Journal of Pharmacy and Allied Health Sciences

ISSN 2224-2503

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

Journal of Pharmacy and Allied Health Sciences 1 (2): 34-48, 2011
ISSN 2224-2503 / DOI: 10.3923/jpahs.2011.34.48

Toxicity Evaluation of a Polyherbal Antihypertensive Mixture in Ghana

[1]G.A. Koffuor, [1]E. Woode, [2]C. Obirikorang and [3]E. Asiamah

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Ghana

[2]Clinical Laboratory, KNUST Hospital, Kumasi, Ghana

[3]Department of Pathology, Komfo Anokye Teaching Hospital, Kumasi, Ghana

Corresponding Author: George A. Koffuor, Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana Tel: +233 277 400 312

ABSTRACT

The aim of the study was to evaluate and assess the safety of a polyherbal antihypertensive commonly used in Ghana. The product was administered by gavage to mice at doses of 36, 72 and 180 mg/kg/day. Organ-to-body weight ratio, liver and kidney function, hematological and lipid profile and histopathological examinations were performed. Physical observation showed no toxic symptoms. Higher doses recorded a decrease in liver weight with increase in treatment periods. White blood cells also increased significantly. Hemoglobin concentration (but not red blood cells and mean corpuscular volume) decreased very significantly after 23 days of treatment. Hematocrit, plateletcrit and platelet distribution width increased very significantly, while mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration significantly reduced. Total plasma protein (albumin and globulin) increased significantly. *Alanine aminotransferase*, *Aspartate aminotransferase* and *Alkaline phosphatase*, increased significantly with onset of treatment but these returned to normal. In the kidney function tests, creatinine increased significantly. Total cholesterol and high density lipoproteins reduced very significantly while triglycerides, very low density lipoproteins and low density lipoproteins increased significantly initially but all these were normal by day 45. Urine analysis showed no significant changes. The liver, kidney and spleen showed some pathological changes. The product had an initial antigenic effect and causes microcytic-anitocytic anaemia. It affects liver function transiently and has effect on the kidney with prolonged used. The no-observable-adverse-effect-level is 32 mg/kg/day. The product is safe to use at reasonably lower doses but liver and kidney function must be monitored.

Key words: Polyherbal, hypertension, liver function, hematology, kidney function

INTRODUCTION

The polyherbal antihypertensive under study is an aqueous preparation made from *Persea americana* Mill (Lauraceae) and *Vernonia amygdalina* L. (Asteraceae) obtained from forests in Ghana. *Persea americana* (avocado, alligator pear or butter pear) is known to have hypotensive or antihypertensive effects (Adeboye *et al*., 1999; Kate and Lucky, 2009). *Vernonia amygdalina* also known as "bitter leaf" is a widely used medicinal plant in Africa for its antihypertensive effects (Lawal *et al*., 2010). Vernonia amygdalina is a shrub that grows predominantly in the tropical Africa. Leaves from this plant serve as vegetable and culinary herb in soup (Aregheore *et al*., 1998).

In traditional Nigerian homes, extracts of the plant are used as tonic, in the control of tick and treatment of cough, feverish condition, constipation and hypertension (Regassa, 2000; Kambizi and Afolayan, 2001; Amira and Okubadejo, 2007).

Cardiovascular disease is the main cause of death in virtually all industrialized countries (Whelton, 1994). WHO (2002) identified hypertension, or high blood pressure, as the third ranked factor for disability-adjusted life years. Hypertension is one of the primary risk factors for heart disease and stroke, the leading causes of death worldwide. Recent, analyses have shown that as of the year 2000, there were 972 million people living with hypertension worldwide and it is estimated that this number will escalate to more than 1.56 billion by the year 2025. Nearly two-thirds of hypertensive patients live in low- and middle-income countries, resulting in a huge economic burden (Chockalingam *et al.*, 2006; Kearney *et al.*, 2005).

Hypertension is now widely reported in Africa and is the most common cause of cardiovascular disease on the continent. It is estimated that more than 20 million Africans are affected, majority of which live mainly in urban areas (Addo *et al.*, 2007). Prevalence ranges from 25-35% in adults aged 25-64 years and its prevention and control could avoid at least 250,000 deaths in Africa per year (WHO, 2002).

In Ghana, hypertension is a major public health problem and is associated with relatively low levels of awareness, drug treatment and blood pressure control The prevalence of hypertension in urban Accra is 28.3% (crude) and 27.3% (age-standardized) (Amoah, 2003). Hypertensive renal disease is a common complication in both Kumasi and Accra (Plange-Rhule *et al.*, 1999). In Kumasi and surrounding communities, the prevalence of hypertension is 28.7% (Cappuccio, 1997) The Ghana Health Service indicates that hypertension is the number one killer disease in Ghana today. It is now the second most reported medical condition but was fifth as at 2006 (GHS News Release, 2007). The upsurge of hypertension has led to an increase in the demand for antihypertensive medicines. Recently, the use of polyherbal antihypertensive is on the ascendancy due to reduced cost, easy accessibility and an increased belief in their efficacy. The drug regulatory agencies in many countries (including Ghana) have raised safety concerns on the use of these products. Up to date, no toxicological effects of the selected polyherbal had been reported. Thus, this study seeks to evaluate the safety profile of a polyherbal anti-hypertensive mixture commonly used in Ghana using qualitative and quantitative scientific methods.

MATERIALS AND METHODS

This study was conducted from March 2008 to August 2010 in the Department of Pharmacology, KNUST in compliance with: OECD Principles of Good Laboratory Practices ENV/MC/CHEM (98)17, EEC Good Laboratory Practices (90/18/EEC) and FDA Good Laboratory Practice Standards (Part 58 of 21 CFR). This study was also designed to meet or exceed the requirements of the Food and Drugs Board as seen in the Food and Drugs law of 1992 (PNDCL 305B).

Animals and husbandry: Imprint Control Region (ICR) mice at 4 weeks of age were obtained from the Department of Pharmacology, Animal House and acclimated for 2 weeks prior to initiation of dosing. During this period, mice were observed (physical; in-life) daily and weighed. At initiation of treatment, animals were approximately 6-7 weeks old. The mean weight for the males was 34.7 g and the females 26.4 g. The females were nulliparous and nonpregnant. Individual weights of mice placed on test were within ±30% of the mean weight for each sex. Animals were housed in

stainless steel wire mesh cages during the acclimation and the experimental periods. The mice were kept under ambient light/dark cycle, room temperature and relative humidity. The animal had free access to pelleted mice chow (GAFCO, Tema, Ghana) and water presented to them daily.

The polyherbal antihypertensive and dosing: The polyherbal product under study is an aqueous preparation made from stem bark and leaves of *Persea americana* and *Vernonia amygdalina* obtained from forests in Ghana. Dosing of the product was done based on the manufacturer's recommendations. ICR mice were grouped (n = 5) and received vehicle or 36, 72, or 180 mg kg^{-1} of the product by gavage. Dosing was once daily for 45 days at a volume of 10 mL kg^{-1} body weight. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used because it is the intended human exposure route.

Antemortem evaluative parameters: During the 45 day dosing period, cage-side observations for mortality and emaciation were made morning and evening. Daily observations of the skin for hair loss, open or closed lesions, or abnormal masses, secretions and excretions from the eye, nose, oral cavity, anus and external genitalia, as well as pupil size and respiratory pattern and "chattering" were made. Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were sought for. Lethargy, aggression and hunch appearance was also noted once daily for any signs of toxicity. Pharmacological toxic symptoms sought for included, constipation, hemorrhage, sedation, diarrhoea, polyuria, polydipsia, polyphagia, anorexia, rhinorrhoea/nasal congestion, loss of autonomic reflexes, decreased locomotory activity, neuromuscular inco-ordination and collapse, hyperesthesia, hypothermia, twitching, spasticity, writhing and convulsion. Mice were weighed twice weekly. Food and water intake were determined weekly.

Blood samples were collected for clinical pathology parameters prior to necropsy on day 23 and 45 post-treatment and compared with the untreated. Hematological analysis was performed on EDTA-preserved blood using KX-21 N Automated Haematology Analyser (Sysmex Corporation, Chuo-ku, Kobe, Japan). Liver and kidney function tests and lipid profile was performed on plasma obtained from coagulated blood using the Flexor Junior clinical chemical analyzer (Vital Scientific B.V, The Netherlands). Immediately following dosing, mice were placed in metabolic cages

Table 1: Parameters monitored in the hematological and clinical chemistry assessment of blood male and female ICR mice treated with the test antihypertensive medication for 45 days

Clinical assessment	Parameters monitored
Hematology	White Blood Cell Count (WBC), Red Blood Cell Count (RBC) Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Blood Cell Distribution Width (RDW-CV and RWD-SD), Platelet Count (PLT)
Liver function tests	Albumin, Globulins, Total protein, *Alanine aminotransferase* (ALT), *Aspartarte aminotransferase* (AST), *Alkaline phosphatase* (ALP), Bilirubin Direct, Bilirubin Indirect, Bilirubin Total, *Gamma glutamyl transferase* (GGT)
Kidney function tests	Creatinine, Urea, Sodium, Potassium, Chloride
Lipid profile	Cholesterol, Triglycerides, High Density Lipoproteins (HDL), Very Low Density Lipoproteins (VLDL) , Low Density Lipoprotiens (LDL)
Urine analysis	pH, Protein (PRO), Specific gravity (SG), Blood (BLO), Bilirubin (BIL), Ketone (KET), Glucose (GLU), Ascorbic acid (ASC), Color, Appearance, Smell

(Ugo Basile Biological Research, Comerio-[va]- Italy). A 24 h urine sample was collected while the mice were allowed food and water *ad libitum*. Urine analysis was also performed using urine test strips (Taytec, Mississauga, Canada). Parameters monitored are listed in Table 1.

Postmortem parameters: Following physical examination and blood sampling, mice were sacrificed by cervical dislocation. The brain, heart, kidney, liver, lung, spleen, testes, uterus, ovaries, were excised, trimmed of fat and connective tissue, blotted dry with filter paper and weighed. The organ weight to body weight ratio was calculated for each organ. Tissue samples for histological evaluation were obtained on all organs and preserved in 10% phosphate-buffered formalin. Tissue for histopathological evaluation was embedded in paraffin, sectioned and hematoxylin/eosin stained specimens prepared. Specimens from all mice in the control and different drug treated dose groups were examined microscopically.

Statistical analysis: Data obtained from organ-to-body weight ratio, semi-quantitative urine analysis, hematology and blood chemistry determination between treated and untreated groups was analyzed using GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Data presented are Mean±SD and analyzed by one-way ANOVA followed by Dunnet's multiple comparisons test (post test). The $p \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Physical (in-life) evaluation: No mortality occurred during the study. Weekly clinical observations recorded during the study were considered common findings in laboratory mice and unrelated to the test antihypertensive product. There were no secretions from the eye, ear, nose, anus and external genitalia, no "wasting", audible "chattering", alopecia and pallor in the eyes. The mice were not lethargic, they fed well and their stool showed no signs of diarrhea. There were no ocular findings, decreased motor activity and neurological conditions. There was no significant test article effect on body weight in either sex.

Organ- to-body weight ratio: There were no significant changes in weights for the heart, uterus, ovaries, testis, lung, kidney and spleen between the control and treated mice. The brain indicated a significant increase in weight ($p \leq 0.05$-0.001) after 23 days of treatment (Table 2). The weight however, reduced after 45 days of treatment showing no significant changes between the control and treated mice (Table 3). There were no significant changes ($p > 0.05$) in liver weight between the control and treated mice at a treatment dose of 36 mg kg^{-1} (Table 3). Higher doses however recorded decreasing weight ($p \leq 0.001$) with increasing treatment periods (Table 4).

Table 2: Organ weight to body weight ratio of ICR mice treated with 36mg/kg of the test polyherbal antihypertensive in a toxicity study

	Organ weight to body weight ratio (%)								
	Testis	Lungs	Kidney	Spleen	Liver	Heart	Brain	Uterus	Ovaries
Control	0.69±0.19	0.60±0.03	1.35±0.08	0.67±0.23	5.82±0.19	0.50±0.05	0.96±0.08	0.50±0.12	0.15±0.02
Day 23	0.76±0.02ns	0.64±0.06ns	1.31±0.04ns	0.63±0.32ns	5.70±0.67ns	0.47±0.04ns	1.18±0.04**	0.61±0.03ns	0.14±0.02ns
Day 45	0.65±0.02ns	0.68±0.03*	1.30±0.08ns	0.57±0.10ns	5.62±0.49ns	0.52±0.04ns	0.92±0.15ns	0.59±0.02ns	0.16±0.02ns

Values are Means±SD. n = 5 * implies $p \leq 0.05$; ** implies $p \leq 0.01$, ns: Not significant

Table 3: Percentage organ weight to body weight ratio of ICR mice treated with 72mg/kg of the test herbal antihypertensive in a toxicity study

	Organ weight to body weight ratio (%)								
	Testis	Lungs	Kidney	Spleen	Liver	Heart	Brain	Uterus	Ovaries
Control	0.69±0.19	0.60±0.03	1.35±0.08	0.67±0.23	5.82±0.19	0.50±0.05	0.96±0.08	0.50±0.12	0.15±0.02
Day 23	0.79±0.05ns	0.57±0.03ns	1.28±0.08ns	0.64±0.11ns	4.86±0.36***	0.42±0.02*	1.21±0.03***	0.55±0.02ns	0.14±0.02ns
Day 45	0.64±0.05ns	0.64±0.07ns	1.31±0.04ns	0.51±0.18ns	4.81±0.24***	0.48±0.04ns	0.98±0.08ns	0.56±0.03ns	0.16±0.02ns

Values are Means±SD. n = 5. *implies p≤0.05; *** implies p≤0.001, ns: not significant

Table 4: Percentage organ weight to body weight ratio of ICR mice treated with 180mg/kg of the test herbal antihypertensive in a toxicity study

	Organ weight to body weight ratio (%)								
	Testis	Lungs	Kidney	Spleen	Liver	Heart	Brain	Uterus	Ovaries
Control	0.69±0.19	0.60±0.03	1.35±0.08	0.67±0.23	5.82±0.19	0.50±0.05	0.96±0.08	0.50±0.12	0.15±0.02
Day 23	0.69±0.11ns	0.58±0.10ns	1.35±0.08ns	0.85±0.25ns	4.32±0.48**	0.43±0.01ns	1.06±0.04*	0.03±0.03***	0.02±0.02***
Day 45	0.71±0.12ns	0.57±0.05ns	1.39±0.09ns	0.42±0.06ns	4.03±0.44***	0.51±0.02ns	0.98±0.05ns	0.53±0.02ns	0.16±0.02ns

Values are Means±SD. n = 5. *p≤0.05; **p≤0.01, ***p≤0.001, ns: Not significant

Table 5: The effects of 36, 72 and 180 mg kg^{-1} of the test herbal antihypertensive on the haematological profile of ICR mice

		36 mg kg^{-1}		72 mg kg^{-1}		180 mg kg^{-1}	
Parameters	Control	Day 23	Day 45	Day 23	Day 45	Day 23	Day 45
WBC (x10^9/L)	4.38±0.61	5.38±0.65ns	5.05±0.85ns	5.55±0.48**	5.3±0.47*	6.4±0.46***	6.05±0.35 **
HGB (g/dl)	13.84±0.90	11.13±0.75†††	12.70±0.22†	11.98±0.35†††	12.73±0.17†	11.10±1.07†††	13.55±0.06ns
RBC (x10^{12}/L)	7.58±0.45	7.57±0.22ns	8.58±0.20*	7.99±0.26ns	8.11±0.30ns	7.65±0.54ns	8.20±0. 27ns
HCT (%)	36.02±3.79	40.1±2.06*	44.45±0.87***	41.95± 1.95**	46.08± 0.71***	42.83±2.52**	48.15±0.52***
MCV (fL)	54.20±1.42	53.40±1.94ns	52.01±0.33ns	50.83±1.73††	55.60±0.22ns	50.78±1.90†††	51.50±1.17†
MCH (pg)	16.80±1.03	14.8±0.68††	14.73±0.17†	14.7±0.61†††	15.33±0.05†	14.30±0.67†††	14.73±0.48††
MCHC (g/dl)	36.74±0.74	27.90±0.48†††	28.40±0.08†††	28.73±0.60†††	27.60±0.08†††	28.20±0.35†††	28.18±0.33†††
RDW-CV (%)	15.30±1.19	14.83±0.71ns	16.45±1.31ns	15.03±1.18ns	15.93±1.63ns	15.15±5.17ns	17.55±1.17ns
RDW-SD (fL)	24.78±2.52	29.70±0.32***	30.35±0.33***	29.03±1.71*	31.13±1.88***	30.75±4.35*	30.78±1.00*
PLT (x10^9/L)	794±70.26	1303±192.26***	1400±84.49***	1087±136.77***	1014±16.10**	1034±90.71**	1214±143.15***
MPV (fL)	6.22±0.68	5.73±0.13ns	5.80±0.22ns	5.78±0.17ns	6.08±0.13ns	6.15±0.21ns	5.55±0.13†
PDW	16.52±1.33	6.50±0.18†††	6.58±0.40†††	6.7±0.22†††	6.95±0.13†††	7. 08±0.46†††	6.38±0.15†††
PCT (%)	0.50±0.09	2.55±0.06***	2.72±0.46***	2.75±0.37***	3.8±0.54***	4.35±1.16***	2.40±0.27**

Values are Means±SD. n = 5 . ns implies p>0.05 for insignificant increments or decrements. For significant increments: *p≤0.05; **p≤0.01, ***p≤0.001. For significant decrements: †p≤0.05; ††p≤0.01, †††p≤0.001

Hematological profile: Hematological evaluation (Table 5) showed that WBCs increased significantly (4.38±0.61 to 6.4±0.46) in the 72 and 180 mg kg^{-1} treated groups over the period. Overall, RBCs were not significantly affected by the treatment. The HGB decreased very significantly (13.84±0.9 to 11.10±1.07) after 23 days of treatment but by day 45, HGB levels were just significantly lower ($p \leq 0.05$) or comparable to the control. The HCT increased gradually to a very significant increase (36.02±3.79 to 48.15±0.52) through the study period. MCV and MCH significantly reduced on treatment with 72, and 180 mg kg^{-1} (53.40±1.94 to 50.78±1.90

and 16.80±1.03 to 14.30±0.67, respectively) after 23 days of treatment. MCV and MCH was just slightly lower ($p \leq 0.05$) than the normal by day 45. MCHC values for the entire study period for all treatment groups were very significantly low (36.74±0.74 to 28.18±0.33). RDW-SD increased very significant increases from 24.78±2.52 to 31.13±1.88 with dose, but for 180 mg kg^{-1} over the period of study. PLT, PDW and PCT increased very significantly throughout the treatment period.

Liver function tests: The liver function tests (Table 6) indicates that albumin levels reduced very significantly in all treatment groups (35.5±0.59 to 38.9±0.30) by 23 days of drug treatment. Albumin level however increased significantly above the normal by day 45. Globulin levels also increased very significantly (12.1±0.70 to 47.2±0.24) in all treatment groups, therefore total protein increased. *ALT*, *AST* and *ALP* levels increased significantly (60.1±2.21 to 81.4±1.72, 195.3±1.98 to 296.8±9.75 and 113.0±8.45 to 200.9±0.3, respectively) in all the treatment groups after 23 days of treatment but either decreased very significantly below the control or remained insignificantly high by day 45. *GGT* levels were high enough to be detected after 45 days of treatment. Direct bilirubin, indirect bilirubin and total bilirubin increases very significantly by day 45 with values changing from 1.68±0.08 to 2.4±0.18, 0.61±0.22 to 1.4±0.21 and 2.34±0.15 to 3.8±0.18, respectively.

Kidney function tests: The kidney function tests (Table 7), indicated very significant decrease (15.04±0.56 to 6.4±0.05) in blood urea in all treatment over the entire study period. Creatinine levels increased very significantly (37.20±0.45 to 81.0±0.61) in all the treatment groups after 45 days but changes in creatinine levels were not significant when measured after 23 days of treatment.

Lipid profile: Lipid profile tests (Table 8) revealed that total cholesterol and HDL reduced very significantly (2.8±0.06 to 2.0±0.02 and 1.6±0.02 to 1.2±0.05, respectively) in the 36 and 72 mg kg^{-1} treated groups after 23 days of treatment but total cholesterol level compare to the control group was not significantly affected in all treatment groups after 45 days. Plasma TAG was very significantly high (1.0±0.08 to 1.7±0.07) in all treatment groups after 23 days. TAG however was insignificant in all the treatment groups after 45 days. VLDL and LDL increased significantly (0.7±0.04 to 0.8±0.02 and 0.5±0.02 to 0.7±0.05, respectively) after 23 days of treatment but plasma VLDL and LDL reduced very significantly (0.8±0.04 to 0.4±0.01 and 0.7±0.05 to 0.2±0.01, respectively) below the control after 45 days.

Urine analysis: In the urine analysis (Table 9) of treated and untreated mice, there were no significant changes over time except for increases in pH (5.4±0.55 to 8.2±1.80) and change of urine color from straw to amber within 24 h of treatment.

Histopathological examination: Photomicrograph from the histological examination showed some changes in the liver, kidney and spleen between control (Fig. 1, 4 and 7) and treated groups of animals. Histological changes observed in the liver at 23 and 45 days of treatment was assessed to be cirrhosis and fibrosis at higher doses (180 mg kg^{-1}) and prolonged administration (45 days) of the test drug (Fig. 2, 3). Photomicrograph of the kidney showed glomerular inflammation

Table 6: The effect of 36, 72 and 180 mg kg^{-1} of the test herbal antihypertensive on liver function tests in ICR mice

		36 mg kg^{-1}		72 mg kg^{-1}		180 mg kg^{-1}	
Parameters	Control	Day 23	Day 45	Day 23	Day 45	Day 23	Day 45
Albumin (g L^{-1})	35.5±0.59	31.5±0.46 †††	38.2±0.55 ***	32.1±0.68 †††	37.4±0.51***	32.8±1.76 ††	38.9±0.30***
Globulins (g L^{-1})	12.1±0.70	28.7±1.26 ***	41.3±1.10 ***	30.9±1.06 ***	43.7±1.41***	26.1±1.11 ***	47.2±0.24***
Total protein (g L^{-1})	48.2±0.37	60.8±1.46 ***	79.2±0.92 ***	63.0±2.57 ***	81.6±1.15***	59.1± 1.35 ***	86.2±0.30***
ALT/GPT (U I^{-1})	60.1±2.21	81.4±1.72 ***	32.1±0.50 †††	67.5±1.52 **	42.3±8.98†††	70.1±7.01 **	38.9±2.33†††
AST/GOT (U I^{-1})	195.3±1.98	296.8±9.75 ***	92.3±0.81 †††	236.7±2.19 **	118.2± 29.79†††	219.3±17.5***	110.9±4.8†††
ALP (U I^{-1})	113.0±8.45	200.9±0.3 0 ***	65.2±8.09 †††	171.2±1.17 ***	126.3±9.21*	156.4±24.42 ***	126.3±9.21ns
Bil. Direct (µmol L^{-1})	1.68±0.08	1.9±0.13 ns	2.5±0.19 ***	1.7± 0.05 ns	2.8±0.22***	1.7±0.20 *	2.4±0.18***
Bil Indirect(µmol L^{-1})	0.61±0.22	0.7±0.18 ns	1.3±0.12 ***	0.6±0.07 ns	1.6±0.11***	0.5±0.20 *	1.4±0.21***
TBil (µmol L^{-1})	2.34±0.15	2.6±0.11 *	3.8± 0.18 ***	2.3±0.13 ns	4.4±0.27***	2.2±0.10 ns	3.8±0.18***
GGT (µmol L^{-1})	R	R	0.3±0.1	R	0.2±0.07	R	0.2±0.07

Values are Means±SD. n = 5. ns implies p>0.05 for insignificant increments or decrements. For significant increments: *p≤0.05; **p≤0.01, ***p≤0.001. For significant decrements: †† p≤0.01, ††† p≤0.001. ALT/GPT: *Alanine Transaminase/Glutamic pyruvate transaminase*, AST/GOT: *Aspartate transaminase/Glutamic Oxaloacetic transaminase*, ALP: *Alkaline Phosphatase*, Bil: Bilrubin; Tbil: Total bilirubun, GGT: Gamma GlutamylTransferase. R: means plasma, Concentration too low to be detected

Table 7: The effect of 36, 72 and 180 mg kg^{-1} of the test herbal antihypertensive on kidney function tests in ICR mice

		36 mg kg^{-1}		72 mg kg^{-1}		180 mg kg^{-1}	
Parameters	Control	Day 23	Day 45	Day 23	Day 45	Day 23	Day 45
Creatinine (µmol L^{-1})	37.20±0.45	40.2±2.96ns	43.6±7.25ns	35.7±0.51ns	78.5±15.85***	36.74±0.40ns	81.0±0.61***
Urea (mmol L^{-1})	15.04±0.56	10.4±1.06†††	5.6±0.36†††	10.6±0.39†††	5.9±0.67†††	8.5±0.58†††	6.4±0.05†††
Sodium (mmol L^{-1})	143.82±1.51	157.2±6.67*		150.2±1.08ns		144.42±3.26ns	
Potassium (mmol L^{-1})	8.19±0.40	9.0±0.3ns		10.3±0.51ns		9.3 ±0.17ns	
Chloride (mmol L^{-1})	111.92±1.25	118.2±6.51**		111.6±1.84ns		109.12±3.26ns	

Values are Means±SD. n = 5. ns implies p>0.05 for insignificant increments or decrements. For significant increments: *p≤0.05; **p≤0.01, ***p≤0.001. For significant decrements: ††† p≤0.001

Table 8: The effect of 36, 72 and 180 mg kg^{-1} of the test herbal antihypertensive on lipid profile in ICR mice

		36 mg kg^{-1}		72 mg kg^{-1}		180 mg kg^{-1}	
Parameters	Control	Day 23	Day 45	Day 23	Day 45	Day 23	Day 45
CHOL (mmol L^{-1})	2.8±0.06	2.0±0.02†††	2.8±0.06ns	2.2±0.07†††	2.7±0.12ns	2.7±0.14ns	2.8±0.12ns
TAG (mmol L^{-1})	1.0±0.08	1.5±0.01***	1.0±0.08ns	1.2±0.05**	0.9±0.12ns	1.7±0.07***	0.9±0.01ns
HDL (mmol L^{-1})	1.6±0.02	1.2±0.03†††	1.6±0.02ns	1.2±0.05†††	1.5±0.07ns	1.6±0.03ns	1.6±0.06ns
VLDL (mmol L^{-1})	0.7±0.04	0.8±0.02***	0.5±0.03†††	0.8±0.02***	0.4±0.06†††	0.8±0.04***	0.4±0.01†††
LDL (mmol L^{-1})	0.5±0.02	0.8±0.06***	0.3±0.11††	0.7±0.03***	0.4±0.02††	0.7±0.05**	0.2±0.01†††

Values are Means±SD. n = 5. ns implies $p>0.05$ for insignificant increments or decrements. For significant increments: *$p\leq0.05$; **$p\leq0.01$, ***$p\leq0.001$. For significant decrements: †† $p\leq0.01$, ††† $p\leq0.001$. CHOL: Cholesterol; TAG: Triacylglycerides, HDL: High density lipoprotein; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein

Table 9: The effects of 36, 72 and 180 mg kg^{-1} of the test herbal antihypertensive on urine of ICR mice

		36 mg kg^{-1}			72 mg kg^{-1}			180 mg kg^{-1}		
Parameters	Control	Day 1	Day 23	Day 45	Day 1	Day 23	Day 45	Day 1	Day 23	Day 45
pH	5.4±0.55	7.8±1.60	5.6±0.54	5.2±0.44	5.6±0.54	5.8±0.44	5.6±0.54	8.2±1.80	5.2±0.44	5.6±0.54
NIT (mg dL^{-1})	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
URO (mg dL^{-1})	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00
PRO (mg dL^{-1})	72±38.3	86±31.3	58±38.3	86±31.3	86±31.3	86±31.3	100±0.00	86±31.3	100±0.00	86±31.3
SG	1.000±0.000	1.004±0.002	1.000±0.000	1.00±0.000	1.003±0.003	1.000±0.000	1.000±0.000	1.004±0.002	1.000±0.000	1.000±0.000
BLO(Ery uL^{-1})	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
BIL (mg dL^{-1})	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
KET (mg dL^{-1})	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
GLU (mg dL^{-1})	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
ASC (mg dL^{-1})	32±11.0	36±8.0	32±11.0	28±11.0	28±11.0	32±11.0	36±8.0	32±11.0	24±8.9	32±11.0
Color	Straw	Amber	Straw	Straw	Amber	Straw	Straw	Amber	Straw	Straw
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Smell	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent
Volume	1.14±0.28	1.24±0.27	1.10±0.20	1.16±0.20	1.22±0.08	1.22±0.22	1.08±0.22	1.18±0.16	1.22±0.08	1.14±0.15

Values are Means±Standard D eviations. n = 5, PRO: Protein, SG: Specific gravity, BLO: Blood, BIL: Bilirubin, KET: Ketone, GLU: Glucose, ASC: Ascorbic acid, (-) = Negative/Trace quantities undetectable by the test strip

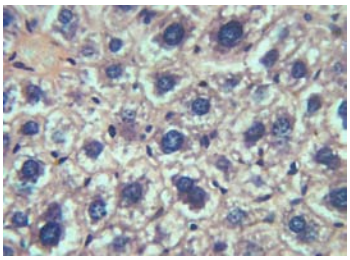

Fig. 1: Photomicrograph of the liver of normal ICR mice in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

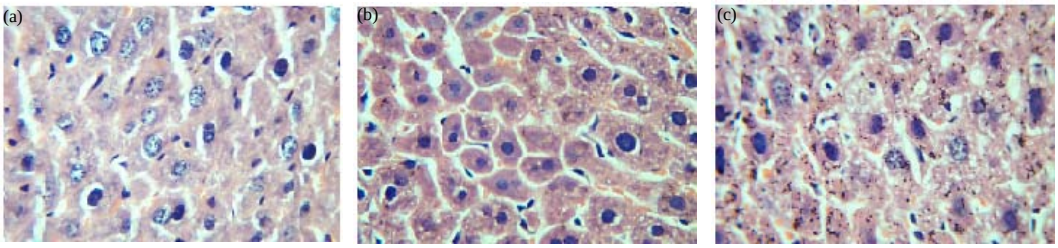

Fig. 2(a-c): Photomicrograph of the liver of ICR mice showing cirrhosis after treatment with 36, 72 and 180 mg kg^{-1} of the herbal antihypertensive preparation for 23 days represented by A, B, C, respectively in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

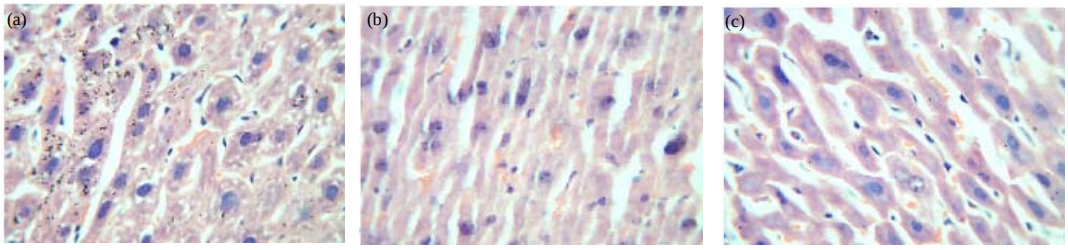

Fig. 3(a-c): Photomicrograph of the liver of ICR mice showing fibrosis after treatment with 36, 72 and 180 mg kg^{-1} of the herbal antihypertensive preparation for 45 days represented by A, B, C, respectively in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

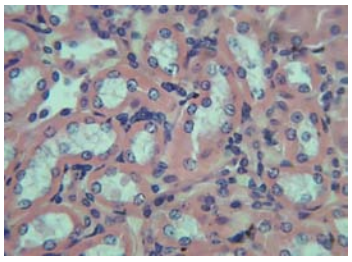

Fig. 4: Photomicrograph of the kidney of normal ICR mice in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

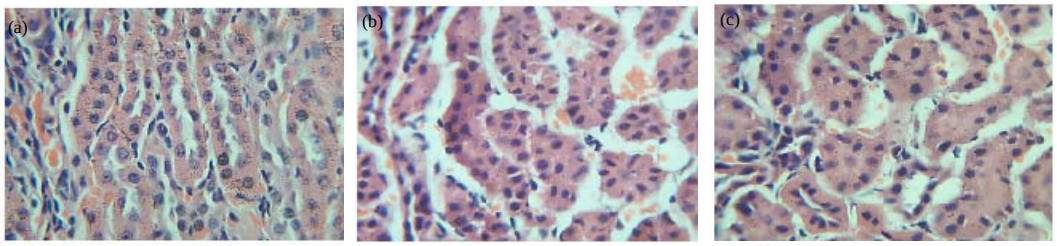

Fig. 5(a-c): Photomicrograph of the kidney of ICR mice showing glomerular inflammation after treatment with 72 and 180 mg kg^{-1} of the herbal antihypertensive preparation for 23 days represented. A, B, C represents treatment with 36, 72 and 180 mg kg^{-1} of PHA, respectively in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

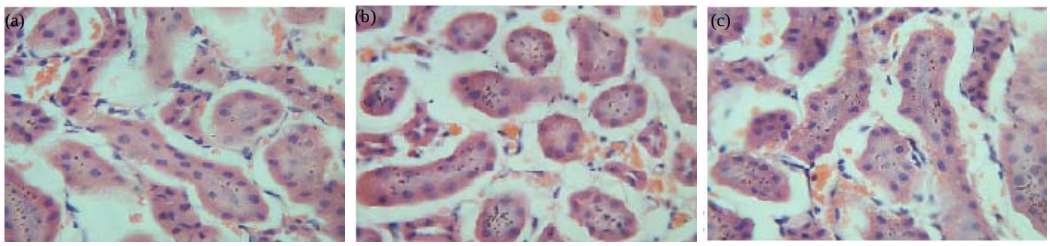

Fig. 6(a-c): Photomicrograph of the liver of ICR mice showing glomerular inflammation after treatment with 36, 72 and 180 mg kg^{-1} of the herbal antihypertensive preparation for 45 days represented by A, B, C, respectively in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

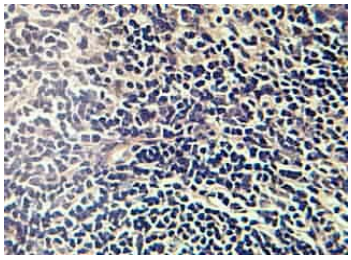

Fig. 7: Photomicrograph of the kidney of normal ICR mice in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

(Fig. 5, 6). The red pulp of the spleen had very mild congestion after 23 days (Fig. 8) but normalized after 45 days (Fig. 9). The heart, lungs, testes and uterus did not show any observable histological changes.

DISCUSSION

The "no effect on body and skin" observed suggests that the product under study may not have any allergic or carcinogenic effect on skin. It may not cause hypersensitization and neurogenic

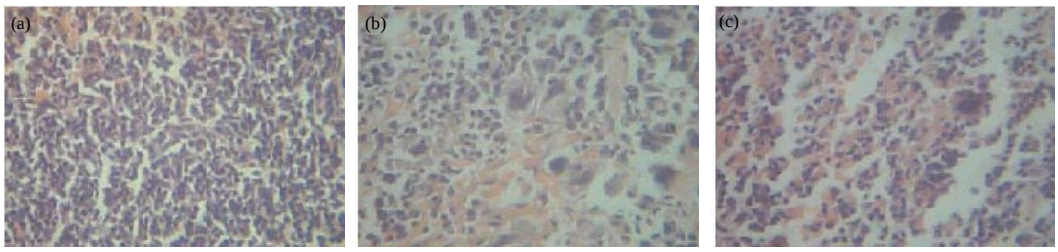

Fig. 8(a-c): Photomicrograph of the spleen of ICR mice showing mild red pulp congestion after treatment with 72 and 180 mg kg^{-1} of the herbal antihypertensive preparation for 23 days. A, B, C represents treatment with 36, 72 and 180 mg kg^{-1} of PHA, respectively in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

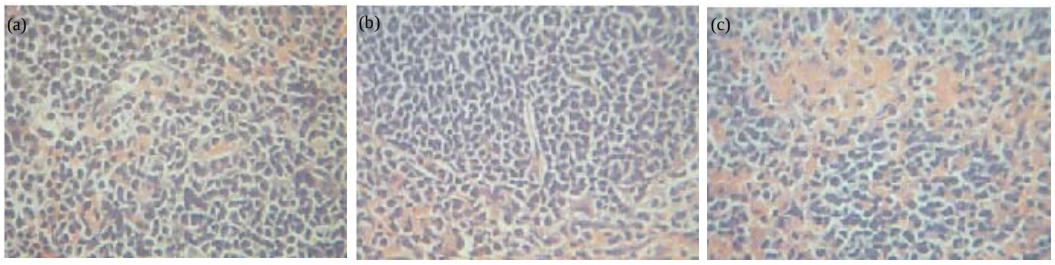

Fig. 9(a-c): Photomicrograph of the liver of ICR mice showing no effect after treatment with 36, 72 and 180 mg kg^{-1} of the herbal antihypertensive preparation for 45 days represented by A, B, C, respectively in histopathological studies in a subchronic toxicity test. Magnification X40; Stain: Haematoxylin and Eosin

inflammation. Allergic reactions and cutaneous neurogenic inflammation can result in hyperesthesia, pruritus and hyperplasia and carcinomas which cause pain at the site of development and may attract scratching, biting, licking and increased locomotory activity. The mice do not sleep, they lose their fur and they look debilitated, unkempt (an evidence of underlying illness) and unhealthy (Richardson and Vasko, 2002; Steinhoff *et al.*, 2003). The product does not cause autonomic nervous system hypereflexia because lacrimation, miosis, rhinorrhoea, salivation, urination, defecation and labored breathing which are signs of muscarinic hyperactivity were not seen.

Observations showed no CNS depression and/or muscle relaxation effects; noticed as a decrease in locomotory activity (Verma *et al.*, 2010) as well as pain and inflammatory effects; realized as writhing, change in gait and body posture and decreased locomotory activity (Saha and Ahmed, 2009). The product did not seem to have any "wasting effect". There was no pallor in the eyes (symptom of anaemia).

Brain weight increased after 23 days of treatment for each of the doses of the polyherbal preparation but returned to normal by day 45. This could have been an indication of hyperplasia of brain cells. Changes in brain weights are rarely associated with neurotoxicity (Sellers *et al.*,

2007). The brain has effective repair mechanisms and immediately repairs any abnormality imposed on it (Zhao *et al.*, 2003). The reduction liver weight with increasing dose could be due to the metabolic end products or the herbal preparation itself inducing hepatocellular atrophy. There were no significant changes observed in the weights of the following organs between control and treated animals: kidney, spleen, heart, lung, testes, uterus and ovaries.

There were significant increases in WBC count in ICR mice receiving the higher dose of the preparation. The high counts recorded however were still within the proposed range for mice quoted as 3.0-14.2×10^3 cells μL^{-1} (Suckow *et al.*, 2001). The increase in WBC count could be caused by the product because it becomes a foreign component of the internal environment when adsorbed into the blood from the gut and this attracts an immune system response. Increases in RBC count were not significant. The significant decrease in haemoglobin concentration recorded after 23 days of product-treatment could possibly be a result of the formation of microcytic red blood cells. The product therefore seems to cause microcytic anaemia in the early days of administration. The possibility of causing microcytic anaemia is confirmed by the significant reductions observed with results obtained for the MCV, MCH and MCHC. Iron deficiency anemia initially presents with a varied size distribution of red blood cells. An elevated RDW is known as anisocytosis. Intense reticulocyte replication always gives rise to anisocytosis. The HCT was elevated; an indication of an increased volume occupied by RBCs. From the RBC count, HCT, RBC indices and RBC morphology it could be deduced that the product cause microcytic-anitocytic anaemic initially.

The results showed an increase in platelet count. The elevated platelet number was confirmed by the elevated plateletcrit as the volume occupied is directly proportional to the number of cell. This suggests that there is product-related activation of thrombocyte production which may increase the risk of thrombosis. Anisocytosis of red blood cells and platelets might co-occur (Wiwanitkit, 2004). The insignificant changes in the Mean Platelet Volume (MPV) however, suggests that the bone marrow is manufacturing platelets normally and therefore reduces the suspected risk of causing thrombosis and other related cardiovascular disorders. The PDW was significantly high. This suggests that there is high variation in platelet width. This is expected because there is drug-induced platelet production and newly produced platelets tend to be larger than older ones.

Combining results obtained from the liver function tests, it may be deduced that the test product causes an inflammatory disorder which leads to the development of some form of cirrhosis in the liver in the initial stages of administration. The cellular destruction reduces albumin levels while the inflammation raises globulin levels with a net increase in total protein levels. Initially, the product affects the functionality of the liver as was evidently seen by the rise in ALT, AST. The product also causes a decrease in the liver's excretory function evidently seen by raising ALP and GGT and total bilirubin (direct and indirect bilirubin). The marked rise observed at the end of the treatment period, may indicate a time dependent hepatic excretory impairment since no significant changes were observed after 23 days post treatment. Also, usually, high bilirubin levels are characteristic of idiosyncratic injury, i.e., drug toxicity unrelated to dosage (Kumar and Clark, 2005).

Urea levels recorded over the entire study period were significantly low. This could suggest a normal kidney function but an impaired liver function because blood urea nitrogen is affected by liver function. It is therefore possible that the product had effect on the liver rather than the kidney. Creatinine is a more specific indicator of kidney function (McClellan, 2009). Even though creatinine was not significantly affected when measured 23 days after initiation of treatment, was significantly high after 45 days of treatment in all dose levels. This gives an indication that the kidney's function was not affected in the early stages of the treatment.

The reduction in cholesterol observed in the first half of the treatment stage could be due to an interference of adsorption from the intestine by the product, or perhaps interference of the synthesis of cholesterol by the liver and/or intestines. It could also be due to an enhanced peripheral utilization of cholesterol (King, 2011). Levels however returned to normal after 45 days of treatment. There could be several factors coming into play e.g., liver function returning to normal (as observed in LFT) with an increase in cholesterol synthesis (homeostatic mechanism), reduction in peripheral utilization or even an increased intake from diet. Looking at the results for total cholesterol alone it can be deduced that the test product cannot be a risk factor for heart and other cardiovascular diseases.

The rise in plasma TAG, VLDL and LDL levels and the lowering of HDL in the first half of the experimental period could have been product related. Based on these alone, the product would not support its antihypertensive claims as it may rather increase the risk for cardiovascular disorders. The conditions however reversed and TAG and HDL levels returned to normal as VLDL and LDL levels dropped very significantly below normal values. A decrease in TAG, VLDL and LDL implies a reduced risk of artherosclerosis, heart disease and cardiovascular disorders (King, 2011).

Histological changes observed in the liver after 23 days and 45 days of test product treatment was described as periportal fibrosis and cirrhosis. Administration of the preparation might have resulted in the derangements in the synthesis and degradation of matrix by injuring mesenchymal cells (Minino *et al*., 2007). This therefore, eventually develops into cirrhosis. Long term use of the preparation at high concentration causes inflammation of the glomerulus. This might have resulted from necrosis of glomerular cells. Renal excretion would therefore be impaired. The preparation would therefore increase the toxicity of other drugs administered concurrently with it. The red pulp of the spleen was congested after 23 days which returned to normal after 45 days. The congestion could have resulted due to increased WBCs and platelets which are present in the pulpasplenica (Junqueira and Carneiro, 2005). These high WBC and PLT coupled with the RBC might have caused the initial blockade of the splenic sinuses which is an integral part of the pulpasplenica. This will eventually impair the filtering ability of the spleen. The absence of significant changes in the heart, testes, uterus and the lungs signifies that the extract has no effect on them.

CONCLUSION

The polyherbal antihypertensive product did not cause any observable CNS adverse effect but causes microcytic-anitocytic anaemia in the early stages of use. It may be detrimental to the liver and kidney when used in higher doses. Use of this antihypertensive could however, be safe to use when used in lower doses The no-observable-adverse-effect-level (NOAEL) is 32 mg/kg/day. Monitoring of the blood, liver and kidney may be required with its use.

ACKNOWLEDGMENTS

Mr. Thomas Ansah of the Department of Pharmacology, CHS, KNUST, Leslie Nii-Djan Mensah, Vivian Nwudu, Ivan Nyarko-Danquah, Afua Odame-Kani and Newman Osafo for helping with laboratory procedures involved in this research.

REFERENCES

Addo, J., L. Smeeth and D.A. Leon, 2007. Hypertension in sub-saharan Africa: A systematic review. Hypertens., 50: 1012-1018.

Adeboye, J.O., M.O. Fajonyomi, J.M. Makinde and O.B. Taiwo, 1999. A preliminary study on the hypotensive activity of *Persea americana* leaf extracts in anaesthetized normotensive rats. Fitoterapia, 70: 15-20.

Amira, O.C. and N.U. Okubadejo, 2007. Frequency of complementary and alternative medicine utilization in hypertensive patients attending an urban tertiary care centre in Nigeria. BMC Complementary Altern. Med., 7: 30-30.

Amoah, A.G., 2003. Hypertension in Ghana: A cross-sectional community prevalence study in greater Accra. Ethn. Dis., 13: 310-315.

Aregheore, E.M.K., H.P.S. Makkar and K. Becker, 1998. Feed value of some browse plants from the central zone of Delta State. Nig. Trop. Sci., 38: 97-104.

Cappuccio, F.P., 1997. Ethnicity and cardiovascular risk: Variations in people of African ancestry and South Asian origin. J. Hum. Hypertens., 11: 571-576.

Chockalingam, A., N.R. Campbell and J.G. Fodor, 2006. Worldwide epidemic of hypertension. Can. J. Cardiol., 22: 553-555.

GHS News Releases, 2007. Hypertension is the number one killer disease in Ghana. http://www.ghanahealthservice.org/articles.php?nd=35&cat=1&tt=.

Junqueira, C.L. and J. Carneiro, 2005. Basic Histology: Text and Atlas. McGraw-Hill Professional, New York, ISBN-10: 0071440917, pp: 274-277.

Kambizi, L. and A.J. Afolayan, 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J. Ethnopharmacol., 77: 5-9.

Kate, I.E. and O.O. Lucky, 2009. Biochemical evaluation of the tradomedical uses of the seeds of *Persia americana* Mill., (Family: Lauraceae). World J. Med. Sci., 4: 143-146.

Kearney, P.M., M. Whelton, K. Reynolds, P. Munter, P.K. Whelton and J. He, 2005. Global burden of hypertension: Analysis of worldwide data. Lancet, 365: 217-223.

King, M.W., 2011. Intestinal uptake of lipids: The medical biochemistry page. [themedicalbiochemistrypage.org, 1996-2011]. http://themedicalbiochemistrypage.org/lipoproteins.html.

Kumar, P. and M. Clark, 2005. Clinical Medicine. 6th Edn., Elsevier Saunders, USA., ISBN-10: 0702027634, pp: 469.

Lawal, I.O., N.E. Uzokwe, A.B.I. Igboanugo, A.F. Adio and E.A. Awosan *et al.*, 2010. Ethnomedicinal information on collation and identification of some medicinal plants in Research Institutes of South-West Nigeria. Afr. J. Pharm. Pharm., 4: 1-7.

McClellan, W., 2009. The kidneys and how they work, National institute of diabetes and digestive and kidney diseases (NIDDK) health information. National Institutes of Health Publication No. 09-3195.

Minino, A.M., M.P. Heron, S.L. Murphy and K.D. Kochanek, 2007. Deaths: Final data for 2004. Nat. Vital. Stat. Rep., 55: 1-119.

Plange-Rhule J., R. Phillips, J.W. Acheampong, A.K. Saggar-Malik, F.P. Cappuccio and J.B. Eastwood, 1999. Hypertension and renal failure in Kumasi, Ghana. J. Hum. Hypertens, 13: 37-40.

Regassa, A., 2000. The use of herbal preparations for tick control in Western Ethiopia. J. S. Afr. Vet. Assoc., 71: 240-243.

Richardson, J.D. and M.R. Vasko, 2002. Cellular mechanisms of neurogenic inflammation. J. Pharmacol. Exp. Therapeutics., 302: 839-845.

Saha, A. and M. Ahmed, 2009. The analgesic and anti-inflammatory activities of the extract of *Albizia lebbeck* in animal model. Pak. J. Pharm. Sci., 22: 74-77.

Sellers, R.S., D. Mortan, B. Michael, N. Roome and J.K. Johnson *et al.*, 2007. Society of toxicologic pathology position paper: Organ weight recommendations for toxicology studies. Toxicol. Pathol., 35: 751-755.

Steinhoff, M., S. Stander, S. Seeliger, J.C. Ansel, M. Schmelz and T. Luger, 2003. Modern aspects of cutaneous neurogenic inflammation. Arch. Dermatol., 139: 1479-1488.

Suckow, M.A., P.J. Danneman and C. Brayton, 2001. The Laboratory Mouse. CRC Press LLC Boca Raton, London, New York, Washington DC., ISBN-13: 9780849303227, pp: 52-55.

Verma, A., G.K. Jana, S. Sen, R. Chakraborty, S. Sachan and A. Mishra, 2010. Pharmacological evaluation of *Saraca indica* Leaves for central nervous system depressant activity in mice. J. Pharm. Sci. Res., 2: 338-343.

WHO, 2002. Reducing risks, promoting healthy life. World Health Organization Report, Geneva.

Whelton, P.K., 1994. Epidemiology of hypertension. Lancet, 344: 101-106.

Wiwanitkit, V., 2004. Plateletcrit, mean platelet volume, platelet distribution width: Its expected values and correlation with parallel red blood cell parameters. Clin. Applied Thromb. Hemost., 10: 175-178.

Zhao, M., S. Momma, K. Delfani, M. Carlen and Cassidy, 2003. Evidence for neurogenesis in the adult mammalian substantia nigra. Proc. Natl. Acad. Sci. USA., 100: 7925-7930.