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Some Toxicological Studies of Methanol Leaves Extract *Lannea acida* in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JN managed the analyses of the study. Author AUA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The current study determined phytochemical constituents evaluated the acute and sub-chronic toxic profiles of *Lannea acida* methanol leaves extract (LAMLE) in Wistar albino rats
Methodology: The phytochemical screening of LAMLE was conducted using standard methods. A total of 31 male albino rats were used for the antioxidant studies. A total of 31 male albino rats were used for the toxicological study. The LD_{50} was determined using six (6) rats according to OECD, 2001 using fixed limit dose. For the sub-chronic study, the rats were divided into five (5) groups of five (5) rats. Control group (group 1) received distilled water orally 2ml/kg. Groups (2-5) received doses of 250, 500, 1000, 2000 mg/kg of the extracts. The experiment lasted for 28 days.
Results: The phytochemical screening revealed the presence of Flavonoids, Phenols, Tannins, Saponins, Alkaloids and Steroids. The LD_{50} of the extract was found to be greater than 5000mg/kg. There were significant reduction in the concentration of ALT, ALP and ALB ($P<.05$) in the group that received the highest dose of the extract when compared to the normal control while other liver biomarkers were not significantly different ($P>0.05$) from the control. The sub-chronic dose of 2000mg/kg of the extracts shows significant ($P<.05$) decrease in all kidney function biomarkers

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except chloride when compared to the control. The haematological parameters (WBC, RBC, HGB, Neutruphils) showed a significant decrease in Group 5 when compared to the normal control group while MCV and lymphocytes showed significant decrease (P<.05) when compared to the control.
Conclusion: The result suggests that the methanol leaves extracts of *Lannea acida* is relatively safe and can be used in medicinal formulations.

Keywords: Phytochemical; acute Toxicity; Lannea acida; Subchronic Toxicity.

1. INTRODUCTION

The study of herbal medicines and the use of plant leaves, stems, roots, seeds and the latex, for human benefits, is an age long event for human benefits [1]. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for the treatment of diseases possibly due to lower cost, availability, fewer adverse effects and perceived effectiveness [2]. The exploitation of cheap agricultural materials to manufacture industrial products will enhance the development of rural agro-based economy [3]. The historic role of medicinal plants in the treatment and prevention of diseases and their role as catalysts in the development of pharmacology do not however, assure their safety for uncontrolled use by an uninformed public [4]. The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants do indeed have medicinal values [3].

The use of traditional medicine in developed as well as developing countries as basis for the treatment of many ailments has been in existence for thousands of years and there is no doubt that their importance has been widely acknowledged [5]. As defined by the World Health Organisation, a medicinal plant is any plant which in one or more of its organs contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. Chukwuma et al. [6] reported that about 80% of the population in Nigeria use traditional medicine. Schmelzer and Gurib-Fakin [7] emphasized that medicinal plants significantly contributes to rural livelihoods of the people and social equilibrium in Africa. Traditional medicine enjoys a wider acceptability among the people of developing countries partly due the fact it blends readily into the socio-cultural life of the people in whose culture it is deeply rooted and also due to the inaccessibility of orthodox medicine [8]. In Africa, the resolution on “Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region”, adopted by the fiftieth meeting of the WHO Regional Committee for Africa in August 2000, states that the African Member States are aware that about 80% of the region’s population depends on traditional medicine for its health care needs. Some reasons advanced for this include affordability accessibility and beliefs [6].

Lanne acida belong to the family anacardiaceae, in Fulani-fulfulde (Nigeria) “faruhi” and in Hausa is known as “faàrú” [9], is a small deciduous tree, leaves exceeding 30 cm. It is used as an important drug in the indigenous system of treatment in North Nigeria. The leaves and bark are used in the management of fever and have been described to be useful in gout, rheumatism, for wounds, swelling and burns [9]. Despite preliminary evidence of therapeutic benefits and traditional use of *Lannea acida*, the scientific validation and systematic safety evaluation of *Lannea acida* has not been comprehensively established so far. This research therefore evaluates the possible acute and sub-chronic toxicity of orally administrated methanol extracts of *Lannea acida* on Wistar rat models.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The plant sample (*Lannea acida*) was collected from Tulluwa Kulafasa from Bodinga local government area, Sokoto State, Nigeria. The plant was taken to the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Kebbi State where it was identified and authenticate by plant taxononist. A voucher specimen with the number (VN 183) that was deposited at the Herbarium of the botany for future reference.

2.2 Experimental Animals

The Wistar albino rats were purchased from Animal House, Usmanu Danfodiyo University Sokoto. Thirty one (31) healthy Wister albino rats

of both sexes weighing 120 – 150 g were used for this study. All protocols were carried out in compliance with NIH guidelines for care and use of Laboratory Animals (Pub. No. 85-23, Revised 1985).

2.3 Qualitative Phytochemical Screening

Test for Flavonoids: Two millimetres (2mls) of the extract was treated with 1mls of 5% lead acetate solution in a test tube. A yellow colour indicates the presence of flavonoids [10].

Test for Phenols: The 2 mL of the extract was mixed with 2 mL of 1% ferric chloride. The formation of deep blue or blue-black coloration is an indication of a positive result [11].

Test for Tannins: Three (3) drops of 5% $FeCl_3$ solution was added to 3mls of the extract. Presence of brownish green or a blue-black precipitate (when viewed on white paper) indicated the presence of tannins (condensed and hydrolysable) [12].

Test for Saponins: Five millimetres (5mls) of the extract was shaken with 15mls of distilled water in a test tube. Persistent frothing on warming confirmed the presence of saponins [12].

Test for Phlobatannins: Exactly 2.5ml of 50% sulphuric acid was added to 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH. Then 5ml of Fehling's solution was added and mixture was boiled. A brick-red precipitate indicated the presence of glycosides [13].

Test for Alkaloids: Five millimetres (5ml) of 1% HCl was added to 1ml of the extract and stirred on a steam bath and filtered. Three portions (1ml each) of the filtrate were treated with 3 drops of Drangendorffs, Mayers and Wagners reagents respectively. Formation of turbidity confirmed the presence of alkaloids [12].

Test for Terpenoids: An amount of 0.8 g of selected plant sample was taken in a test tube, then poured 10 ml of methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform were mixed in extract of selected plant sample and 3 ml of sulphuric acid were added in selected sample extract. Formation of reddish brown colour indicates the presence of terpenoids in the selected plants [11].

Test for Steroids: Two millimetres (2mls) of acetic anhydride was added to 2mls of the extract followed by the addition of 2mls of dilute H_2SO_4. Violet colour which changes to blue indicates the presence of steroids [11].

Test for Anthraquinone: Five grams (5g) of sample extract was boiled with 10ml aqueous H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of benzene. The benzene layer separated and half of its own volume of 10% ammonia solution was added. A pink, red or violet colour indicated the presence of anthraquinone glycosides [11].

2.4 Toxicity Studies

2.4.1 Acute oral toxicity (LD_{50})

The acute oral toxicity study was conducted according to the method of Organization for Economic and Cultural Development for testing of chemicals (OECD) [14] using fixed limit dose. A total of six (6) animals were randomly selected and used for the experiment. The rats were divided into six groups of one rat each. They were fasted overnight providing water only. The first group received distilled water orally and served as control. The remaining groups (2 - 6) were administered single oral doses of 5000mg/kg bw of the extract. The animals were observed for toxicity periodically at 8hrs, 14hrs, 24hrs, and 48hrs and up to 14 days after administration of the extract. The rats were observed for toxic symptoms such as hair loss, loss of appetite, drowsiness, salivation, tremors, aggression, skin change, loss of weight and mortality [15].

2.4.2 Sub-chronic Toxicity

Sub-chronic oral toxicity test was performed according Organization of Economic Co-operation and Development guideline (OECD) [14]. A total of 25 albino rats of either sex weighing between 120 – 150g were randomly divided into 5 (5) groups of five (5) rats each.

The treatment was as follows:

Group I: 2ml/kg of Distilled water,
Group II: 250mg/kg of extract,
Group III: 500mg/kg of extract,
Group IV: 1000mg/kg of extract and
Group V: 2000mg/kg of extract.

2.5 Biochemical Analysis

The rats were fasted overnight on the 28th day and on the 29th day, they were sacrificed, blood samples were collected via cardiac puncture and centrifuged at 3000rpm for 10mins to obtain serum for further analysis. Biochemical parameters measured were serum alanine amino transferase (ALT) and aspartate amino transferase (AST) [16], total proteins [17], Bilirubin (Total and direct) [18], alkaline phosphatase [19], urea [20], and creatinine [21], levels using biochemical assay kits while serum electrolytes sodium and potassium would be measured using flame photometry [22].

2.6 Haematological Analysis

Parked Cell Volume (PCV), Haemoglobin concentration, Red Blood Cells count (RBC), White Blood Cells count (WBC) (neutrophils, lymphocytes, eosinophils, monocytes, basophiles), Haematocrit (HCT), Platelets, Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Haemoglobin (MCH) were analysed using an automated haematological analyser (Sysmex XS800i, Sysmex corporation, USA)

2.7 Statistical Analysis

All data were reported as Means ± Standard Error of Mean (SEM). The values were analysed using Statistical Package for Social Sciences (SPSS) 20.0, Duncan Post Hoc. Comparison test were used to check the differences between the individual groups. Test of significance between means were carried out using one-way analysis of variance (ANOVA). Differences in mean was considered significant if P<.05.

3. RESULTS

3.1 Phytochemical Screening

The phytochemical analysis of *Eucalyptus camaldulensis* stem bark revealed the presence of Flavonoids, Phenols, Tannins, Saponins, Alkaloids and Steroids. (Table 1).

3.2 Acute Oral Toxicity (LD_{50})

The methanol plant extract at a dose of 5000 mg/kg body weight of rats had no adverse effect on the rats such as change in body weight, irritation, restlessness, food and water refusal and respiratory distress were not observed over a period of 14 days.

Table 1. Qualitative phytochemical components of *Eucalyptus camaldulensis* stem bark

Tests	Inference
Flavonoids	+
Phenols	+
Tannins	+
Saponins	+
Phlobatannins	ND
Alkaloids	+
Terpenoids	ND
Steroids	+
Anthraquinones	ND
Glycosides	ND

Key: ND = Not detected + = Present

3.3 Biomarkers of Liver Function

The effect of administration of sub chronic doses of *L. acida* methanol extract on liver function indices is presented in Table 2. The ALT, AST and ALP concentrations showed that there was significant (P<.05) reduction in their concentrations when compared to the normal control. There was no significant (P<.05) difference in TP and DB concentrations. The ALB and TB concentrations were significantly reduced

3.4 Biomarkers of Kidney Function

Urea, creatinine, uric acid, K^+ were significantly (P<.05) reduced in the treated groups when compared to the normal control group. There was no significant change (P<.05) in the concentrations of Na^+, Cl^+, HCO_3^-.

3.5 Haematological Parameters

The effect of administration of sub chronic doses of *L. acida* methanol extract on haematological indices is presented in Table 4. WBC,RBC, HGB were significantly increased in the extract treated groups when compared with the normal control group. There no significant changes in MCV, PCV and platelets.

4. DISCUSSION

Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential efficacy [23]. The healing effect of many medicinal plants has been attributed to the presence of chemical constituents. The phytochemical constituents of this plant include

Table 2. Effect of administration of Sub chronic Doses of *L. acida* Methanol Extract on liver function Indices

PARAMETER	Control (2ml/kg D/water)	MLELA (250 mg/kg)	MLELA (500 mg/kg)	MLELA (1000 mg/kg)	MLELA (2000 mg/kg)
ALT (U/L)	24.01±1.40^{b}	22.40±1.20^{ab}	20.33±1.10^{ab}	18.25±1.59^{a}	16.33±1.76^{a}
AST (U/L)	61.00±1.99^{b}	60.80±1.41^{b}	58.33±0.68^{ab}	55.25±1.05^{a}	51.33±1.84^{a}
ALP (U/L)	199.83±0.37^{c}	192.22±1.56^{b}	188.91±1.63^{ab}	183.67±1.27^{ab}	179.32±1.54^{a}
TP (g/dl)	9.52±0.41^{a}	8.65±0.91^{a}	8.26±0.76^{a}	9.03±0.69^{a}	9.72±0.16^{a}
ALB (g/dl)	6.20±0.36^{b}	5.96±0.29^{ab}	5.25±0.15^{a}	5.10±0.32^{a}	4.19±0.51^{a}
DB (mg/dl)	0.34±0.66^{a}	0.29±0.53^{a}	0.18±0.49^{a}	0.31±0.12^{a}	0.26±0.23^{a}
TB (mg/dl)	0.68±0.61^{b}	0.72±0.44^{b}	0.65±0.37^{b}	0.48±0.24^{a}	0.51±0.11^{ab}

Values are expressed as mean ± standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). Key: ALT- Alanine Amino Transferase, AST- Aspartate Amino Transferase, ALP- Alkaline Phosphatase, TP- Total Protein, ALB- Albumin, DB- Direct Bilirubin, TB- Total Bilirubin.

Table 3. Effect of Administration of Sub chronic Doses of *L. acida* methanol Extract on kidney Function Indices

Parameters	Control (2m/kg D/water)	MLELA (250 mg/kg)	MLELA (500 mg/kg)	MLELA (1000 mg/kg)	MLELA (2000 mg/kg)
Urea (mmol/l)	15.71 ± 1.82^{b}	14.37±1.21^{ab}	13.99±1.19^{ab}	12.99±0.78^{a}	12.86±0.56^{a}
Creatinine (µmol/l)	13.65±1.70^{b}	12.24±1.54^{b}	10.20±1.90^{a}	11.09±1.74^{a}	11.03±1.99^{a}
UA (mg/dl)	3.13±0.30^{b}	2.96±0.72^{a}	2.24±0.38^{a}	2.24±0.38^{a}	2.16±0.72*^{a}
Na^{+} (mg/l)	133.60±9.82^{a}	134.80±9.76^{a}	131.00±9.55^{a}	129.42±9.11^{a}	126.12±9.29^{a}
Cl^{-} (mg/l)	3.40±0.19^{a}	3.30±0.16^{a}	3.27±0.44^{a}	3.21±0.14^{a}	3.13±0.17^{a}
K^{+} (mg/l)	48.60±2.83^{b}	46.80±2.40^{b}	41.33±2.60^{b}	38.75±2.25^{a}	34.33±2.60^{a}
HCO_3^{-} (mg/l)	3.50±0.27^{a}	3.24±0.62^{a}	3.81±0.17^{a}	3.11±0.31^{a}	3.08±0.17^{a}

Values are expressed as mean ± standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). Uric Acid (UA), Potassium (K+), Sodium (Na+), Bicarbonate (HCO_3)

Table 4. Haematological Indices of Rats Administered with Sub-chronic Doses (mg/kg bw) of Methanol Extract of *L. acida*

Parameter	Control (2m/kg D/water)	MLELA (250 mg/kg)	MLELA (500 mg/kg)	MLELA (1000 mg/kg)	MLELA (2000 mg/kg)
WBC (10^9/L)	12.20±1.52 [a]	12.69±1.22 [a]	13.42±1.55 [a]	15.02±1.58 [ab]	17.93±1.01 [b]
RBC (10^{12}/L)	5.88±0.73 [a]	7.39±0.26 [b]	7.44±0.33 [b]	7.27±0.48 [b]	6.78±0.58 [b]
HGB (g/dl)	12.18±0.84 [a]	13.96±0.96 [a]	15.13±1.19 [a]	18.38±1.22 [b]	21.73±1.37 [b]
MCHC (g/dl)	34.22±3.15 [b]	31.14±1.80 [b]	29.97±0.54 [a]	27.68±2.72 [a]	25.90±2.59 [a]
MCH (pg)	22.16±3.54 [b]	18.90±0.31 [b]	17.97±0.27 [a]	15.33±0.21 [a]	14.27±0.18 [a]
MCV (fL)	63.48±3.73 [a]	61.42±3.54 [a]	59.33±5.35 [a]	57.20±6.52 [a]	55.40±9.57 [a]
PCV (%)	41.68±1.29 [a]	42.52±1.84 [a]	41.13±2.28 [a]	40.08±3.19 [a]	42.63±3.92 [a]
Platelets (10^9/L)	687.60±47.14 [a]	689.75±21.22 [a]	692.33±38.68 [a]	695.42±47.69 [a]	698.67±51.56 [a]
Monocytes (%)	3.30±0.01 [a]	3.35±0.02 [a]	3.38±0.04 [a]	3.41±0.07 [a]	3.44±0.09 [a]
Neutrophils (%)	58. 30±2.01 [a]	59.32±2.54 [ab]	63.11±2.89 [b]	66.45±3.56 [b]	69.65±1.99 [b]
Lymphocytes (%)	34.25±3.18 [a]	37.84±1.00 [a]	39.95±1.81 [ab]	42.28±2.26 [b]	45.84±1.95 [b]

Values are expressed as mean ± standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). Key: WBC-white blood cell, RBC-red blood cell, HGB-haemoglobin, MCHC-mean cell haemoglobin concentration, MCH-mean cell haemoglobin, MCV-mean corpuscular volume, PCV-packed cell volume

Flavonoids, Phenols, Tannins, Saponins, Alkaloids and Steroids. Phytochemicals are also known as secondary metabolites. They are important plant constituents which have overlapping functions which may include antioxidant effect, modulation of detoxification enzymes, and stimulation of immune system and modulation of hormone metabolism [24].

Isolated flavonoids have been shown to be effective in the treatment of diarrhoea, gonorrhoea and blood poisoning. Flavonoids have also been reported to possess wound healing potentials [23]. This ability of flavonoids has also been postulated to occur by mechanisms that include activation of defence system through stimulation of prostaglandin synthesis. Tannin-rich plant extracts have been widely used for the bacterial infections [25].

The result of the acute toxicity test (LD_{50}) carried out showed that the plant has a very high safety margin even when administered to a very high dose of 5000mg/kg. In screening drugs, determination of LD_{50} is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of manifestations and it is one of the initial screening experiments performed with all compounds [26]. Data from acute toxicity studies may serve as the basis for classification and labelling, provide initial information on the mode of toxic action of a substance, help arrive at a dose of a new compound, help in dose determination in animal studies. The greater the index, the safer the compound [27]. Since the LD_{50} of *L. acida* was greater than 5000mg/kg in the present study, it goes to suggest that the extract is relatively non-toxic.

ALT, AST and ALP activities are commonly measured to monitor potential plant and drug induced hepatic injury in both pre-clinical studies and human patients and thus serves as indicators of liver toxicity [26]. ALT is localized primarily in the cytosol of hepatocytes, AST is normally found in the cytoplasm and mitochondria of many cells, primarily in cardiac muscle, liver and skeletal muscle. Its concentration is much lower, however, in the kidney, pancreas and erythrocytes [27]. ALP is found in most tissues, including bone, liver and kidney. Changes in ALT and AST activities may indicate alteration of cellular permeability or cellular injury and necrosis. Elevated activity of AST is also observed in myocardial infarction. Increased ALP level is usually a characteristic finding in cholestatic liver disease and biliary obstruction [28]. There was no significant increase in ALT and ALP activity following repeated oral administration of extract of *Lannea acida* at the highest dose. Significant changes were not also observed in AST. The lack of significant alterations in this indicates of liver damage suggests that sub-chronic administration of extract of *Lannea acida* have effect on hepatocyte function only at high concentrations.

Measurement of serum albumin and total proteins are usually performed to evaluate the synthetic capacity of the liver [29]. In the present study, there were no significant changes in TP, DB and TB levels in treated rats when compared to the control. Low serum albumin content may suggest infection or continuous loss of albumin. Malnutrition and dehydration can also cause depletion of these proteins in the serum. The absence of alteration in these parameters is suggestive that the synthetic ability of the liver was not affected buttressing further the absence of toxicity. Bilirubin is a break down product of haemoglobin. It is assayed to measure the binding, excreting, and conjugating ability of a hepatocyte [29]. As presented in the table, there was no significant decrease in total and direct bilirubin in the serum of extract treated rats when compared with the control. This is an indication that the extract might not have interfered with the metabolism of bilirubin in the liver.

Renal function indices such as urea, creatinine and uric acid are usually required to assess the normal functioning of the kidney. Urea and uric acid are the major nitrogen containing metabolic end product of protein catabolism. Creatinine is a waste product of muscle energy metabolism. They are found in the liver and conveyed through the blood to the kidney for excretion. Healthy kidneys remove these compounds from the blood to be excreted in the urine. Accumulation of these metabolites in the blood is a characteristic finding in renal dysfunction [30]. The results of serum levels of urea and creatinine of the extract treated groups showed significant difference ($P>0.05$) only with the highest dose when compared to the control. This may be due to the absence of toxicity of the extract of *Lannea acida* on the kidneys at low concentrations and hence is safe on its subchronic use in various diseases.

Serum electrolytes such as sodium, potassium, bicarbonate and chloride play a major role in intercompartmental water balance and the

exchange of gases. Significant alteration in the concentration of these electrolytes is indicative of poor renal functions or renal impairment. Decrease in sodium level can result from relative increase in the amount of body water relative to sodium. Potassium ion is a cation of the intracellular fluid which plays a vital role in muscle contraction [29]. In the present investigation, there was significant decrease potassium levels in the group treated with the highest dose of the extract when compared with the control; this could be attributed to interference of the extract to the normal functioning status of the kidney. There was no significant change (p>0.05) in chloride values in the treatment group when compared to the normal control. Bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered [28].

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals. The total red blood cell count, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), hemoglobin, and PCV are the most useful indicators in the diagnosis of anemia in humans and animals [30]. In the present study the evaluation of total RBC count, MCHC, MCH, MCV, hemoglobin and PCV did not show significant changes following repeated administration of the extract except in the group treated with the highest dose when compared to the controls. This finding is in agreement with other findings in which the values of the various hematological parameters of extract treated rats were found to be comparable with those of the control [30]. These results indicate that there is no lysis of blood cells, bleeding, anemia and inhibition in blood cells synthesis or bone marrow suppression by any of the active constituents in the extract of *Lannea acida*. White blood count was significantly increased in a dose dependent manner in rats treated with the extract. The rise in white blood count therefore, may be due to enhancement of white cell production, increase in its entrance into the blood, and a reduction in its removal from the circulation in an attempt to defend the system [31]. This may have been achieved by hyperstimulation of haematopoietic regulatory elements by macrophages and stromal cells in the bone marrow such as colony stimulating factors, interleukins (IL- 2, IL-4 and IL-5) which regulate the proliferation, differentiation and maturation of committed stem cells necessary for the production of white blood cells [30].

5. CONCLUSION

It also shows that L. acida leaf extract may lack toxic effect that will compromise its medicinal uses since no major alterations were found in the haematological, hepatic and renal parameters. This also shows that the plant may lack toxic effect that will compromise its medicinal uses since no major alterations were found in the haematological, hepatic and renal parameters. The biological active compounds of the extracts such Flavonoids, Phenols, Tannins, Saponins, Alkaloids, Steroids may be responsible for the observed pharmacological properties of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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