BIU Journal of Basic and Applied Sciences 5(1): 125 – 136, 2020. ©Faculty of Science, Benson Idahosa University, Benin City, Nigeria ISSN: 2563-6424

TOXICOLOGICAL PROFILE OF METHANOL LEAF EXTRACT OF Annogeissus leiocarpus (DC.) GILL AND PEER IN ALBINO WISTER RATS

*INNALEGWU, D. A.,¹ AMUDA, O.,² GARA, T. Y.¹ AND MUHAMMAD, F. M.¹

¹Department of Biochemistry, Federal University of Technology Minna, Nigeria ²Department of Microbiology, Federal University of Technology Minna, Nigeria *Corresponding author: a.daniel@futminna.edu.ng

ABSTRACT

Toxicity of medicinal plants and dose quantification is one of the major challenges confronting its acceptance and usage. This research is aimed at evaluating the toxicological profile of methanol leaf extract of Annogeissus leiocarpus in Albino Wister Rats. Acute and sub-chronic toxicity of the extract was carried out using the methods described by Lorke's and Organization for Economic Co-operation and Development respectively. Histopathology and microscopic examination of the liver and kidney was carried out using standard methods while enzyme assay of the serum was carried out using the standard ready-to-use kits. Acute toxicity of the extract gave an LD_{50} of 1789mg/kg bodyweight while the sub-chronic toxicity profile of the extract for 10 weeks shows mild effects on the haematological, biochemical and renal parameters of the animals with a significant correlation (p<0.01 and p<0.05) between the treated and the control group. There was a significant difference (p<0.05) between the serum enzymes of the control and treated group throughout the 10 weeks of treatment with the extract. Microscopic examination of the liver and kidney shows no alteration in the histoarchitecture of the organs. Toxicological evaluation of A. leiocarpus showed that the plant is safe however, prolonged use of this plant should be discouraged as its accumulation may become harmful particulaly to the liver and the kidney. Further study is therefore required to validate the safety and usage of Annogeissus leiocarpus leaf.

KEYWORDS: Annogeissus leiocarpus, Acute toxicity, Serum enzymes, Histoarchitecture

INTRODUCTION

The major challenge confronting the usage of medicinal plants to treat ailments in traditional medicine is quantification and safety of herbal mixture. There are no empirical methods for traditional practitioners to determine the actual or safe dose and monitor delayed effects, rare or adverse effects such as mutagenicity, arising from long-term administration of herbal mixtures (Sharif *et al.*, 2015).

There has been increasing interest in the use of medicinal herbs for meeting the of primary goal healthcare delivery worldwide in the developing particularly countries who have large percentage of her populace depending on medicinal herbs as their sole source of treatment (Atansuyi et al., 2012).

Accordingly, evaluation of toxicity profile of medicinal plants has been carried out (Ojo et al., 2014; Ekundayo et al., 2011; Kalimuthu et al., 2010) and are ongoing as majority of medicinal plants need to be explored and studied. Plants remain a major source of medicine (Ojo et al., 2014). Phytochemicals or secondary metabolites from plants have been considered as alternatives to synthetic compounds due to their natural sources. Plant extracts have been used for the treatment of various diseases and they are the basis of all traditional systems of medicine (Ojo et al., 2014). The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries due to the poor economic system of these countries and poor maintenance of the health care facilities (Kalimuthu et al., 2010). In recent years, advocates of pharmacological medicinal and professionals have lauded the use of indigenous drugs in the treatment of diseases (Ekundayo et al., 2011). However, the toxic effects produced by the administration of these drugs from plants may be much more severe than that of the disease itself (Ekundayo et al., 2011).

Annogeissus leiocarpus is a deciduous tree which reproduce by seeds as well as vegetative propagation. The plant has been used locally for treating different ailments due to its medicinal potentials (Arbab, 2014). The stem is used majorly by the rural dwellers in Nigeria for maintaining good oral hygiene. In Ivory Coast, it is also used for the treatment of parasitic diseases such as Malaria, Trypanosomiasis, Helminthiasis and dysenteric herbal syndrome. Traditional practitioners in Togo used it for the management of fungal infections such as dermatitis and Mycosis, also the decoction of leaves is used against stomach infections (Arbab, 2014). The plant is also used for the treatment of diabetic ulcers general body pain, blood clots, asthma, coughing and tuberculosis (Victor. 2013). Therefore, aim and objectives of this study is to evaluate the toxicological profile and effects of the methanol extract of Annogeissus leiocarpus in the serum, liver and kidney of albino Wister Rats.

METHODS

Plant Collection and Identification

The leaves of Anogeissus leiocarpus were freshly collected during the raining season in the month of September 2017 and was identified by a botanist at the Herbarium unit of National Institute for Pharmaceutical Research and Development, Idu, Abuja. The voucher number: NIPRD/H/6799 was deposited at the herbarium. The toxicological analysis of the crude methanol extract of A. leiocarpus was carried out at the Pharmacological unit of National Institute for Pharmaceutical Research and Development, Idu. Abuja, Nigeria.

Preparation of Plant Materials

The leaves of *A. leiocarpus* were washed with distilled water and shade dried at room temperature (25°C) for two weeks. The dry leaves were pulverized into fine powder using the kitchen type blender. Five hundred grams (500 g) of powdered sample was extracted using Soxhlet apparatus with 2.5 litres of methanol. The extract was concentrated by rotary evaporator under reduced pressure and stored in the refrigerator at 4°C until it is required for use. The percentage yield of the crude extract was calculated using the following formula:

Percetage yield (%) weight of extract

 $= \frac{weight of extract}{Total weight of sample extracted} X 100$ *Animals*

Male adult Wistar rats (180–250g) were used for the acute and subchronic toxicity profiling. The animals were obtained from National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria. They were fed ad libitum with standard feed and had free access to water. They were also maintained standard conditions under of humidity, temperature, and 12 hours light/darkness cycle. The animals were allowed to acclimatize for two weeks prior to the study. A standard protocol was followed in accordance with the Good Laboratory Practice (GLP). The "principles of laboratory animal care" were also adhered in this approved study as by Federal University of Technology Minna Research Committee on Animal Right. Acute Toxicity Studies

The acute toxicity (LD₅₀) was estimated orally in rats (n = 4) in each case following Lorke's method as reported by (Sharif et al., 2015). Dose levels of 100, 200, 400, 600, 800, 1000 mg/kg body weight were used for the first phase. The number of deaths in each group within 24 hours was recorded. In the second phase which was deduced from the first phase, 16 rats were grouped into four groups of 4 rats each and they were treated with doses of 1200, 1600 and 2000 mg/kg body weight orally. They were also observed for 24 hours as in the first phase, and final LD₅₀ value was determined from Lorke's formula as follows: $LD_{50} = \sqrt{a+b}$. Where *a* is the highest dose at which no death occurred in the second phase and *b* is the least dosage at which death occurred in the second phase (Oyewole *et al.*, 2013).

Sub-chronic Toxicity Studies

Repeated-dose toxicity study was in employed accordance with Organisation for economic co-operation development and (OECD) 2002 guidelines (OECD, 2010) to assess the toxic effects resulting from the accumulation of compounds in an organism. The study was conducted for a period of ten (10) weeks with a total of 40 rats (20 rats for the test and 20 rats for the control) and at the end of week one, five and ten, four rats are randomly selected, anesthetized and their blood samples collected through cardiac puncture into sample bottles containing EDTA for haematological analyses while the remaining blood was kept in plain bottle from which serum was collected and stored for biochemical analysis. At the end of the experiment, the remaining rats were sacrificed and organs (liver and kidney) harvested and preserved in 10% formalin for histopathological study (Dekant and Vamvakas, 2005).

Gross Pathology and Microscopic Examination

The liver and the two kidneys for each rat were fixed and preserved in 10% formaldehyde before subjection to tissue processing procedures for the preparation of permanent mount of each tissue as described by Ojo *et al.* (2014). The tissues were dehydrated through various grades of alcohol comprised of 30, 50, 70, and 95% with a final bath in 100% alcohol (twice) to ensure total elimination of moisture. Clearing was performed in toluene in order to raise its refractive index to that of glass (1.5) to enable of transparency the cellular inclusions. The processes of infiltration and embedding were performed using liquid paraffin and molten paraffin wax using L-shaped mould, respectively. Sections were made using Rotary Microtome and the Hot plate method was used for mounting specimens onto slides. Staining of tissues was performed using iron hematoxylin and eosin stains. Canada balsam was used in mounting the tissues (Ojo et al., 2014). Slides were viewed under the \times 40 objective of the light microscope and photographed using the digital eye piece camera (Model 582, Oplenic optronic Kina) to capture tissues images.

Preparation of Sera Samples

On day 28 of the dosing period, the animals were starved for 24hrs, and on day 29 all the animals were sacrificed under pentobarbital anesthesia and blood samples were drawn from the heart of each sacrificed animal. The samples were collected in sterilized plain plastic test tubes and allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were then centrifuged at 3000 rpm for 10 minutes and clear serum samples were aspirated off and stored and were frozen for the biochemical test (Ojo *et al.*, 2014).

Serum Biochemistry

The parameters were determined spectrophotometrically using the ready-to-use standard kits and methods of Human (Arbab, 2014) for aspartate aminotransferase serum (AST), Serum Alanine Aminotransferase (ALT). serum alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, creatinine, serum urea. and electrolytes (sodium. potassium, bicarbonate, and chloride).The manufacturer's instructions for each biochemical parameter were strictly adhered to in the course of the investigations.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) to check if there were significant differences between the time in weeks for each of the haematological and biochemical parameters at p<0.05 while Least significant difference (LSD) test was used to separate the significant means using SPSS version 20 package. Correlation analysis was used to check the relationship of time (in weeks) of the treated and control group.

RESULTS AND DISCUSSION

Table 1: First phase of acute toxicity screening of crude methanol extract of *A*. *leiocarpus* in rats

Doses (mg/kg	Observations	Mortality
bodyweight)		Wortanty
100	Absence of any visible physical changes in animals. The rats were generally devoid of any apparent impairment. The feed intake was also normal. There was no mortality in 72 hours.	0/4
200	Physical appearance and behaviour apparently normal. There were no alterations or diminished response to external stimuli and no animal died within died within a 72-hour span.	0/4
400	Similar to observations above for 100 and 200 mg/kg bodyweight doses with the animals exhibiting normal physical-activity and feed utilization. No mortality was recorded.	0/4
800	The animals were devoid of any apparent changes in behaviour. No mortality was obtained	0/4
1000	The animals were devoid of any apparent changes in behaviour. No mortality was obtained	0/4

Table 2: Second phase of	acute toxicity	screening of	crude methano	l extract of
A. leiocarpus in	rats			

A. <i>lelocurpus</i> m fats					
Doses (mg/kg	Observations	Mortality			
bodyweight)					
1200	Animals generally healthy with no indications of any distress. Absence of mortality in 72 hours.	0/4			
1600	Slight irritation among two rats but apparently in good health condition. No mortality in 72 hours.	0/4			
2000	Slight reduction in activity but normal feed uptake, two mortality within 72 hours.	2/4			
-					

 $LD_{50} = \sqrt{(Maximum \ tolerated \ dose)(Minimum \ lethal \ dose)}$

 $LD_{50} = \sqrt{(1600)(2000)} = 1789 \text{ mg/kg bodyweight}$

Innalegwu et al. Toxicologica	l Profile of Methanol Leaf	Extract of Annogeissus leiocarpus
-------------------------------	----------------------------	-----------------------------------

parame	eters of rats				
Parameters	Treatments	Week 1	Week 5	Week 10	Correlation coefficients (Weeks vs Parameters)
Hb (g/L)	Test	11.92±0.36°	13.80±0.27°	14.23±0.10°	0.503*
	Control	12.15±0.35°	12.42±0.26°	12.43±0.05°	0.541**
	Significance	NS	NS	NS	
PCV (%)	Test	33.66±0.18 ^e	33.60±0.51 ^e	33.72±0.37 ^e	0.540**
	Control	34.17±0.26 ^e	34.96±0.15 ^e	34.38±0.24 ^e	0.083
	Significance	NS	NS	NS	
MCHC (g/dL)	Test	35.42±1.02 ^e	40.01±1.11 ^f	42.22±0.73 ^f	0.581**
(8,02)	Control	$35.56 \pm 1.04^{\circ}$	35.05±0.80 ^e	36.17±0.37 ^e	0.485*
	Significance	NS	*	*	01100
MCH (pg)	Test	22.60±0.25 ^d	22.96±0.16 ^d	23.77±0.53 ^d	0.068
(P8)	Control	22.86 ± 0.29^{d}	22.92 ± 0.28^{d}	23.29±0.21 ^d	0.500*
	Significance	NS	NS	NS	
MCV (fL)	Test	61.23±0.30 ^g	65.20±0.22 ^h	65.95±0.37 ^h	0.259
	Control	61.49 ± 0.46^{f}	63.92 ± 0.30^{g}	64.08±0.34 ^h	0.807**
	Significance	*	*	NS	0.007
WBC (x 10 ⁹ /L)	Test	8.84±0.18 ^b	8.79 ± 0.07^{b}	9.75±0.09 ^b	0.891**
	Control	7.43±0.18 ^b	7.71 ± 0.15^{b}	7.60 ± 0.04^{b}	0.628**
	Significance	NS	NS	NS	0.020
Neutrophils (%)	Test	60.94 ± 0.44^{g}	61.06 ± 0.37^{g}	62.18 ± 0.39^{g}	0.389
(eu opinio (<i>i</i> o)	Control	$60.46 \pm 0.54^{\text{f}}$	$60.25 \pm 0.09^{\text{f}}$	61.37 ± 0.24^{g}	0.487*
	Significance	*	*	NS	0.107
Lymphocytes (%)	Test	68.71±0.23 ^g	70.37±0.31 ⁱ	71.15 ± 0.46^{i}	0.304
	Control	68.41 ± 0.26^{g}	69.76±0.26 ^h	70.03 ± 0.45^{i}	0.745**
	Significance	NS	NS	NS	017.10
Eosinophiles (%)	Test	0.87 ± 0.01^{a}	0.88 ± 0.01^{a}	0.93 ± 0.01^{a}	-0.278
(0)	Control	0.85 ± 0.01^{a}	0.93 ± 0.02^{a}	0.97 ± 0.01^{a}	0.773**
	Significance	NS	NS	NS	5.175
Platelet (x 10 ⁹ /L)	Test	97.69±0.47 ⁱ	98.50±0.21 ^j	99.13±0.73 ^j	0.233
	Control	96.14 ± 0.60^{i}	98.06±0.37 ⁱ	99.24 ± 0.39^{j}	0.689**
	Significance	NS	NS	NS	0.007

Table 3:	Effect of crude methanol extract of A	<i>leiocarpus</i> on the haematological
	parameters of rats	

Key: Hb: Haemoglobin, PCV: Packed Cell Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean Corpuscular Volume, WBC: White Blood Corpuscles. Values on the same row with different superscript are significantly different at $p \le 0.05$ * Significantly different at $p \le 0.05$, ** Correlation is significant at $p \le 0.01$

NS = Not significant

parameter	is of fat				
Parameters	Treatments	Week 1	Week 5	Week 10	Correlation coefficients (Weeks vs Parameters)
Glucose (mg/dL)	Test	107.37±1.79 ^d	104.41±0.55 ^e	101.20±0.71e	0.564**
	Control	100.46 ± 0.69^{d}	101.40±0.42 ^e	104.41±0.56 ^e	-0.379
	Significance	NS	NS	NS	
Triglycerides (mg/dL)	Test	147.05±1.69e	146.23±0.57 ^f	145.66 ± 0.65^{f}	0.669**
	Control	143.40±1.76 ^e	143.01±1.09 ^f	140.29±0.31 ^f	-0.302
	Significance	NS	NS	NS	
Total protein (g/L)	Test	5.29±0.24 ^a	8.12±0.11 ^b	7.77±0.11 ^b	-0.056
	Control	6.24±0.20 ^b	7.62±0.28 ^b	7.68±0.12 ^b	0.754**
	Significance	*	NS	NS	
Albumin (g/L)	Test	5.31±0.25 ^a	4.57±0.16 ^a	4.65±0.09 ^a	-0.443*
	Control	5.05±0.03 ^a	5.11±0.07 ^a	5.49±0.21 ^a	-0.105
	Significance	NS	NS	NS	
Total Bilirubin (mg/L)	Test	22.31±0.46°	22.95±0.37 ^d	25.78±0.38 ^d	0.275
	Control	22.12±0.70°	22.15±0.41 ^d	24.22±0.24 ^d	0.733**
	Significance	NS	NS	NS	
Conjugated Bilirubin (mg/dL)	Test	8.79±0.04 ^b	9.52±0.18°	9.94±0.17°	-0.074
	Control Significance	9.17±0.10 ^b NS	9.48±0.34° NS	9.83±0.10° NS	0.738**

Table 4: Effect of crude methanol extract of A. leiocarpus on liver and biochemical parameters of rat

OrganizationNSNSValues on the same row with different superscript are significantly different at $p \le 0.05$ * Significantly different at $p \le 0.01$ *** Correlation is significant at $p \le 0.01$

NS = Not significant

Table 5: Effect of crude methanol extract of A. leiocarpus on renal parameter of rat

Parameters	Treatments	Week 1	Week 5	Week 10	Correlation
					coefficients (Weeks
					vs Parameters)
Urea (mg/dL)	Test	34.04±0.43 ^d	35.50±0.68 ^d	35.60±0.63 ^d	-0.029
	Control	34.84 ± 0.88^{d}	35.01±0.43 ^d	35.50±0.43 ^d	0.384
	Significance	NS	NS	NS	
Creatinine (mg/dL)	Test	7.33±0.07 ^b	7.99±0.05 ^b	9.33±0.16 ^b	0.078
	Control	7.49±0.13 ^b	7.98 ± 0.04^{b}	8.82±0.31 ^b	0.895**
	Significance	NS	NS	NS	
Uric acid (mg/dL)	Test	5.57±0.05 ^a	5.55±0.20 ^a	5.91±0.07 ^a	0.084
-	Control	5.44±0.16 ^a	5.46±0.15 ^a	5.98±0.04 ^a	0.587**
	Significance	NS	NS	NS	
Na+ (mmol/L)	Test	134.40 ± 0.62^{f}	134.58±0.20 ^f	141.20 ± 1.17^{f}	0.158
	Control	134.68±0.69 ^f	135.53±0.55 ^f	137.40 ± 0.78^{f}	0.713**
	Significance	NS	NS	NS	
K ⁺ (mmol/L)	Test	5.97±0.04 ^{ab}	6.27±0.18 ^a	6.92±0.02 ^a	0.303
	Control	5.47±0.24 ^a	6.27±0.17 ^a	6.50±0.02 ^a	0.791**
	Significance	NS	NS	NS	
Cl ⁻ (mmol/L)	Test	98.16±0.84 ^e	97.78±0.43 ^e	98.74±0.58 ^e	0.168
	Control	97.63±0.43 ^e	97.71±0.56 ^e	98.16±0.73 ^e	0.194
	Significance	NS	NS	NS	
HCO3 ⁻ (mmol/L)	Test	25.89±0.53°	27.14±0.11°	26.68±0.18 ^c	-0.286
	Control	26.11±0.11°	26.89±0.20°	28.39±0.25°	0.636**
	Significance	NS	NS	NS	

SignificanceINSINSValues on the same row with different superscript are significantly different at $p \le 0.05$ * Significantly different at $p \le 0.01$

NS = Not significant

Parameters	Treatments	Week 1	Week 5	Week 10	Correlation coefficients
					(Weeks vs Parameters)
ALP (U/L)	Test	90.05±0.37°	93.54±0.50cd	101.58±1.15 ^d	0.510*
	Control	87.300.85 ^b	87.24±0.76 ^b	94.84±0.49 ^c	0.755**
	Significance	*	*	*	
AST (U/L)	Test	19.56±0.84 ^b	23.73±0.57°	25.23±0.61 ^d	0.754**
	Control	17.25±0.50 ^a	17.68 ± 0.20^{a}	17.68 ± 0.20^{a}	0.511*
	Significance	*	*	*	
ALT (U/L)	Test	14.31±0.28 ^a	19.13±0.56 ^b	24.57±0.99°	0.364
	Control	14.26±0.28 ^a	18.83±0.14 ^b	18.97±0.32 ^b	0.840^{**}
	Significance	NS	NS	*	

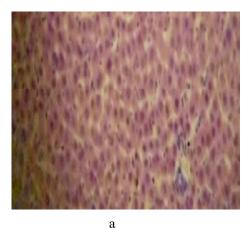
Table 6: Effect of crude methanol extract of A. leiocarpus on serum enzymes of rat

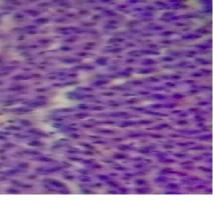
Values on the same row with different superscript are significantly different at p≤0.05

* Significantly different at p≤0.05

** Correlation is significant at p≤0.01

NS = Not significant





b

Figure 1: Micrograph of the liver section of control rat (a); not treated and the treated (b) with 1600 mg/kg body we4ight of the extract showing normal hepatocyte architecture respectively, ×400 magnification.

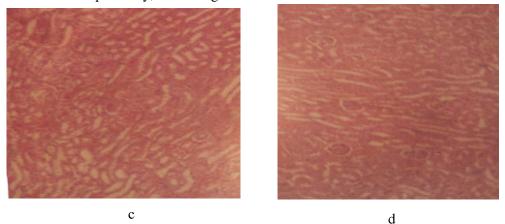


Figure 2: Micrograph of the kidney section of control rat (c); not treated and the treated (d) with 1600 mg/kg body weight of the extract showing Normal glomeruli and collecting duct respectively, x 400 magnification.

DISCUSSION

Plants are natural reservoir of pharmacological active constituents and reports from ancient traditions shows that they have be used for the treatment of different ailments (Amuda et al., 2017). Despites this biological importance of plants, they are not without side effect just like other synthetic drugs hence they must undergo thorough toxicological evaluation to ascertain the level of their safety before usage (Amuda et al., 2017). It has also been justified that any drug used in treatment of any ailments is not absolutely free from side effects either mild or adverse¹². Plant extracts are not exempted as they can only be beneficial after a thorough and careful evaluation of the side effects associated with their uses through assessment of their toxicity using experimental model (Ojo et al., 2014). Indices such as LD₅₀ and any effect on the organs associated with the metabolism and homeostasis such as liver and kidney can be evaluated to ascertain the toxicity status of such plant extract (Ojo et al., 2014).

Advanced toxicity studies such as sub-chronic study in animal model could also assist in predicting the potential toxic state of these plant extracts. from which possible deductions of the response may be correlated with human and in addition may give an idea about the organ system involvement (Ojo et al., 2014; Cotran et al., 2005). Methanol extract of Annogeissus leiocarpus gave a percentage yield of 88.45 17.69 %. The plant is commonly used particularly among the Yoruba of South Wester Nigeria as chewing stick and the roots, stems and leaves is used for the management of ailments such as bacterial infections (Amuda *et al.*, 2017).

Acute toxicity of the extract administered orally gave an LD₅₀ of 1789 mg/kg body weight (Table 1 and 2) which is within the standard range of 500-5000 mg/kg body weight. Hence, the methanol extract of A. leiocarpus may be described as safe and practically nontoxic on the scale proposed by OECD guidelines 2002 (OECD, 2010) and Lorke, 1983 (Sharif et al., 2015). The behavioural profile of all the tested animals revealed response to pain and touch. The animals showed no signs of depression or restlessness. Monitoring the body weight during treatment provides a fair index of the general health status of the animals (Sharma et al., 2009). Sub-chronic toxicity test of the extract carried out for the period of 10 weeks showed that the extract has no significant difference (p>0.05) on the haematological, biochemical and renal parameters of the treated and the control group (Table 3, 4 and 5). The result between the control and the treated groups also shows a significant correlation at p<0.01 in the haematological, biochemical and renal parameters of the animals. However, there was a significant difference (p<0.05) between the serum enzymes of the control and treated group throughout the 10 weeks of treatment with the extract (Table 6). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are diagnostic enzymes used for the detection of any alteration in the architecture of the liver. Although AST is ubiquitous the level of the enzyme is affected once the major organ where it is domicile is affected (Ahmad and Wudil, 2013). The AST

and ALT aid the conversion of amino acids to ketoacids and they are major indicators of liver damage resulting from exposure toxic substances (Chawla, 1999). Elevated level of serum AST maybe due to necrosis and hepatomegaly myocarditis, and myositis while elevated level of ALT may occur during liver damage (Chawla, 1999). Primary and secondary hepatic tumours may also lead to increase ALT and AST level with AST been higher than ALT. From this study, it maybe postulated that there was a mild necrosis, hepatomegaly myocarditis, and myositis as well as primary and secondary hepatic tumours, since there is a significant (p<0.05) increase in both the AST and ALT (Table 6).

Unconjugated bilirubin is used to monitor the synthetic and detoxification role of the liver. Unconjugated bilirubin combines with glucuronic acid in the liver cells leading to increase water solubility which facilitate its easy transport into bile. The level of serum conjugated bilirubin will increase when the liver has lost its ability to excrete waste or toxic substances (Bosma et al., 1995). This study shows that there was no significant difference (p>0.05) in the conjugated bilirubin of the groups administered with the extract and the Control group because an increase in the level of these parameters in the serum after exposure of the experimental animals to the plant extracts may indicate an injury to the liver (Finlayson et al., 1995). Therefore, this study revealed that the extract did not cause any damage on the liver. Finally, the results of the liver and kidney histology of the treated and the control group showed normal histoarchitecture in both organs (Figure 1 and 2). Based on this, the elevated level of this enzymes in the serum may not be attributed to any damage on the liver and kidney caused by the extract but maybe an indication of the health status of the experimental animals prior to usage.

CONCLUSION

Toxicological evaluation of *A*. *leiocarpus* showed that the plant maybe safe however, prolonged use should be discouraged as its accumulation may become harmful particulaly to the liver and the kidney. Further study is therefore required to validate the safety and usage of *Annogeissus leiocarpus* leaf.

ACKNOWLEDGMENTS

The authors are thankful National Institute for Pharmaceutical Research and Drug Development, Idu, Abuja for providing a convenient environment for the study and for all the technical input in ensuring the success of the research.

COMPETING INTEREST

There authors declared there is no competing interest.

REFERENCES

- Ahmad, I. M. and Wudil, A. M. (2013). Phytochemical screening and Toxicological Studies of Aqueous Stem Extract of Anogeissus leiocarpus in rats. Asian Journal of Scientific Research, 6(4): 781-788.
- Amuda, O., Garba, S. A., Galadima, M., and Jigam, A. A. (2017).
 Antibacterial properties of methanol and aqueous extracts of *Anogeissus leiocarpus* and *Terminalia microptera* against

selected oral pathogens. *World Journal of Pharmaceutical and Medical Research*, 3(8): 6-11.

- Arbab, A. H. (2014). Review on Anogeissus leiocarpus a Potent African Traditional African Traditional Drug. International Journal of Research In Pharmacy And Chemistry, 4(3): 496-500.
- Atansuyi, K., Ibukun, E. O. and Ogunmoyole, T. (2012). Antioxidant properties of free and bound phenolic extract of the leaves of *Jatropha tanjorensis in vitro*. *Journal of Medicinal Plants Research*, 6(31): 4667-4674.
- Bosma, P. J., Chowdhury, J. R., Bakker, C., Gantla, S. and de Boer, A. (1995). The genetic basis of the reduced expression of bilirubin UDPglucuronosyltransferase 1 in Gilbert's syndrome. *New England Journal of Medicine*, 333: 1171-1175.
- Chawla, R. (1999). Practical Clinical Biochemistry: Methods and Interpretation. 2nd Edition. Jaypee Brothers Medical Publishers, New Delhi, 106-118.
- Cotran, R. S., Kumar, S., Fausto, V. N., Nelso, F., Robbins, S. L. and Abbas, A. K. (2005). Pathologic Basis of Disease, Elsevier Saunders, St. Louis, Mo, USA, 7th edition.
- Dekant, W. and Vamvakas, S. (2005). Toxicology. In: *Toxicology in Occupational* and *Environmental Setting*. WILEY-VCHVerlag GmbH & Co. KGaA, Weinheim, 87-89.
- Ekundayo, F. O., Adeboye, C. A. and Ekundayo, E. A. (2011). Antimicrobial activities and

phytochemical screening of pignut (Jat- rophas curcas Linn.) on some pathogenic bacteria. *Journal of Medicinal Plants Research*, 5(7): 1261–1264.

- Finlayson, N. D. C., Boucher, I. A. D., Haslet, C., Edward, C. R. W. and Chilverse, E. R. (1995).
 Davidson's Principles and Practice of Medicine. 17th Edition, *Churchill Livingstone Publishers, London,* 484-545.
- Kalimuthu, K., Vijayakumar, S. and Senthilkumar, R. (2010).
 Antimicrobial activity of the biodiesel plant, *Jatropha curcas*L. *International Journal of Pharma and BioSciences*, 1(3): 1–5.
- OECD (2010). Guideline for testing of chemicals (TG 408). Repeated dose 90-day oral Toxicity study in Rodents. OECD/OEDC, 1-13.
- Ojo, O. A., Ajiboye, O. B., Oyinloye, B. E. and Oio, A. B. (2014). Prophylactic Effects of Ethanolic Extract of Irvingia gabonensis Stem Bark against Cadmium-Induced Toxicity in Albino Rats. Advances in Pharmaceutics, Article ID 894610. 8 pages http://dx.doi.org/10.1155/2014/ 894610.
- Oyewole, O. I., Oyedara, O. O., Olabiyi, B. F. and Fasanya, T. S. (2013). Phytochemical, antimicrobial and toxicity studies of *Phyllanthus amarus* whole plant extract. *International Journal of Bioassays*, 2(3): 519–523.
- Sharif, H. B., Mukhtar, M. D., Mustapha, Y., Gabi B. and Lawal, A. O. (2015). Acute and Sub-chronic Toxicity Profile of *Euphorbia pulcherrima*

Methanol Extract on Wistar Albino Rats. *Advances in Pharmaceutics*, Article ID 539646, 9 pages http://dx.doi.org/10.1155/2015/ 539646.

Sharma, S., Sharma, K., Yadav, O. and Sharma, K.P. (2009). Alterations in biochemical and histopathological profile of liver in distillery soil leachate treated Swiss albino mice *Musculus L. Pharmacology online*, 3: 1047-1053.

Victor, Y. A. (2013). *In-Vitro* Assessment of Antioxidant and Antimicrobial Activities of Methanol Extracts of Six Wound Healing Medicinal Plants. *Journal of Natural Sciences Research*, 3(1): 74-82.