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Toxicological, Histopathological and Purity Evaluation of Polyherbal Drug Katoka Mixtures in Rodents

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the experiment and wrote the protocol for the study. He also partook in the write up of the paper. Author GOM undertook the tissue processing and analysis and also partook in the write up and final editing of the manuscript. Author HII undertook the microbial purity evaluation. Author JEE conducted the biochemical analysis. Author DAO conducted the laboratory work as well as managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To conduct toxicological, histopathological and purity evaluation on Katoka® an herbal mixture in rodents.
Materials and Methods: Microbial purity was evaluated on some bacterial and fungal organisms. Toxicity of the drug was on Swiss mice administered with graded doses of the formulation from 1.0

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to 15.0 g/kg body weight (bwt). Wistar rats were fed different doses of the formulation for 30 days to evaluate their biochemical profiles while vital organs histology was conducted.
Results: LD_{50} of the poly-herbal medicine was 7.2 g/kg bwt. Cytotoxic effect was not observed because median lethal concentration (LC_{50}) of the formulation calculated to be 300 μgml^{-1} was higher than 0.288 μgml^{-1} of reference drug, vincristine. Except for little growth of *E. coli* ($0.5x10^2$ cfu/ml) that were within acceptable official limit no growth of other *coli* forms. Hepatic histology of treated animals at highest dose exhibited diffuse presence of lymphocytic cells with onset of edematous changes and focal necro-inflammation. Aspartate aminotransferases (AST) exhibited significant ($p \leq 0.05$) increase while alanine aminotransferases (ALT) decreased markedly compared to the control.
Conclusion: Katoka® is safe for consumption because no cytotoxic effect observed but must be within recommended dose to avoid hepatic inflammation.

Keywords: Histopathology; toxicity; microbial purity; Katoka mixture.

1. INTRODUCTION

The use of plants and plant extracts as therapeutic weapons against various human, animal and even plant diseases has been recognized since prehistoric era [1]. Herbal medicine or phytomedicine as medicine derived from plant, is popularly known, is renowned and is recognized as the most common form of alternative medicine. It is used by about 60 % of the world population both in the developing and in the developed countries where modern medicines are predominantly used [2,3]. The use and increase in the popularity of herbal remedies could be attributed to their advantages of being efficacious and a cheap source of medical care. Secondly, there is a growing disillusionment with modern medicine and also misconception that herbal remedy being natural may be devoid of adverse and toxic effects often associated with allopathic medicines.

Herbal medicine are most often prepared from the combination of many plants product with the belief that the more plant recipes involved the more effective the product would be in the concurrent treatment of many diseases [4]. These drugs are often consumed indiscriminately without appropriate dose monitoring thereby undermining the greater potential for adverse effect. Incidences of harmful effect with marked deleterious changes on tissue morphology have been recorded [5,6]. Herbal preparations could be contaminated with microbiological and foreign materials, such as heavy metals, pesticide residues or even aflatoxins. Contaminants when present in an herbal preparation may give it the capacity to produce prominent health defects underscoring the claimed safety. An increase in the morbidity and mortality associated with the use of herbal or the so called traditional medicines has raised universal attention in the last few years [7]. Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations imperative.

This herbal medicine, Katoka is a polyherbal preparation from mixtures of many plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize. The individual components of the mixture might have been investigated in one way or the other by various authorities but to the best of our knowledge this herbal mixture prepared with various herbal parts has not been scientifically investigated for histotoxicity and microbial purity.

The aim of this study was to evaluate the safety of a polyherbal preparation, Katoka Mixtures®, by carrying out tissue histopathology and toxicity studies in rodents and microbial purity test. The evaluation is required to establish potential adverse effects of this highly valuable polyherbal medicine that is now widely consumed for its physiological benefits.

2. MATERIALS AND METHODS

Katoka Mixtures® with National Agency for Food, Drug Administration and Control (NAFDAC) Registration 04-4763 L were supplied by the manufacturer De-Katoka (WA) ltd for the purpose of histo-toxicity studies and microbial purity evaluations. The mixture supplied in 25cl (centiliter) amber coloured plastic bottles was indicated to have been prepared with specified quantities of plant parts as outlined below: (a) *Olax subscorpioidea* Oliv. (4%) (b) *Erthrophleum sauveolens* Guill. & Perr. Brenan (3% each) (c) *Entandrophragma candollei* Harms (5%)

(d) *Belmoschus esculentus* (3%) (e) *Canarium schweinfurthii* Engl. (5%) (f) *Xylopia aethiopica* (Dunal) A.Rich (2%) (g) *Morinda lucida* Benth (2%) (h) *Alstonia boonei* De Wild (1%) (i) *Nauclea latifolia* (1%) (j) *Parinari curatellifolia* Seeds (2%) (k) Water qs (100%).

Six bottles from the procured pack were randomly selected and stored in the refrigerator at temperature range of 4-6°C from where the quantity needed for purity assessment was taken aseptically. Then 1000 ml of the formulation was filtered using sterilized muslin cloth first and Whatmann's no. 4 filter paper to obtain 835.7 ml which was subsequently lyophilized to obtain 42.5 g gel.

2.1 Purity Assessment

2.1.1 Determination of microbial purity

The microbial load of the preparation was determined using the standard plate method [8]. Various diagnostic media - Tryptone Soy Agar (TSA), Salmonella-Shigella Agar (SSA), Eosin Methylene Blue Agar (EMBA), MacConkey Agar (MAC), Nutrient Agar (NA), Manitol Salt Agar (MSA), Sabouraud Dextrose Agar (SDA) - were used to culture the test product with no specific bacterial or fungal species used for the assay. The essence of the exercise therefore was to find and calculate the presence of inherent organisms and determine whether the inherent quantity was within acceptable official pharmacopoaeas limit. Each of the media was prepared according to the manufacturers' instruction and sterilized at 121°C for 15 minutes. Three fold serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) were made using sterile water and 1 ml each of the dilutions seeded in 25 ml each of the sterile culture media. The media were allowed to cool to 45°C, swirled and left to solidify. The bacterial media were incubated at 37°C for 3 days while the fungal medium (SDA culture) was incubated at 25°C for 7 days.

2.2 Acute Toxicity Study

The toxicity study was carried out using thirty five (35) male and female Swiss albino mice. The animals were randomly distributed into one control group and six treated groups containing five animals per group. After the overnight fasting, the control group received 0.3 mL of distilled water orally. The doses 1.0, 2.5, 5.0, 10.0, 15.0 g/kg were respectively administered orally to the groups from 80 % (w/v) solution of the gel which was prepared by dispersing 16 g of the gel with 7 mL distilled water in a 100 ml beaker and transferred to a 20 mL volumetric flask. The beaker was thoroughly rinsed with distilled water; the content added to the volumetric flask and the volume made to mark. The animals were observed continuously for the first 4 hours and then for each hour for the next 12 hours, followed by 6 hourly intervals for the next 56 hours (72 hrs observations) to observe any death or changes in general behavior and other physiological activities [3,9,10].

2.3 Cytotoxicity: Brine Shrimp Lethality Bioassay

Brine shrimp (*Artemiasalina*) lethality bioassay was carried out to check the cytotoxic activity of the polyherbal formulation. The assay was done according to Meyer's process with some modifications. Simply, brine shrimp eggs were obtained from the department of Clinical Pharmacy and hatched with properly aerated filtered seawater for 48 hrs. After hatching, active nauplii were collected and 10 nauplii were drawn through a dropper and placed in each well of microlitre plate containing 250 µl of seawater. Then 50 µl of the polyherbal extract solution (extract dissolved in distilled water) was added to make final concentration of plant extract as 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml in respective treatments. Vincristine sulphate (Gedeon Richter Ltd., Hungary) was used as positive control and seawater was used as negative control. After 24 hrs, dead and alive nauplii were counted under microscope. Each experiment was performed in three replicas. The percentage mortality was then determined using the formula.

$$\% \text{ Mortality} = N_1/N_0 \times 100$$

N_1 = Number of killed nauplii after 24 hrs incubation.

N_0 = Number of total nauplii transferred.

2.4 Subchronic Toxicity Study

Recommended adult dose of the formulation was 50 ml daily. 1000 ml of the formulation was lyophilized to give 42.5 g gel translating to 2.125 g gel equivalent to 50 ml daily [11].

2.4.1 Dose calculation for the animals

(1) The recommended dose of the herbal formulation for an adult of 70 kg (Average body weight bwt) was 50 ml daily.

(2) 1000 ml of the formulation was lyophilized to yield 42.5 g gel,
(3) 50 ml of the formulation will be an equivalent of 42.5/1000 x50 g=2.125 g.
(4) This dose is for 70 kg bwt, 1 gbwt= 2.125/70000 g = 2.125x1000 mg / 70000 2.125/70 mg.
(5) 150 g rat will be equivalent to 2.125x 150/70 mg=4.55 approximate 5 mg.

2.4.2 Doses administered

(1) 20% w/v of the gel stock solution was made = 20 g in 100 ml or 20000 mg in 100 ml or 200 mg/ml.
(2) 5 mg normal dose will contain in 5/200 ml = 0.025 ml for 150g bwt rat.
(3) Low high dose (normal dose x 10) = 0.025 ml x10 = 0.25 ml.
(4) Medium high dose (normal dose x 25) = 0.025 ml x 25 = 0.625 ml.
(5) Very high dose (normal dose x 50) = 0.025 ml x 50 = 1.30 ml.

Male and female Wister rats weighing 150 g ± 10 g were used after being allowed to acclimatize to the laboratory conditions for seven days, and were maintained on standard animal feeds and provided with water *ad libitum*. The studies were in compliance with the Institute of Laboratory Animal Research (ILAR) guidelines on the use and care of animals, in experimental studies [12]. They were weighed and divided into five groups of five animals each and after fasting the rats overnight the control group received a dose of 0.5 ml of distilled water orally once a day for 30 days. The four treated groups respectively received the following doses of 20 % w/v lyophilized solution of the formulation: 0.025 ml (normal dose), 0.25 ml (low high dose equivalent to 10 times the normal dose) 0.63 ml (medium high dose equivalent to 25 times the normal dose) of the gel orally once a day and 1.30 ml (very high dose, equivalent to 50 times the normal dose) for 30 days [4,5,13,14]. The animals were weighed every five days, from the start of the treatment, to note any weight variation. At the end of the experiment, the animals were starved overnight. On the 31st day, they were made unconscious by cervical dislodgement and blood was collected via cardiac puncture in two tubes: one with EDTA for analysis of haematological parameters and the blood chemistry and the other with heparin to separate plasma for biochemical estimations. The heparinized blood was centrifuged within 5 min of collection at 4000 rpm for 10 min to obtain plasma, which was analyzed for total cholesterol, total triglyceride, and HDL-cholesterol levels by modified enzymatic procedures from Sigma Diagnostics [15]. LDL-cholesterol levels were calculated using Friedwald equation [16]. Plasma was analyzed for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods [17]. Plasma glucose contents and protein contents were determined using enzymatic spectroscopic methods [18]. Haematocrit was estimated using the method as described by Ekaidem et al. [19]. The blood samples were analyzed for red blood cells (RBC) by haemocytometic method [19], the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method [20], packed cell volume (PCV) was estimated using the method as described by Dacie, JC, Lewis [20]. Haematocrit tubes were filled with whole blood to the mark by capillary action and the bottom of the tubes sealed with plasticide and centrifuged for 4-5 minutes using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersected the 100% line. The white blood cell (WBC) count was as described by Dacie and Lewis [20].

2.5 Tissue Histology Preparation

The animals were later sacrificed and the target organs, liver, kidney, heart, and testes harvested from each group and then fixed in 10 % formal saline for seven days before embedding in paraffin wax. The fixed target organs were removed and dehydrated in increasing concentrations of alcohol; 70%, 80%, 90% and absolute alcohol (100%). The organs were treated with acetone and then cleared in xylene for 30 min to enhance the tissue transparency followed by impregnating and embedding in paraffin wax. Each tissue was then sectioned at 5µm and cleared (dewaxed) for staining with haematoxylin and eosin [6]. Slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.6 Statistics

Statistical significant differences were determined using a Student's t-test. Differences were considered significant if $p < 0.05$ or $p < 0.01$. All data were expressed as mean± standard error of mean (S.E.M).

3. RESULTS

3.1 Variation of Weights

The percentage difference in the weight of the treated animals compared to the control is shown in Fig. 1. There was insignificant ($p \geq 0.05$) decrease in the body weight of the animals treated with low dose of the extract while more marked decrease occurred at the highest dose treatment compared to the control. The testicular weight however exhibited more significant decrease in the very high dose of the extract treatment. Macroscopic examinations showed no changes in the colour of the organs of the treated animals compared to the control.

3.2 Purity Test

The microbial purity evaluation of the formulation (Table 1) showed no growth of bacterial and fungal organisms in the first 24 hrs in the various diagnostic media used. There were no growths of either *Bacillus subtilis* (0.0×10^2 cfu/ml) observed in Tryptone Soy Agar culture or of other *coli* forms (0.0×10^2 cfu/ml) observed in MacConkey agar culture after 72 hrs indicating that all the microbial loads were within acceptable official limit . However, *E. coli* (0.5×10^2 cfu/ml) showed a little growth observed in the Eosine Methylene Blue Agar medium which was also within the acceptable microbial limit for aqueous herbal formulations. There was no growth observed on Sabouraud Dextrose Agar on the 6th and 7th day of incubation showing the absence of fungal organisms.

3.3 Acute Toxicity Test

The acute toxicity study of lyophilized extract of Katoka herbal mixtures® (Table 2) indicated no changes in the behaviour and in the sensory nervous system responses in the animals. Also no adverse gastrointestinal effects were observed in male and female mice used in the experiment. All the mice that received 20.0 g/kg dose of the extract died within 4 hrs while the animals that received 5.0 g/kg dose survived beyond the 24 hrs of observation. The median lethal dose (LD_{50}) of the extract was determined to be 9.0 g/kg bwt.

3.4 Cytotoxicity Test

The results of the cytotoxic effects of the polyherbal formulation and the reference standard vincristine were summarized in Table 3 and Table 4. The polyherbal formulation at the dose administered did not exhibit cytotoxic effects since there was quite significant difference ($p < 0.05$) between the median lethal concentrations (LC_{50}) which was calculated to be 300 $\mu g ml^{-1}$ compared to the reference drug, vincristine with the median lethal concentration (LC_{50}) calculated to be 0.288 $\mu g ml^{-1}$.

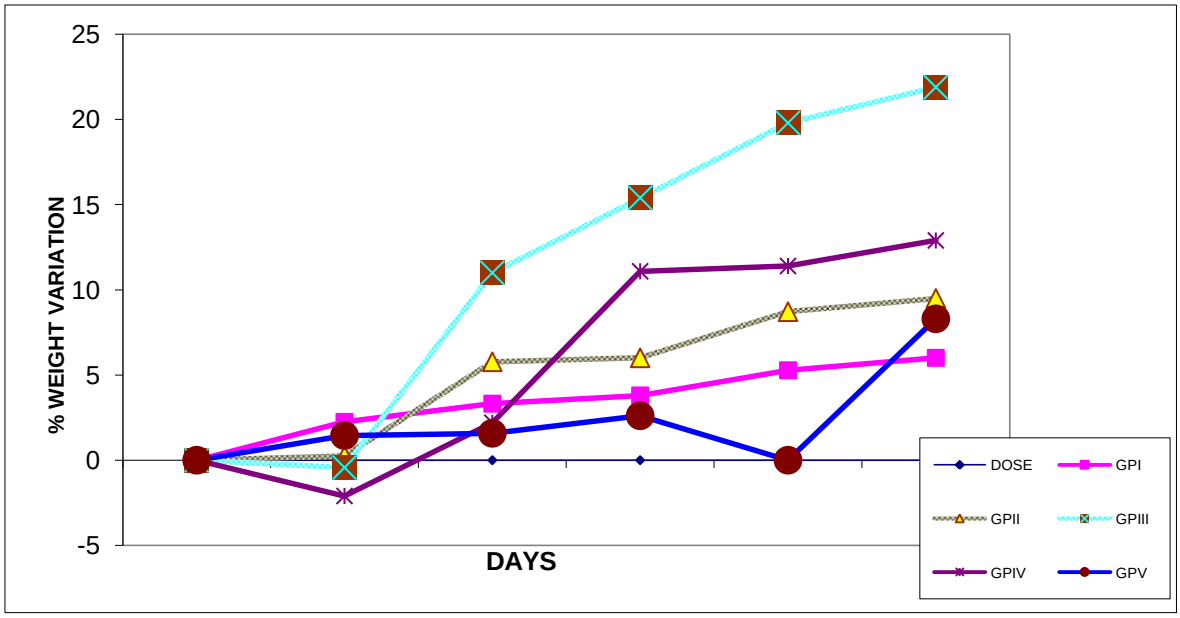

Fig. 1. Percentage weight variation of the control and the treated animals

■GPI Control, ⊿ GPII Normal Dose, X GPIII Low Dose, ✱ GPIV Medium Dose, ● GPV Very High Dose

Table 1. Microbial purity test of the polyherbal formulation

Media	*S. typhi* cfu/ml	*Bacillus* species $x10^2$ cfu/ml	*Shigella* species cfu/ml	Other coliforms cfu/ml	Proteus specie $x10^2$ cfu/ml	*P. aeruginosa* cfu/ml	*S. aureus* $x10^1$ cfu/ml	*E. coli* $x10^3$ cfu/ml	TYMC 10^2	TACC $x10^1$ cfu/ml	Total
SSA	0.00	-	0.00		0.00	-	-	0.00	-	-	0.00
MAC	-	-	-	0.00	-	-	-	0.00	-	-	0.00
NA	-	-	-	-		-	-	0.00	-	-	
CA	-	-	-	-	-	0.00	-	0.00	-	-	0.00
MSA	-	0.95	-	-	-	-	0.00	0.00	-	-	0.00
EMBA	-	-	-	-	-	-	-	$0.5x10^2$	-	-	0.00
SDA	-	-	-	-	-	-	-	-	1.10	-	$1.1x10^2$
TSA	-	-	-	-	-	-	-	-	-	9.50	0.00

Average count from 5 different preparations plus/minus standard error of mean.

Targeted organisms: Salmonella typhi (nil), Shigella species (nil) Other Coliforms (nil) Proteus species (nil), Pseudomonas aeruginosa (nil) Staphylococcus aureus (nil) Escherichia coli ($0.5x10^2$ cfu/ml) Mould and Yeast ($1.1.00x10^2$ cfu/ml), Bacillus species ($0.00x10^2$ cfu/ml; $\leq 10^4$ HPA, FSAI) HPA= Health Protection Agency, United Kingdom; FSAI = Food Safety Authority, Ireland

Table 2. Acute toxicity test of lyophilized extract of Katoka mixture

Doses of Drug g/kg	Number of animals	Log dose	24 hr mortality	72 hr mortality	% Mortality	Probit
0.5	5	-1.698970	0	0	0	0.00
1.0	5	0.000000	0	0	0	0.00
2.5	5	0.3979400	0	0	0	0/00
5.0	5	0.6989700	0	0	0	0/00
10.0	5	1.000000	0	1	12.5	3.2
15.0	5	1.1760913	0	2	37.5	4.67
20.0	5	1.3010300	0	5	100	7.33

Control: 0.3 ml Distilled water

Table 3. The effect of lyophilized Katoka mixture® on Brine Shrimp cytotoxic lethality test

Conc (μg/L)	Log C	Nauplii total	Nauplii dead	% Mortality	Log LC_{50} (μg/L)	LC_{50} (μg/L)
Blank	-	30	0	0.00	-	-
Solvent	-	30	0	0.00	3.00	3000.0
10	1.00	30	9	30.0	-	-
100	2.00	30	12	40.0	-	-
1000	3.00	30	24	80.0	-	-

Table 4. The effect of Vincristine sulphate on Brine Shrimp Naupii lethality

Conc (μg/L)	Log C	Nauplii total dead	% Mortality	Log LC_{50} (μg/L)	LC_{50} (μg/L)
Blank	_		0.00	-	-
Solvent	_		0.00	- 0.539	0.288
1.25	0.096		70.00	-	-
2.5	0.397		83.00	-	-
5	0.698		90.00	-	-

Table 5. The effect of the polyherbal on the biochemical parameters

Parameters	Doses in mg/kg bwt				
	Control	Normal dose (0.025 ml)	Low high dose (0.25 ml)	Medium high dose (0.63 ml)	Very high dose (1.30 ml)
Glucose (mmol/dl)	8.1± 0.5	8.7±0.3	7.3±0.0	7.2±1.0	5.5± 0.1*
AST (IU/L)	204.5±0.3	138.2±0.5*	201.2±1.1	202.6±1.2	215.5±0.1*
ALT (IU/L)	54.3±0.4	59.6±0.3	20.8±0.6*	44.1±0.7*	43.3±0.1*
T. Bil (mg/dl)	3.2±0.1	3.1±0.1	2.4±0.1*	2.7±0.1	3.4± 0.1
Creatinine (mg/dl)	43.1±0.1	49.2±1.2	35.9±0.3**	35.3±0.2**	38.0±1.0
Urea (mg/dl)	5.8±1.1	5.8±0.5	5.8±0.6	4.7±0.2	7.5±3.2
Albumin (mg/dl)	38.6±3.9	34.3±0.9	28.7±1.3*	21.1±3.1*	32.5±0.1**
T. Protein (g/dl)	65.6±0.2	67.1±0.2	65.1±0.2*	62.1±3.5	60.7±2.1
HDL (mg/dl)	1.1±0.1	1.0±0.1	0.6±0.1	0.7±0.1	0.7±0.1
LDL (mg/dl)	0.4±0.0	0.3±0.1	0.2±0.0*	0.3±0.0	0.2±0.0
T. Cholest (mg/dl)	2.0±0.5	1.6±0.3*	1.4±0.2*	1.3±0.0*	1.2±0.0*
T. Trigly. (mg/dl)	1.0±0.1	0.9±0.1	0.8±0.0	0.5±0.0**	0.7±0.0**
Alk Phos.	189.3±3.2	183.5±6.8	185.2±1.2	183.3±1.2	109.3±1.2*

*N=5, *p≤0.05, **p≤ 0.01.control animals were administered with 0.5ml distilled water*

3.5 Subchronic Toxicity Test

Table 5 is a summary of the results of the effects of the drug on the biochemical parameters. AST showed significant decrease ($p < 0.05$) while marked increase in ALT level was observed in the very high dose of the herbal drug. Total plasma protein and albumin showed decrease in very high dose with albumin exhibiting more marked decrease. The plasma creatinine level decreased while urea and bilirubin exhibited insignificant increase ($p \geq 0.01$) compared to the

control. The alkaline phosphate level showed dose dependent decrease. Significant decrease ($p < 0.05$) in the plasma glucose level occurred at very high dose in the treated animals compared to the control. Also, significant decrease ($p < 0.05$) in plasma TC and TG were observed in the group that received the very high dose of the poly-herbal drug while the LDL-cholesterol and HDL-cholesterol levels exhibited insignificant decrease ($p \geq 0.01$).

The effects of the herbal drug on blood parameters are presented in Table 6. An increase was observed in RBC count and Hb level at the highest dose treatment while PCV exhibited marked decrease. The RBC differentials showed insignificant increase ($p \geq 0.01$) at the highest dose treatment while WBC count showed decrease. The platelet exhibited significant increase ($p < 0.05$) which showed dose effect compared to the control.

3.6 Tissue Histology/Histopathology

Figs. 2-5 showed the histological studies of the effects of the phytomedicine on the target organs. The hepatic tissue of the control (Fig. 2a) showed the portal area (red arrowed) with hepatocytes (black arrowed) interspaced by hepatic sinusoids (blue arrowed) forming a concentric arrangement around the central veins (blue arrowed). In the medium high dose treatment (Fig. 2b), the hepatic tissue showed infiltrating leukocytes into the hepatic parenchyma (blue arrowed) and mild edematous changes. In the animals treated with very high dose of the formulation (Fig. 2c), hepatic tissue showed marked inflammatory changes. There was diffuse presence of lymphocytic cells (blue arrowed) with onset of edematous changes and vacuolation (black arrowed) leading to sinusoidal congestion. Also remarkable was focal necro-inflammation (red arrowed).

The photomicrograph of heart tissue (Fig. 3a, control group) showed the myocytes (black arrowed) interspaced by unremarkable interstitium (blue arrowed). In the medium high dose (Fig. 3b) and very high dose (Fig. 3c) treatments, the myocardial tissues showed no abnormal change in the myocytes and at the interstitium.

The photomicrograph of kidney of the control group (Fig. 4a) showed cortical area of the renal tissue with glomerular apparatus (red arrowed) forming rounded mass and separated from the surrounding structures by Bowman's space (blue arrowed). Different sections of the convoluted tubules were equally shown (black arrowed). In the medium high dose (Fig. 4b) the renal tissue showed normal appearance. At the very high dose (Fig. 4c) treatment, mild inflammatory changes occurred. There was slight enlargement in luminal diameter of convoluted tubules while the renal corpuscles exhibited partial shrinkage.

The photomicrograph of testis of the control group (Fig. 5a) showed the seminiferous tubules (blue arrowed) cut at different planes and separated by testicular interstices (black arrowed). The wall of the seminiferous tubules showed thick epithelium comprising compactly arranged differentiating spermatogenic cells with the matured cells, spermatids and spermatozoa forming a cluster in the lumina. In the medium high dose (Fig. 5b) and very high dose (Fig. 5c) treatments, the testicular tissues showed no depletion in spermatogenic cells mass.

Table 6. The effect of the polyherbal formula on the haematological parameters of the control and treated animals

Parameter	Dose mg kg^{-1} body weight				
	Control	Normal dose	Low high dose	Medium high dose	Very high dose
RBC×10^6	6.9±0.4	7.9± 0.2	7.2±0.3	7.6±0.2	9.7±2.3**
Hb (g/dl)	11.9±0.2	11.7± 0.4	12.8±0.8	10.7±1.3	12.2±0.6
PCV (%)	38.6± 1.4	38.3± 0.5	39.3±1.1	46.4±0.5*	33.8±0.7**
WBC×10^3	8.1±0.5	4.8± 0.7*	10.1±0.1**	7.8±0.6	8.0± 0.5
MCV (FL)	59.9±1.4	59.9± 2.5	59.6±3.2	58.1±2.2	61.7± 2.7
MCH (pg)	17.9±0.3	18.2±0.2	19.4±0.4	18.6±0.2	19.7±0.2
MCHC (g/dl)	30.8±2.2	30.4± 0.9	32.7±1.1	32.0±1.1	31.9±1.1
Platelets	433.0± 3.5	538.5± 2.5*	547.3± 4.0*	464.5± 2.8*	780.0± 4.5*

*N=5, *p≤0.05, **p≤ 0.01 control animals were administered with 0.5 ml distilled water*
MCH- Mean corpuscular Haemoglobin, MCHC - Mean corpuscular Haemoglobin concentration, MCV- Mean corpuscular volume

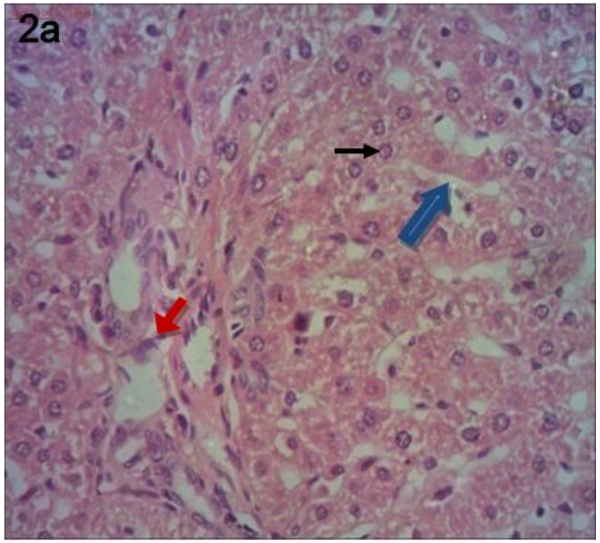

Fig. 2a. Photomicrograph of a cross section of hepatic tissue of the control indicating portal area (red arrowed) and hepatocyte (black arrowed) and sinusoid (blue arrowed)

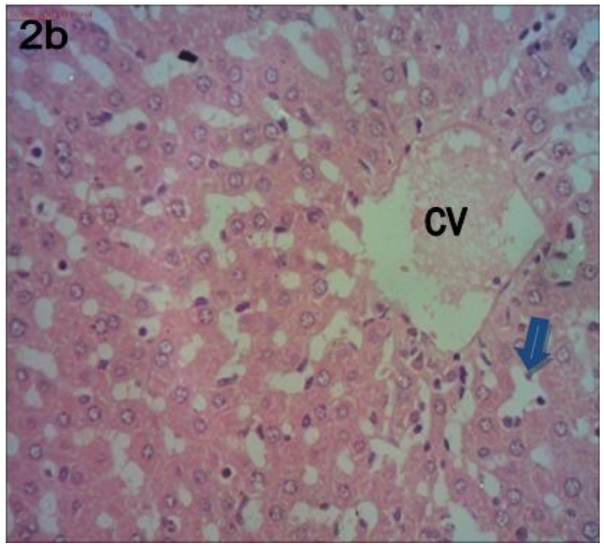

Fig. 2b. The cross section of treated hepatic tissue (medium high dose) showing on set of degerative changes

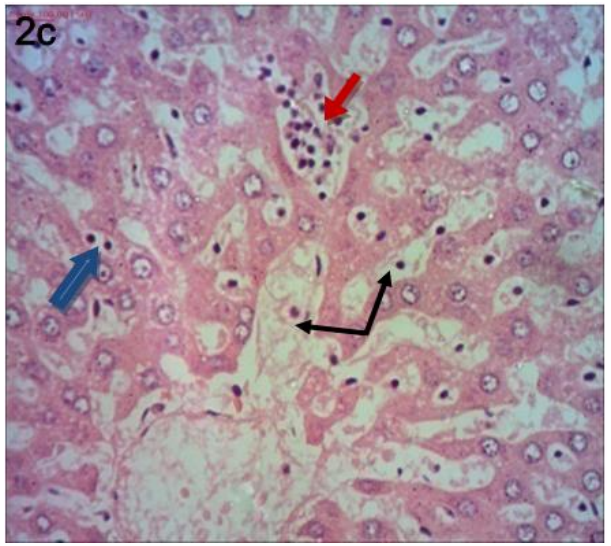

Fig. 2c. The cross section of treated hepatic tissue (very high dose) showing marked degerative changes. Mag. X400

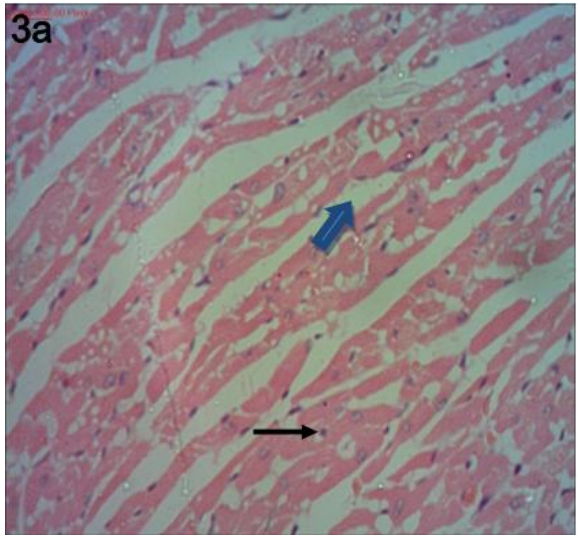

Fig. 3a. The cross section of myocardium indicating myocytes (Black arrowed)

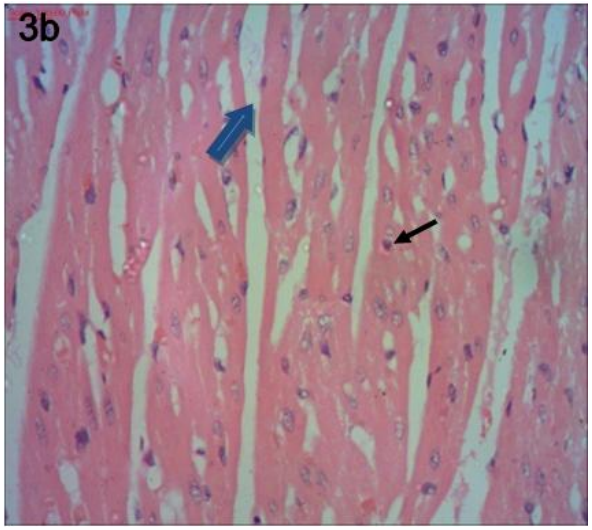

Fig. 3b. The cross section of myocardium treated with medium high dose of the formulation showing no lesion

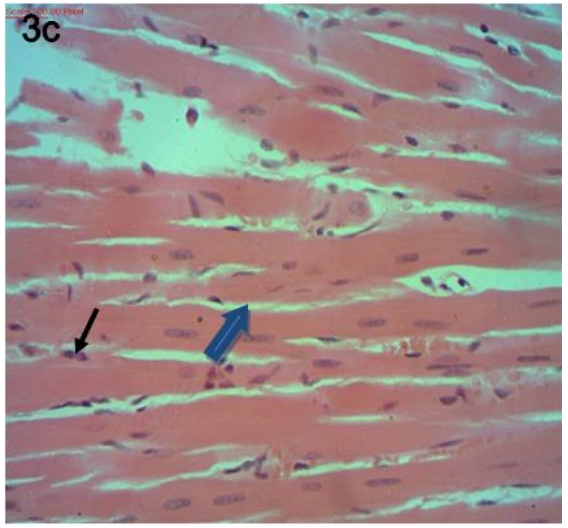

Fig. 3c. The cross section of myocardium treated with very high dose showing no lesion. Mag. X400

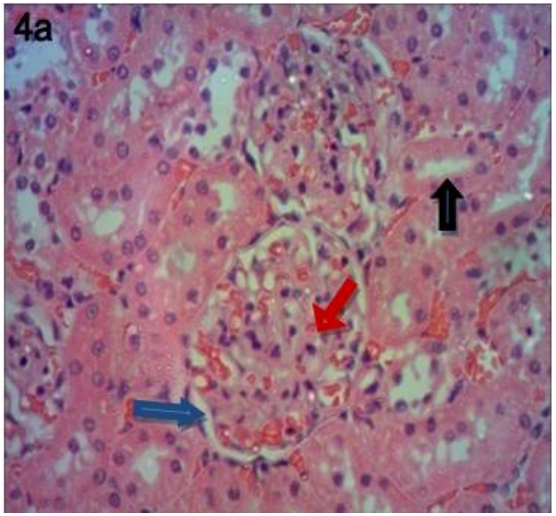

Fig. 4a. The cross section of renal tissue of the control indicating renal corpuscles (red arrowed), Bowman,s capsule (blue arrowed) and convoluted tubules (black arrowed)

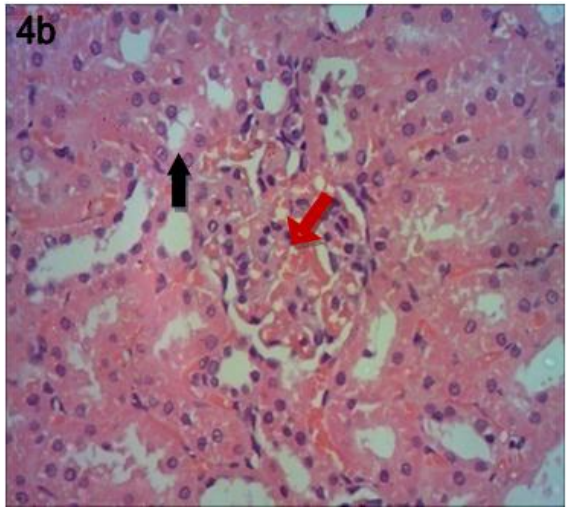

Fig. 4b. The cross section of renal tissue treated with medium high dose showing no lesion

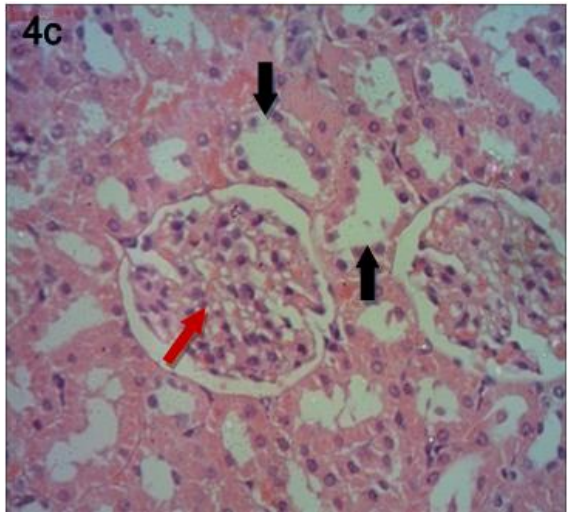

Fig. 4c. The cross section of renal tissue treated with very high dose indicating mild inflammatory changes. Mag. X400

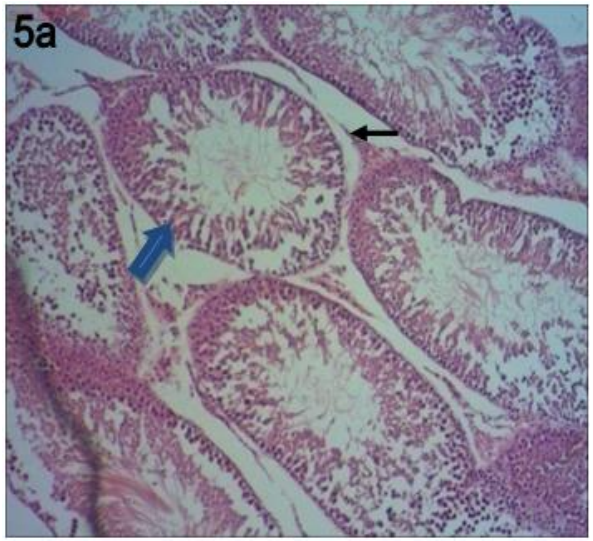

Fig. 5a. The cross section of testicular tissue of the control indicating densely packed spermatogenic cells (blue arrowed) and the interstitium (black arrowed)

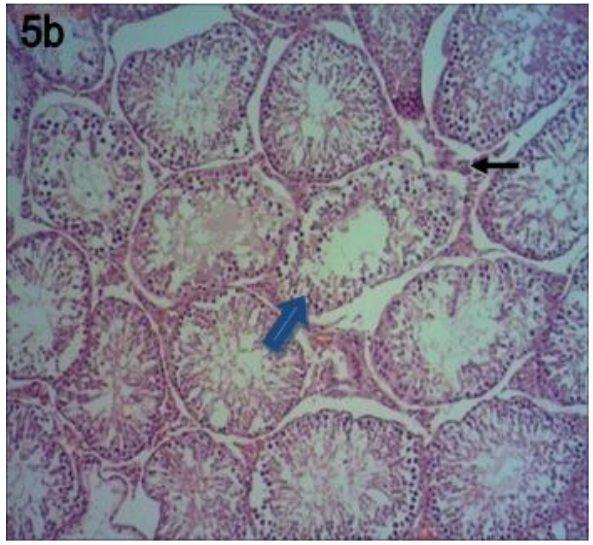

Fig. 5b. The cross section of testicular tissue of the treated with the medium high dose showing no lesion

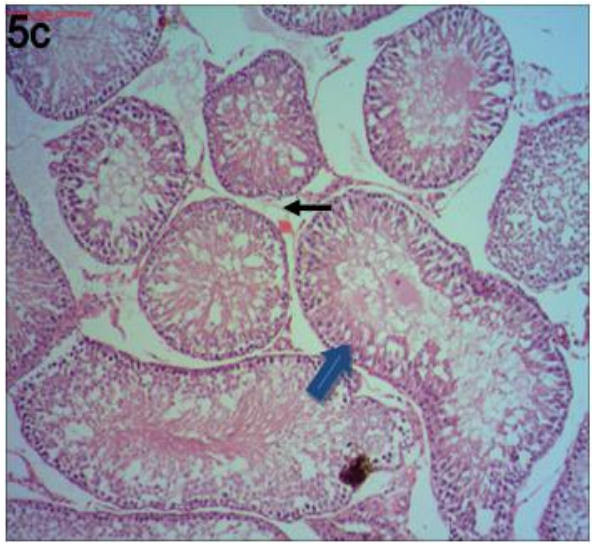

Fig. 5c. The cross section of treated testicular tissue with the very high dose showing no lesion. Mag. X400

4. DISCUSSION

Antibiotic treatment is a preferred choice to control bacterial infections hence the antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world as alternative to antibiotic [21]. However, the emergence of antimicrobial resistance and toxicity issues subside the use of antibiotic [22,23]. A purity test on Katoka mixtures® revealed a little growth of *E. coli* ($0.5x10^2$ cfu/ml) in Eosine Methylene Blue Agar medium. This was however within the acceptable microbial limit for aqueous herbal formulations in accordance with the World Health Organization (WHO) guidelines for the assessment of the safety, efficacy, and quality of herbal medicines [24]. A high level load of *E. coli* above the acceptable limit could cause serious bloody diarrhea infection or watery diarrhea depending on the *E. coli* patho-type of toxin produced [25]. Evaluation for the presence of other enterobacteria and gram-negative bacteria such as *S. typhi, S. aureus, Bacillus cereus*, *Pseudomonas aeruginosa* was conducted as stipulated by WHO guidelines which was necessary depending on the origin of the herbal drug, raw materials and/or the method of preparation adopted [5,26,27]. There was however no growth recorded.

According to Ghosh [25] and Klaasen et al. [28] the extract could be classified as not being toxic, since the LD_{50} was found to be 9.0 g/kg bwt. The gram equivalence of the LD_{50} in an average adult man would translate to 630 g dose of the drug. This is a very high value and makes the preparation relatively safe for use. Also, it was evident that the extract may not have demonstrated significant cytotoxicity activities since there was marked difference between the median lethal concentrations (LC_{50}) of the polyherbal formulation compared to the reference drug, vincristine.

The viscera of the dead animals did not show any macroscopic changes that could point to the cause of the death. However, since the animals did not convulse before death, it could be postulated that the extract did not kill the mice by some action on the nervous system [29]. The effects of the drug on the body weight variation of the treated animals was remarkable only on the groups that received higher doses of the polyherbal drug and no significant increase in weight was observed in the group that received low dose compared to the control (Fig. 1). The decrease in the weights that occurred between day 1 and 7 in the groups treated with higher doses could be attributed to the initial suppression of the animals' appetite by the drug and when this effect was overcome the animals exhibited progressive weight increase especially in the group treated with a dose of 250 mg/kg bwt.

There were no morphological changes in the colour of the various organs of the treated animals compared to the control. Histological studies revealed that the drug had some severe deleterious effects on the liver while mild inflammatory changes were observed in the kidney. The histological study of the hepatic tissue at the low and medium high doses showed no visible morphological changes, but the very high dose of administration exhibited diffuse presence of lymphocytic cells with onset of edematous changes and vacuolation. Also remarkable was focal necro-inflammation. In the biochemical study, the liver and the kidney function profile were examined. ALT and AST are two classical enzymes for assessing liver malfunction or damage. While ALT is considered more specific to the liver, AST could also be seen in the cardiac muscle [30]. An elevation in plasma concentration of these enzymes is an index of liver damage or changes in permeability in hepatocytes membrane [14,31,32]. In this study, there was a significant decrease in the ALT level. AST exhibited marked increase in the very high dose treatment. This enzyme is seen both in the liver and heart. But from the tissue histology, the cardiac tissue showed normal appearance therefore the observed increase in level could be due to the inflammatory changes in the liver which corroborated with the result of the hepatic tissue histology. The total protein showed insignificant decrease ($p \geq 0.05$) with dose. Usually decrease in total protein level implies protein loss which cold be due to mal-absorption resulting from either the liver or the kidney damage [33]. There was marked decrease in albumin level in the low and medium doses of the formulation and less significant decrease in the highest dose. The plasma level of albumin is used to assess the synthesis level of liver [33]. The formulation therefore does seem to have lowered the synthesis level in the liver. The bilirubin level indicated decrease in low and medium high levels but showed considerable increase only in the highest dose treatment. An increase in plasma bilirubin level in very high dose of the herbal drug indicates disturbance in liver function [32]. The observed increase further

confirmed the hepatotoxic effect of the dose. The plasma alkaline phosphate level decreased progressively. The alkaline level is known to increase following obstruction to bile flow [34]. Therefore it is apparent that the drug did not affect the bile flow.

Creatinine and urea are used to assess the renal function profile. The creatinine exhibited significant decrease ($p \leq 0.05$) while the urea showed insignificant changes at low and medium doses but showed considerable increase in very high dose treatment. These are non-protein nitrogenous substances said to be end products of protein metabolism that must be removed continually [32]. The increase in urea level could have been responsible for the inflammatory changes observed at the interstices of the renal tissue.

The formulation ameliorated deranged blood glucose and lipid profile. The decrease in the plasma Total Cholesterol (TC) and Triglyceride (TG) levels might be attributed to the presence of hypolipidemic agents in the polyherbal medicine. There was a reduction in LDL-cholesterol levels observed in all the treated animals which is confirmatory to the fact that the drug can reduce the cardiovascular risk factors that contribute to the death of diabetic subjects. The reduction of the cardiovascular risk factors gave further support as to the traditional use of the herbal formulation. However, the HDL-cholesterol level did not show marked variation compared to the control.

In the haematological study (Table 6), RBC, Hb and PCV exhibited an increase compared to the control. The RBC increased appreciably in the very high dose indicating changes in the rate of the RBCs production. In PCV, the increase was more marked in the medium high dose while decrease occurred in the very high dose treatment. There were insignificant ($p \geq 0.05$) changes in MCHC, MCV and MCH in the treated animals compared to the control. Low MCHC has been associated with iron deficiency anaemia [35]. Therefore, the insignificant change in the level of MCHC suggested that the formulation did not effect a change in the average size of red blood cells and so did not induce anaemia. The platelet count was statistically highly significant ($p \leq 0.05$) compared to the control. The implication is that the herbal formulation may have induced thrombocythemia. The cause of thrombocythemia is not restricted to anaemia only, it may also result from infections and usually associates with complications like blood clot disorder, bleeding and in extreme condition myocardial infarction [36]. The WBC of the treated animals did not show considerable variation compared to the control. The WBC is known to rise as body defense in response to toxic environment [37].

5. CONCLUSION

The formulation exhibited high LD_{50} value (9 g/kg) while the microbial loads were within acceptable official limits which suggest that the polyherbal preparation could be safe for use. However, the study revealed that the drug at very high dose did provoke toxic effect to the animal liver by causing focal necro-inflammation in the tissue. Therefore, its consumption above the recommended dose portends some health risks.

CONSENT

It is not applicable; since the study was on animals and not on humans.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" [38] and ethical guidelines for investigation of experimental pain in conscious animals [39] were strictly followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ogbonnia SO, Odimegwu JI, Enwuru VN. Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats. Afri J Biotechnol. 2008;7:2535-2539.
2. Rickert K, Martinez RR, Martinez. Pharmacist knowledge of common herbal preparations. Proc West Pharmacol Soc. 1999;42:1-2.
3. Ogbonnia SO, Olayemi SO, Mbaka GO, Morgan Y, Usman A, Odusanya PA, Ota D. Evaluation of microbial purity and toxicity profile of two polyherbal formulations used in Nigerian herbal

medicine. Pharmacologia. 2014;357-368. DOI:10.5567/pharmacologia.2014.357.368

4. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngongang J. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L) Roxb (Ceasalpiniaceae). Afr J Biotechnol. 2006; 5:283-289.
5. Ogbonnia SO, Mbaka GO, Emordi J, Nkemhule F, Joshua P, Usman A, Odusanya P. Antimicrobial evaluation and acute and sub-acute toxicity studies on a commercial polyherbal formulation, "Ade & Ade Antidiabetic®" used in the treatment of diabetes in southwestern Nigerian. J Med Med Sci. 2013;4(11):423-432. DOI:http:/dx.doi.org/10.14303/jmms.2013.138
6. Mbaka GO, Ogbonnia SO, Olubamido TO, Awopetu PI, Ota DA. Evaluation of acute and sub-chronic toxicities of aqueous ethanol root extract of *Raphia hookeri* (Palmaceae) on Swiss albino rats. Br J Pharmacol Toxicol. 2014;5(4):129-135. e-ISSN: 2044-2467
7. Bandaranayake WM. Phytomedicine. Turning Medicinal Plants into Drugs. Ahmad, I., Aqil F. and Owais M. (Eds.) Wiley- VCH, Verlag GmbH and Co. KGaA, Weinheim; 2006. ISBN: 3-527-31530-6
8. Fontana R, Mendes MA, de Souza BM, Konno K, Cesar LMN. Jelleines, a family of antimicrobial peptides from the royal jelly of honey bees (*Apis mellifera*). Peptides. 2004;25:919-928. DOI:10.1016/j.peptides.2004.03.016 [PubMed]
9. Shah AMA, Garg SK, Garg KM. Subacute toxicity studies on pendimethalin in rats. Int J Pharmacol. 1997;29:322-324. [PubMed]
10. Bürger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC Soares dos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasilinsis*) (Asteraceae) in mice. J Pharm Sci. 2005; 8:370-373. Available:www.cspsCanada.org [PubMed]
11. Ogbonnia SO, Mbaka GO, Nwozor AM, Igbokwe HN, Usman A, Odusanya PA. Evaluation of microbial purity and acute and sub-acute toxicities of a Nigerian commercial polyherbal formulation used in the treatment of diabetes mellitus. Br J Pharm Res. 2013;3(4):948-962.
12. Institute of Laboratory Animal Research (ILAR). Commission on life science. National Research Council; 1996. Available:www.edu/openbook.php?record_id=5140
13. Joshi CS, Priya ES, Venkataraman S. Acute and subacute studies on the polyherbal anti-diabetic formulation Diakyur in experimental animal model. J Health Sci. 2007;53:245-249. [PubMed]
14. Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. J Health Sci. 2007;53: 351-358. [PubMed]
15. Wasan KM, Najafi S, Wong J, Kwong M. Assessing plasma lipid levels, body weight and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FM-VP4, to gerbils. J Pharm Sci. 2001;4:228-234. Available:www.ualberta.ca/~csps [PubMed]
16. Crook MA. Clinical chemistry and metabolic medicine. 7th Edn., Hodder Arnold, London. 2006;426.
17. Sushruta K, Satyanarayana S, Srinivas N, Sekhar RJ. Evaluation of the blood–glucose reducing effects of aqueous extracts of the selected Umbellifereous fruits used in culinary practice. Trop J Pharm Res. 2006;5(2):613-617. DOI: 10.4314/tjpr.v5i2.14639
18. Hussain A, Eshrat HM. Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa*, Linn and partially purified product from *Abroma augusta*, Linn. in streptozotocin induced diabetes. Indian J Clin Biochem. 2002;17:33-43. [PubMed]
19. Ekaidem IS, Akpanabiatu MI, Uboh FE, Eka OU. Vitamin B12 supplementation: Effects on some biochemical and haematological indices of rats on phenytoin administration. Biokemistri. 2006;18:31-37.
20. Dacie, JC, Lewis SM. Practical hematology. 5th Edn., Churchill Livingstone, London; 1984.
21. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l: An ethnomedicinal plant. J

Adv Pharm Technol Res. 2011;2(2):104–109.
[PubMed]

22. Malini M, Abirami G, Hemalatha V, Annadurai G. Antimicrobial activity of ethanolic and aqueous extracts of medicinal plants against waste water pathogens. Int J Res Pure and Appl Microbiol. 2013;3:40–42.
[PubMed]
23. Zhang R, Eggleston K, Rotimi V, Zeckhauser RJ. Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States. Global Health. 2006;2:6.
[PubMed]
24. WHO. Specifications for identity and purity and toxicological evaluation of food colours. WHO/Food Add/66.25 Geneva, WHO; 1966.
25. Ghosh MN. Fundamentals of experimental pharmacology. 2nd Edn., Scientific Book Agency, Calcutta. 1984;154-157.
26. Shrikumar S, Maheswari MU, Saganthi A, Ravi TK. Review: WHO guidelines for herbal drug standardization. Pharmainfo. Net. 2004;1- 8.
27. Drew KA, Myers SP. Safety issues in herbal medicine: Implications for health professions. MJA. 1997;166.
[PubMed]
28. Klaasen CD, Amdur MO, Doull J. Casarett and Doull's toxicology: The basic science of poison. 8th Edn., Mc Graw Hill, USA. 1995;13-33.
29. Ogwal-Okeng WJ, Obua C, Anokbonggo WW. Acute toxicity effects of the methanolic extract of *Fagara zanthoxyloides* (Lam.) root-bark. Afr Health Sci. 2003;3:124-126.
[PubMed]
30. Abd-Allah GA, El-Bakry KA, Bahnasawy MH, Elmouldi NT. Immunological and physiological studies on the protective and therapeutic effects of *Nigella sativa* oil in albino rats treated with carbon tetrachloride. Adv Environ Biol. 2015; 9(18):14-19.
31. Mbaka GO, Adeyemi OO, Oremosu AA. Acute and sub-chronic toxicity studies of the ethanol extract of the leaves of *Sphenocentrum jollyanum* (Menispermaceae). Agric Biol J N Am. 2010;1(3):265-272.
32. Ahmad M, Farsi E, Lim CP, Asmawi MZ, Yam MF. Acute and subchronic oral toxicity of *Coriolus versicolor* standardized water extract in Sprague-Dawley rats. J Ethnopharmacol. 2011;137(3):1067–1076.
[PubMed]
33. Suckow MA, Stevens KA, Wilson RP. The laboratory rabbit, guinea pig, hamster, and other rodents. Seattle, Wash, USA: Academic Press; 2012.
34. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol. 2007;45(9):1551–1557.
[PubMed]
35. Agbor GA, Oben M, Knight DC, Mills RG, Bry JJ, Crag PA. Human physiology. 4th Edition, Churchill Livingstone. 2005;290-294JE.
36. Perk BO, Ilgin S, Atli O, Duymus HG, Sirmagul B. Acute and subchronic toxic effects of the fruits of *Physalis peruviana* L. Evid Based Complement Alternat Med; 2013.
DOI: 10.1155/2013/707285
[PubMed]
37. Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. Afr J Biotechnol. 2005;4:833-837.
38. NIH. Guidelines for the care and use of laboratory animals. National Academic Press, NIH Publication No. 85. 1996;23.
39. Zimmermann M. Ethical guidelines for investigation of experimental pain in conscious animals. Pain. 1983;19:109-110.

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