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Ethanol leaf extract of *Pterocarpus milbreadii* ameliorates aluminium chloride induced alterations of renal and haematological indices in male Wistar rats

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ABSTRACT: The effect of ethanol leaf extract of *Pterocarpus milbreadii* on aluminium chloride induced renal damage and alterations in some haematological parameters of male Wistar rats were investigated in the present study. Twenty (20) male Wistar rats weighing between 140 and 160 g were randomly divided into four groups with five animals in each group. Animals in Group 1 served as the control while those animals in Group 2 were treated with 100 mg/kg bw of aluminium chloride daily for 14 days. Animals in Group 3 received 400 mg/kg bw of *Pterocarpus milbreadii* leaf extract. Animals in Group 4 were treated with 100 mg/kg bw of aluminium chloride and 400 mg/kg bw of the extract concurrently for a period of 14 days. Evaluation of indices of renal function indicated that aluminium chloride induced a significant increase ($p < 0.05$) in the serum concentrations of urea, creatinine and potassium ions. Histological assessment of the kidney tissues revealed degenerated glomeruli, inflammation of renal tubule and parenchyma. However, concurrent administration of the extract and aluminium chloride was found to normalize the elevated concentrations of renal function indices and restore normal histological features as in the control. The administration of aluminium chloride to the experimental animals was also observed to produce a significant reduction ($p < 0.05$) in the WBC, RBC, HGB, MCV, MCH, MCHC and platelet counts when compared to the control animals. Furthermore, the administration of ethanol leaf extract of *Pterocarpus milbreadii* concurrently with aluminium chloride significantly improved some of these haematological parameters. These observations imply that ethanol leaf extract of *Pterocarpus milbreadii* ameliorated the deleterious effect of aluminium on the kidney and haematology of rats, hence, the leaves could be utilized as food supplement for individuals who are at risk of aluminium toxicity.

Keywords: *Pterocarpus milbreadii,* Aluminium Chloride, Haematology, Renal Function.

Introduction

Aluminium is the most abundant metal and the third most abundant element in the earth's crust (8%) after oxygen (47%) and silicon (28%) [1,2]. It is found in various forms such as aluminium oxide, aluminium silicate, aluminium hydroxide, aluminium chloride and aluminium sulphide. These forms of aluminium contaminate the environment through the weathering of rocks and anthropogenic activities [3]. Aluminium is widely utilized in the production of industrial utensils and humans are therefore exposed to high doses of the metal through mining, ore processing, recycling of metal and welding processes [4]. Exposure to aluminium is further enhanced by the utilization of several products containing aluminium. Aluminium sulphate (alum), aluminium potassium (potash alum) and other forms of the metal are used in

industrial processes such petroleum cracking and refining of crude oil. Industrial products used by man such as cooking utensils, glass, ceramics, electrical insulators, cosmetics, detergents, pharmaceutical products, cements, pottery and foils are manufactured from aluminium [5].

The presence of high concentrations of aluminium in animals often leads to multifaceted cellular and organ toxicity. Specifically, aluminium is associated with disruption of the integrity of cell membranes [6], immunotoxicity and immunosuppression [7], enzymes inhibition and activation [8,9], disruption of iron homeostasis [10], altered gene function and genotoxicity [11]. Furthermore, hepatic and renal toxicity [12,13], pancreatic toxicity [14], cardiac toxicity [15] have also been reported following exposure to various forms of aluminium.

Aluminium toxicity is mediated by free radical generation and oxidative stress [16,7]. Consequently, extracts with antioxidant potentials obtained from plants are being evaluated as antidote against aluminium toxicity [17-19,12,13].

Pterocarpus milbreadii is a rainforest plant that can grow to a height of about 35 m [20]. The leaves of *P. mildbraedii* appear light green in color when young and become deep green upon maturity [21]. The leaves are called "Oha" by the Igbo people of Eastern Nigeria and "Mkpafere" by the Efik/Ibibio people of Southern Nigeria. Traditionally, the young and tender leaves are used as vegetable in the preparation of soups. They have been reported to be rich in nutrients [21,22] and contain bioactive compounds such as flavonoids, alkaloids, saponins and tannins [23]. The in vitro antioxidant activities of *Pterocarpus mildbraedii* leaf extract has been reported [22]. Furthermore, the leaf extract of this plant has been found to be nephroprotective [24] and to positively impact on hamatological parameters in albino rats [25]. Hence, the present study evaluated the effect of ethanol leaf extract of *P. milbreadii* on renal function and haematology of male Wistar rats treated with toxic dose of aluminium chloride.

Materials and Methods

Chemicals

All the chemicals used were of analytical grade. Pure aluminium chloride was obtained from Guangdong Guanghua Sci-Tech Co. Ltd., China.

Collection of leaf samples

Fresh leaves of *Pterocarpus milbreadii* were purchased in December, 2018 at a local market in Onna Local Government Area, Akwa Ibom State, Nigeria. They were authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Thereafter, the leaves were rinsed in clean water, air dried at room temperature for a period of two weeks and were ground using a manual grinder. The pulverized sample (500 g) was macerated in 2 L of 80% ethanol for 72 hours. The resulting extract was filtered with Whatman size 1 filter paper and evaporated to dryness at 40 ^{o}C using a Rotary Evaporator. The crude extract produced was preserved in a refrigerator at -4^{o}C.

Experimental Animals

Twenty (20) male Wistar rats (140-160 g) were used for the study. The animals were obtained from the Animal House Facility, College of Health Sciences, University of Uyo, Uyo, Nigeria. The animals were housed in standard cages. They were fed commercial rat pellets and clean drinking water *ad libitum*. The care and use of the animals were in accordance with the guidelines of the National Research Council (US) Committee for the Update on the Guide for the Care and Use of Laboratory Animals [26]. Permission and approval for the animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Uyo, Nigeria.

Experimental Design

The animals were allowed an acclimatization period of ten days after which they were randomly separated into four experimental groups with five animals per group. Group 1 served as the normal control. Group 2 received 100 mg/kg body weight of aluminium chloride while 400 mg/kg body weight of

Pterocarpus milbreadii leaf extract was administered to Group 3 for 14 days. Group 4 was administered concurrently, 100 and 400 mg/kg body weight of aluminium chloride and *Pterocarpus milbreadii* leaf extract respectively. The administration of aluminium chloride and the leaf extract was carried out by oral gavage.

Collection of Blood Samples and Organs

At the end of the period of administration, the animals were fasted overnight and sacrificed under ketamine anesthesia. Blood was collected by cardiac puncture using sterile 5 ml syringe and needle. The blood sample from each animal was divided into two portions. One portion (2 ml) was transferred to in an EDTA-containing sample bottles and used for the determination of haematological indices while the other portion (3 ml) was transferred into a plain sample bottles, allowed thirty minutes to clot before centrifugation at 3000 rpm for 15 minutes to separate the serum which was used for the determination of the indices of renal function. The kidneys were surgically removed and preserved in 10% buffered formalin for histological studies.

Assessment of Haematological Parameters

The haematological parameters were determined using an automated haematological analyzer (Sysmex® Analyzer KX-21, Japan) at Haematology Unit, University of Uyo Teaching Hospital.

Determination of Indices of Renal Function

The serum concentrations of urea, creatinine and electrolytes (Na^+, K^+, Cl^- and HCO_3^-) were determined using reagent kits from Fortress Diagnostic Ltd at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo, Nigeria.

Histopathological Analysis of the Kidney

The kidneys were processed and stained with Hematoxylin and Eosin (H & E) according to the standard procedures at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo, Nigeria.

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS 22). Data were reported as mean ± SEM and subjected to the one way analysis of variance (ANOVA). Comparison between the means of each group was carried out using Least Significant Difference (LSD) post hoc tool. Differences between means were considered to be significant at $p < 0.05$.

Results

Effect of Ethanol Leaf Extract of *Pterocarpus milbreadii* on Some Parameters of Kidney Function in Aluminium Chloride Intoxicated Male Wistar Rats

The effect of ethanol leaf extract of *Pterocarpus milbreadii* on some indices of renal function in aluminium chloride intoxicated male Wistar rats is presented in Table 1. Administration of aluminium chloride (Group 2) induced a significant increase ($p<0.05$) in the serum concentrations of urea, creatinine and potassium ions. It was also found that administration of ethanol leaf extract *P. milbreadii* alone (Group 3) did not produce any significant alterations in these indices. Concurrent administration of the extract and alumonium chloride was also found to normalize the elevated concentrations of renal function indices (Group 4).

Effect of Ethanol Leaf Extract of *Pterocarpus milbreadii* on some Haematological Parameters in Aluminium Chloride Intoxicated Male Wistar Rats

Effect of the leaf extract on some haematological parameters of aluminium chloride intoxicated male Wistar rats is presented in Table 2. The administration of aluminium chloride to the experimental animals

(Group 2) produced a significant reduction ($p<0.05$) in the WBC, RBC, [Hb], MCV, MCH, MCHC and platelet counts when compared to the control animals. Administration of the ethanol leaf extract alone (Group 3) gave a non-significant improvement in haematological parameters compared with those of the control animals. It was also observed that the administration of ethanol extract of *Pterocarpus milbreadii* concurrently with aluminium chloride significantly improved some of these parameters.

Effect of *Pterocarpus milbreadii* Leaf Extract on the Renal Histology of Aluminium Chloride Intoxicated Male Wistar Rats

The histological evaluation of the kidney tissues in the present study is presented in Figure 1. Photomicrographs labeled G1 – G4 represent Group 1 – Group 4 respectively. Normal cytoarchitecture of the kidney with distinct glomerulus (GM) and renal tubules (RT) was observed in photomicrograph G1.

Similarly, normal histological features as in the control were observed in photomicrographs labeled G3 and G4. However, the photomicrographs of representing Group 2 (G2) which was administered 100 mg/kg bw of aluminium chloride revealed features such as degenerated glomeruli (DG) and inflamed renal tubule (RT) as well as the inflammation of parenchyma (#). The glomerulus was not as distinct as in the control.

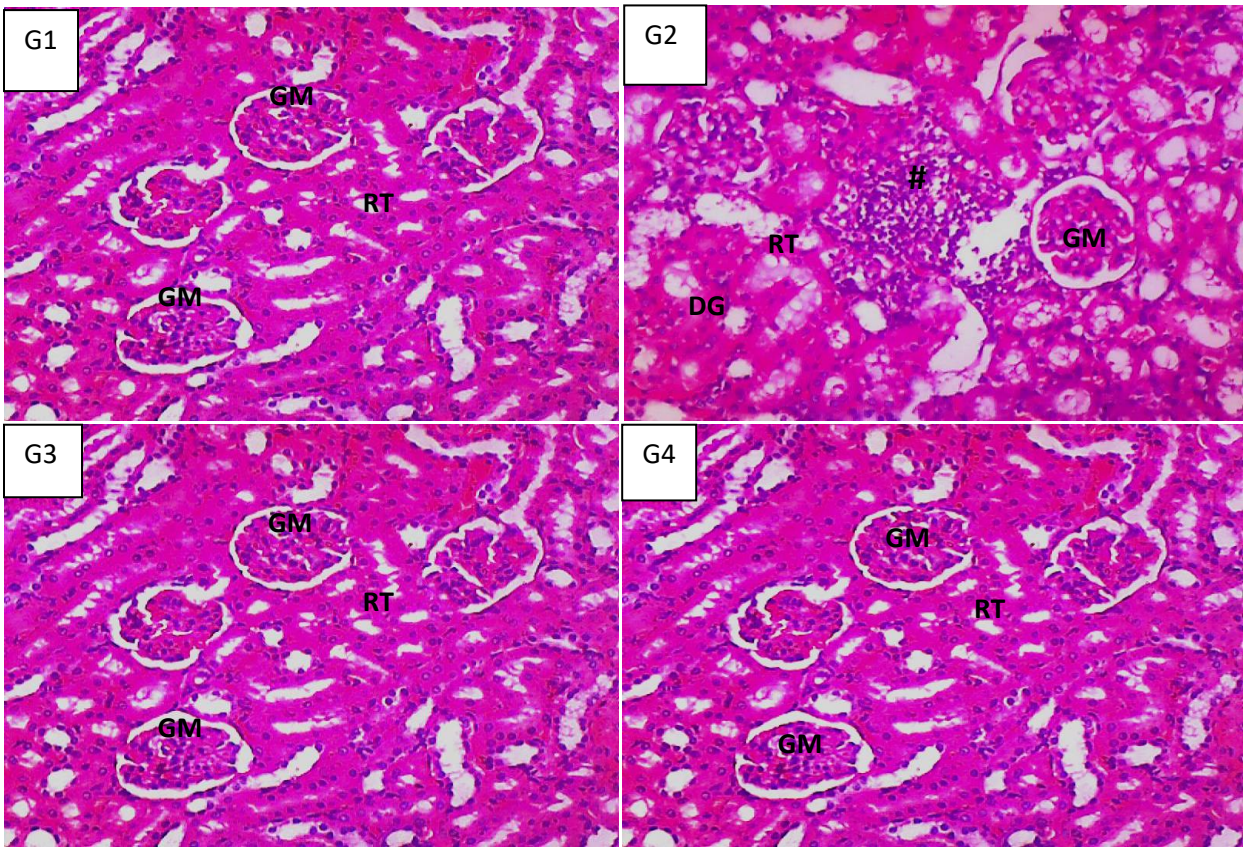

Figure 1: Renal tissue histological architecture evidenced with normal glomeruli (GM) and renal tubule (RT) with no evidence of pathologic lesion seen in photomicrograph labelled G1 (Control). Photomicrographs G3 (*P. milbreadii)* and G4 (*P. milbreadii* and aluminium chloride concomitantly), showed normal cytoarchitecture of renal histology similar to what was observed in G1. However, the histology of kidney of animals in Group 2 (G2) administered 100mg/kg bw of aluminium chloride revealed features such as degenerated glomeruli (DG), renal tubule (RT), and parenchyma inflammation (#).

Discussion

The kidney is one of the target organs in which aluminium accumulates to induce toxic injury [27,28]. Thus, the present study assessed the nephroprotective potential of ethanol leaf extract of *Pterocarpus mildbraedii* against aluminium chloride induced renal damage in male Wistar rats by measurement of serum concentrations of urea, creatinine and electrolytes (Na^+, K^+, Cl^- and HCO_3^-) as well as histological examination of kidney sections.

Urea and creatinine are waste products of metabolism that are normally excreted through the kidneys [29]. Thus, toxic damage to the kidneys would produce an increase in the serum concentrations of these metabolites [30]. Measurement of the serum levels of urea and creatinine, therefore, serves as biomarker of the functional capacity of the kidneys [31]. The results of this study have shown that aluminium chloride induced a significant increase in the serum concentrations of urea and creatinine. This is an indication of damage to the kidney tissues affecting its excretory functions and this is in line with the report of other authors [32,33].

Electrolytes are substances that ionize and exhibit electrical conductivity in aqueous solution [34]. They are involved in the regulation of nerve and muscle functions, hydration of the body and maintenance of blood pressure [35]. The determination of the serum concentrations of the major electrolytes (N^+, K^+, Cl^- and HCO_3^-) is commonly called "electrolyte panel" [36]. Sodium and chloride ions are the major electrolytes in the extracellular fluid while potassium and bicarbonate ions are the main electrolytes in the intracellular fluid. Physiologically, the major role of sodium is to control extracellular fluid volume while potassium is effective in the regulation of muscle and nerve excitability [37]. Chloride ion is important for water balance, acid-base balance and regulation of osmotic pressure. Abnormality in sodium metabolism usually parallels that of chloride because of the close association between these two ions [38]. The bicarbonate ion is best known to act as a buffer for the maintenance of normal levels of acidity (pH) in body fluids [39]. In the present study, aluminium chloride produced a significant increase in the serum concentration of potassium ions but did not affect the serum levels of other electrolytes. Renal excretion is the major route for the elimination of potassium ions from the body and renal damage has been reported to be a common cause of hyperkalemia [40].

When administered alone, ethanol leaf extract of *Pterocarpus mildbraedii* did not produce any significant effect on the serum levels of urea, creatinine and electrolytes. This is in agreement with the findings of Ezekwesili *et al.* [25]. However, concurrent administration of aluminium chloride and the leaf extract was found to normalize the aluminium chloride induced increase in the serum concentrations of all the indices of renal function measured in this study. Hence, the extract is adjudged to be nephroprotective. Al-Kahtani,[41] had earlier observed that aluminium induced renal toxicity was mediated by free radicals. Thus, the nephroprotective effect of the ethanol leaf extract of *Pterocarpus mildbraedii* could be attributed to its antioxidant activities as described by Usunomena and Chinwe [23].

Histological examination of the kidney sections of aluminium chloride intoxicated animals revealed some features of renal damage such as degenerated glomeruli and areas of inflammation. Similar toxic features in the histology of the kidney were reported by other authors [15]. These toxic features were absent in the photomicrograph of the kidney of the control group. Normal histological features of the kidney were restored in the photomicrographs of the animals treated with ethanol leaf extract of *Pterocarpus milbreadii*.

The assessment of haematological indices is valuable in the determination of the impact of xenobiotic substances on blood constituents of animals [42]. In the present study, it was observed that the administration of aluminium chloride caused a significant reduction in the levels of white blood cells, red blood cells, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume as well as platelet count when compared to the control group.

The white blood cells constitute the first line of defense against infectious agents, inflammatory processes and tissue injury [43]. There are inconsistent reports in the literature on the effect of aluminium chloride on WBC count. Some authors have reported that aluminium chloride produced a significant increase in WBC count [19,44] while others observed a significant decrease in WBC count [2,45]. However,

data from Osman *et al.* [20] and Yakubu *et al.* [46] indicate that administration of aluminium chloride did not have any significant effect on WBC count. A decreased count of WBC as observed in this study would imply that aluminium chloride has a suppressive effect on the synthesis of white blood cells in the bone marrow [47]. Ethanol leaf extract of *Pterocarpus mildbraedii* boosted the WBC count indicating that the leaves would be valuable in protecting against microbial infections and inflammatory processes [43,26].

The red blood cell count and related indices are important parameters used in the evaluation of the erythrocytes. These indices are significant in the diagnosis of anemia and in the determination of the capacity of the bone marrow to produce the red blood cells [48]. The reduction of RBC, Hb and Hematocrit by aluminium chloride in this study suggests the induction of anemia [49]. MCV is a parameter that defines the size of the RBCs. When MCV is below normal, the RBC will be smaller than normal and are described as microcytic. When MCV is elevated, the RBCs will be larger than normal and are termed macrocytic [50]. MCH and MCHC define the concentration of hemoglobin in a single RBC [51] and these indices enable the classification of anemia into hypochromic (lower than normal hemoglobin), normochromic (normal hemoglobin) as well as hyperchromic (greater than normal hemoglobin). Igbokwe *et al.* [3] reported that aluminium induced anemia is usually characterized by a decrease in MCV (microcytosis) and MCH (hypochromia). This in line with the results of the present study. Aluminium induced anemia has been proposed to involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with the incorporation of iron into heme [52-54]. Administration of ethanol leaf extract of *Pterocarpus mildbraedii* alone produced a significant increase in the haematological parameters determined in this study. Concurrent administration of the extract and aluminium chloride to the animals was also observed to restore these parameters to the normal values. Victor and Nonyelum [26] had reported that the aqueous and ethanol leaf extracts of this plant induced a significant dose dependent increase in almost all the haematological parameters. The positive effect of the extract on the indices of anemia suggests that the extract contains bioactive components that stimulate the formation or secretion of erythropoietin which is the hormone that induces the stem cells in the bone marrow to synthesize the red blood cells [55]. The leaves of *Pterocarpus mildbraedii* are rich in phytochemicals such as flavonoids, saponins, alkaloids and tannins [23]. Flavonoids and tannins have been observed to exert a positive influence on hematopoiesis [56]. These leaves also contain significant quantities of mineral elements and essential amino acids, some of which are important in the process of erythropoiesis [22,23].

Platelets (thrombocytes) are the blood cells involved in the process of blood coagulation, converting insoluble fibrinogen to fibrin which forms a solid mesh on the surfaces of injured region of the skin to prevent excessive bleeding [57]. Low platelet count would suggest that the process of clot formation will be delayed, risking excessive blood loss in cases of injury [58]. In the present study, administration of ethanol leaf extract of *Pterocarpus mildbraedii* produced a significant increase in platelet count when administered alone and also when administered concurrently with aluminium chloride. This increase in platelet count maybe attributed to the stimulatory effect of the extract on thrombopoietin as earlier documented by Li *et al.* [59].

The present study has provided further evidence in support of aluminium induced anaemia and nephrotoxicity. Additionally, this study indicated that ethanol leaf extracts of *Pterocarpus milbreadii* ameliorated the deleterious effect of aluminium on the kidney and haematology of rats. Hence, dietary supplementation with the extract could be beneficial in the prevention and/or management of renal damage as well as anaemia in susceptible individuals

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