Protective potential of ethanol leaf extract of *Vernonia amygdalina* against aluminium chloride induced renal toxicity in male Wistar rats

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Abstract

**Background:** *Vernonia amygdalina* Del. is used in traditional medical practice for the treatment of various ailments including kidney disorders.

**Objective:** To evaluate the protective potential of ethanol leaf extract of *Vernonia amygdalina* against aluminium chloride induced renal damage in male rats so as to provide some evidence for its ethnomedicinal application in the treatment of renal disorder.

**Methodology:** Male Wistar rats weighing 180-220 g were treated with 100 mg/kg bw of aluminium chloride and 400 mg/kg bw of ethanol leaf extract of *Vernonia amygdalina* for 21 days. Assay for some indices of renal damage in serum and histopathological examination of the kidney were carried.

**Results:** Treatment with aluminium chloride induced a significant increase (*p*<0.05) in serum levels of urea, creatinine, bicarbonate and chloride which became significantly reduced (*p*<0.05) in the presence of ethanol leaf extract of *Vernonia amygdalina*. Histopathological alterations in tissue sections were also reversed by the leaf extract.

**Conclusion:** The results demonstrated the protective properties of ethanol leaf extract of *Vernonia amygdalina* against aluminium chloride induced damage to the kidney of male Wistar rats.

**Keywords:** Aluminium chloride, kidney, nephroprotective, *Vernonia amygdalina*

1. Introduction

Aluminium is one of the most extensively distributed elements on earth [1]. It is a component of industrial products such as cosmetics, cookware, food additives and toothpaste [2] as well as pharmaceutical products including antacids, buffered aspirin, vaccines and injectable allergens [3, 4, 5]. Aluminium is often found in drinking water as a consequence of its being utilized in water purification process [6]. Aluminium is a major component of industrial waste especially in cement producing factories [7]. Furthermore, food substances such as corn, yellow cheese, salt, herbs, spices and tea have been reported to contain aluminium [8]. Human exposure to aluminium is inevitable because of its abundance [9]. Daily normal intake of aluminium for adults has been reported to be within 1-10 mg [10]. Ingestion of high levels of aluminium predisposes to increased deposition of this metal in the heart, kidneys, brain and the liver [11, 12]. This may result in cardiotoxicity, nephrotoxicity, neurotoxicity and hepatotoxicity [13, 14].

Medicinal plants have been used by man since ancient times to treat diverse ailments [15, 16]. Currently, a significant number of persons in third world countries still rely on plants for medicinal purposes [17]. One of such medicinal plants with an increasing role in herbal medicine is *Vernonia amygdalina* Del [18]. This plant is commonly called ‘bitter leaf’ because of its bitter taste. It is a shrub or small tree that can attain a height of 2-5 m when fully grown. It belongs to the family Asteraceae and thrives in most parts of Tropical Africa. The leaves are petiolate, about 6 mm in diameter, elliptical in shape with characteristic color and taste [19]. *Vernonia amygdalina* has been domesticated in many parts of Africa. In Nigeria, it has a variety of local names such as ‘Etidog’ in Ibibio, ‘Ewuro’ in Yoruba, ‘Onugbo’ in Igbo and ‘Chusa-doki’ in Hausa [20]. Almost all the parts of this plant have been found to be pharmacologically important [21]. In ethnomedicine, *Vernonia amygdalina* is used to treat a wide variety of ailments including fevers, hiccups, kidney problems and abdominal discomfort [22]. Many of the traditional medicinal applications of this plant have been confirmed scientifically [18, 23]. Various phytochemical compounds are present in this plant such as...
saponins, sesquiterpenes, lactones and flavonoids, vernoniosides A1, A2, A3, A4, B1 B2 and B3 [23]. The therapeutic benefits of *Vernonia amygdalina* have been attributed to its rich content of bioactive compounds [24]. The protective values of some medicinal plants and plant based products against aluminium toxicity have been reported [13, 25, 26, 27]. There is paucity of information on the protective potential of *Vernonia amygdalina* against aluminium induced toxicity [28]. Therefore, the present study was carried out to assess the effect of ethanol leaf extract of *Vernonia amygdalina* on some indices of renal function in male Wistar rats exposed to toxic dose of aluminium chloride.

### 2. Materials and methods

#### 2.1 Chemicals and reagents

Aluminium chloride was obtained from Guangdong Guanghua Sci-Tech Company Limited, Shantou, Guandong, China. All other chemicals used for this study were of analytical grade. Working solutions were prepared with distilled water.

#### 2.2 Collection and preparation of leaf extract

Fresh leaves of *Vernonia amygdalina* were purchased from a local market in Uyo, Akwa Ibom State, Nigeria. They were authenticated by a Taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State where a voucher specimen was deposited. The leaves were washed with clean water to remove dust particles. They were air dried for two weeks. The dried leaves were pulverized using a manual grinder. The powdered sample (600 g) was macerated in 1.5 L of 70% ethanol for 48 hours with intermittent shaking. The mixture was filtered with white muslin cloth and subsequently with Whatman No 1 filter paper. The filtrate was evaporated to dryness in vacuo using a Rotary Evaporator. The weight of the resulting extract was 30.54 g; yield = 5.09%. The crude extract was reconstituted in distilled water to appropriate concentrations for administration during the experiment.

#### 2.3 Experimental animals

Male Wistar rats weighing 180-220 g were used for the study. The animals were obtained from the Animal House, Faculty of Basic Medical Sciences, University of Uyo, Uyo. The rats were housed in standard cages and were maintained on a standard pelleted feed. They were allowed free access to food and drinking water. They were kept in a well-ventilated room at a temperature of 25±2 degrees Celsius and under a 12 h light/12 h dark cycle. The care and use of the animals were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [29]. Approval for the study was obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Uyo, Nigeria. The rats were allowed to acclimatize for a period of two weeks prior to commencement of the experiment.

#### 2.4 Experimental design

A total of twenty (20) rats were randomly selected into four (4) groups with 5 rats in each group. Group 1 served as the control group and was administered water 10 mL/kg body weight. Groups 2 and 3 were respectively administered with 100 mg/kg body weight of aluminium chloride and 400 mg/kg body weight of ethanol leaf extract of *Vernonia amygdalina*. Group 4 received concomitantly, 100 mg/kg body weight of aluminium chloride and 400 mg/kg body weight of ethanol leaf extract of *Vernonia amygdalina*. Aluminium chloride and ethanol leaf extract of *Vernonia amygdalina* were administered by oral gavage daily (between 8 and 10 am) for a period of 21 days.

#### 2.5 Collection of serum sample

At the end of the experimental period, the animals were fasted overnight, placed under ketamine anesthesia (100 mg/kg body weight) and blood sample was obtained through cardiac puncture. The blood sample (5 mL) was dispensed into plain sample bottles and allowed to clot. Serum was obtained by centrifugation in a bench top centrifuge (MSE Minor, England) at 3000 rpm for 15 minutes. The serum was stored frozen at −20 °C. The kidneys were surgically removed and preserved in 10% buffered formalin for histological studies.

#### 2.6 Determination of indices of renal function

Serum concentrations of urea, creatinine and electrolytes were measured using the automated Clinical Chemistry Analyzer (BS-200E). The analyses were based on standard procedures described in the respective assay kits.

#### 2.7 Histopathological analysis

The kidneys were processed and embedded in paraffin wax. Sections of 5 μm thickness were prepared, stained with haematoxylin and eosin (H&E) and examined for histopathological changes using a light microscope [30].

#### 2.8 Data analysis

Data obtained were expressed as Mean ± SEM and analyzed using the one way ANOVA. Post hoc analysis (comparison across groups) was carried out using the least significant difference (LSD). Values of *p* < 0.05 were considered to be statistically significant.

### 3. Results

#### 3.1 Effect of ethanol leaf extract of *Vernonia amygdalina* on some parameters of renal function in Wistar rats treated with aluminium chloride

The effect of ethanol leaf extract of *Vernonia amygdalina* on some parameters of renal function in Wistar rats treated with aluminium chloride is presented in Table 1. Administration of aluminium chloride resulted in a significant increase (*p*<0.05) in the serum concentrations of urea, creatinine, chloride and bicarbonate. Administration of ethanol leaf extract of *Vernonia amygdalina* alone did not induce any significant difference (*p*>0.05) in the parameters of renal function when compared to the control. The administration of ethanol leaf extract of *Vernonia amygdalina* and aluminium chloride concomitantly produced a significant decrease (*p*<0.05) in the serum concentration of urea, creatinine, potassium, chloride and bicarbonate when compared to the aluminium chloride treated group. The values in this case were comparable to those of the control group.
Table 1: Effect of ethanol leaf extract of *Vernonia amygdalina* on some indices of renal function in male Wistar rats treated with aluminium chloride

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Na⁺ (mmol/L)</th>
<th>K⁺ (mmol/L)</th>
<th>Cl⁻ (mmol/L)</th>
<th>HCO₃⁻ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.58±0.46</td>
<td>69.92±0.83</td>
<td>98.42±0.99</td>
<td>7.11±0.52</td>
<td>100.03±0.61</td>
<td>21.87±1.09</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>34.58±1.52a</td>
<td>85.62±2.11a</td>
<td>100.55±0.71</td>
<td>7.17±0.79</td>
<td>105.71±1.93</td>
<td>34.09±2.06a</td>
</tr>
<tr>
<td>Extract</td>
<td>27.92±0.87b</td>
<td>71.62±1.34b</td>
<td>95.09±1.27</td>
<td>6.66±0.33</td>
<td>102.34±2.16</td>
<td>25.44±2.67b</td>
</tr>
<tr>
<td>AlCl₃ + Ex</td>
<td>28.77±0.23b</td>
<td>70.34±0.29b</td>
<td>97.05±1.19</td>
<td>5.15±0.39b</td>
<td>96.74±0.57b</td>
<td>14.19±0.50b</td>
</tr>
</tbody>
</table>

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.

3.2 Effect of ethanol leaf extract of *Vernonia amygdalina* on the histology of the kidney of male Wistar rats treated with aluminium chloride

The effect of ethanol leaf extract of *Vernonia amygdalina* on the histology of the kidney of male Wistar rats treated with aluminium chloride is presented in Figure 1. Kidney of control group and *Vernonia amygdalina* treated group showed normal renal tissue histiarchitecture. Administration of aluminium chloride caused congested capillaries in some of the glomeruli which were reversed by co-administration of ethanol leaf extract of *Vernonia amygdalina*.

Fig 1.0: Photomicrograph of Kidney of Group 1 (Control) showed normal renal tissue histology architecture evidenced with normal glomeruli (GM) and renal tubule (RT); Group 2 (administered aluminium chloride) showed glomeruli (GM) with reduced cellularity and renal tubule (RT); Group 3 (administered *Vernonia amygdalina*) evidenced with normal glomeruli (GM) and renal tubule (RT); Group 4 (concomitantly administered aluminium chloride and *Vernonia amygdalina*) evidenced with normal glomeruli (GM), renal tubule (RT).

4. Discussion

The present study evaluated the protective potential of ethanol leaf extract of *Vernonia amygdalina* against aluminium chloride induced toxicity to the kidney in male Wistar rats. The kidney is an important physiological organ that plays a vital role in the regulation of intracellular fluid volume, electrolyte and acid-base balance as well as the excretion of waste products of metabolism. Renal functions are compromised when there is toxic injury. Such damage is associated with abnormalities in biomarkers such as serum urea, creatinine and electrolytes [31]. Creatinine and urea are non-protein nitrogenous metabolites that are normally cleared by the body following glomerular filtration [32]. Consequently, the serum concentrations of these two compounds will increase when the functional capacity of the kidney is impaired [33]. In the present study, administration of aluminium chloride precipitated a significant increase in the serum concentrations of urea and creatinine compared to the control. This is an indication of toxic injury to the kidneys induced by aluminium chloride. Other authors have also reported renal toxicity of aluminium characterized by a significant increase in serum levels of these biomarkers [34, 35, 36, 37].

The kidney also plays a major role in the regulation of body fluids and electrolytes [38]. An imbalance in electrolyte levels could have a negative impact on human health [39]. In this study aluminium chloride was observed to cause derangement in electrolyte concentrations characterized by significant increase in serum levels of bicarbonate and chloride ions. Chloride is the major anion in extracellular fluid where it plays a crucial role in water distribution, osmotic pressure acid base balance. Increased plasma concentrations of chloride ions may occur in various conditions including acute renal failure [40]. Bicarbonate constitutes the second largest fraction of plasma anions. The bicarbonate ions act as buffer to maintain the normal levels of acidity in blood and other
 fluids [38]. Conditions that affect the kidneys can influence acid base balance of body fluids [41]. Administration of ethanol leaf extract of Vernonia amygdalina was found to reduce the serum concentrations of urea, creatinine, chloride and bicarbonate ions even in the absence of the toxicant. This is an indication of the nephroprotective potential of the leaf extract of Vernonia amygdalina. Methanol extract of Vernonia amygdalina had earlier been observed to significantly reduce the elevated concentrations of urea and creatinine in sodium chloride induced hypertension [42]. Also a significant reduction in the elevated serum concentrations of urea and creatinine in alloxan induced diabetic rats following the administration of methanol extract of Vernonia amygdalina [43]. Incidentally, Vernonia amygdalina has been found to be rich in phytochemicals such as flavonoids, alkaloids and polyphenols [23]. The ameliorative activities of ethanol leaf extract of Vernonia amygdalina could, therefore, be attributed to its rich content of these bioactive compounds [34, 44].

The histology of the kidney tissues added credence to the nephrotoxic effects of aluminium chloride. Interestingly, the ethanol leaf extract of Vernonia amygdalina effectively restored these histopathological alterations to normal. Histopathological changes in renal tissues induced by aluminium chloride but reversed by the administration of camel’s milk, Lipidium sativum and naringenin respectively have been documented by other authors [36, 44, 45].

5. Conclusion
The present study has demonstrated the protective potentials of ethanol leaf extract of Vernonia amygdalina against aluminium chloride induced renal impairment. The results have also provided experimental evidence for the ethno medicinal use of this plant in the management of renal disorders.

6. Conflict of interest
The authors declare that there is no conflict of interest.

7. References
24. Farombi EO, Owoeve O. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia bflavonoid. International Journal of