Evaluation of In vivo Toxicity of Methanolic Leaf Extract of Vernonia lasiopus (O. Hoffman)

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Abstract

The main objective of this study was to evaluate the in vivo toxicity of methanolic leaf extract of Vernonia lasiopus. To provide information on the safety of V. lasiopus, we evaluated its acute and sub-chronic toxicity in Wistar rats. For evaluation of acute toxicity of the plant extract, five Wistar rats were orally dosed with 2000 mg/kg body weight sequentially. Sub-chronic toxicity was tested in twenty Wistar rats using three extract doses 100, 300 and 1000 mg/kg body weight. They were orally administered for 28 days. Mortality and toxicity signs were monitored during the study period. At the end of the experiment, the animals were sacrificed, their internal organs weighed and blood samples collected for haematology and biochemical analysis. In acute toxicity, no single death was reported; leading to conclusion that the median lethal dose (LD50) of methanolic leaf extract of V. lasiopus is beyond 2000 mg/kg body weight. In sub-chronic toxicity studies, V. lasiopus lowered total proteins in all the study groups significantly. Albumin was also lowered at extract dose of 1000 mg/kg body weight. In addition, it resulted to significant neutropenia, lymphocytosis and thrombocytosis in the group administered with dose extract of 1000 mg/kg body weight (P<0.05). It was therefore concluded that methanolic leaf extract of V. lasiopus is safe for use when administered at therapeutic doses. The plant extract may also be useful in the management of haematological disorders especially thrombocytopenia.

Keywords: Vernonia lasiopus, Toxicity; Wistar rats; In vivo; Methanolic leaf extract

Introduction

Medicinal plants have been helpful in treating both infectious and life style diseases. Knowledge on their use has been preserved by passing it through oral communication and cultural practices by communities around the world [1]. They also play a role in the development of conventional drugs in modern age. However, the main drawback in the use of ethnobotanical medicine is the fact that the dosage is non-standardized and most of the plants have not been evaluated for toxicity [2].

On the other hand, global upsurge in therapeutic application of medicinal plants has been accompanied by various toxicological effects including drug overdose, drug dependence, hypersensitivity and idiosyncratic reactions [3]. The observed adverse reactions have direct effects on various body organs especially the liver and the kidney which are more predisposed to toxic effects of xenobiotics during their metabolism and excretion [3].

A major cause of toxicity includes plant misidentification, use of medicinal plants of unknown toxicity and contamination of medicinal plants with nephotoxic non-herbal drugs [4]. In addition, presence of traces of pesticides and heavy metals, consumption of meat from animal that has grazed on toxic plants and interaction of conventional drugs with herbal compounds, have a time resulted to toxic effects [4]. Consumption of toxic plants leads to bioaccumulation of toxic herbal compounds or altered detoxification processes [5].

Vernonia lasiopus (O. Hoffman) is a plant that grows to a maximum height of about 3 m and has smooth greyish brown bark. It has oval shaped and densely hairy leaves with pale mauve or white flowers on colour [6]. V. lasiopus belongs to the tribe of Vernonieae and the family Asteraceae with about 1500 described species [7]. Most of the Vernonia species are found in temperate, tropical and sub-tropical areas, especially in South America, Asia, Africa and North America [8]. V. lasiopus is widely distributed in bush land, grassland, woodland and forest. It grows in an altitude between 1000 to 2500 m. In Ethiopia, Vernonia species are used to treat eye infections, wounds and bone related problems such as fractures [9]. In Kenya, V. lasiopus is used to treat malaria and helminthic infections by decoction of leaves and bark [10]. V. lasiopus has also been in use traditionally in treating fever, abdominal pain, diarrhoea, sores, venereal diseases, scabies and ascariasis among other ailments [11]. This plant has been shown to have pain relieve ability, sedating power, anti-ulcerogenic and membrane stabilizing activities on reduced red blood cells [12-13]. It also has erythropoiesis and leucopoiesis stimulating power [12-13]. Despite extensive traditional use and scientific evaluated properties of this plant, little has been done in investigating in vivo toxic effects of the plant. It is against this back ground that we designed this study with the objective of investigating acute and sub-chronic oral dose toxicity of methanolic leaf extract of V. lasiopus in Wistar rat models, as part of the safety evaluation.
Materials and Methodology

Plant material collection

Fresh leaves of Vernonia lasiopus plant were collected with the help of a local traditional herbalist in Embu County. The plant materials were transported to Biochemistry and Biotechnology laboratory at Kenyatta University, where they were shade dried for two weeks at room temperature. A voucher specimen was deposited at Kenyatta University Herbarium after botanical authentication by a taxonomist.

Extraction

Dried leaves of V. lasiopus were crashed into a fine powder using electric mill. To obtain an extract, 500 g of the fine powder was soaked in one litre of methanol for 24 hours. After filtration of the extract using Whatman filter paper No. 1, the filtrate was evaporated to dryness under reduced pressure using rotary evaporator at 40°C.

Experimental animals

Male Wistar rats aged between 8-10 weeks were obtained from Kenyatta University Animal house, Department of Biochemistry and Biotechnology. The animals were randomly selected and caged in groups of five. They were kept at standard laboratory conditions of temperature (25 ± 2°C), relative humidity (60 ± 5%) and 12/12-h light/dark cycle. Commercial rodent feed was supplied as food and water provided ad libitum. Five days acclimatization was observed before beginning the experiments. Guidelines from Organization for Economic Cooperation and Development on animal studies were followed on handling the animals [14]. Research permit was obtained from National Commission of Science, Technology and Innovation. Permission from Ethics Committee of the Kenyatta University on research on animal models was also granted.

Acute toxicity studies

Up and down method was used to perform a limit test at dose extract of 2000 mg/kg body weight [15]. Baseline weight after overnight fasting of the rats was used to calculate individual dose for each animal. The test was carried out using 5 male Wistar rats. Extract dose of 2000 mg/kg body weight was sequentially administered to each animal at 48 hours interval by oral gavages. For the first four hours, the animals were denied access to food. Only water was provided ad libitum. Signs of toxicity and fatality were observed up to 14 days and the results recorded. Special attention was given to the first three hours after administration of the extract.

Sub-chronic toxicity testing

The animals were divided into four groups each containing five animals. Group 1 was the control. This group was orally administered with 0.5 ml of 1% DMSO for 28 days. Groups 2, 3 and 4 were orally administered with methanolic leaf extract of V. lasiopus at extract dose of (100, 300 and 1000) mg/kg body weight respectively, daily for 28 days. A progression factor of 3.2 was used to arrive at the three doses [15]. The animals were fed on commercial rodent feed and water was provided ad libitum.

Samples collection

On the 29th day, animals were anaesthetized in air tight dissection jar containing cotton soaked in chloroform and blood collected through cardiac puncture. Whole blood for full hemogram tests was collected in anticoagulated tubes containing Ethylenediaminetetraacetic Acid (EDTA) anticoagulant and kept at 4°C. Blood for Renal Function Tests (RFT) and Liver Function Tests (LFT) was collected in tubes without anticoagulant. After clotting, the blood was centrifuged at 3000 rpm for five minutes to obtain serum which was kept at -20°C awaiting analysis. The tests performed included blood Glucose, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total proteins, albumin, creatinine, urea and uric acid. The anesthetized animals were later laid on a dissection board and opened up by cutting through vertical mid-line from neck to peritoneum using a pair of scissors. Body organs (brain, heart, liver, kidneys and pancreas) were excised blotted and weighed using digital weighing balance. The organs were preserved in plastic containers containing 10% buffered saline.

Statistical data analysis

The data obtained was presented in tables and graphs. Values were expressed as mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) was used to analyze data obtained from each animal body and organs weight, haematological tests and biochemical tests. Minitab statistical computer software v.17 (Minitab Inc., Pennsylvania, USA) was used in analyzing the data. Tukey’s Honest Significant Difference test was used to separate means at a confidence level of 95% (p ≤ 0.05).

Qualitative phytochemical screening

Qualitative phytochemical screening for the presence of phenolic compounds, terpenoids, saponins, sterols, cardiac glycosides, alkaloids and flavonoids was done using methods described by Kotake [16].

Results

Acute toxicity

Administration of 2000 mg/kg body weight of methanolic extracts of V. lasiopus resulted in decreased activity, wheeze, pilo-erection and anorexia in the Wistar rats. However, these symptoms lasted for less than three hours. The severity of these symptoms decreased with time.

Sub-chronic toxicity

Effects of methanolic leaf extract of Vernonia lasiopus on body and organ weight in Wistar rats: The results obtained following administration of methanolic leaf extract of V. lasiopus, clearly indicate that there was no significance difference in weight observed between the treatment groups and the control group. Only a gradual insignificant weekly gain in weight was observed in all the groups (p>0.05; Figure 1). In addition, the differences in weight of organs (brain, liver, kidney, spleen, lungs and heart) of the treated animals was not statistically outstanding when compared to the control group (p>0.05; Table 1).
Figure 1: Effects of methanolic leaf extract of *Vernonia lasiopus* on body weight in Wistar rats.

Table 1: Effects of oral administration of methanolic leaf extract of *Vernonia lasiopus* on organ weight in Wistar rats.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Treatment groups</th>
<th>Control 100 mg/kgbw</th>
<th>300 mg/kgbw</th>
<th>1000 mg/kgbw</th>
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<tr>
<td>Brain</td>
<td>1.97 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.92 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.99 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Liver</td>
<td>11.35 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.63 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.27 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.56 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.72 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.79 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.47 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Spleen</td>
<td>1.01 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.90 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Lungs</td>
<td>2.93 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.69 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.36 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>0.77 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
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Table 2: Effects of methanolic leaf extract of *Vernonia lasiopus* on liver and renal functions in Wistar rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose of <em>Vernonia lasiopys</em> (mg/kgbw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL (mmol)</td>
<td>6.76 ± 1.10&lt;sup&gt;a&lt;/sup&gt; 6.38 ± 0.93± 8.52 ± 0.86± 6.36 ± 0.67±</td>
</tr>
<tr>
<td>PROT (g/l)</td>
<td>58.98 ± 2.32&lt;sup&gt;a&lt;/sup&gt; 54.8 ± 1.76&lt;sup&gt;a&lt;/sup&gt; 54.44 ± 1.36&lt;sup&gt;a&lt;/sup&gt; 58.58 ± 3.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALB (g/l)</td>
<td>31.72 ± 1.61&lt;sup&gt;a&lt;/sup&gt; 27.96 ± 1.18&lt;sup&gt;a&lt;/sup&gt; 27.80 ± 1.74&lt;sup&gt;a&lt;/sup&gt; 24.14 ± 1.84&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>ALT (IU/L)</td>
<td>145.46 ± 3.59&lt;sup&gt;a&lt;/sup&gt; 159.06 ± 5.17&lt;sup&gt;a&lt;/sup&gt; 143.16 ± 2.89&lt;sup&gt;a&lt;/sup&gt; 154.82 ± 4.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>189.44 ± 6.51&lt;sup&gt;a&lt;/sup&gt; 176.8 ± 8.04&lt;sup&gt;a&lt;/sup&gt; 194.56 ± 4.25&lt;sup&gt;a&lt;/sup&gt; 164.04 ± 6.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>1.74 ± 0.24&lt;sup&gt;a&lt;/sup&gt; 1.72 ± 0.12&lt;sup&gt;a&lt;/sup&gt; 1.95 ± 0.17&lt;sup&gt;a&lt;/sup&gt; 1.97 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>D.BIL (µm)</td>
<td>0.44 ± 0.21&lt;sup&gt;a&lt;/sup&gt; 0.24 ± 0.19&lt;sup&gt;a&lt;/sup&gt; 0.56 ± 0.35&lt;sup&gt;a&lt;/sup&gt; 0.26 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>T.BIL (µm)</td>
<td>3.55 ± 0.57&lt;sup&gt;a&lt;/sup&gt; 1.69 ± 0.88&lt;sup&gt;a&lt;/sup&gt; 3.32 ± 0.56&lt;sup&gt;a&lt;/sup&gt; 2.6 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRE (µm)</td>
<td>47.90 ± 5.64&lt;sup&gt;a&lt;/sup&gt; 45.96 ± 8.14&lt;sup&gt;a&lt;/sup&gt; 55.66 ± 4.26&lt;sup&gt;a&lt;/sup&gt; 62.04 ± 7.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>UREA (mmol)</td>
<td>8.29 ± 0.56&lt;sup&gt;a&lt;/sup&gt; 7.28 ± 0.41&lt;sup&gt;a&lt;/sup&gt; 8.68 ± 0.21&lt;sup&gt;a&lt;/sup&gt; 7.14 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>UA (µm)</td>
<td>342.7 ± 49.10&lt;sup&gt;a&lt;/sup&gt; 389.7 ± 44.20&lt;sup&gt;a&lt;/sup&gt; 384.9 ± 30.90&lt;sup&gt;a&lt;/sup&gt; 290.7 ± 42.40&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Table 3: Effects of oral administration of methanolic leaf extract of *Vernonia lasiopus* on haematological parameters in Wistar rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose of <em>Vernonia lasiopys</em> (mg/kgbw/day)</th>
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</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>14.18 ± 0.34&lt;sup&gt;a&lt;/sup&gt; 14.84 ± 0.34&lt;sup&gt;a&lt;/sup&gt; 14.72 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>64.6 ± 2.18&lt;sup&gt;a&lt;/sup&gt; 66.18 ± 2.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>22.00 ± 0.35&lt;sup&gt;a&lt;/sup&gt; 22.60 ± 0.19&lt;sup&gt;a&lt;/sup&gt; 22.20 ± 1.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>32.32 ± 1.16&lt;sup&gt;a&lt;/sup&gt; 35.98 ± 2.03&lt;sup&gt;a&lt;/sup&gt; 33.46 ± 1.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RDW (% )</td>
<td>16.72 ± 0.55&lt;sup&gt;a&lt;/sup&gt; 19.76 ± 0.40&lt;sup&gt;a&lt;/sup&gt; 19.36 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WBC (109/L)</td>
<td>9.18 ± 2.22&lt;sup&gt;a&lt;/sup&gt; 10.40 ± 1.55&lt;sup&gt;a&lt;/sup&gt; 9.66 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>32.40 ± 4.71&lt;sup&gt;a&lt;/sup&gt; 13.88 ± 2.24&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>
found in the liver, other organs including kidney, skeletal muscles and heart contain lesser amounts of the enzyme. The main function of this enzyme is transamination of amino acid alanine. Since ALT is mainly found in the cytosol, upon hepatocytes injury, the enzyme leaks into the extracellular space resulting in its rise in the plasma [23-24]. The insignificant rise in ALT indicates that there was no hepatic injury that occurred following administration of V. lasiopus. This is further supported by insignificant rise in AST, direct bilirubin and total bilirubin.

Aspartate Aminotransferase is found both in the cytosol and mitochondrion and is widely distributed in many tissues compared to ALT [25]. Because of wide distribution of this enzyme in other tissues, it can be used to monitor other disorders in these tissues. However, in combination with ALT, it is a good maker of liver disease [24]. Bilirubin is classified as direct and indirect. Direct bilirubin is due to impaired hepatic secretion and obstruction to bile flow intra-hepatic or extra-hepatic. On the other hand, indirect bilirubin is mainly due to severe red blood cells haemolysis. Reduced uptake of bilirubin by hepatocytes, impaired conjugation, and reduced secretion can result in increase in both direct and indirect bilirubin [26-27].

In this study, both direct and indirect bilirubin rise was insignificant. We can therefore deduce from the study that V. lasiopus extract was safe to the liver at all the three doses administered orally, since it did not cause any significant rise in makers of liver toxicity.

The main function of the kidney is to excrete excess metabolic end products and to retain important substances by re-absorption or by retention, in order to maintain consistency in the extracellular fluid [28]. When this is not possible, such products accumulate in the plasma resulting to increased levels. Urea a product of protein catabolism is excreted mainly through the kidney [29]. Increase in plasma urea is associated with high protein intake, anabolic effects of some drugs (tetracyclines and glucocorticoids), kidney diseases, urinary tract blockage, congestive heart failure, trauma or serious illness. Plasma urea can increase when glomerular filtration rate is low [30]. On the other hand, low levels are seen in surgery, trauma, malnutrition, opioids use and in use of anabolic steroids [31]. As a measure of kidney function test, serum urea level was not affected by administration of methanolic leaf extract of V. lasiopus.

In addition, creatinine a marker of kidney function test was also not significantly affected by administration of the extract. Creatinine is usually not metabolized by the kidney, is not protein bound and is inert physiologically, therefore a good marker of filtration in the kidney. Serum creatinine increases in both acute and chronic kidney failure. However, at times the creatinine levels may be unrelated to kidney injury since creatinine can be influenced by muscle composition, function and activity, diet and health status [30]. Blood urea nitrogen-creatinine ratio is usually elevated in acute renal failure in pre-renal conditions [25].

Furthermore, kidney diseases are associated with rise in uric acid level in the blood [32]. Both the kidney and the gastrointestinal tract plays a role in the elimination of uric acid, where by the kidney excretes about two-third of this product [33]. Uric acid, a product of purine metabolism may increase in plasma due to inherited metabolic disorders, excess purine rich diet intake, alcohol intake, hypoxia, preeclampsia and altered Adenosine Triphosphate (ATP) metabolism. On the other hand, decreased excretion can result from kidney disease, lead poisoning, organic acids and salicylates leading to its rise in blood

Table 3: Effects of methanolic leaf extract of *Vernonia lasiopus* on haematological profiles in Wistar rats.

<table>
<thead>
<tr>
<th>Lymphocyte (%)</th>
<th>58.20 ± 6.00a</th>
<th>71.18 ± 1.19b</th>
<th>67.60 ± 1.17b</th>
<th>75.30 ± 2.25a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes (%)</td>
<td>8.0 ± 1.41a</td>
<td>12.20 ± 1.39a</td>
<td>10.60 ± 0.97a</td>
<td>9.02 ± 2.21a</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.00 ± 0.55a</td>
<td>0.80 ± 0.58a</td>
<td>0.60 ± 0.25a</td>
<td>0.40 ± 0.40a</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.20 ± 0.20a</td>
<td>0.20 ± 0.20a</td>
<td>0.40 ± 0.25a</td>
<td>0.60 ± 0.25a</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>361 ± 47.9b</td>
<td>523.8 ± 41.5b</td>
<td>529.8 ± 48.1b</td>
<td>733.2 ± 28.1b</td>
</tr>
<tr>
<td>PDW</td>
<td>14.38 ± 0.02b</td>
<td>15.16 ± 0.32b</td>
<td>14.04 ± 0.34b</td>
<td>14.98 ± 0.33b</td>
</tr>
<tr>
<td>Platelet%</td>
<td>0.27 ± 0.04a</td>
<td>0.37 ± 0.03b</td>
<td>0.36 ± 0.05b</td>
<td>0.89 ± 0.15b</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p<0.05 (Analysed by ANOVA followed by Tukey’s post hoc test). RBC: Red Blood Cells, Hb: Haemoglobin, PCV: Packed Cell Volume, MCV: Mean Cell Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, RDW: Red Blood Cells Distribution Width, WBC: White Blood Cells, PDW: Platelet Distribution Width.
[25]. Methanolic leaf extract of *V. lasiopus* did not alter uric acid levels significantly, meaning that the extract was safe to the kidney.

In diagnosis of renal disorders, measurement of electrolytes reflects kidney ability to excrete and reabsorb substances. Electrolytes are used to diagnose conditions related to acid-base balance, endocrine conditions and water balance among other conditions. Decrease in potassium excretion in the distal tubule, coincides with its increase in serum [30]. Measurement of both potassium and sodium level in the animals that received the extract did not reflect any toxicity.

Compared to the control group, haemoglobin level, haematocrit and the red blood cell count were insignificantly high in groups administered with *V. lasiopus* extract. This shows that the extract may be containing phytochemicals, which at high doses stimulate erythropoiesis. Moreover, the extract did not cause toxic effects on red blood cell indices.

Methanolic extract of *V. lasiopus* caused significant rise in platelets and plateletcrit. This is in consistence with study done by Muriithi et al., who found out that leaf extract from *V. lasiopus* improved erythrocytic parameter profiles significantly [13]. In a similar study, *Carica papaya*, *Ipomea batatas*, *Alternanthera sessilis*, and *Euphorbia hirta* were found to have the ability to stimulate platelet production [34]. This proves the ability of plant extracts in stimulating platelets production. Saponins, tannins, and alkaloids have been found to act on the bone marrow, thereby enhancing production of platelets and preventing their destruction in the circulation [35]. Since the main regulator of platelet production is thrombopoietin, significant increase in platelets in animals that received methanolic leaf extract of *V. lasiopus* at 1000 mg/kg body weight, is an indication that the extract may be containing substances similar to thrombopoietin or it may be stimulating thrombopoietin production [36].

The red blood cells indices in animals that received *V. lasiopus* extract were not altered significantly in comparison to the control group. This signifies that the extract did not cause any toxic effect in erythrocytes. However, *V. lasiopus* extract at a dose of 1000 mg/kg body weight caused significant rise in lymphocytes and decrease of neutrophils.

The bone marrow plays a key role in production of neutrophils and two thirds of cells production is dedicated to production of monocytes and granulocytes [37]. Neutrophils production is regulated by interleukin (IL) 23, IL-17 and granulocyte colony stimulating factor. This axis of neutrophils production is down regulated by the liver X receptors (LXRs) [38]. Therefore, the plant extract could have resulted in increased expression of LXRs in the liver. Neutrophil death through extrinsic apoptosis is triggered by tumor necrotic factor alpha (TNF-α) which proceeds through cleavage of pro-caspase-8 [39]. Plants extracts have demonstrated the ability to induce production of TNF-α [40–41]. Therefore, this may be a possible mechanism through which the plant extract may have resulted to significant decrease in neutrophils count.

In addition, dendritic and macrophage cells are responsible for engulfing apoptotic neutrophils. When this happens, the phagocytes decrease production of IL-23 subsequently resulting in decrease in IL-17 [37]. Through this process, neutrophil production is down regulated.

Research on nine Chinese medicine herbal extracts did show the ability of plant extracts to stimulate lymphocytes production and proliferation [42]. Similarly, the significant increase of lymphocytes may have been stimulated by phytochemicals in plant extract given. However, chronic inflammation from repeated cell injury following daily oral administration of the plant extract can also result to significant increase in lymphocytes [43]. Similar results were obtained by Antai et al., following oral administration of ethanolic extracts of *Gogolonea latifoliolium* in rats [44]. In the study, neutrophils were significantly reduced in a non-dose dependent manner and lymphocytes increased significantly.

In addition to antimicrobial activities of phenols, some phenolic compounds have activity on platelets and others have been linked to anti-inflammatory activity [45]. They may therefore have contributed to the observed changes in both white blood cells and platelets. On the other hand, saponins have been reported to have anti-inflammatory activities and ability to fortify the immune system [46].

**Conclusion**

The conclusion drawn from this study is that methanolic leaf extract of *V. lasiopus* is practically safe for both acute and sub-chronic use when administered orally. It is therefore recommended to evaluate other different routes of administration (peritoneal, subcutaneous, intradermal and intramuscular). This is to figure out more benefits as well as toxic effects of this plant.

**Study limitations**

The study did not focus on histopathology analysis of the tissues and quantitative phytochemical analyses of the plant extract due to time and cost implications.

**Acknowledgement**

We acknowledge the Department of Biochemistry and Biotechnology Kenyatta University and Kwale Hospital for allowing us to use their facilities in conducting this study.

**References**


