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Ionoregulatory Disruption and Acetylcholinesterase Activity in Aluminium Toxicity: Effects of Vitamins C and E

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Summary: To investigate the effects of vitamin C and E on electrolyte profile and the activity of acetylcholinesterase (AChE) in Aluminium (Al) chloride exposed rats, thirty-six male rats were used for this study. The animals were randomly grouped into six (n=6); group I (Control) was given normal saline. Group II (Al only) was exposed to 20mg/kg body weight (BW) of Al. Groups III (Vitamin C only) and IV (Vitamin E only) were administered 200mg/kg BW of vitamin C and vitamin E respectively. Groups V (Al + Vit C) and VI (Al + Vit E) were exposed to 20mg/kg Al and were treated with 200mg/kg vitamin C and vitamin E respectively. Al exposure resulted in a significant ($P<0.05$) increase in plasma calcium and erythrocyte magnesium concentrations compared with control. The erythrocyte sodium concentration of group treated with Al alone was significantly ($P<0.05$) higher by 2.01folds than the control group. While the two vitamins were unable to correct the disruption in calcium homeostasis, they ameliorated the intracellular levels of sodium and magnesium ions. A reduction in the activity of AChE (1378.90±130.02U/L) was observed in erythrocyte of the group exposed to Al when compared to the control (1968.80±283.72U/L). Treatment with vitamins C and E further inhibited erythrocyte AChE activity by 34% and 39% respectively compared to a 30% inhibition by Al only. Positive associations were observed between erythrocyte magnesium and blood sodium, and plasma calcium and erythrocyte sodium levels. Negative associations were however observed between plasma AChE activity and erythrocyte sodium and magnesium levels. In conclusion, vitamins C and E ameliorated ionoregulatory disruptions caused by sub-acute aluminium on only erythrocyte sodium and magnesium levels but not on plasma calcium level and erythrocyte acetylcholinesterase activity.

Keywords*:* Aluminium toxicity, Acetylcholinesterase activity, Ionoregulation disruption, Vitamin C and Vitamin E

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INTRODUCTION

Aluminum (Al) is well known and is the most abundant metal in the earth's crust. It is widely distributed and its extensive use in daily life provides easy exposure to human beings (Kumar and Gill, 2009). It is found in the rocks, soils, water, air and foods which account for its ubiquity in nature. Al-containing diets are mainly corn, yellow cheese, salts, herbs, spices, tea and Al is also found in cosmetics (Yousef, 2004). Al has also been seen to be incorporated into medications such as buffered aspirin, anti-diarrheal products and antacids (Kalaiselvi *et al*., 2015, Kaehny *et al*., 1997, Lione *et al*., 1985) and also added to drinking water for purification purposes (Ochmanski and Barabasz, 2000).

There is no known physiological role for Al within the body and its adverse effects have greater susceptibility due to the predisposition of some genetic polymorphisms (Basant *et al*., 2017). Al has been shown to exert its effects by disrupting lipid membrane fluidity, perturbing iron (Fe), magnesium and calcium homeostasis, and causing oxidative stress (Mailloux *et al*., 2011). Aluminum promotes oxidative stress by inhibiting scavenger enzymes of free radicals such as superoxide dismutase (SOD) and glutathione peroxidase (Barquero, 2016). An increase in aluminium exposure has been observed to increase the risk of developing Alzheimer's disease a neurodegenerative disorder by some 71% in a recent meta-analysis involving eight cohort- and case-controlled studies (Wang *et al*., 2016)

Acetylcholine, a cholinergic neurotransmitter plays a key role in motor function, cognitive function and memory and it is synthesized in the presynaptic neuron by choline acetyltransferase from choline and acetyl-coenzyme A (Voss *et al*., 2008). Acetylcholinesterase enzyme hydrolyses and inactivates acetylcholine to choline and acetate and it controls the transmission of nerve impulses through the cholinergic synapse (Soreq and Seidman, 2001). Cognitive dysfunctions as a result

of changes in the activity of acetylcholinesterase and the alterations in the cholinergic system have been associated with neurodegenerative disorder such as Schizophrenia (Damazio *et al*., 2017).

Vitamins C and E are effective and have a safe dietary administration in a large range of concentrations without harmful side effects and are the ideal antioxidants to increase tissue protection from oxidative stress (Cadenas and Cadenas, 2002). Vitamin C and E had been suggested to play an important role in electrolyte homeostasis (Iribhogbe *et al.,* 2011) and was reported to be useful in maintaining brain acetylcholinesterase activity to normal level when dementia was induced by scopolamine administration (Lee *et al.,* 2001). Vitamin C has ameliorative properties against free radical injury to brain in neurodegenerative disorders (Uttara *et al*., 2009). Vitamin E is an important component in human diet and considered the most effective lipid soluble antioxidant found in biological systems (Atef, 2011) which has a great protective effect in the brain and other organs mopping up free radicals produced by various environmental stressors (Singh *et al*., 2013)

This study evaluated the orally administration of Al as Aluminium chloride on blood electrolyte profile and acetylcholinesterase activity and determined the effects of vitamins E and C supplementation in the rats exposed to aluminium.

MATERIALS AND METHODS

Thirty-six (36) male Wistar rats weighing between 150-200g were purchased from the Department of Anatomy, Faculty of Veterinary Medicine, University of Ibadan and used for this study. The animals were kept under standard conditions of temperature and natural light-dark cycle. All the animals had access to feed and clean water *ad libitum* and all conditions of animal experimentation conformed to the NIH guidelines as outlined in NIH publication 80-23 (NRC, 1985). The animals were allowed to acclimatize for two weeks before the commencement of the study. The study was approved by the Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta.

The animals were randomly divided into six groups comprising of six animals per group and given all administrations by gavage.

Group I (control): administered normal saline based on weight of animal orally for 28.

Group II (Al-treated): 20mg/kg BW of Al in $AlCl_3$ was administered orally for 28 days.

Group III: 200mg/kg BW of vitamin C was administered orally for 28 days.

Group IV: 200mg/kg BW of vitamin E was administered orally for 28 days.

Group V: 20mg/kg BW of Aluminium in $AlCl_3$ + 200mg/kg BW of vitamin C was administered orally for 28days.

Group VI: 20mg/kg BW of Aluminium in $AlCl_3$ + 200mg/kg BW of vitamin E was administered for 28days.

Preparation of Blood plasma and assays:

At the end of the treatment, the animals were sacrificed and blood samples were collected into heparinized tubes by cardiac puncture method under light ether anesthesia after an overnight fast. The whole blood was centrifuged at 4000rpm for 5 minutes to separates plasma and red blood cells. The red blood cells were washed twice with physiological saline before being stored for further biochemical analyses.

Estimation of plasma and erythrocyte electrolyte

Erythrocyte and Plasma electrolytes (sodium, potassium, calcium and magnesium) were determined colorimetrically using commercial diagnostic kits. Plasma and erythrocyte sodium ion concentrations were determined according to the method of Trinder (1951). Plasma and erythrocyte potassium ion concentrations were determined using the method of Terry and Sesin (1958). Plasma and erythrocyte calcium ion concentrations were determined according to the method of Farell (1984) and Plasma and erythrocyte magnesium ion concentrations were determined according to the method of Abernethy and Fowler (1982).

Determination of acetylcholinesterase activity in plasma and erythrocyte

Activity of acetylcholinesterase was determined in both plasma and erythrocyte according to the methods of Ellman *et al*. (1961). The erythrocyte was diluted in distilled water in the ratio 1:50 before carrying out the assay. Reaction involved, 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) with thiocholine liberated from its esters by enzymatic hydrolysis. The yellow colour complex of 5-thio-2-nitrobenzoate (TNB) which was formed over 30 minutes was measured with spectrophotometer at 412 nm.

Statistical Analysis

The results obtained are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey's test was used to analyze the results. Values with $p<0.05$ were regarded as significant using the Statistical Package for Social Sciences (SPSS) version 16.0. Relationships among the parameters were assessed by Pearson's correlations.

RESULTS

Table 1 depicts the plasma concentrations of calcium, sodium, magnesium and potassium. The plasma calcium concentration was significantly ($p< 0.05$) higher in the Aluminium treated and Al + Vit. E treated rats by 3% and 8% respectively and the concentration was significantly ($p< 0.05$) lower in other groups ranging from 13% to 25%, with the lowest concentration observed in the Al + Vit. C treated rats

Table 1: Effects of vitamin C and vitamin E on plasma electrolyte levels in rats exposed to Aluminium

Group	Ca^{2+}(mg/dl)	Na^{+}(mEq/L)	Mg^{2+}(mg/dl)	K^{+}(mEq/L)
I (Control)	9.06±0.43[a]	122.87±9.51[a]	1.86±0.01[a]	1.59±0.27[a]
II (Al only)	9.33±0.22[b]	107.75±16.62[a]	1.52±0.16[a]	1.75±0.12[a]
III (Vit. C)	7.89±0.72[c]	89.88±7.76[a]	1.56±0.20[a]	2.05±0.25[a]
IV (Vit. E)	7.17±0.36[c]	91.25±10.09[a]	1.29±0.13[a]	1.89±0.30[a]
V (Al+Vit.C)	7.11±0.65[c]	95.00±15.65[a]	1.75±0.10[a]	1.56±0.10[a]
VI (Al+Vit.E)	9.81±0.03[b]	101.24±15.98[a]	1.48±0.12[a]	1.33±0.17[a]

Values are mean ± S.E.M (n = 6). Values in a column having no letter (a-c) in common are significantly different from each other ($p<0.05$).

Table 2: Effects of vitamin C and vitamin E on RBC electrolyte levels in rats exposed to Aluminium.

Group	Ca^{2+}(mg/dl)	Na^{+}(mEq/L)	Mg^{2+}(mg/dl)	K^{+}(mEq/L)
I (Control)	6.87±0.57[a]	25.68±3.87[a]	15.88±1.09[a]	85.39±11.00[a]
II (Al only)	7.75±0.57[a]	51.48±6.56[b]	22.17±2.87[b]	55.97±11.80[a]
III (Vit. C)	6.65±0.61[a]	18.06±2.51[a]	13.70±2.27[a]	48.69±5.59[a]
IV (Vit. E)	6.88±0.76[a]	12.80±1.56[a]	16.57±2.80[a]	73.10±5.45[a]
V (Al+Vit. C)	6.66±0.79[a]	14.82±1.81[a]	15.70±2.26[a]	77.50±10.40[a]
VI (Al+Vit. E)	8.43±0.26[a]	16.71±1.67[a]	13.04±2.82[a]	57.88±7.33[a]

Values are mean± S.E.M (n = 6). Values in a column having no letter (a-b) in common are significantly different from each other ($p< 0.05$).

Table 3: Effects of vitamin C and vitamin E on acetylcholinesterase **(AChE)** activity in rats exposed to aluminium

Group	**AChE Erythrocyte (U/L)**	**AChE Plasma (U/L)**	**AChE Erythrocyte activity (% of control)**	**% Inhibition**
I (Control)	1968.80±283.72[a]	177.73±12.73[a]	100	-
II (Al only)	1378.90±130.02[b]	173.75±12.67[a]	70	30
III (Vit. C)	1539.20±60.45[b]	221.83±19.12[a]	78	22
IV (Vit. E)	1250.60±129.06[c]	229.53±10.73[a]	64	36
V (Al+Vit. C)	1290.10±85.42[b]	230.85±23.23[a]	66	34
VI (Al+Vit. E)	1208.30±165.33[c]	221.44±31.85[a]	61	39

Values are mean± S.E.M (n = 6). Values in a column having no letter (a-c) in common are significantly different from each other ($p< 0.05$).

Table 4: Relationship among the parameters.

	Correlation coefficient(r)	**p-value**
Ery Mg → Ery Na	0.371	0.028
Ery K → Ery Na	-0.363	0.032
Pla Ca →Ery Na	0.404	0.016
Ery Mg →Pla Na	0.375	0.027
Ery Ca → Ery AChE	-0.335	0.049
Ery Na → Pla AChE	-0.478	0.004
Ery Mg →Pla AChE	-0.395	0.019

Ery - Erythrocyte; Pla - Plasma

when compared with the control. The plasma sodium, magnesium and potassium levels showed no significant ($p > 0.05$) difference compared with control.

Table 2 reveals the effects of vitamin C and vitamin E on erythrocyte electrolyte levels in rats exposed to aluminium. The erythrocyte concentrations of calcium and potassium of all the test groups showed no significant difference when compared with the control. Sodium and magnesium concentrations of the Al only group were significantly ($p< 0.05$) higher than the control by 2.01 fold and 1.39 fold respectively. All other groups showed no significant ($p> 0.05$) difference in the sodium and magnesium concentration in the erythrocyte.

Table 3 shows the activity of acetylcholinesterase in both the plasma and erythrocyte. While there was no significant ($p> 0.05$) difference in the plasma acetylcholinesterase activity of all the groups when compared to the control, acetylcholinesterase activity in erythrocyte exhibited a different pattern. Compared to control, erythrocyte acetylcholinesterase activity was significantly ($p< 0.05$) lowered in the test groups. The enzyme activity was inhibited in the erythrocyte of other groups by 22% to 39% compared with control. The lowest activity was observed in rats administered with Al + Vit. E. The percentage inhibition in the enzyme activity was highest in the Al + Vit. E group (39%) and lowest in the Vit. C group (22%).

Table 4 depicts the intensity association between and within electrolyte levels and acetylcholinesterase activity. Significant ($p< 0.05$) positive and negative associations were observed between the electrolytes and acetylcholinesterase parameters. Erythrocyte calcium, sodium and magnesium concentrations were inversely correlated with the activity of acetylcholinesterase.

On the contrary, within the electrolytes, plasma calcium and erythrocyte magnesium showed

significant positive correlation with sodium in the erythrocyte and plasma respectively. Also, there was an inverse significant correlation between erythrocyte potassium and erythrocyte sodium (r= -0.363, p=0.032).

DISCUSSION

Heavy metals are widely distributed in the environment and some of them can cause physiological, biochemical and histological disorder (Atef, 2011). Human are exposed to heavy metals by different means or sources which may include contamination from soil, water and food. It is of great importance to humanity to evaluate the hazardous potential possessed by these contaminants.

Controlling fluid distribution, intra- and extracellular acidobasic equilibrium, maintaining osmotic pressure of body fluids and normal neuro-muscular irritability are the basic functions of electrolyte in the body (Harper, 1977). Electrolytes are also responsible for the proper functioning of all types of tissues (Mohanty and Mishra, 1983) and are essential for the activity of many enzymes and the toxicity of heavy metals such as aluminum can alter the concentration of electrolyte in the blood (Kori-Siakpere, 2007).

Reports have shown that reduction in tubular reabsorption of calcium occurs under conditions that depress the renal reabsorption of sodium and as a result high serum calcium level correlates with high serum sodium level (Ita and Edagha, 2016). Aluminium increased the sodium level in the erythrocyte in this study. The ameliorative effects of Vit. C and Vit. E were not visible in this above-mentioned electrolyte. Etang *et al.* (2006) and Iribhogbe *et al.* (2011) also obtained a non-significant increase in serum sodium ion after Vit. C supplementation which corroborated with this study. These findings also agree with the study of Prasad (2010) who reported that during hypercholesterolemic state, Vit. E showed no effect on serum electrolyte (Na^+). This may be as a result of the degree of manifestation of the heavy metal. According to Roy-Chowdhury (2009), manifestations of different metals depend on dose, duration, route of administration and physiological factors, especially nutrition.

The increase in the concentration of calcium in plasma and magnesium in erythrocyte of the aluminium exposed group as observed in this study might be an indicator that aluminium indeed promotes influx of calcium and magnesium in plasma and erythrocyte respectively. Aluminium has been shown to provoke either an increase or a decrease in cytosolic free calcium concentration (Plieth *et al*., 1999). It is also possible that the aluminium like other toxicant such as Nigerian Bonny light Crude Oil (NBLCO) as reported by Oruambo and Jones (2007) might have altered the structure of the membrane of endoplasmic reticulum (ER) and mitochondria which stores calcium, the alteration might cause calcium to leak out from ER and mitochondria to increase cytoplasmic calcium and in turn increase extracellular calcium level (Ita and Edagha, 2016).

A receptor that binds calcium and magnesium known as calcium-sensory receptor has also been described (Brown *et al*., 1998; Handlogten *et al*., 2000; Abam *et al*., 2008). Intracellular calcium and magnesium concentration are controlled by reversible binding to specific calcium-binding protein, whereas, the calcium and magnesium flux across the external membrane is regulated by Ca^{2+}-Mg^{2+} ATPase in the normal red cell (Katz, 1985; Abam *et al*., 2008). Calcium and magnesium homeostasis may therefore be interfered by toxicants either by promoting their influx into or inhibiting their efflux from the cytoplasm (Abam *et al*., 2008). Also inhibiting the ion pumps or depleting the ion pumps of their driving forces may diminish the efflux of these ions (Timbrell, 2000; Gregus and Klaassen, 2003). In this study, rats administered with Vitamin C and E only, shows a decrease in the plasma calcium concentration and an increase in rats exposed to aluminium and treated with Vitamin E. The vitamins do not have any effect on the magnesium in these findings.

Acetylcholinesterase is an enzyme that is responsible for interrupting normal nerve transmission at the synapses by hydrolyzing the neurotransmitter acetylcholine to acetic acid and choline. This transmission interruption ensures that the nervous system is not unnecessarily stimulated (Ademuyiwa *et al*., 2007). Aluminium ion has been shown to alter properties and structure of cellular membrane, inhibiting enzymes like alkaline phosphatase, acetylcholinesterase and adenylcyclase (Platt *et al*., 2001; Qitu *et al.*, 2002).

The findings from this study indicate a lower erythrocyte acetylcholinesterase activity in the Aluminium groups which might in turn decrease the acetylcholinesterase in the brain since there is supporting evidence that living brain tissue intakes aluminum from bloodstream (Barquero, 2016). Al exposure has been reported to cause a significant decrease in acetylcholinesterase activity of the brain (Madhavan *et al.*, 2015, Kumar *et al*., 2009 and Moshtaghi *et al.*, 1999). Madhavan *et al.* (2015) assumed that a slow accumulation of aluminium and the formation of Aluminium complex resulted in the induction of oxidative stress which then inhibits the acetylcholinesterase activity. Aluminium might also interfere with either synthesis of acetylcholine or inhibit choline uptake by synaptosomes (Sadhana, 2013). An altered cholinergic function can result from changes in the activity of acetylcholinesterase and Aluminium has been reported to cause changes in cholinergic function of the CNS, thereby acting as a

cholinotoxin (Gulya *et al.*, 1990) and cause behavioral changes which can be detrimental to the survival of the organism (Maheswari *et al.* 2014**).**

The groups administered Vit. C only as the lowest percentage inhibition on acetylcholinesterase and Vitamin C increased the activity of this enzyme showing that Vit. C play a protective role against oxidative damage and cellular alterations which is in agreement with Damazio *et al*., (2017) which findings show that Vit. C increases the activity of the acetylcholine enzyme within the hippocampus of the brain of mouse.

In conclusion, an important implication of our study is that sub-acute Aluminium exposure might be associated with inhibition of acetylcholinesterase activity which might lead to neurotoxic effect of Aluminium and reveal to some extent an effect on the homeostasis of calcium, sodium and magnesium in plasma and erythrocyte.

Vitamin E showed a positive impact in ameliorating the ionoregulatory disruption caused by sub-acute Aluminium toxicity; Vit. C showed little or no effect on the electrolyte homeostasis in this study. Further research should be considered to know whether a combination therapy of both vitamins would show a positive outcome.

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