International Journal of Phytopharmacy **Research Article**
ISSN: 2277-2928 (Online)
Journal DOI: https://doi.org/10.7439/ijpp

Evaluation of Acute and Sub-chronic toxicities of a Nigeria polyherbal formulation

Gift Ishioma Abvwoe[1], Chibueze Peter Ihekwereme[1], Mathew Jegbefume okonta[2], Amarachukwu Ukamaka Anwuchaepe[*3] and Sonne Ikechukwu Mbagwu[1]

[1]*Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*
[2]*Department of Clinical Pharmacy and Pharmacy Management, University of Nigeria, Nsukka, Enugu State, Nigeria.*
[3]*Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*

QR Code

***Correspondence Info:**
Amarachukwu Ukamaka Anwuchaepe
Department of Pharmacognosy and Traditional Medicine,
Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

***Article History:**
Received: 04/09/2017
Revised: 27/09/2017
Accepted: 27/09/2017
DOI: https://doi.org/10.7439/ijpp.v7i6.4378

Abstract

This study was carried out to evaluate safety profile of a commercial polyherbal product "Washing and setting" that is highly consumed in South-Eastern Nigeria. Qualitative phytochemical analysis was conducted. Acute toxicity test on selected doses of the polyherbal formulation was carried out in mice and rats. In sub-chronic toxicity study, animals were exposed to the polyherbal product daily for 90 – days at 1, 5 and 10 ml/kg. Five rats from each dose level were selected on the 31st, 61st and 91st days for the determination of biochemical and haematological indices. Histology of the liver and kidney were also carried out. A 28 days post-treatment study was conducted to determine reversibility in toxicological changes. From results of the study, alkaloids were observed to be the most abundant phytocompound in the polyherbal formulation. No sign of acute toxicity was observed. No mortality was observed in the treated groups throughout the 90 days study. At the tested doses, there was significant ($p<0.05$) increase in both alanine aminotransferase and aspartate aminotransferase and blood urea nitrogen at the 91st day at 10 ml/kg group compared with the vehicle treated group. Significant ($p<0.05$) elevations were observed in serum creatinine on the 61st day at the highest dose (10 ml/kg) and on the 91st day for 5 and 10 ml/kg compared to vehicle-treated control group. All toxicological effects were reversible on withdrawal of administration in post treatment studies. The polyherbal formulation may be nephrotoxic and hepatotoxic especially at high doses on long term exposure.

Keywords: Washing and setting, decoction, acute toxicity, sub-chronic toxicity, Phytochemical screening.

1. Introduction

Traditional medicines are important part of most culture and traditions. According to the WHO estimate, about 80% of the world population relies on traditional systems of medicine for primary health care due to the affordability, accessibility and high safety profile assumed by most uses [1]. It is estimated that over 20 million Nigerians depend on traditional medicine for their primary health care needs [2].

Inappropriate methods of collection, processing and storage of safe natural products can result in contamination of products which can lead to toxicity [3]. Toxic effects inherent in the use of some plant product may not elicit obvious signs of toxicity on acute administration. However, prolonged use of these product as common in chronic disease conditions, may lead to bioaccumulation with significant organ damage and toxicity [4]**.** Some plant materials when ingested either in the raw state or their extracts have been reported to be toxic which may result in Schizophrenia, seizure, liver damage etc. [5], e.g., water Hemlock (*Cicuta maculata*), Deadly Nightshade (*Atropa belladonna*), Tobacco (*Nicotiana tabacum*).

The polyherbal product "Washing and Setting" is widely used for the treatment of various ailments ranging from infections to pains and" body weakness in the South-Eastern Nigeria. Its labeled constituents include aqueous root and bark of the following plants: *Uvaria afzelii, Zanthoxylum gilletii, Allium sativum and Acacia nilotica.*

Considering the high reliance on this product by the populace, no scientific information exists on its safety profile. Hence, this study was designed to evaluate its safety profile.

2. Materials and methods

2.1 Herbal Product

The herbal product "Washing and Setting" was bought from a traditional herbal practitioner in Awka, Anambra State, Nigeria. It is popularly called "Washing and Setting".

2.2 Phytochemical Analysis

Phytochemical analysis of the decoction was carried out using standard procedures as described by Trease and Evans [6]. About 5 ml of the decoction was used to test for the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, carbohydrates and proteins.

2.3 Animals

Swiss-Male albino rats (220 ± 20 g) and mice (25 ± 5 g) were used for the study. All the animals were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The animals were housed in standard laboratory conditions of 12 h light\dark cycle, room temperature, and 40-60% relative humidity and were fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water *ad libitum*. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub No: 85-23 Revised 1985) and approved by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes.

2.4 Acute Toxicity Study

The median lethal dose was determined in both albino rats (n = 30) and albino mice (n = 30) each group of 30 was divided into six subgroups namely; 5 test groups (1, 5, 10, 15 and 20 ml/kg doses of the herbal product) and a control group, (20 ml/kg distilled water). The sub-grouping was done after 6 h of fasting for mice and 24 h fasting for rats. The animals were observed for obvious toxic symptoms, behavioral changes and mortality for 24 h post administration [7]. Dosage selection was based on the Organization of Economic Corporation and Development (OECD) guidelines, where 1ml\100g (10ml\kg) body weight of aqueous solution test substance could be considered for acute toxicity testing [8].

2.5 Sub-chronic Toxicological Studies

Eighty (80) albino rats were randomly divided into four groups of 20 rats per group. Blood samples were collected from animals via retro orbital plexus prior to treatment for the determination of basal haematological and biochemical parameters. Groups 1 – 3 received 1, 5 and 10 ml/kg oral doses of the decoction respectively while the control group received 10 ml/kg of distilled water once daily for 90 days.

Five rats from each group were selected on the 31st, 61st and 91st days of the study for periodic toxicity evaluation. Blood samples were collected through retro orbital puncture and were used for the estimation of haematological and biochemical parameters. The remaining 5 rats in each group were used for 28 days post-treatment recovery study where the animals were maintained on normal animal feed and water without drug administration. Physical observations of the animals were done on daily bases.

2.6 Haematological analysis

The blood parameters measurements were carried out using automated heamatological analyzer. These parameters include Packed Cell Volume (PCV), total white blood cell (WBC) count, Red Blood Cell (RBC) count, heamoglobin concentration, among others. Biochemical parameters, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, total bilirubin, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, calcium and lipid profile parameters (total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol) were determined using commercial assay kits procured from Randox Laboratories Limited, United kingdom and Teco diagnostics, California U.S.A.

2.7 Statistical Analysis

Results obtained from the study were presented as mean ± Standard error of mean (SEM) of sample replicates (n = 5). Raw data were subjected to statistical analyses by one way analyses of variance (ANOVA), followed by post hoc Turkey's test using statistical package for social Science (SPSS, version 20). *P* values <0.05 were considered statistically significant.

3. Results

3.1 Phytochemical analysis

This revealed abundant presence of; alkaloids, saponins, flavonoids, glycosides and proteins. Terpenoids, steroids, fats and oil, carbohydrates and resins were present but less abundant. Tannin was absent (**Table 1**).

Table 1: Phytochemistry results

Phytocompounds	Degree of occurrence
Alkaloids	++++
Saponins	+++
Flavonoids	+++
Glycosides	+++
Terpenoids	++
Steroids	+++
Fats and oil	++
Tannins	-
Carbohydrates	++
Proteins	+++
Resins	++

- = not present, + = present in small concentration, ++ = present in moderately high concentration, +++ = present in high concentration, ++++ =abundantly present.

3.2 Acute toxicity studies

There was no sign of death and toxicity up to 20 ml/kg of groups treated with the polyherbal product. All the groups maintained normal physical activities throughout the period of the observation.

3.3 Sub-chronic toxicity

3.3.1 Effect of the herbal formulation on Body weight

At all the tested doses, there was significant increase in body weight on 31, 61, 91 and 28 days post treatment when compared to pre-treatment body weight (**Table 2**).

Table 2: Effect of polyherbal formulation on body weight of Wistar rats

Treatment	Body weight				
	Pre-treatment	Day 31st	Day 61st	Day 91st	Post-treatment
Control	220.6±4.7	248.4±10.5	264.7±5.2	275.4±4.3	289.2±5.5
1 ml/kg	230.3±8.8	253.5±5.6	261.7±4.9	266.5±5.7	280.3±4.8
5 ml/kg	226.0±3.5	254.2±3.9	258.6±4.1	263.1±3.9	277.6±2.5
10 ml/kg	220.6±4.7	245.7±7.0	253.2±3.6	258.3±3.1	269.3±3.0

Values are presented as mean ± Standard error of mean (n =5). p< 0.05: Statistically significantly different from pre-treatment values.

3.3.2 Effect of the herbal formulation on liver biomarkers

There was no significant difference (p>0.05) in pretreatment and post treatment values for alkaline phosphatase, albumin and bilirubin level throughout the period of study. However, there was significant (p<0.05) increase in ALT and AST levels on the 91st day in the group treated with 10 ml/kg of the polyherbal product. These changes were reversed 28 days post-treatment (**Table 3**).

Table 3: Effect of sub-chronic administration of levels of Wistar rats

	Treatment	ALT (U/L)	AST (U/L)	ALP (IU/L)	Albumin (mg/dl)	Total bilirubin (mg/dI)
Pretreatment	**Control**	35.4 ± 1.7	50.6 ± 3.5	65.4 ± 4.7	3.4±0.3	0.40± 0.05
	1mL/kg	34.5 ± 2.5	47.8 ± 2.4	64.2 ± 3.3	3.3± 0.5	0.42± 0.08
	5 mL/kg	35.7 ± 1.6	52.6 ± 1.5	66.5 ± 2.9	3.5± 0.4	0.38± 0.06
	10 mL/kg	34.4 ± 2.6	48.6 ± 4.3	64.7 ± 2.5	3.2± 0.5	0.42± 0.07
Day 31st	**Control**	35.9 ± 2.5	51.6 ± 4.7	65.8 ± 3.8	3.4 ± 0.2	0.42 ± 0.06
	1m L/kg	36.5 ± 3.7	49.5 ± 2.6	65.9 ± 3.5	3.4 ± 0.4	0.45 ± 0.06
	5 mL/kg	37.2 ± 1.8	54.7 ± 3.8	68.8 ± 2.7	3.5 ± 0.3	0.42 ± 0.05
	10 mL/kg	37.3 ± 2.7	52.3 ± 2.5	67.5 ± 2.6	3.4 ± 0.3	0.40 ± 0.04
Day 61st	**Control**	36.7 ±3.6	51.9 ±3.6	67.2 ±4.5	3.5 ± 0.4	0.44± 0.04
	1m L/kg	38.6 ± 2.7	52.6 ± 3.5	68.3 ± 4.5	3.5 ± 0.3	0.44± 0.05
	5 mL/kg	40.7 ± 3.6	57.7 ± 2.8	70.6 ± 5.7	3.4 ± 0.6	0.45± 0.04
	10 mL/kg	43.2 ± 2.9	58.5 ± 4.8	70.5 ± 4.8	3.3 ± 0.5	0.43± 0.06
Day 91st	**Control**	38.2 ± 2.6	52.7 ± 4.8	68.5 ± 3.9	3.6± 0.4	0.45± 0.05
	1m L/kg	42.7 ± 4.8	56.5 ± 3.6	70.6 ± 5.7	3.5± 0.4	0.48± 0.05
	5 mL/kg	43.9 ± 6.9	59.6 ± 3.7	73.9 ± 4.8	3.5± 0.3	0.48± 0.03
	10 mL/kg	*¶50.5 ± 3.2	*¶65.3 ± 2.0	75.3 ± 7.8	3.4± 0.6	0.46± 0.04
Posttreatment	**Control**	37.8 ± 3.8	52.8 ± 3.5	68.7 ± 3.6	3.5± 0.5	0.46± 0.05
	1m L/kg	36.8 ± 3.5	52.6 ± 2.6	66.5 ± 3.6	3.4± 0.5	0.48± 0.04
	5 mL/kg	37.7 ± 2.6	54.9 ± 4.6	67.4 ± 3.7	3.5± 0.3	0.47± 0.05
	10 mL/kg	39.3 ± 4.8	56.3 ± 5.9	69.6 ± 4.0	3.4± 0.3	0.48± 0.06

Values are presented as mean ± Standard error of mean (n =5). *p<0.05: Statistically significantly different from control group.¶ p< 0.05: Statistically significantly different from pre-treatment values.

3.3.3 Effect of the herbal formulation on kidney biomarkers

There was increase ($p<0.05$) in creatinine level in groups treated with 10 ml/kg of test sample on 61st day as well as groups treated with 5 and 10 ml/kg on 91st days. BUN level increased on the 91st day in the group treated with 10 ml/kg when compared to control group. These changes were reversed 28 days post-treatment. There was no ($p>0.05$) difference in the blood levels of sodium, potassium, chloride and calcium groups treated with 1, 5 and 10 ml/kg doses of test sample on 31, 61 and 91st days respectively (**Tables 4**).

Table 4: Effect of sub-chronic administration of levels of Wistar rats

	Treatment	BUN (mg/dI)	Creatinine (mg/dI)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mg/dl)	Calcium (mg/dl)
Pretreatment	**Control**	16.8± 2.2	0.68± 0.16	145.6± 4.6	5.6± 0.6	88.6± 7.5	8.12± 0.9
	1m L/kg	17.3± 3.5	0.67± 0.14	143.3± 4.6	5.7± 0.6	88.6± 3.4	8.15± 1.7
	5 mL/kg	16.5± 1.4	0.69± 0.15	145.9± 3.5	5.6± 0.5	88.7± 6.6	8.14± 0.4
	10 mL/kg	17.2± 2.4	0.65± 0.13	144.7± 5.6	5.5± 0.5	88.3± 4.5	8.17± 0.6
Day 31st	**Control**	17.6 ± 2.4	0.68 ± 0.15	146.8 ± 2.6	5.4 ± 0.5	88.5 ± 4.5	8.16 ± 0.4
	1m L/kg	18.7 ± 2.5	0.75 ± 0.26	142.7 ± 4.5	5.6 ± 0.5	88.6 ± 3.6	8.18 ± 0.4
	5 mL/kg	19.2 ± 2.3	0.79 ± 0.55	144.7 ± 3.7	5.5 ± 1.5	88.8 ± 4.7	8.08 ± 1.3
	10 mL/kg	21.5 ± 2.6	0.78 ± 0.26	143.3 ± 8.4	5.8 ± 0.6	88.9 ± 5.8	8.14 ± 0.7
Day 61st	**Control**	17.6± 3.7	0.86± 0.23	143.5± 5.5	5.8± 0.7	89.2± 6.6	8.21± 0.6
	1m L/kg	19.3± 3.5	0.89± 0.37	140.3± 5.6	5.7± 0.6	88.9± 5.1	8.12± 0.7
	5 mL/kg	23.8± 2.8	0.93± 0.44	146.5± 7.6	5.8± 0.8	89.4± 3.8	8.02± 1.5
	10 mL/kg	28.2± 3.7	*1.34± 0.06	143.2± 4.8	5.9± 0.6	89.6± 3.5	7.86± 0.7
Day 91st	**Control**	18.6± 2.5	0.84± 0.35	143.7± 4.5	5.8± 1.2	88.8± 9.6	8.23± 2.5
	1m L/kg	23.6± 4.6	0.99± 0.45	145.3± 6.5	5.7± 0.8	89.2± 2.6	8.02± 1.1
	5 mL/kg	27.4± 6.7	*1.28± 0.32	146.0± 4.5	5.8± 0.9	89.5± 4.8	7.89± 0.5
	10 mL/kg	*35.9±3.1	*1.59± 0.47	145.6± 6.7	6.2± 1.0	89.8± 6.6	7.57± 0.8
Post treatment	**Control**	18.9± 3.5	0.69± 0.31	143.9± 2.4	5.7± 0.5	88.5± 7.4	8.14± 0.4
	1m L/kg	23.8± 3.5	0.68± 0.26	143.4± 4.5	5.6± 0.5	88.6± 8.6	8.16± 0.6
	5 mL/kg	24.6± 4.5	0.69± 0.22	142.5± 3.7	5.6± 0.6	88.5± 8.5	8.10± 0.8
	10 mL/kg	25.3± 3.4	0.72± 0.36	146.2± 3.8	5.6± 0.5	88.6± 4.7	8.04± 0.9

Values are presented as mean ± Standard error of mean (n =5). *p<0.05: Statistically significantly different from control group.

3.3.4 Effect of the herbal formulation on lipid profile

There was no ($p>0.05$) difference total cholesterol, HDL and LDL level in animals treated with test sample on 31st day. On 61st day, total cholesterol and LDL-cholesterol levels were reduced ($p<0.05$) in the group treated with 10 ml/kg of test sample. HDL-cholesterol level in 5 and 10 ml/kg were significantly reduced on 61st day. On 91st day, there was significant ($p<0.05$) reduction in total cholesterol and LDL-cholesterol in groups treated with 5 and 10 ml/kg of test sample. However, changes were reversible (**Table 5**).

Table 5: Effect of sub-chronic administration of levels of Wistar rats

	Treatment	Total cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)
Pretreatment	Control	76.75± 10.4	45.36± 3.7	8.25± 0.6
	1m L/kg	76.83± 5.1	44.76± 2.6	8.41± 0.7
	5 mL/kg	76.42± 4.8	45.15 ± 4.7	8.28± 0.9
	10 mL/kg	77.24± 9.9	44.85± 4.8	8.53± 0.6
Day 31st	Control	75.53 ± 9.6	45.86 ± 2.9	8.34 ± 0.4
	1m L/kg	76.54 ± 6.8	45.58 ± 1.9	8.12 ± 1.1
	5 mL/kg	76.14 ± 6.5	45.15 ± 4.7	7.63 ± 0.7
	10 mL/kg	74.38 ± 5.8	45.37 ± 6.8	7.02 ± 0.5
Day 61st	Control	76.49± 0.7	45.53± 4.6	8.23± 0.5
	1m L/kg	76.16± 9.5	46.34± 2.8	7.86± 0.5
	5 mL/kg	71.37± 6.4	*48.28± 1.8	7.25± 0.6
	10 mL/kg	*68.26±4.5	*48.16± 3.7	*¶6.63± 1.0
Day 91st	Control	77.26±7.8	45.53± 4.6	8.42± 0.6
	1m L/kg	71.42±11.6	46.34± 2.8	7.23± 0.7
	5 mL/kg	*¶66.26±3.6	*48.28± 1.8	*¶6.48± 0.5
	10 mL/kg	*¶61.43±12.6	*48.16± 3.7	*¶6.23± 0.8
Post treatment	Control	76.13± 8.7	45.29± 3.8	8.35± 0.6
	1m L/kg	76.12± 4.8	45.47± 4.6	8.29± 0.5
	5 mL/kg	74.27± 6.6	45.27± 2.7	8.20± 0.7
	10 mL/kg	72.96± 9.8	45.21± 2.2	8.24± 0.6

Values are presented as mean ± Standard error of mean (n =5). *p<0.05: Statistically significantly different from control group.¶ p< 0.05: Statistically significantly different from pre-treatment values.

3.3.5 Effect of the herbal formulation on haematological parameters

There was no difference ($p>0.05$) in white blood cell (WBC), lymphocytes (LYM), granulocytes (GRA), Mean corpuscular value (MCV), Platelet (PLT), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), mid cell total count (MID), platelet distribution width (PDW), red blood cell distribution width (RDW), Mean platelet volume (MPV), and platelet crit (PCT) levels of animals treated with 1, 5 and 10 ml/kg of test samples on 31, 61 and 91st days respectively. However, there was significant ($p< 0.05$) reduction in hematocrit, hemoglobin and RBC levels in groups treated with 10 ml/kg of test samples on 91st day. On post-treatment studies, these changes were reversed (**Tables 6, 7 and 8**).

Table 6: Effect of the test herbal sample on haematological parameters of Wistar rats

	Treatment	WBC ($x10^3$/ul)	LYM ($x10^3$/ul)	MID ($x10^3$/ul)	GRA ($x10^3$/ul)	LYM% (%)	MID% (%)	GRA% (%)
Pretreatment	**Control**	10.6± 0.4	7.1± 0.3	1.5± 0.05	2.0± 0.05	67.0± 0.7	13.7± 0.4	19.3± 0.4
	1m L/kg	9.8± 0.7	6.6± 0.4	1.4± 0.05	1.8± 0.05	67.3± 0.7	14.3± 0.5	18.4± 0.8
	5 mL/kg	9.5± 0.4	6.5± 0.6	1.3± 0.06	1.7± 0.04	68.4± 0.6	13.7± 0.6	17.9± 0.6
	10 mL/kg	10.2± 0.5	6.8± 0.6	1.6± 0.2	1.8± 0.05	66.7± 0.7	15.7± 0.4	17.6± 0.5
Day 31st	**Control**	9.3± 0.4	6.7± 0.6	1.1± 0.04	1.4± 0.07	72.4± 0.8	12.1±0.5	15.1± 0.4
	1m L/kg	9.2± 0.3	6.5± 0.3	1.4± 0.05	1.3± 0.03	70.7± 0.6	15.2± 0.5	14.1± 0.4
	5 mL/kg	9.0± 0.4	6.6± 0.5	1.0± 0.05	1.4± 0.02	73.3± 0.7	11.1± 0.3	15.6± 0.3
	10 mL/kg	8.9± 0.4	6.7± 0.5	0.9± 0.4	1.3± 0.03	74.8± 0.5	10.5± 0.5	14.7± 0.5
Day 61st	**Control**	8.5± 0.3	5.3± 0.3	1.2± 0.04	2.0± 0.07	62.4± 0.8	14.1± 0.5	23.5± 0.6
	1m L/kg	8.0± 0.5	5.7± 0.6	0.9± 0.04	1.4± 0.06	71.3± 0.7	11.3± 0.5	17.5± 0.6
	5 mL/kg	7.9± 0.7	5.2± 0.5	1.1± 0.02	1.6± 0.03	65.8± 0.8	13.9± 0.6	20.3± 0.4
	10 mL/kg	7.3± 0.3	5.6± 0.6	0.7± 0.06	1.0± 0.04	77.2± 0.4	9.5± 0.3	13.3± 0.4
Day 91st	**Control**	8.9± 0.3	5.5± 0.2	1.2± 0.05	2.2± 0.04	61.8± 0.5	13.5± 0.3	24.7± 0.3
	1m L/kg	7.9± 0.4	5.3± 0.3	1.3± 0.07	1.3± 0.05	67.1± 0.4	16.5± 0.7	16.5± 0.5
	5 mL/kg	7.7± 0.3	5.0± 0.2	1.2± 0.05	1.5± 0.02	64.9± 0.5	15.6± 0.4	19.5± 0.4
	10 mL/kg	7.5 ± 0.5	5.2 ± 0.3	1.0± 0.05	1.3± 0.03	69.3± 0.6	13.3± 0.4	17.3± 0.4
Post treatment	**Control**	8.7± 0.2	5.2± 0.1	1.2± 0.03	2.3± 0.05	59.8± 0.5	13.8± 0.2	26.4± 0.8
	1m L/kg	8.7± 0.3	5.6± 0.2	1.1± 0.03	2.0± 0.04	64.4± 0.8	16.5± 0.3	12.6± 0.1
	5 mL/kg	8.5± 0.2	5.2± 0.2	1.0± 0.04	2.3± 0.09	61.2± 0.8	11.8± 0.6	27.1± 0.9
	10 mL/kg	8.3± 0.2	5.0± 0.09	1.4± 0.02	1.9± 0.03	60.2± 0.5	16.9± 0.4	22.9± 0.5

Values are presented as mean ± Standard error of mean (n =5). *p<0.05: Statistically significantly different from control group. WBC = White blood cell, LYM = Lymphocytes, GRA = Granulocytes, MID = mid cell total count,

Table 7: Effect of the test herbal sample on haematological parameters of Wistar rats

	Treatment	RBC ($x10^6$/ul)	Hgb (g/dl)	HCT (%)	MCV (f l)	MCH (pg)	MCHC (g/dl)
Pretreatment	**Control**	8.4± 0.3	13.1± 0.2	38.7± 0.7	45.8± 0.8	15.5± 0.5	33.8± 0.7
	1m L/kg	8.4± 0.5	12.6± 0.5	38.8± 0.8	45.6± 0.7	15.8± 0.6	36.4± 0.4
	5 mL/kg	8.5± 0.7	13.3± 0.2	39.5± 0.5	45.9± 0.6	16.1± 0.4	33.4± 0.5
	10 mL/kg	8.8± 0.5	13.5± 0.3	39.3± 0.5	46.2± 0.5	15.9± 0.4	35.2± 0.4
Day 31st	**Control**	6.2± 0.2	10.1± 0.3	30.2± 0.5	48.8± 0.5	16.3± 0.6	33.4± 0.4
	1m L/kg	6.1± 0.5	10.0± 0.3	29.0± 0.4	47.1± 0.6	15.9± 0.3	33.4± 0.5
	5 mL/kg	6.0± 0.2	9.9± 0.2	28.7± 0.3	47.5± 0.5	15.6± 0.5	33.5± 0.3
	10 mL/kg	6.1± 0.3	9.6± 0.3	27.9± 0.5	46.4± 0.6	15.4± 0.3	33.3± 0.3
Day 61st	**Control**	6.7± 0.5	10.4± 0.4	38.2± 0.8	49.7± 0.8	18.6± 0.5	36.3± 0.5
	1m L/kg	6.4± 0.7	9.8± 0.6	32.9± 0.2	47.4± 0.5	17.2± 0.5	35.8± 0.7
	5 mL/kg	6.1± 0.4	9.3± 0.7	29.5± 0.6	46.1± 0.6	15.9± 0.6	34.3± 0.3
	10 mL/kg	5.8± 0.2	9.0± 0.3	27.0± 0.5	45.4± 0.4	14.8± 0.4	32.6± 0.5
Day 91st	**Control**	6.8± 0.2	10.9± 0.3	40.0± 0.6	49.9± 0.7	19.2± 0.3	38.6± 0.6
	1m L/kg	5.8± 0.2	8.7± 0.2	31.9± 0.5	46.8± 0.4	15.7± 0.3	36.7± 0.7
	5 mL/kg	5.2± 0.5	7.6± 0.4	27.3± 0.7	44.6± 0.6	14.7± 0.5	34.3± 0.4
	10 mL/kg	*3.9± 0.2	*5.6± 0.4	*19.4± 0.7	41.6± 0.5	13.7± 0.6	30.3± 0.6
Post treatment	**Control**	6.5± 0.1	10.2± 0.7	40.3± 0.8	48.3± 0.6	20.6± 0.6	37.5± 0.4
	1m L/kg	6.7± 0.3	10.5± 0.4	39.3± 0.7	47.3± 0.5	18.5± 0.3	36.6± 0.7
	5 mL/kg	6.2± 0.09	9.6± 0.2	37.9± 0.5	45.7± 0.8	18.5± 0.7	35.2± 0.5
	10 mL/kg	6.0± 0.2	9.2± 0.3	36.7± 0.8	45.1± 0.7	18.9± 0.6	35.0± 0.4

Values are presented as mean ± Standard error of mean (n =5). *p<0.05: Statistically significantly different from control group. Hgb = Haemoglobin, HCT = Haematocrit, MCV = Mean corpuscular value, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration.

Table 8: Effect of the test herbal sample on haematological parameters of Wistar rats

	Treatment	RDW (%)	PLT (x10^3/ul)	PCT (%)	MPV (f l)	PDW (f l)
Pretreatment	**Control**	15.4± 0.7	354± 6.5	0.3± 0.04	7.8± 0.5	6.4± 0.5
	1m L/kg	18.5± 0.6	352± 8.2	0.6± 0.02	7.3± 0.5	6.6± 0.5
	5 mL/kg	16.6± 0.3	358± 6.2	0.4± 0.06	6.9± 0.3	6.2± 0.4
	10 mL/kg	16.4± 0.6	357± 5.1	0.5± 0.03	7.6± 0.7	6.9± 0.5
Day 31st	**Control**	18.1± 0.3	407± 5.3	0.3± 0.04	7.6± 0.4	6.6± 0.3
	1m L/kg	18.2± 0.5	408± 6.2	0.4± 0.04	7.6± 0.2	6.7± 0.4
	5 mL/kg	18.0± 0.2	406± 4.3	0.4± 0.2	7.5± 0.3	6.8± 0.5
	10 mL/kg	17.9± 0.4	405± 6.3	0.3± 0.5	7.5± 0.4	6.6± 0.5
Day 61st	**Control**	17.5± 0.6	319± 7.5	0.3± 0.04	7.5± 0.9	6.5± 0.7
	1m L/kg	17.7± 0.3	323± 6.8	0.3± 0.05	7.5± 0.4	6.5± 0.6
	5 mL/kg	17.3± 0.7	324± 6.2	0.3± 0.04	7.4± 0.6	6.6± 0.5
	10 mL/kg	16.9± 0.2	315± 5.1	0.2± 0.04	7.3± 0.2	6.3± 0.5
Day 91st	**Control**	18.2± 0.6	324± 5.4	0.4± 0.03	7.7± 0.2	6.9± 0.2
	1m L/kg	17.5± 0.4	325± 6.9	0.4± 0.02	7.6± 0.5	6.8± 0.4
	5 mL/kg	16.1± 0.2	322± 5.9	0.3± 0.06	7.4± 0.5	6.8± 0.7
	10 mL/kg	14.5± 0.7	312± 4.8	0.3± 0.02	7.0± 0.3	6.1± 0.3
Post treatment	**Control**	20.9± 0.5	328± 4.5	0.4± 0.02	7.5± 0.1	7.1± 0.2
	1m L/kg	18.9± 0.4	329± 6.4	0.3 0.01	7.7± 0.4	6.9± 0.2
	5 mL/kg	19.2± 0.4	332± 5.3	0.4± 0.03	7.6± 0.3	6.9± 0.4
	10 mL/kg	22.8± 0.7	327± 6.3	0.3± 0.07	7.4± 0.4	6.6± 0.2

Values are presented as mean ± Standard error of mean (n =5). *p<0.05: Statistically significantly different from control group. PDW = platelet distribution width, RDW = red blood cell distribution width, MPV = Mean platelet volume, PCT = platelet crit. PLT: Platelet.

3.3.6 Effects of the herbal formulation on liver histology

The liver parenchyma architecture was preserved in the control group (**Plate A**). Liver photomicrograph of the group that received 5 ml/kg on the 61st day showed normal histology with no sign of inflammation (**Plate B**). Liver photomicrograph of the group that received 10 ml/kg on the 91st day showed cell infiltration around the portal area (**Plate C**). However, in post-treatment study, the liver photomicrograph of the group that received 10 ml/kg showed mild mononuclear cell infiltration indicating a trend of recovery to normal cellular integrity (**Plate D) (Figure 1).**

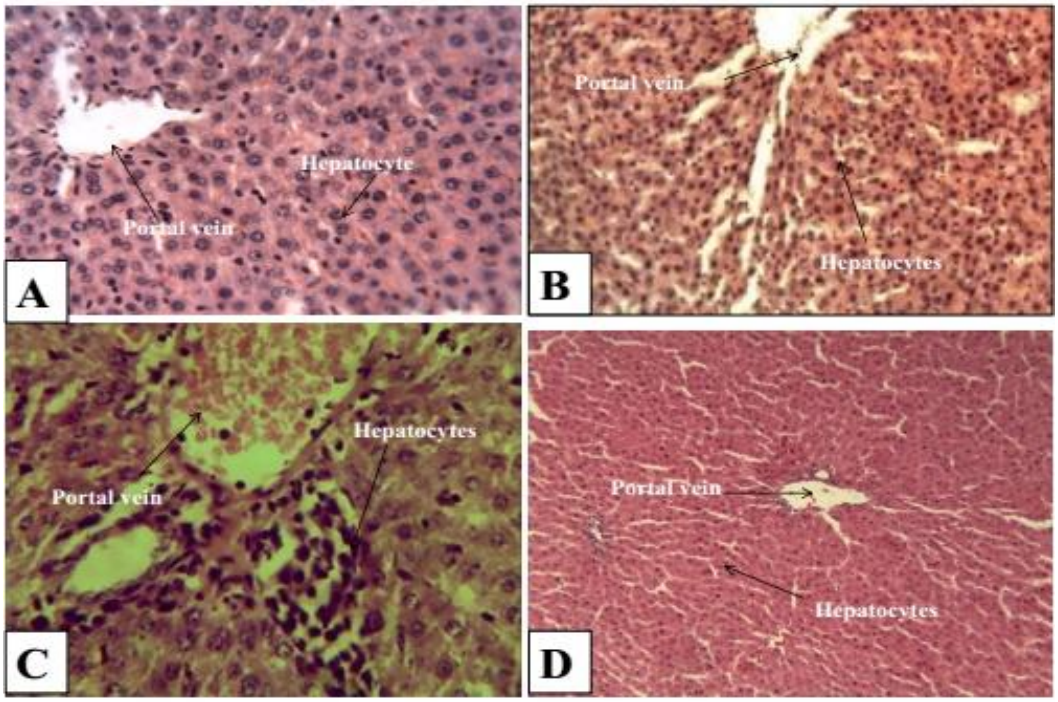

Figure 1: A = Liver photomicrograph for the group that served as the control (liver parenchyma architecture is preserved). **B =** Liver photomicrograph of the group that received 5 ml/kg on the 61st day (showing normal histological changes, no sign of inflammation. **C** = Liver photomicrograph of the group that received 10 ml/kg on the 91st day showing focal area of mononuclear cell infiltration around the portal area (P).**D** =Liver photomicrograph of the group that received 10 ml/kg in post-treatment study showing mild mononuclear cell infiltration indicating a trend of recovery to normal cellular integrity. H and E x400.

3.3.7 Effects of the herbal formulation on kidney histology

Photomicrograph of control kidney showed the glomerulus (G) and the tubules (T) with no observable histological changes. This indicates normal kidney (**Plate A)**. Photomicrograph of kidney that received 5 ml/kg on the 91st day showed mild areas of tubular degenerations (**Plate B)**. Photomicrograph of the group that received 10 ml/kg on the 91st day showed hypercellularity of the glomerulus (**Plate C)**. Photomicrograph of the group that received 10 ml/kg after post treatment study showed less hypercellularity of the glomeruli (**Plate D) (Figure 2).**

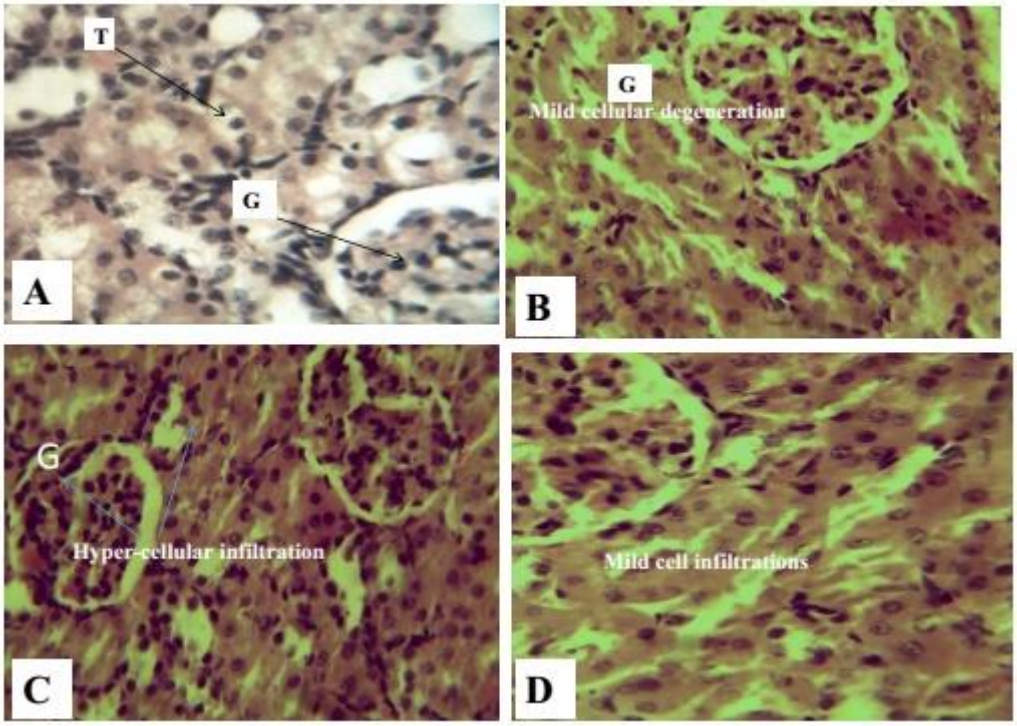

Figure 2: Photomicrograph of control kidney showing the glomerulus (G) and the tubules (T) with no observable histologic changes. This indicates normal kidney. **B =** Photomicrograph of kidney that received 5 ml/kg on the 91st day showing mild areas of tubular degenerations. **C** =Histologic section of the group that received 10 ml/kg on the 91st day showing hypercellularity of the glomerulus (G). **D =** Histologic section of the group that received 10 ml/kg after post treatment study showing less hypercellularity of the glomerulus (G). H and E .x400.

4. Discussion

This study evaluated the phytochemical constituents of the poly-herbal formulation "washing and setting". Its acute oral safety as well as its sub-chronic toxicity effect on body weight, liver functions, kidney function, electrolytes, lipid profile and haematological parameters was also determined. The presence of the phytocompounds (alkaloids, saponins, flavonoids, glycosides, terpenoids, steroids, fats and oil, carbohydrate, proteins and resins) could be attributed to the polyherbal products medicinal uses. Alkaloids have been reported as one of the important groups of phytocompounds obtained from natural sources recorded [9].

Absence of mortality and sign of toxicity up to 20 ml/kg suggests that the polyherbal decoction is safe on single usage for the treatment of diverse disease problems [10]. In sub-chronic toxicity experiment, there was no significant change in body weight, suggesting that the herbal remedy may not likely affect appetite feeding. This corroborated by the absence of tannin (an anti-nutritive phytoconstituent which is known to cause appetite suppression as well as body weight reduction) in *Allium sativum* (Garlic) present in the polyherbal formulation [11]. The liver biomarkers ALT, AST and ALP are currently accepted as the commonly used tool in the diagnosis of liver function [12].

From this study, significant elevations of ALT and AST recorded only on the 91st day of the study is an indication that long term administration of this decoction at higher dose may lead to hepatic damage. Non-significant alteration in ALP level suggests that the decoction may be unconnected with hepatic cholestasis – a major cause of serum ALP elevation in liver disease [13]. Previous study by Gatsing *et al*., [4] revealed that at higher dose or prolonged administration of (4800 mg/kg), *Allium sativum* a major component of this decoction caused elevations in ALT and AST [4]. Histological photomicrograph of the liver at the highest dose at the 91st day revealed cell

infiltration around the portal area in the group that received 10 ml/kg when compared to control groups (figure 1). The reversibility in liver architecture of group treated with 10 ml/kg of the decoction following post-treatment studies suggests that the toxicity caused by high dose is not permanent.

Bilirubin and albumin a major indicator of functional status of the liver were not significantly elevated at all the tested doses throughout this study, indicating the unlikely hood of the decoction to alter liver functions [14].

Blood urea nitrogen (BUN) and creatinine are excreted specifically by the kidney and as such their plasma levels are considered as a strong indicator of kidney function [15]. Significant elevations of BUN and creatinine recorded during this study are indication of possible altered kidney function. Histological photomicrograph of the kidney (figure 2) showing mild areas of tubular degeneration and hypercellularity of the glomerulus supports this inference.

High content of alkaloid observed from the phytochemical analysis of the Product might have accounted for the observed altered kidney function and structure. Alkaloids isolated from *Z. gilletii* – a major component of the product have been documented to be nephrotoxic [16]. The recovery of the kidney architecture in control group to normalcy in post-treatment study suggests that the pathological changes due to the herbal decoction were reversible.

Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance, nerve conduction, blood clothing and muscle contraction and also used as a prognosis to certain disease conditions especially as it concerns cardiac, renal and gastrointestinal functions. The non-significant elevation in serum electrolytes level is an indication that the decoction may not affect electrolyte levels in chronic use of the product [17].

Blood lipid profile directly impacts on the overall cardiovascular health [18]. The ability of the product to maintain good lipid profile through significant reduction of total cholesterol and LDL and elevation of HDL is an indication of its positive effect on the cardiovascular system and may be connected to its folkloric use in general body healing. Blood cholesterol lowering effect of garlic has been reported [19] as its mechanism of both antiatherosclerotic and antiatherogenic effects. Also Thomson *et al* [20] reported that garlic lowers free cholesterol and cholesteryl esters in lipid-overloaded arterial cells. *Acacia nilotica* and *Allium sativum* have been reported to possess powerful antioxidant activity [21] and the phytochemical analysis of the product revealed rich content of antioxidant phytocompounds. The decrease in cholesterol and LDL and corresponding increase in HDL demonstrated by this product might be attributed to multiple integrated mechanisms involved in lipid metabolism [22].

The haematological parameters provide information on the general state of the blood [23]. Significant ($p<0.05$) reduction of red blood cell count, haemoglobin and haematocrit values are all indication of the likelihood of the product to cause anaemia on chronic use. The cytotoxicities of *uvaria afzelii* and *zanthoxylum gilletii* have been documented [24] while the leaves of *Acacia nilotica* have been shown to have both cytotoxic and haemolytic activities [25]. Also aqueous extract of *Allium sativum* bulbs has been documented to induce anaemic conditions with marked decreases in haematocrit values [4]. All these finding in past literatures lend credence to the marked reduction of the haematological parameters recorded in this study.

5. Conclusion

This study evaluated the phytochemical constituents, acute and sub-chronic oral toxicity of a Poly-herbal formulation "Washing and setting". Alkaloid was the most abundant phytocompound in the product. Reduction of cholesterol and LDL with elevated HDL was recorded following sub-chronic administration. Sub-chronic administration decreased heamatological parameters such as red blood cell count, Haemoglobin concentration, heamatocrit.

From the present study, the test sample may be considered safe on acute administration. However, the product may be nephrotoxic and hepatoxic especially at high doses on long term usage. These results portend possible dangers for patients who may be placed on long term therapy on this polyherbal remedy at high dose. Thus, consumers should be cautioned on the dangers of chronic and high dose administration of this product.

Reference

[1]. Duraipandiyan DB, Fontaine KR, Heshka S, Mentore JL, Heymsfield SB. Alternative treatments for weight loss: *Crit Rev Food Sci Nut* 2006; 41:1–28.

[2]. Nwankwo JP. Alternative medicine - The risks of untested and unregular remedies. *N Engl J Med* 1998; 339:839–841.

[3]. Gurib-fakim JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res* 2006; 33:179–189.

[4]. Gatsing D, Aliyu R, Kuiate JR, Garba IH, Jaryum KH, Tedongmo N, *et al*. Toxicological evaluation of the aqueous extract of *allium sativum* bulbs on

laboratory mice and rats. *Cameroon J Exp Biol* 2005; 1(1): 39-45.

[5]. Chan TY. Monitoring the safety of herbal medicines. *Drug Saf 2007*; 17: 209–215.

[6]. Treas GE and Evans WC. Textbook of Pharmacognosy. 12th ed. London: Bailliere Tindal; 1983. p 343-383.

[7]. Miller C, Tainter MC. Estimation of LD_{50} and its error by means of logarithmic-probit graph paper. *Proc Soc Exp Bio Med* 1994; 57: 261-264.

[8]. OECD. Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No.24 (2000).

[9]. Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. *J Phytol* 2011; 3(12): 10-14.

[10]. Desta G, Yalemtsehay M, Wondwosson E, Kahsay H. The effects of *Moringa stenopetala* on blood parameters and histopathology of liver and kidney in mice Desta Ghebreselassie. *Ethiop J Health Dev* 2011; 25(1).51-57.

[11]. Saba F, Mohammad ZQ, Sabiha K, Rukhshan K. Effect of feeding garlic (*Allium sativum*) on body weight and serum cholesterol levels in rats. *Pak J Physiol* 2011; 7(1): 17-19.

[12]. Mohan S, Thiagarajan K, Chandrasekaran R, Arul J. *In vitro* protection of biological macromolecules against oxidative stress and *in vivo* toxicity evaluation of *Acacia nilotica (L.*) and ethyl gallate in rats. *BMC Complement Altern Med* 2014; 14: 257.

[13]. Schlaeger R, Haux P, Kattermann R. Studies on the mechanism of the increase in serum alkaline phosphatase from rat liver. *Enzymes* 2002; 28(1): 3-13.

[14]. Adak M, Shivapuri JN. Enzymatic and non-enzymatic liver function test: A review. *RJPBCS* 2010; 1(4): 593-605.

[15]. Idonije BO, Nwoke EO, Festus O, Oluba OM. Plasma concentrations of kidney function indicators in malaria patients in Ekpoma, South-South Nigeria. *Int J Trop Med* 2011; 6(1): 4-7.

[16]. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, *et al*. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharmacol* 2000; 32(1): 56-67.

[17]. Beck BD, Calabrese EJ, Anderson PD. "The use of toxicology in the regulatory process," in Principles and Methods of Toxicology, 4th ed. New York: Raven Press; 2009. p. 1–28.

[18]. Ottobani MA. "How chemicals cause harm," in The Dose Makes the Poison. New York: 2011. pp. 19–28.

[19]. Rahman K. Historical Perspective on Garlic and Cardiovascular Disease. *J Nut* 2001; 131: 977S-979S.

[20]. Thomson M, Al-Amin ZM, Al-Qattan KK, Shaban LH, Ali M. Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum*) in streptozotocin-induced diabetic rats. *Int J Diabetes Metab* 2007; 15: 108-115.

[21]. Sullivan JB, Krieger GR. "Introduction to hazardous material toxicology," in Hazardous Materials Toxicology: Clinical Principles of Environmental Health, Baltimore: Williams & Wilkins; 2002. p. 2–8.

[22]. Koeman, JH. "Toxicology: History and scope of the field," in Toxicology: Principles and Applications. 2006. p. 2–15.

[23]. Ballantine B, Sullivan JB. "Basic Principles of Toxicology," in Hazardous Materials Toxicology: *Clinical Principles of Environmental Health*, J. B. Sullivan, and G. R. Krieger, Edition. Baltimore: Williams & Wilkins; 2002. p. 9–23.

[24]. Schuppan D, Jai JD, Brinkhaus B, Hahn EG. Herbal products for liver diseases: a therapeutic challenge for the new millennium. *Hepatology* 2009; 30: 1099-1104.

[25]. Subramanion LJ, Zuraini Z, Yeng C, Yee LL, Lachimanan YL, Sreenivasan S. Acute oral toxicity of methanol seed extract of *cassia fistula* in mice. *Molecules* 2011; 16:5268-5282.