Evaluation of the Protective Effect of *Moringa oleifera* Leaf Extract against Aluminium Induced Liver Damage in Male Albino Wistar Rats

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**Abstract:** Aluminium is a hepatotoxic element that is extensively used in the production of household cookware, storage utensils, water purification and in the preparation of some drugs. Conversely, the leaf extract of *Moringa oleifera* is hepatoprotective amongst other medicinal and nutritional benefits. The present study evaluated the protective effect of ethanol leaf extract of *Moringa oleifera* on Aluminium induced hepatotoxicity in male albino wistar rats. Eighteen (18) male albino wistar rats weighing between 140 and 180 g were divided into 3 groups of 6 animals per group. Group 1 served as control and was given normal rat chow and distilled water; Group 2 was administered 100 mg of Aluminium chloride per kg body weight whereas animals in Group 3 received 300 mg/kg body weight of ethanol leaf extract of *Moringa oleifera* and 100 mg per kg body weight of Aluminium chloride by oral gavage. The rats were sacrificed after 28 days of treatment. Blood and liver samples were obtained and used for the analyses of some marker enzymes (ALP, AST, ALT); haematological indices; lipid profile and histopathological assessment using standard techniques. The results show that Aluminium chloride increased the activities of ALP, ALT and AST significantly (P<0.05), TC, TG and LDL also increased significantly whereas HDL showed a significant decrease (P<0.05). The RBC count, Hb, PCV, lymphocytes and platelets decreased while WBC count and neutrophils increased significantly (P<0.05). Aluminium chloride caused alterations in the normal histology of the hepatocytes consistent with observed changes in enzyme activities. Administration of ethanol leaf extract of *Moringa oleifera* moderated the deleterious effects of Aluminium chloride.

**Keywords:** Aluminium, Enzymes, Haematology, Histopathology, Lipid, Moringa

1.0 Introduction

*Moringa oleifera* belongs to the Moringaceae family of perennial angiosperm plants. It is a fast growing tree that can attain a height of about 10 – 12 m with a diameter of about 45 cm (Bosch, 2004; Parrotta, 2005). Although native to the Sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan, it is now cultivated throughout the tropical and subtropical regions of the world because of its numerous benefits (Odee, 1998; Anwar and Bhanger, 2003; Hsu et al., 2006). In Nigeria, *Moringa oleifera* is planted in all parts of the country and is identified by a variety of local names including, ‘Zogale’ (Hausa); ‘ewe igbale’ (Yoruba) and ‘ikwa oyibo’ (Ibo) (Thilza et al., 2010). The leaves, fruit, flowers and immature pods of *Moringa oleifera* are highly nutritious and have also been utilized in ethnomedicine for the treatment of various human ailments (Anwar et al., 2007; Kumar et al., 2010). Specifically, the leaves are reported to be rich in proteins, mineral elements, vitamins A, C, E, β-carotene, various polyphenolic compounds and natural antioxidants (Amaglo et al., 2010; Karthivashan et al., 2013). Moreover, a variety of pharmacological activities have been attributed to the leaf extract of *Moringa* including anticancer, anti-inflammatory, bactericidal, hypocholesterolemic, antiatherosclerotic, antioxidant, neuro and hepatoprotective (Buraimoh, 2011; Peixoto et al., 2011; Saalu et al., 2012; El-bakry et al 2016; Ekong et al., 2017).

Aluminium is the third most abundant element constituting about 8% of the total mineral components of the earth’s crust (WHO, 2010). The element is an essential component of medications such as antacids, vaccines, phosphate binders (Kaechny et al., 1997; Exley, 1998); water purification agents (Newairy et al., 2009); food additives (Yokel, 2000) and tooth paste (Abbasali et al., 2005). Aluminium is also used extensively in the...
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Aluminium chloride was obtained from Guangdong Guanghua Sci-Tech Company Limited, China. Reagent kits used for the determination of biochemical parameters were products of Randox Laboratories Ltd., England. All the other chemicals used in this study were of analytical grade (AR).

**2.2 Collection of Leaf Samples and Preparation of Extract**

Fresh leaves of *Moringa oleifera* were obtained from a local plantation in Uyo, Akwa Ibom State, Nigeria. They were identified and authenticated by the curator in the Pharmacognosy Herbarium, Faculty of Pharmacy, University of Uyo. The leaves were washed clear of dust particles and air dried at room temperature for two weeks. The dried leaves were ground into a powder using a kitchen blender. The powdered sample was extracted in 80% ethanol. The extract was filtered through Whatman No. 1 filter paper. The resulting extract was concentrated and evaporated to dryness using a Rotary Evaporator at 40°C. The extract was further reconstituted in distilled water and preserved in a refrigerator maintained at 4°C.

**2.3 Experimental Animals**

Eighteen (18) male albino Wistar rats were procured from the animal house facility of Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria. They were housed in well ventilated wire-wooden cages and maintained under standard conditions (temperature, 28 ± 3°C; relative humidity, 67 ± 3%; 12 hours light/dark cycle). The animals were allowed unrestricted access to drinking water and rat chow (Livestock Feeds Plc, Lagos, Nigeria). The animals were cared for in accordance with the United States National Institute of Health Guidelines for the Care and Use of Laboratory Animals in Biomedical Research (NRC, 1985). Institutional approval for the study was obtained from the Postgraduate School, University of Uyo, Uyo.

The rats were allowed to acclimatize for a period of fourteen days after which they were randomly divided into three groups of six animals per group. Group 1 served as control and received 1.0 ml of distilled water; Group 2 received 100 mg of AlCl₃ per kg body weight of rat; Group 3 was treated with 300 mg of the ethanol leaf extract of *Moringa oleifera* and 100 mg of AlCl₃ per kg body weight of rat. Administration of extract and AlCl₃ was carried out by oral garage, once daily, between the hours of 8 and 10 am for a period of 28 days.

**2.4 Collection of Blood Samples**

At the end of the experimental period, the animals were allowed to fast for 12 hours and thereafter sacrificed under chloroform anaesthesia. Blood samples were collected by cardiac puncture using sterile needles and syringes. One portion of blood was transferred into EDTA sample bottles and used for the determination of haematological parameters. The second portion of blood was collected in sterile plain bottles and allowed to clot. Serum was separated from the clot by centrifugation at 3000g for 5 mins using a bench top centrifuge (MSE minor, England). The serum samples were stored frozen (−20°C) until required for analysis.

**2.5 Determination of Haematological Parameters**

The automated haematologic analyzer (Sysmex KX-21) was used to analyze the haematological parameters at the Department of Haematology, University of Uyo Teaching Hospital, Uyo, Nigeria.

**2.6 Determination of Biochemical Parameters**

AST, ALT and ALP were analysed using reagent kits from Randox Laboratories, England. Estimation of total serum cholesterol was carried out using the cholesterol oxidase phenol aminophenazone (CHOD-PAP) method whereas high density lipoprotein cholesterol was analysed by the polyethylene glycol glycol cholesterol oxidase phenol aminophenazone (PEG-CHOD-PAP) method. Triacylglycerols (TGs) were determined using the glycerol phosphate oxidase, phenol aminophenozone (GPO-PAP) end point assay. LDL-cholesterol was obtained by calculation (Friedwald et al., 1972).

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Reagent kits for lipid analyses were obtained from Randox Laboratories, England.

2.7 Histopathological Examination of Liver Samples
A portion of the liver was removed, fixed in buffered formalin and embedded in paraffin wax. Tissue sections (5 μm) were prepared and stained with Hematoxylin and Eosin (H&E) for microscopic examination (Bancroft et al., 1996).

2.8 Statistical Analysis
Results were expressed as mean ± standard deviation. The data obtained were analyzed by one-way ANOVA. Duncan’s Multiple Range test (Duncan, 1955) was used to determine the significance of difference between means. Statistical significance was accepted at P< 0.05.

3.0 RESULTS
Table 1 shows the effect of ethanol leaf extract of *Moringa oleifera* on Aluminium chloride induced increase in the activities of AST, ALT and ALP in male rats. This table indicates that treatment with Aluminium chloride (Group 2) precipitated a significant increase (p<0.05) in enzyme activities. Co-administration of ethanol leaf extract of *Moringa oleifera* with Aluminium chloride reversed enzyme activities towards normal values (Group 3). The effect of ethanol leaf extract of *Moringa oleifera* on Aluminium chloride induced alterations in serum lipid profile of male rats is presented in Table 2. Aluminium chloride induced a generalized significant increases (p<0.05) in serum concentrations of total cholesterol (TC), triacylglycerol (TAG) and low density lipoprotein cholesterol (LDL-C), whereas there was a significant decrease (p<0.05) in HDL-C. Administration of aluminium chloride with ethanol leaf extract of *Moringa oleifera* ameliorated the dyslipidemia caused by Aluminium chloride (Group 3).

Table 3 shows the effect of ethanol leaf extract of *Moringa oleifera* on Aluminium chloride induced changes in haematological indices of male rats. There was a significant decrease (p<0.05) in PCV, Hb, RBC, lymphocytes and platelets as a consequence of exposure to Aluminium chloride. The WBC and neutrophils increased significantly (p<0.05). The MCH and MCHC were not affected. Ethanol leaf extract of *Moringa oleifera* moderated most of the haematological effects of Aluminium chloride.

The effects of Aluminium chloride and ethanol leaf extract of *Moringa oleifera* on the histology of the liver are shown in Figures I – III. The liver of control animals demonstrated normal histarchitecture (Figure I). Administration of Aluminium chloride induced histopathological changes including inflammation, vacuolation and vascular degeneration (Figure II) which were reversed by the ethanol leaf extract of *Moringa oleifera* (Figure III).

4.0 DISCUSSION
Aluminium is extensively used in daily life and it is potentially toxic to man. The present study evaluated the protective effects of ethanol leaf extract of *Moringa oleifera* against the toxicity of Aluminium chloride on the liver of male rats by the measurement of some biochemical parameters, haematological indices and histopathological examination.

Toxic injury to the liver is associated with the release of some marker enzymes into circulation (Batzakis and Briere, 1979; Jaeschke et al., 2013). In the present study, hepatic damage was assessed by the assay of liver specific enzymes (AST, ALT and ALP). Administration of Aluminium chloride caused a significant increase (P<0.05) in the activity of these enzymes. This observation is in agreement with other reports which demonstrated that exposure to Aluminium chloride induced necrosis of the liver with elevation in the activities of liver specific enzymes (Abbdel-Wahab, 2012; Onyegeme-Okerenta and Anacetus, 2016; Yakubu et al., 2016). AST and ALT are amino transferase enzymes that are usually released into plasma as a consequence of hepatic damage (Naik, 2010). ALP is a membrane bound enzyme (Larkshmi et al., 1991). The increase in serum activity of ALP has been attributed to membrane damage consequent upon Aluminium chloride intoxication (Nehru and Anand, 2005; Abdeld-Wahab, 2012).

Administration of ethanol leaf extract of *Moringa oleifera* restored the activities of AST, ALT and ALP towards normal. This is an indication of improved liver function and protection against the hepatotoxicity of Aluminium chloride. This observation is in consonance with other studies which reported that the leaf extract of *Moringa oleifera* significantly reduced the elevated activities of liver enzymes induced by toxicants (Saalu et al., 2012; Karthivashan et al., 2013; Shiekh et al, 2014; Toppo et al, 2015). The hepatoprotective effects of *Moringa oleifera* leaves have been observed to follow the antioxidant mediated mechanism provided by various bioactive compounds (Fakurazi et al., 2012; El-bakry et al., 2016).

The liver is pivotal in the metabolism of lipids and lipoproteins (Havel and Kane, 2001). Thus, hepatic damage may induce alterations in the serum concentrations of cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol and triacylglycerol (Halim et al., 1997). The present study has demonstrated that Aluminium chloride...
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precipitates a significant upsurge in total cholesterol, low density lipoprotein cholesterol, triacylglycerol, but significantly lowered the serum concentration of high density lipoprotein cholesterol. This pattern of dyslipidemia has been recognized as a risk factor for the development of atherosclerosis and other cardiovascular diseases (Yakubu et al., 2008; Divi et al., 2012; Singh et al., 2012). The results obtained in this study are in line with those of other authors (Nampoothiri et al., 2015; Dass and Ramoji, 2017).

LDL-cholesterol is the primary carrier of cholesterol from the liver to extrahepatic tissues for utilization (Oparinde et al., 2014). Elevated concentrations of cholesterol and LDL-cholesterol following the administration of AlCl₃ as observed in this study have been attributed to two factors: (i) Enhanced β-oxidation of fatty acids resulting in high levels of acetylCoA which serve as key substrate in the biosynthesis of cholesterol (Yakubu and Afolayan, 2009; Naik, 2010), (ii) Accumulation of AlCl₃ in the liver resulting in loss of membrane integrity which has been observed to be associated with hyperlipidemia and/or hypercholesterolemia in human and animal studies (Sarin et al., 1997; Yuosef, 2004; Newairy et al., 2009).

HDL-cholesterol (the good cholesterol) mediates the reverse transport of cholesterol from extrahepatic tissues to the liver for degradation and excretion as bile acids (Kwitterovich, 2000; Das, 2003). HDL-C is reported to possess antiatherogenic properties. There is an inverse relationship between HDL-C and coronary heart disease (Yakubu and Afolayan, 2009). Administration of AlCl₃ was observed to decrease serum concentrations of HDL-cholesterol which is in agreement with the report of other authors (John et al., 2015; Ugbaja et al., 2015) indicating increased risk of atherosclerosis.

Triacylglycerols represent the major storage forms of lipids. The observed increase in serum concentration of TAGs in response to AlCl₃ has been attributed to its ability to enhance lipolysis (Yakubu and Afolayan, 2009).

Ethanol leaf extract of *Moringa oleifera* ameliorated the dyslipidemia precipitated by Aluminium chloride. The leaves of *Moringa oleifera* are reported to be rich in proteins, vitamins, carotenoids, trace elements and various phenolics (Amaglo et al., 2010). Enebebe et al. (2012) had reported that the supplementation of broiler chicks with *Moringa oleifera* leaves resulted in a significant increase in PCV, Hb and RBC. In ethnomedicine, the consumption of *Moringa* leaves is recommended for the treatment of anaemia (Subadra et al., 1997).

The evaluation of haematological parameters is an important biomarker for the determination of the haematoxic potential of xenobiotics (Kalaiselvi et al., 2015). The observed decrease in Hb, PCV and RBC indicate the potential to develop anaemia following exposure to Aluminium chloride. This result is consistent with the report of other authors (Osman et al., 2012; Anacleriu and Onyegem-Okrenta, 2016; Selvi and Alagesan, 2017; Yakubu et al., 2017). Mechanisms that have been advanced to explain the aluminium induced anaemia include lysis of the erythrocytes (Selvi and Alagesan, 2017) and the inhibition of haem synthesis either by repression of enzyme activity or interference with the incorporation of iron into haem (Kaiser and Schwartz, 1985; Han et al., 2000).

The present study also demonstrated that Aluminium chloride did not induce any significant alterations in the values of MCH and MCHC. This is in line with the findings of other authors (Kalaiselvi et al., 2015; Selvi and Alagesan, 2017).

Administration of ethanol leaf extract of *Moringa oleifera* moderated the negative impact of Aluminium chloride on PCV, Hb and RBC. This may be explained by the fact that the leaf extract of *Moringa oleifera* contains a profile of nutrients such as protein, amino acids, trace elements and various phenolics (Amaglo et al., 2010). The supplementation of broiler chicks with *Moringa oleifera* leaves resulted in a significant increase in PCV, Hb and RBC. In ethnomedicine, the consumption of *Moringa* leaves is recommended for the treatment of anaemia (Subadra et al., 1997).

The WBCs and their differentials are indicators of the ability of an organism to fight infections (Dacie and Lewis, 2006). The significant increase in WBCs in response to Aluminium chloride can be considered to be a normal immune response to toxic insult (Nussey et al., 2002; Igwe et al., 2011). It is noteworthy that an increase in the total number of WBCs does not
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necessarily translate into a generalised increase in all types of leukocytes (Yakubu and Afolayan, 2009).

The neutrophils are the principal phagocytic and microbicidal responders in the immune system (Willey et al., 2013). Increase in neutrophil is usually considered as an index of tissue damage or the entry of foreign bodies into the blood stream (Sakhthivel, 1988). A significant increase in neutrophil count as a consequence of exposure of African cat fish to Aluminium has also been reported by Selvi and Afagesan (2017).

The lymphocytes are the primary cells involved in specific immune reactions to foreign matter (Willey et al., 2013). In the present study, treatment with Aluminium chloride induced a significant decrease in lymphocyte count. Aluminium induced lymphopenia has been attributed to the capacity of this element to induce oxidative stress and exert genotoxic damage to the lymphocytes (Garcia-Medina et al., 2010).

The platelets are blood components involved in coagulation. A decrease in platelet count as observed in this study implies an inhibitory effect on thrombopoietin secretion (Malomo et al., 2002). A similar decrease in platelet count as a consequence of exposure to aluminium has been reported by Anacletus and Onyegeme-Okerenta, (2016).

Administration of ethanol leaf extract of *Moringa oleifera* was observed to restore to normal most of the immunological alterations induced by Aluminium chloride. The immunomodulatory effects of the leaf extract of *Moringa oleifera* is due to the wide range of macronutrients, micronutrient and phytochemicals found in this plant (Nfambi et al., 2015).

Histopathological evaluation plays an important role in the assessment of the adverse effects of xenobiotics (Reddy and Rawat, 2013). The histopathological results of the present study confirm the hepatotoxic effects of Aluminium chloride and recovery consequent upon treatment with ethanol leaf extract of *Moringa oleifera*. Similar histopathological results have been obtained with the use of other bioactive substances against aluminium hepatotoxicity (Mahmoud and Elsoadaa, 2013; Shrivastava, 2013).

5.0 CONCLUSION

The present study has provided supportive evidence that the oral administration of Aluminium chloride in male rats at a dose of 100 mg/kg body weight daily for a period of 28 days induces hepatic dysfunction as evidenced in significant alterations in some biochemical, haematological and histological parameters. The use of ethanol leaf extract of *Moringa oleifera* in combination with Aluminium chloride was observed to attenuate some of the harmful effects of this element. Therefore, supplementation with *Moringa oleifera* leaves may prove useful as protective therapy against the hepatotoxic effects of Aluminium chloride.

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Table 1: Effect of ethanol leaf extract of Moringa oleifera on aluminium chloride induced changes in the activities of some serum enzymes in male rats.

| Group | AST (U/L) | ALT (U/L) | ALP(U/L) |
|-------|-----------|-----------|----------|
| 1     | 13.03 ± 0.25<sup>a</sup> | 18.80 ± 0.55<sup>a</sup> | 58.01 ± 0.80<sup>a</sup> |
| 2     | 37.11 ± 0.37<sup>b</sup> | 30.00 ± 0.46<sup>b</sup> | 97.00 ± 0.44<sup>b</sup> |
| 3     | 14.87 ± 0.60<sup>a</sup> | 20.12 ± 0.57<sup>a</sup> | 60.36 ± 0.52<sup>a</sup> |

Values represent mean ± SD
<sup>a</sup>-<sup>b</sup> Mean values within each column represented by the same alphabet are not significantly different (P>0.05) by the Duncan’s multiple range test.

Table 2: Effect of ethanol leaf extract of Moringa oleifera on aluminium chloride induced alterations in the lipid profile of male rats.

| Group | TC(mg/L) | TAG(mg/L) | HDLC(mg/L) | LDLC(mg/L) |
|-------|----------|-----------|------------|------------|
| 1     | 67.14 ± 0.90<sup>c</sup> | 19.80 ± 0.36<sup>c</sup> | 29.16 ± 0.54<sup>c</sup> | 39.42 ± 1.26<sup>c</sup> |
| 2     | 86.76 ± 2.34<sup>b</sup> | 27.18 ± 0.54<sup>b</sup> | 21.42 ± 0.54<sup>b</sup> | 45.18 ± 1.44<sup>b</sup> |
| 3     | 72.54 ± 5.40<sup>a</sup> | 24.84 ± 0.36<sup>a</sup> | 28.64 ± 0.72<sup>a</sup> | 41.48 ± 1.26<sup>a</sup> |

*Values represent mean ± SD.
<sup>a</sup>-<sup>c</sup> Mean values within each column represented by the same alphabet are not significantly different (P>0.05) by the Duncan’s multiple range test.

Table 3: Effect of ethanol leaf extract of Moringa oleifera on aluminium chloride induced changes in some haematological parameters in male rats.

| Parameter          | Group 1            | Group 2            | Group 3            |
|--------------------|--------------------|--------------------|--------------------|
| PCV (%)            | 48.72±2.42<sup>a</sup> | 43.18±4.77<sup>b</sup> | 44.07±2.68<sup>b</sup> |
| Hb (g/dl)          | 13.26±0.23<sup>a</sup> | 8.97±0.56<sup>b</sup> | 10.92±0.42<sup>b</sup> |
| RBC (x10<sup>5</sup>/μL) | 7.85±0.21<sup>a</sup> | 4.82±0.37<sup>b</sup> | 7.11±0.46<sup>a</sup> |
| MCH (pg)           | 17.43±0.30<sup>a</sup> | 16.90±0.30<sup>a</sup> | 17.22±0.21<sup>a</sup> |
| MCHC (g/dl)        | 30.37±0.31<sup>a</sup> | 28.75±0.44<sup>a</sup> | 28.88±0.49<sup>a</sup> |
| WBC (10<sup>3</sup>/μL) | 6.30±0.06<sup>a</sup> | 9.48±0.05<sup>b</sup> | 7.00±0.08<sup>a</sup> |
| Platelets (x10<sup>3</sup>/μL) | 788.33±1.28<sup>a</sup> | 737.33±0.61<sup>b</sup> | 639.00±0.68<sup>b</sup> |
| Lymphocytes (%)    | 78.47±0.69<sup>a</sup> | 71.18±0.65<sup>b</sup> | 76.90±0.68<sup>a</sup> |
| Neutrophils (%)    | 15.92±0.62<sup>a</sup> | 24.00±0.25<sup>b</sup> | 26.45±0.35<sup>a</sup> |

*Values represent mean ± SD.
<sup>a</sup>-<sup>b</sup> Mean values within each row represented by the same alphabet are not significantly different (P>0.05) by the Duncan’s multiple range test.
Figure 1: Photomicrographs of liver of male albino Wistar rats without treatment (control); A (x100) and B (x400) reveal normal cellular profile of portal triad, bile duct, hepatic artery, hepatic vein, hepatocytes and nucleus within normal cellular architecture.

Key: Portal triad (PT), Bile duct (BD), Hepatic artery (HA), Hepatic vein (HV) Hepatocytes (H), Sinusoidal lining (SL).
Figure II: Photomicrographs of Liver treated with 100 mg of AlCl₃ per kg body weight; C (x100) and D(x400) reveal areas of inflammation, vascular degeneration and vacuolation.

Key: Portal Triade (PT), Inflammation (I), Vascular Degeneration (VD), Pyknotic nucleus (Pn), Hepatocytes (H), Vacuolation (V).
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Figure III: Photomicrographs of liver treated with 100 mg A1C₃ and 300 mg of *Moringa oleifera* per kg body weight at magnification G(x100) and H(x400) reveal that co-administration of ethanol leaf extract of *Moringa oleifera* had some modulatory effect on the Aluminium chloride induced alterations observed in Figure II.

**Key:** Portal Triade (PT), Hepatocytes (H), Hepatic Artery (HA), Hepatic Vein (HV), Cellular Proliferation (CP), Bile Duct (BD).