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# Effects of ethanol leaf extract of *Vernonia amygdalina* on some indices of liver function, oxidative stress and lipid profile in aluminium chloride intoxicated male Wistar rats

**Edet Okon Akpanyung[1], Utibe Evans Bassey[2], Utonne Texubong Noah[1], and Grace Sylvester Effiong[1]**

[1]Department of Biochemistry, University of Uyo, Uyo, Akwa Ibom State, Nigeria
[2]Department of Biochemistry, Obong University, Etim Ekpo, Akwa Ibom State, Nigeria

Author for Correspondence: edetakpanyung@gmail.com; +234(0)7037917688

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ABSTRACT: Aluminium is a widely distributed element with established toxicity to the liver. The present study evaluated the protective potential of ethanol leaf extract of *Vernonia amygdalina* against the toxic effects of aluminium chloride in male Wistar rats by measurement of some indices of liver function, oxidative stress and lipid profile. Twenty-five (25) male Wistar rats weighing 180 – 220g were randomly divided into five groups of five animals per group. Group 1 served as control and was given normal drinking water. Group 2 received 100 mg/kg body weight of aluminium chloride while Group 3 was administered 400 mg/kg body weight of ethanol leaf extract of *Vernonia amygdalina* for 14 days each. Group 4 received 400 mg/kg body weight of ethanol leaf extract of *Vernonia amygdalina* and 100 mg/kg body weight of aluminium chloride concomitantly for 14 days while Group 5 received 100 mg/kg body weight of aluminium chloride for 14 days and was allowed another period of 14 days without treatment. Assay for the activity of some diagnostic enzymes in serum and histological assessment of the liver were carried out. Parameters of oxidative stress and lipid profile were also assayed. Aluminium chloride significantly ($p < 0.05$) increased the activities of ALT, AST, and ALP, concentrations of MDA, total cholesterol, triglyceride and LDL-cholesterol compared to control. However, the leaf extract effectively suppressed the $AlCl_3$-induced increase in enzyme activities, increased the activities of SOD and catalase, reduced the concentrations of total cholesterol and triglyceride as well as MDA in Group 4. Histological assessment of the hepatocytes corroborated the observed changes in enzyme activities. It is therefore concluded that ethanol leaf extract of *Vernonia amydalina* has protective effect against aluminium chloride induced hepatotoxicity, oxidative stress and alterations in lipid profile in male Wistar rats.

**Keywords**: Aluminium Chloride, Liver Function, Lipid Profile, Oxidative Stress, *Vernonia amygdalina*

## Introduction

Aluminium is the third most abundant element in the earth's crust and constitutes about 8% of the total mineral components [1] It is widely used in the manufacture of cosmetics, cookware, food additives and toothpaste [2]. It serves as a component of pharmaceuticals such as antacids, buffered aspirin, vaccines and injectable allergens [3-5]. Aluminium is also used in the purification of drinking water [6, 7]. Industrial waste and particulate matter generated by cement producing factories contain high amount of aluminium

and individuals who reside around the vicinity are exposed to high levels of this metal [8]. Food sources of aluminium include corn, yellow cheese, salt, herbs, spices and tea [9].

Human exposure to aluminium is inevitable because of its presence food, pharmaceuticals and drinking water [10, 11]. The average normal daily intake of aluminium for adults is 1-10 mg [12]. It has been reported that aluminium is poorly absorbed after oral intake and in plasma 80-90% of this element is transported bound to transferrin [13]. Aluminium is usually excreted from the body through urine [14, 15].
Al-Kahtani, [16] reported that the ingestion of excessive amounts of aluminium may cause damage to various organs such as liver, kidneys, bone, lungs and heart. Aluminium has been implicated in the pathogenesis of Alzheimer's and Parkinson's diseases [17]. The toxicity of aluminium was observed to be mediated by the generation of free radicals hence various antioxidant compounds and plant extracts are reported to play a role in ameliorating the toxic effects of this element [13, 18-21].

*Vernonia amygdalina* Del. (commonly known as bitter leaf) is a shrub 2 – 5 m tall that grows predominantly in tropical Africa [22]. The leaves of this plant are green in color, elliptical, 6 mm in length, with characteristic odor and taste [23]. They are rich in nutrients and are used as vegetable in the preparation of soups [24-26]. Bitter leaves extracts have for long been used in ethnomedicine as antimalarial, antimicrobial, anthelmintic, antidiabetic and anticancerous medications [27-31]. The free radical scavenging activities of the leaf extracts of *Vernonia amygdalina* have also been reported by various authors [32-36]

In view of the reported involvement of free radicals in the toxicity of aluminium, the present study investigated for the first time, the protective potential of ethanol leaf extract of *Vernonia amygdalina* against the toxic effects of aluminium chloride in male Wistar rats by measurement of some indices of liver function, oxidative stress and lipid profile.

## Materials and Methods

### Plant Materials and Aluminium chloride

*Vernonia amygdalina* leaves were obtained from Atan Ikot Ekpene village in Ikot Ekpene Local Government Area, Akwa Ibom State, Nigeria. The leaves were identified and authenticated by the curator in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria. Pure crystalline aluminium chloride was obtained from Guangdong Guanghua Sci-Tech Company Limited, Shantou, Guandong, China.

### Preparation of Extract

The leaves of *Vernonia amygdalina* were washed, air dried at room temperature for two weeks and ground into powder using a manual grinder. The powdered sample (200 g) was macerated in 1L of 80% ethanol for 48 hours at room temperature and filtered through a Whatman No.1 filter paper. The filtrate was concentrated in a water bath at 40$^{o}$C and stored in a refrigerator at 4$^{o}$C until required for the experiment.

### Experimental Design

Twenty-five (25) male Wistar rats, weighing 180 – 220 g were obtained from the Animal House, Faculty of Basic Medical Sciences, University of Uyo, Uyo. They were fed standard laboratory diet, housed in wire meshed cages, maintained under standard environmental conditions of temperature, 23 ± 2$^{0}$C, relative humidity, 60% and 12 hour light/dark cycles. The rats were allowed free access to drinking water and rat chow. After an acclimatization period of seven days, the animals were randomly divided into five (5) groups with 5 rats in each group. Group 1 served as control. Group 2 was administered 100 mg/kg of aluminum chloride daily for 14 days. Group 3 received 400 mg/kg of ethanol leaf extract of *Vernonia amygdalina* for 14 days. Group 4 received 100 mg/kg of aluminum chloride and 400 mg/kg of ethanol leaf extract of *Vernonia amygdalina* simultaneously. Group 5 received aluminum chloride for 14 days and was allowed a wash out period of 14 days before sacrifice. Administration of aluminium chloride and extract was carried out by oral gavage between the hours of 8 and 10 am daily. The study was carried out in

accordance with the "Guide for the Care and Use of Laboratory Animals" [37]. Institutional approval for the conduct of this study was obtained from the Postgraduate School, University of Uyo, Nigeria.

**Collection of Serum Samples**

At the end of the experimental period, the animals were allowed to fast overnight and then sacrificed using ketamine as anaesthesia. Blood samples were collected by cardiac puncture into sterile plain bottles and allowed 30 minutes to clot. Serum was separated by centrifugation at 3000 g for 15 minutes using a Bench Top Centrifuge (MSE minor, England). The serum samples were stored frozen until required for analysis.

**Biochemical Assay**

The serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed using the Fortress Diagnostic Reagent Kit.

MDA was determined by the measurement of thiobarbituric acid reactive substances in serum [38]. SOD was assayed by the ability of the enzyme to inhibit the autoxidation of pyrogallol [39]. The assay for catalase was carried out by measurement of the rate of decomposition of hydrogen peroxide [40]. GSH was determined by measuring the reduction of Ellman's reagent at 412 nm [41]. Glutathione peroxidase was determined with the Fortress Reagent kit using the manufacturer's guidelines.

Total cholesterol, triglycerides and HDL-cholesterol were determined using diagnostic kits supplied by Randox Laboratories, England. LDL-cholesterol was estimated based on the principles of Friedewald *et al.*, [42].

**Histopathological Studies**

Sections of the liver were preserved in buffered formaldehyde and fixed in 10% formalin for histological examination as described [43]. The tissues were processed into 5 µm thick sections, stained with hematoxylin-eosin and then observed under a photomicroscope (Olympus BX 41).

## Results

**The Effect of Ethanol Leaf Extract of *Vernonia amygdalina* on the Activity of Some Serum Enzymes in Male Wistar Rats Intoxicated with Aluminium Chloride**

The effect of ethanol leaf extract of *Vernonia amygdalina* on the serum enzyme activity of male Wistar rats treated with aluminium chloride is presented in Table 1.0. Significantly elevated activities of ALT, AST and ALP ($p < 0.05$) were observed following administration of aluminium chloride (Group 2) when compared to the control. Administration of *Vernonia amygdalina* leaf extract along with aluminium chloride modulated the increase in the activities of ALT, AST, and ALP. The activities of the liver enzymes in this group were significantly reduced compared to Group 2 and tended towards the values obtained for the control. After allowing a wash out period of 14 days (Group 5), enzyme activities remained elevated compared to the control.

Table 1: Effect of ethanol leaf extract of *Vernonia amygdalina* on the activity of some serum enzymes in male Wistar rats intoxicated with aluminium chloride

| GROUPS – TREATMENT | ALT (U/L) | AST (U/L) | ALP (U/L) |
|---|---|---|---|
| GROUP 1 – Control | 14.89 ± 1.17 | 38.53 ± 3.91 | 77.03 ± 2.59 |
| GROUP 2 - Aluminum Chloride | 31.10 ± 3.54[a] | 57.12 ± 4.58[a] | 92.36 ± 3.67[a] |
| GROUP 3 – *Vernonia amygdalina* | 19.71 ± 2.36[b] | 44.88 ± 0.60[a,b] | 72.95 ± 0.49[b] |
| GROUP 4 - Aluminum Chloride and *Vernonia amygdalina* | 22.04 ± 2.82[a,b] | 39.07 ± 1.68[b,c] | 78.61 ± 3.72[b] |
| GROUP 5 - Aluminum Chloride then 14 Days without Treatment | 24.77 ± 1.27[a,b] | 52.38 ± 2.04[a,d] | 87.66 ± 5.99[a,b,d] |

Data presented as Mean ± Standard Error of Mean (SEM). Values are considered statistically different at $P<0.05$. a = significantly different when compared to Group 1; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4.

**Effect of Ethanol Leaf Extract of *Vernonia aamygdalina* on Aluminium Chloride Induced Alterations in Some Indices of Oxidative Stress in male Wistar Rats**

The effect of ethanol leaf extract of *Vernonia amygdalina* on aluminium chloride induced changes in some indices of oxidative stress is presented in Table 2.0. This table indicates that oral administration of $AlCl_3$ resulted in a significant decrease ($p < 0.05$) in the activity of GPX and a significant increase ($p < 0.05$) in the concentration of MDA when compared to the control group. However, the decrease was significant ($p < 0.05$) in only in GPx and MDA activities. Treatment with ethanol leaf extract of *Vernonia amygdalina* alone showed a significant decrease in SOD activity and MDA concentration when compared to the control. Simultaneous administration of ethanol leaf extract of *Vernonia amygdalina* and aluminium chloride induced a significant increase in the activities of SOD, CAT and GPx with a significant decrease in concentration of MDA. The administration of $AlCl_3$ for 14 days followed by a wash out period of 14 days resulted in a significant increase in the activities of CAT and GPx as well as MDA concentrations when compared to the $AlCl_3$ treated group.

Table 2: Effect of ethanol leaf extract of *Vernonia amygdalina* on aluminium chloride induced alterations in some indices of oxidative stress in male Wistar rats.

| Groups | SOD (U/ml) | CAT (μm/min/ml) | GSH (U/ml) | GPX (mmol/min/ml) | MDA (μM) |
|---|---|---|---|---|---|
| 1 | 0.76±0.02 | 8.33±0.68 | 0.29±0.01 | 3.83±0.50 | 3.23±0.27 |
| 2 | 0.58±0.06 | 7.49±0.24 | 0.37±0.04 | 1.39±0.12[a] | 18.51±0.92[a] |
| 3 | 0.37±0.11[a] | 8.38±0.76 | 0.27±0.01[b] | 2.75±0.28[b] | 1.76±0.13[a,b] |
| 4 | 0.94±0.12[b,c] | 12.25±0.26[a,b,c] | 0.30±0.03 | 4.95±0.52[b,c] | 1.10±0.09[a,b] |
| 5 | 0.51±0.15[d] | 8.00±0.24[b,d] | 0.29±0.03 | 4.31±0.35[b] | 11.04±0.22[a,b,d] |

Data presented as Mean ± Standard Error of Mean (SEM). a = significantly different when compared to Group 1; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4.

**Effect of Ethanol Leaf Extract of *Vernonia amygdalina* On $AlCl_3$ Induced Changes in Lipid Profile of Male Wistar Rats.**

The effect of ethanol leaf extract of *Vernonia* amygdalina on $AlCl_3$ induced changes in lipid profile is presented in Table 3.0. Oral administration of $AlCl_3$ induced a significant increase ($p < 0.05$) in the serum concentrations of total cholesterol (TCHOL), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) while high density lipoprotein cholesterol (HDL-C) level decreased significantly when compared to the control. There was no significant difference ($p > 0.05$) in the parameters of lipid profile when the animals were treated with the extract alone. Administration of ethanol leaf extract of *Vernonia amygdalina* and $AlCl_3$ resulted in a significant decrease in the lipid profile indices when compared to the control and $AlCl_3$ treated group. Administration of $AlCl_3$ for 14 days followed by a wash out period of 14 days also instigated a significant decrease in the lipid profile parameters when compared to the $AlCl_3$ treated group.

Table 3: Effect of ethanol leaf extract of *Vernonia amygdalina* on aluminium chloride-induced changes in lipid profile of male Wistar rats

| Groups | TCHOL (mmol/L) | TAG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) |
|---|---|---|---|---|
| 1 | 126.93±1.93 | 82.43±1.18 | 28.84±0.26 | 81.61±1.92 |
| 2 | $143.07±2.43^{a}$ | $88.25±1.92^{a}$ | 28.53±0.58 | $96.89±2.23^{a}$ |
| 3 | $122.16±0.55^{b}$ | $80.08±1.63^{b}$ | 28.19±0.59 | $77.95±0.58^{b}$ |
| 4 | $101.64±0.90^{a,b,c}$ | $70.02±0.96^{a,b,c}$ | $22.86±1.30^{a,b,c}$ | $64.78±1.67^{a,b,c}$ |
| 5 | $125.64±2.08^{b,d}$ | $81.02±1.87^{b,d}$ | $25.54±0.70^{a,b}$ | $83.90±2.13^{b,d}$ |

Data presented as Mean ± Standard Error of Mean (SEM). a = significantly different when compared to Group 1; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4.

The effects of $AlCl_3$ and ethanol leaf extract of *Vernonia amygdalina* on the histology of the liver are shown in Figure 1 (L1 – L5). Liver of the control and *Vernonia amygdalina* group (L1 and L3) showed normal lobular architecture with normal hepatic cell, central vein and sinusoid. Administration of $AlCl_3$ caused dilated, congested, widely infiltrated sinusoid, a mild micro vesicular steatosis and foci of degenerative changes (L2) which were reversed by co-administration of ethanol leaf extract and $AlCl_3$ (L4). In group 5, the toxic effects of $AlCl_3$ were observed to persist after a wash out period of 14 days (L5).

**Effect of Ethanol Leaf Extract of *Vernonia amygdalina* on $AlCl_3$ Induced Histopathology of the liver in Male Wistar Rats.**

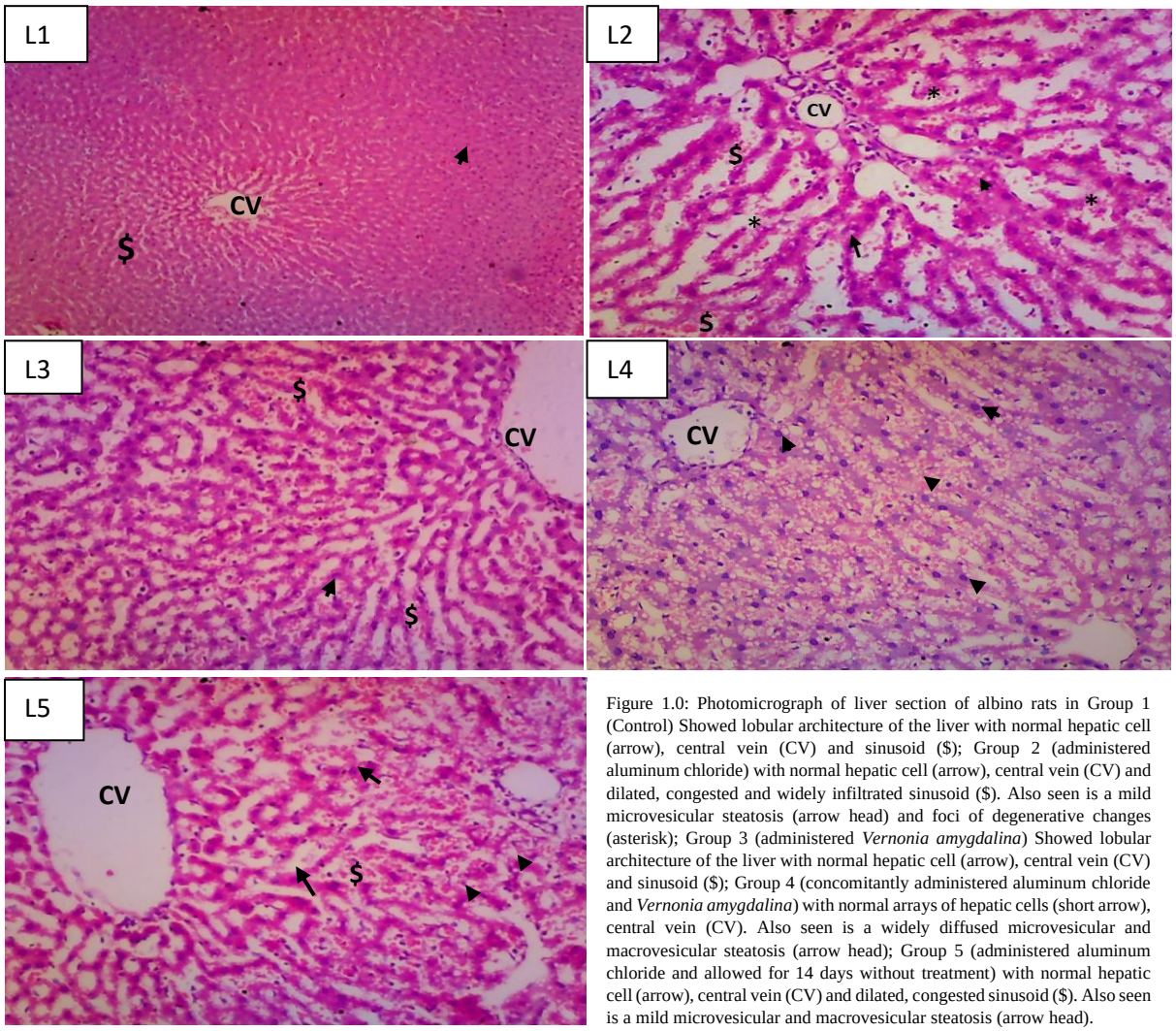


Figure 1.0: Photomicrograph of liver section of albino rats in Group 1 (Control) Showed lobular architecture of the liver with normal hepatic cell (arrow), central vein (CV) and sinusoid ($); Group 2 (administered aluminum chloride) with normal hepatic cell (arrow), central vein (CV) and dilated, congested and widely infiltrated sinusoid ($). Also seen is a mild microvesicular steatosis (arrow head) and foci of degenerative changes (asterisk); Group 3 (administered *Vernonia amygdalina*) Showed lobular architecture of the liver with normal hepatic cell (arrow), central vein (CV) and sinusoid ($); Group 4 (concomitantly administered aluminum chloride and *Vernonia amygdalina*) with normal arrays of hepatic cells (short arrow), central vein (CV). Also seen is a widely diffused microvesicular and macrovesicular steatosis (arrow head); Group 5 (administered aluminum chloride and allowed for 14 days without treatment) with normal hepatic cell (arrow), central vein (CV) and dilated, congested sinusoid ($). Also seen is a mild microvesicular and macrovesicular steatosis (arrow head).

Figure 1: Photomicrograph of liver section of aluminium chloride intoxicated male albino Wistar rats treated with ethanol leaf extract of *Vernonia amygdalina*.

## Discussion

The leaf extract of *Vernonia amygdalina* is reported to be hepatoprotective and to possess antioxidant properties [32, 44, 45]. It has also been observed that the hepatotoxic effect of aluminium chloride is mediated by the generation of free radicals via oxidative stress [46, 47]. Hence, this study evaluated the protective potentials of ethanol leaf extract of *Vernonia amygdalina* against aluminium chloride induced toxicity in male Wistar rats.

The aminotransferase enzymes (AST, ALT) as well as alkaline phosphatase (ALP) are biomarkers of toxic injury to the liver [48]. In the present study, it was observed that the administration of $AlCl_3$ caused a significant increase in the activities of these enzymes indicating hepatotoxicity. This is in line with the report of other authors [46, 49-51]. Ethanol leaf extract of *Vernonia amygdalina* significantly reduced the

activities of these marker enzymes when compared to the aluminum chloride treated group. This is an indication of the protective effect of the extract against the hepatotoxic effect of aluminium chloride. It is noteworthy that administration of ethanol leaf extract of *Vernonia amygdalina* alone induced a significant increase in the activity of AST when compared to control. Such increase in enzyme activity was earlier observed by Ojiako and Nwanjo [52] who attributed the elevated enzyme activity to some unspecified extra hepatic sources. Enzyme activities remained elevated after discontinuation of treatment with $AlCl_3$ indicating that the hepatocytes would require a longer period of time for self-recovery from $AlCl_3$ induced damage. The protective effect of the leaf extract of *Vernonia amygdalina* against chemically induced hepatic damage has been attributed to its rich content of natural antioxidants which confer stability on the cell membrane and protect the tissues from free radical damage thereby preventing further leakage of marker enzymes into circulation [53].

The histological reports of the liver tissues in the present study confirmed the observations from biochemical parameters measured. The liver of the control group revealed normal cyto-architecture of hepatocytes with normal central veins and sinusoids. However, degenerative changes were observed in the photomicrograph of the aluminium chloride intoxicated group with other features such as steatosis and dilated sinusoids indicating toxicity resulting from the administration of aluminium chloride. Similar reports on toxicity of aluminium chloride on the liver of albino rats have been documented by other authors [19]. Normal cellular architecture of the liver was observed in the group co-administered with $AlCl_3$ and ethanol extract of *Vernonia amygdalina.* This was indicative of the protective potential of the extract of *Vernonia amygdalina* against the toxic effect of $AlCl_3$. Hepatoprotective effect of ethanol extract of *Vernonia amygdalina* has been reported [54] and the present study corroborates same. Mild degenerative features were still observed in Group 5 with washout period of 14 days implying that longer period would be required for the toxic effect of $AlCl_3$ to wear off naturally.

The assessment of lipid profile is a vital diagnostic procedure because of the fact that dyslipidemia plays an important role in the pathogenesis and progression of vascular diseases [55]. Various authors have reported adverse effects of aluminium chloride on lipid profile [13, 56, 57]. These authors observed that intoxication with aluminium chloride produced dyslipidemia characterized by increase in total cholesterol, triacylglycerol, low density lipoprotein cholesterol and decreased concentrations of high density lipoprotein cholesterol. In the present study, administration of aluminium chloride precipitated a significant increase in all lipid fractions except HDL-cholesterol. Hyperlipidemia as observed in this study is a risk factor for the development of cardiovascular diseases [55]. However, the altered lipid profile induced by aluminium chloride was reversed in animals treated with ethanol leaf extract of *Vernonia amygdalina*. The hypolipidemic effect of *Vernonia amygdalina* has also been reported by other authors [58-60]. The lipid fractions were also restored to normal values after a wash out period of 14 days (Group 5). This would imply that the animals have the potential for self-recovery from $AlCl_3$ induced dyslipidemia. The lipid lowering effect of *Vernonia amygdalina* leaf extract has been attributed to its phytochemical constituents such as flavonoids, tannins and saponins [33, 60-62].

Oxidative stress has been defined as disturbance in the balance between free radical generation and antioxidative processes in favor of radical production [63]. Lipid peroxidation is one of the hallmarks of oxidative stress. It is a free radical mediated chain reaction which results in oxidative deterioration of polyunsaturated lipids with malondialdehyde as one of its toxic products. Hence, malondialdehyde is used as a biomarker of oxidative stress induced lipid peroxidation [64]. In the present study, administration of $AlCl_3$ induced a significant increase in serum concentrations of MDA. This is an indication of increased lipid peroxidation precipitated by $AlCl_3$ which is in line with the observation of other authors [13, 46, 47]. Ethanol leaf extract of *Vernonia amygdalina* attenuated the $AlCl_3$ induced increase in serum concentrations of MDA due to the antioxidant potential of this extract [32, 37, 59, 63, 65].

There are various defense mechanisms that prevent, limit or ameliorate the harmful effects of free radicals. These include the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) as well as the non-enzyme antioxidants (reduced glutathione) [66]. Reduced glutathione (GSH) plays an important role in scavenging ROS and detoxification of xenobiotics. It participates in non-enzymatic conjugation and becomes depleted during exposure to toxicants [67]. Superoxide dismutase catalyzes the

dismutation of the highly reactive superoxide anion to molecular oxygen and hydrogen peroxide [40]. Catalase is involved in the conversion of hydrogen peroxide to water and oxygen [41]. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids thus effectively removing toxic peroxides from living cells [67].

There are indications that decreased antioxidant levels are the yardstick of oxidative stress in biological systems [68]. In the present study, administration of $AlCl_3$ caused a non-significant decrease in the concentration of GSH as well as decreased activities of superoxide dismutase and catalase. However, the decrease in the activity of glutathione peroxidase was significant compared to control. Other authors had reported a significant decline in all antioxidant parameters following the administration of $AlCl_3$ [13, 46, 69]. The Agency for Toxic Substances and Disease Registry [70] reported that aluminium accumulates mainly in the liver, testes, kidneys and brain. Thus, the manifestations of aluminium toxicity are likely to be more severe at organ/tissue level compared to extracellular fluid. The decrease in antioxidant capacity has been attributed to reduced synthesis of GSH, SOD, Catalase and GPx caused by higher intracellular concentration of aluminium and/or the accumulation of free radicals [46, 71] as well as inhibition of the expression of endogenous antioxidants [72]. Ethanol leaf extract of *Vernonia amygdalina* caused a significant increase in all the antioxidant parameters (SOD, GPx, Catalase, GSH). This is consistent with earlier reports [13, 46, 69]. The beneficial effect of the extract could originate from a host of phytochemical constituents such as polyphenols which have been reported to act as inducers of these antioxidant enzymes [73].

**Conclusion**

It can therefore concluded that ethanol leaf extract of *Vernonia amydalina* has protective effect against aluminium chloride induced hepatotoxicity, oxidative stress and alterations in lipid profile in male Wistar rats.

## References


1. Verstraeten SV, Aimo L, Oteiza PI. Aluminium and Lead: molecular mechanisms of brain toxicity. *Archives of Toxicology*, 2008; 82: 789 – 802.
2. Abbasali KM, Zhila T, Farshad N. Developmental toxicity of aluminium from high doses of aluminium chloride in mice. *Journal of Applied Research*, 2005; 5: 575 – 579.
3. Cannatta J, Briggs J, Junor R, Fell S. Aluminium hydroxide intake: real risk of aluminium toxicity. *British Medical Journal*, 1983; 286: 1937 – 1938.
4. Lione A. Aluminium toxicity and the aluminium containing medications. *Journal of Pharmacology and Therapeutics*, 1985; 29: 255 – 285.
5. Kaehny W, Hegg A, Alfrey A. Gastrointestinal absorption of aluminium from aluminium containing antacids. *New England Journal of Medicine*, 1997; 296: 1389 – 1390.
6. Newairy AS, Salama AF, Hussien HM, Yousef M. Propolis alleviates aluminium induced lipid peroxidation and biochemical parameters in male rats. *Food and Chemical Toxicology*, 2009; 47: 1093 – 1098.
7. Turkez H, Yousef MI, Geyikoglu F. Propolis prevents aluminium induced genetic and hepatic damages in rat liver. *Food and Chemical Toxicology*, 2010; 48: 2741 – 2746.
8. Fatima SK, Prabhavathi PA, Pradmarathi P, Reddy PP. Analysis of chromosomal aberrations in men occupationally exposed to cement dust. *Mutation Research*, 2001; 490: 179 – 186.
9. Pennington JA, Schoen SA. Estimation of dietary exposure to aluminium. *Food Additives and Contaminants*, 12(1): 1995: 119 – 128.
10. Greger JL. Dietary and other sources of aluminium intake. In: Aluminium in biology and medicine. CIBA Foundation Symposium, 1992; 169: 26 – 49.
11. Kumar V, Gill KD. Aluminium neurotoxicity: Neurobehavioral and oxidative aspects. *Archives of Toxicology*, 2009; 83: 965 – 978.
12. Greger JL, Goetz W, Sullivan D. Aluminium levels in foods cooked and stored in aluminium pans, trays and foil. *Journal of Food Protection*, 1985; 48(9): 772 – 777.

13. Onyegeme-Okerenta BM, Anacletus FC. Hepatoprotective and ameliorative effects of selected antioxidants on aluminium induced toxicity in on Wistar rats. *European Journal of Advanced Research in Biological and Life Sciences*, 2016; 4(2): 24 – 34.
14. Stoehr G, Leubbers K, Wilhelm M, Hoelzer J, Ohmann C. Aluminium load in ICU patients during stress ulcer prophylaxis. *European Journal of Internal Medicine*. 2006; 17: 561 – 566.
15. De Voto E, Yokel RA. The biological speciation and toxikinetics of aluminium. *Environmental Health Perspective*, 1994; 102: 940 – 951.
16. Al-Kahtani MA. Renal damage mediated by oxidative stress in mice treated with aluminium chloride: Protective effect of taurine. *Journal of Biological Sciences*, 2010; 10(7): 584 – 595.
17. Madhavi T, Ch N, Lakshmi DM, Raju PDB, Anusha P, Mahitha B, Sushma NJ. Ameliorative role of *Mentha arvensis* against the aluminium induced oxidative stress in albino mice. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 2015: 4(3): 568 – 573.
18. Ekong MB, Ekpo MM, Akpanyung EO, Nwaokonko DU. Neuroprotective effect of *Moringa oleifera* leaf extract on aluminium induced temporal cortical degeneration. *Metabolic Brain Disease,* 2017; 32(5): 1437 – 1447.
19. Akpanyung EO, Nwaokonko DU, Moses BE, Ekpo MM. Evaluation of the protective effect of *Moringa oleifera* leaf extract against aluminium induced liver damage in male albino Wistar rats. *International Journal of Sciences*, 2018a; 4(02): 20 – 30.
20. Ugbaja RN, Olaniyan TO, Afolabi OK, Dosumu OA, Akinloye DI, Mufuatu AO, Nwa-John OP (2015). Vitamins C and E attenuate lipid dystrophy in tissues of rats administered aluminium. *Biokemistri*, 2015; 27(2): 98 – 105.
21. Dass AP, Ramoji PC. The effects of aqueous ginger extract on aluminium chloride induced alteration in lipid profile in male Wistar rats. *International Journal of Basic and Clinical Pharmacology*, 2017; 6(11): 1 – 4.
22. Burkill HM. The useful plants of West Tropical Africa. Second edition. Royal Botanical Gardens. 1985.
23. Singha SC. Medicinal plants in Nigeria. National Press Ltd. Apapa, Lagos, Nigeria, 1966; p49.
24. Oboh G. Nutritive value and haemolytic properties (*in vitro*) of the leaves *of Vernonia amgdalina* on human erythrocyte. *Nutrition and Health*, 2006; 18 (2): 151 – 160.
25. Eleyinmi AF, Sporns P, Bressler DC. Nutritional composition of *Gongronema latifolium and Vernonia amygdalina. Nutrition and Food Science*, 2008; 38: 99 – 109.
26. Olaposi AR, Adunni AO. Chemical composition of three traditional vegetables in Nigeria. *Pakistan Journal of Nutrition*, 2010; 9(9): 858 – 860.
27. Akah PA, Okafor CL. Blood sugar lowering effect of Vernonia amygdalina Del. in an experimental model. *Phytotherapy Research*, 1992; 6: 171 – 183.
28. Abosi AO, Raseroka BH. In vivo antimalarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Science*, 2003; 60(20): 89 – 91.
29. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African Journal of Biotechnology*, 2003; 2: 662 – 671.
30. Izevbighie EB, Bryant JL, Walker AA. A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. *Experimental Biology and Medicine*, 2004; 229: 163 – 169.
31. Ebong PE, Atangwo IJ, Eyong EU, Egbung GE. Antidiabetic efficacy of combined extracts from two continental plants: *Vernonia amygdalina* (A. Juss) and *Vernonia amygdalina* (Del.) (African Bitter leaf). *American Journal of Biochemistry and Biotechnology*, 2005; 4: 239 – 244.
32. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid. International Journal of Environmental Research and Public Health*, 2011; 8(6): 2533 – 2555.
33. Erasto G, Grierson DS, Afolayan AJ. Antioxidant constituents of *Vernonia amydalina* leaves. *Pharmaceutical Biology*, 2007; 45(3): 195 – 199.
34. Johnson M, Olufunmilayo LA, Anthony DO, Olusoji EO. Hepatoprotective effect of ethanolic leaf extract of *Vernonia amygdalina* and *Azadirachta indica* against acetaminophen induced hepatotoxicity in Sprague Dawley male albino rats. *American Journal of Pharmacological Sciences*, 2015; 393: 79 – 86.
35. Adegboye AA, Aadunmo GO, Bolaji M, Jimoh AA, Dan, MI. Hepatoprotective efficacy of methanolic leaf extract of *Vernonia amygdalina* against acetaminophen induced hepatotoxicity in wistar albino rats. *Texila International Journal of Public Health*, 2017; 5(4): 1 – 7.
36. Omede A, Suleiman, MS, Atanu FO, Friday ET, Sheneni VD, Jegede ER. Evaluation of antioxidant and cytotoxic properties of *Vernonia amygdalina*. *International of Cell Science and Molecular Biology*, 2018; 4(4): 001 – 005.
37. National Research Council. Guide for care and use of laboratory animals. Publication of the National Institute of Health. Bethesda, MD. 1985.
38. Ohkawa H, Ohishi N, Yogi K. Assay for lipid peroxidation in animal tissues by barbituric acid reaction. *Annals of Biochemistry*, 1979; 95: 351 – 358.

39. Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 1974; 47: 469 – 474.
40. Aebi H. Catalase *in vitro*. *Methods in Enzymology*, 1984; 105: 121 – 126.
41. Sedlak J, Lindsay RH. Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, 1968; 25: 1192 – 1205.
42. Friedewald WT, Levy IR, Fredrickson DS. Estimation of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. *Clinical Chemistry*, 1972; 18: 499 – 502.
43. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. Churchill Livingstone, Elsevier, China. 2008.
44. Fasakin FC, Udenigwe CC, Aluko RE. Antioxidant properties of chlorophyll-enriched and chlorophyll-depleted polyphenolic fractions from leaves of *Vernonia amygdalina* and *Gongronema latifolium*. *Food Research International*, 2011; 44: 2435 – 2441.
45. Oloyede HOB, Abdulkareem HO, Salawu MO, Ajiboye TO. Protective potentials of *Vernonia amygdalina* leaf extracts on cadmium induced hepatic damage. *Al-Hikmah Journal of Pure and Applied Sciences,* 2019; 7: 9 – 21.
46. Abdel-Wahab WM. Aluminium chloride-induced toxicity and oxidative stress in liver of male rats: *Protection by* melatonin. *Life Science Journal*, 2012; 9(4): 1173 – 1181.
47. Shrivastava S. Amelioration of aluminium induced toxicity by *Allium sativum*. *Scientific Research and Essays*, 2013; 8(4): 168 – 177.
48. Burtis CA, Ashwood ER, Bruns DE. Tietz Fundamentals of Clinical Chemistry. (6th Edition). Elsevier New Delhi. 2008; pp 322 – 329.
49. Nehru B, Anand P. Oxidative damage following chronic aluminium exposure in adult and pup rat brains. *Journal of Trace Elements in Medicine and Biology*, 2005; 19: 203 – 208.
50. Yakubu OE, Nwodo OFC, Abdulrahaman M, Uyeh LB. Effects of *Vitex doniana* leaf extract on aluminium-induced toxicity in male albino Wistar rats. *Journal of Applied Biology and Biotechnology*, 2016a; 4(05): 037 – 040.
51. Yakubu OE, Obot AC, Dawoye Y. Effects of aqueous extract of *Hymenocardia acida* leaves on aluminium chloride-induced toxicity in male albino rats. *Journal of Analytical and Pharmaceutical Research*, 2016b; 3(2): 00049. Doi:10.15406/japlr.2016.03.00049.
52. Ojiako OA, Nwanjo HU. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *African Journal of Biotechnology*, 2006; 5: 1648 – 1651.
53. Ogunlade B, Akunna GG, Fatoba OO, Ayeni OJ, Adebiyi AA, Adelakun SA. Aqueous extract of *Vernonia amygdalina* protects against alcohol induced hepatotoxicity in Wistar rats. *World Journal of Young Researchers*, 2012: 2(5); 70 - 77.
54. Akpanyung EO, Bassey UE, Udofia EE, Effiong GS. Effect of Ethanol Leaf Extract of *Vernonia amygdalina* on Some Indices of Liver, Kidney Function and Lipid Profile in Theobromine Intoxicated Male Albino Wistar Rats. *Journal of Food and Nutrition Sciences*, 2018b; 6(4): 106 – 114.
55. Singh R, Singh S, Singh SP, Jain IP, Singh S. Study of alteration in serum lipids by cardioselective beta blockers in albino rabbits. *Asian Journal of Pharmacy and Life Sciences*, 2012; 292: 166 – 173.
56. Nampoothiri M, John J, Kumar N, Mudgel J, Nampurath GK, Chamaiiamudi MR. Modulatory role of simvastatin against aluminium chloride induced behavioral and biochemical changes in rats. *Behavioral Neurology*, 2015; http://dx.doi.org/10.1155/2015/210169.
57. Al-Qayim MAJ. Propolis cardioprotective role from the impact of aluminium chloride in female rabbits. *Basic Journal of Veterinary Research*, 2015; 14(2): 136 – 149.
58. Owen OJ, Amakiri AO. Karibi-Botoye TA. Lipid lowering Effect of bitter leaf (*Vernonia amygdalina*) in broiler chicken fed finishers' mash. *Agriculture and Biological Journal of North America*, 2011; 2(6): 1038 – 1041.
59. Audu SA, Alemika ET, Abdulraheem RO. A study review of documented of phytochemistry of *Vernonia amygdalina* (Family:Asteraceae) as the basis for pharmacologic activity of plant extract. *Journal of Natural Sciences Research*, 2012; 2(7): 1 – 9.
60. Ajuru G, Onwuli D, Ajuru M. The effect of *Vernonia amygdalina* Del. (Bitter leaf) leaf extract on the lipid profile of wistar albino rats. *Continental Journal of Biomedical Sciences*, 2013; 7(1); 23 – 30.
61. Ezekwe CO, Obidoa O. Biochemical effects of *Vernonia amygdalina* on rat liver microsome. *Nigerian Journal of Biochemistry and Molecular Biology*, 2001; 16: 174S – 179S.
62. Adaramoye AO, Akintayo O, Achem J, Fafunso AM. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vascular Health and Risk Management*, 2008; 4(1): 235 – 241.

63. Cheraghi E, Roshnaei K. The protective effect of curcumin against aluminium chloride induced oxidative stress and hepatotoxicity in rats. *Pharmaceutical and Biomedical Research*, 2019; 5(1): 11 – 18.
64. Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I, Ellouz M, Miled A, Hammami M. Effects of olive oil and its fractions on oxidative stress and liver fatty acid composition in 2, 4-dichlorophenoxyacetic acid treated rats. *Nutrition and Metabolism*, 2010; 7: 80 – 92.
65. Tsao R. Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2010; 2: 1231 – 1246.
66. Daevall DG, Martin EA, Hurner, JM, Roberts R. Drug-induced oxidative stress and toxicity. *Journal of Toxicology*, 2012; 11, 1 – 13.
67. Wu D, Cederbaum I. Alcohol, oxidative stress and free radical damage. *Alcohol Research and Health*, 2003; 27: 277 – 284.
68. Abraham P, Rabi S. Nitrosative stress, protein tyrosine nitration, PARP activation and NAD depletion in kidneys of rats after single dose of cyclophosphamide. *Clinical and Experimental Nephrology*, 2009; 13: 281 – 287.
69. Al-Hashem F. Camel's milk protects against aluminium chloride induced toxicity in the liver and kidney of white albino rats. *American Journal of Biochemistry and* Biotechnology, 2009; 5(3): 98 – 109.
70. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for aluminium, US Department of Health and Human Services, Public Health Service, 2008.
71. Orihuela D, Meichtry V, Pregi N, Pizzaro M. Short term oral exposure to aluminium decreases glutathione intestinal levels and changes enzyme activities involved in its metabolism. *Journal of Inorganic Biochemistry*, 2005; 99: 1871 – 1878.
72. Gonzalez MA, Alvarez ML, Pisani GB. Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminium to rats. *Biological Trace Element Research*, 2007; 116: 329 – 348.
73. Du Y, Guo H, Lou H. Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. *Journal of Agricultural and Food Chemistry*, 2007; 55: 1695 – 1701.