Science World Journal Vol. 17(No 3) 2022
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

TOXICITY STUDY AND EFFECT OF THE LEAF EXTRACT OF *ACACIA NILOTICA* ON SOME BIOCHEMICAL PARAMETERS OF WISTAR ALBINO RATS

*Gabi Baba[1], Hauwa B. Sharif[2], Hauwa Umar[3] and Isyaku Umar[3],

[1]Department of Biochemistry, Kaduna State University Kaduna, Nigeria
[2]Departments of Applied Biology, Kaduna Polytechnic, P.M.B 2021, Kaduna, Nigeria
[3]Departments of Applied Chemistry, Kaduna Polytechnic, P.M.B 2021, Kaduna, Nigeria

*Corresponding Author Email Address: babagabi1969@gmail.com Phone: +2348025255143

ABSTRACT
Plants are the primary source of human medications and knowledge on its toxicity is vital; this work evaluates the acute toxicity and effect of *Acacia nilotica* leaf crude extract on the liver and kidney functions. The mean lethal dose (LD_{50}) was determined by Lorke's method, while the crude extract effect was evaluated by biochemical and histo-pathological assessments. The LD_{50} value was 3807.89 mg/kg for both oral and intra-peritoneal route of administrations. An elevated serum urea above the normal reference value in both control and treated group upon administration of 1000 mg/kg of the extract with mean values of 7.92 ± 1.19 and 7.86 ± 1.14 mmol/l respectively was observed. The results of ALAT, ASAT, ALP, T.protein, Albumin, and bilirubin in all cases were within the normal values. The kidney and liver function parameters at higher extract concentrations of 500 and 1000 mg/kg/day and relative organ weight were statistically significant ($p< 0.05$) and correlates with mild effect indicted on the histopathology of the organs. This study showed that administration of *A. nilotica* extract at 500 and 1000 mg/kg/day for an extended period could prompt hepatic and nephron toxicity.

Keywords: Acute toxicity, LD_{50}, Hepatic, Nephron toxicity, Histo-pathology, *Acacia nilotica*

INTRODUCTION
Plants serve as the primary source of human medications as long as the history of human existence. This implies, many varieties of plants species were routinely used in the diseases treatment and management without much scientific documentations (Prasanth *et al.,* 2015).

A common problem associated to that in ethno-medicine is the inadequate information on the toxicity profiles of such plants employed to that effect (Gathirwa *et al.*, 2008; Jonville *et al.*, 2008; Sathya and Gopalakrishnan, 2012). One of such plants used in many localities is *Acacia nilotica*. The leaf and stem part of *A. nilotica* is presented in figure I.

Figure I: Leaf and Stem Parts of *A. nilotica* (Academy, 2010)

It is a plant belonging to the family *Fabaceae* and sub-family *Mimosoideae*, a medium sized tree widely distributed in tropical and sub-tropical countries (Delile and Willd, 2010).
The plant is called *Baani, Booni* in Yoruba, *bagaruwa* in Hausa and *Gabaruwa* in Nupe, (Blench *et al.*, 2007) traditionally used in the treatment of cancers of different part of body and tumors of mouth, bones (Ali *et al.*, 2012), ears, eyes, skin and testicles (Uguru *et al.*, 2014).

Twigs of the plant are employed as tooth brushes in India and Africa, also in the management of cold, bronchitis, asthma, diabetes, diarrhea, dysentery, blindness, bleeding piles and leukoderma (Oppong-Bekoe *et al.,* 2019). Furthermore its equally employed in treatment of stomach upset, protection against scurvy; as anti-helminthes, antiseptic for open wounds and anti-coughs expectorant(Ali *et al.*, 2012; Olowokudejo *et al.*, 2016).
The plant's activity is attributable to its composite bio-actives and nutrients. Its antioxidant potential is well comparable to antioxidants like quercetin, tocopherol, ascorbic acid and catechin (Audru *et al.*, 2000). The plant and its related species have wide application in veterinary medicine as desirable fodder for cattle, and the young leaves, shoots and pods aid milk production (Atif-Ali, 2012; Uguru *et al.*, 2014; Feedipedia and Stewart, 2016).
Considering the recent resurgence in the natural product research, medicinal plants with such a wide pharmaceutical applications required a thorough investigation of its toxicity profiles (Academy, 2010; Kuriyan *et al.*, 2010;). This present work therefore, aimed at evaluating the acute toxicity profile of *A. nilotica* leaves and its effect on liver and kidney functions of Wistar albino rats.

MATERIALS AND METHODS

Plant Materials

The plant material used for the study is the leaf of the *A. nilotica* obtained in December 2019 from the botanical garden of the Department of Applied Biology, Kaduna Polytechnic. Identified and authenticated by a botanist Alh Musa Kalla in the herbarium unit, Department of Botany, Ahmadu Bello University, Zaria and a voucher specimen number 686 was deposited.

Experimental animals

Thirty Three (33) adult rats of weights ranging between 137 to 181g were used for the research work.

Sample Preparation and Extraction

The leaves were shade dried at room temperature (25^{o}C) for two weeks and grounded into powdered form using pestle and mortar; it was then sieved, weighed, labeled and stored in an air tight polythene bag inside a desiccator for further analysis.

Aqueous extraction was carried out using maceration method. Two hundred grams (200g) of the powdered sample was soaked in 1000mL of distilled water for 24h. The percolate was filtered with (What-man No. 1) filter paper and concentrated to dryness on a rotatory evaporator. The dried extract was kept in air tight bottle for further use. The percentage yield of the extract was calculated using equation below;

$$\% \text{ Yield of Extract} = \frac{\text{Weight of extract}}{\text{Sample weight}} \times 100$$

Phytochemical Screening

Phytochemical screening for the qualitative detection of alkaloids, tannins, saponin glycosides, flavonoids, unsaturated steroids and Triterpenes, carbohydrate, cardiac glycosides, and glycosides from *A. nilotica* leaf crude extract was carried out using the conventional procedures as described by Trease and Evans (2009)

Animals Grouping

Adult rats were obtained from the animal house in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were kept for two weeks to acclimatize before the experiment and fed with growers mash from vital feeds and water *ad libitum.* They were also maintained under standard condition of humidity, temperature and 12h light/darkness cycle. The experimental animals were distinguished and grouped based on the plant crude extract doses administered.

Acute toxicity studies (Determination of LD_{50})

The acute toxicity (LD_{50}) was estimated both orally and intra-peritoneal in rats (n = 3) in each group following Lorke's method (Lorke, 1983). Dose levels of 10, 100 and 1000 mg/kg were used for the first phase. The number of deaths in each group within 24h was recorded. The second phase concentration of extract doses were deduced from the first phase with four rats grouped into four groups of one rat each, treated with doses of 1200, 1600, 2900 and 5000 mg/kg for both oral and intra-peritoneal. The animals were observed for 24h after which the LD_{50} was calculated from Lorke's formula (Lorke, 1983) as follows

$$LD_{50} = \sqrt{a \times b}$$

Where a = highest dose at which no death occurred in the second phase

b= the least dosage at which death occurred in the 2nd phase

The extract was classified using the calculated LD_{50}.

Animal grouping and Biochemical Assay

The rats were weighed and randomly grouped into four groups of five rats each and the administered doses was 1/3 value of LD_{50} obtained accordingly(Diallo *et al*., 2010; Arsad *et al*., 2013; Jn *et al.*, 2019; Loha *et al.*, 2019; Mwandah *et al*., 2019). The dose level thus were 250, 500 and 1000mg/kg body weight for group B, C and D respectively, while group A served as the control treated with normal saline. The extract was administered for the period of 28 days during which food consumption and water intake of the groups were equally observed together with likely physical manifestation of toxicity and mortality. On the 29th day the animals were starved overnight and weighed before sacrifice. The animals were anaesthetized with chloroform and sacrificed by decapitation. The bloods were collected in sample plain bottles for biochemical assays after sacrifice and the liver and kidney were removed and stored in 10% formalin for histopathology study.

Biochemical Assay

Biochemical assay carried out to include liver function test (LFT) which involved assay for serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), alkaline phosphatase (ALP), total proteins, albumin, and total bilirubin. Likewise the kidney function tests were urea; creatinine and electrolytes were evaluated using the ready-to-use Kits as from Sigma Aldrich based on the method described by(Julian, 2000; Vasudevan and Vaidyanathan, 2017) .

Relative Organ Weight and Histopathology

The liver and kidney of each animal was excised, carefully examined for gross pathological changes and weighed. Relative organ weight (ROW) was calculated using the formula below and the two organs were further taken for histopathological examination.

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

(Diallo *et al*., 2010; Nwankwo *et al*., 2015)

Histo-pathological studies

Histo- pathological examination of the liver and kidney was initiated with the removal of organ from the body and stabilized using fixative to prevent organ decay. It was further dehydrated, cleaned, impregnated with wax and embedded into paraffin wax for block formation. The block was then trimmed and sectioned using rotary microtome, fixed onto glass side and de-waxed by placing the slides on a water bath at 40^{o}C (Jn *et al*., 2019; Zainal *et al*., 2020). The slides were then stained with iron haemotoxylin and eosin then mounted on Canada balsam and covered with cover slip for viewing under X10 and X40 objectives of the microscope.

Statistical analysis

The results were statistically expressed as means and standard deviation (SD). The data were analyzed using the IBM SPSS version 20.0 at 95% confidence limit.

Science World Journal Vol. 17(No 3) 2022
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

RESULTS

The Physical Characteristics and Percentage Yield of *A. nilotica* (L) Aqueous Leaf Extract

The physical characteristics of the aqueous leaf crude extract and percentage yield of the *A. nilotica* indicated the extract is dark brown appearance with gummy texture, while the percentage yield obtained was 28.2%.

Phytochemical compositions of *A. nilotica* Aqueous Leaf Extract

The results of the qualitative phytochemical screening of *A. nilotica* Leaf extract indicates the presence of carbohydrates, saponins, steroids, terpenoides, tannis, flavonoids, alkaloids and glycosides in the crude extract, while anthraquinone was absent (Table 1).

Table 1: Phytochemical compositions of *A. nilotica* (L) Crude Leaf Extract

Phyto-constituents	Inference
Carbohydrate	+
Anthraquinone	-
Cardiac-glycoside	+
Saponins	+
Steroids	+
Terpenoids	+
Tannins	+
Flavonoids	+
Alkaloids	+
Glycosides	+

Key: + = Presence - = Absence

Acute Toxicity Profile of the *A. nilotica* Aqueous Leaf Extract

The results of the acute toxicity profile represented in (Table 2) showed that both first and second phases of the test indicated zero mortality except at 5000mg/kg dose level where deaths were recorded for both oral and intra-peritoneal routes of administration examined. The LD_{50} value was therefore estimated to be 3807mg/kg for both routes according to Lorke (1983).

Table 2: Acute Toxicity Profile of the Aqueous Leaf Extract of *A. nilotica* on Wister Albino rats

Oral Doses (mg/kg)	Death / Survival rate (Phase 1)	
	Oral	Intra-peritoneal
10	0/3*	0/3
100	0/3	0/3
1000	0/3	0/3
Orally Doses (mg/kg)	Death / Survival rate (Phase II)	
1200	0/1	0/1
1600	0/1	0/1
2900	0/1	0/1
5000	1/1	1/1

* Survival rate numerator is the number of death animal recorded and denominator is the number of used animals.

LD_{50} is given by the square root of maximum value that did not cause death and lowest value that cause death according to Lorke, (1983).

$LD_{50} = \sqrt{2900 \times 5000} = 3807.89$mg/ (Lorke, 1983)

Effect of *A. nilotica* (L), Aqueous Leaf Extract on Some Serum Biochemical Parameters for Kidney Function Assessment of Rats after Treatment

The results as represented in Table 3 indicated, mean values of groups treated with the highest concentration at 1000mg/kg to be high than the control values for both urea and creatinine with mean values $7.6\ 1\pm 0.5^a$ and 8.3 ± 0.5^a and 82.4 ± 0.7^a and 86.6 ± 0.07^a respectively (Table 3). Urea values for both control and treated groups were higher than the reference values, while creatinine values were lower than the normal reference. But the group treated with highest concentration showed values higher than the control values. However, both parameters were statistically insignificant ($P>0.05$).

The results of the electrolytes indicated a general trend with the mean values of the control significantly higher than that of the groups treated ($P<0.05$), for Na^+, K^+ and Cl^- but with the values generally within the normal reference values except for the bicarbonate that the mean values of the control was lower than that of the treated groups, however, the values were within the normal reference values and statistically insignificant ($P>0.05$).

Table 3: Effect of *A. nilotica* (L) Aqueous Leaf Extract on Some Serum Biochemical Parameters for Kidney Function Assessment of Rats after Treatment with extracts

Parameters (mmol/L)	Concentration (mg/kg body weight)			
	Control	250	500	1000
Urea(mmol/L) 2.5 -6.5	7.6 ± 0.5^a	7.5 ± 0.4^a	7.14 ± 0.5^a	8.3 ± 0.5^a
Creatinine(90.0-126)(mmol/L)	82.4 ± 0.7^a	69.5 ± 0.1^a	73.8 ± 0.8^a	86.6 ± 0.7^a
Sodium (Na)(135-150mmol/L)	148.2 ± 0.7^b	139 ± 0.3^a	145.2 ± 1.6^{ab}	144.3 ± 1.2^{ab}
Potassium (K)(3.4-5.3mmol/L)	4.8 ± 0.1^b	3.9 ± 0.8^a	4.8 ± 0.3^a	4.2 ± 0.3^a
Chloride (Cl) (95-110 mmol/L)	108 ± 0.6^b	98.7 ± 0.8^a	104.8 ± 0.5^{ab}	103.6 ± 0.1^{ab}
Bicarbonate (HCO_3^-) (24-32mmol/L)	25.6 ± 1.1^a	27.0 ± 0.8^a	26.0 ± 1.5^a	25.6 ± 1.6^a

Values are expressed as mean ± SD for n = 5. Values in the same row with different super scripts differ significantly at ($P<0.05$).

Effect of A. *nilotica* (L), Aqueous Extract of Some Serum Biochemical Parameters for Liver Function Assessment of Rats treated

The results of the serum liver function test parameters as represented in (Table 4) showed correlation between the mean total protein and albumin values with the control groups significantly ($P<0.05$) lower than group treated with the highest concentration (1000mg/kg). While those treated with 500mg/kg were significantly lower than the control values for both parameters. The groups treated with the lower concentration (250mg/kg) however showed insignificant differences ($P>0.05$) as compared to the control group.

Alkaline transaminase, aspartate transaminase and alkaline phosphatase values were within the normal reference value and showed no significant variation statistically ($P>0.05$).

The total and conjugate bilirubin values were also within the normal reference value, the mean value of the group treated with 1000mg/kg were significantly higher than the control values for both parameters ($p<0.05$) (Table 4).

Science World Journal Vol. 17(No 3) 2022
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Table 4: Effect of *A. nilotica* (L) Aqueous Leaf Extract on Some Serum Biochemical Parameters for Liver Function Assessment of Wister Albino Rats after Treatment

Parameters	Concentration (mg/kg body weight)			
	Control	250	500	1000
Total protein (g/dl)	76.6±0.4^{ab}	77.0±0.6^{ab}	73.8±0.7^{a}	81.3±0.2^{b}
Albumin (g/dl)	39.2±0.9^{ab}	38.5±0.3^{ab}	37.2±0.4^{a}	41.7±0.3^{a}
Alanine Transaminase(up to 22UI)	30.6±0.6^{a}	27.8±0.8^{a}	26.8±0.3^{a}	31.7±0.7^{a}
Aspatate transaminase(Up to 218 UI)	171.2±1.2^{a}	173.5±3.5^{a}	167.4±2.2^{a}	137.3±1.0^{a}
Alkaline Phosphotase(u/l) (60 - 170)	94.6±0.6^{a}	101.5±0.5^{a}	129.0±0.5^{a}	105.0±0.6^{a}
Total Bilirubin(µmol/l) (1.7-17.1)	9.2±1.1^{a}	9.7±1.1^{a}	9.2±1.0^{a}	11.3±0.6^{ab}
Conjugated Bilirubin(µmol /l)(1.7- 8.5)	5.0±0.7^{a}	4.8±0.9^{a}	4.8±0.8^{a}	6.3±0.6^{b}

Values are expressed as mean ± SD for n = 5. Values in the same row with different super scripts differ significantly at (P<0.05).

Relative organ weight of the rat treated with *A. nilotica* (L), Aqueous Extract.

The result of the relative organ weight (ROW) indicated mean values of the treated groups to be significantly higher than the mean values of the control group (p<0.05) (Table 5). in other words, various dose concentrations of the plant extract seem to have influenced the relative organ weight for both liver and kidney even though the ROW are within the normal reference value, hence the influence might not be of pathological significance.

Table 5: Effect of Aqueous Leaf Extract of *A. nilotica* (L) on Relative organ weight of the Wistar Albino rat treated

	Concentration (mg/kg body weight)			
Organ	Control	250	500	1000
Liver	0.015±0.008^{a}	0.034±0.006^{b}	0.025±0.005^{b}	0.026±0.004^{b}
Kidney	0.002±0.003^{a}	0.003±0.001^{b}	0.003±0.002^{b}	0.003±0.001^{b}

Values are expressed as mean ± SD for n = 5. Values in the same row with different super scripts differ significantly at (P<0.05).

Histopathology of liver and kidney of experimental model treated with *A. nilotica* (L), Aqueous Extracts.

The result of the liver histopathology showed normal hepatocytes for the control group and group treated with 250mg/kg body weight of the extract (Figure 1A and B). While that of the group treated with higher concentration of 500mg/kg and 1000mg/kg showed slight kuffer cell hyper-plasia and slight hepatocellular necrosis respectively (Figure 2A and B). Figure 3A featured the normal tubules and glomerulus of the kidney of control group. While group treated with 250mg/kg showed slight lymphocyte hyper-plasia (Figure 3B). The kidney of rats treated with 500 and 1000mgkg of the extract on the other hand showed moderate lymphocyte hyper-plasia and tubular distortions respectively (Figure 4A and B).

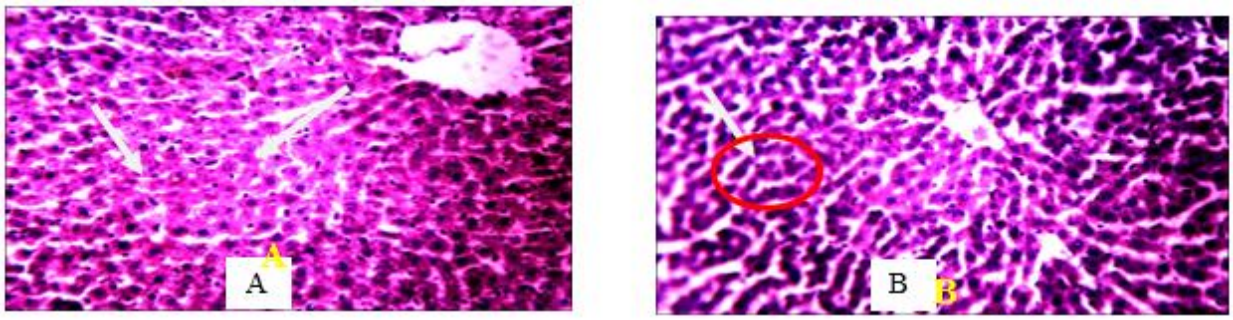

Figure 1: A. Micrograph of the liver from the control rat showing normal hepatocytes
B: Micrograph of the liver from rats treated with 250mg/kg showing normal features

Science World Journal Vol. 17(No 3) 2022
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

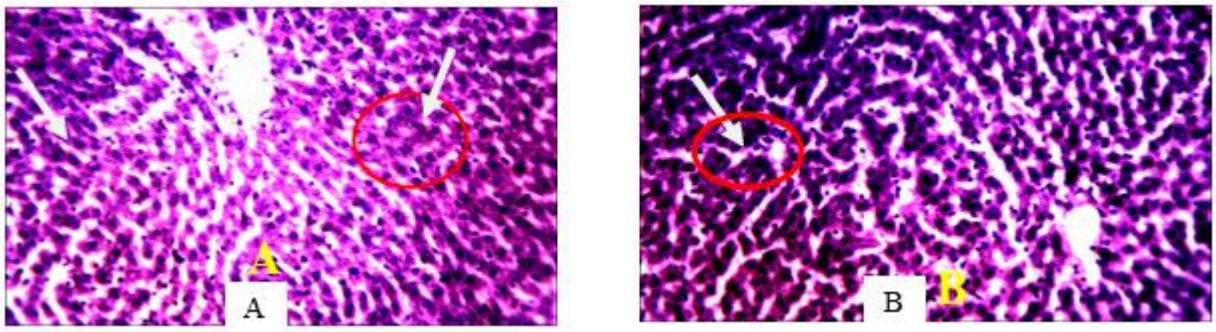

Figure 2: A. Micrograph of the liver from rats treated with 500mg/kg showing slight kufer cell hyper-plasia and lymphocyte hyper-plasia.
B. Micrograph of the liver from rats treated with 1000mg/kg showing slight hepatocellular necrosis.

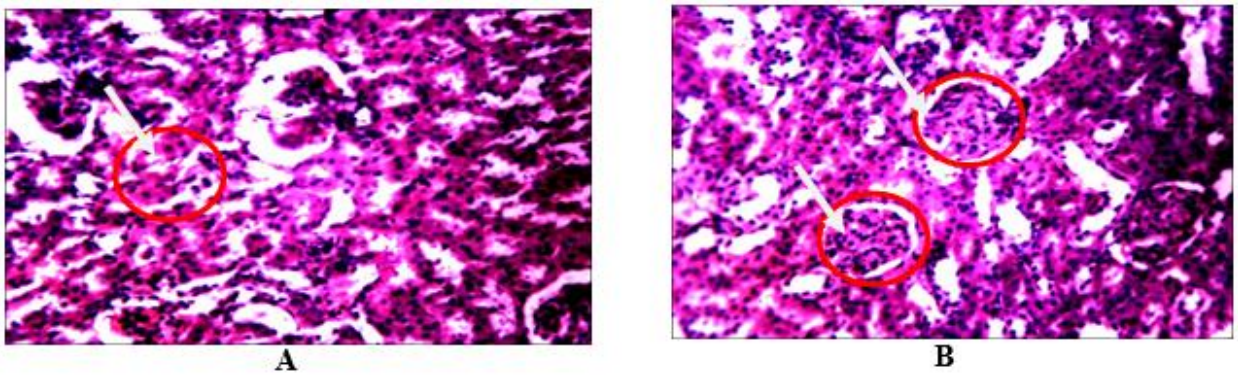

Figure 3: A. Micrograph of the kidney from the control rat showing normal tubules and glomerulus
B: Micrograph of the kidney from rats treated with 250mg/kg showing slight lymphocyte hyper-plasia.

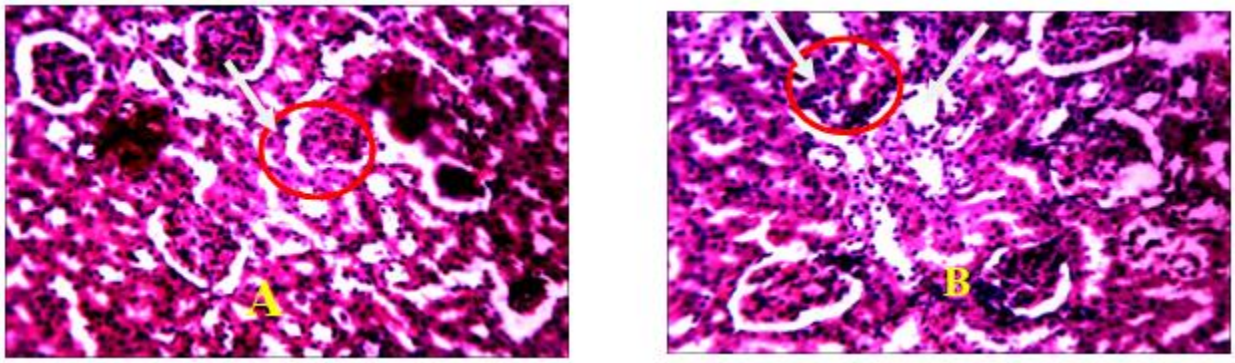

Figure 4: A .Micrograph of the kidney from rats treated with 500mg/kg showing moderate lymphocyte hyper-plasia.
B. Micro-graph of the kidney from rats treated with 1000mg/kg showing moderate tubular distortions and lymphocyte hyper-plasia.

DISCUSSION

For centuries, natural products, such as medicinal plants and the plant derived bioactive compounds have been the basis for the treatment of various ailments (Kamagaté *et al*., 2014), which has contributed immensely to the prevention of many diseases, most of which are not nutrients based. Hence, the knowledge of their safety and side-effects, if any, are important considerations for their effective usage in disease management (Prasanth *et al*., 2015). The assessment and evaluation of the toxic profiles of products such as plant extracts, fractions, or compounds is usually an initial step towards pharmaceutical products development regardless of its benefit. In that regard, the general toxicity of medicinal plants and their derivatives needs to be validated scientifically.

This research work was conducted using the aqueous extract of *A. nilotica*. The percentage yield was found to be 28.2% which indicated relatively high quantity of water soluble phytochemicals been extracted. Consequently, it's a validation of the traditional extraction method using aqueous as solvent. Carbohydrates, cardiac-glucoside, saponins, steroids, terpenoids, tannins, flavonoids, alkaloids and glucosides are phyto-constituents identified in the extract which conferred on the extract the medicinal benefit as reported in the literatures (Abubakar, 2010; An *et al*., 2017; Balamurugan *et al*., 2019; Mondal *et al*., 2019), many of

these phytochemicals were found at higher concentration and could impose a negative effect such as anti-nutritional factors (Arg *et al*., 2022; Isah *et al*., 2015) on animal models.

The acute toxicity profile of the extract for both oral and intraperitoneal routes of *A. nilotica* indicated zero mortality at low doses 10 to 2900 mg/kg/day only at 5000mg/kg dose level a death was recorded, while LD_{50} value was estimated to be 3870mg/kg for both routes according to Lorke (1983). This indicated relative safety of its administration within a short period of 24h (Lorke, 1983).
Acute toxicity had been an indexes used in the measurement of adverse effects from oral or dermal administration of xenobiotics on a single or multiple doses within 24h, or exposure to gases via inhalation within 4h of such test, principally used for safety evaluation of new identified product (Diallo *et al*., 2010).

On the other hand, sub-chronic studies are undertaken in the evaluation of the adverse consequences of continuous or recurrent exposure during a period of one third of the experimental animals' average lifespan. Specifically, it provided needed information on the target organ toxicity mostly designed to identify non-observable adverse effect level. It further helps to evaluate the appropriate dose regimens for longer-term adverse effect studies. Consequently, in this current study the sub-chronic toxicity effect of *A. nilotica* extract was evaluated and no clinical signs of toxicity in the animals' models were noticed. However, significant reduction in food and water intake was suggested as being responsible for the observed loss of body weight (Fraga-corral *et al*., 2020; Isah *et al*., 2015; Marci *et al*., 2022). Loss of appetite is often synonymous to weight loss due to disturbances in carbohydrate, protein or fat metabolisms (Klaasen *et al*., 2001). Moreover, higher doses, of crude plant extracts were reported to metabolize to toxic end product, which could interfere with gastric function decreasing the food conversion efficiency (Chokshi, 2007). Interestingly, the food and water consumption of the animals were found to be slightly altered (result not included). The observed decline in the food intake could be associated to non-nutritional factors such as Tannins composition a potential proteins coagulator (Fraga-corral *et al*., 2020; Liao, 2009).

The serum hematology and clinical biochemistry analyses were done to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. High levels of ALT, AST, and alkaline phosphatase were usually reported in liver diseases or hepatotoxicity (Brautbar and Williams, 2002). But in this research result no significant increase ($P>0.05$) was observed in ALT, AST, and alkaline phosphatase in both male and female rats at all doses. A decrease in total protein, albumin, and globulin is a sign of the reduced synthetic function of the liver or might be due to impaired hepatocellular function. Low serum albumin content may also suggest infection or continuous loss of albumin (Tietz *et al*., 1994). The observed correlation between total protein and albumin mean values, coupled with the significantly higher and lower values ($P<0.05$) of both parameters for 1000mg/kg and 500mg/kg than the control groups can be associated to low food intake earlier reported. Increase or decrease in albumin levels are regulated by protein intake into the body, protein digestion or absorption that is adequate or inadequate, and or diseases (Tietz *et al*., 1994). Since a drop in albumin levels can be utilized as a sign of protein insufficiency in the body.

The conjugate and total bilirubin value were also within normal reference values, the mean value of the group treated with 1000mg/kg were significantly higher than the control values for both parameters ($p<0.05$) which is an indication of adverse effect of high consumption of the extract over extended period of time. .
Renal dysfunction can be assessed by concurrent measurements of urea, creatinine and uric acid and normal levels reflect reduced likelihood of renal problems (Davis and Brett, 1994). In the present study, changes in plasma urea and creatinine levels in *A. nilotica* extract treated groups showed non-significant differences ($P<0.05$) indicating a normal renal function. The mean value of group treated with the highest concentration at 1000mg/kg were slightly higher than the control values, while creatinine values were lower than normal reference values, however, the group treated with highest concentration showed values higher than the control value, but both parameters were statistically insignificant ($p>0.05$). Likewise, the result of the electrolytes indicated a general trend with the mean values of the control significantly higher than that of the groups treated for Na+, K+ and Cl- but all the values were also within the normal range reference values except for the bicarbonate, which mean values of the control was lower than that of the treated groups. However, the values were within the normal range and statistically insignificant ($P>0.05$), hence forth do not translated to any negative effect of the extract on the kidney of the experimental model.

The result for the liver histopathology showed normal hepatocytes for control group and that treated with 250mg/kg body weight of the extract, while that of the group treated with higher concentration of 500mg/kg and 1000mg/kg showed slight kufer cell hyper-plasia and slight hepatocellular necrosis respectively. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood (Davis and brett, 1994). Thus, the significant changes in serum concentration of total protein, and albumin strongly suggest that the sub chronic administration of *A. nilotica* extract did alter the hepatocellular or secretory functions of the liver over a longer period of the higher doses tested.

Equally, there were also no significant increase in urea and creatinine in the sub chronic administration of *A. nilotica* aqueous extract when compared to the control group. Any rise in urea, creatinine and uric acid levels is only observed if there is marked damage to functional nephrons (Slichter, 2004). This finding was further supported by histological examination of the kidney tissue from this investigation, which revealed that rats given extract doses of 500 and 1000 mg/kg each had only very little and inconsequential lymphocyte hyper-plasia and tubular abnormalities. As a result, the findings of this investigation showed that the *A. nilotica* extract may have a minor impact on how well the rats' renal tubules operate.

The usefulness of weighing organs in toxicity studies includes their sensitivity to predict toxicity, enzyme induction, physiologic perturbations, and acute injury; it is frequently a target organ of toxicity; it correlates well with histopathological changes; there is little inter animal variability; historical control range data are available (Michael *et al*., 2007). The relative organs weights have been observed in toxicity studies to be a relatively sensitive indicator for particular organs, and, thereafter, define toxicity as significant changes observed in those particular organs (Kluwe, 1981). The findings of this study demonstrated that key vital

organs, such as the liver and kidneys, were much heavier in the treated groups than in the control group. In other words, although while the ROW of the liver and kidney were within the normal reference range, different dose concentrations of the plant extract appeared to have affected them, suggesting that the influence may not have had any pathogenic relevance.

Conclusion

The result of this study clearly indicated that the LD_{50} is above 3000mg/kg body weight which indicates that the aqueous extract of the *A. nilotica* leaf is practically non-toxic acutely (Lorke, 1983). However, rats given larger doses of 500 to 1000 mg/kg body weight of extracts showed modest portal lymphocytic hyper-plasia and tubular distortion, suggesting that the daily oral administration of the *A. nilotica* (L) aqueous extract over a lengthy period may have a mild effect on the liver and kidney. Therefore, prolonged usage at greater concentrations should be avoided as it may have a mildly toxic effect on the kidney and liver.

REFERENCES

Abubakar, E.-I. (2010). Phytochemical and Histochemical Screening of Aqueous Extract of Root of Asparagus Pubescens Bak. *Ajps*, *3*(2), 65–68.

Academy, T. N. (2010). *The Norwegian Academy of Science and Letters Bioactive compounds in plants – benefits and risks for man and animals Proceedings from a symposium held at* (Issue November 2008).

Ali, A., Ali, A., Akhtar, N., Khan, B. A., Khan, M. S., & Rasul, A. (2012). *Acacia nilotica : A plant of multipurpose medicinal uses Acacia nilotica : A plant of multipurpose medicinal uses*. *January*. https://doi.org/10.5897/JMPR11.1275

An, S., Zhao, L. P., Shen, L. J., Wang, S., Zhang, K., Qi, Y., Zheng, J., Zhang, X. J., Zhu, X. Y., Bao, R., Yang, L., Lu, Y. X., She, Z. G., & Tang, Y. Da. (2017). USP18 protects against hepatic steatosis and insulin resistance through its deubiquitinating activity. *Hepatology*, *66*(6), 1866–1884. https://doi.org/10.1002/hep.29375

Arg, T. M., Wa, M., Blaise, M., Damien, K., Lunkondo, M., Colette, M. A., Jean-jacques, A. D., Mawi, F., & Ngbolua, K. (2022). *A mini-review on ethnomedical uses , chemical constituents , pharmacological activities and toxicological study of Alchornea cordifolia (Schum &. 10*(4), 139–147. https://doi.org/10.15406/ppij.2022.10.00376

Arsad, S. S., Esa, N. M., Hamzah, H., & Othman, F. (2013). Evaluation of acute, subacute and subchronic oral toxicity of Rhaphidophora decursiva (Roxb.) Schott extract in male Sprague Dawley rats. *Journal of Medicinal Plants Research*, *7*(41), 3030–3040. https://doi.org/10.5897/JMPR2013.2611

Atif Ali. (2012). Acacia nilotica: A plant of multipurpose medicinal uses. *Journal of Medicinal Plants Research*, *6*(9). https://doi.org/10.5897/jmpr11.1275

Audru, J., Labonne, M., Guerin, H., & Bilha, A. (2000). Acacia nilotica: a traditional forage species among the Afar of Djibouti. In *Legume trees and other fodder trees as protein sources for lifestock* (pp. 277–292).

Balamurugan, V., Sheerin, F. M. A., & Velurajan, S. (2019). a Guide To Phytochemical Analysis. *International Journal Of Advance Research And Innovative Ideas In Education*, *5*(January), 1.

Blench, R., Dendo, M., Road, G., & Kingdom, U. (2007). *HAUSA NAMES FOR PLANTS AND TREES* (2nd editio). Roger Blench Mallam Dendo 8, Guest Road Cambridge CB1 2AL United Kingdom. http://www.rogerblench.info/RBOP.htm

Delile, A. L., & Willd, L. (2010). Scientific name Family / tribe Common names Morphological description Acacia nilotica. *Acacia Nilotica Tropical Forage*, 1–10. http://www.weeds.crc.org.au/documents/wmg_prickly_acacia.pdf

Diallo, A., Eklu-Gadegkeku, K., Agbonon, A., Aklikokou, K., Creppy, E. E., & Gbeassor, M. (2010). Acute and sub-chronic (28-day) oral toxicity studies of hydroalcohol leaf extract of Ageratum conyzoides L (Asteraceae). *Tropical Journal of Pharmaceutical Research*, *9*(5), 463–467. https://doi.org/10.4314/tjpr.v9i5.61059

Diallo, A., Eklu-gadegkeku, K., Aklikokou, K., & Creppy, E. E. (2010). *Acute and Sub-chronic (28-day) Oral Toxicity Studies of Hydroalcohol Leaf Extract of Ageratum conyzoides L (Asteraceae)*. *9*(January), 463–467.

Feedipedia, A., & Stewart, J. (2016). Feedipedia. *Feedipedia Animal Feed Resources Information System*.

Fraga-corral, M., Garc, P., Pereira, A. G., Lourenço-lopes, C., Jimenez-lopez, C., Prieto, M. A., & Simal-gandara, J. (2020). Technological Application of Tannin-Based Extracts. *Molecules Review*, *25*(614), 1–27.

Gathirwa, J. W., Rukunga, G. M., Njagi, E. N. M., Omar, S. A., Mwitari, P. G., Guantai, A. N., Tolo, F. M., Kimani, C. W., Muthaura, C. N., Kirira, P. G., Ndunda, T. N., Amalemba, G., Mungai, G. M., & Ndiege, I. O. (2008). *combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya*. *115*, 223–231. https://doi.org/10.1016/j.jep.2007.09.021

Isah, A., Abdullahi, M., & Tsado, M. J. (2015). *Evaluation of Phytochemical , Anti-nutritional and Antioxidant Potentials of Flower and Seed Methanol Extracts of Senna alata L . Grown in Nigeria*. *3*(3), 93–100. https://doi.org/10.11648/j.ajac.20150303.12

Jn, N., C, T., N, K., FF, B., SF, L., JP, D., and S, M. (2019). Acute and Sub-Chronic Toxicity Evaluation of Triplotaxis stellulifera (Benth.) Hutch and Crasssocephalum bougheyanum C.D . Adams Methanol Extract on Mice. *Biochemistry & Analytical Biochemistry*, *08*(03), 1–10. https://doi.org/10.35248/2161-1009.19.8.385

Jonville, M. C., Kodja, H., Humeau, L., Fournel, J., Mol, P. De, Cao, M., Angenot, L., & Frédérich, M. (2008). *Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity*. *120*, 382–386. https://doi.org/10.1016/j.jep.2008.09.005

Julian, M. L. (2000). Biochemistry Profile Serum Hitachi Model 917 Multichannel Analyzer. *Laboratory Procedure Manual*, 1–57.

Kamagaté, M., Koffi, C., Mathieu Kouamé, goran, Akoubet, A., Alain Roland Yao, guessan, & Maxime Die-Kakou, H. (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of Cassia siamea Lam. *The Journal of Phytopharmacology JPHYTO*, *3*(31), 57–76. www.phytopharmajournal.com

Kuriyan, R., Kumar, D. R., Rajendran, R., & Kurpad, A. V. (2010). *An evaluation of the hypolipidemic effect of an extract of Hibiscus Sabdariffa leaves in hyperlipidemic Indians : a double blind , placebo controlled trial*.

Liao, F. (2009). Discovery of artemisinin (Qinghaosu). *Molecules*, *14*(12), 5362–5366. https://doi.org/10.3390/molecules14125362

Loha, M., Mulu, A., Abay, S. M., Ergete, W., & Geleta, B. (2019).

Acute and Subacute Toxicity of Methanol Extract of Syzygium guineense Leaves on the Histology of the Liver and Kidney and Biochemical Compositions of Blood in Rats. *Evidence-Based Complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/5702159

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, *54*(4), 275–287. https://doi.org/10.1007/BF01234480

Marci, N., Gromotowicz-pop, A., & Tomczyk, M. (2022). *Tannins as Hemostasis Modulators*. *12*(January), 1–21. https://doi.org/10.3389/fphar.2021.806891

Mondal, M., Hossain, M. S., Das, N., Khalipha, A. B. R., Sarkar, A. P., Islam, M. T., Smrity, S. Z., Biswas, S., & Kundu, S. K. (2019). Phytochemical screening and evaluation of pharmacological activity of leaf Methanolic extract of Colocasia affinis Schott. *Clinical Phytoscience*, *5*(1). https://doi.org/10.1186/s40816-019-0100-8

Mwandah, D. C., Ntulume, I., Aliero, A. A., Kiyimba, K., Ayikobua, E. T., Bwanika, R., Odoki, M., Kwezira, E., Maseruka, R., Odda, J., & Oloro, J. (2019). Evaluation of Sub-acute and Sub Chronic Toxicity Profile of the Aqueous Leaf Crude Extract of Melanthera Scandens (Schumach & Thonn) in Wistar Rats. *Journal of Complementary and Alternative Medical Research*, *8*(March), 1–16. https://doi.org/10.9734/jocamr/2019/v8i330124

Nwankwo, N., Fred, O., Nwodo, C., Amalunweze, A., Oko, F. P., & Agbo, K. U. (2015). Liver and Kidney Function Tests and Histological Study on Malaria Parasite Infected Mice Administered with Seed Extract of Picralima nitida Liver and Kidney Function Tests and Histological Study on Malaria Parasite Infected Mice Administered with Seed Ext. *International Journal of Biochemistry Research & Review*, *8*(2), 1–14. https://doi.org/10.9734/IJBCRR/2015/18841

Olowokudejo, J. D., Kadiri, A. B., & Travih, V. A. (2016). *AN ETHNOBOTANICAL SURVEY OF SOME HERBAL MARKETS AND An Ethnobotanical Survey of Herbal Markets and Medicinal Plants in Lagos State of Nigeria*. *April*, 1–13.

Oppong Bekoe, E., Kitcher, C., Ama Mireku Gyima, N., Schwinger, G., & Frempong, M. (2019). Medicinal Plants Used as Galactagogues. *Pharmacognosy - Medicinal Plants*. https://doi.org/10.5772/intechopen.82199

Prasanth, K. M., Suba, V., Ramireddy, B., & Srinivasa, B. P. (2015). Acute and subchronic oral toxicity assessment of the ethanolic extract of the root of oncoba spinosa (flacourtiaceae) in rodents. *Tropical Journal of Pharmaceutical Research*, *14*(10), 1849–1855. https://doi.org/10.4314/tjpr.v14i10.16

Sathya, V., & Gopalakrishnan, V. K. (2012). *Study of Drug Likeness Activity of Phytochemicals In Medicinal Plants*. *3*(11), 127–128.

Trease, G. E., & Evans, W. C. (2009). Pharmacognosy 16th edition. *W.B. Sanders Company Ltd., New York.*, 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.

Uguru, C., Partial, I. N., Of, F., Requirements, T. H. E., Award, T. H. E., The, O. F., Of, D., Of, D., & Of, F. (2014). *Acacia nilotica (L.)*. *1991*.

Vasudevan, D., & Vaidyanathan, K. (2017). *Chapter 25 Kidney Function Tests Chapter*. *January*, 2–14. https://doi.org/10.5005/jp/books/13014

Zainal, Z., Ong, A., May, C. Y., Chang, S. K., Rahim, A. A., & Khaza'ai, H. (2020). Acute and subchronic oral toxicity of oil palm puree in sprague–dawley rats. *International Journal of Environmental Research and Public Health*, *17*(10). https://doi.org/10.3390/ijerph17103404