



Toxicological, Biochemical and Histological Effect of Heartfiz (a Polyherbal Formulation) on Rodents

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SUMMARY

Keywords:

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Objective: This study was undertaken to evaluate the histotoxicity of a polyherbal drug composed of *Allium sativum* bulbs, *Zingiber officinale* rhizomes, *Cassia tora* leaves extracts in a mixture of ethanol, bee honey and lime juice.

Methods: Acute toxicity of the polyherbal drug was evaluated on Swiss albino mice by feeding with graded doses of the drug in the ranges of 1.0 g to 20.0 g/kg body weight (bwt) orally and observed continuously for the first 4 hrs and hourly for the next 12 hrs, then 6 hourly for 56 hrs. Wistar rats were also fed with different doses of the drug for 30 days and the effects of the drug on vital tissues – liver, kidney, testes and heart were assayed histologically, biochemically and haematologically in sub-chronic toxicity model.

Results: The LD₅₀ of the polyherbal medicine was 13.8g/kg bwt. No significant increase in body weight in the treated groups. The drug had some deleterious effects on the liver and testes in which significant necrotic effect was observed in the hepatic parenchyma with severe hepatocytes vacuolization and sinusoidal congestion. The testes exhibited depletion in cellular mass of spermatogenic cells. Aspartate aminotransferases (AST) and creatinine levels showed insignificant increase while alanine aminotransferases (ALT) increased markedly. The drug significantly reduced ($p \leq 0.05$) plasma glucose and low density lipoprotein (LDL-cholesterol) levels, but increased high density lipoprotein (HDL-cholesterol) in the treated rodents.

Conclusion: Although the drug has some health benefits, it should be taken with precaution because of its potential toxic effect on liver and testes.

INTRODUCTION

Traditional medicine is the earliest known medical practice and its popularity in the developing world has continued to increase in spite of the giant stride and wide application of modern medicine. It has herbal medicine or phytomedicine as its major component. According to WHO, 80% of the population of developing countries rely on traditional medicine, mostly plant drugs, for their Primary Health Care needs.[1,2] Plant drug, therefore, remain the main therapeutic remedy in the developing countries and is widely used by many communities for the management of various human and animal diseases both of pathological and pathogenic origins. They have proved to be very useful especially in the management of certain disease conditions where synthetic drugs may lack curative potentials but employed only to ameliorate or suppress the disease symptoms such as epilepsy, convulsion and diabetes.

Herbal recipes are most often prepared from a combination of two or more plant products with the belief that the more the number of plant products included, the more effective the preparation would be in the concurrent treatment of different disease conditions.[3,4] An herbal recipe, therefore, may have many folklore claims as a result of a numerous organic constituents present in the preparation.[5]

The preparations may be administered in certain disease conditions over a long period of time without a proper dosage monitoring and consideration of toxic effects that might result from such prolonged use. Warning about the potential toxicity of such herbal therapies demands that the practitioners should be kept abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs.[5]

This herbal recipe, Heartfiz, was formulated by local herbalists and has been widely used in the management of certain disease conditions. It is claimed to have antihypertensive and heart beats regulatory effects. It is also used in the management of chest and lung pains and general body pains. The active plant materials include; *Allium sativum* bulb, *Zingiber officinale* rhizome and *Cassia tora* leaf. Each of the plant materials has been employed either alone or in combination with other herbs for the management of diseases and might be administered over a long period. The use of combination therapy is commonly practiced amongst traditional herbalists because of the belief that a mixture has synergistic effects and therefore a wider spectrum of activities. The herbal product contains in addition to the plant materials bee honey and lime juice. Bee honey has been recognized for its food and medicinal values. It has been evaluated for antibiotic effects in wound healing.[6] Also

chemical analyses showed that honey possess significant quantities of antioxidants, non-nutritive agents that can retard biologically destructive chemical reactions that cause rancidity in food and have been linked up to many chronic diseases like diabetes which potentiates free radical release.[7]

The aim of this study therefore was to evaluate the safety of the phytomedicine –Heartfiz- by carrying out histological, toxicological and biochemical studies on rodents, to establish potential adverse effects of this valuable medicinal product.

MATERIALS AND METHODS

Preparation of the medicine

The phytomedicine – Heartfiz: - was ceded by traditional practitioners, Afroherbs Natural Drugs Co, No 10 Emmanuel Taiwo Street, Ije-Ododo, Ijebu, Ikorodu, Lagos, Nigeria. The label claimed that the formulation was prepared with the following compositions; *Allium sativum*, *Zingiber officinale*, *Cassia tora* leaves, ethanol, purified bee honey, lime juice. Five bottles (1.5L) of the un-tampered polyherbal formulations were stored in a refrigerator at 4 – 6°C. Before the commencement of the study, 1000 ml of the formulation was filtered using Whatman's no 4 filter and the resulting 845.6 ml was freeze dried to yield 42.5 g gel. The polyherbal drug, a slightly thick brownish liquid, was stored in an amber plastic bottle. The prescribed dose for human is two table spoonfuls (20 ml) (equivalent to 0.3 ml/kg for an average man of 70 kg) three times a day (60 ml/day).

Animals

Swiss mice (20 – 25 g) and Wistar rats (140 ± 20 g) of either sex obtained from the Laboratory animal Center, College of Medicine, University of Lagos, Idi-Araba were kept under standard environmental condition of 12/12 hr light/dark cycle. They were housed in polypropylene cages (5 animals per cage), and were maintained on standard mouse chow (Feeds Nigeria Ltd) with water *ad libitum*. The animals were allowed to acclimatize for seven days to the laboratory conditions before the experiment. The use of the animals and the experimental protocol was in accordance with approved Experimental Ethics on Animals Use.[8]

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 overnight fasting of the animals, the control group received 0.3 ml of Tween 80 (2 %) solution orally. The doses 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g /kg were respectively administered orally to the groups from 80 % (w/v) solution of the gel which was prepared by dispersing 16g of the gel with 7ml Tween 80 (2%) solution in a 100ml beaker and transferred to a 20 ml volumetric flask. The beaker was thoroughly rinsed with the Tween solution; the content added to the volumetric flask and the volume made to mark with the Tween solution. 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 h observations) to observe any death or changes in general behaviour and other physiological activities.[9]

Sub-chronic toxicity test

A total of 20 male and female wistar rats weighing 140g ± 10 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*. They were weighed and divided into four groups of five animals each. After fasting the rats overnight the control group received a dose of 0.5ml of 2% Tween 80 solution orally once a day for 30 days. The three treated groups respectively received the following doses: 100, 250 and 500 mg/kg body weight (bwt) of the gel orally once a day for 30 days.[3,2,9] The gel suspension (12% w/v) was prepared by dispersing the gel (12g) with 45 ml of Tween 80(2%) solution in a beaker, and transferred to a 100 ml volumetric flask. The beaker was then rinsed with Tween solution and the content transferred to the volumetric flask and the volume made to mark with the 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. On the 31st day they were made unconscious by cervical dislodgement and blood was collected via cardiac puncture in two tubes: one with heparinized tube to separate plasma for the analysis of biochemical and haematological parameters; another with fluoride oxalate tube for blood glucose estimation.

The heparinized blood centrifuged within 5 min of collection at 4000 rpm for 10 min to obtain plasma was used to analyze for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods [10], while protein content was determined using enzymatic spectroscopic method.[11] The blood samples collected with fluoride oxalate tubes were also centrifuged within 5 min of collection at 4000 rpm for 10 min to obtain plasma used for plasma glucose estimation.[12] Total cholesterol (TC), triglyceride (TG) and high density lipoprotein-cholesterol (HDL-cholesterol) levels were estimated with heparinized blood using modified enzymatic procedures from Sigma Diagnostics.[13] Low density lipoprotein-cholesterol (LDL-cholesterol) levels were calculated using Friedwald equation.[14] The blood samples were analyzed for red blood cells (RBC) by haemocytometric method.[15] Haematocrit was estimated as described by Ekaide et al [16]. In brief, 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 (PCV) was read by sliding the tube along a “critocap” chart until the meniscus of the plasma intersected the 100 % line. Haemoglobin (Hb) contents were determined using Cyanmethaemoglobin (Drabkin) method.[16] The white blood cells (WBC) count were as described by Dacie and Lewis.[15]

Slide 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.[17]

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 $\pm$ standard error of the mean.

Results

Acute toxicity test

The acute toxicity study (Table 1) recorded 100 % death for all the animals that received 20.0g/kg bwt of the extract and 37.5% and 12.5% for animals that received 15.0g and 10.0 g respectively, while there was no death in the animals that received 5.0g/kg bwt and less. The median acute toxicity (LD_{50}) of the extract was determined to be

13.8g/kg bwt.

Variation of weights

The effect of the drug on the bwt of the control and treated animals is as shown in Table 2 and the percentage increase in the weight of the treated animals compared with the control is shown in Figure 1. Generally, there was insignificant ($p \geq 0.05$) increase in the bwt of the treated animals compared to the control. A drop in bwt was observed in all the treated animals in the first 5 days of the treatment. From day 10 the bwt increased continually to the end of the experiment. The result of the effect of the phytomedicine on the organs was presented in Table 3. Significant changes ($p < 0.01$) in the weights of various organs were observed only in the animals treated with the highest dose of the extract (500 mg/kg bwt). Macroscopic examinations showed no changes in the colour of the organs of the treated animals compared to the control.

Table 1: The acute toxicity of the phytomedicine in mice

Group	No of Mice	Doses of extract (g/kg)	Number of dead mice	% Cumulative of dead mice
1	5	control	0	0.0
2	5	1.0	0	0.0
3	5	2.0	0	0.0
4	5	5.0	0	0.0
5	5	10.0	1	12.5
6	5	15.0	2	37.5
7	5	20.0	5	100.0

Control group: each mouse received orally 0.3mL Tween 80 (2%) solution

Table 3: The effects of the phytomedicine on kidney, heart, liver and brain of the control and treated animals in the sub-chronic toxicity study

Organ	Control	100 mg/kg	250 mg/kg	500 mg/kg
Heart (g/100g)	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0
Kidney (g/100g)	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	0.4 $\pm$ 0.0**
Liver (g/100g)	3.0 $\pm$ 0.0	3.1 $\pm$ 0.4	3.2 $\pm$ 0.2	4.7 $\pm$ 0.2*
Brain (g/100g)	0.8 $\pm$ 0.0	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.9 $\pm$ 0.0**

Mean $\pm$ SEM, (n=5), * $p < 0.05$; ** $p < 0.01$ vs. control group. Control group received 0.5mL Tween 80 (2%) solution.

Table 2: The effects of the phytomedicine on weight changes in the control and treated rats in the sub-chronic toxicity study

Dose	DAY1	DAY 5	DAY10	DAY15	DAY 20	DAY 25	DAY 30
Control	140.0 $\pm$ 2.7	141.0 $\pm$ 2.2	145.1 $\pm$ 1.8	147.5 $\pm$ 1.1	150.6 $\pm$ 2.5	153.8 $\pm$ 2.7	154.5 $\pm$ 2.2
100mg/kg	134.0 $\pm$ 2.1	132.2 $\pm$ 0.4*	136.3 $\pm$ 1.2*	140.1 $\pm$ 1.8*	141.5 $\pm$ 0.2*	144.5 $\pm$ 0.2	146.3 $\pm$ 0.3
250mg/kg	132.0 $\pm$ 0.3	129.2 $\pm$ 1.5*	130.0 $\pm$ 7.5**	134.2 $\pm$ 6.3	138.8 $\pm$ 5.0*	140.6 $\pm$ 1.2*	142.0 $\pm$ 1.5*
500mg/kg	130.1 $\pm$ 4.2	126.3 $\pm$ 6.9**	128.1 $\pm$ 4.8	131.8 $\pm$ 5.0	134.8 $\pm$ 5.0	138.1 $\pm$ 2.3*	138.5 $\pm$ 1.2*

Mean $\pm$ SEM, (n=5) * $p < 0.05$; ** $p < 0.01$ vs. control group. Control group received 0.5mL Tween 80 (2%) solution

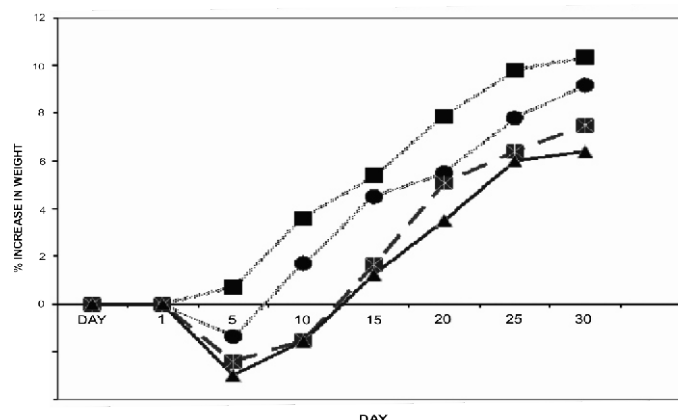


Figure1: Percentage increase in weight of the control and treated animals in the sub chronic study

■control ●100mg/kg bwt, ✕ 250mg/kg bwt ▲ 500mg/kg bwt

Histological studies

Figures 2-9 showed the histological studies of the effects of the phytomedicine on target organs. Tissue morphological alterations were observed only in the highest dose treatment.

The hepatic tissue of the control (Figure 2) showed the portal tract with poorly defined hepatic lobulation. The hepatocytes which showed radial arrangement around the central veins were interspaced by well defined hepatic sinusoids. In the animals (Figure 3) treated with 500mg/kg bwt of the formulation, hepatic tissue showed pathologic changes in which significant necrotic effect was observed. There was a diffuse mass within the hepatic parenchyma with severe hepatocytes vacuolization and sinusoidal congestion.

The kidney of the control group showed cortical area of renal tissue with glomerular apparatus (Figure 4) forming a rounded mass and separated from the surrounding structures by Bowman's space. In the animals treated with 500 mg /kg bwt of the formulation (Figure 5), no histological changes were observed compared to the control.

Figure 6 showed normal testis, the seminiferous tubules cut at different planes showed distinct boundary separated by interstitial spaces. The wall of the seminiferous tubules showed thick epithelium. Close to the thick epithelium is compactly arranged differentiating spermatogenic cells while the matured cells, spermatids and spermatocytes formed a cluster in the lumina. In the treated animals (Figure 7) morphologic changes were observed. The seminiferous tubules exhibited depletion in cellular mass of spermatogenic cells which was more marked at the basement.

The cardiac muscle of the control animals (Figure 8) in the longitudinal section showed deeply stained muscle fibres forming a branched network. The treated animals (Figure 9) showed no indication of cellular lesion or distortion.

Sub-chronic toxicity test

Table 4 is a summary of the results of the effects of the drug on the biochemical parameters. There was significant decrease ($p < 0.05$) in the plasma glucose level especially at the highest dose in the treated rats compared with the control. There was an increase in plasma protein in the two lower doses of the formulation whereas marked decrease was observed in the highest dose of administration. Creatinine level increased in the lowest dose of treatment while exhibiting significant decrease ($p < 0.05$) in the two higher doses compared to the control. There was no significant increase in AST level while a marked increase in ALT level was observed in all treated animals especially in the group treated with the highest dose. The drug significantly reduced ($p \leq 0.05$) plasma glucose. Also, significant decrease ($p < 0.05$) in the plasma total cholesterol level was observed in the group that received the highest dose of poly-herbal drug. Decrease in triglyceride and LDL-cholesterol levels occurred while significant increase ($p < 0.05$) in HDL-cholesterol level was observed.

The effects of the drug on the blood components and the electrolytes were presented in Table 5. Significant decreases ($p < 0.01$) were observed in the Haemoglobin content while RBC count also indicated appreciable increase with the exception of highest dose treatment which surprisingly recorded a decrease. PCV also showed an increase in all the doses with the exception of highest dose

treatment while WBC increase was only noticeable in the groups that received the lowest and highest doses of the formulation. There was insignificant decrease ($p \geq 0.01$) in calcium level in all the treated animals compared to the control while phosphorus level exhibited an increase only in lowest and highest dose treatments.

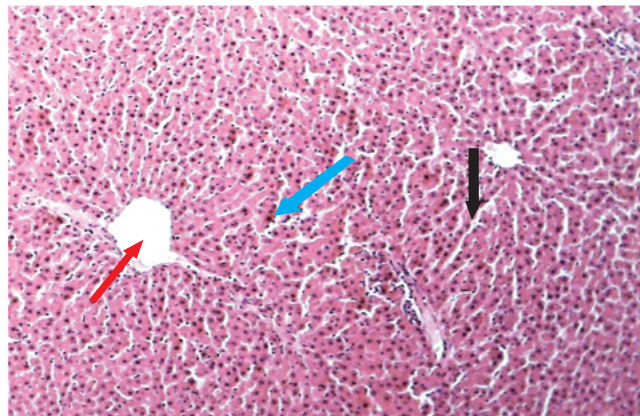


Figure 2: The histology of the cross section of hepatic tissue of the control group showing normal hepatocytes (Blue arrowed) radially arranged around the central vein (Red arrowed) interspaced by well-defined sinusoids (Black arrowed) (H and E stain) mag. x100.

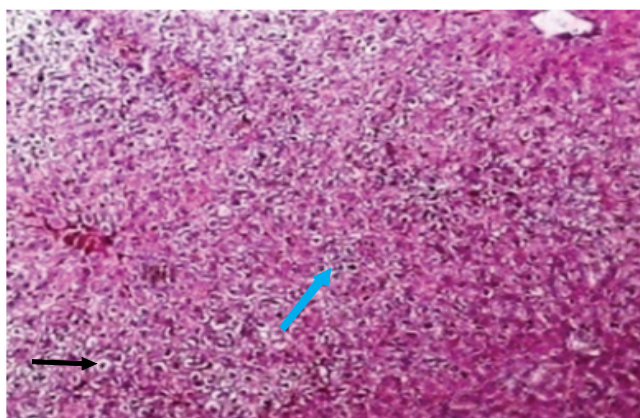


Figure 3: The histology of the cross section of hepatic tissue administered with 500mg of the polyherbal drug showing partial distortion in hepatic parenchyma (Blue arrowed) with severe hepatocytes vacuolization (thin arrowed) (H and E stain) mag. x100.

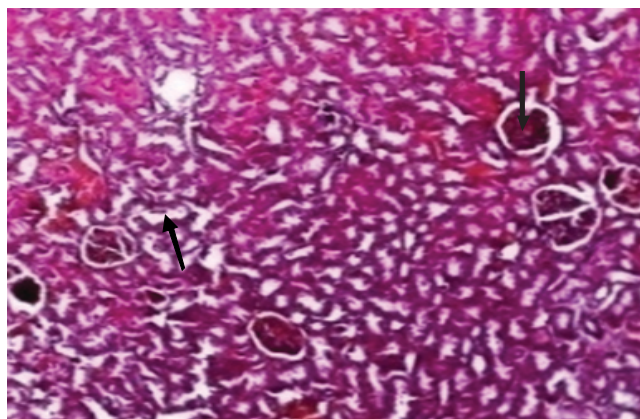


Figure 4: The histology of a cross section of renal tissue (control group) showing renal corpuscles (Thick black arrowed) and convoluted tubules (Thin black arrowed) (H and E stain) mag. x100.

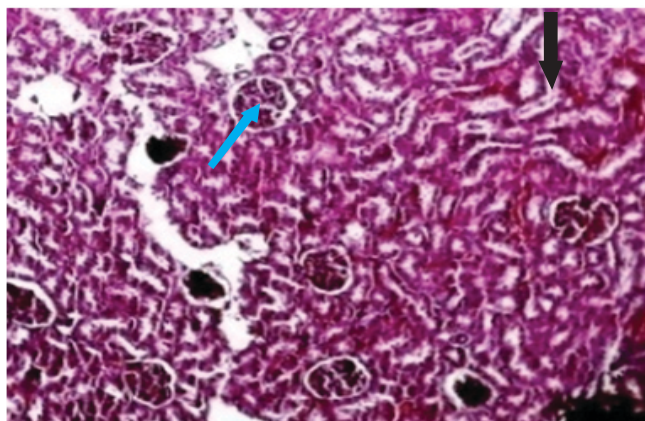


Figure 5: The cross section of renal tissue of the group treated with 500mg of the drug. No renal abnormality was observed at renal corpuscles (Blue arrowed) and the interstices (Black arrowed) (H and E stain) mag. x100.

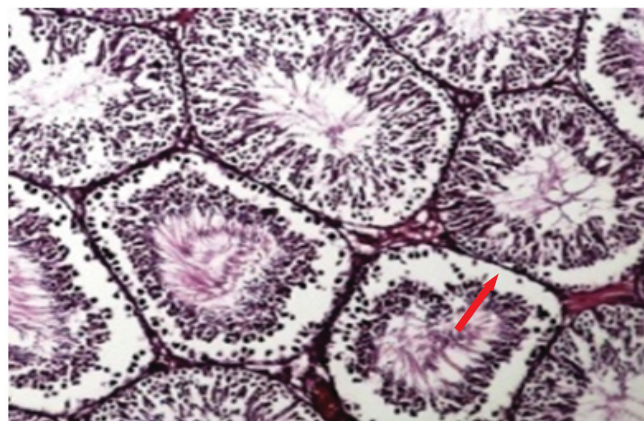


Figure 7: The cross section of seminiferous tubules of testis treated with 500mg of the polyherbal drug showing scanty sperm cells (Red arrowed) at the basement (H and E stain) mag. x100.

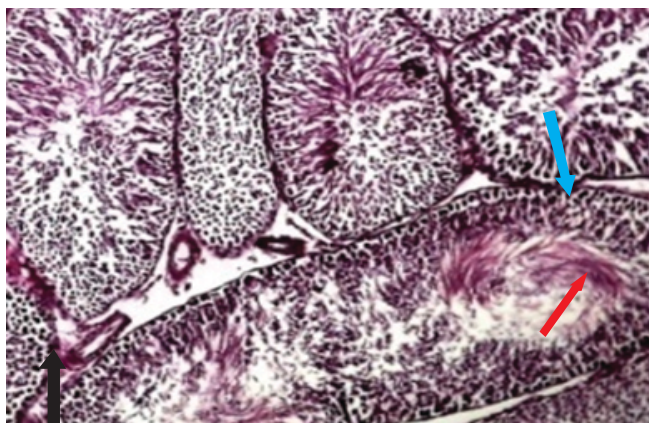


Figure 6: The cross section of testes of the control group showing seminiferous tubules and the interstitium (Black arrowed). Close to the basement are compact spermatogonia cells (Blue arrowed) while the tail of spermatozoa form wavy appearance at the centre (Red arrowed) (H and E stain) mag. x100.

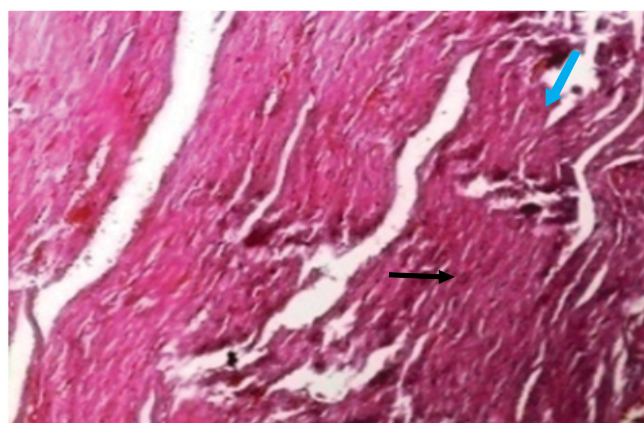


Figure 8: The cross section of the cardiac muscle of the control group showing the myocyte (Thin black arrowed) and unremarkable interstitium (Blue arrowed) (H and E stain) mag. x100.

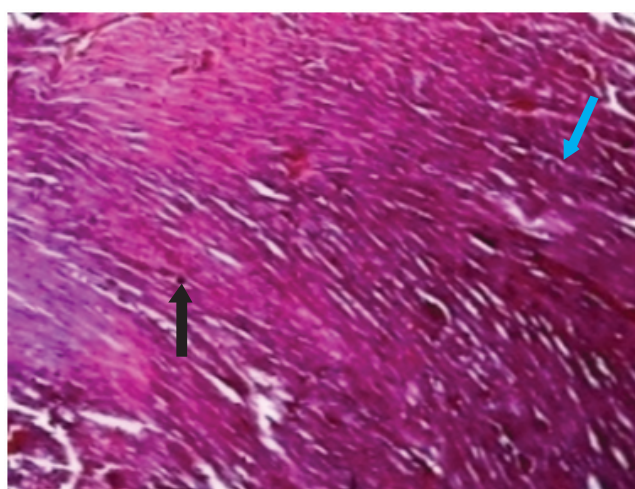


Figure 9: The cross section of the cardiac muscle of the group treated with 500mg of the drug indicating normal myocytes (Black arrowed) and interstitium (Blue arrowed) (H and E stain) mag. x100.

Table 4: Effect of daily administration of the drug for 30 days on biochemical profiles of control and treated rats in the sub-chronic toxicity study

Parameter	control	100 mg/kg	250 mg/kg	500 mg/kg
Glucose (mg/dl)	92.3±0.1	74.2±0.6*	52.5±0.1*	54.1±0.3*
Cholesterol (mg/dl)	62.6±0.6	51.8±0.0*	33.6±0.4*	9.6±1.4*
Triglyceride (mg/dl)	10.6±0.4	61.7±1.5*	26.6±0.0*	14.4±0.0*
HDL (mg/dl)	44.3±1.6	49.5±0.0	46.7±0.1	27.3±3.1
LDL (mg/dl)	74.9±0.3	68.8±2.6	50.8±0.6	47.4±1.6
Protein (g/dl)	7.5±0.3	8.3±0.3	8.6±0.6	6.0±0.2**
Creatinine (mg/dl)	3.5±0.5	5.4±0.0	1.5±0.1**	0.9±0.0*
AST (IU/L)	10.2±1.2	10.5±2.5	10.6±0.0	16.5±2.3**
ALT (IU/L)	12.5±1.8	15.6±2.5**	22.2±2.6*	34.0±3.6*

Mean±SEM (n=5), *p<0.05; **p<0.01 vs. control group. Control group received 0.5mL Tween 80 (2% solution).

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

HDL: High density lipoprotein-cholesterol

LDL: Low density lipoprotein-cholesterol

Table 5: Effect of the drug on haematological parameters of the control and treated animals in the sub-chronic toxicity study

Parameter	control	100 mg/kg	250 mg/kg	500 mg/kg
Haemoglobin (mg/dl)	14.5±0.3	16.0±0.1**	15.1±0.2**	11.5±0.4**
RBC (10 ⁶ /mm ³)	5.9±0.0	7.2±0.1	6.3±0.1	5.5±0.1
WBC (10 ³ /mm ³)	6.4±0.0	7.3±1.7**	4.8±0.0**	8.7±0.0*
PCV %	43.0±2.2	49.1±1.5	46.9±2.5*	35.1±0.5*
Calcium (mg/dl)	9.7±0.0	8.3±0.1	8.3±0.0	9.2±0.0
Phosphorus (mg/dl)	16.0±0.0	18.8±1.0	13.3±0.0**	20.7±0.0**

MeanSEM (n = 5), *p<0.05; ** p<0.01 vs. control group. Control group received 0.5mL normal saline solution.

RBC: Red blood cell

WBC: White blood cell

PCV: Percentage cell volume

DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand.[10,4] In rural Nigerian communities, the exclusive use of herbal drugs prepared and dispensed by herbalists without formal training for the treatment of diseases is still very common. This practice is usually characterized by improper dose prescription. Besides, some of the herbal formulations though may be effective in treating certain disease conditions have exhibited harmful effect with marked deleterious changes on tissue morphology of some vital organs.[18,19] Experimental screening method is therefore important for ascertaining the safety and efficacy of these herbal products as well as to establish their active components.[1]

The acute toxicity study of the extract 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 hr while the animals that received 5.0 g/kg dose survived beyond the 24 hr of observation. The median acute toxicity value (LD₅₀) of the extract was determined to be 13.8 g /kg bwt. According to Klaasen *et al.*, [20] the extract could be classified as being slightly toxic, since the LD₅₀ was found to be between 5.0 g and 15.0 g/kg.

The gram equivalence of the LD₅₀ in an average adult man would translate to 898.9 g dose of the drug.[18] This is a very high value and makes 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. However, since the animals did not convulse before dying, it postulated that the extract did not kill the mice by some action on the nervous system.[21]

The effects of the drug on the body weight variation of the test animals was remarkable since no significant increase in weight was observed compared with the control. This effect was clearly shown in the mean percentage increase in the weights of the treated animals compared to the control. The observed decrease in the weights could be attributed to the suppression of the animals' appetite by the drug which showed dose dependent decrease.

There were no morphological changes in the colour of the various organs of the treated animals compared to the control but histological studies revealed that the drug had some deleterious effects on the liver and testes but not in the kidney and heart. The severe hepatocytes vacuolization and sinusoidal congestion observed in the hepatic tissue was established only in the highest dose of the formulation. Although this suggested dose effect but considering that the formulation showed high safety margin of 13.8 g /kg bwt, other considerations like drug interaction from different plant

components could be the probable cause. Also, animals that received the highest dose of the drug exhibited significant changes in their various organs weights especially the kidney, liver and brain.

Although there was a slight increase in the plasma protein level in the two lower dose treatments, the highest dose treated recorded marked decrease. Besides, the kidney tissue histology showed no morphological changes indicating that there was no sign of impaired renal function.[22] Decrease in plasma creatinine concentration at higher doses further suggested that no kidney damage took place specifically by renal filtration mechanism.[13] The finding corroborated with the result of the photomicrograph study where no morphological change was observed. The liver and heart release AST and ALT, thus an elevation in their plasma concentrations is an index of the organs damage.[2,14] The ALT which is more specific to the liver showed marked increase particularly at the highest dose which suggested that inflammatory changes might have occurred in the liver. This was confirmed by the histological study. However, AST which is also an index for heart damage[14] showed no significant changes compared to the control which implied that the formulation did not exhibit harmful effect to the heart as was evident in the tissue histology.

The poly-herbal medicine though was not indicated for the treatment of diabetes had pronounced plasma glucose level lowering effect especially at the highest dose in the treated rats compared to the control. This indicated the presence of hypoglycaemic components in the drug. The decrease in the plasma TC and TG levels might be attributed to the presence of hypolipidemic agents in the poly-herbal medicine. The increase in HDL-cholesterol level (anti-atherogenic agent) and a reduction in LDL-cholesterol level observed in all the treated animals are confirmatory to the fact that the drug can reduce the cardiovascular risk factors that contribute to the death of atherogenic subjects.[14] The reduction of the cardiovascular risk factors gave further support as to the traditional use of the herbal formulation.

The observed significant ($p \leq 0.01$) decrease in the Hb level in the group treated with the highest dose might be due to the decreased absorption of iron. The calcium levels were not affected in all the treated animals while marked increase in the level of phosphorus was observed only in the animals treated with the highest dose of the extract. It has been reported that increase in the level of phosphorus may be associated with renal problems.[23,24] But since protein levels were not affected, the increase might be due to an unknown cause. The formulation on the other hand caused depletion in the cellular mass of testicular tissue.

CONCLUSION

The high LD₅₀ value (13.8g/kg) obtained was a clear indication that the poly-herbal preparation could be safe for use. However, the study revealed that the drug at the highest doses investigated did provoke toxic effects to the animals' liver and testes but without harmful effect on the kidney and heart. Therefore in the long term treatment of diseases the liver and the testicular functions must be carefully monitored to prevent damage. On the other hand, the study showed that the drug has some hypoglycaemic activity and good reducing effects on cardiovascular factors thus validating its use by herbalists.

Conflict of Interest

The authors declare no competing interest.

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