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TOXICITY PROFILING REVEALED BENEFICIAL EFFECTS OF THE ETHANOL STEM BARK EXTRACT OF *ADANSONIA DIGITATA* (MALVACEAE) AT LOW DOSE

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Abstract

Background: The increasing patronage of *Adansonia digitata* for medicinal and cosmetics values makes it a potential source of indiscriminate uses. This study aims at establishing the toxicity potential of the ethanol stem bark extract with a view to providing scientific evidence for the safe use of the plant.
Materials and Methods: The stem bark of *Adansonia digitata* was identified, collected, dry, milled to powder, extracted in absolute ethanol, filtered and concentrated *in vacuo* with rotary evaporator at 45°C. The oral median lethal dose (LD_{50}) was determined using Lorke's method. Male and female rats (100 – 140 g) were used in this study. Toxicity effects on haematological and plasma biochemical indices, as well as histopathological effect in the selected organs was done using single (1000, 3000 and 5000 mg/kg) and repeated dose (250, 500 and 1000 mg/kg) toxicity profiling, and *in silico* toxicity profiling of its selected phytoconstituents.
Results: The oral LD_{50} was greater than 5000 mg/kg, suggesting non-toxic potential. Our results show varying degree of significant alterations in heamatological, biochemical and histopathological indices following single and repeated doses. These alterations were dose and sex dependent, more pronounced at doses ≥ 1000 mg/kg and correlated well with *in silico* toxicity profiling. Though some of the phytoconsituents were predicted to show some elements of toxic potentials, our *in silico* toxicity profiling revealed that none has potential for mutagenicity, genotoxicity, nor inhibit major P450 cytochrome enzymes.
Conclusion: The study concluded that the extract may be more beneficial at lower repeated doses than high doses with toxic potential.

Keywords: *Adansonia digitata*; Toxicity; *In silico*; Haematology; Biochemical; Histolopathology

Abbreviations: OECD, Organization for Economic Co-operation and Development; TG, Test Guidelines; FOB, Functional observatory batteries; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; WBC, White blood count; RBC, Red blood count; Hb, Hemoglobin concentration; HCT, Hematocrit; PLT, Platelets; MCV, Mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW-CV and RDW-SD, Red blood cell distribution width variation coefficient and standard deviation respectively; MPV, Mean platelet volume; PDW, Platelet distribution width; PCT, Plateletcrit.

Introduction

Plants have been used for medicinal purposes globally for many centuries (Gurib-Fakim, 2006; Iwu, 2014). Although, quite a number of medicinal plants have been shown to be relatively safe, we, as well as others have also reported the toxic effects of some herbs (Ozolua et al., 2009; Jaganathan et al., 2013; Agbaje et al., 2019; Olayode et al., 2019; Shobo et al., 2019). These reports, couple with increasing patronage of medicinal plant products as a means of solving unabated human health challenges, underscore the need to properly establish the safety profile of these plants. Toxicological assessment identifies adverse effects and determines limits of exposure levels at which adverse effects occur (Parasuraman, 2011; Asare et al., 2012).

Adansonia digitata L. (Malvaceae) also known as African Baobab is one of the many plants with medicinal and economic values (Kamatou et al., 2011; Adeoye and Bewaji, 2015; Sundarambal et al., 2015; Eltahir and Elsayed, 2019;). It is a deciduous African tree, popularly referred to as "igi-ose" in South-western Nigeria, and naturally distributed in most part of tropical Africa (Kamatou et al., 2011; Sundarambal et al., 2015). It grows up to 25 meters in height, 28 meters in width and lives for several years (Adeoye and Bewaji, 2015). The leaves can be used to prepare soup, while the seeds can be roasted and eaten as snacks (Kamatou et al., 2011; Sundarambal et al., 2015). Several parts of this plant have traditionally been used as immuno-stimulant, anti-inflammatory, analgesic, insect-repellent, anti-diarrhoea and pesticides (Braca et al., 2018; Eltahir and Elsayed, 2019). Other documented uses include the use of leaves and fruit

pulp as febrifuge, and immunostimulant, anti-inflammatory, hepatoprotective, anti-diabetic and anti-oxidant; oil extracted from the seeds to treat diarrhoea; stem bark as antibacterial, anti-sickling and anti-diabetic agents (Adeoye and Bewaji, 2015; Braca et al., 2018; Magashi and Abdulmalik, 2018; Eltahir and Elsayed, 2019). While anti-viral property was found in the root bark, the leaves and seed oil have shown insecticidal and enzyme inhibition activities, respectively (Abiona et al., 2015; Magashi and Abdulmalik, 2018; Eltahir and Elsayed, 2019).

Several phytoconstituents have been identified from various parts of the plant and are mostly terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (Kamatou et al., 2011; Abiona et al., 2015; Braca et al., 2018; Sharma et al., 2019). The alkaloid, adansonin, found in the bark is thought to be the active principle for treatment of malaria and other fevers and its high fat, calcium, copper, iron and zinc content, may explain its use in promoting weight gain in infants (Sundarambal et al., 2015; Kamanula et al., 2018). In a study, eight, fifteen and twenty-two components were identified in aqueous, petroleum ether and ethanol extracts of stem bark respectively by GC-MS and HPLC analysis (Magashi and Abdulmalik, 2017, 2018). While Friedelin, lupeol and baurenol (terpenoids) and betulinic acid were identified in the bark of the plant, linoleic acid, the most frequently used fatty acid as skin moisturizer in cosmetic products and which aids in the healing of dermatoses and suburns, and in treatment of *Acne Vulgaris*, was found in the seed oil (Kamatou et al., 2011; Magashi and Abdulmalik, 2017, 2018; Eltahir and Elsayed, 2019).

However, despite these several pharmacologic and economic importance, the toxicity potential of the stem bark or *A. digitata* is yet to be fully evaluated. In this study we report the evaluation of the toxicity profile of the ethanol stem bark extract of *A. digitata*.

Materials and methods

In vivo experimental design and procedures

Plant material: collection and extraction

The stem bark of A. digitata was obtained from the Ajibode area of the University of Ibadan. The plant's identity was confirmed at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria with a voucher number Ife - 17650. The plant name was also checked with http://www.theplantlist.org for further confirmation. Fresh stem samples were cut into small pieces and then dried under shade for two weeks. Exactly 5 kg of the dried sample was ground and soaked in 3 liters of absolute ethanol for one week. The extract was thereafter filtered with muslin cloth and then with Whatman filters paper 1. The filtrate was concentrated in a rotary evaporator in vacuo at a 45^{o}C. The concentrated extract was stored in a desiccator to complete drying, weighed and kept in a refrigerator at 4^{o}C until required.

Care and use of experimental animal

Healthy male and nulliparous female Wistar rats (100-140 g, and bred locally in the animal holding of the Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife) were used in both the single and repeated dose toxicity tests. The animals were housed in standard plastic cages for at least 7 days prior to the start of toxicity studies to allow for acclimatization. The animals were allowed access to standard laboratory pellets (Grand Cereals, United African Company Plc, Nigeria) and water ad libitum except on the day prior to the start of experiment (OECD, 2008). The procedure for the animal care was based on the "Guide for the Care and Use of Laboratory animals - Eight Edition" (National Research Council USA, 2011) and the National Centre for the Replacement, Refinement, and Reduction of animals in research (NC3Rs) guidelines on humane animal care and use (Franco et al., 2012; Singh, 2012) and was granted institutional ethical approval number IPH/OAU/12/1759.

Median lethal dose (LD_{50}) determination and sighting study

The median lethal dose (LD_{50}) was determined using Lorke's method (Lorke, 1983). With no mortality observed at 5000 mg/kg, a confirmatory test was conducted to validate the observation. Using LD_{50} as guide, and monitoring functional observational battery (FOB) (Gauvin et al., 2016; Mathiasen and Moser, 2018), the sighting study (OECD, 2008) was conducted to determine the optimal doses for the acute and repeated dose toxicity studies, and humane endpoint criteria.

Single dose (acute) toxicity study

Twenty-four each of males and females (nulliparous non-pregnant) rats were used in this study. Each category (male or female rats) was randomly allotted into four groups (control and three test groups, n = 6) and the study procedure was as earlier reported (Olayode et al., 2019). The graded doses of the ethanol stem bark extract of *A. digitata* (1000, 3000, 5000 mg/kg body weight) and distilled water (control) were orally administered to rats following an overnight fast. The volume of administered doses was not more than 1 ml/100 g body weight. Monitoring of cage side observation using FOB and weight gains were performed as earlier reported (Olayode et al., 2019; Shobo et al., 2019). On day 7, rats were sacrificed for samples collection.

Repeated dose toxicity/ recovery studies

Forty-eight each of male and female nulliparous non-pregnant rats were randomly allotted into four groups of twelve rats (male or female) each. The procedure for the repeated dose toxicity study follows OECD Test Guideline 407 as earlier reported (OECD, 2008; Olayode et al., 2019; Shobo et al., 2019). Groups 1-3 were daily orally administered 1000, 500, and 250 mg/kg body weight of the extract, while the control was given distilled water. The volume of administered doses was not more than 1 ml/100 g body weight. Following the 28 days of repeated daily dosing for both male and female rats, the surviving male and female rats were sub-grouped into Toxicity (TS) and Recovery (RS) sets as earlier reported (Agbaje et al., 2019; Olayode et al., 2019). The RS rats were monitored for

further 21 days of non-dosing recovery period for obvious signs of toxicity. Sample collections for TS and RS sub-groups are as detailed below.

Collection, Treatment and Analysis of Samples and Data Presentations
Collection and treatment of samples

All surviving rats in single dose and repeated dose TS were humanely euthanized using cervical dislocation. Procedures for blood and organ samples collection and treatment were as earlier reported (Olayode et al., 2019; Shobo et al., 2019). Plasma samples were kept in freezer at -20^{o}C until analysis and kidney and liver preserved in 10% formal saline for histological assessment.

Relative body and organ weights analysis

Relative weight change is the daily weights of rats expressed as percentage of the initial animal weight prior to start of extract administration. Average relative weight change, organ - body weight and organ - brain weight ratios over the period of experiments were measured.

Assay of plasma samples for biochemical indices

Plasma samples were assayed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine were performed using commercial biochemical assay kits (Randox Laboratories Limited, Crumlin, County Antrim, BT294QY, United Kingdom), as per manufacturer's instructions.

Assay of blood samples for heamatological indices

Complete blood count was performed using an Haematology Auto-Analyzer as earlier reported (Ode et al., 2017; Olayode et al., 2019; Shobo et al., 2019).

Histopathological evaluation of kidney and liver

Histopathological evaluations of kidney and liver were performed using standard procedures as earlier described (Greaves, 2012; Feldman and Wolfe, 2014; Olayode et al., 2019; Shobo et al., 2019). Sectioning was done using a rotary microtome (Leica Bright B5143 Huntington, England), and slides were mounted with cover slips and viewed under the microscope with photomicrograph taken with a Camera Microscope (Leica DM750) (magnification x400).

***In silico* Experimental Designs and Procedures**

The phytochemicals of *A. digitata* were sourced from literatures and 3D SDF files of the selected phytochemicals were downloaded from PubChem database (Kim et al., 2018) and used to construct an in-house database with Aceryl discovery studio visualizer (Dassault Systèmes BIOVIA, 2015). Two-phase toxicity screening approach (Daniyan and Ojo, 2019) was adopted for the *in silico* toxicity screening of the phytochemicals. All screenings were performed using VEGA *in silico* platform version 1.1.5-b22 (Ferrari and Gini, 2010; Fjodorova et al., 2010), Toxicity Estimation Software Tool (T.E.S.T) version 4.2.1 (Martin et al., 2012), Toxtree version 3.1.0 (Patlewicz et al., 2008), and pkCSM and vNN ADMET online servers (Pires et al., 2015; Schyman et al., 2017) as earlier described (Agbaje et al., 2019).

Statistical analysis and Data Presentation

Data were expressed as mean ± standard error of mean (Mean ± SEM) and compared using Students' T-test for pairwise comparison and/or One way analysis of variance (ANOVA) followed by Dunnet posthoc test. Differences were considered significant at $p < 0.05$.

Results

Cage-side observations with FOBs

The preliminary assessment during sighting study revealed that the extract did not show any critical effects that could lead to death. The LD_{50}, which was greater than 5000 mg/kg, may suggest that the extract could be practically non-toxic. As such, none of the FOB was selected as endpoint criterion for the purpose of monitoring adverse effects that could lead to death and the highest acute dose was therefore selected to be 5000 mg/kg. However, taken into consideration the potential cumulative effects of the extract following repeated administration, the doses used for repeated dose toxicity study were taken as one-fifth of the acute toxicity study as earlier reported (Olayode et al., 2019; Shobo et al., 2019). Expectedly, continuous monitoring during single and repeated dose administration indicated that at the tested doses, none of the observed adverse effects led to death. Specifically, following single and repeated dose administration, the results show no overt sign of intoxication in both sexes, across the dose levels (5000, 3000 and 1000 mg/kg for acute, and 1000, 500 and 250 mg/kg for repeated doses) within the first 24 h. However, normal behavioural changes (lethargy, mouth scratching and transient hypokinesia) which appeared within the first 4 h and gradually wore off were observed. No mortality was recorded throughout the periods of the experiments, in both sexes and across all the dose levels.

Effects of the extract on relative body weights and organ - weight ratios

Following acute dose administration, changes in liver:body, kidney:brain, and heart:brain weight ratios were similar in both male and female rats, indicating significant reduction in liver:body and kidney:brain at 3000 and 5000 mg/kg, and significant increases in heart:brain ratio at all dose levels (Table 1). However, male rats also showed significant reduction in kidney:brain ratio at 1000 mg/kg, and while female rats showed significant reduction in relative body weights at 3000 and 5000 mg/kg and significant increase in liver:brain ratio at all dose levels, the significant changes, though similar, are opposite in male rats (Table 1). In addition, while female rats showed significant reduction in kidney:body and brain:body weights ratio at all dose levels, male on the other hand showed significant increase in heart:body ratio at all dose levels and in brain:body only at 5000 mg/kg. On the other hand, the levels of significant changes observed in acute study were significantly reduced following repeated administration. Apart from relative weight change, where female rats showed significant dose dependent reduction in weights, and male rats weights were significantly increased, there were noticeable significant dose dependent reduction in kidney:brain, heart:brain and liver:brain ratios at all dose levels. In addition, male rats also showed significant dose dependent reduction in liver:body at all dose levels. It appears that in both acute and repeated doses, there are noticeable sex differential effects of the extracts on rats (Table 1).

Effect of the extract on haematological indices

There are varying changes in heamatological indices in male and female rats following single and repeated doses (Table 2). The changes, which increases dose dependently, were more pronounced following acute doses when compared to repeated doses. Aside from MCV, MCH, RDW-CV, MPV, PDW and PCT that were either not altered or slightly altered in male and female rats, all other indices were significantly altered with increasing doses following acute dose administration (Table 2), thus suggesting a toxic effect of the extract. Following repeated doses, however, and interestingly, most indices were not altered. Apart from significant dose dependent changes in WBC, HGB, PLT and PCT in female rats, other indices were not altered. Also, male rats showed significant dose dependent alteration in WBC, HGB, RBC, HCT, MCH and RDW-CV, supporting the idea of sex deferential effects of the extracts (Table 2).

Table 1: Effects of *A. digitata* ethanol extract on relative weight gain, organ - body and organ - brain weights ratio in male and female rats following single and repeated doses

	Relative Body weights (%)	Organ-Body weights ratios				Organ-Brain weights ratios		
		Kidney:Body	Heart:Body	Liver:Body	Brain:Body	Kidney:Brain	Heart:Brain	Liver:Brain
FEMALE RATS – SINGLE DOSE TOXICITY STUDY (n = 6)								
Control	102.78 ± 0.99	0.0060 ± 0.0003	0.0049 ± 0.0004	0.0392 ± 0.0009	0.0138 ± 0.0004	0.431 ± 0.007	0.353 ± 0.012	2.844 ± 0.076
1000 mg/kg	104.15 ± 0.68	0.0046 ± 0.0002*	0.0055 ± 0.0006	0.0386 ± 0.0006	0.0104 ± 0.0006*	0.450 ± 0.009	0.529 ± 0.015*	3.156 ± 0.022*
3000 mg/kg	98.89 ± 0.79*	0.0045 ± 0.0001*	0.0054 ± 0.0001	0.0323 ± 0.0007*	0.0120 ± 0.0001*	0.379 ± 0.010*	0.447 ± 0.012*	3.221 ± 0.037*
5000 mg/kg	99.08 ± 1.10*	0.0041 ± 0.0001*	0.0051 ± 0.0002	0.0348 ± 0.0005*	0.0110 ± 0.0002*	0.376 ± 0.010*	0.461 ± 0.016*	3.136 ± 0.048*
MALE RATS – SINGLE DOSE TOXCIITY STUDY (n = 6)								
Control	101.11 ± 0.79	0.0042 ± 0.0003	0.0035 ± 0.0003	0.0352 ± 0.0012	0.0107 ± 0.0006	0.392 ± 0.006	0.325 ± 0.011	3.286 ± 0.070
1000 mg/kg	98.67 ± 0.94	0.0042 ± 0.0001	0.0054 ± 0.000*	0.0316 ± 0.0015	0.0123 ± 0.0008	0.347 ± 0.003*	0.449 ± 0.017*	2.286 ± 0.015*
3000 mg/kg	109.56 ± 0.91*	0.0044 ± 0.0002	0.0058 ± 0.0006*	0.0278 ± 0.0007*	0.0126 ± 0.0008	0.313 ± 0.008*	0.409 ± 0.018*	2.235 ± 0.010*
5000 mg/kg	107.60 ± 1.39*	0.0041 ± 0.0003	0.0049 ± 0.0002*	0.0317 ± 0.0009*	0.0142 ± 0.0005*	0.328 ± 0.011*	0.392 ± 0.006*	2.553 ± 0.052*
FEMALE RATS - REPEATED DOSE TOXICITY STUDY (n = 6)								
Control	116.06 ± 0.64	0.0027 ± 0.0002	0.0036 ± 0.0003	0.0317 ± 0.0005	0.0100 ± 0.0002	0.274 ± 0.002	0.358 ± 0.006	3.162 ± 0.011
250 mg/kg	117.67 ± 1.12	0.0028 ± 0.0001	0.0038 ± 0.0000	0.0329 ± 0.0005	0.0086 ± 0.0001*	0.471 ± 0.001*	0.441 ± 0.002*	3.824 ± 0.012*
500 mg/kg	113.34 ± 0.18*	0.0041 ± 0.0001*	0.0041 ± 0.0002	0.0345 ± 0.0009*	0.0096 ± 0.0002	0.348 ± 0.009*	0.429 ± 0.003*	3.597 ± 0.014*
1000 mg/kg	112.05 ± 0.30*	0.0033 ± 0.0001*	0.0030 ± 0.0002	0.0321 ± 0.0006	0.0096 ± 0.0004	0.287 ± 0.008	0.313 ± 0.005*	3.346 ± 0.012*
MALE RATS - REPEATED DOSE TOXCIITY STUDY (n = 6)								
Control	104.36 ± 0.60	0.0030 ± 0.0001	0.0032 ± 0.0001	0.0365 ± 0.0008	0.0098 ± 0.0003	0.304 ± 0.006	0.325 ± 0.009	3.735 ± 0.017
250 mg/kg	105.39 ± 0.79	0.0031 ± 0.0001	0.0037 ± 0.0004	0.0382 ± 0.0005*	0.0097 ± 0.0001	0.369 ± 0.003*	0.390 ± 0.004	3.947 ± 0.023*
500 mg/kg	108.64 ± 0.85*	0.0034 ± 0.0002	0.0035 ± 0.0001	0.0292 ± 0.0006*	0.0098 ± 0.0004	0.329 ± 0.001*	0.355 ± 0.008*	2.993 ± 0.014*
1000 mg/kg	107.50 ± 0.59*	0.0035 ± 0.0002*	0.0028 ± 0.0003	0.0328 ± 0.0003*	0.0102 ± 0.0004	0.313 ± 0.008	0.272 ± 0.006*	3.220 ± 0.016*

* Significantly different when compared with their respective controls at $p < 0.05$

Table 2: Effects of *A. digitata* ethanol extract on heamatological indices on male and female rats following single and repeated doses

	FEMALE RATS – SINGLE DOSE (n = 6)				MALE RATS – SINGLE DOSE (n = 6)			
	Control	**1000 mg/kg**	**3000 mg/kg**	**5000 mg/kg**	**Control**	**1000 mg/kg**	**3000 mg/kg**	**5000 mg/kg**
WBC (x 10^9)	6.80 ± 0.05	8.93 ± 0.17*	8.87 ± 0.10*	9.63 ± 0.24*	6.47 ± 0.09	6.53 ± 0.07	7.23 ± 0.17*	7.23 ± 0.24*
HGB (g/dl)	132.33 ± 1.78	143 ± 1.60*	144.67 ± 1.10*	142.00 ± 1.45*	104.67 ± 1.61	137.67 ± 2.52*	140.33 ± 1.90*	134.00 ± 2.22*
RBC (x 10^{12})	7.55 ± 0.12	8.42 ± 0.08*	8.58 ± 0.17*	8.38 ± 0.14*	5.96 ± 0.02	7.91 ± 0.12*	7.59 ± 0.36*	7.81 ± 0.39*
HCT (%)	49.30 ± 0.28	52.67 ± 0.17*	47.27 ± 0.43*	52.60 ± 0.10*	37.87 ± 0.29	51.53 ± 0.43*	46.20 ± 0.60*	48.57 ± 0.52*
MCV (fL)	63.73 ± 0.56	62.60 ± 0.48	60.33 ± 0.33*	62.90 ± 0.17	63.97 ± 0.41	65.23 ± 0.05*	61.53 ± 1.90	63.23 ± 0.35
MCH (pg)	17.07 ± 0.13	16.90 ± 0.19	16.73 ± 0.19	16.90 ± 0.12	17.50 ± 0.09	17.37 ± 0.05	17.00 ± 0.19*	16.83 ± 0.12*
MCHC (g/L)	268.00 ± 0.33	270.67 ± 1.02*	272.00 ± 1.67*	269.67 ± 1.91	274.67 ± 1.42	266.67 ± 1.07*	260.67 ± 2.04*	268.00 ± 1.33*
RDW-CV (%)	17.57 ± 0.12	16.70 ± 0.20*	18.87 ± 0.15*	17.07 ± 0.16*	17.73 ± 0.32	18.13 ± 0.10	17.63 ± 0.30	17.70 ± 0.23
RDW-SD (fL)	37.63 ± 0.27	35.97 ± 0.32*	38.03 ± 0.12	36.33 ± 0.37*	39.33 ± 0.02	39.00 ± 0.00*	38.20 ± 0.27*	37.73 ± 0.37*
PLT (x 10^9)	449.33 ± 2.27	608.00 ± 1.90*	603.33 ± 2.6*	611.67 ± 3.78*	333.67 ± 1.69	595.67 ± 4.86*	501.33 ± 2.80*	441.33 ± 3.67*
MPV (fL)	8.73 ± 0.09	8.57 ± 0.08	8.30 ± 0.03*	8.20 ± 0.07*	8.57 ± 0.13	8.40 ± 0.12	8.40 ± 0.10	8.50 ± 0.06
PDW	14.93 ± 0.13	14.87 ± 0.11	14.67 ± 0.10	14.60 ± 0.03*	14.87 ± 0.05	14.93 ± 0.13	14.73 ± 0.25	14.67 ± 0.14
PCT (%)	0.32 ± 0.01	0.52 ± 0.02*	0.31 ± 0.03	0.51 ± 0.04*	0.29 ± 0.05	0.35 ± 0.10	0.42 ± 0.04	0.39 ± 0.04
	FEMALE RATS - REPEATED DOSE (n = 6)				**MALE RATS - REPEATED DOSE (n = 6)**			
	Control	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
WBC (x 10^9)	8.50 ± 0.17	7.68 ± 0.18*	7.30 ± 0.35*	3.37 ± 0.13*	8.07 ± 0.14	6.80 ± 0.22*	4.80 ± 0.18*	8.70 ± 0.12*
HGB (g/dl)	119.67 ± 0.38	124.67 ± 1.07*	128.00 ± 0.88*	114.00 ± 0.58*	124.33 ± 0.19	129.33 ± 1.02*	133.00 ± 1.20*	124.33 ± 0.38
RBC (x 10^{12})	7.51 ± 0.21	7.57 ± 0.24	7.17 ± 0.54	7.09 ± 0.03	7.14 ± 0.18	8.03 ± 0.14*	8.06 ± 0.12*	7.56 ± 0.17
HCT (%)	42.37 ± 1.37	43.89 ± 0.92	41.10 ± 0.52	40.47 ± 0.37	40.50 ± 0.58	46.67 ± 0.31*	47.30 ± 1.42*	44.83 ± 0.39*
MCV (fL)	56.43 ± 0.52	58.25 ± 0.68	57.70 ± 0.59	57.17 ± 0.67	57.10 ± 0.7	58.27 ± 0.76	58.60 ± 0.95	60.07 ± 1.27
MCH (pg)	15.87 ± 0.15	16.50 ± 0.40	15.83 ± 0.3	16.03 ± 0.16	15.43 ± 0.11	16.10 ± 0.15*	16.43 ± 0.24*	15.80 ± 0.20
MCHC (g/L)	282.00 ± 0.58	284.13 ± 3.82	285.33 ± 2.34	281.33 ± 2.04	271.67 ± 5.17	276.67 ± 1.02	280.67 ± 0.38	273.67 ± 3.02
RDW-CV (%)	17.00 ± 0.22	17.08 ± 0.31	17.13 ± 0.42	17.03 ± 0.23	17.60 ± 0.3	18.20 ± 0.07	16.77 ± 0.12*	16.57 ± 0.18*
RDW-SD (fL)	33.60 ± 0.17	34.01 ± 0.36	33.30 ± 0.17	33.67 ± 0.69	36.30 ± 0.71	36.27 ± 0.37	35.00 ± 0.58	36.47 ± 0.17
PLT (x 10^9)	483.00 ± 3.48	671.60 ± 4.98*	608.00 ± 1.45*	507.67 ± 2.22*	590.33 ± 3.95	580.00 ± 4.36	588.67 ± 2.36	584.33 ± 4.74
MPV (fL)	8.53 ± 0.08	8.63 ± 0.16	8.50 ± 0.12	8.30 ± 0.23	8.60 ± 0.09	8.37 ± 0.07	8.40 ± 0.12	8.50 ± 0.07
PDW	14.53 ± 0.13	14.87 ± 0.09	14.67 ± 0.10	14.50 ± 0.1	14.53 ± 0.11	14.57 ± 0.04	14.60 ± 0.00	14.57 ± 0.07
PCT (%)	0.41 ± 0.00	0.61 ± 0.02*	0.51 ± 0.03*	0.63 ± 0.01*	0.51 ± 0.01	0.49 ± 0.01	0.46 ± 0.03	0.48 ± 0.00

* Significantly different when compared with their respective controls at $p < 0.05$

Effects of the extract on biochemical indices

There were significant dose dependent reductions in AST and ALT activities with corresponding significant increases in AST/ALT ratio at all dose levels when compared with control following single dose administration (Table 3). On the other hand, urea and creatinine increased significantly at all dose levels when compared with the control, suggesting potential toxic effects on the kidney (Table 3). Furthermore, contrary to what was observed in acute toxicity study, following repeated doses, AST and ALT activities increased with increasing doses. Interestingly however, the AST/ALT ratios were less than 1. Also, though decreases with increase in doses, urea was significantly increased and creatinine significantly decreased when compared to control.

Effects of the extract on organs histology
Effects of the extract on kidney

Assessment of the effect of the test substances on the cellular architecture of the kidney revealed that the control shows good histoarchitecture, clear distinct proximal and distal tubules (Figures 1 and 3). Following administration of the acute doses of the ethanol extract of *A. digitata* stem bark, the renal histology revealed tissue haemorrhage across all doses (1000 mg/kg, 3000 mg/kg and 5000 mg/kg body weight), and localized clogging of tubules and mild glomerulonephritis at higher doses in female rats compared to the control (Figure 1). The effects were mild following repeated doses of the test substances showing mild tubular congestion, diffuse tissue haemorrhage and mild glomerulonephritis at 500 mg/kg and 1000 mg/kg body weight (Figure 3).

Effects of the extract on liver

The histopathological assessment of the liver shows clear sinusoids, cords of hepatocytes, well outlined central vein and portal triad; and conspicuous nucleoli in the controls (Figures 2 and 4). Upon administration the acute doses of the ethanol extract of *A. digitata* stem bark, the liver histology revealed sinusoidal congestion and disrupted histoarchitecture at 3000 mg/kg body weight, and extensive tissue haemorrhage, vascular congestion, clogged sinusoids and disrupted histoarchitecture at 5000 mg/kg body weight (Figure 2). However, at 1000 mg/kg body weight, there were evidence of mild vascular congestion, clear sinusoids and distinct cords of hepatocytes (Figure 2). Following repeated doses of 250 and 500 mg/kg body weight of the test substance, the liver histology was essentially normal, with isolated mild vascular congestion (Figure 4). At 1000 mg/kg body weight, there were mild sinusoidal congestion and mild vascular congestion of the liver (Figure 4).

Consensus analysis of *In silico* profiling of selected compounds from *Adansonia digitata*

The detailed structural and physicochemical data, as well as the detailed analysis of *in silico* predictions are provided in Tables S1 – S6. The consensus analysis of the *in silico* toxicity screening show that none of the selected compounds has any potential for mutagenicity and genotoxicity (Table 4). Although a number of the compounds appear to show potentials for developmental toxicity and skin sensitization, the potential for carcinogenicity was generally low. Furthermore, some of the compounds show potentials for hepatotoxicity, but their reported relative abundance were very low (Magashi and Abdulmalik, 2017). Except for AG6 (2-Palmitoylglycerol) and AG21 (Phthalic acid), all other compounds were not positive for micronucleus assays. Also, while few of the compounds were predicted to bind to proteins, none showed potential to bind DNA. A good number of the selected compounds showed abilities to cross the blood brain barrier, had no inhibitory activity against major P_{450} cytochrome enzymes, as well as lack capacity to bind DNA and cause the inhibition of hERG I and II.

Table 3: Effects of *A. digitata* ethanol extract on serum biochemical indices in male and female rats following single and repeated doses

		AST (IU/L)	ALT (IU/L)	AST/ALT	Urea (mg/dl)	Creatinine (mg/dl)
SINGLE DOSE TOXICITY						
FEMALE RATS (n = 6)	Control	67.52 ± 0.70	76.35 ± 1.65	0.89 ± 0.02	7.99 ± 1.04	0.94 ± 0.09
	1000 mg/kg	60.58 ± 1.06*	47.02 ± 2.14*	1.30 ± 0.08*	15.99 ± 1.72*	1.43 ± 0.11*
	3000 mg/kg	61.20 ± 2.06*	50.01 ± 4.46*	1.25 ± 0.08*	17.22 ± 2.81*	1.71 ± 0.33*
	5000 mg/kg	58.21 ± 1.59*	48.82 ± 0.07*	1.38 ± 0.18*	15.22 ± 1.35*	1.94 ± 0.11*
MALE RATS (n = 6)	Control	65.35 ± 0.82	71.07 ± 0.96	0.92 ± 0.02	6.18 ± 0.70	1.26 ± 0.07
	1000 mg/kg	45.14 ± 1.07*	37.99 ± 3.24*	1.14 ± 0.07*	17.79 ± 1.46*	1.74 ± 0.15*
	3000 mg/kg	53.79 ± 1.21*	49.90 ± 2.59*	1.08 ± 0.03*	18.55 ± 2.43*	2.17 ± 0.04*
	5000 mg/kg	61.20 ± 1.52*	58.07 ± 2.67*	1.07 ± 0.01*	13.32 ± 1.24*	3.34 ± 0.30*
28-DAY REPEATED DOSE TOXICITY						
FEMALE RATS (n = 6)	Control	74.16 ± 1.78	89.25 ± 1.00	0.83 ± 0.05	5.81 ± 0.11	1.19 ± 0.08
	250 mg/kg	81.05 ± 0.73*	107.81 ± 0.25*	0.75 ± 0.01	9.69 ± 0.13*	1.70 ± 0.16*
	500 mg/kg	92.14 ± 0.35*	105.36 ± 0.80*	0.87 ± 0.01	7.99 ± 0.18*	1.70 ± 0.24
	1000 mg/kg	117.27 ± 1.94*	182.97 ± 3.70*	0.64 ± 0.00*	6.62 ± 0.08*	0.93 ± 0.13
MALE RATS (n = 6)	Control	80.22 ± 0.67	91.81 ± 1.60	0.88 ± 0.02	8.89 ± 0.15	1.76 ± 0.09
	250 mg/kg	90.79 ± 2.15*	96.49 ± 1.47	0.94 ± 0.03	9.98 ± 0.11*	1.31 ± 0.23
	500 mg/kg	112.30 ± 0.57*	160.22 ± 2.41*	0.71 ± 0.05*	6.06 ± 0.08*	1.12 ± 0.05*
	1000 mg/kg	133.01 ± 0.68*	217.21 ± 1.82*	0.65 ± 0.05*	5.19 ± 0.07*	0.89 ± 0.04*

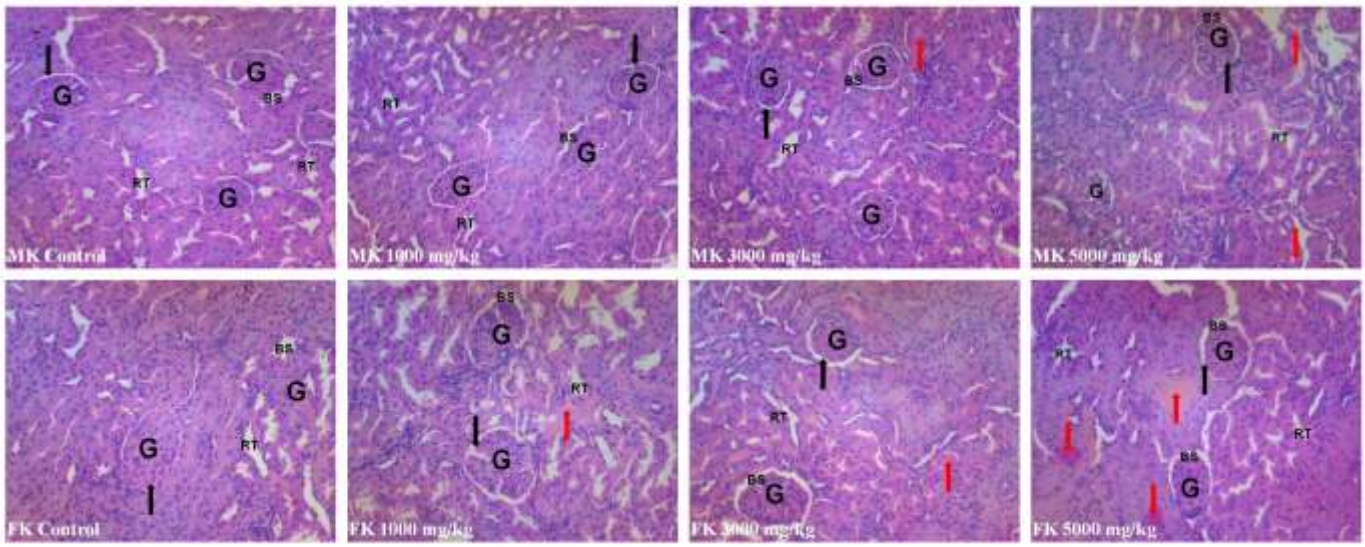

Figure 1: Photomicrographs of Kidney following single oral administration of the suspension of the aerial part of *Adansonia digitata*. G, glomeruli; BS, bowman's capsule and bowman's space; RT, renal tubules; Red arrows were used to identify pathological changes in the kidney including presence of tissue haemorrhages (Magnification x 400).

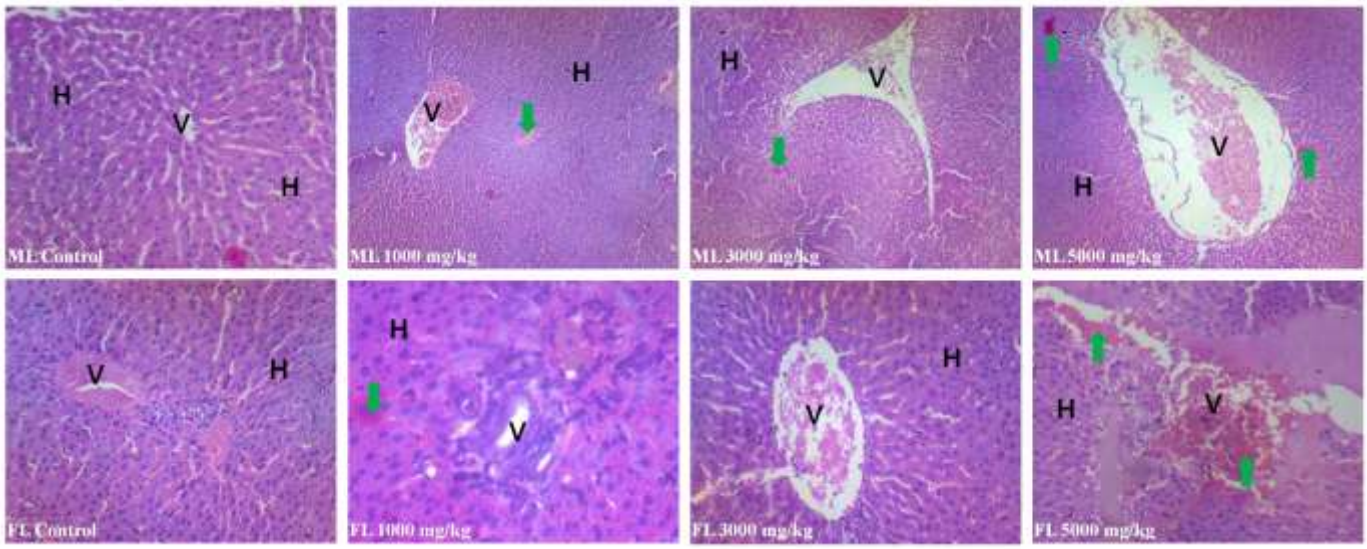

Figure 2: Photomicrographs of Liver following single oral administration of the suspension of the aerial part of *Adansonia digitata*. H, hepatocytes; V, cenral vein. Green arrows were used to identify pathological changes in the liver including presence of tissue haemorrhages (Magnification x 400).

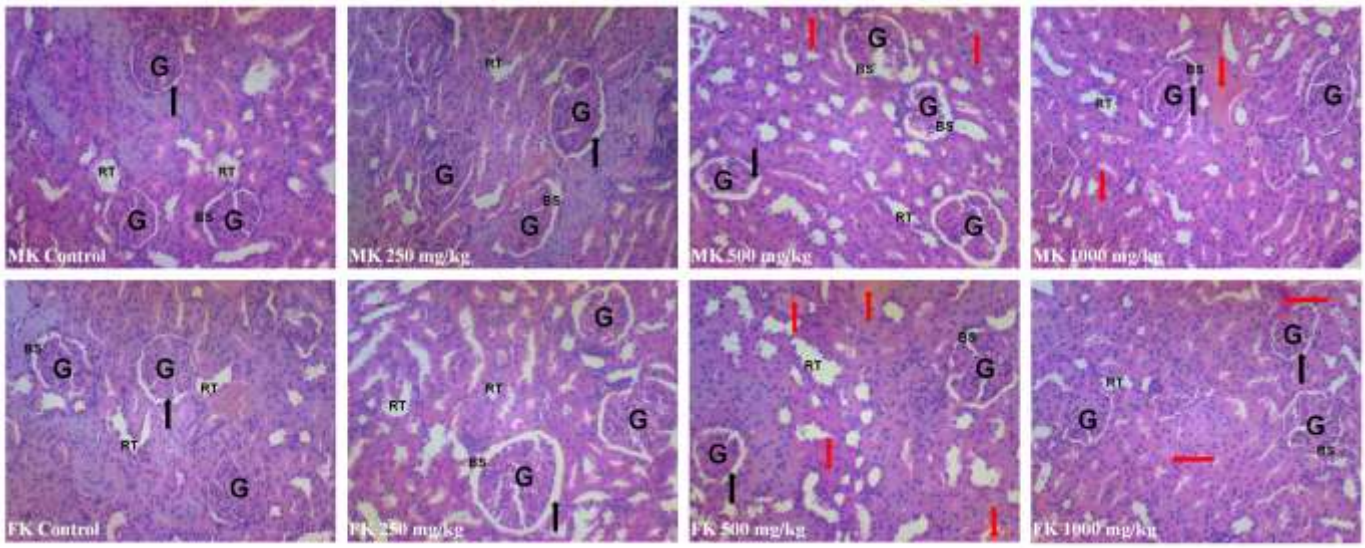

Figure 3: Photomicrographs of Kidney following repeated oral administration of the suspension of the aerial part of *Adansonia digitata*. G, glomeruli; BS, bowman's capsule and bowman's space; RT, renal tubules; Red arrows were used to identify pathological changes in the kidney including presence of tissue haemorrhages (Magnification x 400).

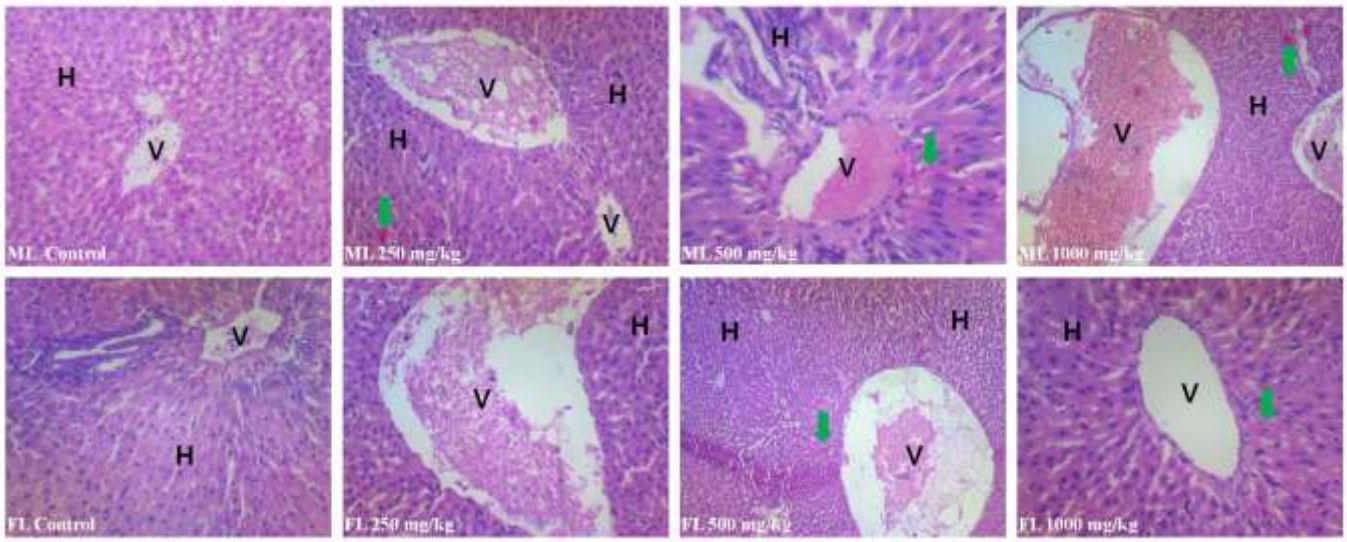

Figure 4: Photomicrographs of Liver following repeated oral administration of the suspension of the aerial part of *Adansonia digitata*. H, hepatocytes; V, cenral vein. Green arrows were used to identify pathological changes in the liver including presence of tissue haemorrhages (Magnification x 400).

Table 4: Consensus analysis of the *In silico* toxicity predictions of selected compounds from *Adansonia digitata*

ID	COMPOUNDS NAMES	Mutagenicity	Carcinogenicity		Development al Toxicity	Skin Sensitization	Hepatotoxicity	*In vivo* micronucleus assay	Binding to:		BBB	hERG I inhibitor	hERG II inhibitor
			Potential[#]	Genotoxic					DNA?	Protein?			
AG1	(E)-9-Octadecenoic acid ethyl ester	NO	1	NO	NO	YES	NO	Class II	NO	YES	YES	NO	NO
AG2	1-octadecyne	NO	3	NO	YES	YES	NO	Class II	NO	YES	YES*	NO	YES
AG3	1-Tetradecene	NO	2	NO	NO	YES	NO	Class II	NO	YES	YES*	NO	NO
AG4	2,4-Dimethyl-docosanoic acid	NO	1	NO	YES	YES	YES	Class II	NO	NO	YES	NO	NO
AG5	2-Methly-Z,Z-3,13-octadecadienol	NO	3	NO	YES	YES	YES	Class II	NO	YES	YES	NO	YES
AG6	2-Palmitoylglycerol	NO	1	NO	NO	YES	YES	Class I	NO	NO	YES	NO	NO
AG7	9-Octadecenoic acid	NO	2	NO	YES	YES	NO	Class II	NO	YES	YES	NO	NO
AG8	9-Octadecynoic acid	NO	3	NO	YES	YES	NO	Class II	NO	YES	YES	NO	NO
AG9	Docosanoic acid, ethyl ester	NO	1	NO	YES	YES	NO	Class II	NO	NO	YES*	NO	YES
AG10	Docosanoic acid	NO	1	NO	YES	YES	YES	Class II	NO	NO	YES*	NO	NO
AG11	Eicosane, 2-methyl-	NO	2	NO	NO	YES	NO	Class II	NO	NO	YES*	NO	YES
AG12	Ethyl palmitate	NO	0	NO	NO	YES	NO	Class II	NO	NO	YES*	NO	NO
AG13	Ethyl stearate	NO	0	NO	NO	YES	NO	Class II	NO	NO	YES*	NO	NO
AG14	Hexadecane-1,2-13C2	NO	2	NO	YES	YES	NO	Class II	NO	NO	YES*	NO	NO
AG15	n-Cetane	NO	2	NO	YES	YES	NO	Class II	NO	NO	YES*	NO	NO
AG16	Nonadecane	NO	2	NO	NO	YES	NO	Class II	NO	NO	YES*	NO	YES
AG17	Nonanoic acid	NO	0	NO	YES	YES	NO	Class II	NO	NO	YES*	NO	NO
AG18	Oleic acid	NO	2	NO	YES	YES	NO	Class II	NO	YES	YES	NO	NO
AG19	Palmitic acid	NO	1	NO	NO	YES	NO	Class II	NO	NO	YES*	NO	NO
AG20	Phenol-2,6-bis-(1,1-dimethylethyl)	NO	1	NO	YES	YES	NO	Class II	YES	YES	YES	NO	NO
AG21	Phthalic acid	NO	4	NO	YES	YES	YES	Class I	YES	YES	YES	NO	YES
AG22	Phytol	NO	2	NO	YES	YES	NO	Class II	NO	YES	YES	NO	YES
AG23	Squalene	NO	2	NO	YES	YES	YES	Class II	NO	YES	YES	NO	YES
AG24	Tetracosanoic acid	NO	1	NO	YES	YES	YES	Class II	NO	NO	YES*	NO	NO
AG25	Friedelin	NO	2	NO	YES	NO	NO	Class II	YES	YES	NO	NO	YES
AG26	Lupeol	NO	2	NO	YES	NO	NO	Class II	YES	NO	NO	NO	YES
AG27	Baurenol	NO	4	NO	YES	NO	YES	Class II	YES	NO	NO	NO	YES
AG28	Betulinic acid	NO	3	NO	YES	NO	NO	Class II	YES	NO	NO	NO	NO

[#]Carcinogenicity is scored based on the number of models that produced positive predictions (Caesar, ISS, IRFMN/Antares, IRFMN/ISSCAN-CGX, Oral IRFMN and Inhalation IRFMN). Therefore, potential for carcinogenicity range from 0 (none) to 6 (certain)

*Indicate application of both restricted and unrestricted applicability domains as implemented in vNN ADMET online server

Class I indicate positive alert for at least one of the micronucleus assay and Class II indicates no alert

Discussion

Adansonia digitata is a plant with many cosmetics, economic and medicinal benefits and like most commonly used medicinal plants, its use is gaining increasing patronage as sources of alternative therapy. The global acknowledgement of the values of medicinal, not only in bridging the gap in healthcare delivery occasioned by poverty and lack of access to affordable health care services, but also as long-term sources of drug candidates, make it imperative to establish their safety. More so, because of their ease of access and affordable financial implications, these plants are prone to abuse occasioned by indiscriminate or unguarded uses (alone or in combinations). Here, we present our evaluation of the toxicity profiling of *Adansonia digitata,* and show that at high doses, there is the possibility of sex differential and dose dependent toxic effects of the plants.

Our oral LD_{50} in this study was consistent with earlier report (Abdulmalik and Magashi, 2016), provided an initial indication that the plant extract may be practically non-toxic. We further determined the effects of the extract on organ weights and weight ratios which are among the critical parameters in accessing the toxicological effects of a given test agents (Agbaje et al., 2019; Olayode et al., 2019; Shobo et al., 2019). However, while body and organ weights have their place in toxicity assessments (Michael et al., 2007), they are often difficult to interpret and thus may not be very predictive. On the other hand, organ:body and organ:brain weight ratios are more predictive and are likely to accurately detect target organ toxicity (Bailey et al., 2004). Therefore, the observed significant changes in body weights and weight ratios (Table 1) may be of toxicological significance. However, apart from sex differential and dose dependent effects of the extract on weight and weight ratios, to a large extent our data revealed a direct correlation between body weights change and weight ratios in both male and female rats following single and repeated doses, similar to earlier reports of Bailey et al., (2004) and Nirogi et al., (2014).

The application of haematological parameters in toxicity assessments has been well articulated by Arika et al., (2016). As observed in this study, the significant changes in heamatological indices following single dose of the extract may not constitute any toxicity concerns. However, it is important to note that at high doses the result show a greater tending toward severe toxicity (Arika et al., 2016; Olayode et al., 2019), especially as the significant increases in WBC could suggest toxic effects. With most of the heamatological indices remaining unchanged or better improved at lower doses following repeated administration (Table 2), it is tempting to speculate that the user of the plant may benefit more from lower doses.e

Furthermore, though the significant increase in urea and creatinine relative to control may be a reflection of toxic activities, AST/ALT ratios of less than 1 and reduction in urea and creatinine concentrations following repeated doses (Table 3), may suggest potential protective effects of the extracts on organ functions. However, it is also possible that the dose dependent reductions in urea and creatinine may be indicative of underlying dysfunction or homeostatic response to the effects of the extract. Meanwhile, AST/ALT ratios were greater than 1 at all dose levels following single dose administration of the extract, suggest alteration in enzymes activities, despite the reduction in AST and ALT activities when compared with control (Table 3). It is to be noted that the observed alteration in histoarchitectures of liver and kidney (Figures 1 – 4) were consistent with the effects seen on weights, weight ratios, heamatological and biochemical indices with administration of high doses of test agent. There were essentially no alterations in liver and kidney architectures at 250 mg/kg and with only mild alterations at 500 and 100 mg/kg repeated doses.

Further evaluation of the toxicity potentials of selected phytoconstituents contained in *A. digitata* was done, with a view to correlating their predicted toxic potentials with our observed *in vivo* toxicity assessment. The results indicated that considering the relative abundance of the selected compounds, which had also been reported in some other plants with demonstrable and/or predicted biological activities, including antioxidant, antidiarrheal, hypoglycemic, anti-inflammatory, antimicrobial, anthelmintic and antibacterial activities (Adnan et al., 2019; Gunathilaka et al., 2019; Osuntokun and Cristina, 2019; Kim et al., 2020), the use of the extract at low does may not be of any toxicological concerns. Earlier report of GC-MS analysis of the ethanol extract of *A. digitata* revealed that oleic acid (AG18) was the most abundant components (27.32%), followed by ethyl palmitate (AG12 - 15.47%) and (E)-9-Octadecenoic acid ethyl ester (AG1 - 12.63%) (Magashi and Abdulmalik, 2017). In general, and consistent with our *in vitro* results, the *in silico* analysis revealed that the use of *A. digitata* may not portend any serious toxicological concern, especially when used at lower doses.

Conclusion

In conclusion, our results on the significant alterations in some key biochemical, heamatological and histopathological indices, show that sex differential and dose dependent toxic effects of the extract are very evident and demonstrated that dose and frequencies of dosing may have played critical roles in the toxic effects of the extracts in rats. For instance, acute administration of higher doses of the extract caused significant alteration in key weights and hematological parameters, while repeated administration of lower doses did not. The same effects were observed in the histology results, showing pronounced alterations following single higher doses when compared to lower repeated doses. Also, lack of obvious signs of toxicity during recovery period was indicative of complete recovery and lack of persistent toxic effects. It is pertinent to note that effective doses of the extracts reported in the literature to have produced significant pharmacological activities fall within the range of doses used for our repeated doses (250 – 1000 mg/kg) experiments (Adeoye and Bewaji, 2015; Yakubu et al., 2020;

Shehu et al., 2021), thus suggesting that adherence to lower effective doses may not only be beneficial, but also prevent or lower the risk of toxicity.

Conflict of Interest/Competing Interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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