Special Conference Edition, April, 2022

http://dx.doi.org/10.4314/bajopas.v13i1.72S

Bayero Journal of Pure and Applied Sciences, 13(1): 478 - 484
ISSN 2006 – 6996

TOXICITY STUDIES OF ETHANOLIC LEAF EXTRACT OF *Adansonia digitata*

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ABSTRACT
In Ayurvedic system of healing, Adansonia digitata is used as a medicinal plant for the treatment of several ailments and diseases. This study is aimed to evaluate the toxicological, hematological, biochemical and histological parameters of A. digitata Leaf (ADL) extract in mice. Ethanolic leaf extract was administered to the mice using the Lork's method, after which the toxicity effects, liver and kidney functions, as well as hematological and histological parameters were evaluated. The result showed that there was 25% mortality in both phases which occurred at high concentrations. There was significant difference in Red Blood Cell (RBC) and Packed Cell Volume (PCV) counts and also there was a slight increase in the White blood Cell (WBC) and Platelet counts. For the liver function test, Alkaline transaminases (ALT), Aspartate transaminases (AST) and Alkaline Phosphatase (ALP) were elevated while Total Protein (TP) decreased at high concentration (800mg/kg). The results of kidney function showed that ADL extract caused decrease in sodium and potassium levels in low concentration and increased in high concentration and there was significant difference in the creatinine level. The histology of organs after the treatment showed no effect on the heart and lung tissues even at 800mg/kg body weight but showed slight Glomerular and Hepatic necrosis and Hyperplasia of inflammatory cells in spleen tissue. The extracts showed normal morphologies on tissue histology of organs from mice at lower concentrations however, all the abnormalities seen were found at higher doses. This suggest that the extract is non-toxic at low doses as such should be used with caution at high doses.
Keywords: Adansonia digitata, Toxicity, Hematological, Biochemical and Histological.

INTRODUCTION

Adansonia digitata also known as the baobab tree is the most widespread species of the genus Adansonia and is native to the African continent. It is a deciduous, massive majestic tree up to 25m high, it has thick, angular, wide spreading branches and a short, stout trunk which attains 10 – 14m or more in girth and often becomes deeply fluted. It may live for hundreds of years up to 1500 years. Its parts are used as food, medicine, shelter etc (Orwa *et al*., 2009; Kaboré *et al*., 2011; Yusha'u *et al*., 2010 and Jackson, 2015). In Nigeria, the leaves are locally known as "Kuka" in Hausa, 'ɓokko' in Fulfulde and in Yoruba as "Luru" which is a staple food source for rural populations because they are important source of protein, glutamic and aspartic acids. They are eaten both fresh and as a dry powder to make soup (Yusha'u *et al.,* 2010). Different parts of the plant have been reported to have variety of medicinal benefits such as antiperspirant, fever reducing, blood clearing, asthma in humans, diarrhea, inflammation, dysentery, guinea worm diseases, insect bite and to treat parasitic skin infections (Yagoub, 2008; Masola *et al*., 2009; De Caluwé *et al*., 2010; Yusha'u *et al.,* 2010; Jackson, 2015; Gupta and Saxena, 2015). The leaves have anti-histamine and hyposensitizing properties thus are used to treat kidney and bladder infections (Yusha'u *et al.,*2010). The plant kingdom holds many species of plants containing substances of medicinal value which are yet to be discovered. Therefore, the aim of this study is to evaluate the toxicological effects of *A. digitata* plant.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The leaves of *A. digitata* were collected from Munture Agro-allied farms, Rano LGA of Kano state and identified at the Herbarium section of the Department of Plant Biology, Bayero University Kano (Accession number BUKHAN 36).

Extraction of Plant Materials

The plant materials were extracted using the method described by Salim and Imam (2016), where 500g was grinded to powder and percolated with 96% ethanol (2.5L). The percolates were then evaporated to dryness using a rotary evaporator (R110) at 40˚C. The crude residue was weighed and labeled.

Acute Toxicity Assay

Acute toxicity assay was conducted following the Lorke's method as described by Muhammad *et al.*, (2016). Test Animals (Albino mice 18-22kg of age between 6-12 weeks) were collected from the animal house of the Department of Pharmacology and Therapeutics, Bayero University Kano, for the analysis. The mice were maintained at 22^0C at 50-70% humidity, fed with diet containing P-aminobenzoic acid 45mg/kg and water.

Experiment was conducted in two phases;

Phase 1: Three doses 10, 100, 1000mg/kg of extract were administered interperitoneally to 3 groups of three rats each. Then signs of toxicity such as paw licking, salivation, stretching of the entire body/abdomen, weakness, sleep, weight loss, food and water consumption, respiratory distress, coma and/or death were observed in the first 4hours and subsequently 24 hours.

Phase 2: Since the extract caused mortality at 1000mg/kg dose, 3 lower doses (that is 200, 400 and 800mg/kg) and 1 higher dose (1600mg/kg) were administered to 4 rats (1 rat per group) so as to make sure the death was caused by the extract. Then signs of toxicity, coma and/or death were observed in the first 4hours, 24 hours and subsequently 14 days.

After the 14th day (of phase 2), blood samples were collected for hematology and chemical pathology, then the test was terminated by sacrificing the animals and histopathology of organs (Kidney, liver, heart, lungs and spleen) were carried out. Calculation of Lethal dose $LD_{50}$was done as geometric mean of the highest non-lethal dose and the lowest lethal dose.

The LD_{50} was calculated by the formula: $LD_{50} = \sqrt{D_0 \times D_{100}}$

D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality

*Each dose group of phase I was made up of 3 mice each while those in phase II had 1 mice per group.

Hematology test

Hematological parameters such as Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Hemoglobin (Hb), Platelet Count (PLT) and Total White Blood Cell Count (WBC) were determined using hematology analyzer as described by Igbe *et al.,* (2016).

Biochemistry Analysis

Serum Biochemistry Analysis was carried out following the methods of Godkar and Godkar, (2014). Blood sample was collected and allowed to clot at room temperature and centrifuged for 10 minutes to obtain the serum which was used for the analysis. For liver Function Test (LFT); Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatase (ALP), Albumin and total protein tests were carried out while for Renal Function Test (RFT); Urea, creatinine and electrolytes (Sodium, Potassium, Chloride and Bicarbonate) tests were determined Spectrophotometrically using standard diagnostic kits by Randox Laboratories Ltd United Kingdom.

Histology Analysis

Histological investigation of the organs was done according to the method of (Salim and Imam, 2016). The organ pieces (3-5 micro meters thick) were fixed in 10% buffered formalin for 24 h and washed in running water. Samples were dehydrated with serial ethanol cycles (70% to absolute), followed by clarification in xylene and then embedded in paraffin. Duplicate slides of each block were obtained. Slices of 5 µm were produced with a rotation microtome. Deparaffination was performed with the following protocol: Xylene for 4 min; 100% ethanol for 2mins; 90% ethanol for 2mins; 70% ethanol for 2mins. Afterwards slices were stained with Mayer Hematoxylin and Eosin stain (H&E) and mounted with mounting medium (DPX). Sections were then screened under the light microscope for histological study.

Statistical Analysis

The values were expressed as mean ± Standard Deviation. P values < 0.05 were considered as significant.

RESULTS

Table 1: Acute Toxicity of Ethanolic Leaf Extract of *Adansonia digitata* in Mice (*Mus musculus)*

Extract	Phase 1* (mg/Kg)		Phase 2 (mg/Kg)				
	Doses	Mortality	Doses	Mortality	D_0	D_{100}	LD_{50}
ADL	10	0/3	200	0/1	800	1000	894
	100	0/3	400	0/1			
	1,000	2/3	800	0/1			
			1600	1/1			

Key: ADL- *A. digitata* Leaves, D_{0-} Highest dose that gave no mortality, D_{100-} Lowest dose that produced mortality, LD_{50} - Lethal Dose $_{50}$.

Table 2: Acute Toxicity of Leaf Extract of *Adansonia digitata* of the Hematological parameters in Mice (*Mus musculus)*

Extract	Dose (mg/kg)	Hb (g/dl)	RBC (10^6/µl)	WBC(10^3/µl)	PLT (10^6/µl)	PCV (%)
Control	N/S	*14.33±1.53	6.63±0.49	4.23±0.91	198.00±1.40	43.00±0.70
ADL	200	13.66±1.53	6.67±2.08[a]	3.83±0.72	186.00±1.00	40.33±4.16 [a]
	400	12.00±1.00	6.67±1.11	5.36±0.85	207.33±7.50	39.00±3.00
	800	14.50±3.50	8.03±1.22	5.70±0.44	217.00±6.81	41.66±3.05

Key: Hb-Hemoglobin, RBC- Red Blood Cells, WBC- White Blood Cells, PLT- Platelets and PCV- Packed Cell Volume, Control= 10mls/Kg Normal saline. *Values are expressed as mean ± SD of 3 rats in each group. [a] Values showed significant difference compared to control group. P values <0.05 were considered as significant.

Table 3: Acute Toxicity of *Adansonia digitata* Leaf Extract on the Liver Function Test (LFT) in Mice (*Mus musculus)*

Extract	Dose (mg/kg)	Liver Function Test (LFT)				
		ALT(IU/L)	AST(IU/L)	ALP(IU/L)	TP(G/DL)	ALB((G/DL)
Control	N/S	24.66±1.41 [a]	15.00±0.70	27.67±2.08	13.3±1.52	1.90/0.20
ADL	200	20.33±10.11	10.33±2.51	9.33±2.51	2.60±0.55	0.73±0.05
	400	39.00±3.00	33.33±2.08	19.66±8.96	2.80±0.26	0.73±0.05
	800	41.66±3.05	40.00±1.00	41.66±3.05	3.16±0.40	1.23±0.25

Key: ADL- *A. digitata* Leaves, ALT-Alkaline transaminases, AST- Aspartate transaminases, ALP- Alkaline Phosphatase, TP- Total Protein and ALB- Albumin. [a]Values showed no significant difference compared to control group. P values < 0.05 were considered as significant.

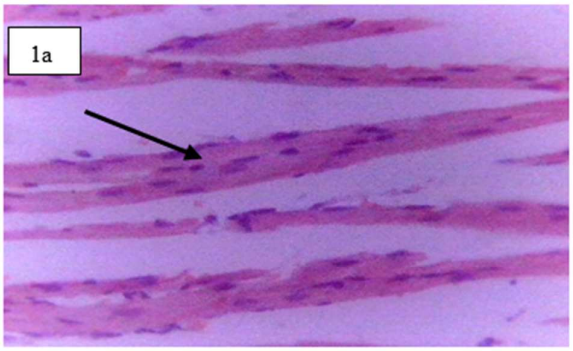
Table 4: Acute Toxicity of *Adansonia digitata* Leaf Extract on the Renal Function Test (RFT) in Mice (*Mus musculus)*

Extracts	Dose(mg/kg)	Renal Function Test (RFT)					
		UREA (mg/dl)	Sodium (mmol/l)	Potassium (mmol/l)	Creatinine (mg/dl)	Chloride (mg/dl)	Bicarbonate (mg/dl)
Control	N/S	8.40±0.14	51.33±2.12	4.86±0.49	0.86±0.15	31.1±2.00	101.00±3.00
ADL	200	6.93±0.00	20.00±2.00	0.86±0.15	0.66±0.20 [a]	21.66±3.05	89.33±6.50
	400	7.36±0.89	37.33±1.52	5.00±2.00	0.80±0.10	27.33±2.51	90.66±6.02
	800	12.66±1.15	37.33±1.52	10.66±2.08	1.06±0.20	30.00±2.00	100.33±6.02

Key: ADL- *A. digitata*Leaves, Renal Function Test (RFT). [a]Values showed significant difference compared to control group. P values < 0.05 were considered as significant.

Histological Analysis

The slides in Plates 1-5 shows the histological changes on the effect of extract of ADL after 14 days treatment on heart, Kidney, Liver, Spleen and Lung tissues.



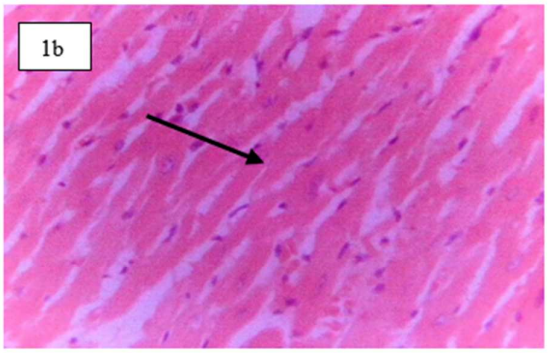
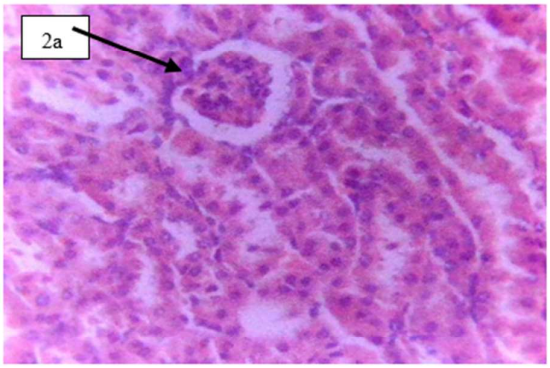


Plate 1a: Heart Tissue Showing Normal Myocardium (Control - 10ml NS/Kg)

Plate 1b: Heart Tissue Showing Normal Myocardium at 800mg/Kg Body Weight



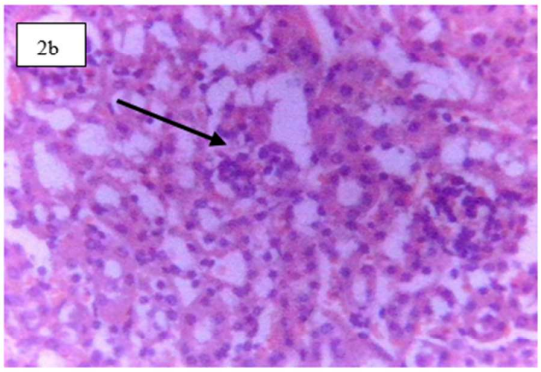
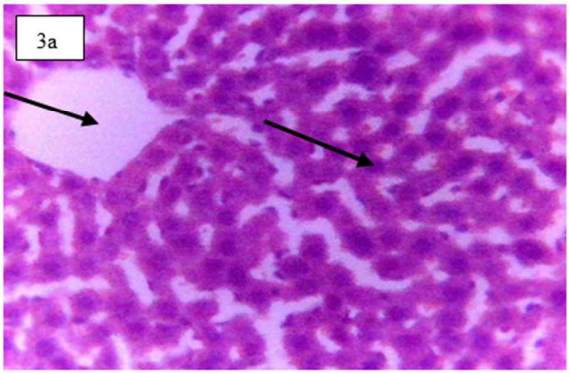
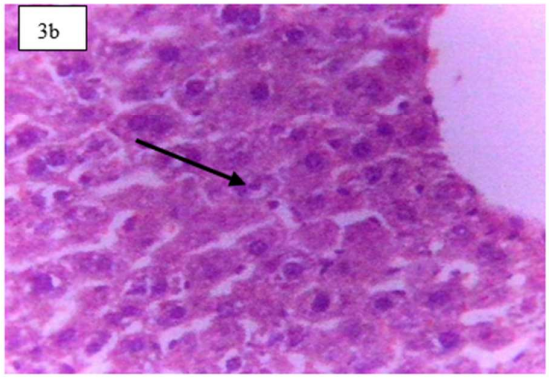
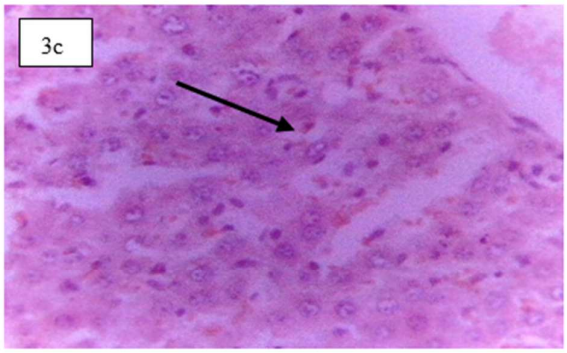


Plate 2a: Kidney Tissue Showing Normal Glomerulus (Control)

Plate2b: Kidney Tissue Showing Slight Glomerular Necrosis at 800mg/kg body weight







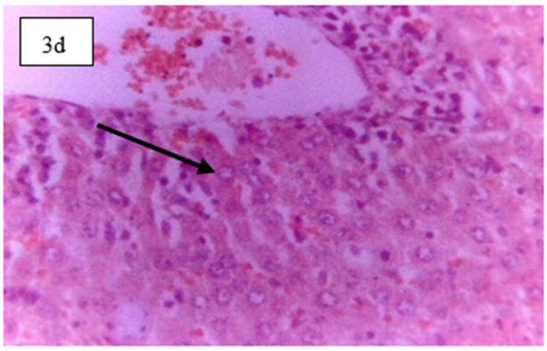
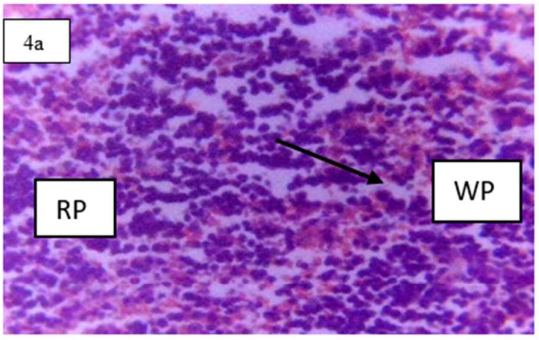


Plate 3a: Liver Tissue Showing Normal Hepatocytes and Central Vein (control)
Plate 3b: Liver Tissue Showing Slight Hepatic Necrosis at 200mg/kg body weight
Plate 3c: Liver Tissue Showing Slight Hepatic Necrosis at 400mg/kg body weight
Plate3d: Liver Tissue Showing Slight Hepatic Necrosis at 800mg/kg body weight



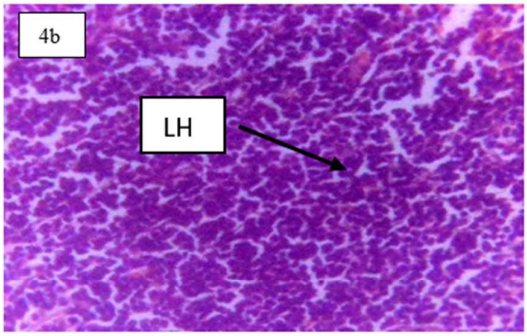
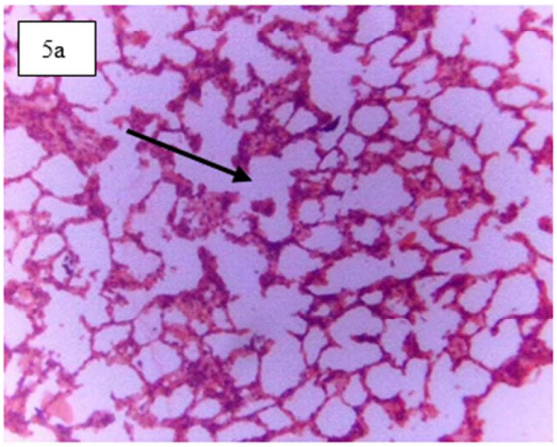


Plate 4a: Spleen Tissue Showing Normal White Pulp and Red Pulp (Control)

Plate 4b: Spleen Tissue Showing Slight Hyperplasia of Inflammatory Cells at 800mg/kg body weight



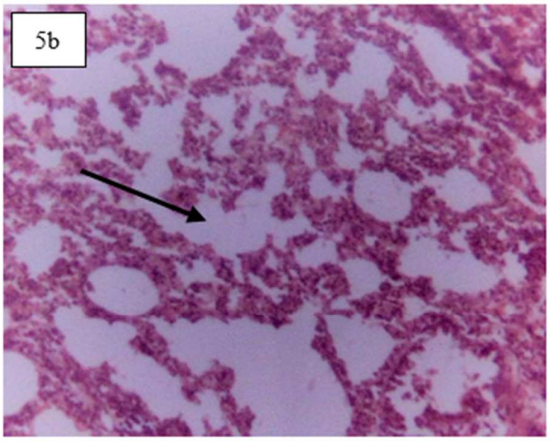


Plate 5a: Lung tissue showing Normal Alveoli (Control)

Plate 5a: Lung tissue showing Normal Alveoli at 800mg/kg body weight

DISCUSSION

The result of Acute toxicity test showed that there was death at 1000mg/Kg dose in first phase of the test while in the second phase, death occurred at dose 1600 mg/Kg. The LD_{50} was calculated as 894, as presented in Table 1. This indicates that the toxicity occurs at high doses, since there was no death at lower doses. This may have been responsible for its widespread use by humans as nutritional source and as remedy of different diseases. The result of this study is in line with the experiments performed by Anele *et al.,* (2014), on rats which showed that administration of *A. digitata* fruit pulp extract at a dose between 400 and 800 mg/ml had a marked anti-inflammatory effect and reduce formalin-induced edema in the animals, but is in contrast to the results of Christian *et al*., (2012), which showed that *Adansonia africana* leaf extract has negligible toxicity as shown in the LD_{50} value of 5000 mg/kg.

The result of hematological studies showed that there is significant difference in RBC count, PCV and slight increase in the WBC and Platelet counts. This is presented in Table 2. These suggest a tendency by the extracts to activate the immune system and can be attributed to slight changes found such as inflammation and necrosis. This observation is in concordance with other findings (Igbe *et al.,* 2016; Aliyu and Samaila, 2016). The results of Liver Function Test (LFT**)** showed that ALT, AST and ALP were elevated, while TP decreased at high concentration (800mg/kg) compared with the control. This is in line with the study of Salim and Imam (2016), were the ALP and ALT were significantly increased after administration with extract of *Vitex simplicifolia* and in a study by Igbe *et al.,* (2016) were the biochemical parameters are within normal for lesser doses compared to the doses used in this study. These enzymes are found in liver cells and leak out to make their way into the blood when there is damage (Aliyu and Samaila, 2016).

The results of Renal Function Test (RFT) showed that ADL extract caused decrease and increase in sodium and potassium levels in low concentration and high concentrations respectively. There is significant difference in the creatinine level. The elevation of serum urea, creatinine, sodium and chloride may have resulted from kidney damage from exposure to high concentrations of the extracts. It is an established fact that a wide variety of renal diseases with different permutation of glomerular, tubular, interstitial or vascular damage can cause an increase in these parameters (Salim and Imam, 2016). Histological results of the organs substantiated these observations.

Plates 1-5 represents the histology slides of organs after 14 days treatment with the plant extracts. Some toxicity signs were recorded from the mice at high doses (1000 and 1600mg/kg) such as weakness, stretching of the abdomen and reduction of food intake but consumed more water and subsequent death. All the extracts showed no effect on the heart and lungs tissue even at higher concentrations, but was able to produce slight Glomerular Necrosis (GN) in kidney, hepatic necrosis, slight and moderate hyperplasia of inflammatory cells in spleen tissue at higher concentrations. This may be a transient physiological response due to the extract which may be reserved by renal regeneration as also showed by the serological test. This is an indication that the extract might be slightly hepatotoxic and the result shows healing process due to the kupfer cells hyperplasia. Also, there is slight and moderate hyperplasia in spleen tissues at higher concentrations, the intense red pulp signifies normal destruction of red blood cells either from the effect of the extract or aging while hyperplasia signifies normal proliferation of matured cells. Therefore, the extract may have not caused deleterious effect on the spleen. All the abnormalities were seen at higher doses with lower doses showing normal morphologies in all the organs. In a study by Salim and Imam (2016), the liver and kidney architecture of control group did not show any pathological changes after sub chronic toxicity testing with ethyl acetate extract of *Vitex simplicifolia* while the tested groups at 1000mg/kg body weight showed mild fatty change, moderate lymphocytosis with inflammatory cells in liver and hydropic swelling in kidney. Igbe *et al.,* (2016) reported that the administration of *Brachystegia eurycoma* at 800mg/kg concentration in the sub-acute toxicity test resulted in mild vascular congestion, inflammation and Kuffer cells activation in liver, mild activation of lymphoid follicles and histocytes activation in spleen and mild interstitial congestion in kidney and lungs.

CONCLUSION

The extracts showed normal morphologies on tissue histology of organs from mice at lower concentrations however, all the abnormalities seen were found at higher doses. This shows that the extract is non-toxic at low doses as such should be used with caution at high doses. Therefore, *Adansonia digitata* plants have been a source of safe, less toxic, low price, easy access, available and reliable natural drugs in many parts of the world.

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