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An Evaluation of Acute and Subchronic Toxicities of a Nigerian Polyherbal Tea Remedy

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Abstract: The evaluation of the acute and subchronc toxicities of a Nigerian polyherbal tea remedy, prepared with *Anthocleista vogelii*, *Ficus exasperata* leaves and *Viscum album* (mistletoe), was carried out in Swiss albino mice and Wistar rats of both sexes. The lyophilized extract in the dose ranges of 1.0 g to 20.0 g/kg body weight was administered orally to the mice and observed continuously for the first 4 h and hourly for the next 12 h, then 6 hourly for 56 h (72 h, acute toxicity). Wistar rats were also fed with different doses of the lyophilized formulation for 30 days and the effects on some tissues - heart, liver and testes - were histologically evaluated. Also the effects on the biochemical and haematological parameters were evaluated (subchronic toxicity model). The median acute toxicity value (LD_{50}) of the polyherbal tea was determined to be 8.970 g/kg body weight. The tea 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 groups compared to the control. No significant increase in the body weight was observed in the treated groups. Aspartate Aminotransferases (AST) and creatinine levels were not significantly increased while significant decrease in Alanine Aminotransferases (ALT) levels were observed in all the treated groups. The high LD_{50} value of the drug implies that the drug could be safe in one dose treatment. The study also revealed that the polyherbal tea may have good hypoglycemic effects and favourable reducing effects on the cardiovascular risk factors.

Key words: Acute toxicity, sub-chronic toxicity, *Anthocleista vogelii*, *Ficus exasperata* leaves, *Viscum album* (mistletoe)

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 (Ogbonnia *et al*., 2008a). Herbal medicine or phyto-medicine 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 (Rickert *et al*., 1999; Ogbonnia *et al*., 2008b). The use of herbal remedies especially in the form of teas for the treatment of various diseases is gaining increasing popularity making them the main stay of health care system especially among the rural populace in the developing countries. Their increasing popularity could be attributable 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 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 (Bandaranayake, 2006). 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.

Herbal medicine is most often a 'polyherbal' prepared always 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 bioactive principle(s) in most herbal

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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 on a clear scientific definition of the raw materials used for such preparations. Also herbal medicine may have multiple physiological activities and an herbal preparation could be used in the treatment of a variety of disease conditions (Pieme *et al*., 2006). They may be 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 (Tedong *et al*., 2007).

Serena stress free herbal tea is a popular polyherbal medicine used generally for well being. It is prepared with *Anthocleista vogelii*, *Ficus exasperata* leaves and *Viscum album* (Mistotoe). These are cherished medicinal plants and different parts of the plants are widely used by the traditional herbalists in the treatment of various diseases. Herbal combination therapy is more common, since most traditional medical practitioners believe that a combination of many plant products would create the desired synergy.

The aim of this study was to evaluate the safety of a polyherbal preparation, Serena Stress Free Tea, by carrying out the acute and subchronic toxicity studies in animals. Subchronic toxicity evaluation is required to establish potential adverse effects of this highly valuable polyherbal medicine that is now widely consumed for its physiological benefits.

MATERIALS AND METHODS

The powdered polyherbal tea was conceded by Herbal Fountain Nigeria Ltd of No 16 Light Peace Street, Alagbado Lagos, Nigeria. The polyherbal medicine was prepared with dried *Anthocleista vogelii*, *Ficus exasperata* leaves and *Viscum album* (mistletoe).

Preparation of the extract: The polyherbal tea, a moderately fine powder, was made of the three plants and mixed in equal proportions The prescribed dose for human is two sachets of 20 g each daily. For acute and subchronic studies, 500 g of the powder was subjected to extraction by decoction with 1000 ml of distilled water, then filtered using Whatman's no 4 filter and the resulting 485 ml filtrate was subsequently freeze dried to 34.7 g gel which was stored in an air tight container in a fridge until it was needed.

Animals: Swiss mice (20-25 g) and Wistar rats (160±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 h light/dark cycle. They were housed in polypropylene cages (5 animals per cage) and were maintained on mouse chow (Livestock Feeds Nigeria Ltd), provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. The use and care of the animals and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies (ILAR, 1996).

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 the control group and six treated groups with each group comprising five animals. Following the 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 body weight (bw) were respectively administered orally to the other groups from 80% (w/v) solution. The animals were observed continuously for the first 4 h and then for each hour for the next 12 h, followed by 6 hourly intervals for the next 56 h giving a total of 72 h observations (Shah *et al*., 1997; Burger *et al*., 2005). Death or changes in general behaviour and other physiological activities were noted.

Subchronic toxicity tests: Male and female Wistar rats weighing 160±20 g were used. The animals were weighed and divided into four groups of five animals each. After fasting the rats overnight the control group received a dose of 0.5 ml 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 bw of the gel orally once a day for 30 days (Pieme *et al*., 2006; Joshi *et al*., 2007; Mythilypriya *et al*., 2007). They were weighed every five days, from the start of the treatment, to note any weight changes. At the end of the experiment the animals were starved overnight before being made unconscious using mild diethyl ether anaesthesia and blood was collected via cardiac puncture in two tubes: one with EDTA for analysis of haematological parameters while heparinized tube was used to collect blood for biochemical estimation. 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 (Wasan *et al*., 2001). LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and creatinine by standard enzymatic assay methods (Horder and Sampson, 1991). Plasma glucose contents and protein contents were

determined using enzymatic spectroscopic methods (Hussain and Eshrat 2002). Haematocrit was estimated using the methods described by Ekaidem *et al.* (2006). 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 min 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. Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem *et al.*, 2006).

Tissue histology: The vital organs; liver, kidney, heart and testes were fixed in 10% formal saline for six days before embedding in paraffin wax. A section of each organ tissue at 5 µm was stained with Haematoxylin and Eosin (H and E). Each section was examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

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.

RESULTS

Acute toxicity test: The acute toxicity study (Table 1) recorded 100% death for all the animals that received 20.0 g/kg bw of the extract and 37.5% and 12.5% for animals that received 15.0 g and 10.0 g respectively, while no death occurred in the animals that received 5.0 g/kg bw and less. The median acute toxicity (LD_{50}) of the extract was determined to be 9.0 g/kg bw.

Table 1: Acute toxicity of a Nigerian polyherbal remedy extract in mice

Doses of drug g/kg	No. of animals	No. of animals dead	Cumulative death (%)
0.5	0	0	0.0
1.0	5	0	0.0
2.5	5	0	0.0
5.0	5	0	0.0
10.0	5	1	12.5
15.0	5	2	37.5
20.0	5	5	100.0

Control group received orally 0.3 ml each of normal saline

Variation of weights: The effect of the drug on the body weights of the control and treated animals is shown in Table 2 and the percentage increase in the weight of the treated animals compared with the control is shown in Fig. 1. Generally, there was insignificant ($p\geq0.05$) increase in the body weights of the treated animals compared to the control. A drop in body weight was observed in all the treated animals in the first 5 days of the treatment. From day 10, the body weights increased continually to the end of the experiment.

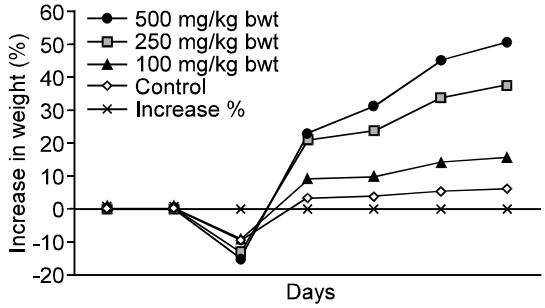

Fig. 1: Percentage weight variations of the control and treated animals in the subchronic study

The effects of the polyherbal medicine on the weight variation of the vital organs of the control and treated animals were presented in Table 6. There were no significant changes observed in the weights of the organs of the control and the animals treated with various doses but significant increase ($p<0.05$) was observed in the heart and kidney of the group treated with 500 mg/kg bw.

Biochemical parameters: Table 3 is a summary of the results of the effects of the polyherbal tea on the biochemical parameters. There was significant decrease ($p<0.05$) in Alanine Amino Transferase (ALT) levels in the treated animals compared to the control. But Aspartate Amino Transferase (AST), plasma protein and creatinine showed marginal increase in animals treated with the highest dose of the tea compared to the control. In the lipid profile study, there was insignificant increase in total cholesterol and High Density Lipoprotein (HDL) levels in the treated groups compared to the control. In contrast, triglycerides and Low Density Lipoprotein (LDL) decreased markedly in animals that received the highest dose of the polyherbal drug. There was however no changes in the total bilirubin, urea and albumin of the treated groups compared to the control while significant increase in alkaline phosphate level occurred in all the treated groups.

Haematological and blood chemistry studies: The effects of the drug on the blood components and white blood cell differentials were presented in Tables 4 and 5 respectively. Significant increases ($p<0.05$) were observed in the haemoglobin content and Red Blood Cells (RBC) in all the groups compared to the control while significant increase in Packed Cell Volume (PCV) was noticeable only in the group that received 500 mg/kg bw. There was however no significant change in Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) level in all the treated animals compared to the control while White Blood Cells (WBC) increased marginally in the groups that received lower doses (100 and 250 mg/kg bw) of the herbal tea and

Table 2: The effects of the polyherbal remedy extract on body weight changes in the control and treated rats in the subchronic toxicity study

Dose	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Control	165.0±2.1	148.7±1.8	170.5±1.1	171.3±2.5	173.7±2.7	175.1±2.2
100 mg/kg	150.9±0.1	150.1±2.8	158.7±0.7**	159.0±1.4**	163.1±1.3*	164.3±2.3**
250 mg/kg	130.0±0.3	125.2±1.5**	145.0±7.5**	148.2±6.3**	155.7±3.7*	158.6±1.2*
500 mg/kg	140.2±6.1	137.2±6.9**	143.1±4.8**	150.7±4.9**	156.1±4.6*	158.3±2.3*

N = 5, *p≤0.05, **p≤0.01. Control animals were administered with 0.5 ml Tween 80 (2%) solution

Table 3: Effect of daily administration of the tea extract for 30 days on biochemical profiles of the control and the treated rats

	Dose mg/kg body weight			
Parameter	Control	100	250	500
Glucose (mg/dl)	95.2±0.1	100.3±0.1	70.1±0.3*	102.7±1.5
AST (IU/L)	53.8±0.3	53.7±0.5	53.9±1.1	54.9±1.2
ALT (IU/L)	8.4±0.4	7.9±0.3	4.5±0.6*	4.1±0.7*
T. Bil (mg/dl)	4.8±0.1	5.6±0.1	4.7±0.1	4.7±0.1
Creatinine (mg/dl)	8.1±0.1	7.9±1.2	7.9±0.2	8.5±0.2
Urea (mg/dl)	6.5±1.1	5.8±0.5	6.6±0.6	7.0±0.2
Albumin (mg/dl)	4.5±3.9	4.5±0.9	4.6±1.3	4.7±3.1
T. Protein (mg/dl)	88.3±0.1	88.9±0.2	88.3±0.2	90.2±0.1
HDL (mg/dl)	24.7±0.1	25.4±0.4	26.1±0.3	28.7±0.1
LDL (mg/dl)	98.2±0.1	87.2±0.1	89.1±0.9	78.3±0.1*
Total cholesterol (mg/dl)	81.8±0.5	83.8±0.3	87.9±0.2	87.2±0.1
Total triglycerides (mg/dl)	59.6±3.1	53.0±0.1	55.1±0.1	49.1±0.2*
Alk Phos	175.5±13.2	45.3±6.8*	53.5±1.2*	65.3±13.2*

N = 5, *p≤0.05, **p≤0.01. Control animals were administered with 0.5 ml Tween 80 (2%) solution

Table 4: Haematological values of control and treatment rats with the polyherbal tea for 35 days in subchronic study

	Dose mg/kg body weight			
Parameter	Control	100	250	500
RBC x 10^6	6.0±0.1	7.9±0.4*	7.8±0.7*	7.9±0.8*
Hb (g/dl)	13.3±0.2	15.4±0.4*	15.8±0.8*	15.7±1.3*
PCV (%)	39.1±1.0	41.2±0.5	39.3±1.1	49.9±4.5*
WBC x 10^3	4.4±0.5	5.4±0.6	4.9±0.8	7.7±0.9*
MCV (FL)	6.9±1.4	6.5±2.5	5.8±2.2	6.7±2.3
MCH (pg)	19.3±0.3	19.2±0.2	17.5±0.4	20.2±0.6
MCHC (g/dl)	34.1±2.2	32.5±0.9	35.1±1.1	33.8±0.7

N = 5, *p≤0.05, **p≤0.01. Control animals were administered with 0.5 ml Tween 80 (2%) solution. MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration, MCV = Mean Corpscular Volume

Table 5: White blood cells differential for control and the rats treated for 35 day with the polyherbal remedy

	Dose mg/kg body weight			
Parameter	Control	100	250	500
Lymphocyte (%)	79.60±8.3	78.60±2.4	86.10±1.9	85.1±2.6
Neutrophil (%)	25.20±2.9	26.00±2.9	39.80±0.9*	38.0±5.6*
Monocytes (%)	2.00±0.1	1.30±0.3	2.00±0.2	3.9±0.8**
Platelet (%)	65.03±8.9	83.07±5.8*	78.03±10.1**	81.9±8.6*

N = 5, *p≤0.05, **p≤0.01. Control animals were administered with 0.5 ml Tween 80 (2%) solution

Table 6: Weight variation of organs of the control and rats treated with polyherbal tea remedy doses in the subchronic study

	Dose mg/kg body weight			
Parameter	Control	100	250	500
Heart	0.4±0.1	0.4±0.2	0.4±0.1	0.8±0.1*
Lungs	1.8±0.5	2.1±0.3	2.2±0.2	2.6±0.2
Liver	5.6±0.3	5.9±0.3	6.0±0.4	6.1±0.6
Kidney	1.3±0.1	1.8±0.1	1.6±0.1	1.9±0.1*

N = 5, *p≤0.05, **p≤0.01. Control animals were administered with 0.5 ml Tween 80 (2%) solution

showing significant increase at the dose of 500 mg/kg. Significant increase ($p<0.05$) in the level of lymphocyte, neutrophil and platelet were observed in all the treated animals with the exception of neutrophil which level was comparable to the control at the dose of 100 mg/kg. The monocyte level on the other hand increased significantly at the highest dose of the drug.

Tissue histology: A cross section of the normal testicular tissue (Fig. 2A1) showed the seminiferous tubules cut in different planes having distinct boundary

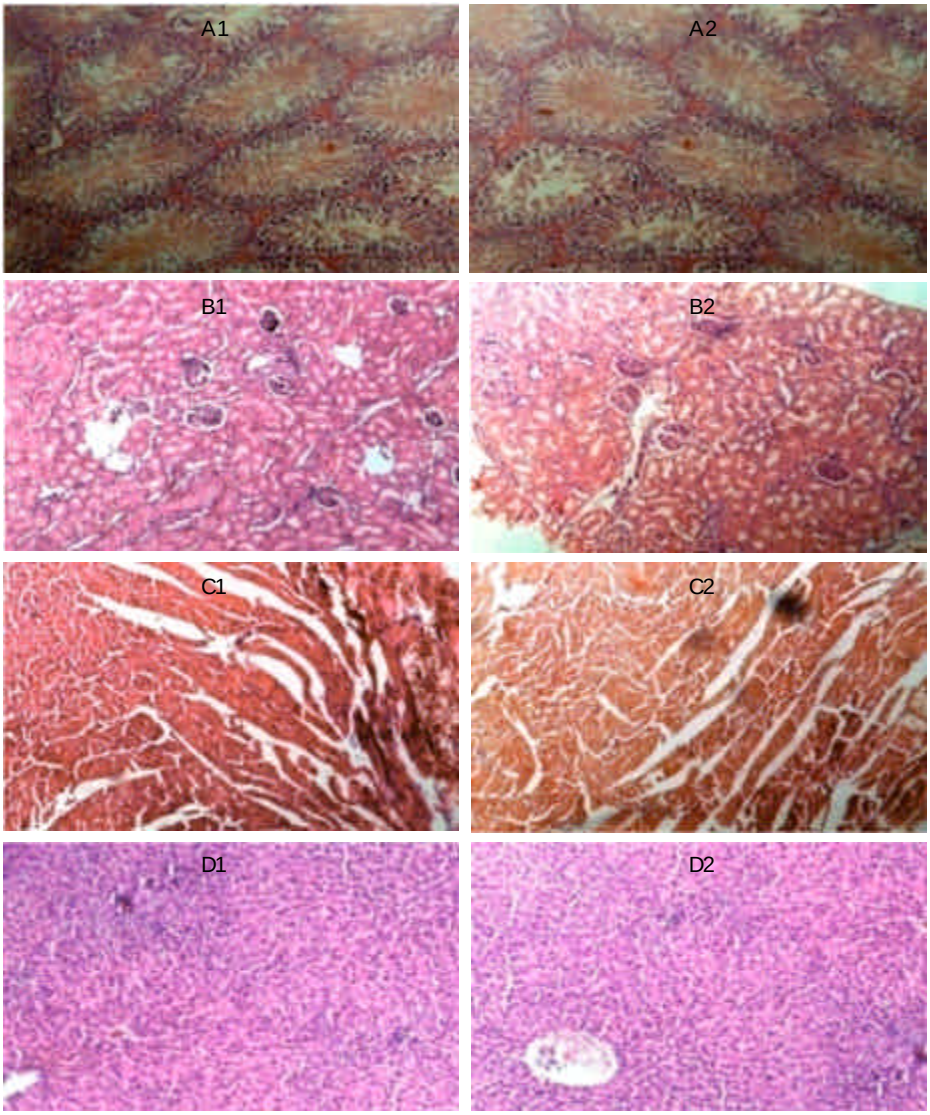

Fig. 2: (A-D) Histological studies of some organs-testes, liver, kidney and heart of the control and animal treated with 500 mg/kg bwt of serena stress free extract. The histology of testes of the control group A1 and A2 animals treated with 500 mg/kg body weigh of the extract showing cross sections of seminiferous tubules (H and E stain) mag. x100. The histology of a cross section of renal tissue of the control group B1 and B2 animals treated with 500 mg/kg bodyweight of the extract showing renal corpuscles and Bowman's spaces (H and E stain) mag. x100. The cross section of the cardiac muscle of the control group C1 and C2 of the animals treated with 500 mg/kg body weight of the extract showing the branched network of muscle fibres (H and E stain) mag. x100. The cross section of liver of the control group D1 and D2 of the animals treated with 500 mg/kg body weight of the extract showing central vein and hepatocytes radially arranged (H and E stain) mag. x100

and between which were the interstitium. Close to the outer wall of the seminiferous tubules were the differentiating germ cells while the spermatozoa formed a cluster at the lumina. The photomicrograph of treated animal testes (Fig. 2A2) showed no evidence of abnormal features both in the seminiferous tubules and at the interstitium.

The photomicrograph of the normal renal tissue (Fig. 2B1) showed the cortical area. The renal corpuscles appeared as a dense rounded mass surrounded by narrow space, the Bowman's space. No morphological changes were observed in the extract treated (Fig. 2B2). The photomicrograph of the normal cardiac muscle (Fig. 2C1) in longitudinal section demonstrated the normal

arrangement of cardiac muscle fibres which branched to give appearance of three dimensional networks. The extract treated (Fig. 2C2) showed no indication of cellular lesion or distortion. The photomicrograph of normal hepatic tissue (Fig. 2D1) showed the portal tracts located at the periphery of the hepatic lobule. The hepatocytes radially arranged, continued from the lobular margins to the centre with each column interspaced by sinusoids. In the extract treated (Fig. 2D2), no evidence of pathologic changes was observed.

DISCUSSION

The acute toxicity study of serena stress free herbal tea 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 h while the animals that received 5.0 g/kg dose survived beyond the 24 h of observation. The median acute toxicity value (LD_{50}) of the extract was determined to be 9.0 g/kg bw. According to Ghosh (1984) and Klaasen *et al.* (1995) the extract could be classified as being slightly toxic, since the LD_{50} was found to lie between 5.0 g and 15.0 g/kg. The gram equivalence of the LD_{50} in an average adult man would translate to 627.9 g dose of the drug. 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 death, it postulated that the extract did not kill the mice by some action on the nervous system (Ogwal-Okeng *et al.*, 2003).

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 (Table 2 and 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 there was a dramatic gain in weight especially in the group treated with a dose of 250 mg/kg bw.

There were no morphological changes in the colour of the various organs of the treated animals compared to the control. Furthermore, histological studies revealed no deleterious effects on the vital organs examined. However, factors responsible for the increase in weight of some vital organs of animals that received the highest dose of the drug were quite unclear.

There was no increase in the plasma protein level in all the treated animals, which suggested that there was no sign of impaired renal function (Tilkian *et al.*, 1979), while the insignificant increase in the plasma creatinine concentration indirectly suggested no kidney damage, specifically by renal filtration mechanism (Wasan *et al.*, 2001). This is a clear indication that the drug at doses employed did not cause renal impairment. The liver and heart release AST and ALT and an elevation in their plasma concentrations are an indicator of liver and heart damage (Mythilypriya *et al.*, 2007; Wasan *et al.*, 2001). The significant decrease observed in the ALT level coupled with insignificant change in AST level suggested that the polyherbal tea did not possess hepatotoxic effect and equally could not have caused some toxic effects on the heart tissue (Crook, 2006).

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. The increase in HDL-cholesterol levels (anti-atherogenic agent) and a reduction in LDL-cholesterol levels observed in all the treated animals 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.

The observed significant ($p \leq 0.05$) increase in the haemoglobin levels in all the treated groups might be due to the increase absorption of iron. There were no significant changes in MCHC in the treated animals compared to the control. Low MCHC has been associated with iron deficiency anemia (Agbor *et al.*, 2005). Therefore, the insignificant change in the level of MCHC in this study suggested that the polyherbal tea did not effect a change in the average size of red blood cells and so did not induce anaemia. The observed slight increase in WBC count in the treated groups could be as a result of body defense in response to toxic environment (Teguia *et al.*, 2007). Lymphocyte, the main effector's cell of the immune system (McKnight *et al.*, 1999) recorded increase that fluctuated with dose suggesting mild challenge on the immune system of the animals.

Conclusion: The high LD_{50} value (12.8 g/kg) obtained was a clear indication that the polyherbal preparation could be safe for use. The study showed that the drug possess hypoglycaemic activity and good reducing effects on cardiovascular factors. The study also revealed that the drug at doses investigated did not provoke toxic effects to the animals' liver at high dose. The polyherbal tea was also observed not to have harmful effect on as well as testes.

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