



Full Length Research Paper

Antimicrobial evaluation and acute and sub-acute toxicity studies on a commercial polyherbal formulation, “Ade & Ade Antidiabetic®” used in the treatment of diabetes in southwestern Nigerian

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ABSTRACT

This study evaluated the microbial purity, acute and sub-chronic toxicities in rodents of a polyherbal formulation, Ade & Ade Antidiabetic®, an antidiabetic formulation, prepared with *Ocimum gratissimum*, *Citrullus lanatus*, *Momordica charantia*, *Chrysophyllum delevoiyi* and *Uncaria tomentosa* leaves. Microbial purity was evaluated on some bacterial and fungal organisms using appropriate diagnostic media. Acute toxicity of the polyherbal formulation was evaluated in Swiss albino mice by administering orally graded doses of the lyophilized formulation in the ranges of 1.0 g to 20.0 g/kg body weight to the animals and observed continuously for the first 4 hours and hourly for the next 12 hours then 6 hourly for 56 hours (72 hr). Wistar rats were also fed with different doses of the lyophilized formulation for 30 days and the effects on the body weight, biochemical and hematological profiles and tissue histology were evaluated (sub-acute toxicity model). The presence of *E.coli* bacterial organisms with loads above officially accepted limit were observed in the formulation. The median acute toxicity value (LD₅₀) of the polyherbal formulation was determined to be 15.0 g/kg body weight. Significant increase in the body weight was observed in all the treated compared to the control. Significant increase ($p \leq 0.05$) in creatinine level occurred while aspartate aminotransferases (AST) and alanine aminotransferases (ALT) levels showed marked decrease. Total cholesterol (TC) and low density lipoprotein (LDL)-cholesterol levels decreased appreciably while triglyceride (TG) and high density lipoprotein (HDL)-cholesterol levels exhibited marked increase. The extract significantly increased ($p < 0.05$) hemoglobin and RBC contents in all the treated groups compared to the control. There was also an increase in WBC which was complemented by an increase in lymphocyte cells.

Keywords: *Ocimum gratissimum*, *Citrullus lanatus*, *Momordica charantia*, *Chrysophyllum delevoiyi* and *Uncaria tomentosa*, acute, sub-acute toxicity.

INTRODUCTION

Plants and herbs derived medicines are popularly known as “Herbal medicine” and are generally regarded as safe;

based on their long-standing use in various cultures (Mosihuzzaman and Iqbal, 2008). Herbal medicines have

been employed since prehistoric era by the traditional medical practitioners for the treatment of various diseases. They remain the main stay of health care system in the developing countries and are gaining increasing popularity in the developed countries where orthodox medicines are predominantly used. Herbal medicines are currently being employed in the management of diabetes mellitus and other diseases that could not be effectively managed with orthodox medicines.

Diabetes mellitus (DM) is a heterogeneous disease of carbohydrate, protein and lipid metabolism characterized by hyperglycaemia and is due to an absolute or a relative lack of insulin. Significant success in the management of diabetes has been recorded with the introduction of insulin therapy (Goldfine and Youngren, 1998). Also oral hypoglycaemic agents such as sulphonylureas and biguanides are currently available for the management of type 2 diabetes. The usefulness of oral hypoglycaemic agents may be limited by their side effects and high rate of secondary failure (Xie et al., 2005). For these reasons attention is now focused on the use of alternative therapy for the management of the disease with plants and plant derived medicines considered as the best option.

The herbal medicine has the advantages of being efficacious as well as being a cheap source of medical care (Ogbonnia et al., 2010a). Also there is growing disillusion with modern medicine coupled with the misconception that herbal products being natural could be assumed of being devoid of adverse and toxic effects associated with convectional and allopathic medicines. However, herbal medicines could be contaminated with microbial and foreign materials such as heavy metals, pesticide residues or even aflatoxins resulting from the unhygienic manners by which they were prepared. The presence of any of the possible contaminants may lead to a potential health risk to the vast population that depends on these preparations for their health care needs. Increased morbidity and mortality associated with the use of herbs 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 polyherbal, being prepared from mixtures of different plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize (Ogbonnia et al., 2010b). The bioactive principles 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. Also herbal medicine may have

multiple physiological activities and could be used in the treatment of a variety of disease conditions (Pieme et al., 2006). It could be administered in most disease states over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage (Ogbonnia et al 2010c). The danger associated with the potential toxicity of herbal therapies employed 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 (Tédong et al., 2007; Ogbonnia et al., 2008). Ade and Ade antidiabetic formulation is one of such popular polyherbal formulation used in the treatment of diabetes. It is prepared with *Ocimum gratissimum*, *Citrullus lanatus*, *Momordica charantia*, *Chrysophyllum delevoiyi* and *Uncaria tomentosa* leaves.

The aim of this study was to evaluate the safety of the polyherbal formulation, by investigating its microbial purity, and also the acute and sub-acute toxicities in rodents. Sub-chronic toxicity evaluation is required to establish potential adverse effects of this highly valuable polyherbal formulation that is now widely consumed for its physiological benefits.

MATERIALS AND METHODS

The liquid formulation, Ade and Ade Antidiabetic® formulation is one of such popular preparations widely consumed locally for the treatment of diabetes. The preparation (1.5 L) was obtained from a popular Mushin market in Lagos suburb, Nigeria. The label claimed that the formulation was prepared with unspecified quantities of *Ocimum gratissimum*, *Citrullus lanatus*, *Momordica charantia*, *Chrysophyllum delevoiyi* and *Uncaria tomentosa* leaves and the prescribed dose for human adult is 30 ml daily. The production date and batch number of the respective formulation were also not indicated. For purity evaluation, five bottles (1.5 L) were obtained from different sellers and the result expressed as average from the five sources. The un-tampered polyherbal formulations were stored in a refrigerator at 4 – 6 °C until the quantity needed for the purity test was aseptically taken. Then 1000 ml of the formulation was filtered and the resulting 845.6 ml freeze dried to yield 42.5 g gel.

Animals

Swiss mice (22.5± 2.5g g) and Wistar rats (130 ± 10 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

mouse chow (Feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment.

Determination of microbial purity

The microbial load of the preparation was determined using the standard plate method (Fontana et al., 2004). 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 °C 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 45 °C and 1 ml each of the dilutions seeded in 25 ml each of the sterile culture media were swirled and left to solidify. The bacterial media were incubated at 37 °C for 3 days while the fungal medium (SDA culture) was incubated at ambient temperature for 7 days. They were examined 24 hourly during this period for the colonies and the results were 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 °C and observed daily for 3 days for swarming of proteus.

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 (Raghavendra et al., 2006). 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 °C for 24 hours for bacterial cultures and at ambient temperature for 7 days for fungal cultures and observations were made for zones of inhibitions (NCCLS, 1997).

Acute Toxicity Study

The toxicity study was carried out using thirty-five (35) male and female Swiss albino mice (each 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. 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 fast of the animals, the control group received 0.3 ml of acacia solution orally. The extract gel suspension was prepared by dispersing 16 g of the gel with 7 ml acacia (2 %w/v) solution in a 100 ml beaker and transferred to a 20 ml volumetric flask. The beaker was thoroughly rinsed with the acacia solution transferred to the volumetric flask and the volume made to mark with the acacia solution. The following doses 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg were respectively administered orally to the groups. The animals were observed continuously for the first 4 hours and then for each hour for the next 24 hours and at 6 hourly interval for the next 48 hours after administering the extract to observe any death or changes in general behavior and other physiological activities (Shah et al., 1997; Bürger et al., 2005).

Sub-acute Toxicity Study

Male and female Wistar albino rats weighing 130 ± 10 g were used. They were allowed to acclimatize to the laboratory conditions for seven days and were maintained on standard animal feed and provided with water *ad libitum*. The animals were weighed and randomly divided into four groups of five animals each containing both sexes. After fasting the animals overnight the control group received a dose of 0.6 ml acacia (2 %w/v) solution and the treated groups received 200 mg/kg, 300 mg/kg and 600 mg/kg body weight of the extract gel prepared in the same manner as in above for 30 days. The animals were then weighed every five days from the start of the treatment, to note any weight variation. At the end of the experiment, they were made unconscious under mild diethyl ether and blood collected via cardiac puncture in three tubes. One with EDTA for immediate analysis of hematological parameters the second with heparin to separate plasma for biochemical estimations and the third with fluoride oxalate for glucose analysis. The liver, kidney and heart were dissected out, washed in ice-cold saline solution and weighed (Ogbonnia et al., 2010c). The brains were also dissected out and weighed. The collected blood was centrifuged within 20 min of collection at 4000 g for 10 min to obtain plasma, which was analyzed for total cholesterol, triglyceride, HDL-cholesterol levels by precipitation and

Table 1. Microbial Purity Test of the Polyherbal formulations

MEDIA	S. typhi	Bacillus species x10 ²	Shigella Species	Other Coliforms	Proteus Specie x10 ²	P.aeruginosa	S. aureus x10 ¹	E. coli x10 ³	TYMC10 ¹	TACC	TOTAL
SSA	0	-	0		4.00±0.3	-	-	-	-	-	4.00±0.3 x10 ²
MAC	-	-	-	TNTC	-	-	-	-	-	-	TNTC
NA	-	-	-	-	Proteus swarmed	-	-	-	-	-	
CA	-	-	-	-	-	0	-	-	-	-	0
MSA	-	1.00±0.03	-	-	-	-	8.0± 0.1	-	-	-	1.8x10 ²
EMBA	-	-	-	-	-	-	-	1.370± 0.3	-	-	1.370±0.3 x10 ³
SDA	-	-	-	-	-	-	-	-	2.0± 0.02	-	2.0±0.02 x10 ¹
TSA	-	-	-	-	-	-	-	-	-	-	TNTC

N=5, value =(Mean±sem), Average count from 5 different preparations plus/minus standard error of mean.

Targeted organisms: *Salmonella typhi* (nil), Shigella species (nil) Other Coliform were too numerous to count, Proteus species (4.00x10² cfu/ml), *Pseudomonas aeruginosa* (nil) *Staphylococcus aureus* (8.00x10¹ cfu/ml) *Escherichia coli* (1.370x10³ cfu/ml). Mould and Yeast (2.00x10¹ cfu/ml), Bacillus species (1.00x10² cfu/ml), CA - Cetrimide Agar, EMBA - Eosine Methylene Blue Agar, MAC- MacConkey Agar, NA-Nutrient Agar, SDA- Sabouraud Dextrose Agar, SSA - Salmonella Shigella Agar, TSA- Tryptone Soy Agar, TNTC-To numerous to count TYMC Total yeast and mould count.

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 (Sushruta et al., 2006). Plasma glucose and protein contents were determined using enzymatic spectroscopic

methods (Hussain and Eshrat, 2002). Hematocrit was estimated using the methods of Ekaidem et al., (2006). Hematocrit tubes were filled by capillary action to the mark with whole blood and the bottom of the tubes sealed with plasticide and centrifuged for 4 - 5 min using hematocrit centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersects the 100 %

line. Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem et al., 2006).

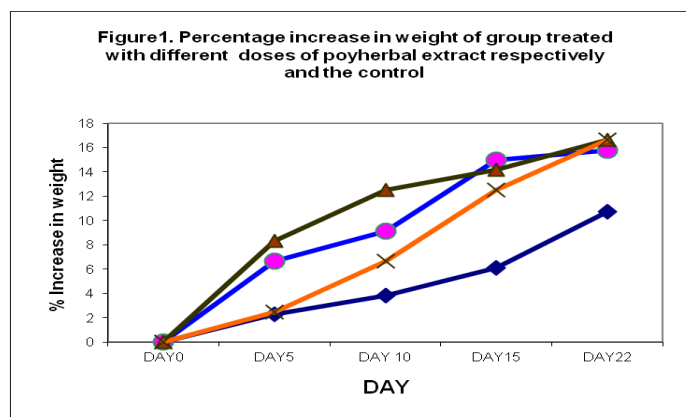
Tissue histology

The hepatic, renal, cardiac and testicular tissues harvested from each group were fixed in 10 % formal saline for ten days before embedding

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

Doses g/kg	Number of Animals	Number of animals dead	%Cumulative death of mice
0.5	0	0	0
1.0	5	0	0
2.5	5	0	0
5.0	5	0	0
10.0	5	1	16.6
15.0	5	2	50
20.0	5	3	100

Control received 0.3ml each of 2% acacia suspension



◆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

in paraffin wax. The embedded tissues were sectioned at 5µm, mounted on a slide and stained with Haematoxylin and Eosin (H and E) (Mbaka et al., 2012). Each section was examined under light microscope at high power magnification for structural changes and photomicrographs were taken.

RESULTS

The microbial purity evaluation of the preparations (Table 1) showed no growth in the various diagnostic media used for bacterial and fungal organisms in the first 24 hr. In 72 hr *Bacillus subtilis* ($1 \pm 0.03 \times 10^2$ cfu/ml) were observed in Tryptone Soy Agar and MacConkey agar cultures media respectively while after 6 days of incubation moulds and yeast load of $2 \pm 0.02 \times 10^2$ cfu/ml were observed on Sabouraud Dextrose Agar medium. *Escherichia coli*, $1.370 \pm 0.3 \times 10^3$ cfu/ml load were observed on Eosine Methylene Blue Agar medium. A swarmed growth of *Proteus* species was observed on Nutrient Agar, and on Salmonella Shigella Agar medium

($4 \pm 0.3 \times 10^2$ cfu/ml). The presence of *Staphylococcus aureus*, $8 \pm 0.1 \times 10^1$ cfu/ml was also observed on Manitol Salt Agar.

The acute toxicity study (Table 2) showed that all the animals that received the lyophilized extract dose of 5 g/kg body weight (bwt) survived beyond 24 hr while in 72 hr, 20 %, 40 % and 60 % death were respectively recorded for the groups that received 10.0, 15.0 and 20 g/kg bwt of the lyophilized extract. Significant ($p \leq 0.05$) increase in the body weight variation (Figure 1) was observed in all the treated groups from day 10 to the end of the experiment compared to the control. Summarized in Table 3 were the effects of the lyophilized extract on the organ weights of the treated animals compared to the control. There was significant ($p < 0.05$) increase in the weight of the liver and kidney of the animals treated with various doses of the formulations compared to the control while the weight of testes showed marked decrease. The weights of the other organs- heart, lungs, pancreas, spleen and brain however showed insignificant changes.

The effects of the extract on the biochemical parameters are summarized in Table 4. Significant

Table 3. Weight variation of organs of control and the animals treated with various doses of the extract

Parameter/ 100g bodyweight	DOSE mg kg⁻¹ body weight			
	Control	200	300	600
Liver	3.34± 0.2	5.14± 0.4*	4.31±0.4**	5.34± 0.3*
Kidney	0.63±0.3	1.20±0.3*	0.83±0.2**	1.18±0.5*
Heart	0.47± 0.1	0.51±0.2	0.48±0.2	0.52±0.1
Pancreas	0.12±0.1	0.10±0.02	0.18±0.02	0.15±0.1
Spleen	0.56±0.09	0.49±0.09	0.45± 0.01	0.60±0.3
Brain	1.46± 0.05	1.48±0.02	1.46±0.06	1.51±0.3
Lungs	0.81±0.02	0.90±0.3	0.80± 0.1	1.00±0.04
Testes	1.56±0.05	1.02±0.2**	0.89±0.3**	0.85±0.05**

N=5, value =(Mean±sem), *p < 0.05 significant **p < 0.01 vs control

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, GPIV- Animals treated with the extract 600mg/kg body weight

Table 4. Plasma glucose level and other biochemical profiles of the control and the animals treated with various doses of extract for 21days in the subchronic study

Parameter	Group I	Group II	GroupIII	Group IV
AST (U/l)	304.8±2.1	240.0±4.7*	238.2±3.4*	225.1±3.1*
Bilirubin (mmol/l)	15.05±0.7	13.4±1.1*	13.4±0.4*	13.5±0.5*
CREA(mg/dl)	6.0±2.2	6.8±2.0	7.6±0.9*	7.7±1.8*
ALT (U/l)	98.7±1.2	71.7±1.0*	80.9±1.1*	81.1±0.9*
CHOL(mmol/l)	2.02±0.6	1.72±0.3*	1.62±0.9*	1.60±1.0*
TG(mmol/l)	0.45±0.3	0.67±0.5*	0.56±0.7*	0.51±0.2*
HDL(mmol/l)	1.45±0.8	1.51±0.6*	1.67±0.5*	1.80±0.3*
LDL(mmol/l)	1.28±1.2	0.85±1.0*	0.87±1.2*	0.90±1.4*
GLU (mmol/l)	4.1±1.1	3.5±0.9**	4.0±0.6	3.50±1.1**
TP(g/dl)	8.0± 0.2	7.3± 0.1	7.7± 0.3	7.8± 0.5

n=5, value =(Mean±sem), *p < 0.05 significant **p< 0.01 vs control group

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, GPIV- Animals treated with the extract 600mg/kg body weight

CREA - Creatinine, CHOL – Cholesterol, TG - Triglycerides, HDL- High density Lipoprotein, AST- Aspartate aminotransferase, LDL - Low density lipoprotein, ALT- Alanine aminotransferase, GLU – Glucose and TP-Total protein

(p<0.01) decrease in the plasma glucose level was observed in the groups treated with the lowest and highest doses of the formulation while insignificant change ($p \geq 0.05$) occurred in the group treated with intermediate dose (300 mg/kg bwt) compared to the control. Significant increase ($p < 0.05$) in creatinine level was observed in the groups treated with the medium and highest doses of the formulation while the group treated with the lowest dose indicated insignificant increase compared to the control. There was significant ($p < 0.05$) decrease in AST, ALT, TC, and LDL-cholesterol levels respectively and significant increase in TG and HDL-cholesterol level in all the treated groups. TP showed insignificant decrease in the treated groups compared to the control while the bilirubin level decreased markedly.

The effects of the lyophilized extract on the red blood cells (RBC) components and white blood cell differentials were summarized in Table 5. A significant ($p < 0.05$) increase in haemoglobin and RBC and WBC levels was observed compared to the control. No significant ($p \geq 0.05$) changes were observed in the levels of procalcitonin (PCT), the mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) respectively in all the treated animals compared to the control while significant decrease occurred in mean corpuscular volume (MCV).

Tissue histology

The photomicrograph of normal hepatic tissue (Figure 2a)

Table 5. Haematological and blood differential values of control and treatment rats with the phytomedicine for 21 days in subchronic study

Parameter	Group I	Group II	Group III	Group IV
Haemoglobin (g/dl)	14.8±0.3	16.4±1.1*	15.4±0.26**	16.7±1.0*
RBC (10 ⁶ /l)	6.87±0.02	8.49±0.2*	7.69±0.1**	7.39±0.2**
WBC (10 ³ /l)	4.5±0.17	6.9±3.1*	7.7±0.3*	9.2±2.2*
PCT (%)	0.27±0.1	0.23±0.4	0.29±0.5	0.26±0.2
PLT (10 ⁹ /l)	451.0±2.3	381.0±2.0	444.0±1.89	499.0±1.6*
MCHC (g/dl)	33.7±1.4	37.8±1.1	37.9±1.0	36.6±1.1
LYM (%)	69.2±2.0	81.4±0.9*	82.2±1.8*	83.8±1.6*
MCH (pg)	21.6±0.9	19.3±1.4	20.0±1.1	18.6±1.0
HCT (%)	44.0±1.4	43.5±0.8	40.6±1.2	37.5±1.1
MCV (fl)	64.1±3.2	51.2±2.1*	52.8±2.6*	50.7±2.1*

n=5, value = (Mean±sem), *p < 0.05 significant **p < 0.01 vs control

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, GPIV- Animals treated with the extract 600mg/kg body weight

MCHC- Mean Corpuscular Haemoglobin concentration, MCH- Mean Corpuscular Haemoglobin

HCT- Haematocrit MCV- Mean Corpuscular Volume, RBC-Red Blood Cell, PCT- Procalcitonin, PLT- Platelet

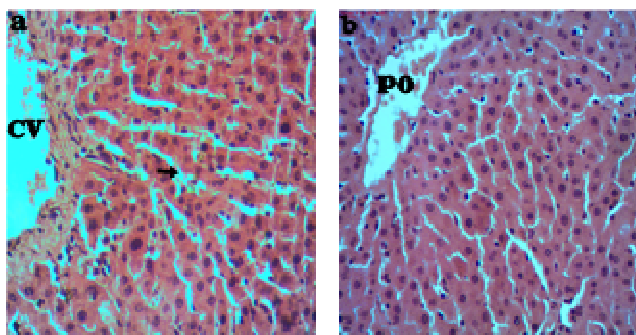


Figure 2a. The normal hepatic tissue indicating the central vein (cv) with the hepatic sinusoids interspersing the hepatic cords (x400). **Figure 2b.** Hepatic tissue post-treatment with the highest dose of the formulation showed normal appearance and indicated (po) is the portal area (x400).

showed the central vein (cv) and radially arranged cords of hepatic cells interspaced by hepatic sinusoids (arrowed). In the extract treated (Figure 2b), there was no evidence of pathological changes.

A cross section of the normal testicular tissue (Figure 3a) showed the seminiferous tubules cut in different planes having distinct boundary and between (d) were the interstitium. The basement zone comprised of darkly spotted compactly arranged primitive germ cells (p) (spermatogonia) while the spermatozoa (s) formed a cluster round the lumina. Post-treatment with the formulation (Figure 3b) indicated no morphological changes. The photomicrograph of the normal renal tissue (Figure 4a) showed the cortical area. Indicated were the renal corpuscles (n) and the convoluted tubules (t). The

renal tissue of treatment with the formulation (Figure 4b) equally indicated normal appearance. The photomicrograph of normal cardiac muscle (5a) in longitudinal section demonstrated the normal arrangement of cardiac muscle fibres which branched to give appearance of three dimensional networks (p). Arrowed was the intercalated disc. Post-treatment with the formulation (Figure 5b) showed no morphological changes.

DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to

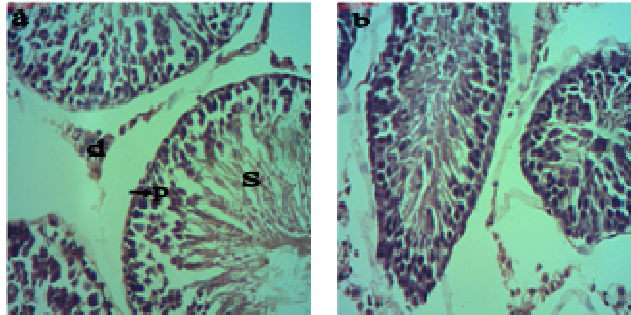


Figure 3a. The normal testicular tissue showed a cross section of seminiferous tubules, the darkly spotted cells arrowed (p) are the primitive germinal cells while the spongy part (s) indicated the matured sperm cells. The interstitium(d) is an area between the seminiferous tubules x400. **Figure 3b.** The testicular tissue post- treatment with the highest dose of the formulation showed no lesion x400.

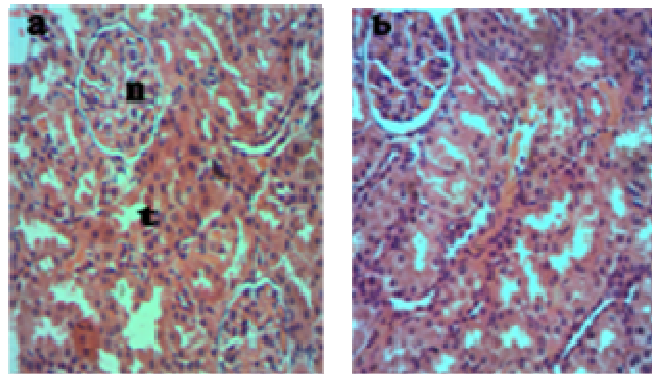


Figure 4a. Showed the cross section of normal renal tissue indicating the renal corpuscles (n) and convoluted tubules (t) x400. **Figure 4b.** The post- treatment with the highest dose of the formulation showed no abnormal feature x400.

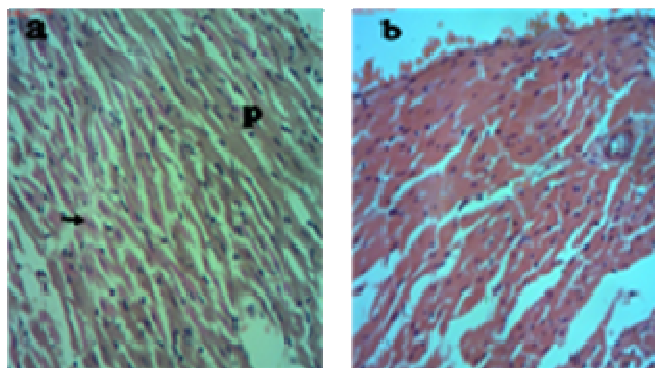


Figure 5a. The cross section of normal cardiac tissue showing the branch network of muscle fibres (p) with the arrow pointing to the intercalated disc x400. **Figure 5b.** The cardiac tissue post-treatment with the highest dose of the formulation indicated normal appearance x400.

subsequent increase in their demands (Sushruta et al., 2006). In rural communities, the exclusive use of herbal medicines, prepared and dispensed by herbalists without formal training, for the management of various diseases is still a very common practice, requiring that experimental screening method be established to ascertain the safety and efficacy of these herbal products as well as to establish their active components (Ogbonnia et al., 2008). The poly-herbal preparation "**Ade & Ade Antidiabetic**" is one of such herbal remedies prepared as a mixture from the leaves of different plants species and is a popular herbal medicine used locally in the treatment of diabetes.

The microbial purity evaluation of the formulation showed the presence of *Bacillus subtilis* as well as some moulds and yeast organisms, though with their loads within an acceptable official limit (Fontana et al., 2004). Evaluation for the presence of enterobacteria and other gram-negative bacteria such as *E. coli*, *S. typhi*, and *S. aureus* was specifically stipulated by the WHO guidelines of microbial limit for herbal formulation (Shrikumar et al., 2004). Also, evaluation for the presence of other microorganisms such as *Bacillus cereus*, *Clostridia*, *Pseudomonas*, *Burkholderia*, *Asperigillus* and *Enterobacter* species may be necessary depending on the origin of the herbal drug, raw materials and/or the method of preparation adopted (Drew and Myers, 1997). All the microorganisms found in the formulation except *Escherichia coli* were within the official microbial limit in accordance with WHO guidelines for herbal formulations. The high load of *E. coli* in the formulation could be attributed to the use of faecal contaminated water for the preparation. It was noted that the adverse effects of herbal medications may be classified as intrinsic or extrinsic effects (Drew and Myers, 1997). Extrinsic effects are usually not related to the herb itself, but to a problem experienced in commercial manufacture or extemporaneous compounding. The high load of *E. coli* in the formulation could suggest failure to adhere to a code of Good Manufacturing Practice (GMP) in the production of the herbal formulation.

There were no noticeable changes in the nervous system responses or adverse gastrointestinal effects observed in the male and female mice used during the experimental period in the acute toxicity study. According to Ghosh (1984) and Klaasen et al., (1995), the poly-herbal medicine could be classified as being non-toxic since the LD₅₀ by oral route of the lyophilized extract was calculated to be 15 g/kg which was much higher than WHO toxicity index of 2 g/kg body weight. There was markedly higher weight gain in the treated animals compared to the control group implying that the formulation stimulated appetite for eating. Some of the organs such as liver, kidney and testes of the treated animals also recorded significant weight gain. However, the gross examination of internal organs revealed no

detectable inflammation. The lyophilized extract effected decrease in plasma glucose levels in the animals treated with the lowest and highest doses indicating the presence of hypoglycaemic agent but the insignificant change in the glucose level on the group treated with the intermediate dose (300mg/kg body weight) could be attributed to unidentified factor. The increase in the creatinine levels coupled with decrease in the protein levels observed in all the treated animals compared to the control was a sign of impaired renal function (Tietz, 1990). The formulation effect on the renal filtration mechanism must have been implicit since renal tissue histology showed no inflammatory changes. The decrease in AST and ALT levels observed in all the treated animals signified that the lyophilized formulation had no deleterious effects on either the heart or liver tissues. The non harmful effect of the formulation was further confirmed by the tissue histology of the liver and heart which showed no abnormal features. The decrease in plasma total cholesterol (TC) and LDL cholesterol levels respectively in all the treated groups and increase in the HDL level demonstrated the likelihood of hypolipidaemic agents' presence in the poly-herbal medicine. The ability of the poly-herbal medicine to manage dyslipidaemia showed that it had a potential beneficial effect on cardiovascular risk factors which are a major cause of death in diabetes mellitus subjects (Valli and Giardina, 2002). The hypoglycaemic and hypolipidemic effects of the formulation gave credence to its use locally as an antidiabetic agent.

The effects of the lyophilized formulation on the red blood cells (RBC) components and white blood cell differentials as indicated in the result showed significant increase ($p < 0.05$) in the haemoglobin and RBC contents in all the treated groups compared to the control. The observed significant ($p \leq 0.05$) increase in the haemoglobin level might be due to increase in the absorption of iron. There was also significant change in white blood cells (WBC) signifying an increase in activity usually associated with response to toxic environment (Crook, 2006) or to an infection. The level of procalcitonin has been found to rise in a response to a proinflammatory stimulus, especially of bacterial origin and is produced mainly by the cells of the lung and the intestine while it does not rise significantly with viral or non-infectious inflammations. The increase in blood level of procalcitonin during systemic inflammation has made it to be regarded as a possible better maker of bacterial sepsis than C- reactive protein (Meisner et al., 1999). Since there was no appreciable changes in the values of procalcitonin in the treated animals it could be assumed that the formulation did not produce any systemic inflammation. However, no significant changes in MCHC and MCH levels were observed in the treated animals compared to the control thus signifying that the polyherbal formulation could not have caused regenera-

tive anemia (Ogbonnia et al., 2010c; Mbaka et al., 2010). The increase in WBC was complemented by an increase in lymphocyte observed in the treated animals which might have clearly indicated response to the contaminants present in the formulation and not necessarily due to toxic environment.

CONCLUSION

The polyherbal formulation contained some microbial contaminants which were above acceptable limits and could therefore be considered microbiologically unsafe for human consumption. Although high LD₅₀ value (15.0g/kg) was obtained, the high loads of microorganisms present negated the safety of the formulation. The formulation however exhibited hypolipidaemic and hypoglycaemic activities and good reducing effects on cardiovascular factors. The tissue histology also revealed that the formulation did not provoke toxic effect on the animals organs at the doses administered. Therefore, improvement in the production process is desirable to make the polyherbal formulation safe for consumption.

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