IN VIVO CYTOGENOTOXIC AND HAEMATOTOXIC SCREENING OF A TRI-HERBAL PILL PRODUCED FOR THE TREATMENT OF HEMORRHOIDS AMONG NIGERIANS IN *ALLIUMCEPA* AND *MUS MUSCULUS*

Alimba, C. G.[1*], Adeyemo, O. A.[2], Uzoma, I. U.[2], Bamigboye, T. V.[2]

[1]Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Nigeria.

[2]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria.

*E-mail: cg.alimba@ui.edu.ng /chivoptera@yahoo.com

(Received: 13th August, 2015; Accepted: 2nd November, 2015)

ABSTRACT

There is unprecedented increase in the use of medicinal plants as source of therapeutics for human sustenance due to their efficacies. The assumption that these plants are very safe had increased their indiscriminate consumption, which can lead to human exposure to some harmful phytochemicals and possible contaminants. The cytogenotoxic and hematotoxic effects of a tri-herbal pill claimed to cure hemorrhoids and backache among Nigerians were investigated in *Allium cepa* (onion plant) and *Mus musculus* (mice). *Allium cepa* was treated with 800, 1200, 1600, 2500 and 0 mg/L (control; tap water) while *Mus musculus* with 600, 900, 1200, 1500 mg/L of the tri-herbal pill and distilled water (negative) and cyclophosphamide (20 mg/kg bwt; positive) served as controls. Mice were examined for signs of toxicity, alterations in hematological indices and frequency of bone marrow micronucleated polychromatic erythrocytes (MNPCE), while *Allium cepa* for root length inhibition, mitotic index and chromosome aberrations. Diarrhea, weakness and sluggishness in movement were common clinical signs observed in mice exposed to 1500 mg/L of the pill. There was significant ($p<0.05$) increase in erythrocytes, haemoglobin, haematocrits, leucocytes, mean corpuscular volume and mean corpuscular haemoglobin, but insignificant ($p>0.05$) decrease in mean corpuscular haemoglobin concentration in the treated mice compared to the control (distilled water). Tri-herbal pills significantly increased frequency of MNPCE by 2.13 to 6.45 folds in the treated mice compared to the control. Also significant decrease in root growth and mitotic index, and increase percentage chromosome aberrations was observed in the *A. cepa* compared to the control (tap water). The tri-herbal pill induced cytotoxicity and DNA damage in *A. cepa* and mice, and altered haematological parameters in mice. This suggests public health issues in case of indiscriminate consumption of such supplementary.

Keywords: *Allium* Test, Cytogenotoxicity, Haematology, Herbal Drugs, Mice, Micronucleus Test.

INTRODUCTION

Many countries in Africa, Asia, America and Europe depend on plant materials for their nutrition and medicinal folklore from antiquity(Farnsworth *et al*., 1985). The discovery of penicillin (group of antibiotics) from *Penicillium* fungus and plethora of other drugs from these natural products(Harvey, 2008),shifted the interest from these natural products to synthetic drug analogs. In recent times, the use of plant materials in medicinal folklore is increasing due mainly to the significant toxic effects from the synthetic drugs (Patwardhan and Vaidya, 2010; Begriche *et al*., 2011), with about 80% of the world populations relying on medicinal herbs for their primary health care (Ouedraogo *et al*., 2012).Herbal materials are readily available and easy to acquire at low cost. Moreover, most people believe they possess high curative properties without elicitingany toxic effect (Egharevba and Ikhatua, 2008). This in part may be responsible for the unprecedented increase in the processing and packaging of many of the plant materials into herbal supplements used in complementary and alternative medicine for the treatment of various ailments. Considering that plants contain numerous chemicals, the World Health Organization (WHO) recommended that medicinal herbs be evaluated for evidence of toxicity to ascertain their safety before consumption (WHO, 2003). Despite this requirement from WHO, majority of these plants are yet to be screened for their possible toxicological effects and are consumed indiscriminately. This attitude is capable of increasing human exposure to harmful phytochemicals and possible contaminants the consumption of such plants.

It is a common practice in Nigeria for mixture of herbal materials to be macerated and boiled or

tinctured using organic solvents into decoctions for the treatment of various diseases. Also some of these herbs are reprocessed into pills that are packaged using polyethene materials and sold as herbal drugs. One among such preparations is a tri-herbal decoction branded as "Ogun Jedi Jedi" common among the Southwestern Nigerians. "Ogun Jedi Jedi" which is translated to mean "drug against hemorrhoid" contains *Manihot esculenta*, *Cassia podocarpa*, *Croton penduliflorus* and mucilage. It is believed to be effective in the treatment of hemorrhoids. The producer also acclaimed it is effective against dysentery, chronic gonorrhea, female menstrual pain and male genital weakness with outside effects. This suggests that the tri-herbal pill is a multi-purpose therapeutic drug. Components of the tri-herbal pill possess some ethno-pharmaceutical properties. For instance *M. esculenta* Crantz is rich in phytochemicals and antioxidants responsible for its anti-hemorrhoid and anti-inflammatory properties (Afolabi *et al*., 2008), but it also contains high concentration of cyanogenic glycosides which are harmful to humans and other mammals (Hidayat *et al*., 2002; Ojo *et al*., 2013).*Cassia podocarpa* and *Croton penduliflorus* possess laxative properties that can be effective against headache when rubbed on forehead (Olusola *et al*., 2011). Mucilage possesses anti-inflammatory and antioxidant properties and is usually added to the herbal preparation to enhance its efficiency (Ameri *et al*., 2015). Okpuzor and Oloyede (2009) used carrageenan-induced oedema, 2,4-Dinitrophenol-induced pyrexia and castor oil-induced diarrhea in rats and mice to confirm that the tri-herbal pill possess anti-inflammatory, anti-pyretic, anti-diarrhoeal properties but is not devoid of some toxic effects. However, easy accessibility to "Ogun Jedi Jedi" from hawkers around homes, market and public places, including at motor parks, coupled with its relatively low cost compared to orthodox drugs may increase its indiscriminate consumption.

There are increasing reports that several plants contain toxic, genotoxic and carcinogenic compounds (Qin *et al*., 2006; Rietjens *et al*., 2008; van den Berg *et al*., 2011). For instance Qin *et al*. (2006) isolated concentricolide (a benzofuranlactone), aromatic steroids and cytochalasin (spindle inhibitor) from a species of mushroom and suggested that these phytochemicals may possess anti-HIV properties. However, it is also known that cytochalasin, a phytochemical in the mushroom, is a spindle inhibitor that can increase DNA damage in biological systems. There is need to screen the tri-herbal pill for its toxicological potentials in both plant and animal models. Haematological indices are considered pathophysiological parameters of the total vertebrate body. Its analysis in mammal systems is useful in diagnosing changes in body physiology and pathology during disease conditions (Evans, 2008). Chromosome aberrations in plants and animals are hallmarks of genome instability which may lead to genetic related diseases and congenital abnormalities (Fenech *et al*., 2011).This study aims at screening the tri-herbal pill "Ogun Jedi Jedi" for its cytogenotoxicity using chromosome aberrations in *Allium cepa* and murine bone marrow micronucleus test in mice and haematotoxic potentials using alterations in haemogram of mice.

MATERIALS AND METHODS

Tri-herbal Pill Preparations

Tri-herbal pills were acquired from hawkers around Iwo Road Motor Park in Oyo State, Nigeria. Each sachet contains four brownish pills wrapped together in nylon with each pill weighing 0.5g. Following the drug prescriptions, the four pills are expected to be dissolved in warm water and orally swallowed. The compositions of the tri-herbal pill as described on the producer's list is 30 % *Croton penduliflorus*, 25 % *Cassia podocarpa*, 15 % *Manihot esculenta*, 20 % Potash and 10 % mucilage. The pills were dissolved in water at 40º,C and stored in amber bottle until used for the experiment within 24 h.

Animals and Experimental Design

Male mice (mean ± SD weight; 21.53 ± 2.39g) were acquired and acclimated to laboratory conditions of 26 ±1 ºC and 12/12 h dark/light mode for 14 days prior to the animal exposure in the animal colony, Department of Cell Biology and Genetics, University of Lagos, Nigeria. They were randomly selected into 6 groups (5 mice per group); 0 (distilled water; vehicular solvent) as negative control,600, 900,1200 and 1500mg/kg

body weight according to Okpuzor and Oloyede (2009). 20 mg/L cyclophosphamide (Endoxan™ Mfg Lic. No.186. Frankfurt am Main, Germany) was used as positive control. The animals were fed standard rodent chow (Ladokun Feed Nigeria®) and drinking water *ad libitum*. All mice were orally administered 0.5 ml of the tri-herbal pill solution, distilled water and cyclophosphamide for 14 consecutive days. Mice in each group were monitored before and after each treatment for signs of clinical toxicity. These signs include appearance of their skin and fur, eyes and mucous membrane, behavioral pattern, morbidity and mortality. *Guide for Care and Use of Laboratory Animals* published by US National Institutes of Health (NIH Publication No. 85-23, revised in 1996) was carefully adhered to.

Micronucleus and Haematological Analysis

At post treatment, mice were fasted overnight and blood was collected from the retro-orbital plexus using heparinized 70 ml micro-haematocrit capillary tubes into Ethylene Diamine Tetraacetic Acid (EDTA) coated bottles. Blood was immediately analyzed for full blood counts using automated analyzer (Abbott Hematology Analyzer Cell-Dyn 1700, Abbott Laboratories, Abbott Park, Illinois, USA). Mice were sacrificed by cervical dislocation and femoral bones surgically excised and bone marrow cells aspirated into Eppendorf tubes using Foetal Bovine Serum (Sigma Aldrich Cheme GmbH, Germany).The cells were centrifuged at 2000 g for 5 min and supernatant decanted to collect the residue for micronucleus preparations. Three slides per animal were smeared with the bone marrow cells, air-dried, fixed in absolute methanol for 30 min and counter-stained with May-Grunwald and Giemsa stains (Schmid, 1975). 2000 cells per mouse were scored for micronuclei polychromatic erythrocytes (MNPCE) (index of genotoxicity) at x100 objective.

***Allium cepa* Root Growth Inhibition and Cytological Analysis**

Equal-sized onion bulbs (*Allium cepa*; 2n = 16) were acquired and air-dried for 14 days. The outer dry, brown scales and the bottom plates (dead roots) were carefully removed leaving the rings of the primordial roots intact. Twelve onion bulbs were used for each of the selected concentrations of the tri-herbal pill; 800, 1200, 1600, 2500 mg/L and tap water (control). The onions were placed directly on the solutions prepared from the various concentrations of the tri-herbal pill in 50 ml beakers at room temperature in the dark. Similar treatment was used to grow bulbs in tap water (control). The tri-herbal solution was replaced daily to ensure continuous exposure of the bulbs. The root lengths of ten bulbs were measured daily for eight (8) days using measuring rule. The values were used to determine the percentile root growth restriction in relation to the negative control to determine the EC_{50} and root growth inhibition (cytotoxicity indices) (Fiskesjo, 1990; Alimba *et al*., 2013).Five onion bulbs with good root growth were selected from each treatment and 0.5–1 cm of the root tip cut and fixed in ethanol: glacial acetic acid (3:1, v/v) for 24 h. The fixed roots were hydrolyzed with 1N HCl at 60°C for 5 min, rinsed in distilled water, squashed on micro-glass slides and stained with aceto-carmine for 10 min. Cover slip was carefully lowered onto each slide to exclude air bubbles and sealed on the sides with finger nail polish. Five slides per onion were prepared for each concentration and 1000 cells/slide were scored microscopically at 1000X magnification, for mitotic index analysis (cytotoxicity) and induction of chromosome aberrations (genotoxicity). The occurrence and frequency of aberrant cells were examined in all the stages of cell division and percentage aberrations were determined relative to the total number of dividing cells. The mitotic index (MI) was determined by counting the number of dividing cells per treatment and the control.

Statistical Analysis

Statistical analysis was conducted with Graphpad prism 5.0® computer program. The percentage root growth, frequency of chromosomal aberrations and mitotic index in the treated onions were compared with the control. Alsomean of MNPCE and haematological parameters in mice were analysed using One-way Analysis of Variance (ANOVA) for significance at $p < 0.05$.Comparison between treated groups and negative control was determined using Dunnett multiple post-hoc test at $p<0.05$.

RESULTS

Clinical Signs of Toxicity and Mortality in Tri-herbal Pill Treated Mice

During the exposure period, there was no recorded mortality in all the treated concentrations of the tri-herbal pills. However diarrhea, weakness and sluggishness in movement and inability to finish the provided feed were the common clinical signs observed mainly in the 1500 mg/L treated mice.

Bone Marrow Micronucleus Formationin Tri-herbal Pill Treated Mice

Figure 1 presents the genotoxic effects of the tri-herbal pill on mouse bone marrow cells. There was significant ($p < 0.001$) increased in the induction of MNPCE frequency in the treated mice. The treatment groups: 600, 900, 1200 and 1500 mg/L were higher than the negative control by 2.13, 1.33, 2.91 and 6.45-folds respectively however only 1200 and 1500 mg/L were significantly different from the negative control. MNPCE induction correlated positively (r=0.97) with increase in concentration of the pill solutions.

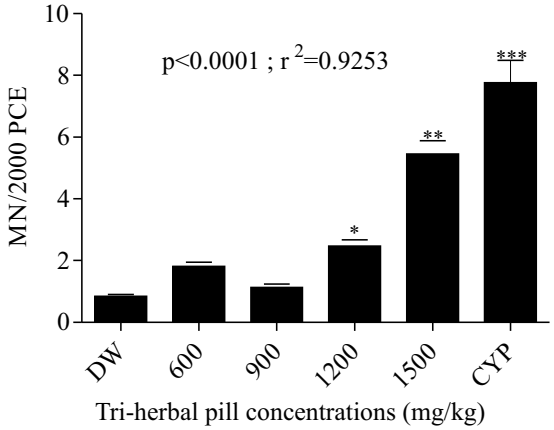

Figure 1. Mean± SE of micronucleated polychromatic erythrocytes in bone marrow of mice treated with various concentrations of the tri-herbal pill ($^{*}p < 0.05$; $^{**}p > 0.01$; $^{***}p > 0.001$).

Alterations in Haematological Parameters of the Tri-herbal Pill Treated Mice

Table 1 presents the effects of tri-herbal pill treatment on the haematological indices in mice. Tri-herbal pill significantly ($p<0.05$) increase red blood cell (RBC), haematocrit (HCT), haemoglobin (HGB),white blood cell (WBC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH),but decrease mean corpuscular haemoglobin concentration (MCHC) in the treated mice. Although only 1500 mg/L treated group showed significant ($p< 0.05$) difference from the negative control group (Dunnette multiple posthoc test).

Table 1. Effects of tri-herbal pill treatment on hematological parameter of mice

Conc (mg/kg)	RBC (^{6}µL)	HCT (%)	HGB (g/dL)	MCV (fl)	MCH (pg)	MCHC (gldL)	WBC (x 10^3µL)
H_2O	6.45±0.29	34.53±2.10	10.60±1.10	53.47±1.85	16.37±1.12	30.53±1.47	8.30±4.46
600	7.32±0.26	36.79±2.14	10.40±2.14	52.17±1.99	15.88±0.50	30.37±0.59	8.60±1.21
900	6.84±0.49	38.27±2.64	11.60±0.67	54.10±2.29	15.73±0.26	29.10±1.03	7.88±2.77
1200	7.60±0.28	37.23±0.88	10.77±0.88	55.40±3.47	16.60±1.53	30.00±1.96	8.40±0.87
1500	9.31±1.43*	44.33±4.21*	14.20±2.73*	60.09±3.17*	19.43±2.74*	25.01±1.03*	10.76±0.92*
CYP	8.17±2.11*	41.79±2.67*	14.58±1.25*	59.62±2.63*	19.10±1.02*	27.86±0.99*	10.80±1.19*
p value	(p=0.0501)	(p=0.0291)	(p=0.0137)	(p=0.0213)	(p=0.0516)	(p=0.3495)	(p=0.0448)

End points represent mean ± SD for 5 mice. RBC (Red blood cell); HGB (Hemoglobin); HCT (Hematocrit); WBC (White blood cell); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration). H_2O (Distilled water; negative control), CYP (Cyclophosphamide; 20 mg/kg; positive control). Superscripts differ significantly (*$p<0.05$) from corresponding control using Dunnett's multiple post hoc test, H_2O = Tap water.

Daily Root Growth Inhibition in the Tri-herbal Pill Treated *Allium cepa*

Table 2 presents the daily root growth inhibition in *A. cepa* grown in tap water (control) and the various concentrations of the tri-herbal pill. The root growth of onions cultivated in the various concentrations of the tri-herbal pill on days 1 and 2 was insignificantly ($p>0.05$) different from the tap water (control), with root growth in 800 and 2500 mg/L higher than the tap water. At days 3 and 4 of the root growth, the control had significantly ($p = 0.0301$ and 0.0024 respectively)longer root length than all the treated groups. After wards root growth was inhibited in some concentrations and was highly significantly ($p<0.001$) different from the control. The effective concentration of 35% ($EC_{50} = 35\%$) was obtained for the tri-herbal pill solution. The tri-herbal pill induced concentration-dependent significant ($p < 0.05$) decrease in mitotic index in the treated *A. cepa* compared to the control (Table 3). Also different types and frequencies of chromosome aberrations were induced by the tri-herbal pill (Table 3). There was significant ($p < 0.05$) increase in the percentage chromosome aberrations in the treated onions compared to the control. Figure 2a-d presents the various stages {(a) prophase, (b) metaphase, (c) anaphase and (d) telophase} of the dividing onion roots cells in the control. Sticky (Figure 2e), multipolar (Figure 2g) and vagrant (Figure 2i) chromosomes were the most common chromosome aberrations observed in the treated groups. Other chromosome abnormalities are c-mitosis (Figure 2f), fragmented and Laggard chromosomes (Figure2h).

Table 2. Effects of tri-herbal pill treatment on daily root growth of *Allium cepa*.

Conc (mg/L) /Days	Control	800	1200	1600	2500	p values
1	0.67±0.15	0.80±0.26	0.57±0.09	0.67±0.21	0.77±0.25	p=0.6515
2	2.43±0.12	2.53±0.15	2.00±0.12[a]	2.43±0.08	2.53±0.18	p=0.1000
3	4.90±0.17	3.83±0.14[a]	4.13±0.09	3.87±0.24[a]	3.17±0.15[a]	p=0.0301
4	6.40±1.00	4.50±0.16[a]	4.47±0.06[a]	4.47±0.15[a]	3.83±0.18[b]	p=0.0024
5	7.40±0.13	4.53±0.15[b]	4.47±0.06[b]	4.47±0.23[b]	4.17±0.11[b]	p<0.0001
6	7.73±0.24	4.80±0.20[b]	4.47±0.32[b]	4.47±0.06[b]	4.17±0.15[b]	p<0.0001
7	8.43±0.12	5.87±0.27[b]	4.53±0.88[b]	4.47±0.15[c]	4.17±0.75[c]	p<0.0001
8	9.83±0.15	6.10±0.10[b]	4.53±0.15[c]	4.47±0.89[c]	4.47±0.27[c]	p<0.0001

End point represents mean ± SD for ten root lengths. Values are significantly different from the controls as determined by Dunnette Multiple Posthoc Test ([a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$).

Table 3. Effects of tri-herbal pill treatment on the induction of chromosome aberrations and mitotic inhibition in *Allium cepa* meristems

Conc. (mg/L)	Cells in division (Mean ± SE)	Mitotic Index	Chromosome Aberration per 1000 cells						Total aberrant cells	% aberration based on cells in division
			Laggards	Fragments	Vagrants	Stickiness	multipolar	C mitosis		
Control	500.5 ± 2.3	50.05 ± 2.3	1	1	0	0	2	0	4	0.80
800	407.2 ± 1.8[a]	40.72 ± 1.8[a]	0	3	4	2	3	1	13	3.19[a]
1200	358.5 ± 2.1[a]	35.85 ± 2.1[a]	2	5	2	5	2	0	16	4.46[a]
1600	345.9 ± 2.5[b]	34.59 ± 2.5[b]	0	0	7	8	7	0	22	6.36[b]
2500	305.2 ± 1.5[c]	30.52 ± 1.5[c]	0	3	6	7	6	2	24	7.87[b]

End point represents mean ± SE for five root meristems. Values are significantly different from the controls as determined by Dunnette Multiple Posthoc Test ([a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$).

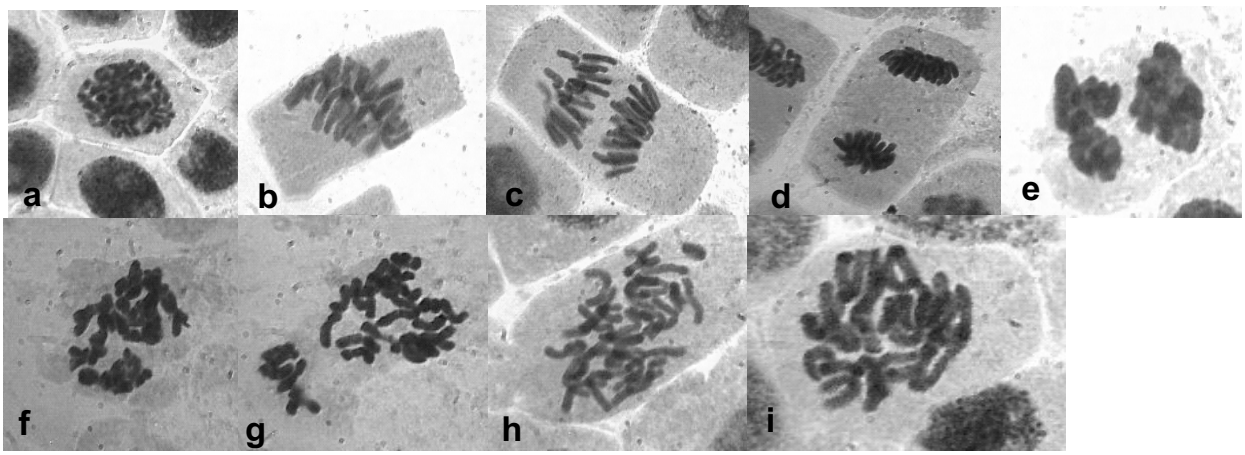

Figure 2: Aberrations observed in *Allium cepa* root tip cells after tri-herbal pill treatment. (a–d) Normal cells at (a) prophase, (b) metaphase, (c) anaphase and (d) telophase; (e) stickiness at anaphase, (f) C-mitosis, (g) multipolar chromosomes, (h) fragment and laggard chromosomes, and (i) Vagrant chromosomes (×1000).

DISCUSSION

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 (Adedapo *et al.*, 2007; van den Berg *et al.*, 2011; Kasim *et al.*, 2013; Bakare *et al.*, 2015).Hence, 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. This study presents the cytotoxic and genotoxic effects and hematological alterations induced by a tri-herbal preparation used in the treatment of hemorrhage and related health issues in Nigeria in *Allium cepa* and mice.

Clinical signs of toxicity observed mainly at the 1500 mg/kg of the tri-herbal pill treated mice showed systemic toxicity. Anorexia (loss of appetite), diarrhea, weakness and sluggishness in movement are common symptoms of chemical-induced toxicosis. These signs along with bloody eyes were similarly observed in rats and mice exposed to 300, 600 and 900 mg/btw of the tri-herbal solutions (Okpuzor and Oloyede, 2009). These symptoms may be attributed to the activities of the individual phytochemicals of the tri-herbal pills or the synergistic interactions among these phytochemicals.

Haematological testing in rodents during toxicity and safety evaluation is generally acknowledged as integral part of systemic toxicity assessment(Brown, 1992). Significant increase in RBC, HCT, HGB, MCV, MCH and WBC and decrease in MCHC at the 1500 mg/kg treated mice showed that the tri-herbal pill induced haematotoxic effects in the mice. Alterations in haematological indices suggest that the phytochemicals of the component plants in the tri-herbal pill affected haematopoiesis is in the bone marrow system of the treated mice (Adedapo *et al.*, 2007; Uboh *et al.*, 2010; Kasim *et al.*, 2013).

The tri-herbal pill solution induced cytotoxicity and genotoxicity on the cell cycle and chromosome structure of *A. cepa* root tips and bone marrow cells of mice. 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(Krishna and Hayashi, 2000).Micronuclei test is routinely used as genetic marker of exposure to clastogens and anuegens, and increased chromosome instability. Significant MNPCE induction in the bone marrow of mice treated with 1200 and 1500 mg/kg of the tri-herbal pill suggests genotoxicity. Hence, indicating the presence of clastogens and/or aneugens in the tested tri-herbal pill. These

chemicals are capable of increasing somatic mutations in mammalian systems which may predispose cells to chromosome-related disorders and carcinogenesis. This further corroborated haematological findings to suggest that higher concentrations of the tri-herbal pill increased haematopoietic disturbance in the mouse bone marrow system.

The Allium test is a universally recognized toxicological assay for assessing the effects of individual and mixture of xenobiotics on root growth, mitotic depression and chromosome structure (Nelson and Rank, 2004).Positive results from studies utilizing this test may suggest the presence of cytotoxic, genotoxic and/or mutagenic agents that can pose direct or indirect risks to life. Furthermore, it has good correlation with different *in vitro* and *in vivo* mammalian reports (Fiskesjo, 1985).The various concentrations of the tested tri-herbal pill induced significant decrease in root growth and mitotic index, and increase percentage chromosome aberrations in the *A. cepa* compared to the tap water. Mitotic index (MI) is an indicator of cell proliferation that shows the proportion of cells in the mitotic phase of the cell cycle. Concentration-dependent decrease in MI of the tri-herbal pill treated *A. cepa* indicates cytotoxic and mito-depressive effects of the pill on the *A. cepa* root tip cells. Reduction in MI has been linked to inhibition in DNA synthesis, blockage of the G2-phase of the cell cycle and/or halting of metabolic processes capable of preventing cells from dividing during M phase of the cell cycle (Schneiderman *et al.*, 1971; Sudhakar *et al.*, 2001; Oyeyemi and Bakare, 2013). Similar observations were reported from treating onions with tinctures and aqueous extracts from different medicinal plants, and cytotoxicity and mito-depressive effects of the extracts on the *A. cepa* root tip cells were suggested (Camparoto *et al.*, 2002; Teixeira *et al.*, 2003; Akintonwa *et al.*, 2009; Pastori *et al.*, 2013).

Root growth inhibition in the treated *A. cepa* corroborates decreased MI to suggest anti-proliferative effects of the tri-herbal pill in the *A. cepa* root tip meristems. This has been attributed to cell death or delay in cell proliferative kinetics (Rojas *et al.*, 1993). Significant increase in percentage chromosome aberrations in the tri-herbal pill treated *A. cepa* is attributed to cell division disturbances caused by genotoxins present in the pill. The occurrence of c-mitosis in cells indicates inhibition of spindle formation and has been associated with lyphophylic effects on the chains of the spindle proteins via disturbances on the polypeptide structure (El-Ghamery *et al.*, 2000; van den Berg *et al.*, 2011). C-mitosis formation, when not reversed in cells, leads to polyploidy (Fiskejo, 1985). Fragmented chromosomes are attributed to the clastogenic effects of xenobiotics on DNA strands. Chromosome stickiness is caused by abnormal physiological functioning of proteins associated with chromosome folding and / or condensation, and its occurrence in cells is associated with high toxic effects of xenobiotics and is usually irreversible leading to cell death (Fiskesjo, 1985; Turkoglu, 2007). The presence of vagrant chromosomes showed unequal distribution of chromosomes due to nondisjunction errors of paired chromatids at anaphase. This may increase incidence of aneuploidy in daughter cells (Turkoglu, 2007).The findings herein is in agreement with other studies that high concentrations of the phytochemicals in medicinal plants elicited chromosome abnormalities in plant and animal test systems (Nabeel *et al.*, 2008; Akintonwa *et al.*, 2009; Oyeyemi and Bakare, 2013; Pastori *et al.*, 2013).

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(Qin *et al.*, 2006; Rietjens *et al.*, 2008; van den Berg *et al.*, 2011). In addition, many of these herbal supplements and medicinal herbs contain high concentrations of numerous hazardous metals that are of public health concern (Razic *et al.*, 2005; Obi *et al.*, 2006; Arpadjan *et al.*, 2008; Arumugam *et al.*, 2012; Aissi *et al.*, 2014). 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 (Ozdemir *et al*., 2013). High concentrations of cyanogenic glycosides (toxins) contained in the leaves and tubers of *Manihot esculenta crantz* (Hidayat *et al*., 2002; Ojo *et al*., 2013), one of the component plants in the tri-herbal pills may have induced the cytogenotoxic effects in the *Allium cepa*, and DNA damage and hematological alterations in *Mus musculus*. Indiscriminate consumption of tri-herbal pill mostly at high concentrations should be discouraged. Although these medicinal plants may possess profound therapeutic advantages, many of them are yet to be screened by drug regulating agencies for toxicological profiles to ascertain suitable concentrations with minimal risk to health. It is advisable they are avoided or consumed with caution to prevent adverse health effects on the public.

REFERENCES

Adedapo, A. A., Abatan, M. O. and Olorunsogo, O. O. 2007.Effects of some plants of the spurge family on haematological and biochemical parameters in rats. *Veterinarski Archives* 77: 29–38.

Afolabi, L., Adeyemi, O. O. and Yemitan, O. K. 2008. Cassava leaves have anti-inflammatory and analgesic principles, which justify its use in traditional African medicine. *Journal of Ethnopharmacology* 119: 6–11.

Aissi, A. K., Pazou, E. Y., Ahoyo, T.A., Fah, L., Fanou, B., Koumolou, L., Koudokpon, H., Agbangla, C., Gnandi, K., Loko, F. and Edorh, P.A. 2014. Evaluation of toxicological risk related to presence of lead and cadmium in *Moringa oleifera* Lam. leaves powders marketed in Cotonou (Benin). *Food and Nutritional Sciences* 5: 770–778.

Akintonwa, A., Awodele, O., Afolayan, G. and Coker, H.A.B. 2009. Mutagenic screening of some commonly used medicinal plants in Nigeria. *Journal of Ethnopharmacology* 125:461–470.

Alimba, C. G., Ogunkanmi, L. A. and Ogunmola, F. J. 2013.Cytotoxic and genotoxic assessment of textile effluent using *Allium* test. *Current Topics in Toxicology* 9: 65–74.

Ameri, A., Heydarirad, G., MahdaviJafari, J., Ghobadi, A., Rezaeizadeh, H. and Choopani, R. 2015. Medicinal plants contain mucilage used in traditional Persian medicine (TPM). *Pharmacological Biology* 53: 615–623.

Arumugam, R., Kannan, R. R. R.,Jayalakshmi, J., Manivannan, K., Devi, G. K. and Anantharaman, P. 2012. Determination of element contents in herbal drugs: Chemometric approach. *Food Chemistry* 135: 2372–2377.

Bakare, A. A., Oguntolu, G. O., Adedokun, L. A., Amao, A. A., Oyeyemi, I. T., Alimba, C. G.,Alabi, O. A. 2015. *In vivo* evaluation of genetic and systemic toxicity of aqueous extracts of *Phyllanthus amarus* in mice and rats. *International Journal of Toxicological* and *Pharmacological Research* 7(4):1–9.

Begriche, K., Massart, J., Robin, M., Borgne-Sanchez, A. and Fromenty, B. 2011. Drug-induced toxicity on mitochondria and lipid metabolism: Mechanistic diversity and deleterious consequences for the liver. *Journal of Hepatology* 54: 773–794.

Brown, G. 1992. Hematological tests in toxicology: time for a re-think? *Comparative Hematology International* 2: 231–235.

Camparoto, M. L., Teixeira, R. D., Mantovani, M. S. and Vicentini, V. E. P. 2002. Effects of *Maytenusilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells. *Genetics* and *Molecular Biology* 25: 85–89.

Egharevba, R. K. A. and Ikhatua, M. L. 2008. Ethno-medical uses of plants in the treatment of various skin diseases in Ovia North East, Edo State, Nigeria. *Research Journal of Agricultural and Biological Sciences* 4: 58–64.

Evans G. O. 2008 Animal Haematotoxicology: *A Practical Guide for Toxicologists and Biomedical Researchers*. CRC Press.(ISBN 142 0080091, 97814400).pp 14–78.

El-Ghamery, A. A., El-Nahas, A. I. and Mansour, M. M. 2000. The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acids content in root meristems of *Allium cepa* and *Viciafaba*. *Cytologia* 65: 277–287.

Farnsworth, N. R., Akerele, O. O., Bingel, A. S., Soejarta, D. D., Eno, Z. 1985. Medicinal plants in therapy. *Bulletin of World Health Organisation* 63: 965–981.

Fenech, M., Kirsch-Volders, M.,Natarajan, A. T., Surralles, J., Crott, J. W., Parry, J., Norppa, H., Eastmond, D. A., Tucker, J.D. and Thomas, P. 2011. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis*26: 125–132.

Fiskesjo, G. 1985. The *Allium* test as a standard in environmental monitoring. *Hereditas* 102:102–112.

Fiskesjo, G. 1990. Occurrence and degeneration of 'Al-structures' in root cap cells of *Allium cepa* L. after Al-treatment. *Hereditas* 112:193–202.

Harvey, A. 2008. Natural products in drug discovery. *Drug Discovery Today* 13: 894–901.

Hidayat, A., Zuraida, N. and Hanarida, I. 2002. The cyanogenic potentials of roots and leaves of ninety nine cassava Cultivars. *Indonesian Journal of Agricultural Science* 3: 25-32.

Kasim, L.S., Okunowo, K., Olaitan, O.J. and Fajemirokun, T.O. 2013. The effects of the leaf water extract of *Struchium sparganophora* (Linn.) *Ktzeasteraceae* on the hematopoietic parameters and the organ system of rats .*Pakistan Journal of Pharmaceutical Science* 26: 1203–1207.

Krishna, G. and Hayashi, M. 2000. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. *Mutation Research* 455: 155–166.

Nabeel, M., Abderra-hman, S. and Papini, A. 2008. Cytogenetic effect of *Arum maculatum* extract on the bone marrow cells of mice. *Caryologia* 61: 383–387.

Nielson, M.H. and Rank, J. 1994. Screening for toxicity and genotoxicity in wastewater using the *Allium* assay. *Hereditas* 121: 249–254.

Obi, E., Akunyili, D. N., Ekpo, B. and Orisakwe, O. E. 2006. Heavy metal hazards of Nigerian herbal remedies. *Science of the Total Environment* 369: 35–41.

Ojo, R. J., Obi N. P., Akintayo, C. O. and Adebayo-Gege, G. I. 2013. Evaluation of cyanogen contents of cassava and cassava based food products in Karu, Nasarawa State, North central Nigeria. *Journal of Environmental Science Toxicology and Food Technology* 6:47–50.

Okpuzor, J. and Oloyede, A. M. 2009.Anti-inflammatory, anti-pyretic and anti-diarrhoeal properties of an anti-haemorrhoid tri-herbal pill. *Nature Science* 7: 89–94.

Olusola, A., Olutayo, O., Michael, A., Olakunle, F. and Edah, A. O. 2011. Elemental analysis and anti-microbial potentials of the leaf extract of *Cassia arereh* Del. *International Research on Pharmacy and Pharmacology* 1: 188–193.

Ouedraogo, M., Baudoux, T., Stévigny, C., Nortier, J., Colet, J., Efferth, T., Qu, F., Zhou,J., Chan, K., Shaw, D., Pelkonen, O. and Duez, P. 2012. Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenicity and nephrotoxicity) of herbal medicines and mushrooms. *Journal of Ethnopharmacology* 140: 492–512.

Oyeyemi I. T. and Bakare A. A. 2013. Genotoxic and anti-genotoxic effects of aqueous extracts of *Spondias mombin* L., *Nymphea lotus* L. and *Luffa cylindrical* L. on *Allium cepa* root tip cells. *Caryologia* 66: 360–367.

Ozdemir, B., Sahin, I.,Kapucu, H.,Celbis, O.,Karakoc, Y., Erdogan, S.and Onal, Y.2013. How safe is the use of herbal weight-loss products sold overthe Internet? *Human and Experimental Toxicology*32:101–106.

Pastori, T., Flores, F. C., Boligon, A. A., Athayde, M. L., da Silva, Cde B., do Canto-Dorow, T. S. and Tedesco, S. B. 2013. Genotoxic effects of *Campomanesiaxanthocarpa* extracts on *Allium cepa* vegetal system. *Pharmacological Biology* 51: 1249–1255.

Patwardhan, B. and Vaidya, A. 2010. Natural products drug discovery: Accelerating the clinical candidate development using reverse pharmacology. *Indian Journal of Experimental Biology* 48: 220–227.

Qin, X., Dong, Z., Liu, J., Yang, L., Wang, R., Zheng, Y., Lu, Y., Wu, Y. and Zheng, Q. 2006. Concentricodiole an anti-HIV agent from the Ascomycete

Daldimaconcentrica. *Helvetica Chimica Acta* 89: 127–133.

Razic, S., Onjia, A., Đogo, S., Slavkovi, L. and Popovic, A. 2005.Determination of metal content in some herbal drugs: Empirical and chemometric approach. *Talanta* 67: 233-239.

Rietjens, I.M.C.M., Slob, W., Galli, C. and Silano, V. 2008. Risk assessment of botanicals and botanical preparations intended for use in food and food supplements: emerging issues. *Toxicology Letter* 180: 131–136.

Rojas, E., Herrera, L.A., Sordo, M., Gonsebatt, M.E., Montero, R., Rodriguez, R. and Ostrosky-wegman, P. 1993.Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. *Anti-Cancer Drugs* 46: 637–640.

Schneiderman, M. H., Dewey, W. C. and Highfield, D. P. 1971. Inhibition of DNA synthesis in synchronized Chinese hamster cell treated in G1 with cycloheleximide. *Experimental Cell Research* 67: 147–155.

Sudhakar, R., Gowda, N. and Venu, G. 2001. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa Cytologia* 66: 235–239.

Teixeira, R. D., Camparoto, M.L., Mantovani, M.S. and Vicentini, V.E.P. 2003. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* assays. *Genetics and Molecular Biology* 26: 551–555.

Turkoglu, S., 2007. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutation Research* 626: 4–14.

Uboh, E. F., Okon, I. E. and Ekong, M. B. 2010. Effect aqueous extract of *Psidium guavaja* leaves on liver enzymes, histological integrity and hematological indices in rats. *Journal of Gastrointestinal Research* 3: 32–38.

van den Berg, S. J., Restani, P., Boersma, M. G., Delmulle, L. and Rietjens, I. M. 2011. Levels of genotoxic and carcinogenic compounds in plant food supplements and associated risk assessment. *Food and Nutritional Science* 2: 989–1010.

World Health Organization (WHO). 2003. *Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants.* World Health Organization Geneva. pp 1–36.