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Evaluation of purity, acute, cyto and subchronic – toxicities of a polyherbal Foraiammulation "FEM-Q®" used in Nigeria in the treatment of female infertility

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Abstract
Background: Plants have served as potential therapeutic weapons making them a sine qua non to lives.
Objective: This study evaluated the microbial purity, and toxicities of FEM Q ELIXIR®, used in treatment of female infertility.
Materials and Methods: Microbial purity was evaluated on some bacterial and fungal organisms. Acute toxicity of the extract was evaluated in Swiss albino mice orally in graded doses, and observed for 72hr while cytotoxicity was evaluated using brine shrimps and potassium dichromate in different concentrations as standard over 24 hr. Wistar rats were fed with the extract doses for 30 days and the effects on biochemical, histological and haematological parameters observed (Subchronic study).
Result: Micro-organisms were absent or within official limits. The LD_{50} was found to be over 20.0g/kg bwt. There is a significant difference ($p \leq 0.05$) between the LC_{50} of the formulation compared to LC_{50} of the reference standard (rf). There was no significant increase ($p \geq 0.05$) observed in body weight of the treated animals compared to control group. Also no significant ($p \geq 0.05$) difference in the organs weight variation while a significant decrease ($p \leq 0.005$) in AST and ALT levels of the animals that received the highest dose compared to the control. The histological examination revealed that the extract exerted no deleterious effects on the evaluated organs.
Conclusion: LD_{50} and LC_{50} values were above WHO toxicity index and no deleterious effects. Observed also were decreases in ALT and AST levels and no toxic effects to the hearts and other tissues.

Keywords: acute, cytotoxicity, subchronic, toxicity and Fem-Q elixir

Introduction
Plants have, from the outset, played an invaluable and indispensable roles as veritable sources of food for the sustenance of human and animal lives and their recognition as a good source of medicines employed as therapeutic weapons in the prevention and treatment of various human, animal and even plant diseases had made plants a sine qua non to human and animal lives (Ogbonnia *et al*., 2008a) [20]. Plant and plant derived drugs, known as herbal medicine, remain the main stay of primary health care system in developing countries where about 60% or more of the population rely on herbal medicine (Rickert *et al*., 1999, Ogbonnia *et al*., 2008b) [26, 20]. The use and increase in the popularity of herbal remedies could be attributed to their efficacy, availability and cheap source of health care. Also, herbal medicines are assumed to be associated with higher safety margins compared to the synthetic drugs, as there is a general misconception that herbal products obtained naturally may be devoid of adverse and toxic effects often encountered in allopathic medicines (Ogbonnia *et al*., 2014a) [23]. Recently witnessed is an increase in the patronage and consumptions of herbal products, especially on the long term basis, for the management of some disease states. This, therefore, calls for an urgent need for both scientific and clinical data making it imperative for safety evaluation of herbal preparations (Pieme *et al*., 2006; Tédong, *et al*., 2007) [25, 31]
Herbal medicines are most often prepared from polyherbal of different botanical origins and most often un-hygienically processed by traditional practitioner without formal education giving rooms to end products contamination. Contaminants when present in herbal preparations might cause prominent health defects, such as renal and hepatic toxicity, underscoring the claimed benefits and safety (Mythilypriya, *et al*., 2007; Ogbonnia *et al*., 2011a) [18]. The resulting clinical toxicity caused by contaminants vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations imperative (Ogbonnia *et al*., 2011b).

Contaminates in herbal medicine could be of microbiological organisms, foreign materials such as heavy metals, pesticide residues or even aflatoxins (Bandaranyake, 2006, Wickramasinghe 2006, Vandana *et al*., 2012). Polyherbals are usually prepared with many plants or plant derivatives from various botanical sources as a result standardization or evaluation of their ingredients which are highly complex may be difficult (Ogbonnia *et al*., 2010).

One of such polyherbal formulation is Fem Q elixir® a popular female anti-fertility treatment formulation from medicinal plants. Fem Q elixir® is a tincture of different herbs based on a traditional African long time usage and the elixir was prepared with the leaves of Jatropha gossypfollia, Turnera diffusa var afrodisiaca, *Ocimum* gratissium, fruits of *Rubusidacus* and bark of *Rhamnus purshianus*. The aim of this study, therefore, was to evaluate the safety of polyherbal preparation, Fem Q elixir®, by carrying out the microbial load evaluation and cytotoxicity, acute and sub-chronic toxicity studies in animals.

Materials and Methods

Materials: The Polyherbal formulation (Fem-Q®) was supplied by Emsyl Healthcare Limited, No 5 Gladys Misan Street, Victory Estate, Iba, Lagos, Nigeria. The label claimed contain the following plant materials in the given proportions *viz*., Jatropha Gossypifolia leaves (5g), *Rhamnus purshianus* bark (2g), Turnera diffusa ver afrodisiaca leaves (3g), RubusIdacus fruits (15g) and *Ocimum gratissium* leaves (20g) in 100ml of 12.5% hydro ethanol solution.

Animals: Swiss albino mice (22.5 ± 2.5 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 12/12hr 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 and were allowed to acclimatize for seven days to the laboratory conditions before the experiment.

Purity evaluation

Determination of Microbial Purity

The microbial load of the preparation was determined using the standard plate method (Fontana *et al*., 2004) [8]. 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) (all Biotec products)- were used to culture the test organisms. 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 sterile culture media swirled and left to solidify. The bacterial media were incubated at 37 ºC 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 the presence of proteus organisms was evaluated using the 1/10 dilution. A loopful was taken and dropped aseptically at the center of nutrient agar plate, the site of inoculation 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 (Ogbonnia *et al*., 2013) [21].

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. 106 cfu/ml of the overnight clinical cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Shigella* species were 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 hr for bacterial cultures and at ambient temperature for 7 days for fungal cultures and observations were made for zones of inhibitions (NCCLS, 1997) [19].

Acute toxicity study

The toxicity study was carried out using twenty (25) 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 four 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 (Bihde, and Ghosh, 2004). After the overnight fasting, the control group received 0.3 ml of acacia solution (2%) orally. The doses 5.0, 10.0, 15.0 and 20g/kg 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 100ml 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 were administered orally 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 hr and then for each hour for the next 24 hr 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 (Shah *et al*., 1997; Burger *et al*., 2005, Ogbonnia *et al*., 2013) [22, 3, 21].

Determination of LD_{50}

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

Brine Shrimp Lethality Assay

Preparation of Sea Water

The sea water collected from bar beach, Victoria Island Lagos, filtered to remove dirt and sand particles.

Hatching of the Shrimps

Artemia saline Leach (Brine shrimp) eggs were supplied by Dr (Mrs.) A.A. Sowemimo of the Department of

Pharmacognosy, Faculty of Pharmacy, University of Lagos, and were hatched in a shallow rectangular container of (7 by 15cm) filled to three quarter with the filtered sea water. The bowl was partly covered to allow light to penetrate through the open space and allowed to stay in an undisturbed environment at a room temperature. After 48hrs, the phototropic shrimp larvae (nauplii) moved towards the illuminated side of the container, the newly hatched free-swimming pink-coloured nauplii were harvested from the bottom outlet as the cyst capsule floated on the surface, this collection method ensured pure harvest of the nauplii. The freshly hatched free-swimming nauplii were used for the bioassay.

Brine Shrimp Lethality Testing

100mg of the prepared plant extract and Fem-Q formulation were respectively dissolved in 20ml of sea water to give a stock solution of 5000µg from which various concentration of 10, 100 and 1000µg/ml solution were prepared. Potassium permanganate in concentrations of 40, 60 and 80µg/ml were used as a positive control. All doses were calculated by serial dilution technique, while sea water served as negative control (Colegate and Molyneux, 1993) [5]. All these concentrations were in triplicate vials making 9 vials of 5ml each for each of the samples. In each of the vials, 20nauplii were transferred and the set-up was allowed to remain for 2hr., under constant illumination. After 24hrs, the survived nauplii in each vial were counted with the aid of hand lens and the average number of survived larvae was determined. The graph of probit against logarithm of dose was plotted to determine the LC_{50} value. The percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula

% Mortality= $N_1 * 100/N_0$

Where N_0= Number of killed nauplii after 6hrs of incubation, N_0=Number of total nauplii transferred. (Sam-Wah-Teng, 1993) [27].

Subchronic toxicity

Male and female Wistar rats weighing 160 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 (ILAR, 1996) [10]. The animals were weighed and divided into four groups of five animals each, containing both sexes. The three treated groups respectively received the following doses: 100, 250 and 500 mg/kg body weight of the gel and the control group received a dose of 0.6 ml acacia (2 %w/v) solution orally once a day for 30 days. 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 optic puncture in two tubes: one with EDTA for analysis of haematological parameters and the blood chemistry and the other with heparin to separate plasma for biochemical estimations. The heparinized blood was centrifuged within 5 min of collection at 4000 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) [32]. LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2007). Plasma was analyzed for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods (Sushruta *et al*., 2006) [30]. Plasma glucose contents and protein contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002) [9]. Haematocrit was estimated using the method as described by Ekaidem *et al*., (2006) [7]. 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. Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem *et al*., 2006; Joshi *et al*., 2007; Mythilypriya *et al*., 2007) [7, 11, 18].

Results

The results of microbial purity evaluations of the formulation were shown on Table I. There were no microbial growths observed in the various diagnostic media used for bacterial and fungal organisms in the first 24 hours and after 6 days incubation.

The acute toxicity study or single dose administration toxicity study gives the idea about time of onset and duration of action. The acute toxicity results showed on Table II revealed that all the animals that received 20g/kg body weight survived beyond 24hr observation. There were no death, behavioural changes, or physical changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate and noticeable distress (drowsiness, gait, tremors and convulsion) observed in all the treated animals. The LD_{50} of the extract therefore will be assumed to be in excess of 20g per kilogram body weight.

The cytotoxicity results were shown on Table III, and figures 1-3. There was a significant difference ($p \leq 0.05$) between the median lethal concentration (LC_{50}) of the formulation compared to the control (Formulation LC_{50} =7.94 ug/ml; Control LC_{50} = 1.99 ug/ml). The effects of the extract on the variations of the body weights of the control and treated animals were shown on Table IV and Figure 4. Significant ($p \leq 0.05$) decrease in the body weight variation (Figure 4) observed in the group treated with the highest dose of 500mg/kgbwt while animals in the other treated groups showed no significant ($p \geq 0.05$) changes in their body weights variation compared with the control.

Summarized in Table V were the effects of the lyophilized extract on the organ weights. There was a significant ($p<0.05$) decrease in the weight of the liver of the animals treated with 100 and 250 mg/kgbwt doses of the extract while the weight of the liver of the animals treated with 500 mg/kg showed a significant ($p \leq 0.01$) increase compared to the control. There were no significant ($p \geq 0.05$) changes observed in the weights of other organs *viz*: ovary and kidney.

The biochemical results were shown on Table VI. There were significant ($p \leq 0.05$) increases in the plasma levels of triglycerides, total protein, ALP, creatinine and albumin in the animals treated with 250 and 500mg/kgbwt compared with control. While a significant ($p<0.01$) decrease in ALT and AST levels were observed in the groups that received the highest dose 500mg/kg bwt of the formulation. There was a significant decreased ($p \leq 0.05$) in urea level). The animals that received the extract doses of 100 and 250 mg/kg, showed a significant ($p \leq 0.05$) decreased in conjugated bilirubin and the total bilirubin levels while a significant ($p \leq 0.05$) increased in their levels was observed in the animals that received 500 mg/kg compared with control. The results also showed that

doses of100 and 250 mg/kg did not increase the serum total plasma protein compared to the control.

The results of haematological studies were shown in Table VII there were no significant c ($p \geq 0.05$) changes in the values of the red blood cell (RBC) and white blood cell (WBC) components except a slight change observed in the animals treated with 100 mg/Kg of the extract compared to the control. There was significant change in the mean corpuscular haemoglobin concentration (MCHC) level in all the treated animals compared to control. Significant decrease in the level of lymphocyte was also observed, also platelet level showed significant changes in all the treated animals compared to the control.

The histology results were presented in Figures V (a to c) The photomicrograph of the kidney of the control animal (a) revealed a normal renal tissue showing the cortical area of renal corpuscles. The corpuscles contained dense round mass of glomerular apparatus separated by Bowman's space from the surrounding structures. At the interstices were the convoluted tubules cut in different sections. There were no abnormalities observed in the photomicrographs of the kidneys (a2 and a3) treated 250 and 500mg/kg bwt of the extract respectively.

There was no abnormality observed in the liver of the animals treated with 250 and 500mg/kg of the extract compared with control (b1 to b3). Also the photomicrograph of the ovary of the control showed the cortical region were follicles at various stages of development which contains the female gametes. The ovary of the animals treated with 250mg/kg body weight showed a normal ovary indicating corpus albicans while the ovary of the animals treated with 500mg/kg body weight showed a: normal ovary indicating developing follicle.

Table 1: Microbial Purity evaluation of the FEM Q ELIXIR 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	-	-	-	0	-	-	-	-	-	-	0
NA	-	-	-	-	0	-	-	-	-	-	0
CA	-	-	-	-	-	0	-	-	-	-	0
MSA	-	0	-	-	-	-	0	-	-	-	0
EMBA	-	-	-	-	-	-	-	0	-	-	0
SDA	-	-	-	-	-	-	-	-	0	-	0
TSA	-	0	-	-	-	-	-	-	-	0	0

N=5; values = m ± sem * $p<0.05$; ** $p<0.01$ vs. Control Group Targeted Organisms: Salmonella typhi 0, Shigella species 0 (nil), Other Coli forms 0(nil), Proteus species 0(nil), Pseudomonas aeruginosa 0(nil), Staphylococcus aureus 0(nil), Escherichia coli 0(nil), Mould and Yeast 0 (nil), and Bacillus species 0(nil), 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- (too numerous to count) TYMC (Total yeast and mould count)

Table 2: Acute Toxicity evaluation of the lyophilized extract of the polyherbal formulationin Mice

Doses of drugs g/kg	Number of animals	Number of animals dead	% cumulative death
1.0	5	0	0
2.5	5	0	0
5.0	5	0	0
10.0	5	0	0
20.0	5	0	0

Control group each animal received 0.3mL acacia 2%w/v solution

Table 3: Cytotoxic lethality assay evaluation of the lyophilized extract on Brine Shrimp after 6 hours

	FEM-Q ELIXIR®	$K_2Cr_2O_7$ Positive Control	Sea Water Negative Control
Log Dos	1 2 3	1 2 3	3
Average Survival After 6 Hrs.	16 9 0	6 2 0	19
% Mortality	20 55 100	70 90 100	5
Probit	4.16 5.13 8.09	5.526.28 8.09	7.87

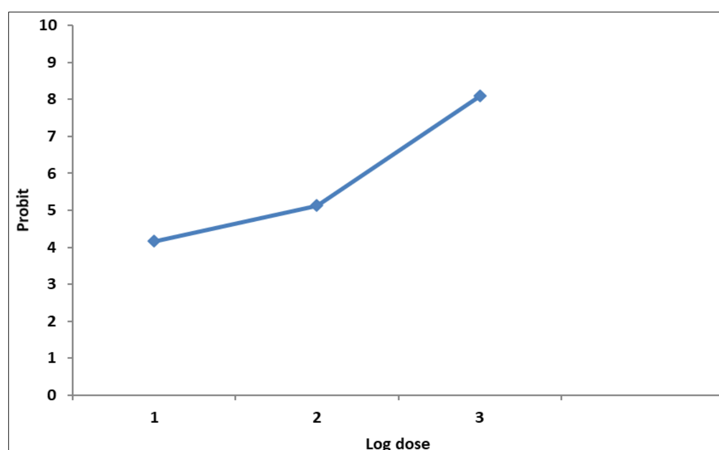


Fig 1: Probit against Logarithm of Dose in Cytotoxic lethality assay evaluation of Fem Q Elixir

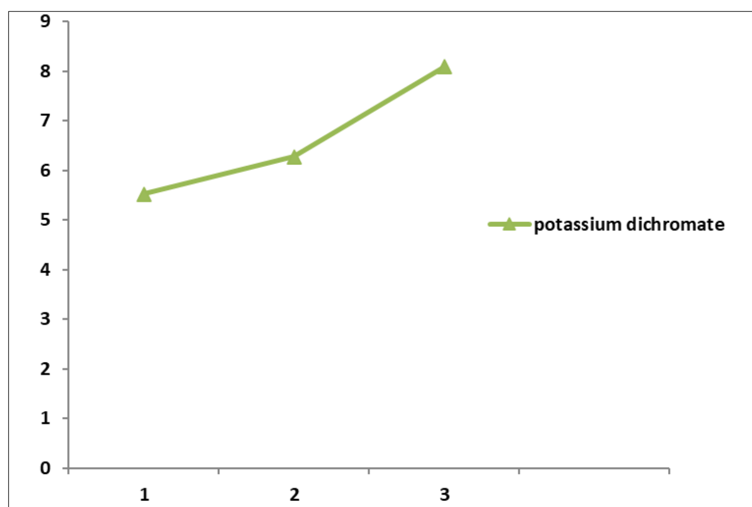


Fig 2: Probit against Logarithm of Dose in Cytotoxic lethality assay evaluation of potassium dichromate

Table 4: Weight variation of the control, animals treated with acacia 2% w/v and animals treated various doses of thelyophilized extract

Time (Days)	Group1(Control A)	Group 2 Control B	Group3(100mg/kg)	Group4(250mg/kg)	Group5(500mg/kg)
1	89.2 ± 0.37	88.2 ± 0.07	84.6 ± 0.01	88.9 ± 0.11	94.2 ± 0.07
5	94 ± 0.06	96.05 ± 0.6	90.6 ± 0.15	93.2 ± 0.03	96 ± 0.02
10	100.4 ± 0.14	101.4 ± 0.14	97.4 ± 0.08	101 ± 0.07	98.6 ± 0.17
15	100.8 ± 0.07	101.8 ± 0.07	98.4 ± 0.02	101.4 ± 0.01	98.6 ± 0.01
20	102.7 ± 0.02	103.7 ± 0.02	98.9 ± 0.25	103.6 ± 0.20	99 ± 0.04
25	104.5 ± 0.05	1O4.02 ± 0.05	99.4 ± 0.05	104.8 ± 0.01	99.6 ± 0.21
30	105.6 ± 0.21	105.6 ± 0.21	100.4 ± 0.07	106.1 ± 0.01	100.8 ± 0.01

N=5 *significant @ $p \leq 0.05$, ** significant @ $p \leq 0.01$

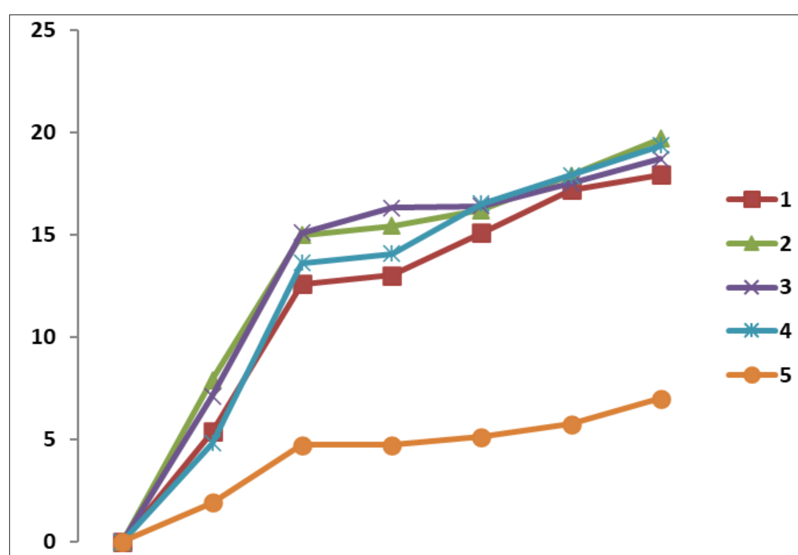
Group1: Control, Group 2 animals treated with acacia(2%w/v) Group3 animals treated with the extract dose of 100mg/kgbw, Group4: animals treated with the extract dose of 250mg/kgbwt, Group5: animals treated with the extract dose of 500mg/kgbwt

Table 5: Weight in variation of organs of control animals and animals treated with various doses of the extract per 100g body weight

Parameters	/100 g^{-1} body weight				
	Group 1	Group 2	Group3	Group 4	Group 5
Liver	0.046 ± 0.01	0.051 ± 0.01	0.037 ± 0.01	0.033 ± 0.01	0.04 ± 0.03
Ovary	0.02 ± 0.00	0.021 ± 0.01	0.02 ± 0.02	0.03 ± 0.04	0.03 ± 0.01
Kidney	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
Heart	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01•	0.06 ± 0.01	0.05 ± 0,01

N=5 *significant @ $p \leq 0.05$, ** significant @ $p \leq 0.01$

Group1: Control, Group 2 animals treated with the reference drug (glibenclamide at the dose of 600µg/kgbwt) Group3 animals treated with the extract dose of 100mg/kgbw, Group4: animals treated with the extract dose of 250mg/kgbwt, Group5: animals treated with the extract dose of 500mg/kgbwt



DAYS→ ■Gp.1 Control,▲Gp 2 animals treated with 2% w/v acacia solution XGp3 animalstreated with the extract 100mg/kg bwt Ж Gp4animals treated with the extract 250mg/kg bwtand • Gp5animals treated with the extract 500mg/kg bwt

Fig 4: Percentage weight variation of the control, animals treated with acacia solution 2%w/v and different extract doses of the extract

Table 6: The biochemical parameters of the control animald, the animals treated with acacia solution(2% w/v) and various doses of the extract respectively

	Group1	Group2	Group3	Group4	Group5
AST (U/l)	132 ± 0.1	135 ± 0.2	138 ± 0.04	145 ± 0.10*	125 ± 0.42*
ALT(U/l)	31 ± 0.07	32 ± 0.15	33 ± 0.53	32 ± 0.03	23± 0.07*
ALP	54.94 ± 0.0 4	55.5 ± 0.21	56.47± 0.1 7	58.64 ± 0.04	55.14 ± 0.04
TP(mmol/l)	82.51 ± 0.03	84.5 ± 0.11	96.63 ± 0.07	99.32 ± 0.61*	118.8± 0.52*
ALB	48.95 ± 0.04	53.2 ± 0.3	49.36 ± 0.13	49.82 ± 0.17	54.98 ± 0.03**
T-BIL(mmol/l)	3.6 ± 0.07	3.2 ± 0.1	3.2 ± 0.21	2.9 ± 0.01	3.9 ± 0.02
D-BIL(mmol/l)	0.7 ± 0.09	0.7 ± 0.02	0.5 ± 0.05	0.6 ± 0.01	0.8 ± 0.11
UREA(mmol/l)	11.77 ± 0.01	12.04 ± 0.5	7.26 ± 0.04	7.63 ± 0.34	5.72 ± 0.02
CREAT(mg/dl)	122.6 ± 0.04	122.01 ± 0.3	158 ± 0.17	192 ± 0.05*	167.7 ± 0.07*
CHOL(mmol/l)	3.46 ± .04	3.2 ± 0.1	3.43 ± 0.06	3.87 ± 0.02	3.07 ± 0.06*
TG(mmol/l)	0.6 ± 0.10	0.7 ± 0.2	1.37 ± 0.13	1.53 ± 0.33	1.71 ± 0.30*

N=5 *significant @ $p \leq 0.05$, ** significant @ $p \leq 0.01$

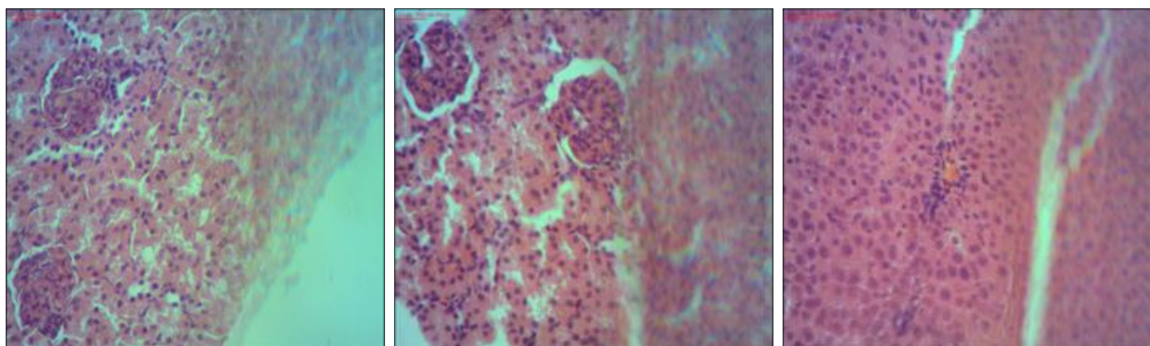
Group1: Control, Group 2 animals treated with the reference drug (glibenclamide at the dose of 600µg/kgbwt) Group3 animals treated with the extract dose of 100mg/kgbw, Group4: animals treated with the extract dose of 250mg/kgbwt, Group5: animals treated with the extract dose of 500mg/kgbwt

Table 7: Haematological values of control and treated rats with polyherbal formulation for 30days in subchronic study

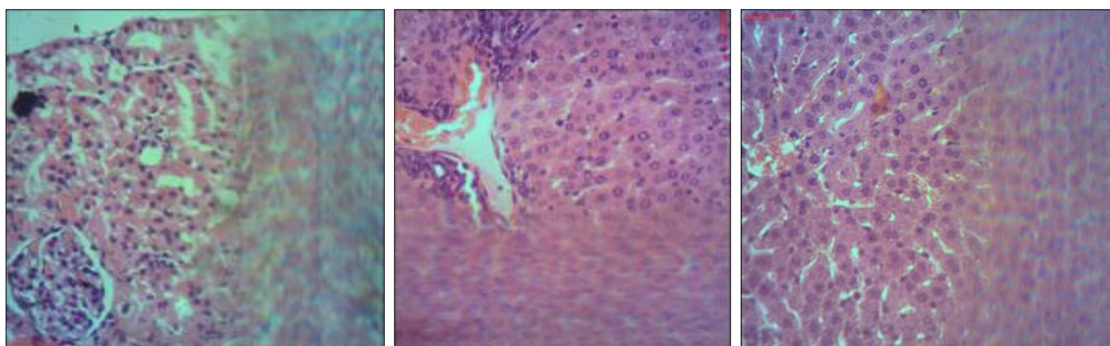
	Group 1	Group 2 100mg/kg	Group 3 250mg/kg	Group 4 500mg/kg	Group 5 Control
RBC(10^12/L)	5.83 ± 0.04	5.83 ± 0.04	7.61 ± 0.06	7.54 ± 0.4	7.48 ± 0.01*
WBC(10^9/L)	6.4 ± 0.04	6.4 ± 0.04	6.1 ± 00.4	5.9 ± 0.08	6.4 ± 0,17
HCT(PCV)%	38.8 ± 0.07	38.8 ± 0.07	45.2 ± 0.01	45.7 ± 0.03	46.5 ± 0,02
PLT(10^9/L)	488 ± 0.13	488 ± 0.13	663 ± 0.01	546 ± 0.06	604 ± 0.41
MCV(fl)	56.1 ± 0.28	56.1 ± 0.28	59.4 ± 0.01	66.8 ± 0.20	62.2 ± 0.03*
MCH(pg)	18.3 ± 0.07	18.3 ± 0.07	18.8 ± 0.3	19.7 ±0.07	14.9 ± 0.06
MCHC(g/dl)	36.6 ± 0.03	36.6 ± 0.03	29.2 ± 0.22	22.5 ± 0.12	28.8 ± 0.23
NEU#	0.9 ± 0.01	0.9 ± 0.01	1.1 ± 0.01	0.9 ± 0.08	1.3 ± 0.02
NEU%	20.7 ± 0.05	20.7 ± 0.05	23.1 ± 0.09	18.9 ± 0.03	27.6 ± 0.02
LYMPH%	31.3 ± 0.07	31.3 ± 0.07	31.6 ± 0.05	28.3 ± 0.02	48.6 ± 0.03*
Haemoglobin(g/dl)	12.8 ± 0.13	12.8 ± 0.13	13.2 ± 0.10**	13.9 ± 0.07**	10.4 ± 0.01*

N=5 *significant @ $p \leq 0.05$, ** significant @ $p \leq 0.01$

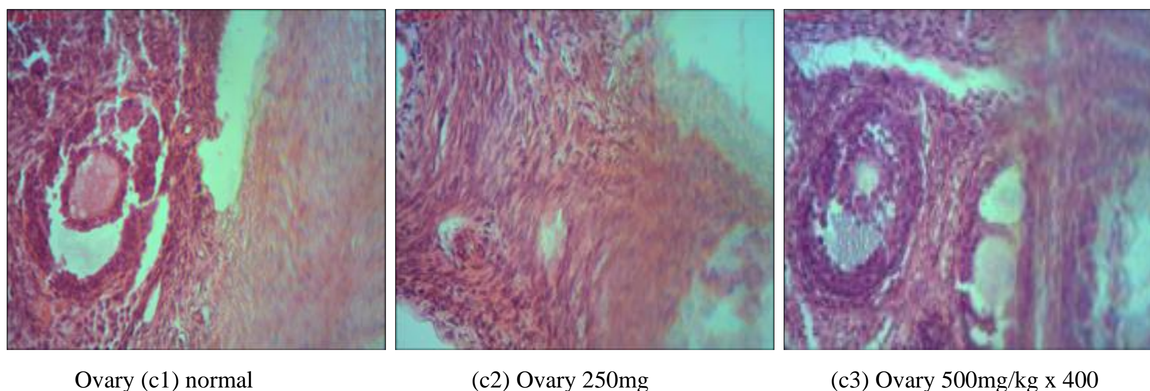
Group1: Control, Group 2 animals treated with the reference drug (glibenclamide at the dose of 600µg/kgbwt) Group3 animals treated with the extract dose of 100mg/kgbw, Group4: animals treated with the extract dose of 250mg/kgbwt, Group5: animals treated with the extract dose of 500mg/kgbwt



Kidney(5a) control(5a2) kidney treated with 250mg extract/kgbwt (5a3) kidney treated withextract500mg/kgbwt



Liver control Liver (5b2) treated with extract 250mg/kg bwt Liver (b3) treated with 500mg/kgbwt x40



Ovary (c1) normal (c2) Ovary 250mg (c3) Ovary 500mg/kg x 400

Fig 5: The photomicrographs of kidney, liver and ovary of the control group and the group treated with 250 and 500mg body weight respectively

Discussion

The used of herbal formulations to treat all sort of diseases is widely practice amongst the rural populace worldwide especially in the development countries and is gaining more ground and acceptance because of the success recorded in their uses (Ogbonnia *et al*., 2014b) [24]. Currently, it was estimated that about 80% of people in developing countries still rely on traditional medicine, based largely on various species of plants and animals for their primary healthcare (Steven, 2010) [29]. Of a great concern is the safety expressed by the purity the contents of pathogenic and nonpathogenic microorganisms in these formulations especially commercial liquid formulations widely consumed by increasing number of patients (Ogbonnia *et al*., 2014c).

In this study, the formulation FEM Q ELIXIR®, used the microbial purity evaluation showed no growth of bacterial organisms in the various diagnostic media used for bacteria after 24 hr of incubation. Also no growth of fungal organisms was observed in Sabouraud Dextrose Agar (SDA),medium used for fungi after seven days incubation. This demonstrated high degree of hygienic status and good manufacturing practice (GMP) observed in the treatment of raw materials and water used for the preparation as well as cleanliness of the surroundings.

The medium acute toxicity value of the polyherbal formulation was found to be above 20g/kg body weight. The polyherbal could be classified as being nontoxic (Klassen *et al*., 1995) [12] since LD_{50} by oral route was found to be much higher than WHO toxicity index of 2g/kg body weight (Mbaka *et al*., 2014a). The median lethal concentration value (LC_{50}) of the preparation was determined to be 7.94 ug/ml which was much higher above LC_{50} valve of the reference standard potassium dichromate calculated to be 1.99µg/ml. The extract could be considered to possess no cytotoxic effects (Colegate and Molyneux, 1993) [5].

In the sub-chronic study of FEM Q ELIXIR®, the lyophilized extract of the formulation in different doses were administered orally to the animals daily for 30 days. A significant ($p \leq 0.05$) decrease in the body weight of the animals that received the highest dose was observed and it could be explained succinctly that this dose of the extract could have suppressed appetites and haemoglobin absorption resulting in the significant ($p \leq 0.05$) decrease in the haemoglobin level observed. There were no significant ($p \geq 0.05$) changes in the body weights of all the animals treated with other doses compared with the control, signifying that the extract doses neither stimulated nor suppressed appetites in the animals (Table IV).

Also there were no significant ($p \geq 0.05$) changes in the organs weights studied except for the significant decrease ($p \leq 0.05$) observed in the heart weight (Table V) of the group treated with 100mg/kg bwt compared with the control. The reason for this reduction in weight cannot be instantly deduced but the fact that the extract did not generally affect the organs at bigger doses meant that it had no deleterious effects in the animals.

The effect of the lyophilized extract on the biochemical parameters showed that there was no significant ($p \geq 0.05$) changes in the bilirubin levels of the treated animals compared with the control observed signifying the extract could not have exerted toxic effect capable of impairing the functions of the liver transaminases AST and ALT are produced by the heart and liver and their increases are good indices of heart and liver damages. There were significant decreases in the plasma levels of AST and ALT observed in the animals treated with highest dose of the extract suggesting the extract had no deleterious effects in the animals at this dose. High levels of AST in the plasma indicates that the heart is damaged which might result from toxicants and muscle injury whereas increase in plasma ALT level is more specific to the liver for detecting hepatocellular damage. There were no changes in ALP levels observed in all the treated animals compared with the control. Significant ($p \leq 0.05$) increases in the creatinine and in the protein plasma levels were observed in the treated animals compared to the control and are indicative of impaired renal functions. An increase in observed in creatinine level could be attributed to kidney illness resulting from loss of normal excretive function or where there is either muscular damage or an incompatible medications interfering with the normal functioning of kidneys. There were significant ($p \leq 0.05$) increase in the triglyceride levels observed in all the treated animals and this might contribute to development of cardiovascular disease even though the level of cholesterols was not affected.

The study revealed significant ($p \leq 0.05$) decrease in heamatological and increase in the RBC, Platelets and PCV contents of the animals treated with 500 mg/kg body weight dose compared to the control. The significant increase in the RBC was an indication of the ability of the formulation to potentiate or stimulate the erythropoietin release in the kidney. A significant ($p \leq 0.05$) increase in the mean corpuscular volume (MCV) while decrease in a mean corpuscular heamoglobin concentration (MCHC) and mean corpuscular heamoglobin (MCH) were observed. The importance of MCH, MCV and MCHC in anaemia diagnosis has been reported by Coles, (1986) [4]. The observed a significant ($p \leq 0.05$) decrease in the Hb, MCH and MCHC

might be due to decrease in the absorption of iron, which could be an indicative of the extract potential to induce anaemia (Ogbonnia *et al*., 2010b).

The histological studies of the organs Figure 5(a to c) kidney, liver and ovary showed no abnormalities in the animals treated with 250 and 500mg/kg body weight compared with the control. The extract therefore did not exert any toxic or deleterious effects in the organs especially the ovary as the formulation is being used ethnobotanically as an anti-fertility agent.

Conclusion

The polyherbal formulation, Fem Q elixir®, was found to contain no microbial contaminants that was above WHO standard acceptable limits. It could be considered microbiologically safe for human consumption. The LD_{50} value (20.0g/kg) obtained which was far higher than WHO toxicity index of 2g/kg body weight was an indication that the polyherbal preparation could be safe for use internally. The tissue histology showed that there were no adverse effects on various organs of the animals evaluated. The hypolipidaemic and hypoglycaemic activities of the formulation indicate that it can be an alternative as well as supportive medication in the management of diabetes.

References

1. Bandaranayake WM. Modern Phytomedicine: Turning Medicinal Plants into Drugs. In: Ahmad I, Aqil F, M Owais (Eds.), Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim 2006.
2. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002;106:1930-1937.
3. Bürger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC, Soares dos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (Acmelabrasilinsis) (Asteraceae) in mice. J Pharm. Sci 2005;8(2):370-3 Bandaranyake.
4. Coles EH. Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, USA 1986, 10-42.
5. Colegate SM, Molyneux RJ. Bioactive Natural Products: Detection, Isolation and Structural Determination. CRC Press Inc. USA 1993, 442-480.
6. Crook MA. Clinical Chemistry and Metabolic Medicine. 7th Edn., Hodder Arnold, London 2006, 426.
7. Ekaidem IS, Akpanabiatu MI, Uboh FE, Eka OU. Vitamin B12 supplementation: Effects on some biochemical and haematological indices of rats on phenytoin administration. Biokemistri 2006;18:31-37.
8. Fontana R, Mendes MA, de Souza BM, Konno R, Ceasar LMM, Malaspina O *et al*. Jelleines: A Families of antmicrobial peptides from the Royal Jelly of honey bees (*Apis mellifera*). Peptides 2004;25:919-9928.
9. 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.
10. ILAR, (Institute of Laboratory Animal Research). Commission on Life Science. National Research Council, Retrieved from 1996: www.edu/openbook,php?record_id=5140.
11. 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.
12. Klaasen CD, Amdor MO, Doull J. Casarett and Doill's Toxicology: The Basic Science of Poison. 5th Edn., McGraw Hill, USA. ISBN-13:9780071054768 1995, 13-33.
13. Mbaka GO, Ogbonnia SO, Banjo AE. Activity of *Raphia hookeri* root extract on blood glucose, lipid profile and glycosylated haemoglobin on alloxan induced diabetic rats. J Morphol. Sci 2012a;29(3):1-9.
14. 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. Br. J Med. Med. Res 2012b;2(4):621-635.
15. Mbaka GO, Ojewale AO, Ogbonnia SO, Ota DA. Antihyperglycaemic and Antihyperlipidaemic Activities of Aqueous Ethanol Root Extract of *Pseudocedrela kotschyion* Alloxan-Induced Diabetic RatsBritish Journal of Medicine & Medical Research 2014;4(36):5839-5852.
16. Mbaka GO, Ogbonnia SO, Olubamido TO, Awopetu PI, Ota DA. Evaluation of Acute and Sub-chronic Toxicities of Aqueous Ethanol Root Extract of Raphiahookeri (Palmaceae) on Swiss Albino Rats British Journal of Pharmacology and Toxicology 2014;5(4):129-135.
17. Miller LC, Tainter ML. Estimation of the LD_{50} and its error by means of logarithmic probit graph paper. P. Soc. Exp. Biol. Med 1944;24:839-840.
18. 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.
19. NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 4thEdn., National Committee for Clinical Laboratory Standards, Wayne, PA., USA 1997.
20. Ogbonnia SO, Odimegwu JI, Enwuru VN. Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of Treculiaafricana Decne and *Bryophyllum pinnatum* Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats. Afr. J Biotechnol 2008a;7:2535-2539.
21. Ogbonnia SO, Mbaka GO, Nwozor AM, Igbokwe AM, Usman HNA, Odusanya PA. Evaluation of Microbial Purity and Acute and Sub-acute Toxicities of a Nigerian Commercial Polyherbal Formulation Used in the Treatment of Diabetes Mellitus. British Journal of Pharmaceutical Research 2013a;3(4):948-962.
22. Ogbonnia SO, Mbaka GO, Emordi J, Nkemhule F, Joshua P, Usman A *et al*. Antimicrobial evaluation and acute and sub-acute toxicity studies on of a commercial polyherbal formulation, Ade & Ade Antidiabetic® used in the treatment of diabetes in southwestern Nigerian. Journal of Medicine and Medical Sciences 2013b;4(11):423-432.
23. Ogbonnia S, Olayemi S, Mbaka G, Morgan Y, Usman A, Odusany P *et al*. Microbial purity and Toxocoty profiles of Two Polyherbal Formulations Used in Nigerian Herbal Medicines. Pharmacologia 2014a, 357-368.
24. Ogbonnia SO, Mbaka GO, Nkemehule FE, Emordi JE, Okpagu NC, Ota DA. Acute and Subchronic Evaluation of Aqueous Extracts of *Newbouldia laevis* (Bignoniaceae) and *Nauclea latifolia* (Rubiaceae) Roots

used Singly or in Combination in Nigerian Traditional Medicines 2014b.
25. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX *et al*. Evaluation of acute and sub-acute toxicities of aqueous ethanolic extract of leaves of (L) Roxb (Ceasalpiniaceae). Afr. J Biotechnol 2006;5:283-289.
26. Rickert K, Martinez RR, Martinez TT. Pharmacist Knowledge of Common Herbal Preparations. Proc. West. Pharmacol. Soc 1999;42:1-2.
27. Sam Wah Teng. Toxicity Testing Using Brine Shrimp-Artemia Salina: In Bioactive Natural Products Detection, Isolation and Structural Determination. Colgegate M. steven and Molyneurej Russsell (Eds). CRC Press USA 1993, 441-447.
28. Shah AMA, Garg SK, Garg KM. Subacute toxicity studies on Pendimethalin in rats. Indian J Pharmacol 1997;29:322-324.
29. Steven K. Introduction to traditional medicine. Pharmaceutical Press 2010;1:1.
30. Sushruta K, Satyanarayana N, Srinivas, Sekhar RJ. Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbellifereous fruits used in culinary practice. Trop. J Pharm. Res 2006;5(2):613-617.
31. Tédong L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF *et al*. Acute and Subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr. J Tradit. Complem 2007;4(2):140-14.
32. 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 FMVP4, to gerbils. J Pharm. Sci 2001;4(3):228-233.
33. WHO. Specifications for identity and purity and toxicological evaluation of food colours. WHO/Food Add/66.25 Geneva 1966.
34. World Health Organization, Geneva. WHO Policy Perspectives on Medicines - Traditional Medicine - Growing Needs and Potential (MAY) PG 2002, 1.