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Research Article

Vitamins C and E attenuate lipid dystrophy in tissues of rats administered aluminium

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ABSTRACT: **To investigate the effects of aluminum chloride ($AlCl_3$) in the deviation of tissue lipid profiles and ways to reduce its effect using antioxidant vitamins C and E, thirty-six male albino rats (120-150g) were divided into six groups with six rats each. Group (1) received normal saline and served as control, Group (2) was administered with $AlCl_3$ (20mg/kg body weight b.wt)), Group (3) was administered with vitamin C (200mg/kg b.wt), Group (4) was administered with vitamin E (200mg/kg b.wt), Groups (5) and (6) were administered aluminium (20mg/kg b.wt) along with vitamins C and E (200mg/kg b.wt) respectively. At the end of the experiment, blood samples and organs (liver, testis, heart, kidney and brain) were harvested and used for lipid profile determination. The results showed that oral administration of aluminum significantly ($p<0.05$) increased cholesterol level in plasma and VLDL+LDL and significantly decreased in erythrocyte, HDL and testis. Cholesterogenesis was induced in the brain, liver, kidney and heart. Plasma and VLDL+LDL triglyceride were significantly ($p<0.05$) increased while erythrocyte and brain triglyceride were significantly decreased. Plasma, VLDL+LDL and brain phospholipid levels were significantly ($p<0.05$) decreased and that of erythrocyte significantly increased. There was no significant difference ($p>0.05$) in rats supplemented with vitamin C and vitamin E compared with control. The vitamins significantly attenuated the affected lipid levels in the tissues affected. It was concluded that administration of vitamin C and vitamin E supplements may be used as therapies against the effects of Aluminium exposure on lipids.**

KEYWORDS: **Aluminium exposure, lipid dystrophy, vitamin C, vitamin E, tissues.**

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INTRODUCTION

In past years, there has been an increased interest on Aluminium (Al) and health. Al is ubiquitous in the environment and its widespread use has provoked an interest in its toxicity. Al is usually deposited naturally into the environment either by weathering and erosion or from anthropogenic sources and thus human are frequently exposed to Al by the inhalation of ambient air and the ingestion of food and medications and water (Soni *et al*., 2001; Fatima *et al.,* 2001; Yokel and McNamara, 2001).

High level of Al in diet has led to an increase in the deposition of this metal in tissues such as heart, kidneys, brain and liver which may lead to cardiotoxicity, nephrotoxicity, neutrotoxicity and hepatic dysfunctions (Oteiza *et al.,* 1993; Chinoy and Patel, 1999; Reinke *et al.,* 2003; Al-Demerdash, 2004).

Al is suggested to be involved in the pathogenesis of neurodegenerative diseases like Alzheimer's and Parkinson's diseases (Erasmus *et al.,* 1995; Flaten *et al.,* 2001; John *et al.,* 2015). Al toxicity can lead to Reactive Oxygen species (ROS) formation (Bondy *et al*., 1998) and can also lead to oxidative damage of membrane lipids, proteins and DNA (Shohda *et al.,* 2001; Gonzalez *et al*., 2007; Newairy *et al*., 2009). The generation of these ROS have been related to diseases such as atherosclerosis, inflammatory conditions, neurodegenerative diseases, cancer, diabetes mellitus, renal, pulmonary, cardiac diseases and the process of aging (Young and Woodside, 2001). Experimental animals that are exposed to Al have developed among many dysfunctions, lipid dystrophy (El-Demerdash,, 2004; John *et al.,* 2015).

The use of antioxidants such as vitamins E and C, coenzyme Q, glutathione and selenium ions have been used to prevent lipid peroxidation and cell damage by acting synergistically (Escott-Stump and Mahan 2000). Vitamin C is an effective antioxidant owing to its high electron donating power and its ability to readily convert back to its reduced form (Devesh et al., 2013). It has been reported to play a role in the amelioration of some heavy metal toxicity (Onunkwor *et al.,* 2004; Ugbaja *et al.,* 2013a). Vitamin E (ά- Tocopherol and its derivatives) a potent antioxidant has been found to scavenge lipid peroxyl radical with formation of tocopheroxyl radical thereby inhibiting peroxidation of membrane lipid (Arita *et al*., 1998). It enhances immunity by maintaining the functional and structural integrity of membranes and immune cells (El-Demerdash, 2004).

Several studies on this metal have been done but the exposure has been interperitoneal and the major routes of human exposure to aluminium are actually pulmonary and orally (Testolin et al., 1996). In view of this, the present study investigated the effects of orally administered Al as Aluminium Chloride on lipid profiles of tissues and determined the attenuating properties of vitamin C and vitamin E on aluminum toxicity in rats.

MATERIALS AND METHODS

Experimental design

Thirty-six adult male albino rats (Wistar strain) weighing between 120-150g and divided into six groups (n=6) were used for the study. They were obtained from the Department of Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The animals were kept housed 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). They were allowed two weeks for acclimation prior to experimental treatment.

The rats were divided into six groups and were given all oral administration of the repective treatments. The duration of experiment was 28 days.

Group 1 – Control (administered normal saline based on weight of animal)

Group 2 – Aluminum Chloride ($AlCl_3$) (20 mg/kg body weight (BW))

Group 3 – Vitamin C (200 mg/kg BW)

Group 4 – Vitamin E (200 mg/kg BW)

Group 5 – $AlCl_3$ (20 mg/kg BW) + Vitamin C (200 mg/kg BW)

Group 6 – $AlCl_3$ (20 mg/kg BW) + Vitamin E (200 mg/kg BW)

Blood and organ collection

After twenty-eight days, animals were mildly anaesthetized with diethyl ether and the blood samples were collected with syringes containing heparin into heparinized tubes. Blood samples were separated into plasma and RBC and stored in Eppendorf tubes for further analyses while the RBC were washed thrice with ice-cold physiological saline solution before using for analysis. The brain, heart, testis, kidney and liver were excised, trimmed of connective tissues and rinsed in ice-cold physiological saline solution. They were then blotted dry and 0.2g of each was weighed out and stored at -20 ^{o}C until analyzed.

Plasma and lipoprotein lipid profiles

The plasma concentrations of total cholesterol and triglyceride were determined by spectrophotometric methods using Cypress diagnostic kits. HDL cholesterol and triglyceride were determined in plasma with the same diagnostic kits for total cholesterol and triglyceride after Very Low Density Lipoproteins and Low Density Lipoproteins (VLDL+LDL) were precipitated using the method described by Gidez *et al.* (1982).

For cholesterol, the method is based on the enzymatic hydrolysis of cholesteryl esters to cholesterol and fatty acids by cholesterol esterase. Cholesterol oxidase then oxidizes

cholesterol to produce hydrogen peroxide which condenses with phenol in a reaction catalysed by peroxidase. In the presence of the former and 4-animoantipyrine, a quinoneimine dye is formed which is proportional to the concentration of cholesterol in the sample.

For triglyceride, the method is based on the enzymatic hydrolysis of triglyceride to glycerol and free fatty acids by lipoprotein lipase. The glycerol is phosphorylated by adenosine triphosphate in the presence of glycerol kinase to form glycerol-3-phosphate and adenosine diphosphate. Glycerol-3-phosphate is then oxidized by glycerophosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. A pink chromogen complex, quinoneimine, is produced by a peroxidase catalyzed coupling of 4-aminoantipyrine and phenol with the hydrogen peroxide, and this is proportional to the concentration of triglyceride in the sample.

Briefly, to 0.01ml of sample (plasma/HDL) was added 1.0ml of the cholesterol/triglyceride kit reagent, and incubated for 10 minutes after which absorbance was read at 505nm with a spectrophotometer. Total phospholipids in plasma/HDL were extracted with chloroform-methanol mixture (2:1, v/v) as described by Folch *et al.* (1957). Phospholipid concentration was then assessed with ammonium ferrothiocyanate by the method of Stewart (1980). The method is based on complex formation between ammonium ferrothiocyanate and phospholipids. It allows for phospholipid measurement in the range of 0.01 - 0.10mg (15 - 150nmol). An aliquot of the extract (0.1 ml) was evaporated to dryness at 60°C. After cooling, 2 ml of chloroform was added to the dried extract, mixed and 2 ml of ammonium ferrothiocyanate was then added and then mixed for 1 min. The mixture was left for 10 min for separation to occur. The chloroform layer was then taken and the absorbance read at 488 nm. Phospholipid concentrations were determined using a phospholipid standard as reference.

Organ, VLDL+LDL and erythrocyte lipid profiles

Lipids were extracted from erythrocytes using the method of Rose and Oklander (1965) and from the organs (liver, kidney, heart, testes and brain) and VLDL+LDL as described by Folch *et al.* (1957). Homogenates of the organs (10%) were prepared in choloroform-methanol (2:1v/v) mixture. The mixtures were then centrifuged at 4000rpm for 10 minutes and the supernatants containing the lipid were removed into clean Eppendorf tubes. After washing the extracts with 0.05M KCl solution, aliquots of the extracts (0.1ml) were then used for the determination of cholesterol, triglycerides and phospholipid concentrations as described by Ugbaja *et al.* (2013a, b). Briefly, for cholesterol determination, 0.1 ml of the extract was evaporated to dryness at 60^{o}C and 20µl of Triton X-100/chloroform mixture (1:1, v/v) was added to the dried extract for resolution. This was evaporated again and then 1 ml of the cholesterol kit reagent was added, mixed and incubated for 30 minutes before reading the absorbance at 505nm in a spectrophotometer. For triglyceride determination, 0.1ml of extract was evaporated to dryness and 0.1 ml of 97% ethanol was added to re-suspend the dried lipid. To this, 1 ml of the triglyceride kit reagent was added, mixed and incubated for 30 minutes before absorbance was read. For phospholipid determination however, the method of Stewart (1980) was employed.

Statistical analysis

The results obtained are expressed as mean ± SD. 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.

RESULTS

Table 1 shows the effect of the treatments on plasma and erythrocyte lipid profiles. While significant increases ($p<0.05$) were observed in the plasma levels of cholesterol (50%) and triglyceride (30%), significant decreases of 42% and 21% were observed in the erythrocyte compared with control. A decrease ($p<0.05$) of 40% was however observed in plasma phospholipid level while it increased by 37% in the erythrocyte when compared with control. On the part of the antioxidants, they did not cause a significant ($p>0.05$) perturbation in the plasma and erythrocyte rather they attenuated the effects of Al in the compartments. Vitamin E proved to be a better agent against the effect of Al than vitamin C, though not significantly ($p<0.05$).

Table 1: Plasma and erythrocyte lipid profiles (mg/dl) of animals in the different treatment groups.

Groups	Triglyceride	Cholesterol	Phospholipid
Plasma			
1 (Control)	99.28±5.91^{a}	81.14±7.31^{a}	158.99±25.33^{a}
2 ($AlCl_3$)	128.61±9.03^{b}	121.47±8.67^{b}	95.87±20.55^{b}
3 (Vit. C)	88.72±7.25^{a}	91.14±7.63^{a}	148.36±24.92^{a}
4 (Vit. E)	97.42±9.13^{a}	86.28±9.10^{a}	135.35±27.65^{a}
5 ($AlCl_3$ + Vit. C)	110.87±10.27^{a}	95.22±5.14^{a}	152.84±21.13^{a}
6 ($AlCl_3$ + Vit. E)	106.55±10.60^{a}	93.73±6.90^{a}	135.85±29.77^{a}
Erythrocyte			
1 (Control)	36.23±3.02^{a}	98.37±2.59^{a}	101.77±4.49^{a}
2 ($AlCl_3$)	21.05±1.76^{b}	74.24±5.58^{b}	140.18±8.06^{b}
3 (Vit. C)	38.06±1.24^{a}	94.61±5.44^{a}	105.16±7.74^{a}
4 (Vit. E)	35.62±1.36^{a}	88.78±5.54^{a}	97.55±6.69^{a}
5 ($AlCl_3$ + Vit. C)	36.45±2.92^{a}	96.04±7.00^{a}	108.51±6.92^{a}
6 ($AlCl_3$ + Vit. E)	32.95±1.28^{a}	84.26±6.54^{a}	119.51±5.62^{a}

Values are mean ± SD (n=6). Values having different superscripts are significantly different from one another at $p<0.05$

Table 2: HDL and VLDL+LDL lipid profiles (mg/dl) of animals in the different treatment groups.

Groups	Triglyceride	Cholesterol	Phospholipid
HDL			
1 (Control)	25.57±2.14^a	36.42±1.40^a	196.24±12.65^a
2 ($AlCl_3$)	25.78±4.66^a	25.94±1.77^b	201.15±10.71^a
3 (Vit. C)	21.62±4.40^a	35.74±1.11^a	180.28±5.65^a
4 (Vit. E)	28.90±9.29^a	40.37±0.83^a	207.03±8.76^a
5 ($AlCl_3$ + Vit. C)	26.07±3.03^a	33.76±1.63^a	186.29±4.79^a
6 ($AlCl_3$ + Vit. E)	27.98±6.75^a	39.29±2.94^a	192.41±9.38^a
VLDL+LDL			
1 (Control)	69.40±5.80^a	59.25±2.00^a	211.94±10.48^a
2 ($AlCl_3$)	107.89±2.70^b	90.40±3.28^b	174.74±16.45^b
3 (Vit. C)	69.12±5.15^a	60.62±2.69^a	210.26±16.45^a
4 (Vit. E)	77.75±4.31^a	54.18±4.96^a	213.31±5.66^a
5 ($AlCl_3$ + Vit. C)	82.44±4.28^a	63.09±1.95^a	190.69±12.75^{ab}
6 ($AlCl_3$ + Vit. E)	80.89±3.36^a	68.15±2.72^a	184.16±13.99^{ab}

Values are mean ± SD (n=6). Values having different superscripts are significantly different from one another at p<0.05.

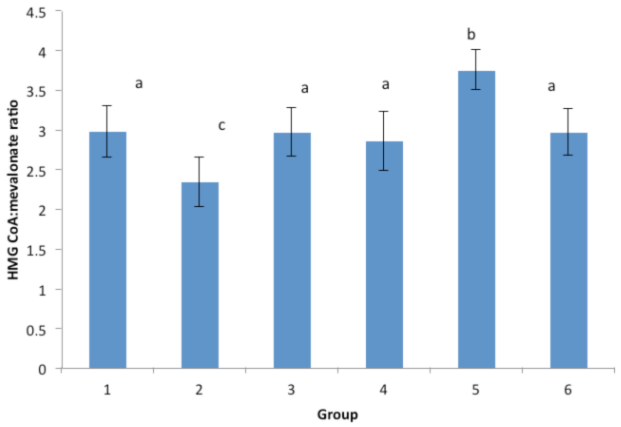

Figure 1: Liver HMG CoA: mevalonate ratio as an index of HMG CoA reductase activity. Each bar represents mean ± SD (n=6). Bars with different alphabets are significantly different at p<0.05. (1= Control, 2 = AlCl3, 3 = Vitamin C, 4 =Vitamin E, 5 = $AlCl_3$ + Vitamin C, 6 = $AlCl_3$ + Viatmin E).

Table 3: Brain and heart lipid profiles (mg/g tissue) of animals in the different treatment groups.

Groups	Triglyceride	Cholesterol	Phospholipid
Brain			
1 (Control)	1.45±0.13^a	12.15±0.98^a	46.24±1.55^a
2 ($AlCl_3$)	1.08±0.14^b	18.81±0.69^b	29.95±2.12^c
3 (Vit. C)	1.48±0.11^a	9.85±0.42^a	38.10±2.52^b
4 (Vit. E)	1.53±0.16^a	10.22±0.55^a	36.17±1.13^b
5 ($AlCl_3$ + Vit. C)	1.40±0.13^a	10.29±0.55^a	34.34±1.12^b
6 ($AlCl_3$ + Vit. E)	1.39±0.16^a	11.35±1.70^a	34.95±1.03^b
Heart			
1 (Control)	3.45±0.25^a	0.48±0.07^a	13.83±1.85^a
2 ($AlCl_3$)	3.25±0.49^a	0.86±0.04^b	16.29±2.78^a
3 (Vit. C)	3.28±0.49^a	0.39±0.06^a	9.81±1.60^a
4 (Vit. E)	3.45±0.39^a	0.38±0.05^a	12.33±1.48^a
5 ($AlCl_3$ + Vit. C)	3.11±0.78^a	0.46±0.06^a	14.31±3.07^a
6 ($AlCl_3$ + Vit. E)	3.23±0.76^a	0.39±0.09^a	15.34±2.29^a

Values are mean ± SD (n=6). Values having different superscripts are significantly different from one another at p<0.05.

Table 2 depicts the effect of the treatments on HDL and VLDL+LDL lipid profiles. Only cholesterol level was affected in HDL. Al intoxication affected a 29% decrease in the reverse cholesterol transport compared with control. The effect on VLDL-LDL was very similar to what was observed in the plasma. Al intoxication resulted in 35% and 30% significant increases in cholesterol and triglyceride level respectively while reducing the VLDL+LDL phospholipid level by 17% compared with control. The two antioxidants attenuated the effects of Al in these lipoprotein fractions to almost the same levels which were not significantly different (p>0.05) from the other even compared with control.

Tables 3 and 4 show the effects of the treatments on the organs. Triglyceride level in the Al administered group was increased significantly (p<0.05) by 13% and 26% in the liver and brain respectively while it was not perturbed in the heart, testis and kidney compared with control. Cholesterol level was however only increased in the heart, liver and brain of the Al intoxicated group by 79%, 49% and 41% respectively and decreased by 52% in the testis compared with control. In all the organ compartments, only the brain phospholipid level was significantly (p<0.05) altered by a 35% decrease in comparison with control. Vitamins C and E prevented the effects of Al in the organs of the animals as observed by the insignificant differences (p>0.05) of the levels of the lipids.

However, in the brain, the antioxidants were unable to effectively attenuate the decreased phospholipid level as the resultant levels were still significantly different ($p<0.05$) from the control.

Figure 1 shows the effect of the treatments on liver HMG CoA: mevalonate ratio as an index of HMG CoA reductase activity. An increase in this ratio signifies an inhibition of cholesterogenesis while a decrease indicates enhanced cholesterogenesis. HMG CoA reductase activity in the liver was significantly increased ($p<0.05$) at a rate of 23% in the Al-administered group compared with control. While the activity of the enzyme was not disrupted by the antioxidants (Figure 1), only vitamin C further significantly ($p<0.05$) inhibited cholesterogenesis by 42% from the Al induced level while vitamin E reversed the effect of Al to a non-significant activity compared with control.

Table 4: Liver, kidney and testis lipid profiles (mg/g tissue) of animals in the different treatment groups.

Groups	Triglyceride	Cholesterol	Phospholipid
Liver			
1 (Control)	2.08±0.19^a	2.91±0.77^a	19.23±1.33^a
2 ($AlCl_3$)	1.81±0.06^b	4.34±0.20^b	20.58±1.76^a
3 (Vit. C)	2.00±0.08^a	2.95±0.13^a	18.13±2.07^a
4 (Vit. E)	2.14±0.09^a	3.01±0.57^a	18.77±2.02^a
5 ($AlCl_3$ + Vit. C)	2.25±0.11^a	3.04±0.51^a	18.33±1.46^a
6 ($AlCl_3$ + Vit. E)	2.14±0.02^a	2.62±0.24^a	17.87±0.41^a
Kidney			
1 (Control)	6.63±0.03^a	8.82±0.65^a	17.01±2.28^a
2 ($AlCl_3$)	6.91±0.05^a	9.08±0.50^a	21.88±2.06^a
3 (Vit. C)	6.54±0.15^a	8.01±0.39^a	17.49±1.17^a
4 (Vit. E)	6.92±0.07^a	8.27±0.28^a	18.96±2.12^a
5 ($AlCl_3$ + Vit. C)	7.02±0.08^a	8.51±0.90^a	19.11±1.27^a
6 ($AlCl_3$ + Vit. E)	6.91±0.19^a	8.77±0.72^a	19.56±0.79^a
Testis			
1 (Control)	2.11±0.71^a	4.94±0.37^a	21.54±1.22^a
2 ($AlCl_3$)	2.72±0.60^a	2.39±0.18^b	21.40±1.11^a
3 (Vit. C)	1.81±0.48^a	4.26±.27^a	20.86±1.01^a
4 (Vit. E)	2.27±0.54^a	4.83±0.36^a	20.66±1.77^a
5 ($AlCl_3$ + Vit. C)	1.96±0.36^a	4.36±0.14^a	18.54±0.79^a
6 ($AlCl_3$ + Vit. E)	1.98±0.34^a	4.14±0.41^a	20.26±1.26^a

Values are mean ± SD (n=6). Values having different superscripts are significantly different from one another at $p<0.05$.

DISCUSSION

Aluminum (Al) is known to be toxic to humans and animals. Its toxicity results to generation of reactive oxygen species which leads to oxidative damage of biomolecules in an organism (Shohda *et al.,* 2001; Gonzalez *et al.,* 2007; Newairy *et al.,* 2009). Of these biomolecules are the lipids which play important roles in the body system whether in circulation or in organs. A lipid dystrophy which could be lipotoxic or non-lipotoxic could play a role in the pathogenesis or progression of a plethora of diseases. The present study investigated the effects of natural antioxidants against the harmful effects of $AlCl_3$ in the blood and organ of rats.

The findings of this study were that Al perturbed the metabolism of lipids (cholesterol, triglyceride and phospholipid) in different compartments in the organism. These perturbations were reflected in the up-/down-regulation of the levels of these lipids. This suggests that the metabolism of these lipids by their respective enzymes may have been up- or down-regulated due to exposure to Al. These perturbations were presented in the plasma as hypertriglyceridemia, hypercholesterolemia and hypophospholipidemia. The same trend was showcased in the VLDL+LDL fraction while only the reverse cholesterol transport mechanism indicated as HDL cholesterol, was significantly decreased. Also, the reverse of the effects of Al exposure in plasma was manifested in the erythrocyte while in the organs; cholesterogenesis was induced in the heart, liver and brain. A decreased cholesterol level was indicated in the testis. On the other hand, a dystrophy in the triglyceride level was presented in only the liver and brain. Treatments with the antioxidants (vitamin C and E) proved effective in reversing the effects of Al exposure in the various tissues.

The increase in plasma cholesterol as a result of ingestion of aluminum agrees with the results of other researchers (El-Demerdash, 2004; Fahid, 2009; Abdel Aziz and Zabut, 2011 and John et al., 2015). This increase probably indicates a loss of membrane integrity (Sarin *et al.,* 1997). This assumption of loss of membrane integrity as a result of Al has been confirmed by Newairy et al. (2009), where they reported Al significantly affecting various membrane-bound enzymes.

HDL cholesterol of the animals administered with Al was decreased compared to other groups including the control. This agrees with the report of John et al. (2015). The decrease in the HDL cholesterol indicated that the reverse cholesterol transport is affected and presents a risk factor for cardiovascular disease, atherosclerosis and ischemia (a reduction in blood flow to the brain) (EL-Kholy *et al*., 2010). HDL is a lipoprotein which picks cholesterol from extrahepatic tissues to the liver for degradation (Murray *et al.,* 2003). The decreased level of cholesterol with an observed concomitant increase in plasma cholesterol indicates the increase associated with the plasma is arising from the increased VLDL+LDL cholesterol level. LDL fraction of the VLDL+LDL takes up cholesterol from the liver (with 49% increase) to extrahepatic tissues hence the accumulation of cholesterol in heart and brain and not testis. The decreased cholesterol level observed in this study corroborates the findings of Kutlubay et al. (2007). Several mechanisms could be adduced for this; (1) Al ingestion probably caused a preferential activation of receptor sites on the cells of the heart and brain for the uptake of cholesterol but not the testis,

(2) Al could have favoured the synthesis of cholesterol in these organs apart from the testis by up-regulating hydroxymethylglutaryl coenzyme A reductase (a rate-limiting enzyme in cholesterol synthesis pathway) since virtually all cells can synthesize cholesterol (3) Al could have compromised the integrity of the cell membrane thereby causing a constipation of cholesterol in the organs by modification of the composition, structure and stability of the cell membranes, such that HDL could not pick up this lipid from these organs.

The decreased level of cholesterol in the testis indicates that Al exposure could lead to a reproductive dysfunction and consequently infertility (Yousef *et al.,* 2005). Al intoxication has been linked to various diseases like cardiovascular diseases (Young and Woodside, 2001). This is confirmed in this study as seen in increased levels of cholesterol in plasma, LDL and heart.

In the erythrocytes, the levels of lipids were presented as a reverse of what is obtained in the plasma (Table 1). A major pathway for the replacement of erythrocyte lipids is through exchange with plasma lipoproteins (Czarnecka and Yokoyama, 1996). Nikolic et al. (2007) reported that there is a rapid exchange of lipids between the HDL fraction and erythrocytes and that HDL cholesterol may be a determinant factor in the lifespan of erythrocytes. Decreased HDL cholesterol was observed in this study. Our result is in line with this report as the same pattern of decreased cholesterol level was observed in these two compartments.

Al exposure resulted in increased triglyceride levels in the plasma. This is in agreement with the findings of El-Demerdash (2004), Abdel Aziz and Zabut (2011) and John *et al.* (2015). VLDL-LDL fraction had an increased level of triglyceride. The major lipid in VLDL is triglyceride which is endogenously synthesized by the liver. Contrary to expected result for liver triglyceride level, a decrease was observed. This result could not be elucidated as yet, but it could be considered thus; that the membranes of the cells have actually been compromised such that a leakage of the lipid ensued from the organ into the extracellular pool giving rise to one of the cardiovascular risk factor (hypertriglyceridemia). The increased levels of VLDL and LDL (ß-lipoproteins) in Al treated rats have been shown to be cytotoxic to cells and tissue, presumably due to enhanced levels of associated lipid peroxidation (Zenker *et al*., 1986). Previous study reported that during hyperlipemia, cell injury and lipid accumulation may result so as to initiate and maintain atheromatous lesions by lipoprotein (Harker *et al*., 1976).

A decrease in the level of phospholipid which is in accordance with the results of Yousef (2004) was observed. Al has high affinity for phosphate group and binds to the phospholipid head groups using electrostatic force disturbing the dynamic parameters of the lipid bilayer (Martin, 1986).This resulted to a decrease in VLDL+LDL phospholipid level, implying that Al induced a failure in hepatic provision of phospholipid in the lipoprotein (Murray *et al.,* 2003). This could also be the reason why the brain also had a decreased level of phospholipid.

This report demonstrated that there was no significant difference in the total lipids in the blood and organs of albino rats administered Al and supplemented with vitamin E and vitamin C indicating that the two antioxidant vitamins could attenuate the effects of exposure to Al. This is in accordance with the reports of Ithayarasi and Devi (1997), Chinoy and Memon (2001), El-Demerdash (2004), Yousef et al. (2005), Kutlubay *et al.* (2007) and Atef (2011) but disagrees with that of Abdel Aziz and Zabut (2011). The difference in the results could be to the dose of supplement administered. While they administered 150mg/kg body weight to the rats, we administered 200mg/kg body weight. While a lot of researches abound for vitamin E in Al intoxication studies, a paucity of literature is found for vitamin C in this metal study. Al has been proved to alter the oxidant status in organisms (Abubakar et al., 2003). Vitamin C as an antioxidant could prevent Al from forming lipid peroxidation products or once formed, scavenge them a render the harmless in the organism (Onunkwor *et al.,* 2004). Vitamin E on the other hand, allows free radicals to abstract hydrogen from its molecule rather than from the polyunsaturated fatty acids, thus breaking the chain of free radical reactions (Pascoe *et al.,* 1987) and neutralizing lipid peroxidation and unsaturated membrane lipids (Aldana et al., 2001; Tuiguta et al., 2006). This therefore maintains the structure and functional integrity of the membrane.

In conclusion, the present study demonstrated that oral administration of $AlCl_3$ at a dose of 20 mg/kg daily for 28 days caused a dystrophy in lipid in blood and organs. Administration of vitamins C and E at doses of 200mg/kg each tended to minimize the effects of Aluminium. Hence, supplementation with these vitamins may be useful as therapies in persons exposed with Aluminium.

REFERENCES

Abdel-Aziz, I. and Zabut, B. (2011) Determination of blood indices of albino rats treated with aluminium chloride and investigation of antioxidant effects of vitamin E and C. *Egyptian J. Biol.* 13:1-7.

Abubakar, M. G., Taylor, A. and Ferns, G. A. A. (2003) Aluminium administration is associated with enhanced hepatic oxidant stress that may be offset by dietary vitamin E in the rat. *Int. J. Exp. Pathol.* 84(1): 49-54.

Aldana, L., Tsutsumi, V., Craigmill, A., Silveira, M. I. and De Mejia, E. G. (2001) α-tocopherol modulates liver toxicity of the prethroid cypermethrin. *Toxicol. Lett.* 125: 107-116.

Arita, M., Sato, Y., Arai, H. and Inoue, K. (1998) Binding of alphatocopherylquinone, an oxidized form of alpha-

tocopherol, to glutathione S-transferase in the liver cytosol. *FEBS Lett,* 436: 424–426.

Atef, M. A. (2011) Antioxidant effects of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. *Saudi J. Biol. Sci.* 18: 63-72.

Bondy, S. C., Guo-Ross, S. X. and Truong, A. T. (1998) Promotion of transition metal-induced reactive oxygen species formation by beta-amyloid. *Brain Res.* 799(1): 91-96.

Chinoy, N. J. and Patel, T. N. (1999) Reversible toxicity of fluoride and aluminium in liver and gastrocnemium muscle of female mice. *Fluoride.* 32: 215-229.

Chinoy, N. J. and Memon, M. R. (2001) Beneficial effects of some vitamins and calcium on fluoride and aluminium toxicity on gastrocnemium muscle and liver of male mice. *Fluoride.* 34: 21-33.

Czarnecka, H. and Yokoyama, S. (1996) Regulation of cellulat cholesterol efflux by lecithin:cholesterol acyltransferase reaction non-specific lipid exchange. *J. Biol. Chem.* 271: 2023-2028.

Devesh, K. J., Manisha, C., Sandeep, T., Amit, P. and Abbas, A. M. (2013) Docosahexaenoic acid reverses aluminium induced alteration in vitamins and lipid profiles in rat serum. Int. J. Pharm. Biol. Sci. 4(1): (B) 485 – 493.

El-Demerdash, F. M. (2004) Antioxidant effects of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *J. Trace Elem. Med. Biol.* 18: 113-121.

El-Demerdash, F. M., Yousef, M. I., Kedwany F. S. and Baghdadi, H. H. (2004) Role of α-tocopherol and β-carotene in ameliorating the fenvalerateinduced changes in oxidative stress, hematobiochemical parameters and semen quality of male rats. *J. Environ. Sci. Health Bull.* 39: 443-459.

EL-Kholy, W. M.., EL-Habibi, E. M. and Mousa, A. T. (2010) Oxidative stress in brains of rats intoxicated with aluminum and the neuromodulating effect of different forms of sage. *Journal of American Science.* 6 (12).

Erasmus, R. T., Kusnir, J., Stevenson, W. C., Lobo, P., Herman, M. M., Wills, M. R. and Savory, J. (1995) Hyperaluminemia associated with liver-transplantation and acute-renal-failure. *Clin. Transplant.* 9:307-311.

Escott-Stump, S. and Mahan, L. K. (2000) Krause's Food, Nutrition, and Diet Therapy. 10th ed. WB Saunders, Philadelphia, PA.

Fahid, A. (2009) Camel's mill protects against aluminium chloride induced toxicity in the liver and kidney of white albino rats. *American Journal of Biochemistry and Biotechnology.* 5(3): 98-109.

Fatima, S. K., Prabhavathi, P. A., Padmavathi, P. and Reddy, P. P. (2001) Analysis of chromosomal aberrations in men occupationally exposed to cement dust. *Mutation. Res.* 490: 179-186.

Flaten, T. P. (2001) Aluminium as a risk factor in Alzheimer's disease with emphasis on drinking water. *Brain Res. Bull.* 55: 187-196.

Folch, M., Lees, M. and Stanley, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 97-509.

Gidez, L. L., Miller, G. J., Burnstein, M., Slagle, S and Eder, H. A. (1982) Separation and quantitation of subclass of human plasma high density lipoproteins by a simple precipitation procedure. *J. Lipids Res.* 23: 1206-1223.

Gonzalez, M. A., Alvarez, M. L. and Pisani, G. B. (2007) Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminum to rats. *Biol. Trace Elem. Res.* 116: 329-348.

Harker, L. A., Ross, R., Slichter, S. J. and Scott, C. R. (1976) Homocystine-induced arteriosclerosis: The role of endothelial cell injury and platelet response in its genesis. *J. Clin. Invest.* 58(3): 731-741.

Ithayarasi, A.P. and Devi, C. S. (1997) Effects of alpha-tocopherol on lipid peroxidation in isoproterenol induced myocardial infarction in rats. *Indian J. Physiol. Pharmacol.* 41: 369-376.

John, J., Nampoothiri, M., Kumar, N., Mudgal, J., Nampurath, G. K. and Chanmallamudi, M. R. (2015) Sesamol, a lipid lowering agent, ameliorates aluminium chloride induced behavioral and biochemical alterations in rats. *Pharmacog. Mag.* 11(42): 327-336.

Kutlubay, R., Oguz, E. O., Car, B., Guven, M. C., Sinik, Z. and Tuncay, O. L. (2007) Vitamin E protection from testicular damage caused by intraperitoneal aluminium. *Int. J. Toxicol.* 26: 297-306.

Martin, R. B. (1986) The chemistry of aluminum as related to biology and medicine. *Clin. Chem.* 32: 1797–1806.

Murray, R. K., Granner, D. K., Mayes, P. A. and Rodwell, V. W. (2003) Harper's illustrated Biochemistry. . 26th edition. Pp: 111-235 McGraw Hill, Asia.

National Research Council. (1985) Guide for the care and use of laboratory animals. Publication no. 85-23 (Rev). National Institute of Health, Bethesda, MD.

Newairy, A. A., Salama, A. F., Hussein, H. M. and Yousef, M. I. (2009) Propolis alleviates aluminium induce lipid peroxidation and biochemical parameters in male rats. *Food Chem. Toxicol.* 47: 1093-1098.

Nikolic, M., Stanic, D., Baricevic, I., Jones, D. R., Nedic, O. and Niketic, V. (2007) Efflux of cholesterol and phospholipids derived from the haemoglobin-lipid adduct in human red blood cells into plasma. *Clinical Biochemistry,* 40:305-309.

Onunkwor, B., Dosumu, O., Odukoya, O. O., Arowolo, T. and Ademuyiwa, O. (2004) Biomarkers of lead exposyre in petrol station attendants and auto-mechanics in Abeokuta, Nigeria:

effects of 2-week ascorbic acid supplementation. Environ. *Toxicol. Pharmacol.* 17: 169-176.

Oteiza, P. L., Keen, C. L., Han, B. and Golub, M. S. (1993) Alumiinium accumulation and neurotoxicity in swiss-webster mice after long-term dietary exposure to aluminium and citrate. *Metabolism.* 42: 1296-1300.

Pascoe, G., Olafsdottier, F. and Read, D. (1987) Vitamin E protection against chemical-induced cell injury. 1. Maintenance of cellular protein thiols as a cytoprotective mechanism. *Arch. Biochem. Biophys.* 256: 150-158.

Reinke, C. M., Breitkreutz, J. and Leuenberger, H. (2003) Aluminium in over the-counter drugs: risks outweight benefits? *Drug Safe.* 26: 1011-1025.

Rose, H. G. and Oklander, M. (1965) Improved procedure for the extraction of lipids from human erythrocytes. *Journal of Lipid Research.* 6: 428–431.

Sarin, S., Gupta, V. and Gill, K. D. (1997) Alterations in lipid composition and neuronal injury in primates following chronic aluminium exposure. *Biol. Trace Elem. Res.* 59: 133-143.

Shohda, A. E., Mohamed, Z. G., Atef, T. F. and Mohamed, A. H. (2001) Effects of cadmium and aluminium intake on the antioxidant status and lipid peroxidation in rat tissues. *J. Biochem. Mol. Toxicol.* 15(4): 207-214.

Soni, M. G., White, S. M., Flamm, W. G. and Burdock, G. A. (2001) Safety evaluation of dietary aluminum. *Regulatory Toxicology and Pharmacology.* 33(1): 66–79.

Stewart, J. (1980) Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal. Biochem.* 104: 10-14.

Testolin, G., Ciappelliano, D. and Bermano, G. (1996) Influence of organic acid on aluminium absorption and storage in rat tissues. *Food additives and Contamination.* 13: 21-27.

Turguta, G., Enlib, Y., Kaptanoglub, B. Turguta,S. (2006) Changes in the level of MDA and GSH in mice serum, liver and spleen after aluminium administration. *Eastern J. Med.* 11: 7-12.

Ugbaja, R. N., Onunkwor, B. O. and Omoniyi, D. A. (2013a) Lead induced dyslipidemia: The comparative effects of ascorbate and chelation therapy. *African Journal of Biotechnology* 12(15): 1845-1852.

Ugbaja, R. N., Owoeye, F. D., Dosumu, O. A., Onunkwor, B. O., Rotimi, S. O., Ademuyiwa, O., Fayemi, A. E., Oginni, F. F. and Ogundana, D.A. (2013b) Beneficial effects of omega-3 fatty acid on dyslipidemia in organs of alloxan-induced diabetic rats. *European Journal of Experimental Biology*. 3(6): 303-310.

Yokel, R. A. and McNamara, P. J. (2001) Aluminium toxicokinetics: an updated minireview. *Pharmcol. Toxicol.* 88(4): 159-167.

Young, I. S. and Woodside, J. V. (2001) Antioxidants in health and disease. *J. Clin. Pathol.* 54: 176–186.

Yuosef, M. I. (2004) Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. *Toxicol.* 199:47-57.

Yousef, M. I., El-Morsy, A. M. and HHassan, M. S. (2005) Aluminium-induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: protective role of ascorbic acid. *Toxicology.* 215: 97-107.

Zenker, G., Koltringer, P., Bone, G., Niederkorn, K., Pfeiffer, K. and Jurgens, G. (1986) Lipoprotein(a) as a strong indicator for cerebrovascular disease. *Stroke.* 17(5): 942-945.