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Drug Discovery

Acute and sub-acute toxicity study of *Lonchocarpus laxiflorus* leaf methanol extract on albino rats

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ABSTRACT

Toxicology is the vital part of pharmacology which deals with the undesirable effect of phytocompounds on living organisms before they are use as drug or chemical in clinical use. This study is aimed at evaluating the acute and sub-acute toxicity effects of *Lonchocarpus laxiflorus* leaf methanol extracts (LLMLE). Standard OECD guidelines were used to conduct acute and subchronic toxicity evaluations. The results of acute oral administration of the leaf extracts indicated no mortality up to 14 days after treatment. There was also no sign of toxicity, and the LD50 was assumed to be greater than 3000mg/kg. In sub-chronic toxicity, there was no significant (P>0.05) alteration in animal bodyweight throughout the experimental period suggesting that the extract did not interfere with feeding or regulate appetite. There was also no significant (P>0.05) alteration in all liver biomarkers of all *Lonchocarpus laxiflorus* leaf methanol extracts treated groups compared to control. *Lonchocarpus laxiflorus* leaf methanol extracts (LLMLE) did not cause any significant (P>0.05) alteration in kidney function parameters. Similarly, there were no significant (P>0.05) alteration in haematological parameters of all *Lonchocarpus laxiflorus* leaf methanol extracts treated groups compared to control. Hence, *Lonchocarpus laxiflorus* leaf methanol extract is not hepatotoxic, nephrotoxic and do not have a haematological effect, thus this research validates the safety of this plant at acute and subchronic administrations

Keywords: Acute, sub-acute, toxicity, *Lonchocarpus laxiflorus*

1. INTRODUCTION

About 80% of the world's population relies on traditional medicine for primary health care (Ekor, 2014; Ugwah-Oguejiofor and Ugwah, 2018). Even though the utilization of these plants has shown promising potential with high global demand, there are still concerns about not only their use but also their safety profile (Ifeoma and Oluwakanyinsola, 2013). Herbal medicine are considered relatively safe or are they have low toxicity profile due to their long history of use by humans (Yuet et al., 2013; Ibrahim et al., 2016). Nevertheless, the latest documented scientific evidence

indicated that many of these products used in folklore medicine revealed adverse effects (Koduru et al., 2006). Toxicology is an essential part of pharmacology that deals with the undesirable effect of compounds on living organisms previous to their use as drugs or chemicals in clinical use (Aneela et al., 2011).

Toxicity analysis is essential, the majority of herbs ingested might have some toxic effects, and several reports have are documented for toxicity due to long term consumption of herbs. The occurrence of toxicity mechanism differs depending on the cell membrane and chemical properties of the xenobiotic in biological system. It might happen within the cell membrane or on the cell surface or tissue underneath, as well as at the extracellular matrix. According to OECD guidelines, to ascertain the protection and effectiveness of a new drug, toxicological studies are highly significant in lower like mice, rat, guinea pig, dog, rabbit, monkey etc. Toxicological studies aid to extend decision whether a new drug should be adopted for clinical use. OECD guidelines such as 401, 423 and 425 do not permit the use of drug clinically without its clinical trial as well as toxicity studies (Ecobichon, 2007).

2. MATERIALS AND METHOD

Collection and Identification of Plant Sample

The plant sample is collected in August 2021 from Aleiro town, Aleiro Local Government Area of Kebbi State. And was identified by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology Aleiro. The plant was identified as *Lonchocarpus laxiflorus*.

Sample Preparation and Extraction

Lonchocarpus laxiflorus leaves were washed with clean water and allowed to dry under shade for two weeks. It was then grinded to coarse powder using mortar and pestle. Five hundred grams (500g) of the powdered leaves was soaked in 2000mls of methanol for 72hrs (Dupont et al., 2002). It was then filtered using muslin cloth and the extract was concentrated in an oven set at 45oC. The dried extract was stored in an air tight container and kept in refrigerator at 4oC. The percentage yield of the extract was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of samlpe}} \times \frac{100}{1}$$

Acute Oral Toxicity Studies (LD50)

Acute oral toxicity study of extract was conducted according to Organization for Economic Co-operation and Development guidelines for testing of chemicals. The rats were fasted overnight, and the weight of each rat was recorded before use. Five Animals received the crude plant extracts at a dose of 5000 mg/kg body weight. Animals were observed for 1hr, 4hrs, 6hrs and 12hrs after administering the extracts, and then observed daily for 14 days for any change in general behaviour and other physical activities (Hickie et al., 1995).

Sub-chronic Oral Toxicity Study

Sub-chronic oral toxicity study was carried out according to the Organization for Economic and Cultural Development (OECD 407, 2008) guideline. Fifteen female albino rats are grouped into five groups containing three animals each. The rats are treated as follows:

Group I: Served as the normal control. No extract treatment.

Group II: It was administered with extract 250 mg/kg bodyweight daily basis for 28 days.

Group III: It was administered with extract 500 mg/kg bodyweight daily basis for 28 days.

Group IV: It was administered with extract 750 mg/kg bodyweight daily basis for 28 days.

Group V: It was administered with extract 1000 mg/kg bodyweight daily basis for 28 days.

The extract was administered to the animals orally. Bodyweight changes is also monitored weekly throughout the experimental period. The rats were sacrificed on the twenty-ninth day of the experiment. Blood samples were collected in heparinised bottles for biochemical analysis while organs (livers and kidney) were collected for histopathology evaluation.

Liver Function Parameters (Biochemical Analysis)

Alkaline phosphatase activity was estimated using the method of (Sood, 2006). Aspartate aminotransferase catalytic activity and alanine aminotransferase activity are determined by the methods of (Reitman and Frankel, 1957). Albumin was determined according

to the method modified by (Doumas et al., 1971). Total protein was estimated using the Biuret reaction method by (Lowry et al., 1951). Total and Direct Bilirubin were determine by the calorimetric method of (Jendrassik and Grof, 1938).

Kidney Function Parameters

Serum urea was determine using the Berthelot colorimetric method of (Young, 1997). Serum creatinine was determined using Jaffe's method as described by (Bartels and Bohmer, 1971). Serum uric acid concentration was determined using the method of (Henry et al., 1957). Serum sodium and potassium ions were measured using the method of (Cheesbrough, 1991). Serum bicarbonate and chloride ions were measured using titration/volumetric method of (Chapman and Parker, 1961).

Haematological Analysis

Hematological parameters, white blood cells count (WBC), hemoglobin concentration, packed cell volume (PCV), lymphocytes, neutrophils, eosinophils, monocytes, and basophils, are analyzed using an automated hematological analyzer Sysmex XS800i (Sysmex corporation, USA), (Theml et al., 2004).

Histopathological Examination

Histopathology was conducted using the method of (Drury et al., 1967). The liver, kidney, and pancreas of the rats were harvested and preserved in 10 % formalin. The organs were fixed in 10 % buffered formalin for 72 hours. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. Embedded tissues are cut into sections of 5 μm thick, and these were stained with hematoxylin and eosin for photo microscopic assessment and placed on a clean labelled microscope glass slide. The slide was mounted on an electric light microscope to examine any possible histopathological features. Photomicrographs of the samples are then taken.

Data Analysis

All data generated from the study were presented as Mean ± Standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were separated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered significant at $P<0.05$.

3. RESULTS

Weight and Percentage Yield

The weight and percentage yield of *Lonchocarpus laxiflorus* methanol extract is 37g and 7.4% respectively, and the extract is soluble in water, dark brown in colour with a gummy texture.

Acute Toxicity (LD50) Profile of *L. laxiflorus* Leaf Methanol Extract

The results of acute oral administration of the leaf extracts indicated no mortality up to 14 days after treatment. There was also no sign of toxicity, and the LD50 was assumed to be greater than 3000mg/kg.

Sub-chronic Toxicity Effect of L. laxiflorus Methanol Leaf Extract on Albino Rats

Effect of L. laxiflorus Methanol Leaf Extract on Bodyweight of Rats

The effect of *Lonchocarpus laxiflorus* methanol leaf extract on bodyweight revealed a non-significant ($P> 0.05$) difference at weeks 0, 1, and 3 in all extract-treated groups compared to normal control. However, at week 2 of extract administration only group administered with extract 500mg/kg significantly ($P< 0.05$) reduced in bodyweight compared to normal control. While at week 4 of extract administration only group administered with extract 750mg/kg significantly ($P< 0.05$) increase in bodyweight compared to normal control (Table 1).

Table 1 Effect of *Lonchocarpus laxiflorus* Methanol Leaf Extract on Bodyweight

Treatments	Bodyweight (g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control Distilled H2O 5ml/kg	139.67±6.23ab	152.00±7.23ab	151.67±8.21b	143.67±6.67a	145.67±7.13a
LLMLE (250mg/kg)	118.25±4.46a	128.50±6.00a	92.00±23.64a	133.75±7.22a	143.25±5.22a
LLMLE (500mg/kg)	122.25±10.45a	128.00±14.08a	122.5±18.11ab	161.75±31.89a	157.75±21.48a
LLMLE (750mg/kg)	138.25±9.47ab	154.50±7.66ab	146.5±8.19b	183.50±6.59a	212.00±4.14b
LLMLE (1000mg/kg)	139.67±6.23b	152.00±7.23b	151.67±8.21b	143.67±6.67a	145.67±7.13ab

Values are presented as mean ± SEM (n = 4). Values having the same superscript down the column are not significantly different at (*P*>0.05) analyzed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. LLMLE= *Lonchocarpus laxiflorus* Methanol Leaf Extract

Effect of L. laxiflorus Methanol Leaf Extract on Liver Function Parameters of Rats

The effect of *Lonchocarpus laxiflorus* methanol leaf extract on liver function parameters revealed non-significant (P> 0.05) difference in aspartate aminotransferase (AST), total protein (TP) and total bilirubin (TB) in all extract-treated groups compared to normal control. However, at week 2 of extract administration only group administered with extract 500mg/kg significantly (P< 0.05) reduced I bodyweight compared to normal control. While at week 4 of extract administration only group administered with extract 750mg/kg significantly (P< 0.05) increase in bodyweight compared to normal control.

However, a significant (P< 0.05) increase in alanine aminotransferase (AST) was only observed in extract-treated group 500mg/kg compared to normal control. Also, there were significant (P< 0.05) increases in albumin (ALB) in extract-treated groups 500, 750 and 1000mg/kg body weight compared to normal control. In contrast, a significant (P< 0.05) decrease in direct bilirubin was observed in extract-treated group 1000mg/kg body weight compared to normal control (Table 2).

Table 2 Effect of *L. laxiflorus* Methanol Leaf Extract on Liver Function Parameters of Rats

Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)	ALB (G/l)	TP (G/l)	TB (mg/dL)	DB (mg/dL)
Normal Control Distilled H2O 5ml/kg	72.94±7.89a	27.26±4.01a	223.56±114.56b	0.12±0.043a	3.34±0.53a	4.30±0.25a	1.80±0.15b
LLMLE (250mg/kg)	67.79±14.54a	36.61±5.52a	74.22±8.36a	0.86±0.45ab	4.07±0.24a	5.36±1.00a	2.32±0.43b
LLMLE (250mg/kg)	67.79±14.54a	36.61±5.52a	74.22±8.36a	0.86±0.45ab	4.07±0.24a	3.62±1.02a	1.48±0.55ab
LLMLE (750mg/kg)	79.97±21.94a	34.73±2.79a	57.27±23.35a	1.91±0.19b	3.70±0.61a	3.07±1.07a	1.23±0.53ab
LLMLE (1000mg/kg)	31.41±5.24a	30.14±3.92a	101.20±15.96ab	1.85±0.19b	3.96±0.70a	5.42±1.52a	0.17±0.0a

Values are presented as mean ± SEM (n = 3). Value having the same superscript in column are not significantly different at (*P*>0.05) analyzed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. AST-Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, ALB- Albumin, TP- Total Protein, TB- Total Bilirubin and DB- Direct Bilirubin. LLMLE= *Lonchocarpus laxiflorus* Methanol Leaf Extract

Effect of L. laxiflorus Methanol Leaf Extract on Kidney Function Parameters of Rats

The effect of *Lonchocarpus laxiflorus* methanol leaf extract on kidney function parameters revealed non-significant (P> 0.05) differences in creatinine, urea, uric acid, sodium ions (Na+), calcium ions (K+), chloride ions (Cl-) and bicarbonate (HCO3-) in all extract-treated groups compared to control (Table 3).

Table 3 Effect of *L. laxiflorus* Methanol Leaf Extract on Kidney Function Parameters of Rats

Treatments	Creatinine (mg/dl)	Urea (mmol/l)	Uric acid (mg/dl)	Na+ (mmol/l)	K+ (mmol/l)	Cl- (mmol/l)	HCO3- (mmol/l)
Normal Control Distilled H2O 5ml/kg	0.29±0.04a	1.30±0.07a	0.06±0.01a	70.00±7.64ab	20.00±2.89a	0.72±0.15ab	0.37±0.03a
LLMLE (250mg/kg)	0.37±0.03a	1.47±0.10a	0.08±0.01a	75.00±3.54ab	17.50±1.44a	0.85±0.09b	0.38±0.09a
LLMLE (500mg/kg)	0.44±0.09a	1.06±0.19a	0.10±0.02a	82.50±2.50b	16.25±1.25a	0.69±0.07ab	0.20±0.07a
LLMLE (750mg/kg)	0.39±0.05a	1.41±0.05a	0.09±0.02a	76.25±2.40ab	17.50±2.50a	0.65±0.04ab	0.38±0.06a
LLMLE (1000mg/kg)	0.50±0.09a	1.22±0.15a	0.12±0.04a	60.00±15.00a	13.33±1.67a	0.53±0.09a	0.27±0.03a

Values are presented as mean ± SEM (n = 3). Value having same superscript in column are not significantly different at (*P*>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. Potassium (K+), Sodium (Na+), Bicarbonate (HCO-3). LLMLE= *Lonchocarpus laxiflorus* Methanol Leaf Extract

Effect of L. laxiflorus Methanol Leaf Extract on Haematological Indices of Rats

The effect of *Lonchocarpus laxiflorus* methanol leaf extract on hematological indices revealed non-significant (P> 0.05) differences in white blood count (WBC), red blood count (RBC), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT) in all extract-treated groups compared to control. In contrast, a significant (P< 0.05) increase in lymphocytes (LYM) was observed in extract-treated groups 750 and 1000mg/kg compared to normal control. Also, a significant (P < 0.05) increase in mean corpuscular hemoglobin was observed in extract-treated groups 250 and 500mg/kg compared to normal control. However, a significant (P< 0.05) decreases was observed in granulocytes (GRA) in all extract-treated groups compared to normal control (Table 4).

Table 4 Effect of *L. laxiflorus* Methanol Leaf Extract on Haematological Indices of RatsValues are presented as mean ± SEM (n = 3).

Treatments	WBC (x103/uL)	RBC (x106/uL)	HGB (g/dL)	LYM (%)	GRA (%)	MCH (pg)	MCHC (g/dl)	PLT (x103/uL)
Normal Control (D. H2O 5ml/kg)	16.28±55.12ab	6.80±1.13a	12.80±1.81a	68.20±2.81a	18.40±0.38b	18.97±0.55a	32.27±0.18a	384.23±301.01a
LLMLE (100mg/kg)	12.98±2.32ab	1.46±0.21a	7.48±0.40a	74.13±4.21ab	8.35±0.51a	61.45±0.66b	20.43±0.25a	32.93±0.96a
LLMLE (100mg/kg)	20.29±1.16b	3.06±1.13a	8.31±1.71a	79.95±4.49ab	9.20±3.43a	51.43±10.19b	23.48±3.32a	26.80±6.71a
LLMLE (200mg/kg)	13.10±2.53ab	6.33±0.31a	12.98±0.59a	85.40±1.94b	3.93±1.37a	20.48±0.23a	33.58±0.19a	379.50±114.10a
LLMLE (400mg/kg)	7.51±3.62b	26.63±22.83a	8.63±3.47a	78.23±3.62b	9.63±3.19a	18.47±0.61a	21.40±10.71a	99.37±30.31a

Value having the same superscript in column are not significantly different at (*P*>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0, RBC- Red blood count, LYM- Lymphocytes, HGB- Hemoglobin, PLT- platelets, MCH- Mean cell volume, MCHC-mean corpuscular haemoglobin concentration, WBC- White Blood Count. LLMLE= *Lonchocarpus laxiflorus* Methanol Leaf Extract.

Effect of Lonchocarpus laxiflorus Leaf Methanol Extract on Histology of Liver

The effect of *L. laxiflorus* methanol leaf extract on the liver histology is presented in (Plates 1– 5). The liver of normal control showed normal central vain (blue arrow) and normal hepatocytes (black arrow) (Plate 1). Group treated with extract 250mg/kg showed normal,

central vein (blue arrow) and normal hepatocytes (black arrow) (Plate 2). Group treated with extract 500mg/kg showed normal, central vein (blue arrow) and normal hepatocytes (black arrow) (Plate 3). Group treated with extract 750mg/kg showing Normal central vein (blue arrow), regular portal triad (green arrow) and normal hepatocytes (black arrow) (Plate 4). Group treated with extract 1000mg/kg showed normal central vein (blue arrow) and normal hepatocytes (black arrow) (Plate 5).

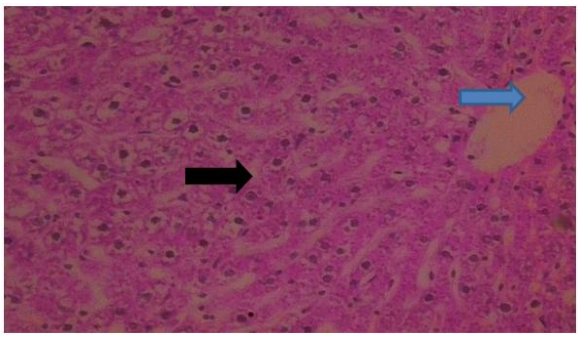

Plate 1 Photomicrograph of rat's liver obtained from control

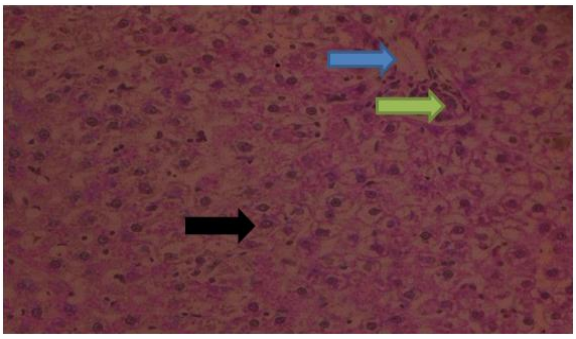

Plate 4 Photomicrograph of rat's liver obtained from group administered with 750 mg/kg of *L. laxiflorus* leaf methanol extract.

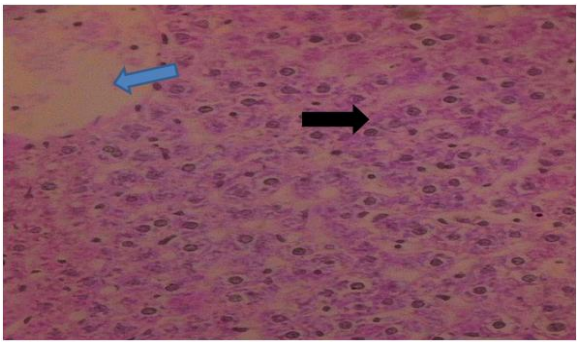

Plate 2: Photomicrograph of rat's liver obtained from group administered with 250 mg/kg of *L. laxiflorus* leaf methanol extract.

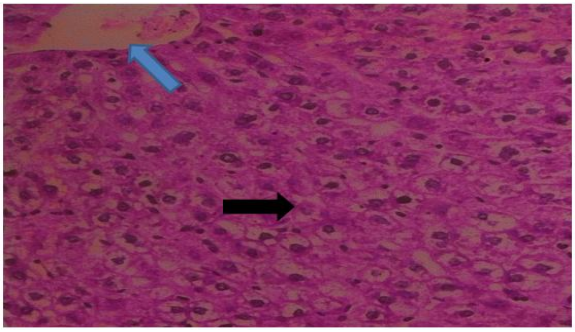

Plate 5 Photomicrograph of rat's liver obtained from group administered with 1000 mg/kg of *L. laxiflorus* leaf methanol extract.

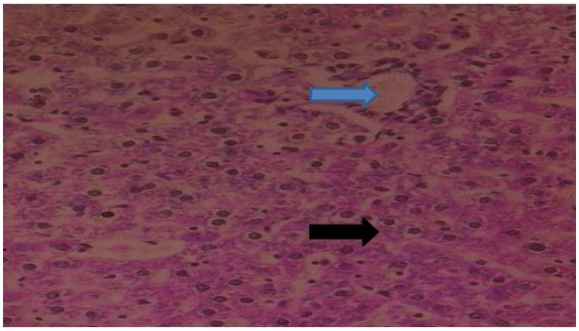

Plate 3 Photomicrograph of rat's liver obtained from group administered with 500 mg/kg of *L. laxiflorus* leaf methanol extract.

Plate 1 Section showing normal, central vain (blue arrow) and normal hepatocytes (black arrow). **Plate 2**: Section show Normal, central vain (blue arrow) and normal hepatocytes (black arrow). **Plate 3**: Section showed normal, central vain (blue arrow) and normal hepatocytes (black arrow). **Plate 4:** Section showing Normal central vain (blue arrow), normal portal triad (green arrow) and normal hepatocytes (black arrow). **Plate 5**: Section showing normal, central vain (blue arrow) and normal hepatocytes (black arrow). All Plates are stained with H & E X 100 magnification

Effect of Lonchocarpus laxiflorus Leaf Methanol Extract on Histology of Kidney

The effect of *L. laxiflorus* methanol leaf extract on histology of the kidney is presented in (Plates 6-10). The kidney of normal control group showed normal glomerulus (blue arrow) with normal tubule (black arrow) (Plate 6). Group treated with extract 250mg/kg showed normal glomerulus (blue arrow) with slightly dense tubule (black arrow) (Plate 7), Group treated with extract 500mg/kg showed normal, central vain (blue arrow) and normal hepatocytes (black arrow) (Plate 8). Group treated with extract 750mg/kg, and 1000mg/kg showed normal glomerulus (blue arrow) with normal tubule (black arrow) Plate 9, and 10 respectively.

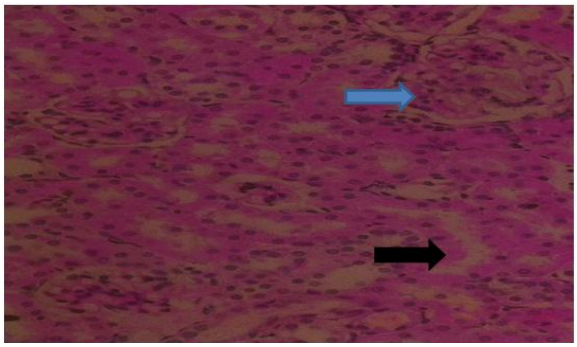

Plate 6 Photomicrograph of rat's kidney obtained from control

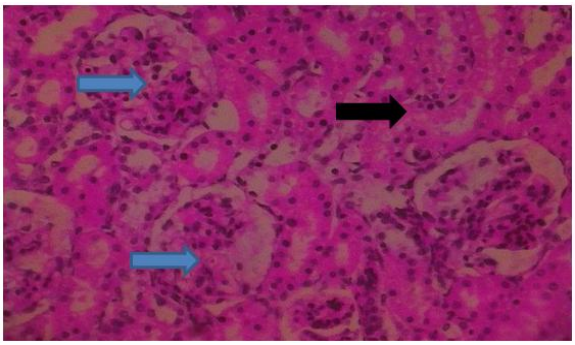

Plate 9 Photomicrograph of rat's kidney obtained from group administered with 750 mg/kg of *L. laxiflorus* leaf methanol extract.

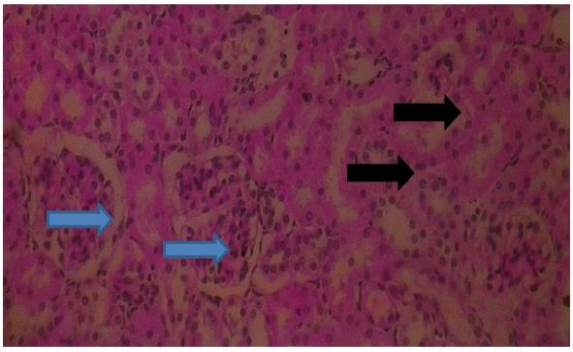

Plate 7 Photomicrograph of rat's kidney obtained from group administered with 250 mg/kg of *L. laxiflorus* leaf methanol extract.

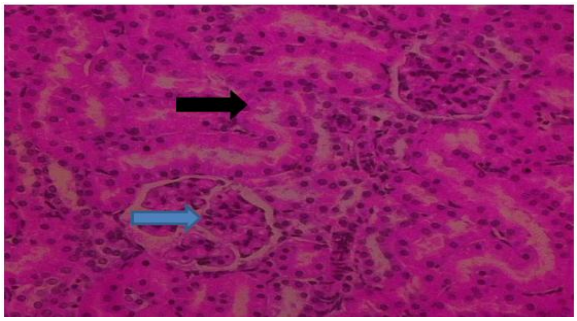

Plate 10 Photomicrograph of rat's kidney obtained from group administered with 1000 mg/kg of *L. laxiflorus* leaf methanol extract.

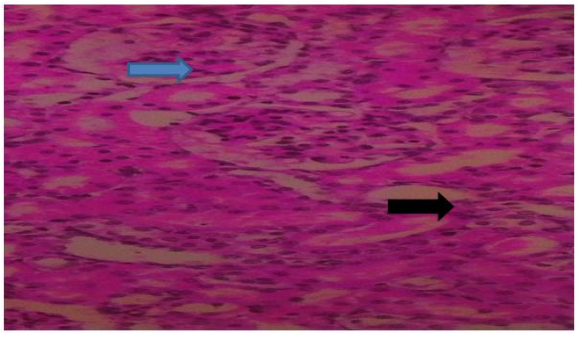

Plate 8 Photomicrograph of rat's kidney obtained from group administered with 500 mg/kg of *L. laxiflorus* leaf methanol extract.

Plate 6 Section showing normal glomerulus (blue arrow) with normal tubule (black arrow). **Plate 7**: Section showing normal glomerulus (blue arrow) with slightly densed tubule (black arrow). **Plate 8**: Section showed normal, central vain (blue arrow) and normal hepatocytes (black arrow). **Plate 9 and 10**: Sections showed normal glomerulus (blue arrow) with normal tubule (black arrow) respectively. All Plates are stained with H & E X 100 magnification

4. DISCUSSION

Acute toxicity refers to those adverse reactions that occur following oral or dermal administration of a single dose of a substance, or several doses given within one day, or an inhalation exposure of 4 hours (Bradberry et al., 2005). According to Prieto et al., (2013) substances with LD50 below 5 mg/ kg are classified to be highly toxic, substance with LD50 between 3000-5000mg/kg are relatively non-toxic, while substances with LD50 above 15,000 mg/kg are termed relatively harmless. In the present study, the LD50 *Lonchocarpus laxiflorus* methanol leaf extract is assumed to be above 3000mg/kg bodyweight, suggesting that the extract is relatively non-toxic at acute dose. Sub-chronic toxicity testing is essential in investigating target organ and haematological or biochemical effects of extracts since these effects are usually not observable in acute toxicity testing (Dasgupta et al., 2019).

According to Ugwah-Oguejiofor and Ugwah, (2018) changes in feed and water intake and body weight gain is used as indicator of the general health status of experimental animals. Feed consumption is regulate through several complex biological mechanisms that ensure relatively constant body weight over long periods (James, 2008). Appetite governs the body's desire for food and plays essential role in weight regulation. In the present study there was no alteration in animal bodyweight throughout the experimental period suggesting that the extract did not interfere with feeding or regulate appetite. Serum liver function tests revealed information about the status of the liver. The liver enzymes (aminotransferases; ALT and AST) describe its cellular integrity, while albumin and total protein levels describe its functionality (Adeoye and Oyedapo, 2004).

AST and ALT are primary synthesized by the liver cells and any injury to the liver may lead to an increase in the serum level of these enzymes (Adedapo et al., 2004). High levels of liver enzymes are signs of hepatocellular toxicity, whereas a decrease may indicate enzyme inhibition (Brautbar and Williams, 2002). The functionality of the liver is assessed by the serum total protein, bilirubin and albumin. A reduction in serum levels of total proteins, bilirubin, and albumin indicates reduced synthetic function, which is evident in liver damage or diseases. An increase in these parameters is usually observe in cancerous conditions or following high protein diet (Yousef et al., 2010). In the present study, there was no alteration in all liver biomarkers suggesting that *Lonchocarpus laxiflorus* leaf methanol extract is not hepatotoxic.

The usual serum test which determines the functionality of the kidneys measures the levels of urea, creatinine and certain dissolved salts (serum electrolyte), (Ugwah-Oguejiofor and Ugwah, 2018). A high serum level of urea indicates that the kidneys may not be working correctly, or that the animal is dehydrated whereas, low urea levels is seen in acute liver failure or overhydration (Njinga et al., 2020). Creatinine clearance, an indicator of glomerular filtration rate is used for assessing kidney function (Traynor et al., 2006). *Lonchocarpus laxiflorus* leaf methanol extracts did not cause any apparent alteration in kidney function parameters, suggesting that the extract is not nephrotoxic.Analyses of haematological parameters are used to study the extent of toxicity of drug substances including plant extracts (Ibrahim et al., 2016).

Haematopoiesis is the process of blood cell formation. Alterations in haematopoietic system have a higher predictive value for human toxicity when data is translate from animal studies. All blood cells are believe to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet), (George-Gay and Parker, 2003). Lymphocytes are dynamic cells and mediate immune response to foreign substances (George-Gay and Parker, 2003; Lisman and Porte, 2010). In the present study, there was no alteration in all haematological parameters, suggesting that *Lonchocarpus laxiflorus* leaf methanol extracts do not have adverse effect on haematological parameters.

5. CONCLUSION

Acute and sub-chronic toxicity evaluation revealed that *Lonchocarpus laxiflorus* methanol leaf extract is relatively non-toxic at acute and sub-chronic administration. Hence this study establishes scientific explanation for the safety of *Lonchocarpus laxiflorus* methanol leaf extract.

Informed consent

Not applicable

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Ethical approval
The Animal ethical guidelines are followed in the study for experimentation. Acute oral toxicity study of extract was conducted according to Organization for Economic Co-operation and Development guidelines for testing of chemicals. Sub-chronic oral toxicity study was carried out according to the Organization for Economic and Cultural Development (OECD 407). The ethical guidelines for plants & plant materials are followed in the study for plant collection, identification & experimentation.

Data and materials availability
All data associated with this study are present in the paper.

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