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EVALUATION OF ACUTE AND SUB-CHRONIC ORAL TOXICITY STUDY OF BAKER CLEANSERS BITTERS - A POLYHERBAL DRUG ON EXPERIMENTAL RATS

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ABSTRACT

Baker Cleanser Bitters (BCB) - a polyherbal formula commonly used in the treatment of diabetes, liver cirrhosis, kidney failure, rheumatism and arthritis was evaluated in an acute and sub-chronic toxicity study in Wistar albino rats. A single administration of BCB was given orally at the highest dose level of 2000 mg/kg body weight in the acute toxicity study. Signs of toxicity were observed every hour for the first 6 h and every day for 7 days. In the sub-chronic oral toxicity study, BCB was administered to rats at doses of 50, 100 and 200 mg/kg body weight for 28 days. Mortalities, clinical signs, body weight changes, biochemical and haematological parameters were monitored during the study period. There were no mortalities or clinical signs observed in rats in the acute toxicity study. In the sub-chronic study in rats, daily oral administration of BCB at the dose of 200 mg/kg body weight resulted in a drop in percentage increase in body weight at the end of the 4^{th} week. Alanine amino transferase (ALT), aspartate amino transferase (AST), fasting blood sugar and packed cell volume (PCV) decreased significantly ($p \leq 0.05$) whereas alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and platelets increased significantly ($p \leq 0.05$) when compared to control. The high no-observed adverse effects level (NOAEL) value of 2000 mg/kg body weight implies that the drug could be safe. The study also revealed that the polyherbal drug may have good hypoglycemic effects and favourable reducing effects on the cardiovascular risk factors and explains the basis for the continual use of this plant by traditional medical practitioners.

Keywords*:* Baker Cleanser Bitters, acute toxicity, sub-chronic toxicity, polyherbal formula

INTRODUCTION

Herbal medicine or phytomedicine is recognized as the most common form of alternative medicine (Ogbonnia et al., 2011). The World Health Organization (WHO) estimates that 80 % of the world's population relies on these "alternative" plant-based medicines as their primary medical intervention especially in the developing and in the developed countries where modern medicines are predominantly used (Rickert et al., 1999; Kroll and Shaw, 2003; Ogbonnia et al., 2008). Over the years, the use of herbs in the treatment of illnesses has been very successful and its historic usage has been useful in drug discovery development. Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1989; Zhu et al., 2002). The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and

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responsibility of practitioners using traditional remedies (Chan, 1995). Herbal remedies are considered safer and less damaging to the human body than synthetic drugs (Alam et al., 2011). However, the lack of standardization has been a major concern regarding use of herbal medicines (Angell and Kassierr 1998; NIEHS, 1998). Although herbal supplements may be considered to be safe, some are known to be toxic at high doses and others may have potentially adverse effect after prolonged use. The general public is largely unaware that adverse health effects can be associated with the use of herbal supplements resulting from overdosing, contaminated formulations to the inherent toxicity of the herbs of choice (Hazel et al., 1999).

Baker Cleanser Bitters is a polyherbal formulation used for various ethnomedicinal purposes in Nigeria. The constituents of Baker Cleanser Bitters (*Aloe vera*, *Acinos ravens*, *Chenopodium murale*, *Cinnoamomum aromaticum, Citrus aurantifolia* and purified water) have been studied extensively (Grieve, 1971; Crawford, 2009). Ethnomedicinally, 30 ml of the liquid product is taken daily after meal preferable at night for seven days for the treatment of diabetes mellitus, waist pain, arthritis, rheumatism, infertility, liver cirrhosis, kidney failure, problems as well as typhoid fever and haemorrhoids. The aim of this study was to evaluate the safety profile of the polyherbal anti-diabetic preparation Baker Cleanser Bitters commonly used in Nigeria after a single or 28-day repeated dosing on selected biochemical and haematological parameters.

MATERIALS AND METHODS

Collection of samples

The herbal remedy Baker Cleanser Bitters (BCB), manufactured by Baker Water and Beverages Ventures situated at 3-4 Sam Balogun close, off May and Baker Avenue, Aina Estate, Egusi Village, opposite Cannan land, Otta, Ogun State, Nigeria was obtained from Isaac Boro Park, Mile1, in Port Harcourt City LGA of Rivers State, Nigeria.

Experimental animals

Healthy Wistar albino rats of both sexes weighing between 120-150 g, obtained from the Animal House of the Department of Physiology, University of Nigeria, Enugu Campus, Enugu State, Nigeria were used for the sub-chronic study. They were divided into four groups of 10 rats per group. Each group comprised of five (5) male and female rats respectively. The animals were housed in a cross-ventilated room and kept under standard environmental condition of 12/12 h light/dark cycle. They were housed in polypropylene cages (5 animals per cage) and were fed with standard rat pellet and water *ad libitum*. They were allowed to acclimatization for 7 days to the laboratory conditions before the experiment. The experiment was performed in accordance with the guidelines established by the European Community for the Care and Use of Laboratory Animals and were approved by Institutional Animal Ethical Committee (IAEC).

Acute toxicity test

Healthy Wistar albino rats of both sexes weighing between 120-150 g maintained under standard laboratory conditions were used for the acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD guideline, 2002). A total of ten animals of equal numbers of male and female rats were used and each received a single oral-dose of 2000 mg kg^{-1} body weight of BCB. Animals were kept overnight fasting prior to drug administration by oral gavage. After administration of drug sample, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 7 days. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure),

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autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted (OECD, 2002).

Experimental design

Forty healthy Wistar albino rats of both sexes were used for the sub-chronic study. They were divided into four groups of 10 rats per group with each having equal numbers of male and female rats. Group I was fed with standard diet and water only. Group II was fed with standard diet, water and 50 mg/kg body weight of BCB, group III was fed with standard feed, water and 100 mg/kg body weight of BCB while group IV rats were fed with standard feed, water and 200 mg/kg body weight of BCB only (Table 1). The drugs were administered using a curved, ball-tipped stainless steel feeding needle for a period of 28 days.

Table 1: Feeding study

Group	Treatment	Duration (Days)	No of rats*
I	Normal feed + water	28	10 rats
II	Feed + **50 mg/kg** bwt Baker Cleanser Bitters	28	10 rats
III	Feed + **100 mg/kg** bwt Baker Cleanser Bitters	28	10 rats
IV	Feed + **200 mg/kg** bwt Baker Cleanser Bitters	28	10 rats

*10 rats (5 males + 5 females)

Measurement of body weight

The body weights of the animals were evaluated weekly and recorded using a sensitive balance (OECD, 1995).

Sample collection

At the end of the experimental period, the animals were sacrificed using cervical dislocation method. Blood samples were obtained by cardiac puncture from each rat by means of a 2 ml hypodermic syringe and needle. The blood samples were introduced into clean dry bottles (EDTA bottles) for haematological parameters while heparinized tubes were used to collect blood for biochemical estimation. The heparinized blood was centrifuged within 5 min of collection at 2500 rpm for 10 minutes. Serum was collected into a clean dry sample container. The levels of biochemical parameters (ALT, AST, ALP, LDH and bilirubin) were estimated using the Humazym MUV-test kits. The white blood cells (WBC) and the differentials were estimated using the improved Neubauer counting chambers as described by Dacie and Lewis (1991). The haemoglobin (Hb) concentration was determined by the Cyameth-haemoglobin method while the Packed Cell Volume (PCV) was determined by the micro method also as described by Dacie and Lewis (1991).

Lipid peroxidation assay

The assay method of Hunter et al. (1963) modified by Gutteridge and Wilkins (1982) was adopted in the estimation of liver malondialdehyde (MDA). One gram of liver was homogenized in 10 ml saline solution and 1.2 ml of the liver homogenate was added to 6ml of glacial acetic acid and 6ml of 1 % of Thiobarbituric acid (TBA) dissolved in 0.2 % sodium hydroxide solution in a test tube. This mixture was placed into boiling water bath at 100 °C for 15 min, then allowed to cool and centrifuged for 10 min and supernatant were collected. In the assay, the clear supernatant (pink colored) was carefully transferred into a cuvette and absorbance read on a spectrophotometer at wavelength of 532 nm against the reagent blank. A molar estimation coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ was used according to the expression of Adam-Vizi and Seregi (1982).

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Statistical analyses

The results are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was employed for between and within group comparison while student's t-test was used for paired comparison. 95 % level of significance ($p \leq 0.05$) was used for the statistical analysis.

RESULTS

There were no BCB extract-treatment related mortalities recorded in animals treated with a single dose of 2000mg/kg body weight. Therefore, the approximate lethal dose (LD_{50}) of BCB extract in the experimental rats was higher than 2000 mg/kg. There were no clinical signs in the skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000 mg kg^{-1} body weight of BCB.

The effects of BCB on the percentage increase in body weight of the control and treated rats are shown in Figure 1. Generally, a steady increase in the body weight was observed in all the treated rats compared with control up to the 3rd week. However, a drop in the body weight was observed in all the treated rats from the 3rd week till the end of the 4th week in rats fed 100 and 200 mg kg^{-1} body weight of BCB. The drop in body weights was from 75.71 % and 83.33 % in the 3rd week to 74 % and 66 % at the end of the 4th week in rats fed 100 and 200 mg kg^{-1} body weight of BCB respectively. Surprisingly, the group fed 50 mg kg^{-1} body weight of BCB showed a percentage increase in body weight from 75 % in the 3rd week to 80 % at the end of the 4th week in a similar fashion as the control rats from 85 % in the 3rd week to 90 % at the end of the 4th week. Table 2 shows a summary of the results of the effects of BCB on the serum biochemical parameters. There was a significant decrease ($p \leq 0.05$) in the activities of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and fasting blood sugar (FBS) levels in the treated rats when compared with the control. However, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) showed a significant ($p \leq 0.05$) increase in rats treated with BCB when compared with the control. There were however, no significant ($p \leq 0.05$) changes in total bilirubin, conjugated bilirubin, malondialdehyde (MDA), total protein, cholesterol, triglycerides, creatinine and urea of the treated groups compared with control.

The effect of sub-chronic administration of BCB on haematological parameters is presented in Table 3. The packed cell volume (PCV) in treated groups showed a marginal decrease when compared with control. The platelets of rats administered 200 mg/kg body weight were significantly ($p \leq 0.05$) higher when compared with the control group. The lymphocytes of rats administered 100 mg/kg body weight showed a marginal increase whereas a marginal decrease in the level of monocytes was also observed in the same group when compared with the control. However, there was no significant difference in WBC and neutrophils (Neu) in rats treated with BCB as compared to control.

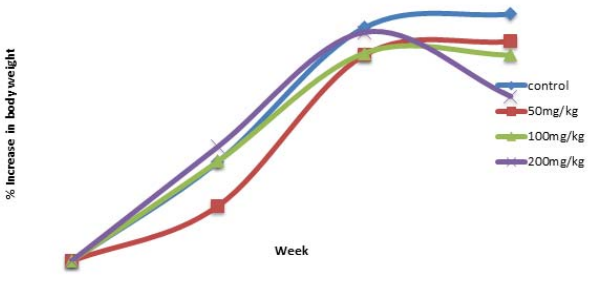

Figure 1: Percentage increase in body weight of the control and rats treated with different doses of BCB

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Table 2: Serum biochemical parameters in rats treated orally with BCB extracts for 28 days

	Dose mg/kg body weight			
Parameters	**0**	**50**	**100**	**200**
ALT	144.50±50.20	143.60±44.12	125.80±46.75*	111.80±18.79*
AST	344.50±14.85	325.28±0.16*	255.80±80.38*	269.20±56.95*
ALP	20.40±1.70	17.51±3.25*	36.51±2.65	35.80±5.52
LDH	405.00±21.21	451.01±62.56*	481.41±95.22*	501.40±175.81*
T.B (µmol/L)	8.75±1.34	6.3±3.5	7.84±4.5	8.26±6.6
C.B (µmol/L)	2.65±0.64	1.34±0.98	1.80±1.64	1.96±1.49
MDA(µmol/L)x10^{-6}	2.38±6.1	2.05±3.6	1.91±6.7	1.30±2.2
Total protein (g/dl)	41.80±0.85	41.74±5.06	41.64±1.65	41.18±2.42
Cholesterol (µmol/L)	2.45 ± 0.35	1.93 ± 0.81	1.93 ± 0.39	2.30 ± 0.53
Triglycerides(mMol/L)	0.90±0.1	0.90±0.26	0.98±0.12	1.27±0.24
Creatinine (µMol/L)	66.70±4.16	70.00±1.73	69.30±3.79	67.00±3.0
Urea (µMol/L)	2.20±0.36	1.97±0.21	1.93±0.57	1.90±0.36
Fasting blood sugar (µMol/L)	9.60±5.98	8.60±0.10	6.73±0.15	5.10±1.08

Values are expressed as mean ± S.E.M of 10 animals (one-way ANOVA); p>0.05 vs. control group (student's t-test). *significantly different from control, p<0.05.

Table 3: Haematological parameters in rats treated orally with BCB extracts for 28 days

	Dose mg/kg body weight			
Parameters	**0**	**50**	**100**	**200**
Hb (mg/dl)	14.25±0.21	14.39±0.38	14.37±0.01	14.75±0.13
PCV (%)	41.50±2.1	33.50±3.5	33.00±1.42	33.50±3.5
WBC (x10^6/µL)	8.15±1.63	9.20±0.42	10.20±0.57	10.70±1.20
Platelets (x10^3/µL)	161.00±73.53	169.00±12.73	173.00±9.90	184.00±8.49*
Neu (%)	13.00±1.41	15.00±2.83	13.00±4.24	16.50±3.53
Lym (%)	70.50±3.54	70.00±2.83	76.00±4.24	71.50±2.12
Mon (%)	16.50±2.12	15.00±4.23	11.00±2.83	13.50±3.54

Values are expressed as mean ± S.E.M of 10 animals (one-way ANOVA); p>0.05 vs. control group (student's t-test). *significantly different from control, p<0.05.

DISCUSSION

Traditional medicine has maintained greater popularity all over developing world and the use is rapidly on the increase (Daswani et al., 2006; Ogbonnia et al., 2010). Despite this, the safety of herbal medicine use has recently been questioned due to reports of illness and fatalities (Stewart et al., 1999; Ernst, 2002; Veiga-Junior et al., 2005; Park et al., 2010); hepatotoxicity (Saad et al., 2006) and nephrotoxicity (Cosyns, 2003; Colson and De Broe, 2005; Debelle et al., 2008). Although there are many traditional herbal medicines available, only a few have been verified by clinical trials, their efficacy and safety are still questioned by consumers (Cheng et al., 2009).

In the present study, single dose of oral administration of BCB to Wistar albino rats at 2000 mg/kg body weight had no effect on mortality and clinical signs such as changes in the skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion). Generally, there was no test substance related mortality observed at 2000 mg/kg (Wallace Hayes, 2001). Therefore, no acute toxicity was found in rats treated with BCB and the approximate medium acute toxicity lethal value (LD_{50}) were determined to be

higher than 2000 mg/kg and as such could be generally regarded as safe (GRAS). This finding is in agreement with Clarke and Clarke (1967), who reported that any compound or drug with oral LD_{50} estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. However, Zbinden and Roversi (1981) suggested that variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the LD_{50} values obtained and as such are considerable uncertainties in extrapolating the LD_{50} obtained for species to other species. This finding is suggestive that LD_{50} may not be considered as a biological constant (Zbinden and Roversi, 1981).

The effect of the drug on the percentage increase in the body weight was remarkable in all the treated groups up till the 3rd week. On the other hand, the significant decrease observed at the end of the 4th week in the groups treated with BCB could be attributed to the suppression of the animals' appetite by the extract leading to reduced food intake (Ogbonnia et al., 2010).

Serum marker enzymes are biochemical parameters associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health. Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) are largely used in the assessment of liver damage by drugs or any other hepatotoxin (Ramaiah, 2011). The liver and heart release ALT and AST and an elevation in their plasma concentrations are indicators of liver and heart damage (Wasan et al., 2001; Mythilypriya et al., 2007). However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Ozer et al., 2008). The significant decrease observed in the level of ALT and AST is suggestive that the polyherbal formula may not possess hepatotoxic effect and equally could not have caused some toxic effects on the heart tissue (Crook, 2006). The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage (Pari and Murugan, 2004). The observable increase in the level of alkaline phosphatase (ALP) in the group administered 100 and 200 mg/kg body weight of BCB may be as a result of congestion or obstruction of biliary tract, which may occur within the liver. ALP activity on the other hand is related to the functioning of hepatocytes and an increase in its activity may be due to its increased synthesis in the presence of increased pressure (Manjunatha et al., 2005). However, the increased level of lactate dehydrogenase (LDH) observed in the present investigation apparently indicated the toxic effect of Baker Cleansers Bitters in rat. There were no significant changes in total protein in rats treated with BCB, which suggested that there was no sign of impaired renal function (Tilkian et al., 1979). Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney and liver diseases and many other conditions (Thierry et al., 2011). The near–normal levels of total cholesterol and triglycerides observed in groups treated with BCB may be attributed to the presence of hypolipidemic agents in the polyherbal drug (Ogbonnia et al., 2011). Similarly, the drug had no adverse effect on the concentration of creatinine and urea. This is suggestive of no kidney damage specifically by renal filteration mechanism (Crook, 2006) or probably indicates that BCB did not interfere with the renal capacity to excrete these metabolites. Therefore, it was evident that the drug at doses employed did not cause renal impairment or kidney damage. Increase in platelets observed in rats treated with 200 mg/kg body weight may be attributed to enhanced production and secretion of thrombopoetin the primary regulator of platelet production (Kaushansky, 1995) by BCB indicating that it has haemostatic property (Olaleye et al., 1998).

In conclusion, the present investigations could be regarded as preliminary probes, necessitating further studies. The present findings have shown that Baker's Cleanser

Bitters is not likely to produce severe toxicological risk. However, a conclusive remark can only be made on the safety profile of the drug after further investigations of the observations made in this study at the cellular level in a chronic study.

REFERENCES

Adam-Vizi V, Seregi A. Receptor independent stimulatory effect of noradrenaline on Na, K-ATPase in rat brain homogenate. Role of lipid peroxidation. Biochem Pharmacol 1982;34:2231–6.

Alam MB, Hossain MS, Chowdhury NS, Mazumder MEH, Haque ME. In vitro and in vivo antioxidant and toxicity evaluation of different fractions of *Oxalis corniculata* linn. J Pharmacol Toxicol 2011;6:337-48.

Angell M, Kassierr JP. Alternative medicine – the risk of untested and unregulated remedies. N Engl J Med 1998;339:839-41.

Chan I. Progress in traditional Chinese edicine. Trends Pharm Sci 1995;16:182-7.

Cheng CW, Bian ZX, Wu TX. Systematic review of Chinese herbal medicine for functional constipation **.**World J Gastroenterol 2009;15:4886–95.

Clarke ML, Clarke EGC. Veterinary toxicology. London: Bailliere Tindall, 1967.

Colson CR, De Broe ME. Kidney injury from alternative medicines. Adv Chronic Kidney Dis 2005;12:261–75.

Cosyns JP. Aristolochic acid and 'Chinese herbs nephropathy': A review of the evidence to date. Drug Saf 2003;26:33–48.

Crawford P. Effectiveness of cinnamon for lowering hemoglobin A1C in patients with type 2 diabetes: a randomized, controlled trial. J Am Board Fam Med 2009;22:507-12.

Crook MA. Clinical chemistry and metabolic medicine. 7th ed. London: Hodder Arnold, 2006.

Dacie JV, Lewis MS. Practical haematology. 7th ed (pp 227-57). London: Churchill Livingstone, 1991.

Daswani GP, Brijesh S, Birdi JT. Preclinical testing of medicinal plants: advantages and approaches. Workshop proceedings on approaches towards evaluation of medicinal plants prior to clinical trial. Organized by the Foundation for medical Research at Yashwantrao Chavan Academy of Development Administration (YASHADA) (pp 60-77). Pune, India, 2006.

Debelle FD, Vanherweghem JL, Nortier JL. Aristolochic acid nephropathy: a worldwide problem. Kidney Int 2008;74:158–69.

Ernst E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends Pharmacol Sci 2002;23:136–9.

Grieve M. A modern herbal: the medicinal, culinary, cosmetic and economic properties, cultivation and folklore of herbs, grasses, fungi, shrubs & trees with their modern scientific uses (Vol.1 A-H). New York: Dover Publ. 1971.

Gutteridge JMC, Wilkins C. Copper dependent hydroxyl radical damage to ascorbic and formation of a thiobarbituric acid reactive products. FEBS Lett 1982;137: 327-40.

Hazel BM, George WL, Kenneth DF. Medicinal herbs in the United States: research Needs. Environ Health Perspect 1999;107: 773-8.

Hunter FE, Gbebicki JM, Hoffstein PE, Weinstein J, Scott A. Swelling and lysis of rats liver mitochondria induced by ferrous ions. J Biol Chem 1963;238:828-35.

Kaushansky L. Thrombopoietin, the primary regulator of megakaryocyte and platelets production. Thrombosis and Haemostasis 1995;74:521-5.

Kroll DJ, Shaw HS. (2003). Complementary and alternative medicine (CAM): relevance to laboratory medicine. Clin Laboratory Int 2003;27(3):14-6.

Manjunatha BK, Vidya SM, Dhiman P, Pallavi R. Hepatoprotective activity of *Leucas hirta* against CCl_4 induced hepatic damage in rats. Indian J Exp Biol 2005;43:722-7.

Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. J Health Sci 2007;53: 351-8.

NIEHS. News: herbal health. Environ Health Perspect 1998;106:A590-2. Web resource. http://en.wikipedia.org/wiki/Aloe Vera.

OECD, Organisation for Economic Cooperation and Development. Repeated dose 28-day oral toxicity test method guideline 407 adopted 23.03.1996. In: OECD, Guidelines for testing of chemicals. Paris: Organisation for Economic Co-Operation and Development, 1995.

OECD, Organisation for Economic Cooperation and Development. Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 423: Acute oral toxicity - acute toxic class method. Paris: Organization for Economic Cooperation and Development, 2002.

Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr J Biotechnol 2008;7:701-5.

Ogbonnia SO, Mbaka GO, Anyika EN, Osegbo OM, Igbokwe NH. Evaluation of acute toxicity of hydro-ethanolic extract of *chromolaena odorata* (L.) king and robinson (Fam. Asteracea) in rats. Agric Biol J North Am 2010;1:859-65.

Ogbonnia SO, Mbaka GO, Anyika EN, Emordi JE, Nwakakwa N. An evaluation of acute and subchronic toxicities of a Nigerian polyherbal tea remedy. Pak J Nutr 2011; 10:1022-8.

Olaleye SB, Akinniyi JA, Salami HA, Mbajiorgu FE, Ameh A. Preliminary study on the toxicity and antimicrobial effects of extracts of *Securidaca longipedunculata*. Afr J Biomed Res 1998;1:39-44.

Ozer J, Ratnerb M, Shawc M, Baileya W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology 2008;245:194-205.

Pari L, Murugan P. Protective role of tetrahydrocurcumin against Erythromycin estolate-induced hepatotoxicity. Pharmacol Res 2004;49:481-6.

Park M, Choi H, Kim J, Lee H, Ku S. 28 days repeated oral dose toxicity test of aqueous extracts of Mahwangyounpae-tang, a polyherbal formula. Food Chem Toxicol 2010;48:2477–82.

Ramaiah SK. Preclinical safety assessment: current gaps, challenges and approaches in identifying translatable biomarkers of drug-induced liver. Clin Laboratory Med 2011; 31:161-72.

Rickert K, Martinez RR, Martinez TT. Pharmacist knowledge of common herbal preparations. Proc West Pharmacol Soc 1999;42:1-2.

Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal. Evid Based Complement Alternat Med 2006;3: 433–9.

Sofowora EA. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books, 1989.

Stewart MJ, Moar JJ, Steenkamp P, Kokot M. Findings in fatal cases of poisoning attributed to traditional remedies in South Africa. Forensic Sci Int 1999;101:177–83.

Thierry TA, Acha AE, Paulin N, Aphrodite C, Pierre K, Tazoacha A. Subacute toxicity study of the aqueous extract from *Acanthus montanus*. Electronic J Biol 2011;7(1):11-5.

Tilkian SM, Conover BM, Tilkian AG. Clinical implications of laboratory tests. 2nd ed. St. Louis, MO: Mosby, 1979.

Veiga-Junior VF, Pinto AC, Maciel MAM. Medicinal plants: safe cure? Quim Nova 2005;28:519–28.

Wallace Hayes A: Principles and methods of toxicology, 4th ed (pp 871-3). Boca Raton: CRC Press, 2001.

Wasan KM, Najafi S, Wong J, Kwong M. Assessing plasma lipid levels, body weight and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FM-VP4 to gerbils. J Pharm Sci 2001;4:228-34.

Zbinden G, Roversi F. Significance of the LD_{50} test for the toxicological evaluation of chemical substances. Arch Toxicol 1981; 47:77–99.

Zhu M, Lew KT, Leung P. Protective effects of plant formula on ethanol-induced gastric lesions in rats. Phytother Res 2002; 16:276-80.