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Potential Genotoxicity, Hematotoxicity and Clastogenicity of Three Commonly Consumed Polyherbal Formulations in Abeokuta South-West Nigeria

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Abstract: Background: The current upsurge in the use of polyherbal remedies and coupled with loose regulation on public access to these products underscore research efforts to evaluate their biochemical effect, noting also that many of the polyherbal medicines lack scientific evidence to support their medicinal claims. Objective: We therefore investigated the potential genotoxicity, hematotoxicity and clastogenicity of three commonly consumed polyherbal formulations (OsomoBitters™, Ogidiga™ and BabyOku™) in South-West Nigeria, in experimental rats. Methods: Two hundred and fifty (250) adult female wistar rats were randomly selected and distributed into 10 groups of 25 rats each. Two mL/kg body weight of distilled water, a non-alcoholic non-polyherbal formulation (Fanta®), a polyherbal non-alcoholic formulation (YoyoBitters™) and an alcoholic non-polyherbal drink (Sabrina™) were orally administered to the control groups while BabyOku™, OsomoBitters™ and Ogidiga™ herbal formulations were administered to the experimental groups at doses of 2 mL/kg and 3 mL/kg body weights. Results: A dose- and tissue-dependent increase in induction of apoptotic DNA fragmentation was observed in the polyherbal groups relative to control groups. Also, an increase in micronucleated polychromatic erythrocytes was formed in a dose-dependent manner in the polyherbal groups when compared with the control groups. Conclusion: From our findings, polyherbal formulations may possess clastogenic, hematotoxic and genotoxic potentials in rats.

Keywords: Polyherbal Formulations, Genotoxicity, Hematotoxicity, Clastogenicity

1. Introduction

Medicinal plants are a major source of active drugs from nature. The use of plant parts in treating diseases is universal, it is often more affordable and believed to be effective than the conventional drugs. Most of these medicinal plants are eaten or used for their rich phytochemical constituents, which provide both preventive and curative properties to consumers against various diseases [1]. In recent years, there has been increase in the popularity enjoyed by herbal remedy usually prepared by mixing various medicinal plant species [2].

In Nigeria, the last few years have witnessed an upsurge in the patronage of herbal remedies. In spite of the wide patronage enjoyed by herbal remedies, little or no empirical data exist to support medicinal claims or otherwise. Also, there are no scientific data on safety and toxicity profiles of these herbals [2-4]. Herbal remedy mixtures such as YoyoBitters™,

Swedish Bitters™, Fijk™, OsomoBitters™, Alomo™, Oroki™ among others have become a common sight in many Nigerian homes. All of these herbals have acclaimed medicinal benefits but only few, if any, have empirical data to support medicinal claims. However, recent studies have demonstrated the need to subject some of the herbal mixtures to scientific scrutiny, at least in part to ascertain safety limits [2-6], more so government regulation of herbal medicine is not as stringent when compared to conventional drugs. Furthermore, microbial contaminants and higher level of heavy metals which could be detrimental to human health have been demonstrated in several herbal remedies [4, 7]. Even for efficient and documented herbal medicinal products, the toxicity can be relatively unexplored; indeed, in contrast with conventional drugs research and development, the toxicity of traditional herbal medicines is not often evaluated [8-10]. Most of the population however does not pay attention, believing that, if these products have been used so far, they should be devoid of toxicity [11-14]. All of these factors serve to fuel the imperativeness for empirical data on either the safety or toxicity margin of herbal mixtures being marketed and promoted to the Nigerian public.

OsomoBitters™, Ogidiga™ and BabyOku™ herbal mixtures are very popular among the Nigerian populace. OsomoBitters™ is an alcoholic-based herbal formulation comprising different herbs; *Callichilai barteri*, *Pachylobus edulis* (Canarium), *L. cupanioides*, *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Monodora myristica* (*C*alabash nutmeg), *Khaya ivorensis* (African mahogany), *Piper nigrum* (Black pepper), *Eugenia caryophyllus* (cloves) [4]. BabyOku™ (produced by Chuby-Zion Industries Nigeria Limited, Ogun State, Nigeria) contains carene, a medicinal component of herbal drugs, eugenol (alcohol), fatty acids such as propenoic acid and nonanoic acid and cyclohexanemethanol in it. Other active ingredients in it are water, ethanol, caramel, herbal flavour, extracts such as angelia root, cassia sanna (sic) leaf, rhuherb root and aloe. The constituents of Ogidiga™ (produced by Bang Amos, Ikorodu, Lagos state, Nigeria) according to its label include ethanol, water, sugar, lemon, garlic, ginger and *combretaceae*. Their bitterness are claimed to boost libido, cure pile, malaria, clear toxins among others, but there are no scientific data on any of these herbals to support these medicinal claims or otherwise [4]. There are increasing reports that several plants contain toxic, genotoxic and carcinogenic compounds [15-17]. Chromosome aberrations in plants and animals are hallmarks of genome instability which may lead to genetic related diseases and congenital abnormalities [18, 19].

The carcinogenicity of polyherbal formulations has been a "hot topic" for over a century. The earliest epidemiological observations were provided in 1910 in France, when 80% of the patients diagnosed with oesophageal cancer were heavy drinkers of absinthe, a polyherbal with high alcoholic strength [20]. The epidemiological evidence about a causality between the lifestyle choice of alcohol consumption and cancer of several sites (oral cavity, pharynx, larynx, oesophagus and liver) was corroborated in numerous studies during the twentieth century, which led the WHO International Agency for Research on Cancer (IARC) to classify "alcohol drinking" as carcinogenic to humans in 1988 [21]. Well-designed long-term experiments have later found both ethanol [22-25] and acetaldehyde as the first metabolite of ethanol as carcinogenic for animals [21, 26, 27].

This study therefore investigated in rats, the potential genotoxicity, hematotoxicity, and clastogenicity of three commonly consumed polyherbal formulations (Osomo bitters™, Ogidiga™ and Baby Oku™) in Abeokuta south-west Nigeria.

2. Materials and Methods

2.1. Experimental Animals

Two hundred and fifty (250) adult female wistar rats weighing 200-250g, used for this study, were purchased at the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. They were acclimatized for two weeks before the onset of the experiment. The animals were housed in wooden cages with good aeration, in a room with average illumination with 12:12-hour light:dark cycle and they were given free access to water and supplied with standard pellet *ad-libitum*.

2.2. Test Substances/Formulations

The polyherbal formulations were purchased at a liquor store at Camp settlement area, Abeokuta, Nigeria. The formulations used for the experiment were Fanta® (a non–alcoholic non-polyherbal formulation), YoyoBitters™ (a polyherbal non-alcoholic formulation), Sabrina™ (an alcoholic non-polyherbal formulation), BabyOku™, Ogidiga™ and OsomoBitters™ (a polyherbal alcoholic formulations).

2.3. Experimental Design

The rats were randomly selected and assigned into 10 groups based on the type and amount of formulation/test substance administered. Each group is further subdivided into 5 subgroups containing 5 rats each based on the duration of administration. Two (2) mL/kg body weight of distilled water, Fanta®, YoyoBitters™ and Sabrina™ were orally administered to the control groups while BabyOku™, OsomoBitters™ and Ogidiga™ polyherbal formulations were administered to the experimental groups at doses of 2 mL/kg and 3 mL/kg body weights. The experimental rats were sacrificed at intervals of day 0, day 8, day 16, day 24 and day 32 of the administration of the test substances by cervical dislocation. Two (2) hours prior to sacrifice, each rat was injected with colchicine (prepared in distilled water) at a dose of 1 mL/100 g body weight intraperitoneally, for mitotic arrest. Rats were dissected and blood samples were collected with heparinized syringes via the abdominal artery and immediately transferred to heparinized tube and kept on ice for full and differential blood counts. Tissues (liver, kidneys, heart, brain, lungs, ovaries, uterus, stomach and spleen) were harvested, washed in ice-cold normal saline and stored at -20 °C for

genotoxicity experiments. Femur from both legs were quickly harvested and immediately used for the micronucleus assay.

2.4. Full and Differential Blood Counts

Samples of EDTA-anticoagulated blood were collected and stored in a cool box, at approximately 4 °C, and delivered to a local processing field laboratory within two hours of collection. Full and differential blood counts were analyzed on a Coulter LH700 series Hematology analyzer (Beckman Coulter, Miami, USA).

2.5. Micronucleus Assay

The femurs from each of the animals were removed and bone marrow was aspirated with a syringe and microscopic slides prepared according to Matter and Schmid [28]. The slides were then fixed in absolute methanol (BDH Chemical Ltd, Poole, England), air-dried, pretreated with May-Grunwald solution (Sigma-Aldrich, procedure No GS-10) and air-dried. The dried slides were stained in 5% Giemsa solution, and immersed in phosphate buffer 0.01 mol L^{-1} (pH 6.8) for 30 s. Thereafter, they were rinsed in distilled water, air-dried, and mounted. The slides were scored at x 100 magnification under a Nikon E200 light microscope (Opto-Edu Co., Ltd, Beijing, China) for micronucleated polychromatic erythrocytes (mPCEs).

2.6. Apoptotic DNA Fragmentation Assay

The method of Wu et al. [29] was used. The tissues were homogenized in 10 volumes of a lysis buffer (pH 8.0) consisting of 5 *mM* Tris-HCI, 20 *mM* EDTA and 0.5% (w/v) t-octylphenoxypolyethoxyethanol (Triton X-100). 1 mL aliquots of each sample were centrifuged at 27,000 *g* for 20 minutes to separate the intact chromatin (pellet) from the fragmented DNA (supernatant). The supernatant was decanted and saved, and the pellet was resuspended in 1 mL of Tris buffer (pH 8.0) consisting of 10 *mM* Tris-HCI and 1 *mM* EDTA. The pellet and supernatant fractions were assayed for DNA content using a diphenylamine reaction. Optical density was read at 620 nm with spectrophotometer. The results were expressed as a percentage of fragmented DNA divided by total DNA.

2.7. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan Multiple Range Test to test for significant differences among the groups of rats using Statistical Package for Social Sciences Program Version 16.0. Data were expressed as mean ± standard error of mean. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Bone Marrow Clastogenicity Potentials of Baby Oku™, Ogidiga™ and Osomo Bitters™

There was a significant increase in the induction of micro-nucleated polychromatic erythrocytes (mPCEs) by all the polyherbal formulations compared with the control (DH_2O) group (Figures 1, 2, and 3) ($p<0.05$). A dose dependent increase (but not statistically significant) in the induction of mPCEs were observed (2ml/kg body weight and 3ml/kg body weight) among rats treated with BabyOku™, Ogidiga™ and OsomoBitters™ polyherbal formulations ($p<0.05$). It was also observed that the degree of induction of mPCEs by the poly-herbal formulations is Ogidiga™ > Osomo bitters™ > BabyOku™. There was a significant increase in the degree of induction of mPCEs by the poly-herbal formulations relative to Fanta®, Yoyo bitters™ or Sabrina™ treated groups ($p<0.05$).

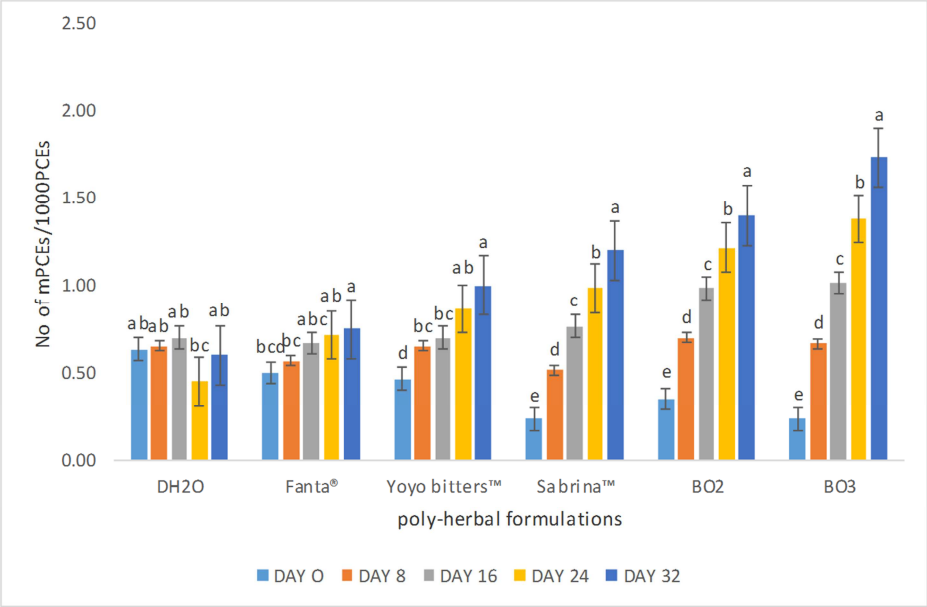

Figure 1. *Bone marrow micro-nucleated polychromatic erythrocytes (mPCEs) of rats treated with BabyOku™. Values are expressed as mean ± standard deviation (n=5). Bars bearing different alphabets are significantly different while those with similar alphabets are insignificantly different (p< 0.05). BO2= 2ml/kg body weight of BabyOku™, BO3= 3ml/kg body weight of BabyOku™.*

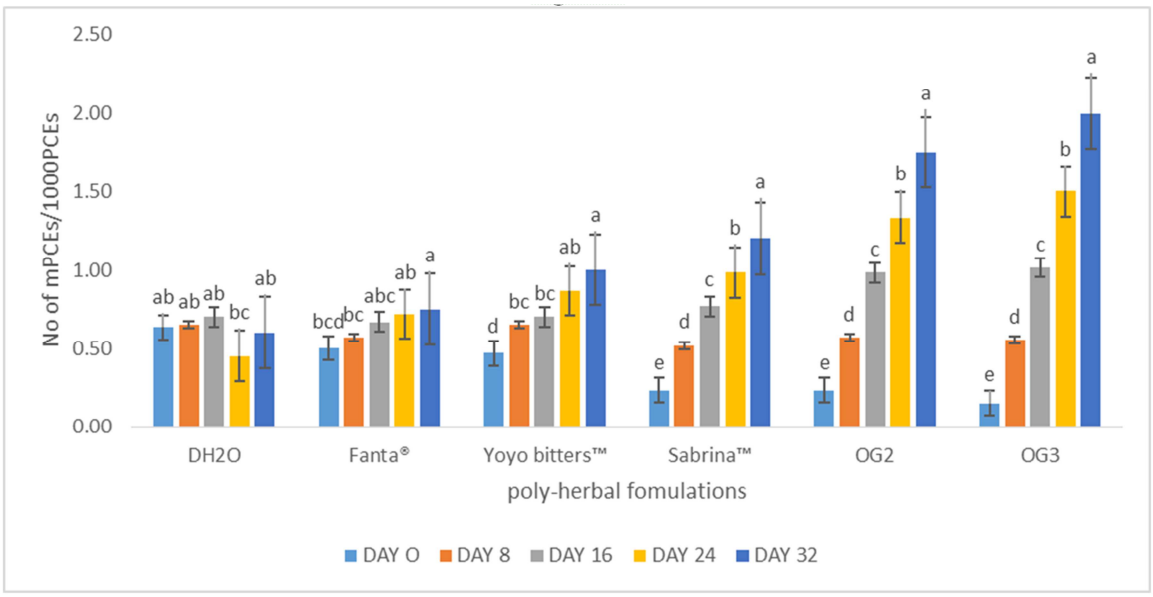

Figure 2. *Induction of bone marrow micro-nucleated polychromatic erythrocytes (mPCEs) of rats treated with Ogidiga™. Values are expressed as mean ± standard deviation (n=5). Bars bearing different alphabets are significantly different while those with similar alphabets are insignificantly different (p< 0.05). OG2= 2ml/kg body weight of Ogidiga™, OG3= 3ml/kg body weight of Ogidiga™.*

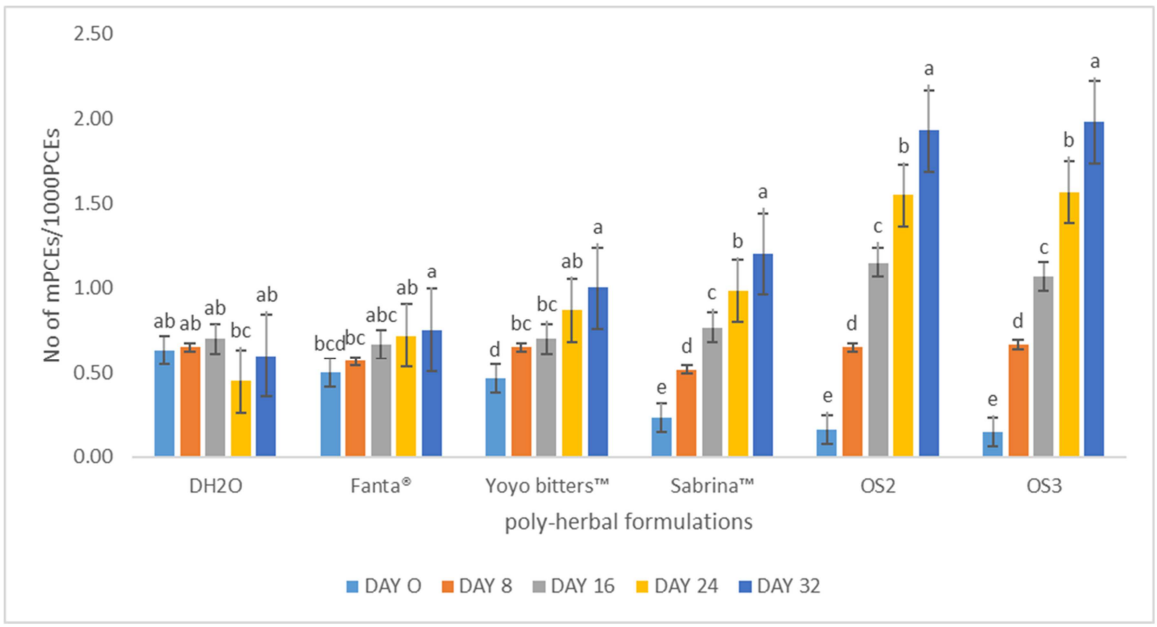

Figure 3. *Bone marrow micro-nucleated polychromatic erythrocytes (mPCEs) of rats treated with Osomo bitters™. Values are expressed as mean ± standard deviation (n=5). Bars bearing different alphabets are significantly different while those with similar alphabets are insignificantly different (p< 0.05). OS2= 2ml/kg body weight of OsomoBitters™, OS3= 3ml/kg body weight of OsomoBitters™.*

3.2. Alterations in Haematological Parameters in Polyherbal Formulations Treated Rats

There was a significant decrease in haematological parameters (Table 1, 2, 3 and 4) by all the poly-herbal formulations compared with the control (DH_2O) group ($p<0.05$). A statistical dose dependent decrease in the hemoglobin concentration and percentage packed cell volume; red blood cell, white blood cell, lymphocytes, neutrophil and platelets counts were observed (2 mL/kg body weight and 3 mL/kg body weight) among rats treated with BabyOku™, Ogidiga™ and OsomoBitters™ polyherbal formulations ($p<0.05$). It was observed that the degree of alteration produced by the polyherbal formulations is Osomo bitters™ > Ogidiga™ > BabyOku™. There was a significant difference in the degree of alterations in haematological parameters induced by the polyherbal formulations relative to Fanta®, Yoyo bitters™ or Sabrina™ treated groups ($p<0.05$).

Table 1. *Hemoglobin concentration, red blood cells and platelets counts in polyherbal formulations treated rats.*

	HEMOGLOBIN CONCENTRATION (g/dl)			RED BLOOD CELLS (x 10^{12} g/L)			PLATELETS COUNTS (x10^3 cells/L)		
	DAY 0	DAY 16	DAY 32	DAY 0	DAY 16	DAY 32	DAY 0	DAY 16	DAY 32
DH_20	16.26±0.11 ^{ab}	16.38±0.31^{b}	16.26±0.11^{b}	7.86±0.01 ^{a}	7.47±0.01^{a}	7.90±0.02^{a}	756.80±1.92 ^{e}	853.80±1.48^{b}	811.40±2.30^{b}
Fanta®	16.36±0.15 ^{ab}	16.78±0.08^{a}	16.74±0.15^{a}	7.63±0.01 ^{bc}	7.37±0.02^{b}	7.07±0.02^{b}	803.00±2.23 ^{d}	860.60±1.95^{a}	851.80±1.79^{a}
Yoyo bitters™	16.36±0.11 ^{ab}	15.26±0.11^{c}	14.26±0.18^{c}	7.55±0.01 ^{cd}	6.73±0.05^{c}	5.67±0.02^{c}	904.80±1.48 ^{a}	789.80±1.30^{c}	767.20±41.69^{c}
Sabrina™	16.32±0.14 ^{ab}	14.30±0.16^{d}	13.24±0.11^{d}	7.19±0.05 ^{e}	6.07±0.03^{d}	5.43±0.02^{d}	843.00±1.87 ^{c}	760.80±1.48^{d}	733.00±11.05^{d}
BO2	16.30±0.15 ^{ab}	11.70±0.16^{e}	4.26±0.11^{f}	7.69±0.01 ^{b}	5.81±0.06^{e}	2.55±0.04^{e}	912.40±1.81 ^{a}	604.80±2.05^{f}	393.60±1.14^{e}
BO3	16.30±0.15 ^{ab}	10.36±0.11^{g}	3.56±0.11^{g}	7.56±0.19 ^{cd}	5.67±0.01^{f}	2.42±0.01^{f}	878.20±46.94 ^{b}	585.20±2.77^{h}	302.20±2.49^{f}
OG2	16.48±0.21 ^{ab}	10.30±0.16^{g}	3.26±0.15^{h}	7.93±0.01 ^{a}	5.32±0.01^{h}	2.43±0.02^{f}	756.80±1.92 ^{e}	403.80±2.28^{j}	212.40±2.07^{h}
OG3	16.40±0.22 ^{ab}	10.74±0.11^{f}	3.24±0.11^{h}	7.16±0.04 ^{e}	5.40±0.02^{g}	2.14±0.02^{h}	703.00±2.23 ^{f}	454.60±1.67^{i}	202.00±1.58^{h}
OS2	16.42±0.13 ^{ab}	9.26±0.11^{h}	4.76±0.11^{e}	7.06±0.01 ^{f}	4.16±0.03^{i}	2.23±0.02^{g}	902.40±2.07 ^{a}	650.80±1.92^{e}	303.60±1.14^{f}
OS3	16.20±0.10 ^{b}	8.72±0.13^{i}	3.42±0.24^{gh}	7.48±0.02 ^{d}	4.19±0.03^{i}	1.63±0.03^{i}	855.80±2.16 ^{c}	590.00±2.24^{g}	251.80±1.48^{g}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 2. *Packed cell volume, white blood cells and lymphocyte counts in poly-herbal formulations treated rats.*

	PACKED CELL VOLUME (%)			WHITE BLOOD CELLS (x10^9 cells/L)			LYMPHOCYTE COUNTS (x10^9 cells/L)		
	DAY 0	DAY 16	DAY 32	DAY 0	DAY 16	DAY 32	DAY 0	DAY 16	DAY 32
DH_2O	84.20±0.83^{abc}	83.80±0.84^{a}	85.80±1.30^{a}	4.62±0.08 ^{ab}	3.32±0.08^{c}	4.30±0.14^{a}	41.20±0.83 ^{c}	46.20±1.48^{a}	45.40±2.19^{a}
Fanta®	82.80±0.83^{bc}	86.20±1.64^{a}	86.00±1.58^{a}	3.40±0.07 ^{e}	4.36±0.11^{b}	3.80±0.10^{b}	42.00±1.58^{bc}	42.80±0.84^{b}	42.20±3.35^{b}
Yoyo bitters™	82.60±1.14^{c}	70.80±2.86^{b}	56.20±1.48^{b}	3.50±0.33 ^{e}	3.30±0.10^{c}	3.36±0.11^{d}	41.60±1.14^{bc}	25.00±1.00^{g}	25.00±1.00^{c}
Sabrina™	83.60±1.14^{bc}	61.00±2.92^{c}	47.80±0.84^{c}	4.68±0.14 ^{a}	4.62±0.16^{a}	3.56±0.11^{c}	43.40±1.14^{ab}	34.40±1.14^{d}	23.20±2.39^{cd}
BO2	85.00±1.58 ^{ab}	61.60±2.97^{c}	46.20±1.30^{cd}	4.38±0.08 ^{bc}	2.50±0.16^{e}	1.26±0.11^{e}	43.00±1.00^{abc}	38.00±0.71^{c}	21.60±0.89^{d}
BO3	84.60±1.51^{abc}	51.00±1.58^{d}	26.00±1.58^{e}	4.78±0.08 ^{a}	2.76±0.11^{d}	1.42±0.16^{e}	43.40±1.81 ^{ab}	21.20±0.84^{h}	15.80±1.48^{e}
OG2	83.80±1.4 ^{bc}	64.00±2.65^{c}	45.00±1.58^{d}	4.14±0.23 ^{c}	2.60±0.12^{de}	1.24±0.11^{e}	41.20±0.83 ^{c}	38.00±1.22^{c}	22.20±1.92^{cd}
OG3	84.80±1.48^{abc}	52.40±1.14^{d}	27.40±1.82^{e}	3.78±0.08 ^{d}	2.46±0.11^{e}	1.28±0.15^{e}	42.20±0.83^{bc}	22.40±1.14^{h}	15.80±2.59^{e}
OS2	83.80±1.92^{bc}	52.20±1.92^{d}	27.20±1.48^{e}	3.36±0.31 ^{e}	2.20±0.10^{f}	0.94±0.15^{f}	44.60±2.72^{a}	32.00±1.22^{e}	22.20±1.92^{cd}
OS3	86.20±2.58^{a}	43.60±2.70^{e}	13.60±2.70^{f}	3.28±0.34 ^{e}	1.96±0.24^{g}	0.70±0.16^{g}	44.60±1.34^{a}	28.60±1.14^{f}	14.20±1.92^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 3. *Neutrophil count, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) in poly-herbal formulations treated rats.*

	NEUTROPHIL COUNT (x10^9 cells/L)			MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (g/dl)			MEAN CORPUSCULAR HEMOGLOBIN (PG)		
	DAY 0	DAY 16	DAY 32	DAY 0	DAY 16	DAY 32	DAY 0	DAY 16	DAY 32
DH_2O	55.20±1.30 ^{bcd}	54.60±1.82^{b}	56.60±3.78^{a}	19.31±0.19 ^{bc}	19.55±0.20^{cd}	18.95±0.28^{b}	20.7±0.026^{e}	21.9±0.04^{c}	20.6±0.01^{c}
Fanta®	54.80±0.83 ^{cd}	57.00±1.58^{a}	54.40±1.14^{a}	19.96±0.73 ^{a}	19.47±0.43^{cd}	19.47±0.26^{b}	21.4±0.024^{cd}	22.8±0.01^{b}	23.7±0.03^{b}
Yoyo bitters™	54.00±0.70 ^{de}	42.80±0.84^{d}	34.40±1.14^{c}	19.81±0.34 ^{ab}	21.59±1.02^{b}	25.39±0.88^{a}	21.7±0.011^{c}	22.7±0.01^{b}	25.2±0.03^{a}
Sabrina™	56.60±1.51 ^{abc}	46.20±1.48^{c}	31.00±1.00^{e}	19.52±0.26 ^{ab}	23.49±1.20^{a}	27.70±0.46^{a}	22.7±0.025^{b}	23.6±0.02^{a}	24.4±0.03^{ab}
BO2	55.40±1.34 ^{bcd}	47.80±1.30^{c}	37.60±1.14^{b}	19.18±0.42 ^{bc}	19.03±0.98^{d}	9.23±0.31^{d}	21.2±0.016^{d}	20.2±0.03^{e}	16.7±0.06^{d}
BO3	56.80±1.92 ^{ab}	35.60±1.34^{e}	21.60±1.14^{f}	19.27±0.31 ^{bc}	20.33±0.80^{c}	13.71±0.42^{c}	21.6±0.062^{c}	18.3±0.02^{g}	14.7±0.05^{e}
OG2	57.40±1.51 ^{a}	47.80±1.10^{c}	33.40±1.52^{cd}	19.67±0.59 ^{ab}	16.11±0.57^{f}	7.25±0.32^{d}	20.8±0.029^{e}	19.4±0.02^{f}	13.4±0.06^{f}
OG3	55.20±0.83 ^{bcd}	30.80±0.84^{f}	21.60±1.14^{f}	19.34±0.09 ^{abc}	20.50±0.44^{c}	11.88±1.09^{c}	22.9±0.042^{ab}	19.9±0.02^{e}	15.2±0.06^{e}
OS2	51.60±1.14 ^{f}	48.00±1.22^{c}	31.40±1.14^{de}	19.60±0.51 ^{ab}	17.75±0.51^{e}	17.56±1.39^{b}	23.3±0.015^{a}	22.2±0.03^{c}	21.3±0.05^{c}
OS3	53.00±1.58 ^{ef}	28.60±1.95^{g}	23.20±2.39^{f}	18.80±0.47 ^{c}	20.05±1.03^{cd}	25.97±5.81^{a}	21.7±0.019^{c}	20.8±0.03^{d}	21.0±0.02^{c}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 4. *Alterations in mean corpuscular volume (MCV) in poly-herbal formulations treated rats.*

	MEAN CORPUSCULAR VOLUME (FL)		
	DAY 0	**DAY 16**	**DAY 32**
DH_2O	107 ± 1^{cd}	112 ± 1.10^{c}	109 ± 1.46^{c}
Fanta®	108 ± 3^{cd}	117 ± 2.17^{bc}	122 ± 2.23^{b}
Yoyo bitters™	109 ± 2^{bcd}	105 ± 4.78^{e}	99.2 ± 2.65^{c}
Sabrina™	116 ± 2^{a}	101 ± 5.09^{de}	88 ± 1.66^{d}
BO2	111 ± 2^{bc}	106 ± 5.48^{d}	181 ± 5.60^{a}
BO3	112 ± 4^{b}	89.9 ± 3.01^{f}	107 ± 6.91^{c}
OG2	106 ± 2^{e}	120 ± 5.03^{ab}	185 ± 6.55^{a}
OG3	119 ± 3^{a}	97 ± 2.42^{e}	128 ± 7.90^{b}
OS2	119 ± 3^{a}	125 ± 4.81^{a}	122 ± 6.86^{b}
OS3	115 ± 3^{a}	104 ± 6.70^{d}	84 ± 17.72^{d}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

3.3. Genotoxic Potentials of Osomo Bitters™, Baby Oku™ and Ogidiga™ on Experimental Rats

There was a significant increase in induction of apoptotic DNA fragmentation (Tables 5 to 13) by all the polyherbal formulations compared with the control (DH_2O) group ($p<0.05$). A statistical dose dependent increase in induction of apoptotic DNA fragmentation were observed (2 mL/kg body weight and 3 mL/kg body weight) among rats treated with BabyOku™, Ogidiga™ and OsomoBitters™ polyherbal formulations ($p<0.05$). It was observed that the degree of induction of apoptotic DNA fragmentation by the polyherbal formulations is in the following order: BabyOku™ > Ogidiga™ > OsomoBitters™. There was a significant increase in the degree of induction of apoptotic DNA fragmentation by the polyherbal formulations relative to Fanta®, Yoyo bitters™ or Sabrina™ treated groups ($p<0.05$). A varying degree of percentage DNA fragments were observed for each polyherbal formulation. The trend for BabyOku™ was stomach > ovaries > heart > kidney > spleen > uterus > liver > brain > lungs, Ogidiga™ had stomach > ovaries > heart > kidney > liver > spleen > uterus > brain > lungs while OsomoBitters™ had stomach > ovaries > heart > liver > kidney >spleen > uterus > brain > lungs.

Table 5. *Induction of brain apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	**DAY 8**	**DAY 16**	**DAY 24**	**DAY 32**
DH_20	2.28 ± 0.10	2.24 ± 0.03^{h}	2.25 ± 0.10^{h}	2.26 ± 0.09^{h}	2.24 ± 0.11^{h}
Fanta®	2.29 ± 0.29	5.22 ± 0.13^{g}	5.26 ± 0.24^{g}	5.34 ± 0.20^{g}	5.38 ± 0.26^{g}
Yoyo bitters™	2.39 ± 0.24	27.35 ± 1.04^{f}	29.98 ± 1.14^{f}	31.40 ± 1.19^{f}	33.28 ± 1.26^{f}
Sabrina™	2.39 ± 0.24	27.93 ± 0.48^{f}	30.62 ± 0.54^{f}	32.07 ± 0.56^{f}	33.99 ± 0.60^{f}
BO2	2.36 ± 0.30	54.43 ± 0.75^{b}	59.66 ± 0.83^{b}	62.48 ± 0.87^{b}	66.22 ± 0.92^{b}
BO3	2.37 ± 0.29	61.65 ± 1.17^{a}	67.58 ± 1.29^{a}	70.77 ± 1.35^{a}	75.01 ± 1.43^{a}
OG2	2.29 ± 0.20	46.59 ± 1.05^{d}	51.07 ± 1.15^{d}	53.48 ± 1.21^{d}	56.68 ± 1.28^{d}
OG3	2.38 ± 0.10	49.61 ± 2.20^{c}	54.38 ± 2.24^{c}	56.95 ± 2.52^{c}	60.36 ± 2.67^{c}
OS2	2.24 ± 0.14	35.96 ± 1.14^{e}	39.42 ± 1.25^{e}	41.29 ± 1.31^{e}	43.76 ± 1.39^{e}
OS3	2.34 ± 0.41	37.98 ± 2.29^{e}	41.63 ± 3.24^{e}	43.60 ± 3.40^{e}	46.21 ± 3.60^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 6. *Induction of hepatic apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	**DAY 8**	**DAY 16**	**DAY 24**	**DAY 32**
DH_20	2.18 ± 0.07	2.18 ± 0.08^{h}	2.15 ± 0.05^{h}	2.17 ± 0.05^{h}	2.14 ± 0.07^{h}
Fanta®	2.38 ± 0.37	4.00 ± 0.24^{g}	3.96 ± 0.15^{g}	3.99 ± 0.21^{g}	3.97 ± 0.22^{g}
Yoyo bitters™	2.37 ± 0.10	17.58 ± 0.06^{f}	21.66 ± 0.82^{f}	27.62 ± 1.05^{f}	31.24 ± 1.18^{f}
Sabrina™	2.37 ± 0.31	17.95 ± 0.31^{f}	22.12 ± 0.39^{f}	28.21 ± 0.49^{f}	31.90 ± 0.56^{f}
BO2	2.37 ± 0.30	34.98 ± 0.49^{b}	43.10 ± 0.60^{b}	54.98 ± 0.76^{b}	62.16 ± 0.86^{b}
BO3	2.32 ± 0.12	39.63 ± 0.75^{a}	48.82 ± 0.92^{a}	62.27 ± 1.18^{a}	70.41 ± 1.34^{a}
OG2	2.31 ± 0.34	29.94 ± 0.67^{d}	36.90 ± 0.83^{d}	47.06 ± 1.06^{d}	53.21 ± 1.20^{d}
OG3	2.31 ± 0.12	31.89 ± 1.41^{c}	39.29 ± 1.74^{c}	50.11 ± 2.22^{c}	56.66 ± 2.51^{c}
OS2	2.34 ± 0.34	23.12 ± 0.73^{e}	28.48 ± 0.90^{e}	36.33 ± 1.15^{e}	41.08 ± 1.30^{e}
OS3	2.30 ± 0.11	24.41 ± 1.90^{e}	30.08 ± 2.34^{e}	38.36 ± 2.99^{e}	43.38 ± 3.38^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2

Table 7. *Induction of splenic apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	0.82 ± 0.02^{a}	0.81 ± 0.03^{h}	0.79 ± 0.01^{h}	0.80 ± 0.01^{h}	0.82 ± 0.01^{h}
Fanta®	0.81 ± 0.08^{abc}	1.46 ± 0.09^{g}	1.50 ± 0.10^{g}	1.49 ± 0.11^{g}	1.49 ± 0.10^{g}
Yoyo bitters™	0.87 ± 0.35^{abc}	6.38 ± 0.24^{f}	8.28 ± 0.31^{f}	12.13 ± 0.45^{f}	19.51 ± 0.74^{f}
Sabrina™	0.89 ± 0.19^{abc}	6.52 ± 0.11^{f}	8.45 ± 0.14^{f}	12.38 ± 0.22^{f}	19.92 ± 0.35^{f}
BO2	0.75 ± 0.16^{ab}	12.69 ± 0.17^{b}	16.47 ± 0.23^{b}	24.13 ± 0.33^{b}	38.81 ± 0.54^{b}
BO3	0.85 ± 0.03^{bc}	14.38 ± 0.27^{a}	18.66 ± 0.35^{a}	27.33 ± 0.52^{a}	43.96 ± 0.83^{a}
OG2	0.82 ± 0.30^{abc}	10.87 ± 0.24^{d}	14.10 ± 0.31^{d}	20.65 ± 0.46^{d}	33.22 ± 0.75^{d}
OG3	0.88 ± 0.10^{a}	11.57 ± 0.51^{c}	15.01 ± 0.66^{c}	21.99 ± 0.97^{c}	35.38 ± 1.57^{c}
OS2	0.84 ± 0.13^{abc}	8.39 ± 0.27^{e}	10.88 ± 0.34^{e}	15.94 ± 0.51^{e}	25.65 ± 0.81^{e}
OS3	0.83 ± 0.07^{abc}	8.86 ± 0.69^{e}	11.49 ± 0.89^{e}	16.84 ± 1.13^{e}	27.09 ± 2.11^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 8. *Induction of stomach apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	1.54 ± 0.05	1.55 ± 0.05^{h}	1.53 ± 0.07^{h}	1.53 ± 0.07^{h}	1.54 ± 0.04^{h}
Fanta®	1.51 ± 0.43	2.84 ± 0.15^{g}	2.84 ± 0.15^{g}	2.84 ± 1.23^{g}	2.85 ± 0.14^{g}
Yoyo bitters™	1.81 ± 0.17	13.81 ± 0.52^{f}	30.45 ± 1.15^{f}	32.34 ± 0.58^{f}	35.79 ± 1.36^{f}
Sabrina™	1.38 ± 0.19	14.10 ± 0.24^{f}	31.10 ± 0.55^{f}	33.02 ± 0.90^{f}	36.55 ± 0.64^{f}
BO2	1.51 ± 0.35	27.49 ± 0.38^{b}	60.60 ± 0.84^{b}	64.35 ± 0.09^{b}	71.22 ± 0.99^{b}
BO3	1.45 ± 0.12	31.13 ± 0.59^{a}	68.64 ± 1.30^{a}	72.89 ± 1.39^{a}	80.67 ± 1.54^{a}
OG2	1.31 ± 0.26	23.53 ± 0.53^{d}	51.87 ± 1.17^{d}	55.08 ± 1.24^{d}	60.96 ± 1.37^{d}
OG3	1.55 ± 0.26	25.05 ± 1.11^{c}	55.23 ± 2.45^{c}	58.65 ± 2.60^{c}	64.92 ± 2.88^{c}
OS2	1.32 ± 0.31	18.16 ± 0.57^{e}	40.04 ± 1.27^{e}	42.52 ± 1.35^{e}	47.06 ± 1.49^{e}
OS3	1.14 ± 0.09	19.18 ± 1.49^{e}	42.28 ± 3.30^{e}	44.91 ± 3.50^{e}	49.70 ± 3.87^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 9. *Induction of lungs apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	1.29 ± 0.04	1.29 ± 0.04^{h}	1.30 ± 0.04^{h}	1.30 ± 0.02^{h}	1.29 ± 0.05^{h}
Fanta®	1.37 ± 0.13	2.34 ± 0.12^{g}	2.35 ± 0.14^{g}	2.37 ± 0.11^{g}	2.36 ± 0.14^{g}
Yoyo bitters™	1.31 ± 0.35	10.39 ± 0.39^{f}	11.20 ± 0.42^{f}	14.56 ± 0.55^{f}	15.46 ± 0.58^{f}
Sabrina™	1.51 ± 0.16	10.61 ± 0.19^{f}	11.44 ± 0.19^{f}	14.87 ± 0.26^{f}	15.79 ± 0.27^{f}
BO2	1.53 ± 0.25	20.68 ± 0.28^{b}	22.30 ± 0.31^{b}	28.98 ± 0.40^{b}	30.77 ± 0.43^{b}
BO3	1.32 ± 0.18	23.42 ± 0.44^{a}	25.25 ± 0.48^{a}	32.83 ± 0.62^{a}	34.85 ± 0.66^{a}
OG2	1.53 ± 0.34	17.71 ± 0.40^{d}	19.08 ± 0.43^{d}	24.81 ± 0.56^{d}	26.33 ± 0.60^{d}
OG3	1.56 ± 0.17	18.85 ± 0.83^{c}	20.32 ± 0.90^{c}	26.42 ± 1.17^{c}	28.04 ± 1.24^{c}
OS2	1.58 ± 0.29	13.67 ± 0.43^{e}	14.73 ± 0.46^{e}	19.15 ± 0.60^{e}	20.33 ± 0.64^{e}
OS3	1.60 ± 0.16	14.43 ± 1.12^{e}	15.56 ± 1.21^{e}	20.23 ± 1.57^{e}	21.47 ± 1.67^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 10. *Induction of cardiac apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	0.72 ± 0.25	0.70 ± 0.02^{h}	0.71 ± 0.02^{h}	0.73 ± 0.06^{h}	0.72 ± 0.03^{h}
Fanta®	0.74 ± 0.07	1.39 ± 0.08^{g}	1.33 ± 0.04^{g}	1.37 ± 0.04^{g}	1.34 ± 0.08^{g}
Yoyo bitters™	0.77 ± 0.20	9.90 ± 0.38^{f}	19.62 ± 0.76^{f}	19.72 ± 0.74^{f}	24.37 ± 0.92^{f}
Sabrina™	0.77 ± 0.09	10.12 ± 0.18^{f}	20.03 ± 0.35^{f}	20.13 ± 0.35^{f}	24.89 ± 0.43^{f}
BO2	0.78 ± 0.14	19.71 ± 0.27^{b}	39.04 ± 0.54^{b}	39.24 ± 0.54^{b}	48.51 ± 0.67^{b}
BO3	0.78 ± 0.18	22.33 ± 0.42^{a}	44.22 ± 0.84^{a}	44.45 ± 0.84^{a}	54.94 ± 1.05^{a}
OG2	0.82 ± 0.14	16.87 ± 0.38^{d}	33.41 ± 0.75^{d}	33.59 ± 0.76^{d}	41.52 ± 0.94^{d}
OG3	0.71 ± 0.02	17.97 ± 0.79^{c}	35.58 ± 1.58^{c}	35.76 ± 1.58^{c}	44.21 ± 1.96^{c}
OS2	0.75 ± 0.16	13.02 ± 0.41^{e}	25.79 ± 0.81^{e}	25.93 ± 0.82^{e}	32.05 ± 1.06^{e}
OS3	0.75 ± 0.03	13.76 ± 1.07^{e}	27.24 ± 2.12^{e}	27.38 ± 2.13^{e}	33.85 ± 2.63^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 11. *Induction of ovarian apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	1.06±0.03	1.06±0.04 ^{h}	1.05±0.03 ^{h}	1.05±0.06 ^{h}	1.04±0.06 ^{h}
Fanta®	1.93±0.10	1.96±0.09 ^{g}	1.97±0.10 ^{g}	1.99±0.08 ^{g}	1.95±0.10 ^{g}
Yoyo bitters™	1.35±0.29	18.21±0.69 ^{f}	29.66±1.26 ^{f}	34.22±1.30 ^{f}	38.67±1.47 ^{f}
Sabrina™	1.85±0.13	18.59±0.32 ^{f}	30.30±0.53 ^{f}	34.94±0.61 ^{f}	39.44±0.69 ^{f}
BO2	1.30±0.21	36.23±0.50 ^{b}	59.04±0.82 ^{b}	68.10±0.95 ^{b}	76.84±1.07 ^{b}
BO3	1.66±0.24	41.03±0.78 ^{a}	66.87±1.27 ^{a}	77.13±1.47 ^{a}	87.04±1.66 ^{a}
OG2	1.43±0.33	31.01±0.70 ^{d}	50.53±1.14 ^{d}	58.28±1.32 ^{d}	65.77±1.48 ^{d}
OG3	1.61±0.05	33.02±1.46 ^{c}	53.81±2.38 ^{c}	62.06±2.75 ^{c}	70.04±3.10 ^{c}
OS2	1.44±0.32	23.94±0.76 ^{e}	39.01±1.23 ^{e}	45.00±1.43 ^{e}	50.77±1.51 ^{e}
OS3	1.34±0.33	25.28±1.97 ^{e}	41.19±3.21 ^{e}	47.52±3.70 ^{e}	53.62±4.18 ^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 12. *Induction of renal apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	1.87±0.06	1.88±0.05^{h}	1.88±0.04^{h}	1.87±0.06^{h}	1.88±0.06^{h}
Fanta®	1.45±0.19	3.43±0.17^{g}	3.44±0.16^{g}	3.46±0.17^{g}	3.43±0.18^{g}
Yoyo bitters™	1.16±0.11	20.56±0.77^{f}	24.80±0.94^{f}	29.20±1.10^{f}	33.91±1.29^{f}
Sabrina™	1.78±0.24	21.00±0.37^{f}	25.33±0.44^{f}	29.82±0.52^{f}	34.63±0.61^{f}
BO2	1.53±0.30	40.92±0.57^{b}	49.35±0.69^{b}	58.10±0.81^{b}	67.47±0.94^{b}
BO3	1.76±0.07	46.35±0.88^{a}	55.90±1.06^{a}	65.81±1.25^{a}	76.42±1.45^{a}
OG2	1.32±0.25	35.02±0.79^{d}	42.24±0.95^{d}	49.73±1.12^{d}	57.75±1.31^{d}
OG3	1.81±0.09	37.30±1.65^{c}	44.98±1.99^{c}	52.95±2.24^{c}	61.49±2.72^{c}
OS2	1.42±0.32	27.04±0.85^{e}	32.61±1.03^{e}	38.39±1.22^{e}	44.58±1.41^{e}
OS3	1.41±0.31	28.55±2.22^{e}	34.44±2.26^{e}	40.54±3.16^{e}	47.08±3.67^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

Table 13. *Induction of uterine apoptotic DNA fragmentation by BabyOku™, Ogidiga™ and OsomoBitters™ in experimental rats.*

	DAY 0	DAY 8	DAY 16	DAY 24	DAY 32
DH_20	1.59±0.05	1.58±0.04^{h}	1.58±0.04^{h}	1.59±0.04^{h}	1.59±0.02^{h}
Fanta®	1.59±0.42	2.88±0.12^{g}	2.83±0.10^{g}	2.85±0.10^{g}	2.91±0.18^{g}
Yoyo bitters™	1.45±0.43	18.84±0.71^{f}	24.02±0.91^{f}	24.02±0.91^{f}	28.41±1.07^{f}
Sabrina™	1.70±0.20	19.24±0.33^{f}	24.53±0.43^{f}	24.53±0.43^{f}	29.01±0.50^{f}
BO2	1.47±0.33	37.48±0.52^{b}	47.79±0.67^{b}	47.79±1.67^{b}	56.54±0.79^{a}
BO3	1.16±0.11	42.46±0.81^{a}	54.13±1.03^{a}	54.13±1.03^{a}	64.04±1.22^{a}
OG2	1.52±0.44	32.08±0.72^{d}	40.90±0.92^{d}	40.90±0.92^{d}	48.39±1.09^{d}
OG3	1.45±0.34	34.17±1.51^{c}	43.56±1.93^{c}	43.56±1.93^{c}	51.53±2.28^{c}
OS2	1.40±0.26	24.77±0.79^{e}	31.58±1.00^{e}	31.58±1.00^{e}	37.36±1.19^{e}
OS3	1.58±0.11	26.16±2.03^{e}	33.35±2.60^{e}	33.36±2.60^{e}	39.46±3.07^{e}

Values are expressed as mean ± standard deviation (n=5); values bearing different alphabets are significantly different while those with similar alphabets are insignificantly different ($p< 0.05$). BO2= 2 mL/kg body weight of BabyOku™, BO3= 3 mL/kg body weight of BabyOku™, OG2= 2 mL/kg body weight of Ogidiga™, OG3= 3 mL/kg body weight of Ogidiga™, OS2= 2 mL/kg body weight of OsomoBitters™, OS3= 3 mL/kg body weight of OsomoBitters™

4. Discussion

Medicinal plants have been widely used by both ancient and modern man of all cultures for treating different ailments. A single plant processed in different formulations can be used to cure a wide range of diseases [30]. However, the historic role of medicinal herbs in the treatment and prevention of diseases and in the development of pharmacology do not assume their safety for uncontrolled use by an uninformed public [31, 32]. The use of herbal materials in alternative medicine plays important roles in primary health care for most African countries, mainly due to their culture and beliefs. Despite the profound therapeutic advantages presented by many of these medicinal plants, some still exhibit some systemic toxicity, genotoxicity and carcinogenicity potentials [17, 33-35]. Therefore, there is the need for more information on the toxicological profile of many of the herbal supplements used in the complementary and alternative medicine in Nigeria and most other countries of the world [19].

This study presents the hematological alterations, clastogenic and genotoxic effects induced by the three commonly consumed polyherbal formulations (PHFs) (OsomoBitters™, Ogidiga™ and BabyOku™) in Abeokuta, southwest Nigeria on experimental rats. Clinical signs of toxicity observed mainly due to the administration of polyherbal formulations in rats are systemic toxicity.

Hematological testing in rodents during toxicity and safety evaluation is generally acknowledged as integral part of systemic toxicity assessment [36]. Significant decrease in packed cell volume; hemoglobin, white blood cells, lymphocytes, neutrophils and platelet counts in OsomoBitters™, Ogidiga™ and BabyOku™ exposed rats showed that the polyherbal formulations induced marked hematotoxic effects in the rodents. Alterations in hematological biomarkers suggest that the component phytochemicals of the various polyherbal formulations affected hematopoiesis in the bone marrow system of the PHFs exposed rats [33, 34, 37].

Anemia is a reduction in the number of erythrocytes, hemoglobin, or both, in the circulating blood. It resulted from excessive red blood cell (RBC) destruction, RBC loss, or decreased RBC production and is a manifestation of an underlying disease process. Therefore, the response to treatment of anemia is transient unless the underlying disease process is addressed [38]. Although it was stated that toxic plants do not produce a direct effect on white blood cells, such as neutrophils, lymphocytes, eosinophils, and monocytes [39], the results of this study showed otherwise. Excessive consumption of a wide variety of plants or their products has been found to cause hypo-proliferative or non-regenerative anemia, which is a stem cell disorder characterized by reduced bone marrow production of all blood components in the absence of a primary disease process infiltrating the bone marrow or suppressing hematopoiesis [40]. It shows that continuous consumption of these formulations may produce these effects in animals. It may also mean that the principal function of white blood cells, which is to defend against invading organisms, will be compromised [38, 39, 41].

The polyherbal formulations induced clastogenicity on bone marrow cells of rats. Micronuclei (MN) are formed in addition to the main nucleus in cells as a result of acentric fragments or lagging chromosomes that failed to be incorporated into either of the daughter nuclei during M phase of the cell cycle [42]. Micronuclei test is routinely used as genetic marker of exposure to clastogens and anuegens, and increased chromosome instability. Significant micro-nucleated polychromatic erythrocyte induction in the bone marrow of rats exposed to 2 mL/kg body weight and 3 mL/kg body weight suggests clastogenic potentials. Hence the possibility of the presence of clastogens and/or aneugens in the tested formulations. These chemicals are capable of increasing somatic mutations in mammalian systems which may predispose cells to chromosome-related disorders and carcinogenesis. This further corroborated hematological findings to suggest that polyherbal formulations increased hematopoietic disturbance in the mouse bone marrow system. Significant increase in percentage chromosome aberrations in the bone marrow of the rats is attributed to cell division disturbances caused by clastogens present in the formulations [19]. The findings herein are in agreement with other studies that proved that high concentrations of inherent phytochemicals in polyherbal formulations elicited chromosome abnormalities in animal test systems [43-46]. The use of herbal supplements and medicinal herbs is not strictly regulated in Nigeria and many other countries. Numerous genotoxic and carcinogenic compounds; alkenylbenzenesestragole, methyleugenol, safrole, β-asarone, unsaturated pyrrolizidine, aromatic steroids, cytochalasin, concentricolide, are increasingly being reported in many of these medicinal botanicals [15-17]. In addition, many of these herbal supplements and medicinal herbs contain high concentrations of numerous hazardous metals that are of public health concern [7, 47-49]. These metals possibly originated during the cultivation of these plants on contaminated soils and the unhygienic and/or illegal processing and packaging of the herbal drugs [50].

Ene-Obong [51] reported a consensus that many of the components of medicinal plants bind onto tubulin, inhibit tubulin assembly or cause the depolymerization of already assembled microtubules. The spindle so disturbed is reduced and may eventually disappear, resulting in the blockage of cell division at prophase or even metaphase. He also speculated that there may be a possible amplification of anti-spindular effects where two or more anti-spindle compounds may be present in the same medicinal plant since different binding sites in tubulin may exist for different anti-spindle compounds. Binucleate cell formation is accepted to be due to inhibition of cytokinesis [52]. Iwalokun et al. [53] in their study reported that Majewska et al. [54] attributed such inhibition to phlamogram inhibition at the early stage of telophase. The presence of nuclear lesions and nuclear dissolution in cells [55] offer cytological evidence for the inhibitory action of chemicals on DNA biosynthesis. The DNA lesions are classified into DNA adducts, DNA intra-strand crosslinks, DNA inter- strand crosslinks and DNA-protein crosslinks [56]. Inhibition of DNA synthesis [51] could occur in two ways: either directly by affecting the incorporation of precursors into DNA or through an influence on the biosynthesis of DNA precursors. The inhibition results in the total failure of mitosis. According to Evandri et al. [57], chromosome bridges and fragments are signs of clastogenic effects resulting from chromosome and chromatid breaks. The formation of bridges has been attributed to several causes which include: breaks that may occur in both chromatids of the same chromosome and incorrect rejoining of the sticky ends to form a sister union [58, 59], incomplete replication of chromosomes by defective or less active replication enzymes [60] or through breaks that may occur late in the cell cycle (in G2) after the chromosomes have replicated [59] or late replicating DNA sequences of the telomeric heterochromatin [61]. Kaltsikes [62] according to Yildiz and Evrim [63] reported that chromosome bridges could occur if heterochromatic DNA sequences do not complete DNA replication when the nucleus is ready to divide. It was discovered from the apoptotic DNA fragmentation results that the polyherbal formulations: Baby Oku™, Ogidiga™ and OsomoBitters™ are potential inducers of genotoxicity in the tissue samples (brain, kidney, liver, lungs, spleen, heart, ovary, stomach and

uterus) of the experimental rats; this might have resulted from the active components of the formulations as well as improper processing or unhygienic methods adopted in the course of processing. Chandra and Khuda-Bukhsh [64] and Klopman et al. [65] suggested that during the metabolism of *Azadirachta* (a medicinal plant), electrophilic ions and radicals are produced and that these interact with the nucleophilic sites in DNA, leading to breaks and other related damage in DNA. Hall and Garcia [59] also noted that anaphase bridge is one of the three types of aberrations that are lethal to the cell, the other two being dicentric and the ring chromosomes. Bridges cause structural chromosome mutations (duplications or deletions in DNA double-strand) [57, 66]. Since one of the pathways leading to apoptosis involves DNA degradation, it is worth stating with emphasis that polyherbal formulations might trigger apoptosis by damaging genetic material.

5. Conclusions

We therefore concluded that Ogidiga™, OsomoBitters™ and BabyOku™, the commonly consumed polyherbal formulations in Abeokuta, South-Western Nigeria has strong potential to induce genotoxicity, hematotoxicity and clastogenicity in experimental rats as evident from increased apoptotic DNA fragmentation, induction of micro-nucleated polychromatic erythrocytes (mPCEs) and reduction in hematological biomarkers.

Conflict of Interest

They authors have no conflict of interest to declare.

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