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Original Research Article

Acute and Sub-Acute Effects of *Morinda lucida* Benth Stem Bark Methanolic Extract on Blood Cytometry, Blood Chemistry and Histoarchitecture of Wistar Rats

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ABSTRACT

Morinda lucida Benth is a valuable plant used in traditional medicine for the management of diarrhea, dysentery, malaria and other illnesses. The study evaluated the safety of *Morinda lucida* stem bark methanol extract (MLSE) in Wistar rats. The stem part was air-dried and extracted by maceration in 70% methanol. Phytochemical compounds in MLSE were determined by qualitative methods and Gas chromatography/Mass spectroscopy (GC/MS). The single oral dose and repeated 28-day oral toxicity were employed for acute and sub-acute toxicity studies respectively. Signs of convulsion, sedation, lacrimation, changes in fur, respiratory distress and mortality were recorded. Alterations in haematological, biochemical and histoarchitectural indices were investigated. Phytochemical analysis on MLSE confirmed the presence of phenolic compounds, flavonoids, alkaloids, phytosterols and glycosides. Hexadecenoic acid, oleic acid and its derivatives and two anthraquinones, digitolutein and rubiadin 1-methyl ether were detected. There was no mortality at doses ≤5000 mg/kg of MLSE. Treatment with MLSE significantly increased ($p<0.05$) percentage mean body weight gain in rats. However, percentage mean relative weight of the liver, kidney, heart of the rats that received MLSE were not significantly different ($p>0.05$), compared to control. MLSE did not adversely affect haematological parameters investigated. There was no derangement in fasting blood sugar, serum electrolytes, biochemical parameters and liver enzymes of rats treated with MLSE. Histopathology of target organs showed no relevant anatomical change. The findings indicate that *Morinda lucida* stem bark methanol extract was non-toxic at oral doses ≤5000 mg/kg and thus represents a potentially valuable product for further investigations.

Keywords *Morinda lucida* Benth, Biochemical parameters, Haematological, Histoarchitectural indices

Introduction

Plants produce an array of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have *in vitro* and *in vivo* pharmacologic properties. [1] In Nigeria *M. lucida* is one of the plants used in traditional medicine against febrile illnesses adjudged to be malaria fever. [2] Petroleum ether extract and fractions of *M. lucida* leaf has been reported to inhibit maturation of animal model of chloroquine sensitive *Plasmodium*. [2] The bitter-lasting roots are used as flavouring for food and alcoholic beverages and as chewing sticks. *M. lucida* is also used in ethnomedicine for the treatment of trypanosomiasis, gastrointestinal congestion, stomach ache, ulcers, diabetes, leprosy, dysentery, and gonorrhea. [3]
Common practice in traditional medicine involves repeated administration of extracts beyond a single day, hence the need to assess delayed toxicity that may arise from the use of such remedy. There is paucity of information on the sub-acute effects of the extracts 'adjudged' to be safe.

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The long-term effects of plant extract used in human as medicine is critical, hence the necessity to perform both acute and sub-acute toxicological assessment in an animal model exposed to such substances to determine the possible extent of organ or cell damage.[4] This study was designed to assess the acute and sub-acute toxicity profile of *Morinda lucida* stem bark methanol extract on haematological parameters, blood chemistry and histoarchitechure in Wistar rats. In addition, the phytochemical screening to detect compounds in the *Morinda lucida* stem bark methanol extract was undertaking using non-specific screening and Gas-chromatography-mass spectroscopy.

Materials and Methods

Plant identification and authentication
Morinda lucida Benth plant was collected from the suburb of Makurdi Town, Benue State, Nigeria (7^o 42′ N, 8^o 29′E), identified and authenticated at Forestry Research Institute of Nigeria Ibadan where a voucher specimen was deposited (FHI NO 110471).

Collection and preparation of plant extract
Morinda lucida stem was collected in June, 2016, air-dried in the laboratory for three weeks, and afterwards pulverized. Briefly, 2 g of the stem part was exhaustively extracted in 5 L of 70% methanol by cold maceration. The methanol extract was concentrated under reduced pressure at a temperature of 50°C and at 60 revolutions per minute using rotary evaporator, RE300/MS (Bibby Sterilin Ltd UK).

The resulting concentrate was subsequently oven-dried to constant weight and then refrigerated at 4^{o}C until used for the study.

Preliminary phytochemical screening
Qualitative phytochemical analysis of *M. lucida* stem extract was performed by the methods previously described. [5]

Gas chromatography-mass spectroscopic analysis
The chemical profile of *Morinda lucida* stem bark extract was determined by injecting 8.0 µL of methanol extract (1 mg/mL) into a gas chromatography system with a micro bore column (Agilent J & W DB-W 8270D) and a triple axis detector (quadruple). A capillary column of 0.25 mm by 30 mm and particle size 0.25 µm [Agilent Technologies, Palo Alto, California, USA] was used to separate the constituents at a gradient flow rate of 1.58 mL/min throughout the process. Column temperature and pressure were maintained at 80^{o}C and 108.0 kpa, respectively. Helium was used as the carrier gas. A continuous scanning of the UV spectra was done at a speed of 1250 scan/min and, the corresponding value for detected compounds recorded. The mass spectrophotometer, QP-2010 MS (Shimadzu Corporation, Tokyo, Japan) was operated at a temperature of 250^{o}C with collision gas helium at a collision rate of 6.2 mL/min. Optimized voltages were set at a negative ionization working mode and appropriate polarity. Fragmentation pattern of mass spectrometry was acquired over a range of mass-charge ratio (m/z) from 40 to 600 Da with the instrument automated at a constant collision energy of 70 eV. Data obtained was integrated and elaborated using RTE integrator and macromolecular structure data chemo-informatics package (MSDchem), while spectra database NBS75K library base was used to identify the compounds.

Experimental animals
Forty healthy young male Wistar rats weighing between 90.0 - 95.0 g were procured, housed in groups of ten (n=10) in cages lined with dry wood shavings and allowed to acclimatize for one week. The animal house was well ventilated, maintained at room temperature, illuminated by sunlight for 12 hours. The animals were fed standard pelletized animal feed (Vital Animal Feeds, Grand cereals Limited, Jos, Nigeria) with access to clean drinking water *ad libitum.* Ethical approval for the use of laboratory animals was obtained from Animal Care, Use and Research Ethics Committee (ACUREC) of the University of Ibadan, Ibadan Oyo State, Nigeria (Ethical approval no.: UI-ACUREC/App/10/2016/005).

Acute toxicity study
A modification of the protocol of Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG420) which explains acute oral toxicity study in rodents for the assessment of the safety of herbal medicines was used in this study.[6] The study was performed in three phases. In the first phase, five (5) young adult male Wistar rats weighing 90.5 – 100.0 g were randomly divided into five groups (n=1) and received graded doses of MLSE per oral after an overnight fast. Group A (50 mg/kg), group B (200 mg/kg), group C (400 mg/kg), and group D (800 mg/kg) of MLSE. Animals in group E served as the negative control and received the vehicle (50% DMSO/water). All experimental animals were allowed access to food and water *ad libitum* 2 hours post MLSE administration. The animals were closely observed for signs of convulsion, sedation, lacrimation, changes in fur, respiratory distress (behavioral toxicity) and possible mortality within the first one hour post-administration, and then 2-hourly for a total period of 24 hours.
In the second phase, four rats weighing 90.5 – 110.8 g were randomly distributed into four groups of one animal each (n=1), and received graded oral doses of MLSE. The designations were group A (1000 mg/kg), group B (1500 mg/kg), and group C (2000 mg/kg) of *M. lucida* stem bark methanol extract. The fourth group (group D) served as the negative control and received the vehicle. Prior to the oral administration of the extract, the experimental animals were fasted overnight and afterwards allowed access to food and water *ad libitum* 2 hours post MLSE administration.
The third phase of the study was performed using four animals weighing 90.5 – 110.8 g which were distributed into four groups (n=1) and given graded doses of MLSE orally. Animals in the designated groups received 3000 mg/kg (group A), 4000mg/kg (group B) and 5000 mg/kg (group C) of MLSE. The animals in group D received the vehicle, and served as the negative control. Experimental animals were closely observed for an initial one (1 hour) and, periodically for 24 hours. Behavioral signs of toxicity and mortality were recorded.
The outcome of the test was validated by confirmatory test carried out with a single dose of 5000 mg/kg of MLSE. Briefly, three animals were weighed and distributed into three groups: animals in group A and B received 5000 mg/kg of the extract, while animals in group C received the vehicle serving as the negative control. Prior to the administration of the extract, the experimental rats were fasted for 18 hours and afterwards closely observed for an initial one (1 hour) followed by a periodic observation for 24 hours. Any behavioral sign of toxicity and mortality were recorded. The LD_{50} was calculated using the mathematical expression below.[7]

$$LD_{50} = \left(\frac{[Mo + M1]}{2}\right)$$

M_0 = Highest dose of test substance that gave no mortality
M_1 = Lowest dose of test substance that gave mortality

Sub-acute toxicity study
Sub-acute toxicity study was performed using a modification of a previously described method as outlined in Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG407) for repeated Dose 28-day Oral Toxicity Study in Rodents.[4]
Twenty (20) young healthy rats were randomly distributed into four groups of five animals each (n = 5) and received orally administered MLSE daily as follows: 250 mg/kg (group A), 500 mg/kg (group B) and 1000 mg/kg (group C) for 28 days. The fourth group (group D) served as the negative control, and received the vehicle. Thirty minutes after administration of MLSE by gavage, the rats were allowed access to food and water *ad libitum*. The rats were observed daily for apparent changes in appearance, fur coloration, passage of loose or bloody stool and constipation, loss of appetite and thirst. The body weight of the experimental animals was determined daily before administration of MLSE, and the data for day 0, 7, 14, 21 and 28 subjected to descriptive statistics.
On day 28, surviving rats were fasted, weighed and the animals were sacrificed under chloroform anesthesia. Blood samples were collected from rats in each group by cardiac puncture into two sets of pre-labeled vacutainer tubes: heparinized and non-heparinized. The blood samples were used for haematological, serum biochemical and liver function tests using standard techniques. The organs (liver, heart, spleen and kidney) were harvested, weighed and preserved in 10% neutral buffered formalin to prevent autolysis of tissue before histopathological assessment.
Relative organ weight was determined using the following formula,

$$\text{Relative organ weight} = \left[\frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrificed day (g)}}\right] \times 100$$

Haematological and biochemical analysis
The red blood cells (RBC), packed cell volume (PCV), haemoglobin (Hb), white blood cells (WBC) count, neutrophils, lymphocytes and eosinophils in the blood samples collected in heparinized vacutainer tubes were analyzed [8] using Sysmex Kx-2w automated haematology analyzer (Sysmex Corporation, New York, USA). Analysis for sodium, potassium, chloride, fasting blood glucose, total protein, albumin, total cholesterol, serum glutamate oxaloacetatic transaminase (SGOT), serum glutamate pyruvic transaminase (SGPT), alkaline phosphatase, total bilirubin, creatinine and urea [7] was done using automated bench-top biochemistry analyzer, Selectra Pros (EliTech group, France). The results of the different variables were compared with normal values to determine significant alterations.

Histoarchitectural investigation
Preserved tissues were processed with automated tissue processor (Ernst Leitz Wetzlar GMBH 530497 No. 537, Germany), fixed in 10% paraformaldehyde, embedded in paraffin wax blocks, sectioned into 5 µm thick and stained with hematoxylin and eosin. [9] Prepared tissues were examined under light microscope to determine histological changes in the structures [10] and the micrographs captured with a digital camera (Kodak, USA) attached to the eyepiece objective. Semi-quantitative scoring systems of Knodel score system and Banff criteria were used to assess for pathological changes.[10]

Statistical analysis
Statistical analysis was performed on the data generated using one-way analysis of variance (ANOVA) and Student's *t*-test using Sigma Pilot Statistical Software (SPSS) version 16.00, (IBM, Chicago USA). Graph was plotted using GraphPad Prism 5 (GraphPad Software Incorporation, California, USA). Level of significance $\alpha = 0.05$ and Duncan Post Hoc test was used for mean separation.

Results and Discussion

Phytochemical screening
The presence of numerous bioactive secondary metabolites in the *Morinda lucida* stem bark methanol extract may account for the diverse pharmacological activities and its use in traditional medicine for the treatment of various illnesses. *Morinda lucida* stem bark methanol extract tested positive for phenolic compounds, flavonoids, phytosterols, alkaloids and anthraquinone glycosides as previously reported. [11] However, saponins and tannins reported to be present in ethanol leaves extract and root bark methanol extracts [12] were not detected in the methanol stem bark extract of *M. lucida* in this study.

Gas chromatography-Mass spectroscopy analysis
Using GC-MS method of analysis, sixteen compounds were identified in the methanol stem bark extract of *M. lucida*. The retention time, mass charge/charge (m/z) ratio, and molecular formula of the compounds are shown in Figure 1 and Table 1 respectively. Hexadecanoic acid, oleic acid and its derivatives (9,12-octadecadienoic acid, 9-octadecenoic acid, octadecadienoic acid and 9-octadecenal), anthraquinones (9,10-anthacenedione, 3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone and 2-hydroxy-1-methoxy-3-methyl-9,10-anthraquinone) were present in significant amounts. Other compounds detected were pregn-5-ene-20-one and its derivative pregn-4-ene-3,20-dione, cyclododecnoic acid, pipoxolan, chola-5,22-dien-3-ol, 1-naphthalenepropanol, cyclopentane, and quinazoline. A novel anthraquinone 1-Hydroxy-3-methoxy-6-methyl anthraquinone was detected in ethyl acetate fraction of *M. lucida* stem bark extract in addition to the anthraquinones, digitolutein and rubiadin-1-methyl ether. Findings from this study corroborates with those of Kouame *et al*. (2019) [13] who isolated and characterized useful anthraquinones including, damnacanthal, damnacanthol, rubiadin-1-methylether, digitolutein amongst other phytochemicals from stem bark and root ethanol and petroleum ether extracts of *Morinda lucida* using H-NMR.

Acute toxicity study:
The safety of plant products frequently used in traditional medicine is of grave concern, hence, the necessity for toxicological assessment of such products with efficacy testing. [14] *In vivo* acute testing of oral administration of series of fixed dose levels of *Morinda lucida* stem bark methanol extract to Wistar rats show that no rat that received fixed dose ≤5000 mg/kg manifested signs of acute respiratory, circulatory, autonomic, central nervous system and behavior toxicity (such as convulsion, sedation, lacrimation, unusual respiration and changes in fur) or death. This observation agrees with findings of Makinde *et al*. (1985) [15] who reported that ethanol leaf extract of *M. lucida* was non-toxic up to a dose of 6400 mg/kg body weight. It is widely accepted that extracts administered orally with LD_{50} of ≥5000 mg/kg are of very low toxicity or non-toxic.[16] The LD_{50} of MLSE was >5000 mg/kg with no acute adverse treatment-related effects on Wistar rats. This suggests that the methanol stem bark extract of *M. lucida* has low acute toxicity potential. However, the fact that the extract was administered orally may have contributed to the absence of gross signs of toxicity as the absorption of orally administered substances may be hampered in the gastrointestinal tract by drug-related and physiological factors, or the substance may undergo a first pass effect.[17] It is imperative that other routes of administration of MLSE are explored to fully substantiate the low acute toxicity profile of MLSE.

Table 1: Profile of compounds identified in *M. lucida* stem bark methanol extract using GC-MS

Retention time (Min)	Proposed compound	Mol. Formula	Mol. Weight (g/mol) m/z	% Area	Nature of compound
7.616	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.52	Carboxylic acid
8.123	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	1.01	Fatty acid
8.142	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	1.01	Fatty acid
8.341	Oleic acid	$C_{18}H_{34}O_2$	282	24.94	Fatty acid
8.405	Octadecadienoic acid	$C_{18}H_{32}O_2$	284	4.01	Fatty acid
8.498	Cyclododecenoic acid	$C_{12}H_2O$	164	1.19	Fatty acid
8.997	Pipoxolan	$C_{22}H_{25}NO_3$	351	1.37	Diphenyl methane
10.538	3-Hydroxy-1-methoxy-2-methyl-9,10-anthraquinone (Rubiadin-1-methylether)	$C_{16}H_{12}O_4$	268	2.34	Anthraquinone
10.616	9,10-Anthracenedione	$C_{14}H_8O_2$	208	5.41	Anthraquinone
10.793	2-Hydroxy-1-methoxy-3-methyl-9,10-anthraquinone (Digitolutein)	$C_{16}H_{12}O_4$	268	2.39	Anthraquinone
14.241	Pregn-5-en-20-one	$C_{21}H_{32}O$	300	4.95	Steroid
14.530	Chola-5,22-dien-3-ol	$C_{24}H_{38}O$	343	5.43	Steroid
15.070	1-Naphthalenepropanol	$C_{13}H_{14}O$	186	9.56	Poly hydroxyl
15.478	Pregn-4-en-3,20-dione	$C_{21}H_{32}O_2$	314	2.00	Steroid
15.789	Cyclopentane	C_5H_{10}	70	1.29	Benzoic acid
15.984	Quinazoline	$C_8H_6N_2$	130	1.29	Alkaloid

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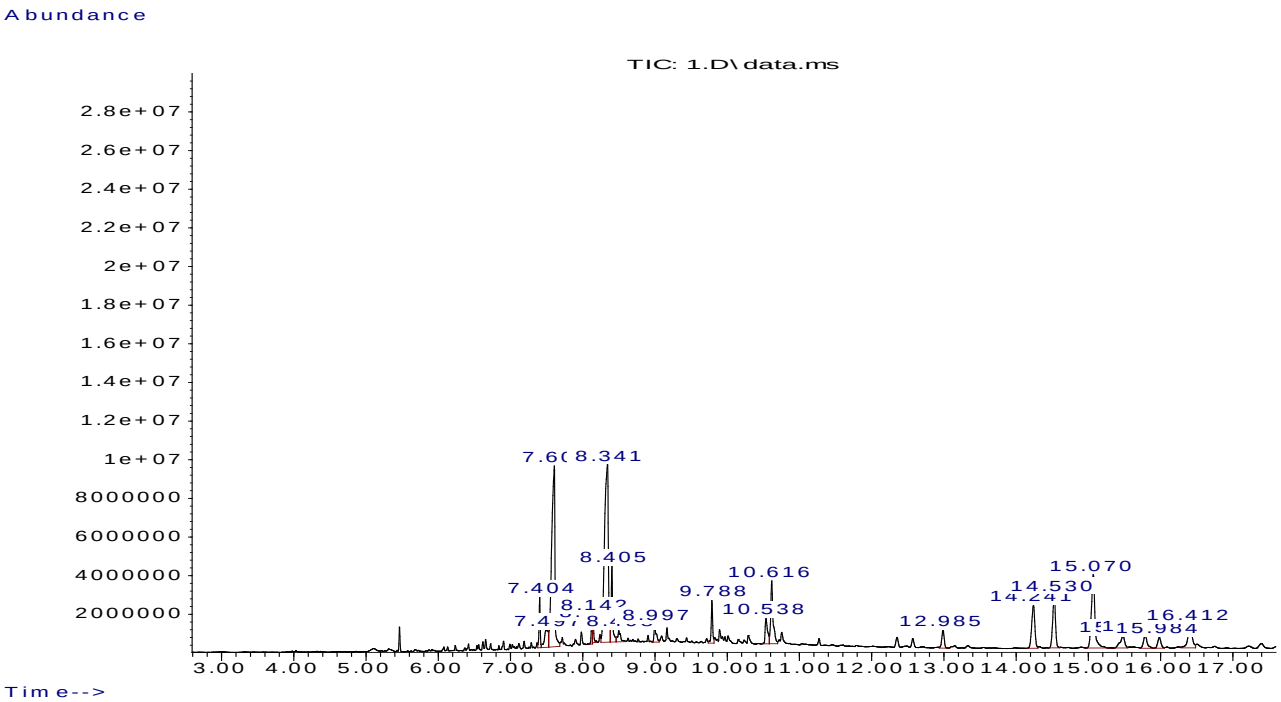

Figure 1: GC chromatogram of chemical constituent of *M. lucida* stem bark methanol extract

Sub-acute toxicity study

Changes in body weight and feed intake are often used as indices to evaluate adverse effects of xenobiotics in experimental animals[18] in which anorexia and decrease in body weight correlates with adverse effect of the test agent. The percentage mean body weight gain of Wistar rats over 28 days repeated oral administration of varying doses of *Morinda lucida* stem bark methanol extract (MLSE) is shown in Figure 2. There was no significant difference ($p>0.05$) in mean body weight of the animals at the beginning of the study. However, the percentage mean body weight gain significantly increased ($p<0.05$) steadily from day 7 to day 28 in group of rats that received MLSE and the control animals. From day 14, the body weight gain of rats increased with increasing dose. Sub-acute administration of 250 mg/kg to 1000 mg/kg MLSE resulted in increased body weight of the experimental animals with corresponding increase in dose of MLSE. Increase in the mean body weight of the animals corresponded with increased feed intake suggesting that MLSE has orexigenic effect. Agbor and collaborators (2012) [18] reported that sub-acute oral administration of 100 - 5000 mg/kg of *M. lucida* stem bark aqueous extract prevented increase in body weight of experimental rats in a dose-dependent manner. The difference between observations made in this study and those of Agbor *et al*. (2012) [18] who used adult male albino rats and aqueous extract could be associated with the age of rats and perhaps variations in the extracts. The oral gavage route used in the present study and the study carried out by Agbor *et al* (2012)[18] has been shown to be better in the delivery of test agents compared to dietary administration.[19] However, esophageal trauma and stress that are sometimes observed in experimental animal during gavage may result in anorexia leading to impaired weight gain that could be misinterpreted as adverse effect of the test agent. [19] Poor animal housing, quality of feed and increased lipid metabolism can also affect feed intake and weight gain.[19]

Mean relative organ weight of Wistar rats 28 days after repeated oral administration of varying doses of MLSE expressed as percentages is shown in Table 2. The mean relative weight of liver, heart and kidney in the rats that received 250 mg/kg, 500 mg/kg and 1000 mg/kg doses of extract were not statistically different ($p>0.05$) compared to the control. This indicates that MLSE did not adversely affect these vital organs. The spleen weight to body weight ratio in rats in the control group were within the normal value. However, the ratio of spleen weight to weight in rats that received 1000 mg/kg body weight of MLSE was twice the upper limit of 0.2%, and significantly higher ($p<0.05$) compared to rats that received vehicle which might indicate splenomegaly.[20] Animals in the group that received 1000 mg/kg of MLSE that presented with enlarged spleen appeared healthy with the PCV within the normal range, neither did the histoarchitectural analysis of the spleen of animals in this group. Therefore, enlargement of spleen of animals in this group of animals suggests adaptive response that is known to sometimes cause increased in relative organ weight[20] with no clinical relevance.

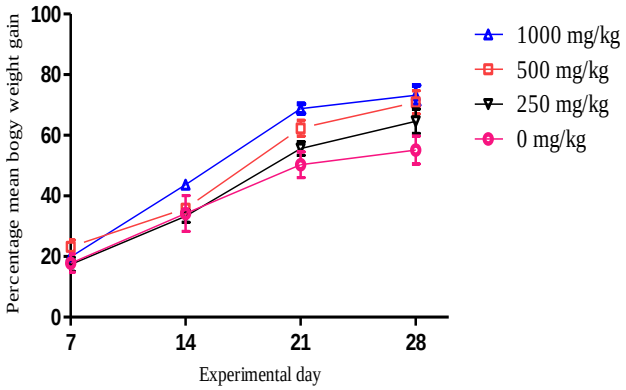

Figure 2: Percentage mean body weight gain of Wistar rats over 28-day repeated oral administration of *M. lucida* stem bark methanol extract, Error bars represent standard error of the mean, $p<0.05$= Significantly different

Haematological and biochemical analyses

Assessment of the extent of deleterious effects of a substance on haematological system and derangement of biochemical parameters of animal is crucial, as it provides the opportunity to predict the potentially safe doses for humans. [21] Mean levels of haematological parameters of Wistar rats after 28-day repeated oral administration of varying doses of *Morinda lucida* stem bark methanol extract is shown in Table 3. Blood cytometry of parameters such as RBC, PCV, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, WBC, lymphocytes, and

eosinophils in the groups of rats treated with varying doses of MLSE were not statistically different ($p>0.05$) compared to the control. PCV values observed for all treatment groups and the control in this study were within the limit of the reference value of 8.39% for 8 – 16 weeks old male Wistar rats as was previously reported.[22] Therefore, MLSE did not negatively affect the red blood cells suggesting that administration of the extract did not cause anaemia in experimental animals. Assessing WBC count in mouse model is integral to evaluation of possible human immunological disorders that may arise with the use of therapeutics intended for the treatment of human illnesses.[23,24] Although percentage segmented neutrophils was significantly higher ($p<0.05$) in rats that received doses ≥500 mg/kg of the MLSE compared to animals that received doses of MLSE ≤500mg/kg, but were within the normal reference range reported by Bolliger *et al*. (2010). The lymphocytes and eosinophils were also within the normal reference range (Table 3). MLSE did not cause depletion of WBC (leucopenia) that is associated with increased risk of infection and other immunological responses or elevation of WBC (leukocytosis) above normal range, an indication of adverse effect induced by therapeutic agents among others. This implies that MLSE did not decrease the ability of the experimental animal to fight infection or impair immunological response.

Biochemical analysis of serum constituents of Wistar rats after 28-day repeated oral administration of varying doses of MLSE is shown in Table 4. The mean fasting blood sugar (FBS) and the electrolytes: sodium, potassium and chloride ions were not statistically different ($p>0.05$) in rats that received varying doses of MLSE compared to the control. Blood glucose reflects the nutritional, emotional and endocrine status of an organism.[24] In this research, fasting blood sugar (FBS) in group of rats that received MLSE were within the reference range[3] and were not significantly different ($p>0.05$) across the various treatments. This observation agrees with the findings of Abdulkareem *et al*. (2019)[3] who reported that aqueous leaf extract of *M. lucida* did not adversely alter glucose levels in normal rats but lowers glucose levels in alloxan-induced hyperglycaemia in rats. This implies that MLSE did not adversely affect α- amylase, an enzyme involved in carbohydrate hydrolysis,[24] insulin secretory organs or glucose utilization as this would have resulted in blood glucose levels above the normal values.

In mammalian cells, sodium and potassium are the primary extracellular and intracellular cations respectively. Evaluation of these electrolytes gives information on the functionality of the renal system that is responsible for re-absorption and excretion of these ions.[25] In some cases, they reflect the clinical status of the cardiovascular system.[25] There was no significant variation in serum electrolytes (sodium ion, potassium ion and chloride ion) of experimental rats that received MLSE compared to the control animals. This suggests that *Morinda lucida* stem bark methanol extract may not have caused metabolic disorders or alteration in renal function.

The mean blood urea nitrogen, creatinine, total cholesterol, total protein, albumin and total bilirubin of rats that received varying doses of MLSE were not statistically different ($p>0.05$) compared to the control. Blood urea nitrogen is the amount of nitrogen in the circulatory system, while creatinine is a nitrogenous metabolite of muscle tissues used to evaluate glomerular filtration rate. Both urea and creatinine are excreted renally and allows evaluation of renal function.[26]

Table 2: Relative organ weight of Wistar rats after 28-day repeated oral administration of varying doses of *Morinda lucida* stem bark methanol extract

	Treatment group/Dose (mg/kg)			
Organ	**0**	**250**	**500**	**1000**
Liver (n = 5)	3.33 ± 0.170 %	3.4 ± 0.250 %	3.8 ± 0.190 %	3.8 ± 0.100 %
Spleen (n = 5)	0.30 ± 0.013 %	0.20 ± 0.033 %	0.30 ± 0.011 %	0.40 ± 0.085 %
Heart (n = 5)	0.30 ± 0.012 %	0.30 ± 0.011 %	0.30 ± 0.010 %	0.40 ± 0.034 %
Kidney (n = 5)	0.30 ± 0.012 %	0.40 ± 0.018 %	0.40 ± 0.002 %	0.40 ± 0.017 %

Key: Data are expressed as ± mean and standard error of the mean (SEM)

Table 3: Haematological profile of Wistar rats after 28 day repeated oral administration of varying doses of of *M. lucida* stem bark methanol extract

	Treatment group/Dose (mg/kg)			
Parameters	**0**	**250**	**500**	**1000**
RBC (x 10^6/ µl)	5.89 ± 0.13	6.18 ± 0.30	5.90 ± 0.44	6.00 ± 0.31
Hb (g/dL)	11.00 ± 0.17	11.37 ± 0.47	12.57 ± 0.98	11.80 ± 0.66
PCV (%)	33.00 ± 0.58	35.33 ± 1.86	38.33 ± 3.18	37.00 ± 3.21
MCV (fl)	56.03 ± 9.43	57.17 ± 9.44	64.96 ± 2.92	61.67 ± 6.66
MCH (pg)	18.67 ± 0.20	18.40 ± 0.15	21.31 ± 2.38	19.67 ± 0.36
MCHC (g/dl)	33.33 ± 0.25	32.18 ± 0.54	32.79 ± 0.38	31.89 ± 0.01
WBC (x 10^3/ µl)	8.80 ± 1.22	7.70 ± 1.00	9.60 ± 1.06	10.70 ± 1.14
Lymphocytes (%)	87.67 ± 2.91	87.00 ± 0.58	83.00 ± 3.61	80.67 ± 1.20
Eosinophils (%)	3.33 ± 1.33	2.67 ± 0.33	3.33 ± 0.67	4.00 ± 1.15
Neutrophils (%)	10.33 ± 0.67	10.33 ± 0.33	16.00 ± 0.58	15.33 ± 0.33

Key: Data are expressed as ± standard error of the mean (SEM), $p<0.05$= significantly different, $p>0.05$= not significantly different, Hb= haemaglobin, RBC= red blood cells, PCV= packed cell volume, MCH= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, WBC= white blood cells

The blood urea nitrogen and creatinine across all the groups were not significantly different ($p>0.0$) from the normal, implying that renal and hepatic functions were not adversely affected by MLSE. In laboratory animals, low levels of blood urea and nitrogen have been attributed to low protein-containing feed and pregnancy.[25] High BUN and creatinine levels are indices of poor renal excretion that correlates

with renal damage. However, blood urea and creatinine were within the normal range, and thus suggests that MLSE was non-toxic to renal and hepatic systems of the experimental animals. Cholesterol is a sterol present in all animal tissues and plays important role in the biosynthesis of steroid hormones and bile.[22] However, hypercholesterolemia increases the iatrogenic index of the subject to cardiovascular diseases. Total cholesterol of experimental animals was within normal reference range for Wistar rats.[3] This implies that four weeks use of *Morinda lucida* stem bark methanol extract may not cause any significant elevation of cholesterol which predisposes to cardiovascular diseases.

The liver is an important organ that aids in digestion, metabolism, detoxification and storage of nutrients.[26] Hepatic damage is most often evidenced by gross changes of hepatic architecture, elevation of liver enzymes and alkaline phosphatase and an index of alteration in hepatic function.[27] The serum glutamic oxaloacetic transaminase were significantly lower ($p<0.05$) in rats treated with varying doses of MLSE compared to control (32.00 ± 2.31 - 39.00 ± 11.00 versus 101.00 ± 1.73). In contrast, mean serum glutamic pyruvic transaminase were not significantly different ($p>0.05$) in groups of rats treated with MLSE compared to the control (3.39 ± 2.90 - 4.50 ± 1.70 versus 2.64 ± 3.84). And alkaline phosphatase range from 52.43 ± 4.10 – 78.93 ± 3.12 U/L. In this study, the liver enzymes and alkaline phosphatase were within acceptable physiological range and no gross relevant pathological changes were observed at a dose of 1 g/kg. However, some authors conducted a 14 and 28 day oral sub-acute toxicity studies of aqueous extract of *Morinda lucida* stem bark and reported hepatic steatosis in albino rats that received 5 g/kg.[21] The dose employed was five times the highest dose used in this present study. This suggests the possibility of occurrence of hepatic damage with long term oral administration of extremely high doses of MLSE.

Bilirubin above or below the normal range reflects toxicant-induced hepatic damage.[27] In this study, the range of total bilirubin in the experimental animals was between 2.47 ± 0.09 to 4.37 ± 0.09 µmol/L, while the albumin level was between 3.63 ± 0.88 to 4.47 ± 3.28 g/L (Table 4). Both bilirubin and albumin levels were within the normal range. This demonstrates that MLSE did not damage hepatic architecture or adversely alter liver function which otherwise would have been a significant health concern.

Histoarchitectural examinations

The histoarchitecture of the kidneys excised from rats that received varying doses of *Morinda lucida* stem bark methanol extract are presented in Figure 3.

Table 4: Serum biochemistry of Wistar rats after 28 day repeated oral administration of varying doses of *M. lucida* stem bark methanol extract

	Treatment group/Dose (mg/kg)			
Parameters	**0**	**250**	**500**	**1000**
FBS [mmol/L]	7.80 ± 0.25	3.73 ± 0.01	6.67 ± 1.82	4.53 ± 0.18
Na^+ [mmol/L]	140.33 ± 1.45	143.00 ± 3.00	147.00 ± 3.79	139.00 ± 1.73
K^+ [mmol/L]	9.57 ± 0.86	8.20 ± 0.15	7.53 ± 0.64	6.50 ± 0.80
Cl^- [mmol/L]	96.67 ± 1.20	93.67 ± 0.88	94.00 ± 0.58	93.00 ± 2.08
Urea [mmol/L]	5.77 ± 0.20	4.80 ± 0.15	5.13 ± 0.09	5.37 ± 0.03
Creatinine [mmol/L]	42.00 ± 1.15	50.00 ± 3.00	48.33 ± 3.93	45.33 ± 1.86
Total Cholesterol [mmol/L]	1.56 ± 0.18	1.84 ± 0.13	1.76 ± 0.20	2.03 ± 0.01
SGOT [U/L]	101.00 ± 1.73	39.00 ± 11.00	32.00 ± 2.31	37.33 ± 7.88
SGPT [U/L]	2.64 ± 3.84	4.50 ± 1.70	4.46 ± 3.50	3.39 ± 2.90
Total Protein [g/L]	56.67 ± 1.20	73.10 ± 4.58	74.33 ± 8.25	72.67 ± 4.91
Albumin [g/L]	3.63 ± 0.88	4.17 ± 1.20	3.97 ± 2.91	4.47 ± 3.28
Alkaline phosphatase [U/L]	62.70 ± 1.31	61.20 ± 5.45	52.43 ± 4.10	78.93 ± 3.12
Total bilirubin [µmol/L]	2.47 ± 0.09	3.17 ± 0.38	4.37 ± 0.09	3.43 ± 0.33

Key: Data are expressed as ± Data are expressed as ± standard error of the mean (SEM), $p < 0.05$ = significantly different, $p > 0.05$ = not significantly different, SGOT = Serum glutamic oxaloacetic transaminase; SGPT = Serum glutamic pyruvic transaminase

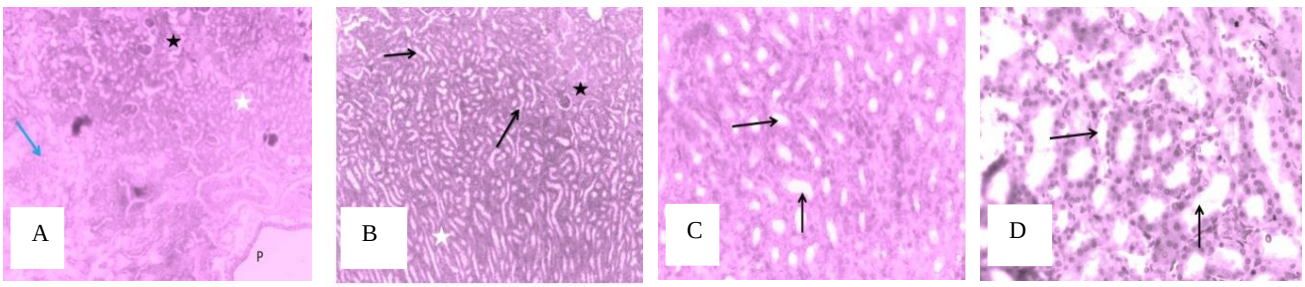

Figure 3A-D: Longitudinal section of kidney of Wistar rats after 28 days repeated oral administration of varying doses of MLSE (A= 250 mg/kg, B= 500 mg/kg, C= 1000 mg/kg, D= 0 mg/kg), Magnification x 100. Histoarchitecture showing, renal glomeruli (black star), collecting ducts (white star), convoluted tubules (black arrows). Images labelled B-D are microscopic sections of normal kidney. The area of necrosis and relative loss of structural integrity (blue arrow) and a portion of the renal pelvis is visible (P) are shown in A. MLSE= *Morinda lucida* stem bark methanol extract

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The kidney histoarchitechture was assessed using a modification of the Banff criteria and scored for the absence or presence of glomerular changes, tubular interstitial atrophy, necrosis, inflammation or vascular changes such as interstitial fibrosis, hyalinosis or lumen occlusion.[10] The kidneys of rats that received 500 mg/kg, 1000 mg/kg or the vehicle did not show changes in the parameters examined. However, one of the five animals that received 250 mg/kg showed tubular interstitial inflammation and necrosis. This observation is in consonance with normal urea and creatinine levels, and thus suggests that MLSE at the highest tested dose may not be no-toxic to the renal system of the experimental animals.

Histological examination of sectioned cardiac muscle for the presence or absence of necrosis and myophagocytosis, inflammation, interstitial fibrosis, fat infiltration, and vacuoles is shown (Figures 4). No animal in the groups that received 0 mg/kg, 250 mg/kg or 500 mg/kg of the MLSE presented with pathological changes (Figures 4). A modified semi-quantitative scoring system was used to evaluate splenic changes and the result shown in Figure 5. Enlargement of T and B lymphocytes parameter was scored on the scale as absent, mild, moderate and severe. [10] One animal that received 250 mg/kg or 1000 mg/kg presented with mild or moderate enlargement of T and B lymphocytes, and increased macrophages, respectively. Conversely, no rat that received 500 mg/kg MLSE or vehicle showed any change in the parameters assessed. Chemical agents that damage hematopoietic organs such as the spleen [28] would most times cause derangement of haematological parameters and gross pathological changes of the spleen and the heart.[18,20,21] The absence of relevant severe pathological changes in the architecture of the heart or spleen of the experimental animals suggests that MLSE may not be toxic to these organs at the maximum dose used for the sub-acute study.

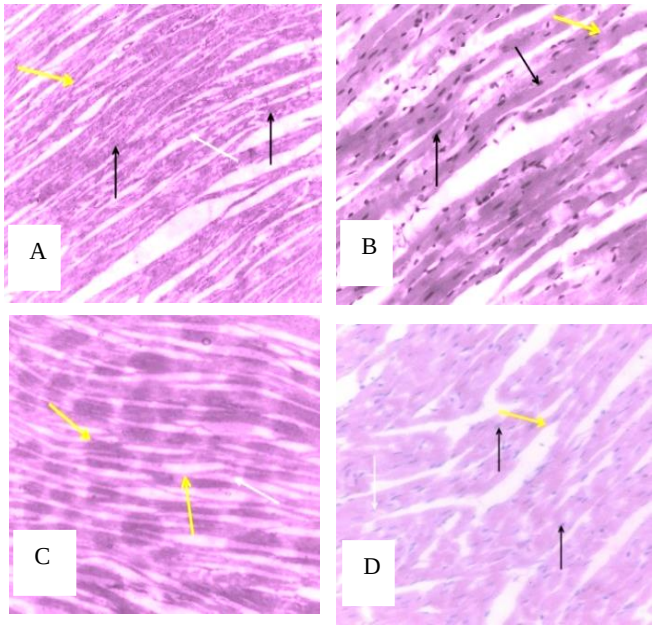

Figure 4A-D: Longitudinal section of the heart of Wistar rats after 28 days repeated oral administration of varying doses of crude extract of MLSE **(**A= 250 mg/kg, B= 500 mg/kg, C= 1000 mg/kg, D= 0 mg/kg), Magnification x 100. Histoarchitecture showing normal cardiac muscles, branching bundle of cardiac muscle fibre (yellow arrow), central nuclei (black arrow) and a faintly visible intercalated disk (white arrow). MLSE= *Morinda lucida* stem bark methanol extract.

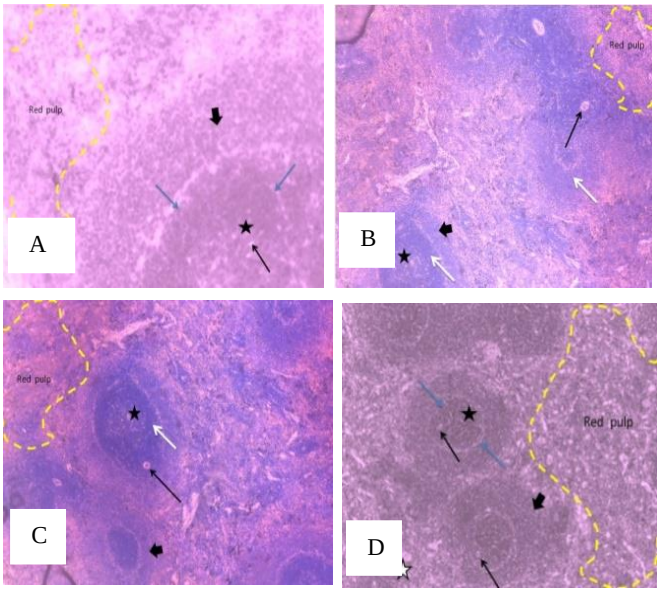

Figure 5A-D: Longitudinal section of spleen of Wistar rats after 28 day repeated oral administration of varying doses of MLSE **(**A= 250 mg/kg, B= 500 mg/kg, C= 1000 mg/kg, D= 0 mg/kg), Magnification x 100. Histoarchitecture of normal spleen and it showing the germinal center (black star), mantle zone (blue arrows) central arteriole (black arrow), trabeculae (white star), marginal zone (black arrow head), and area of red pulp marked (yellow dash line). MLSE= MLSE= *Morinda lucida* stem bark methanol extract

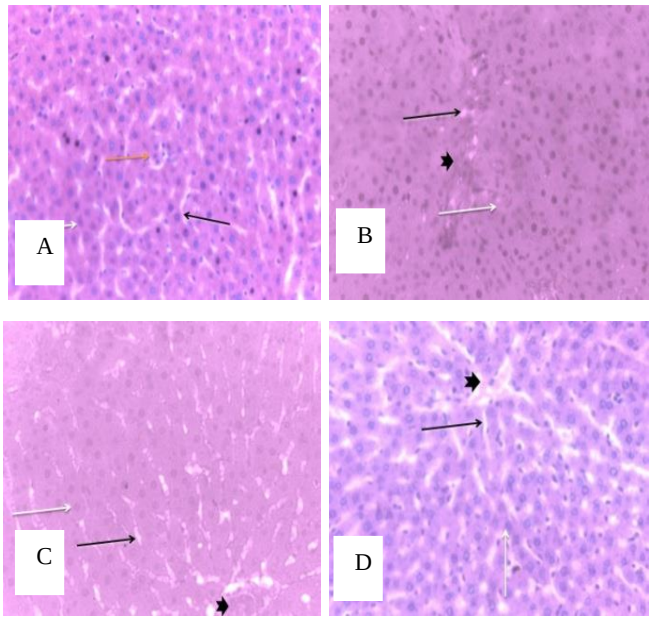

Figure 6A-D: Longitudinal section of liver of Wistar rats after 28 days repeated oral administration of varying doses of MLSE **(**A = 250 mg/kg, B = 500 mg/kg, C = 1000 mg/kg, D= 0 mg/kg), Magnification x 100. Histoarchitecture showing radiating cords of hepatocytes (white arrow) with abundant cytoplasms and centrally located nuclei, the cords of hepatocytes are separated by sinusoids (black arrow), portal region (black arrow head), a sprinkling of lymphocytes and hepatocyte drop out (red arrow) indicating intra-lobular degeneration were observed in (A). MLSE = MLSE = *Morinda lucida* stem bark methanol extract.

Liver damage is most often evidenced by gross changes of hepatic architecture.[27] Histological assessment of sectioned liver after 28 days of administration of *Morinda lucida* stem bark methanol extract based on Knodel score system for the absence or presence of changes such as periportal bridging with or without necrosis, intra-lobular degeneration and focal necrosis, portal inflammation and fibrosis[10] is shown Figures 6. Animals that received 500 mg/kg (B) and 1000 mg/kg (C) did not show periportal bridging with or without necrosis, intra-lobular degeneration and focal necrosis, portal inflammation or fibrosis. However, all but one animal that received 250 mg/kg MLSE presented with normal liver histology. This suggests that MLSE may not have been toxic to the liver of the experimental animals is consistent with the result of the serum biochemistry which did not show relevant elevation of liver enzymes.

Conclusion

The safety of *Morinda lucida* extracts used in ethnomedicine for the treatment of various diseases is of grave concern. In this study, *Morinda lucida* stem bark methanol extract did not show any detrimental alteration in haematology, serum biochemistry and histoarchitecture after daily oral administration of 1000 mg/kg body weight for 28 days in Wistar rats, thus the extract may not be toxic in sub-acute administration. It is hoped that the outcome of this study will serve as an important reference for the future development and potential use of *Morinda lucida* in complementary and alternative medicine.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Manandhar S, Luitel S, Dahal RK. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med. 2019; 2019: Article ID 1895340.
2. Afolabi OJ and Abejide AE. Antiplasmodial activities of *Morinda lucida* (Benth) and *Alstonia boonei* (De wild) in mice infected with *Plasmodium berghei*. Bull Natl Res Centre. 2020; 44(1):1-6.
3. Abdulkareem AO, Igunnu A, Ala AA, Olatunji LA. Leaf extract of *Morinda lucida* improves pancreatic beta-cell function in alloxan-induced diabetic rats. Egypt J Basic Appl Sci. 2019; 6(1):73-81.
4. OECD. Repeated Dose 28-day oral Toxicity study in rodents; in guidelines for testing chemicals. Monograph series on test guidelines 407 section 4. (Online). 2008 [cited 2018 Aug 28]. Available from: https://doi.org/10.1787/978926407068-en
5. Tiwari P, Kumar B, Kaur M, Kaur H. Phytochemical screening and extraction: A review. Int Pharm Sci. 2011; 1(1):98-106.
6. OECD. Acute oral toxicity- acute toxic class method in Guidelines for testing chemicals. Monograph series on test guideline 423. (Online). 2001 [cited 2018 Aug 28]. Available from: https://doi.oecdorg/chemicalsafety/risk-assessment/1948378.pdf
7. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20(3):224-226.
8. Bolliger AP, Everds NE, Zimmerman KL, Moore DM, Smith SA, Barnhart KF. Haematology of laboratory rodents: Mouse (*Mus musculus*) and Rat (*Rattus norvegicus*) in Schalm's veterinary haematology, (6th ed), edited by Weiss, D.J and War drop, Iowa, USA: K.J John Wiley and Sons Limited, 2010; 852-854p.
9. Ganter P and Jolles G. In conjunctive tissues in normal and pathological histochemistry, Gauthier-Villars, Paris. Sci. 1971; 1-2(65):938.
10. Liapis H, Gaut JP, Lein C, Bagnasco S, Kraus E, Farris III AB, Honsova E, Perkowska-Ptasinska A, David D, Goldberg J, Smith M, Mengel M., Haas M, Seshan S, Pegas KL, Horwedal T, Paliwa X, Landsittel D, Randhawa P. Banff histopathological consensus criteria for preimplantation kidney biopsies. Am J Trans. 2017; 2017(17):140-150.
11. Ajayeoba FO, Abiodun OO, Falade MO, Ogbole NO, Ahidie JS, Happi CT, Akimbiye DO. *In vitro* cytotoxicity studies of twenty plan used in Nigerian antimalarial ethnomedicine. Phytomed. 2006; 13(4):295-298.
12. Adomi PO and Umukoro GE. Antibacterial activity of aqueous and ethanol crude extracts of root barks of Alstonia boonei and preliminary phytochemical test of *Morinda lucida*. J Med Plant Res. 2010; 4(8): 644-648.
13. Kouame BFP, Kablan L, Kabran AF, Adiko NM, Akoubet OA, Diomande GGD, Akpa SJ, Bedi G, Tea I, Robins R, Adjou A, Tonzibo FZ. A novel anthraquinone from *Morinda lucida* Benth (Rubiaceae). J Pharmacogn Phytochem. 2019; 8(4):11-14.
14. de Sena Periera VS, de Oliveira CBS, Fumagali F, da Silva Emery F, da Silva NB, de Andrade-Neto VF. Cytotoxicity, hemolysis and *in vivo* acute toxicity of 2-hydroxy-3-anilino-1,4-naphthoquinone derivatives. Toxicol Rep. 2016; 3(2016):756-762.
15. Makinde JM and Obih PO. Screening of *Morinda lucida* leaf extract for antimalarial action on *P. berghei* in mice. Afr J Med Med Sci. 1985; 14(1-2):59-63.
16. Globally harmonized system of classification and labeling of chemical, (7th revised ed). United Nations New York and Geneva. (Online). 2017. [cited 2018 Aug 28]. Available from: https://unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev07/English/ST_SG_AC10_30_Rev7e.pdf
17. Chordiya M, Gangurde H, Borkar V. Technologies, optimization and analytical parameters in gastroretentive drug delivery systems. Curr Sci. 2017; 112(5):946-953.
18. Agbor GA, Tarkang PA, Fogha JVZ, Biyiti LF, Tamze V, Messi HM, Tsabang N, Longo F, Tchinda AT, Dongmo B, Donfagsiteli NT, Mbing JN, Joseph K, Ngide RA, Simo D. Acute and sub-acute toxicity studies of aqueous extract of *M. lucida* stem bark. J Pharm Toxicol. 2012; 7(3):158-165.
19. Beale KEL, Murphy KG, Harrison EK, Kerton AJ, Ghatei MA, Bloom SR, Smith KL. Accurate measurement of body weight and food intake in environmentally enriched male Wistar rats. Obes. 2011; 19(8):1715-1721.
20. Cesta MC. Normal structure, function and histology of the spleen. Toxicol Pathol. 2006; 34(5):455-465.
21. Haytham MD. Evaluation of haematological and biochemical activity of ethanolic extract of *Zygophyllum simplex* Linn. in Wistar rats. Pak J Biol Sci. 2016; 19(4):179-184.
22. Teixeira MA, Chaguri LCA, Carissimi AS, Souza NL, Mori CMC, Gomes VMW, Poli Neto AP, Nonoyama K, Merusse JLB. Haematological and biochemical profiles of rats (Rattus norvegicus) kept under micro environmental ventilation system. Braz J Vet Res Anim Sci. 2000; 37(5):341-347.
23. Simon H, Yousefia S, Germic N, Arnold IC, Haczku A, Karaulov AV, Simon D, Rosenberg HF. The cellular functions of eosinophils: Collegium international allergologicum (CIA) update 2020. Int Arch Allerg Immunol. 2020; 181(1):11-23.
24. Chokki M, Cudalbeanu M, Zongo C, Dah-Nouvlessounon D, Ghinea IO, Furdui B, Raclea R, Savadogo A, Baba-Moussa L,

Avamescu SM, Dinica RM, Baba-Moussa F. Exploring antioxidant and enzymes (A-amylase and B-glucosidase) Inhibitory activity of *Morinda lucida* and *Momordica charantia* leaves from Benin. Foods. 2020; 9(4):434.
25. Ellison DH, Terker AS, Gamba G. Potassium and its discontents: new insight, new treatments. J Am Soc Nephrol. 2016; 27(4):981-989.
26. Rodrigues S, Atunes SC, Nunes B, Correia AT. Histological interactions in gills and liver of rainbow trout (*Oncorhynchus mykiss*) after exposure to the antibiotic oxytetracycline. Environ Toxicol Pharm. 2017; 53(2017):164-176.
27. Benziane AB, Bouras AD, Mezaini A, Belhadri A, Benali M. Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. Drug Chem Toxicol. 2019; 42(2):157–166.
28. Rivadeneyra-Dominguez E, Becerra-Contreras Y, Vazquez-Luna A, Diaz-Sobac R, Rodriguez-Landa JF. Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. Toxocol Rep. 2018; 5(2018):1124-1128.