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Evaluation of Microbial Purity and Acute and Sub-acute Toxicities of a Nigerian Commercial Polyherbal Formulation Used in the Treatment of Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the experiment, the protocol for the study and also partook in the manuscript preparation and statistical analysis. Author GOM undertook the tissue processing and analysis as well as partook in the write up and editing of the manuscript. Author AMN conducted the laboratory work and did part of the literature search. Authors HNI, AU and PAO evaluated the microbial purity of the formulations and did part of the literature search. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Objective: This study evaluated acute and sub-acute toxicities in rodents and microbial purity of a polyherbal formulation, Bobwell® popular among the natives for the management of diabetes mellitus (DM). It was prepared with unspecified quantities of the following plant materials viz. *Gongronema latifolium. Garcinia kola, Vernonia amgydalina*, *Sphenocentrum jollyanum* and *Kigelia africana* leaves.
Materials and Methods: Microbial purity was evaluated on some bacterial and fungal organisms using appropriate diagnostic media. Toxicity of the polyherbal preparation was evaluated in Swiss albino mice by administering to the animals graded oral doses of the

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lyophilized preparation in the ranges of 1.0 to 20.0 g/kg body weight (bwt) and observed for changes. Wistar rats were also fed with different doses of the lyophilized formulation for 30 days and the effects on the biochemical profiles and haematological parameters were evaluated.

Results: The purity evaluation test revealed presence of some bacterial organisms with the load within officially acceptable limits except *Escherichia coli* having a load of $1.50x10^2$ cfu/ml while no fungal organisms were observed. The median acute toxicity value (LD_{50}) of the polyherbal medicine was determined to be 15.2 g/kg bwt. There was significant increase ($P \leq 0.05$) in the body weight of the animals treated with the highest dose of the formulation compared to the control. The biochemical parameters showed marked decrease in the plasma glucose level compared to the control. Increase in creatinine level was observed only in the animals that received the highest dose of the formulation while aspartate aminotransferase (AST) decreased significantly. On the other hand, alanine aminotransferase (ALT) exhibited significant increased ($P \leq 0.05$) at the highest dose. The photomicrograph of hepatic tissue showed focal necro-inflammation around the portal hepatics. There was marked increase in the haemoglobin level and in the red blood cell (RBC) count at the highest doses. There was also significant increase in white blood cells (WBC).

Conclusion: The high LD_{50} value indicated that the polyherbal preparations could be safe for use but its safety was negated by high presence of *E coli* load. Although the formulation showed good hypoglycaemic activity and beneficial effects on cardiovascular risk factors, at the highest dose, the formulation exhibited deleterious effect on the hepatic tissue.

Keywords: Microbial purity; acute; sub-acute; toxicity; polyherbal formulation.

1. INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disordered diseases resulting from absolute or relative defects in insulin secretion or action or both. It is defined as “a state of premature cardiovascular death which is associated with chronic hyperglycemia and also associated with blindness and renal failure” [1]. Oral hypoglycemic drugs including suphonylureas, biguanides and thiazolidinediones, have been employed in the treatment of the disease without achieving a total cure and are without some undesired side effects. Attention is, therefore, now focused on the use of alternative therapy for the disease treatment with plant and plant derived medicines as the best option. Plant derived medicine is known as herbal medicine and is currently being 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 increasing popularity in the use 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 associated with allopathic medicines. More often, and due to the misconception, herbal drugs are administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage. The danger associated with the potential toxicity of such therapy and other herbal therapies used over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs [4].

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 lead to serious 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 [5,6]. 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. Also lack of standardization is a major concern regarding the use of medicinal herbal medicines [7].

Herbal medicine is most often 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 [6]. The bioactive principle(s) in most herbal preparations are not always known and there could be possibilities of interaction with each other in solution. The quality as well as the safety criteria for herbal drugs may be based, therefore, on a clear scientific definition of the raw materials used for such preparations.

The aim of the study was to evaluate the safety of a polyherbal preparation, Bobwell,® an antidiabetic, formulation sold openly in the markets and widely consumed locally for the treatment of DM in most Nigerians south western states for microbial purity and also to carry out its toxicity profile studies in rodents. Bobwell® was claimed to be prepared with unspecified quantities of the following plant material constituents: *Gongronema latifolium* (Asclepiadaceae), *Garcinia kola* Heckle (Guttiferaceae), *Vernonia amgydalina* Del(Asteraceae), *Sphenocentrum jollyanum* (Menispermaceae), and *Kigelia africana* (Lam). Benth (Bignoniaceae).

2. MATERIALS AND METHODS

2.1 Material

The antidiabetic polyherbal formulation Bobwell® (a liquid dosage form, 1.5L) a slightly thick, non viscous brownish coloured liquid was selected on the basis of its local consumption rate and was procured from the Mushin market in Lagos suburb. The un-tampered procured polyherbal formulation bottle was stored in a refrigerator at 4- 6ºC until the quantity needed for the purity test was aseptically taken. 1000 ml of the formulation was filtered and the resulting 875 ml was freeze dried which yielded 37.5 g gel. The prescribed dose for human adult was 30 ml daily. The label indicated only the plant material constituents used in the formulation without specifying the quantity of each used, and also there was no indication of the batch number, the manufacturing and expiring dates.

2.2 Animals

Swiss albino mice (20 – 25 g) of either sex were used for the acute toxicity study, while adult Wistar rats (130±15g) were used for the sub-acute toxicity profiling. The animals were obtained from the animal house of the College of Medicine of the University of Lagos. They were randomly selected with no preference for sex and were fed with a standard animal diet (Pfizer Feeds Ltd, Nigeria) and had access to water *ad libitum*. The animals were maintained in separate (both sexes) spacious polypropylene cages in well ventilated animal house with 12 hrs dark and light cycle and were acclimatized for a week before the commencement of the study. The use and care of the animals, and the experimental protocol were in strict

compliance with the Institute of Laboratory Animal Research (ILAR) guidelines on the use and care of animals, in experimental studies [8].

2.3 Determination of Microbial Purity

The microbial load of the preparation was determined using the standard plate method [9]. Various diagnostic media-Tryptone Soya 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 products. Each of the media was prepared according to manufacturers' instruction and sterilized at 121 ºC for 15 minutes.

Three fold serial dilutions ($10^{-1,}$ 10^{-2} and 10^{-3}) were made using sterile distilled water. The media were allowed to cool to 45ºC and 1ml each of the dilutions seeded in 25 ml each of the sterile culture media swirled and left to solidify. The bacterial media were incubated at 37ºC for 3 days while the fungal medium (SDA culture) was incubated at ambient temperature for 7 days. They were examined 24 hourly during this period for the colonies and the results recorded (Table 1). The purity of the formulations for proteus organisms was evaluated using the 1/10 dilution, a loopful was taken and dropped aseptically at the centre of nutrient agar plate. The site of inoculation was swabbed. The triplicate plates were prepared, covered and incubated in inverted position at 37 ºC and observed daily for 3 days for swarming of proteus.

2.4 Assay of Antimicrobial Activity

The antimicrobial activity of the preparation was investigated using the cup diffusion method on Mueller Hinton Agar for bacterial organisms and Sabouraud Dextrose Agar (SDA) for fungal organisms [10]. 10^6 cfu/ml of the overnight clinical cultures of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Shigella species* was seeded in 25 ml Mueller Hinton Agar respectively while *Candida albican* was seeded in Sabouraud Dextrose Agar. Wells were bored in each of the culture media using a sterile 12 mm cork borer and various dilutions (100%, 50%, 25% and 12.5%) of the test material were prepared using sterile water. 0.5 ml of each dilution was respectively seeded in wells made in inoculated plates with a blank well in each of the plates seeded with 0.5 ml sterile distilled water to serve as a control standard. The cultures were incubated at 37 ºC for 24 hrs for bacterial cultures and at ambient temperature for 7 days for fungal cultures and observations were made for zones of inhibitions [11].

2.5 Acute Toxicity Study

The toxicity study was carried out using thirty-five (35) male and female Swiss albino mice (weighing 20 – 25 g) obtained from the Laboratory Animals Center, College of Medicine, University of Lagos. The animals were randomly distributed into: one control group and six treated groups, containing five animals per group. The rationale for five mice per group was to obtain more reliable mortality information following the polyherbal administration. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After the overnight fasting, the control group received 0.3 ml of acacia solution (2%) orally. The doses 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg bwt were respectively administered orally to the groups from acacia solution of the formulation gel. The stock solution was prepared by dispersing 16 g of the gel with 7 ml of the acacia solution in a 100

ml beaker and then transferred to a 20 mL volumetric flask. The volume was made to mark with the acacia solution to give a stock solution of 800 mg/mL (80% w/v). For mice of average weight of 22.5 g administered 20,000 mg/kg bwt (20mg/g), the total volume consumed was 0.56 mL (450÷800mL) while for 15,000mg/kg bwt (15mg/g) the total volume received was 0.42 mL. The animals were observed continuously for the first 4 hrs and then for each hour for the next 24 hrs and at 6 hourly interval for the next 48 hrs after administering the extract to observe any death or changes in general behaviour and other physiological activities [12,13].

2.6 Determination of LD_{50}

The median lethal dose (LD_{50}) was estimated for each group by log dose – probit analysis [14].The LD_{50} was calculated as the geometrical mean of the maximum dose producing 0% mortality and the minimum dose producing 100 % mortality.

2.7 Sub-acute Study

Male and female Wistar rats weighing 130 g ± 20 g were used. They were allowed to acclimatize to the laboratory conditions for seven days. The animals were maintained on standard animal feeds and provided with water *ad libitum.* The animals were weighed and divided into four groups of five animals each and after the overnight fast of the animals the control group received a dose of 0.6 ml of acacia solution (2%) orally once a day for 30 days. The three treated groups respectively received the following doses: 200 mg/kg, 300 mg/kg and 600 mg/kg bwt of the gel orally once a day for 30 days [15,16,17]. The gel suspension (12 %w/v) was prepared by dispersing the gel (12 g) with 45 ml of acacia (2%) solution in a beaker, and transferred to a 100 mL volumetric flask. Then the beaker was rinsed with the solution and the content transferred to the volumetric flask and volume made to mark with the acacia solution.

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 and on the 26th day, they were made unconscious by cervical dislodgement. The blood was collected via cardiac puncture in three tubes: one with EDTA for analysis of hematological parameters and the blood chemistry, Fluoride oxalate tube for glucose analysis and with heparin to separate plasma for biochemical profiles. The heparinized blood was centrifuged within 5 min of collection at 4000 g 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 [18]. LDL-cholesterol levels were calculated using Friedwald equation [19]. Plasma was analyzed for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods [20]. Plasma glucose contents and protein contents were determined using enzymatic spectroscopic methods [21]. Haematocrit was estimated using the method as described by [22]. 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. Hemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method [22].

2.8 Tissue Histology

The organs were fixed in 10% formal saline for ten days before embedding in paraffin wax. Each organ tissue was sectioned at 5 µm and stained with Haematoxylin and Eosin (H and E) stain [23]. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.9 Statistical Analysis

Significant differences were determined using a Student's t-test. Differences were considered significant if $p < 0.05$. All data were expressed as mean ± standard error of the mean.

3. RESULTS AND DISCUSSION

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. The growth of *Bacillus subtilis* (1.0×10^2 cfu/ml) were observed in Tryptone Soy Agar culture and of other coli forms (2.25×10^2 cfu/ml) observed in MacConkey agar culture after 72 hrs but all were within acceptable official limit [9]. There was no growth observed on Sabouraud Dextrose Agar even on the 6th and 7th day of incubation indicating the absence of fungal organisms. In the Eosine Methylene Blue Agar medium showed the growth of *E. coli* (1.5×10^2 cfu/ml) which was above the acceptable microbial limit for aqueous herbal formulations according to World Health Organization guidelines for the assessment of the safety, efficacy, and quality of herbal medicines as a prerequisite for global harmonization [24]. An increase in *E. coli* level above the acceptable limit can cause serious diarrheal infection which could be a bloody or watery diarrhea depending on the *E. coli* patho-type of toxin produced [25,26].

In the acute toxicity study (Table 2) of the formulation no changes in the behaviour and in the sensory nervous system responses were observed in the animals. Also no adverse gastrointestinal effects were observed in the male and female mice used in the experiment. All the animals that received 5.0 g/kg bwt dose survived beyond the 24 hrs of observation while four of the mice that received 20.0 g/kg bwt dose of the extract died within 4 hrs. The median acute toxicity value (LD_{50}) of the formulation was determined to be 15.2 g/kg bwt. According to World Health Organization (WHO) toxicity index of 2 g/kg bwt [27,28] the extract could be classified as being non toxic, since the LD_{50} was found to be above 15.0 g/kg bwt translating to 1064 g equivalence dose in human adult. This is a very high value making the preparation relatively safe for use. The viscera of the dead animals did not show any macroscopic changes that could point to the cause of the death neither did the animals convulse before dying. It could therefore, be postulated that the formulation did not kill the mice by the action on the nervous system [29].

Table 1. Microbial purity test of the polyherbal formulation

Media	*S. typhi*	*Bacillus* species x 10^2	*Shigella* species	Other Coliforms x 10^2	Proteus species	*P aeruginosa*	*S. aureus*	*E. coli* x 10^2	TMYC	TACC x 10^2	TOTAL
SSA	0	-	0			-	-	-	-	-	0
MAC	-	-	-	2.25	-	-	-	-	-	-	$2.25x10^2$
NA	-	-	-	-	0	-	-	-	-	-	0
CA	-	-	-	-	-	0	-	-	-	-	0
MSA	-	0	-	-	-	-	0	-	-	-	0
EMBA	-	-	-	-	-	-	-	1.50*	-	-	1.50x10
SDA	-	-	-	-	-	-	-	-	0	-	0
TSA	-	$1.0x10^2$	-	-	-	-	-	-	-	9.93	$1.093x10^3$

*N=5; values = m ± sem * P<0.05; ** P<0.01 vs. control group*

Targeted organisms: Salmonella typhi 0, Shigella species 0, Other Coli forms $2.25x10^2$, Proteus species 0, Pseudomonas aeruginosa 0, Staphylococcus aureus 0, Escherichia coli $1.50x10^2$, Mould and Yeast 0 and Bacillus species$1.0x10^2$.

CA - Cetrimide Agar, EMBA - Eosine Methylene Blue Agar, MAC- MacConkey Agar , NA-Nutrient Agar, SDA- Sabouraud Dextrose Agar, SSA - Salmonella Shigella Agar, TSA- Trytone Soya Agar, TNTC-To numerous to count TYMC Total yeast and mould count

Table 2. Acute toxicity evaluation of the polyherbal formulation in mice

Doses of drugs g/kg	Number of animals	Number of animals dead	% Cumulative Death
Control	0	0	0
0.5	5	0	0
1.0	5	0	0
2.5	5	0	0
5.0	5	0	0
10.0	5	1	14.3
15.0	5	2	24.8
20.0	5	4	100

Control received 0,3ml of Acacia (2%w/v) solution

The effects of the formulation on the body weight of the animals treated with various doses are summarized in Fig. 1. Significant ($P \leq 0.05$) increase in the body weight was observed only in animals that received the highest dose (600 mg/kg bwt) of the lyophilized extract while no significant ($P \geq 0.05$) increase occurred in the weight of animals treated with lower doses of the polyherbal formulation compared to the control. The appreciable weight gain of the animals that received the highest dose of the formulation suggested the ability of the polyherbal formulation to have the tendency to stimulate appetite at high dosage. Although the amount of food and water made available to the animals were not quantified, the animals that received the highest extract dose were observed to have consumed more food and as well as had more water intake. There were also organs weight changes (Table 5) in the extract treated which were insignificant ($P \geq 0.05$) compared to the control.

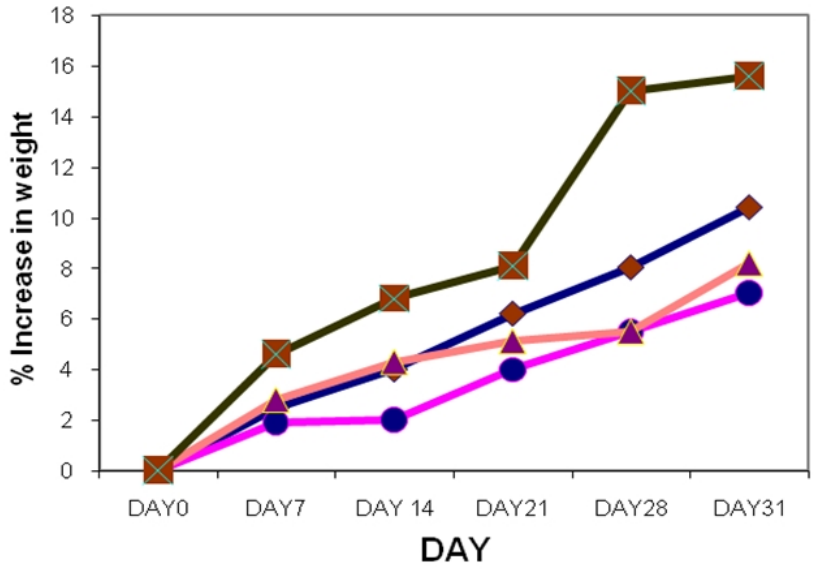

Fig. 1. Percentage weight variation of different groups treated with various doses of the polyherbal extract and the control

♦GPI –Control group treated with 0.5ml Acacia (2%w/v) solution., ●GPII Animals treated with the extract 200mg/kg body weight,▲ GPIII Animals treated with the extract 300mg/kg body weight, X GPIV Animals treated with the extract 600mg/kg body weight

Effects of the polyherbal formulation on the biochemical profiles were summarized in Table 3. There was a remarkable decrease in the plasma glucose levels especially in the animals treated with the highest dose of the formulation compared to the control. This clearly indicated the presence of hypoglycaemic components in the formulation and gives credence to the use of the polyherbal formulation as a hypoglycaemic agent. Significant ($P \leq 0.05$) increase in creatinine level was observed only in the animals that received the highest dose of the formulation (600 mg/kg bwt). The elevation in the plasma creatinine concentration could suggest inflammatory activity in the kidney, specifically by renal filtration mechanism [18]. There was significant decrease in AST level at all the doses. The ALT level except at the lowest extract dose showed progressive increase that was significant at the highest dose compared to the control. An elevation in plasma concentration of ALT is usually due to liver damage while increase in AST level could be linked to damage to either cardiac or hepatic tissues or damage to both [18,19]. The marked increase in ALT at the highest dose was therefore indicative of inflammatory challenge of the formulation on the liver. The decrease in the plasma total cholesterol (TC) and triglyceride (TG) levels might be attributable to the presence of hypolipidaemic agents in the extract. A significant increase in HDL-cholesterol levels and a reduction in LDL-cholesterol levels observed in all the treated animals was an indication that the formulation has the tendency to reduce the cardiovascular risk factors which contribute to death of diabetic subjects [1]. The ability of the formulation to exert a decrease in cardiovascular risk factors lent further support for its use as a hypoglycaemic agent.

Table 3. Plasma glucose level and other biochemical profiles of animals treated respectively with various doses of polyherbal formulation extract for 30 days and the control

Parameter	Group I	Group II	Group III	Group IV
Glucose(mmol/l)	4.1±0.2	3.2±0.4**	2.7±0.2*	2.2±0.3*
Cholesterol(mmol/l)	2.0±0.3	1.4±0.1*	1.6±0.2**	1.3±0.5*
Triglyceride(mmol/l)	0.5±0.0	0.4±0.0	0.3±0.0*	0.2±0.0*
HDL(mmol/l)	1.5±1.0	2.0±0.5**	2.0±0.6**	2.0±0.7**
Creatinine(mmol/l)	59.6±0.3	67.6±0.1	57.8±0.0	130.9±2.5*
AST(IU/L)	304.0±0.5	244.7±5.0**	208.7±0.4*	205.3±0.0*
ALT(IU/L)	66.7±2.0	59.9±0.2	68.8±0.3	79.4±0.2*
LDL(mmol/l)	1.3±0.5	0.8±0.3**	1.0±0.2**	0.6±0.2*

*N=5, values = m ± sem *P <0.05; ** P <0.01 vs. control group. Control group received 0.5 ml Acacia (2 %w/v) solution.*

GP I (Control), GPII (200mg/kg), GP III (300mg/kg), GP IV (600mg/kg)

HDL- High density lipoprotein; LDL - Low density lipoprotein; AST - Aspartine aminotransferease; ALT- Alanine aminotransferease.

Fig. 2a showed the photomicrograph of normal hepatocytes radially arranged from the margins of the portal area towards the central vein with each column interspaced by hepatic sinusoids. Fig. 2b showed the photomicrograph of hepatic tissue of the animals administered with the highest dose of the formulation in which was focal necro-inflammation around the portal hepatics. It was obvious the inflammatory changes in the hepatic tissue precipitated an increase in the ALT level. Fig. 3a showed the normal photomicrograph of renal tissue indicating the cortical area with the renal corpuscles that appeared as a dense rounded mass separated from surrounding structures by Bowman's space. The photomicrograph (Fig. 3b) of the renal tissue treated with the highest dose of the formulation showed normal

appearance. Therefore, the cause of marked increase in creatinine level at the highest dose of the formulation is likely due to other remote factors. The photomicrograph of normal cardiac tissue (Fig. 4a) showed the arrangement of muscle fibres which branched to give the appearance of three dimensional networks. Indicated were deeply stained nuclei. In the extract treated (Fig. 4b), no pathological changes was observed. Fig. 5a showed the cyto-architecture of normal testicular tissue with the seminiferous tubules cut in transverse plane. Close to the basement of the epithelium were more primitive spermatogenic cell series while the matured sperm cells formed a cluster close to the lumina with their tails projecting to form wavy appearance. In the extract treated (Fig. 5b), the testicular tissue showed no lesion.

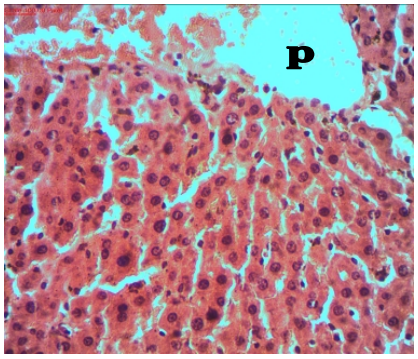

Fig. 2a. Photomicrograph of normal hepatic tissue indicating portal area (p) and radially arranged cords of hepatocytes separated by sinusoids. (H&E stain) Mag. X400

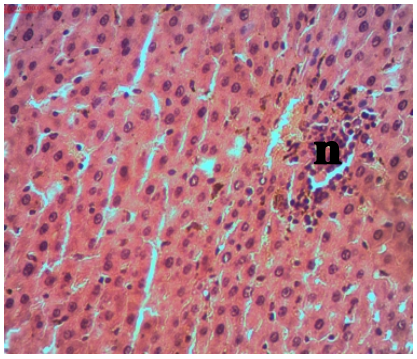

Fig. 2b. Photomicrograph of hepatic tissue treatment with 600mg/kg of the formulation indicating focal necro-inflammation (n) around portal hepatics. (H&E stain) Mag. X400

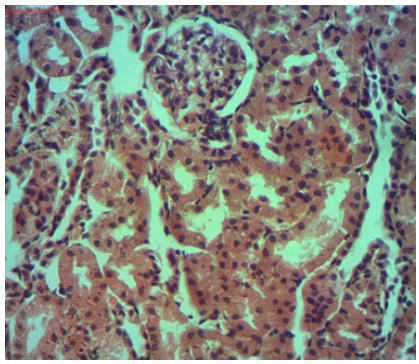

Fig. 3a. Photomicrograph of a cross section of cortical region of the renal tissue of the control indicating renal corpuscles and convoluted tubules. (H&E stain) Mag. X400

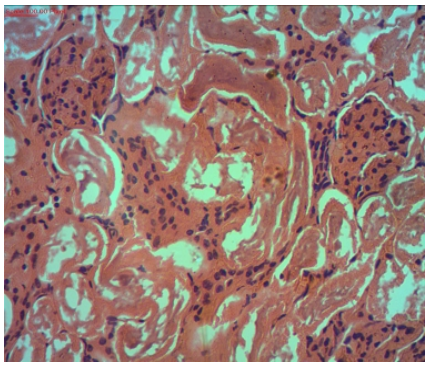

Fig. 3b. Photomicrograph of a cross section of cortical region of the treated animals (600mg/kg) showing normal appearance. (H&E stain) Mag. X400

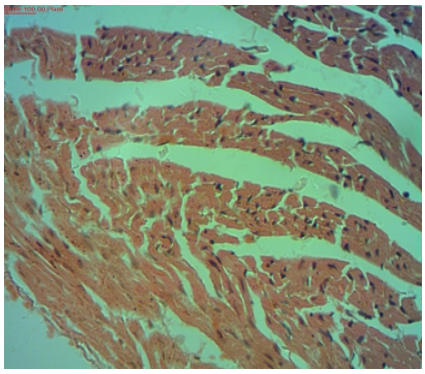

Fig. 4a. Photomicrograph of a cross section of cardiac muscle of the control group showing the branched network of muscle fibres. (H&E stain) Mag. X400

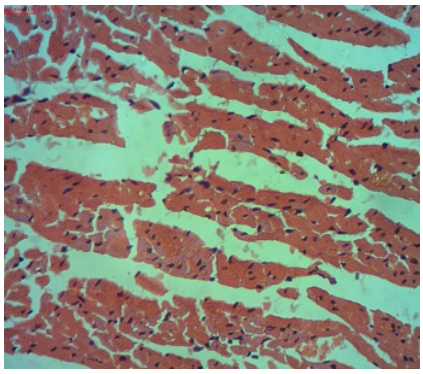

Fig. 4b. The cross section of cardiac muscle of animal treated with 600mg/kg of the formulation indicating no abnormality. (H&E stain) Mag. X400

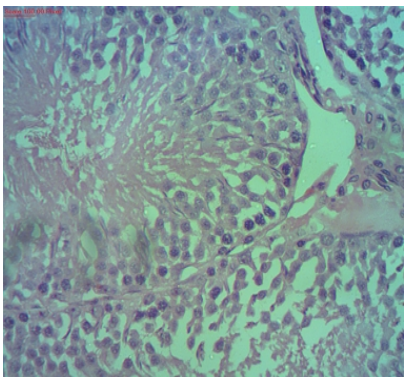

Fig. 5a. The histology of testes of the control group showing cross sections of seminiferous tubules and interstitial cells. (H&E stain) Mag. X400

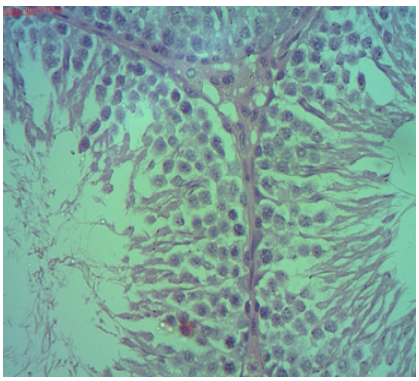

Fig. 5b. The cross section of seminiferous tubules of testis treated with 600mg/kg of the polyherbal drug showing no abnormality. (H&E stain) Mag. X400

The activity of the polyherbal drug on blood parameters were summarized in Table 4. Although the haematocrit level at the highest dose of treatment showed no significant variation compared to the control, the marked increase in the haemoglobin level and in the RBC count at the highest dose was indicative that the polyherbal formulation does possess active principle that can enhance RBC production (erythropoiesis) [30]. This potential haematinic property emphasized the beneficial effect of the formulation to the general well being of the animals. The haematinic effect of the formulation could be due to the activity of *Sphenocentrum jollyanum,* a constituent of the formulation reported to be rich in haematinic property [31,32]. This study showed that there was no significant change in MCHC in the treated animals compared to the control. Low MCHC is associated with iron deficiency anaemia where microcytic hypochromic red cells are produced as a result of lack of iron to support haemoglobin synthesis [33]. There was also no significant change in MCV. However, there was a significant increase in WBC count which is known to rise as body defense in response to toxic environment [34]. Also, lymphocyte, the main effector cell of the immune system [35,36] recorded marginal increase implying that the formulation might not have exerted challenge on the immune system of the animals.

Table 4. Haematological and blood differential profiles of animals treated respectively with various doses of polyherbal formulation extract for 30 days and the control

Parameter	Group I	Group II	Group III	Group IV
RBC x 10^3	6.0±0.0	6.7±0.3	6.3±0.2	8.1±0.4*
WBC(10^6)	4.5±0.1	9.0±0.1*	7.0±0.3*	8.9±0.2*
MCV(fl)	64.1±0.3	52.8±0.1*	54.6±0.2*	53.9±0.5*
HCT (%)	44.0±1.0	39.7±0.5	38.9±0.7	44.6±0.6
PLT (%)	451.0±2.0	527.0±5.0	292.1±0.5	502.0±0.7
PCT (%)	0.3±0.0	0.3±0.1	0.2±0.0	0.3±0.0
Hb (g/dl)	12.8±0.2	61.9±1.5	14.9±0.1	15.9±0.5**
MCH(pg)	21.6±1.0	19.8±0.5	20.5±0.7	19.7±0.6
MCHC (g/dl)	33.7±0.5	37.4±0.6	37.5±1.0	36.6±0.5
LYM (%)	69.2±2.0	61.9±1.5	81.3±0.5	70.2±0.3

*N=5 m ± sem*P <0.05; ** P <0.01 vs. control group. Control group received 0.5 Acacia (2 %w/v) solution; KEY GP I (Control), GPII (200mg/kg), GP III (300mg/kg), GP IV (600mg/kg)*

Table 5. The effects on the weights on kidney, heart, liver and brain of animals treated with various doses of the polyherbal formulation extract for 30 days and the control in the sub-acute toxicity study

Organ /100gkgbwt	GPI	GPII	GPIII	GPIV
Heart(g)	0.5±0.2	0.5±0.1	0.5±0.0	0.5±0.0
Liver(g)	3.4±0.1	3.6±0.5	3.6±0.5	3.7±0.6
Kidney(g)	0.8±0.0	0.9±0.0	0.7±0.0	0.9±0.0
Brain(g)	1.5±0.1	1.5±0.1	1.3±0.0	1.5±0.0

*N=5 values= (m ± sem)*P <0.05; ** P <0.01 vs. control group. Control group received 0.5 Acacia (2%w/v) solution.*
GPI (Control), GPII (200mg/kg), GPIII (300mg/kg), GPIV (600mg/kg).

4. CONCLUSION

The high LD_{50} value (15.2 g/kg) obtained clearly indicated that the polyherbal preparations could be safe for use but its safety was negated by the presence of *E coli* load above officially accepted limit for liquid herbal preparation. The study showed that the formulation had some hypoglycaemic activity and good reducing effects on cardiovascular factors and did not provoke toxic effects to the animals' heart tissues. Furthermore, the presence of haematinic agent emphasized the beneficial effect of the formulation. However, at highest dose used, the formulation exhibited deleterious effect on the hepatic tissue thus necessitating for a cautious use. It could therefore be estimated that 300mg/kg bwt was the highest dose the herbal formulation did not exert an observed toxic effect to the animals.

CONSENT

This was not applicable since the study was on animals and not on humans.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of our Institution". All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that there is no conflict of financial interest in connection with the submitted manuscript.

REFERENCES

1. Barnett HA, O'Gara G. Diabetes and the heart. clinical practice series. Churchill Livingstone. Edinburgh UK. 2003;11-32.
2. Rickert K, Martinez RR, Martinez TT. Pharmacist knowledge of common herbal preparations. Proc West Pharmacol Soc. 1999;42:1-2.
3. 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. Afr J Biotech. 2008;(15):2535-2539.
4. Tédong L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF, Callard P, Kamtchouing P. Acute and Sub chronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr J Tradit Altern Med. 2007;4(2):140-147.
5. Bandaranayake WM. Modern Phytomedicine. Turning medicinal plants into drugs. Ahmad I, Aqil F and Owais M. Edn. Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim; 2006.
6. Ogbonnia SO, Mbaka GO, Igbokwe NH, Anyika EN, Alli P, Nwakakwa N. Antimicrobial evaluation, acute and subchronic toxicity studies of Leone Bitters, a Nigerian polyherbal formulation, in rodents. Agric Biol J N Am, 2010;1(3):366-376.
7. Angell M, Kassier JP. Alternative medicine –the risk of untested and unregulated remedies. N Engl J Med. 1998;339:839-841.
8. ILAR, (Institute of Laboratory Animal Research). Commission on life science. National research council. www.edu/openbook. php?record_id=5140; 1996.
9. 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.
10. Raghavendra MP, Satish S, Raveesha. In-vitro evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica*. J Agric Tech. 2006;2(1):77 – 88.
11. National Committee for Clinical Laboratory Standards (NCCLS) (1997). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th Edition. Approved standard M7-A4, 17, No. 2, Villanova PA.
12. Shah LP, Patil SP, Patil J. Observations on clinical evaluation of indigenous herbal drugs in the treatment of mental illness. Indian J Pharmacol. 1997;29:347-349.

13. Bürger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC, Soares dos Santos AR. Acute and sub-acute toxicity of the hydro alcoholic extract from *Wedelia paludosa* (Acmela brasilinsis) (Asteraceae) in mice. J Pharm Sci. 2005;8(2):370-373. www.cspsCanada.org)
14. Miller LC, Tainter ML. Estimation of the LD50 and its error by means of logarithmic probit graph paper. Proc Soc Exp Biol Med. 1944;24:839- 840.
15. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwe EM, Etoa FX, Ngongang. Evaluation of acute and subacute toxicities of aqueous ethanol extract of leaves of *Senna alata* (L.) Roxb (*Ceasalpiniaceae*). Afr J Biotech. 2006;5(3):283-289.
16. Joshi CS, Priya ES, Venkataraman S. Acute and sub acute studies on the polyherbal antidiabetic formulation Diakyur in experimental animal model. J Health Sci. 2007;53(2):245-249.
17. Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and sub acute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats. J Health Sci. 2007;53(4):351-358.
18. 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(3):228-234. (www.ualberta.ca/~csps)
19. Crook MA. Clinical chemistry and metabolic medicine. 7th Edition. Hodder Arnold, London. 2006;426.
20. 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 Pharmaceutical Res. 2006;5(2):613- 617.
21. 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(2):33-43.
22. 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(1):31-37.
23. Mbaka GO, Ogbonnia SO, Oyeniran KJ, Awopetu PI. Effect of *Raphia hookeri* seed extract on blood glucose, glycosylated haemoglobin and lipid profile of alloxan induced diabetic rats. British J Med & Med Res. 2012;2(4):621-635.
24. WHO. Specifications for identity and purity and toxicological evaluation of food colours. WHO/Food Add/66.25 Geneva, WHO; 1966.
25. Antai SP, Anozie SO. Incidence of infantile diarrhea due to enteropathogenic *Escherichia coli* in Port Harcourt metropolis. J Applied Microbiol. 2008;62(3):227-229.
26. Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic *Escherichia coli* by multiplex PCR. Indian J. Med. Microbiol. 2012;30:279-284.
27. Ghosh MN. Toxicity studies .In: Fundamentals of experimental pharmacology, 2nd ed. Scientific Book Agency, Calcutta. 1984;153-158.
28. Klassen CD, Amdur, MO, Doull J. Casarett and Doull's Toxicology: The basic science of poison. 8th ed. Mc Graw Hill, USA. 199513-33.
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(3):124-126.
30. Sanchez-Elsner T, Ramirez JR, Rodriguez-Sanz F, Varela E, Bernabew C, Botella LM. A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and SMADS. J Mol Biol. 2004;36:9-24.

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. Mbaka GO, Adeyemi OO. Toxicity study of ethanol root extract of *Sphenocentrum jollyanum* (Menispermaceae) Pierre. Asian J Exp Biol Sc. 2010;1(4):860-874.
33. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. Afri J Biotech. 2005;4:833-837.
34. Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. Afr J Biotech. 2005;4:833-837.
35. Mc Knight DC, Mills RG, Bray JJ, Crag PA. Human Physiology. 4th Edition, Churchill Livingstone; 1999.
36. Teguia A, Telefo PB, Fotso RG. Growth performances, organ development and blood parameters of rats fed graded levels of steeped and cooked taro tuber (*Colocasia esculenta var esculenta*) meal. Livestock Res Rural Dev. 2007;19(6):1-7.

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