rats exhibited a significant increase in the discrimination index following comparison to the $AlCl_3$-exposed rats. The improvements in cognitive function in the ascorbic acid pretreated rats suggest a potential neuroprotective effect of ascorbic acid which aligns with reports demonstrating the cognitive-enhancing properties of antioxidants [38, 39].

Antioxidant enzymes play a significant role in maintaining the health and proper functioning of the brain. Antioxidant enzymes play a pivotal role in defending cells and tissues against the harmful effects of oxidative stress by neutralizing free radicals and reactive oxygen species. The brain is highly vulnerable to oxidative stress due to its high oxygen consumption and abundant lipid content, making it reliant on efficient antioxidant defences [40]. SOD acts as a crucial defence against highly reactive superoxide radicals by catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide [41]. CAT is essential in breaking down hydrogen peroxide produced into less harmful oxygen and water molecules [42]. High levels of hydrogen peroxide can lead to oxidative stress, which can damage brain cells and impair brain function. GPx is critical for the reduction of hydrogen peroxide to water and lipid hydroperoxide to corresponding alcohols using GSH as an electron donor [43], thus protecting the neuronal cell membranes from oxidative damage. Findings from this study showed that $AlCl_3$-exposed rats exhibited a significant decrease in SOD, CAT, GPx and GSH when compared to the control, thus indicating an impairment in antioxidant activity. These results are consistent with previous studies demonstrating that aluminium exposure can disrupt antioxidant defences, leading to oxidative stress [5, 32, 44]. However, ascorbic acid pretreated rats showed a significant increase in these antioxidant enzymes following comparison to the $AlCl_3$-exposed rats, suggesting that ascorbic acid enhances the activity of antioxidants and protects against $AlCl_3$-induced disruption of antioxidant defences. In addition, $AlCl_3$-exposed rats exhibited a significant increase in MDA concentration when compared to the control. These findings are consistent with previous studies demonstrating aluminium-induced lipid peroxidation in the brain [4, 44]. However, ascorbic acid pretreated rats showed a significant decrease in MDA when compared to the $AlCl_3$-exposed rats, suggesting that ascorbic acid may protect against $AlCl_3$-induced oxidative damage to lipids.

AChE is an enzyme that breaks down acetylcholine, a neurotransmitter essential for cognitive processes, particularly learning, memory, and attention [45]. Dysregulation of acetylcholine levels due to changes in AChE activity can influence cognitive function. In conditions like Alzheimer's disease, for instance, increased AChE activity can lead to reduced acetylcholine levels contributing to cognitive impairment [46]. Elevated AChE activity causes a rapid breakdown of acetylcholine and prevents its accumulation in the synaptic cleft [45]. Consequently, there is a shorter duration of acetylcholine's action and a reduction in the availability of acetylcholine for nerve signal transmission. Findings from this study showed a significant increase in AChE concentration in the cerebrum of $AlCl_3$-exposed rats when compared to the control. These findings are consistent with previous studies demonstrating increased AChE activity following aluminium exposure [46, 47]. On the other hand, ascorbic acid-pretreated rats exhibited a significant reduction in AChE activity when compared to the $AlCl_3$-exposed rats, suggesting that ascorbic acid may improve cognitive function via the preservation of acetylcholine levels in the brain possibly through its antioxidant properties. Histological analysis of the cerebral cortex revealed significant morphological changes in the $AlCl_3$-exposed rats, these include several cytoplasmic vacuolization and degenerated neuronal cell bodies and nuclei, thus indicating neuronal damage. These alterations are reported to be associated with excessive reactive oxygen species production and endoplasmic reticulum stress, ultimately leading to cell death [48]. These findings are consistent with previous studies demonstrating alterations in the histology of the brain following aluminium exposure [4, 5, 32, 49]. However, in the ascorbic acid pretreated rats, there were

significantly fewer alterations in the cortex when compared to the $AlCl_3$-exposed rats, suggesting a protective effect of ascorbic acid against $AlCl_3$.

Taken together, results from this study show that ascorbic acid attenuates $AlCl_3$-induced cerebral toxicity primarily through its potent antioxidant and anticholinesterase activity. This suggests that ascorbic acid may be useful in the development of novel therapeutic agents relevant to the management and treatment of $AlCl_3$-associated disorders such as dementia and Alzheimer's disease.

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REFERENCES

[1] Krewski D., Yokel R.A., Nieboer E., Borchelt D., Cohen J., Harry J., et al. (2007).Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. Journal of Toxicology Environmental Health, Part B, 10(S1): 1-269.

[2] Xue Y., Tran M., Diep Y.N., Shin S., Lee J., Cho H., et al. (2024).Environmental aluminum oxide inducing neurodegeneration in human neurovascular unit with immunity. Scientific Reports, 14(1): 744.

[3] Vlasak T., Dujlovic T., Barth A. (2024).Aluminum exposure and cognitive performance: A meta-analysis. Science of the Total Environment, 906: 167453.

[4] Okhah A.A., Enogieru A.B. (2023).Antioxidant and protective activities of aqueous Theobroma cacao seed extract against aluminium-induced hippocampal toxicity in Wistar rats. Nigerian Journal of Biochemistry Molecular Biology, 38(4): 197-205.

[6] Amhanyunose P., Enogieru A. (2023).Improvement in Cognition, Memory and Hippocampal CA1 microstructure in Aluminium Chloride-exposed Rats Following Pretreatment with Aqueous Psidium guajava Leaf Extract. Journal of Experimental Clinical Anatomy, 20(1): 1-9.

[7] Bryliński Ł., Kostelecka K., Woliński F., Duda P., Góra J., Granat M., et al. (2023).Aluminium in the human brain: routes of penetration, toxicity, and resulting complications. International Journal of Molecular Sciences, 24(8): 7228.

[8] Srinivasan P., Viraraghavan T., Subramanian K. (1999).Aluminium in drinking water: An overview. Water SA, 25(1): 47-55.

[9] Reinke C.M., Breitkreutz J., Leuenberger H. (2003).Aluminium in over-the-counter drugs: risks outweigh benefits? Drug Safety, 26: 1011-25.

[10] Gupta Y., Meenu M., Peshin S. (2019).Aluminium utensils: Is it a concern. The National medical journal of India, 32(1): 38-40.

[11] O.A., Adeoluwa Y.M. (2020).Production usage, and potential public health effects of aluminum cookware: a review. Annals of Science Technology, 5(1): 20-30.

[12] Abdulrazzaq A.M., Badr M., Gammoh O., Abu Khalil A.A., Ghanim B.Y., Alhussainy T.M., et al. (2019).Hepatoprotective actions of ascorbic acid, alpha lipoic acid and silymarin or their combination against acetaminophen-induced hepatotoxicity in rats. Medicina, 55(5): 181.

[13] Yadav A., Kumari R., Yadav A., Mishra J., Srivatva S., Prabha S. (2016).Antioxidants and its functions in human body-A Review. Res Environ Life Sci, 9(11): 1328-31.

[14] Marino A.L., Consoli D.C., Harrison F.E. Vitamin C and neuroinflammation. Vitamins and Minerals in Neurological Disorders: Elsevier; 2023. p. 439-54.

[15] Leskovec J., Levart A., Perić L., Stojčić M.Đ., Tomović V., Pirman T., et al. (2019).Antioxidative effects of supplementing linseed oil-enriched diets with α-tocopherol, ascorbic acid, selenium, or their combination on carcass and meat quality in broilers. Poultry Science, 98(12): 6733-41.

[16] Aboul-Einien M.H., Kandil S.M., Abdou E.M., Diab H.M., Zaki M.S. (2020).Ascorbic acid derivative-loaded modified aspasomes: formulation, in vitro, ex vivo and clinical evaluation for melasma treatment. Journal of liposome research, 30(1): 54-67.

[17] Moretti M., Fraga D.B., Rodrigues A.L. (2017).Preventive and therapeutic potential of ascorbic acid in neurodegenerative diseases. CNS neuroscience therapeutics, 23(12): 921-9.

[18] Zhang X.-Y., Xu Z.-P., Wang W., Cao J.-B., Fu Q., Zhao W.-X., et al. (2018).Vitamin C alleviates LPS-induced cognitive impairment in mice by suppressing neuroinflammation and oxidative stress. International immunopharmacology, 65: 438-47.

[19] Enogieru A., Omoruyi S. (2022).Exploration of Aqueous Phyllanthus amarus Leaf Extract as a Protective Agent in Mercury Chloride-Exposed Wistar Rats: A Neurobehavioural Study. Journal of Applied Sciences and Environmental Management, 26(4): 629-37.

[20] Enogieru A., Egbon F. (2022).Neurobehavioural and Histological Alterations in Lead Acetate-Exposed Rats Pretreated with Aqueous Leaf Extract of Vernonia amygdalina. African Journal of Biomedical Research, 25(2): 205-13.

[21] Enogieru A., Momodu O. (2022).Neuroprotective effects of Zingiber officinale against lead-induced toxicity in Wistar rats. Nutrire, 48(1): 2.

[22] Montilla P., Barcos M., Munoz M.C., Bujalance I., Munoz-Castaneda J.R., Tunez I. (2005).Red wine prevents brain oxidative stress and nephropathy in streptozotocin-induced diabetic rats. Journal of biochemistry and molecular biology, 38(5): 539.

[23] Cohen G., Dembiec D., Marcus J. (1970).Measurement of catalase activity in tissue extracts. Analytical biochemistry, 34(1): 30-8.

[24] Misra H.P., Fridovich I. (1972).The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry, 247(10): 3170-5.

[25] Nyman M. (1959).Serum hatoglobin; methodological and clinical studies. Scandinavian journal of clinical and laboratory investigation, 11: 1-169.

[26] Ellman G.L. (1959).Tissue sulfhydryl groups. Archives of biochemistry and biophysics, 82(1): 70-7.

[27] Buege J.A., Aust S.D. [30] Microsomal lipid peroxidation. Methods in enzymology. 52: Elsevier; 1978. p. 302-10.

[28] Ellman G.L., Courtney K.D., Andres Jr V., Featherstone R.M. (1961).A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology, 7(2): 88-95.

[29] Drury R., Wallington E. (1980).Carleton's histological technique 5th ed. New York: Churchill Livingstone.

[30] Bondy S.C., Campbell A. Aluminum and neurodegenerative diseases. Advances in neurotoxicology. 1: Elsevier; 2017. p. 131-56.

[31] Loto C.A., Joseph O., Loto R.T., Popoola A. (2014).Corrosion inhibitive behaviour of camellia sinensis on aluminium alloy in H2SO4. International Journal of Electrochemical Science, 9(3): 1221-31.

[32] Ating E., Umoren S., Udousoro I., Ebenso E., Udoh A., Reviews. (2010).Leaves extract of Ananas sativum as green corrosion inhibitor for aluminium in hydrochloric acid solutions. Green Chemistry Letters, 3(2): 61-8.

[33] Usen D.U., Enogieru A.B. (2023).Aluminium Chloride-induced Prefrontal Cortex Toxicity in Wistar Rats: Evidence From Pretreatment with Aqueous Psidium guajava Leaf Extract Dutse Journal of Pure and Applied Sciences (DUJOPAS), 9(3b): 1-10.

[34] Leal F.A., Gonçalves G.D., Soncini J.G.M., Staurengo-Ferrari L., Fattori V., Verri Jr W.A., et al. (2022).Exposure to aluminium chloride during the peripuberal period induces prostate damage in male rats. Acta Histochemica, 124(1): 151843.

[35] Lueptow L.M. (2017).Novel object recognition test for the investigation of learning and memory in mice. Journal of Visualized Experiments, (126): e55718.

[36] Fuster J.M. (2009).Cortex and memory: emergence of a new paradigm. Journal of cognitive neuroscience, 21(11): 2047-72.

[37] Sivakumaran M.H., Mackenzie A.K., Callan I.R., Ainge J.A., O'Connor A.R. (2018).The discrimination ratio derived from novel object recognition tasks as a measure of recognition memory sensitivity, not bias. Scientific reports, 8(1): 11579.

[38] Agrawal M., Singhal M., Semwal B.C., Arora S., Singh B., Sikarwar V., et al. (2024).Neuroprotective Action of Hordenine against the Aluminium Chloride (AlCl3) Induced Alzheimer's Diseases & Associated memory impairment in experimental rats. Pharmacological Research-Modern Chinese Medicine, 12: 100492.

[39] Enogieru A.B., Momodu O.I. (2021).African Medicinal Plants Useful for Cognition and Memory: Therapeutic Implications for Alzheimer's Disease. The Botanical Review, 87(1): 107-34.

[40] Semwal B.C., Singh B., Murti Y., Singh S. (2024).Therapeutic Potential of Ascorbic Acid in the Management of Alzheimer's Disease: An Update. Current Pharmaceutical Biotechnology, 25(2): 196-212.

[41] Lee K.H., Cha M., Lee B.H. (2020).Neuroprotective effect of antioxidants in the brain. International journal of molecular sciences, 21(19): 7152.

[42] Rezayian M., Niknam V., Ebrahimzadeh H. (2019).Oxidative damage and antioxidative system in algae. Toxicology reports, 6: 1309-13.

[43] Gallego-Ríos S.E., Peñuela G.A., Martínez-López E. (2021).Updating the use of biochemical biomarkers in fish for the evaluation of alterations produced by pharmaceutical products. Environmental Toxicology Pharmacology, 88: 103756.

[44] Rai R., Singh S., Rai K.K., Raj A., Sriwastaw S., Rai L. (2021).Regulation of antioxidant defense and glyoxalase systems in cyanobacteria. Plant Physiology and Biochemistry, 168: 353-72.

[45] Kadhim A., Ben Slima A., Alneamah G., Makni M. (2024).Assessment of Histopathological Alterations and Oxidative Stress in the Liver and Kidney of Male Rats following Exposure to Aluminum Chloride. Journal of Toxicology, 2024(1): 3997463.

[46] Haam J., Yakel J.L. (2017).Cholinergic modulation of the hippocampal region and memory function. Journal of neurochemistry, 142: 111-21.

[47] Iyiegbu M.E., Enogieru A.B. (2024).Antioxidant and anticholinesterase activity of rutin in aluminum chloride-exposed Drosophila melanogaster. Comparative Clinical Pathology: 1-8.

[48] Khan K.A., Kumar N., Nayak P.G., Nampoothiri M., Shenoy R.R., Krishnadas N., et al. (2013).Impact of caffeic acid on aluminium chloride-induced dementia in rats. Journal of Pharmacy and Pharmacology, 65(12): 1745-52.

[49] Shubin A.V., Demidyuk I.V., Komissarov A.A., Rafieva L.M., Kostrov S.V. (2016).Cytoplasmic vacuolization in cell death and survival. Oncotarget, 7(34): 55863.

[50] Elizabeth M.A., Samson P., Itohan O.R. (2020).Histomorphological evaluations on the frontal cortex extrapyramidal cell layer following administration of N-Acetyl cysteine in aluminum induced neurodegeneration rat model. Metabolic Brain Disease, 35: 829-39.