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Cytological and Toxicological Properties of a Decoction Used for Managing Tumors in Southwestern Nigeria

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Abstract: The cytological and toxicological potentials of an ethanol extract of a decoction used in South Western Nigeria for the management of breast tumors were evaluated using mice and *Allium cepa* models. The oral administration of the extract to the mice within the range of 400-1600 mg kg⁻¹ b. wt., dosage did not result in any mortality until 2000 mg kg⁻¹ body weight (b. wt.), when 60% mortality occurred. The LD₅₀ of the extract administered intraperitoneally, was 400 mg kg⁻¹ b. wt. Furthermore, the extract induced macroscopic and microscopic changes causing a dose-related root growth inhibition and chromosomal aberrations in *Allium cepa*. The effect of the extract was more pronounced at 1600 mg kg⁻¹, dose while the EC₅₀ was at 380 mg after 72 h. This decoction may present cytological and toxicological potential for managing breast tumors and corroborates its use in ethno medicine.

Key words: Cytotoxicity, *Allium cepa*, chromosomal aberrations, macroscopic and microscopic changes

INTRODUCTION

Herbal medications are used widely in developing countries for the treatment of various diseases and ailments. This is because they are seen as alternatives to orthodox medicines in terms of costs and perceived side effects (Okochi *et al.*, 2003; Zhu *et al.*, 2002). Unfortunately, in spite of its wide usage, there is limited scientific evidence regarding the standards, safety and efficacy of these herbs. The rationale for their utilisation has rested largely on historical clinical experience of local traditional medicine practitioners. However, one major setback of traditional medicine practice generally, is that it is common for unorthodox practitioners to prescribe complex formulations containing multiple herbs to patients. This approach amounts to the administration of several chemical entities at once. The underlying theory therefore, may be that the interactions among the chemicals present within a single plant as an entity and within the different herbs in a formula, exerts synergistic pharmaco-dynamic actions not present when administered in the form of an isolated active ingredient (Odugbemi, 2006). Therefore, with the emerging recourse to the use of herbal medicines amongst the elites, a thorough scientific investigation of these plants becomes pertinent to validate their folkloric claims of efficacy and safe usage.

Allium test has been extremely useful in biological monitoring and determination of toxicity and pollution. It has been widely used for the evaluation of cytotoxic and anti-mitotic activity of various compounds (Sehgal *et al.*, 2006). *Allium cepa* bulbs are easy to store and handle and the root tip cells constitute a convenient system for macroscopic growth (EC₅₀ values) as well as for microscopic parameters (c-mitosis, stickiness, chromosome breaks). Odeigah *et al.* (1997) reported that the *Allium* test has many advantages as a genotoxicity screening assay and that the root cells possess mixed function oxidase system that is capable of activating promutagens or genotoxic chemicals. *Allium* cells possess important plant activation enzymes. Therefore, *Allium* test has a wide area of application and is in agreement with results from other test systems such as eukaryotes and prokaryotes (Fiskesjo, 1988). The most important advantage of the *Allium* test is that it is a low budget method, which besides being fast and easy to handle, it also yields reliable results (Rank, 2003).

In this study, we seek to further the research into the attributes of JOLOO decoction earlier reported to possess anti-inflammatory and analgesic properties in rats (Oloyede *et al.*, 2008) and believed to be effective in management of breast tumours in rural communities. The result of this study may therefore, present overdue scientific justification for its use in ethno medicine.

MATERIALS AND METHODS

Plant materials: Plant materials, *Allium ascalonicum* Linn. (Liliaceae: Alliaceae) PCGH 440, *Butyrospermum paradoxum* Gaertn (Sepotaceae) PCGH 473, *Hoslundia opposita* Vahl (Labitae) PCGH 322, *Olex subscorpioidea* Olive (Olacaceae) PCGH 438, *Xylopia aethiopica* Dunal A. Richard (Annonaceae) PCGH 441, *Securidaca longepedunculata* Fresen (Polygalaceae) PCGH 439 and *Tetrapleura tetraptera* Schum and Thonn (Leguminosae: Mimosidae) PCGH 382. were collected in August 2007 from local medicine practitioners in Totoro village, Abeokuta in Ogun State of Nigeria. They were identified and authenticated at the Department of Pharmacology of the College of Medicine, University of Lagos, Nigeria where the voucher specimens were also deposited.

Allium cepa bulbs: Healthy purple variety of *Allium cepa* bulbs (28-30 g) were obtained from Oyingbo market in Lagos metropolis, Nigeria. Sixty of these *Allium cepa* bulbs were grown in the dark for 48 h in a beaker containing 100 mL tap water at ambient temperature until the roots have grown to approximately 2-3 cm. The viable bulbs were then selected and used for subsequent studies. The water was changed daily throughout the period of the experiment.

Animals: Swiss mice (20-32 g) of either sex bred in the animal house of the University of Lagos, Nigeria, were used for the study. Approval was obtained from the University of Lagos ethical committee on the use of animals for experimental purposes. They had free access to standard diet (Ladokun feeds Plc. Ibadan) and water *ad libitum*. They were also maintained under standard conditions of humidity, temperature and 12 h light/darkness cycle (Bishayee and Chanterjee, 1994).

Preparation of the decoction: The decoction was prepared as described by Oloyede *et al.* (2008). The plant materials *Butyrospermum paradoxum*, *Hoslundia opposita*, *Olex subscorpioidea*, *Xylopia aethiopica*, *Securidaca longepedunculata*, *Allium ascalonicum* and *Tetrapleura tetraptera* were air dried, mixed in the ratio of 5:2:1:4:1:3:3, respectively to give the desired pharmacological action. The plants materials (380 g) were subsequently powdered and soaked in 500 mL of 95% cold ethanol for 72 h. Thereafter the ethanol extract obtained was filtered with muslin cloth, evaporated to dryness at a temperature of 40°C. The dried extract (19 g) reconstituted according to the required concentration, served as the decoction used for the experiments.

Acute toxicity test: Acute toxicity (LD₅₀) was estimated in mice (n = 50 in each case) by intraperitoneal (i.p.) and oral (p.o.) administration of the decoction. The animals were randomly divided into 5 groups of 10 mice each for i.p. and p.o., respectively. The control group received only distilled water p.o. while the different test groups received intraperitoneal and oral doses of 400, 800, 1200, 1600 and 2000 mg kg⁻¹ b. wt., of the decoction. The general symptoms of toxicity observed within 24 h in each group were monitored and recorded. The number of mortality was recorded within 24 h for the i.p. group and 72 h for the p.o. group. The median lethal dose (LD₅₀) estimation was by log dose/probit analysis as described by Nwanjo (2005).

Genotoxicity test

Allium cepa assay: The method described by Odeigah *et al.* (1997) was adopted. Fresh and healthy looking onions with fresh primordial roots were selected and divided into four groups of ten onions each.

Three concentrations of the decoction (400, 800 and 1600 mg kg⁻¹) and distilled water as control, were used. Test solutions were changed daily and the length of roots were measured daily for seven days. After 96 h, some roots from each group were harvested for cytological studies by the conventional aceto-orcin squash technique described by Fiskesjo (1988).

Microscopic examination and determination of mitotic index:

The root tips (2-3 mm) were collected, placed in 1 N HCl for 5 min, squashed and stained with 2% aceto-orcin. For each root tip, the numbers of mitotic and total meristematic cells were counted in 5-8 fields of view using high power (100x) light microscope (Badria *et al.*, 2001). In all, 350-500 cells were counted and cells manifesting different stages of mitosis were recorded. The mitotic index was calculated using the formula below:

$$\text{Mitotic index} = \frac{P+M+A+T}{\text{Total Cells}}$$

Where:

P = Prophase

M = Metaphase

A = Anaphase

T = Telophase

Statistical analysis: Student's t-test was used for statistical analysis and results were expressed as Mean±SEM. Results were considered significant when p<0.05.

RESULTS

Acute toxicity studies: Mortality was not observed in the different groups of mice that received the decoction orally, until the maximum dose of 1600 mg kg⁻¹ b. wt. However, 60% mortality was recorded after 72 h for this same orally treated group at 2000 mg kg⁻¹ dosage. In addition, within the (i.p.) group, mortality rate progressed from 50% at 400 mg kg⁻¹ b. wt., to 100% at 1600 and 2000 mg kg⁻¹ b. wt., dose of the decoction (Fig. 1). The LD₅₀ value was 400 mg kg⁻¹ (i.p.) and 2000 mg kg⁻¹ (p.o.).

Toxicity test: Macroscopic and Microscopic parameters. No structural changes were observed on the *Allium cepa* root tips but the decoction caused significant decrease ($p<0.05$) on the root length with increasing concentrations as shown in Table 1. However, a slight increase in root length was observed from day 1-7 at concentrations of 400 and 800 mg while there was growth cessation on day 4 at 1600 mg. There was 81% root growth inhibition (Table 2) at concentration of 1600 mg. The EC₅₀ value was 380 mg.

Table 1: Effect of extract on root length

Concentration	Days (cm)							Mean±SEM
	1	2	3	4	5	6	7	
Control	0.80	1.70	3.00	3.16	4.70	4.80	4.90	3.20±0.60
400 mg	0.34	0.90	1.10	1.53	1.90	2.43	2.50	1.53±1.30*
800 mg	0.30	0.66	0.76	1.10	1.20	1.40	1.50	0.90±0.20**
1600 mg	0.20	0.40	0.50	0.70	0.70	0.70	0.70	0.50±0.10***

$p<0.05$ significantly different from control (students t-test), *: Significant, **: More significant, ***: Most significant

Table 2: Percentage root growth inhibition

Treatment	Dose (mg kg ⁻¹)	Root length (Mean±SEM)	Root growth inhibition (%)
Control	-	3.20±0.60	-
Ethanol extract	400	1.50±0.30*	53.6
	800	0.90±0.20**	68.3
	1600	0.50±0.10***	81.3

*, Significant, **, More significant, ***: Most significant

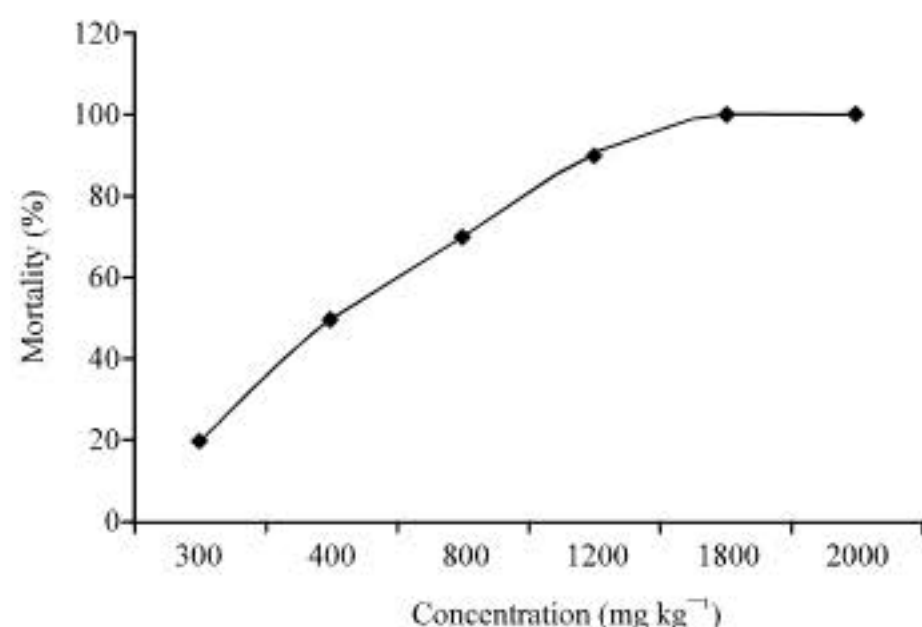


Fig. 1: Percentage mortality of mice treated (i.p.) with the decoction

The microscopic analysis of the effect of the decoction on cell division showed that it interfered with mitosis. All the concentrations of the decoction induced chromosome aberrations which were statistically significant ($p<0.05$) from the control. Different types of chromosomal aberrations such as sticky chromosomes, c-mitosis, vagrant chromosomes, bridges, fragment and attached chromosomes are shown in Fig. 2-5. However, most of the aberrations occurred at anaphase stage and mostly as bridges or sticky chromosomes. At the lower concentration of 400 mg, out of the 412 cells examined, four sticky chromosomes, four bridge fragments

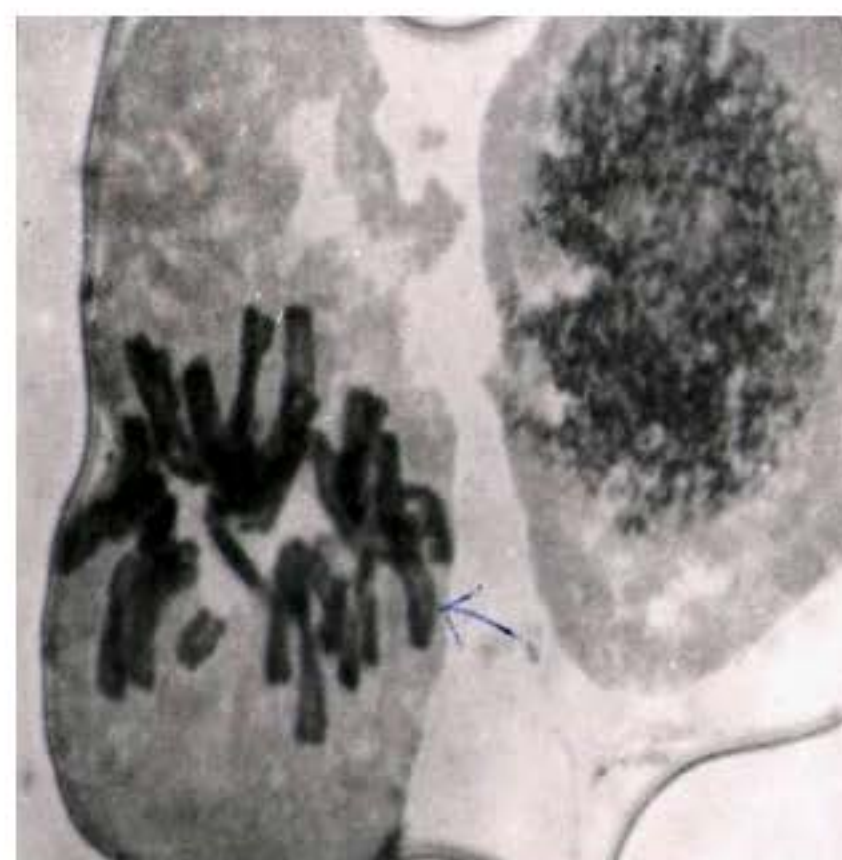


Fig. 2: Vagrant chromosomes (400 mg)

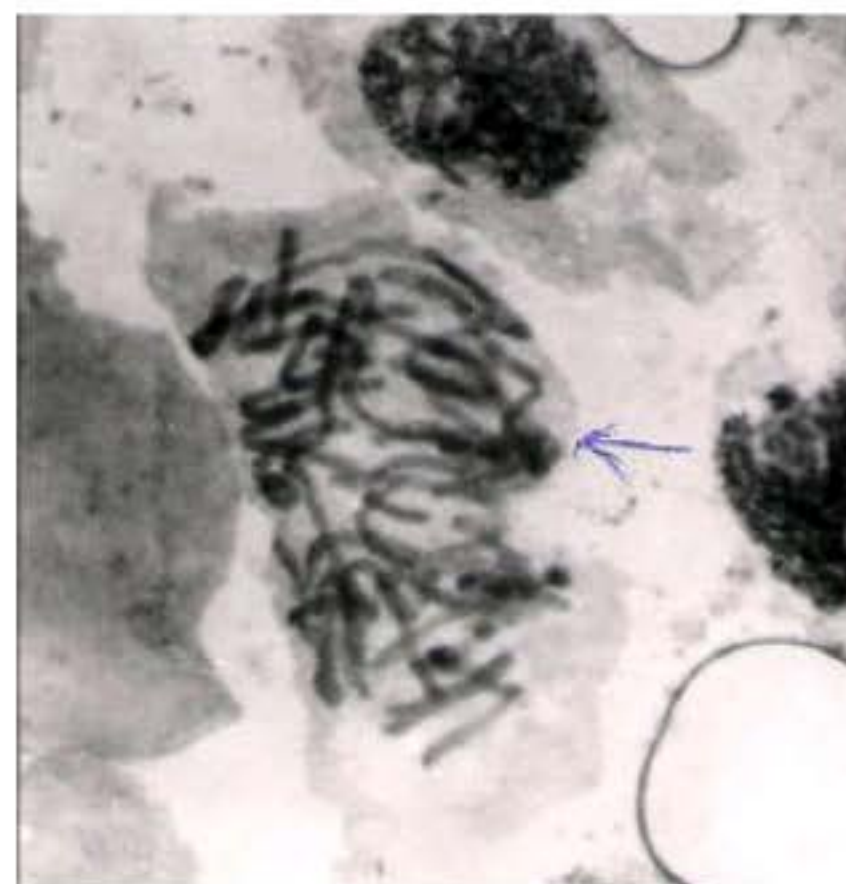


Fig. 3: Vagrant chromosome (800 mg)

Table 3a: Effect of extract on cell division

Concentration	No. of cells	Dividing cells	Mean±SEM	Stickiness	C-mitosis	Vagrant	Bridges fragment
Control	500	31	6.0±0.04	1	0	0	1
400 mg	412	16	3.8±0.02	4	0	0	4
800 mg	360	14	3.8±0.02	3	1	2	3
1600 mg	354	15	4.2±0.01	3	0	1	3

Table 3b: Effect of extract on cell division

Concentration	No. of cells	Dividing cells	Binucleus	Multipolar anaphase	Attached	Total aberration Mean±SEM
Control	500	31	0	0	0	0.29±0.10
400 mg	412	16	0	0	1	1.29±0.70
800 mg	360	14	0	0	1	1.43±0.40*
1600 mg	354	15	2	1	3	1.86±0.40**

*: Significant, **: More significant, ***: Most significant

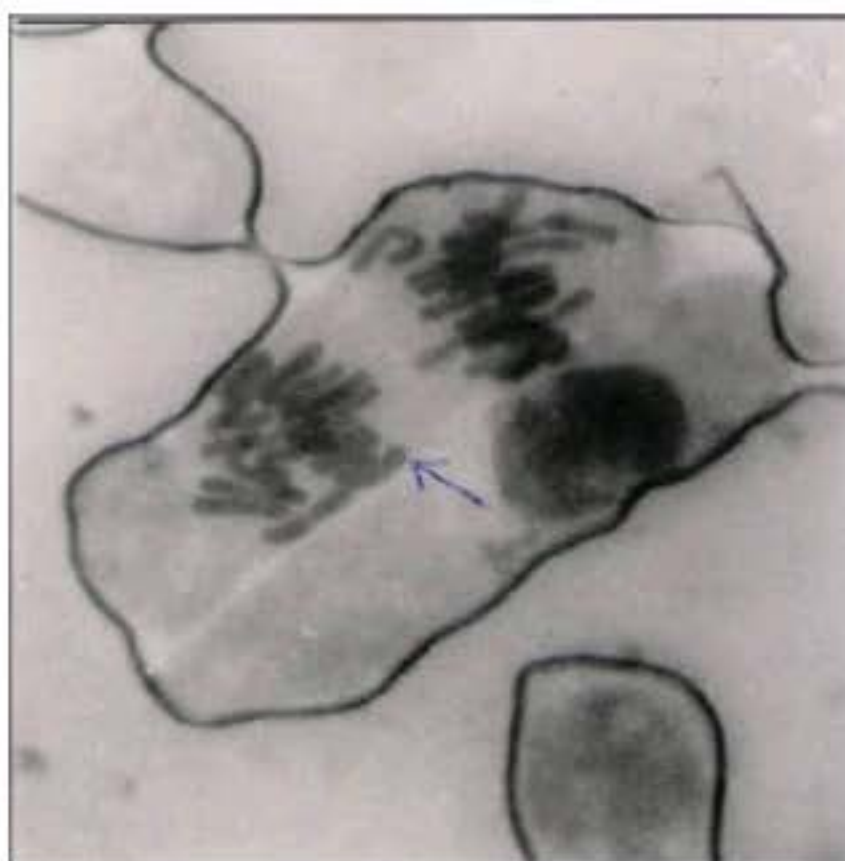


Fig. 4: Anaphase with laggards (1600 mg)

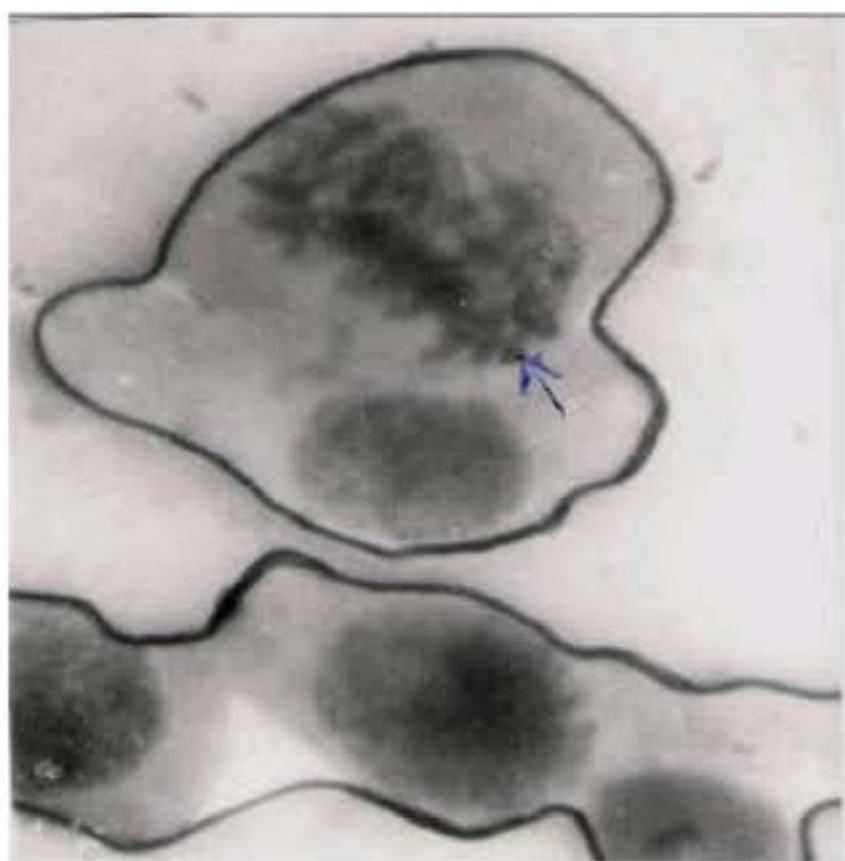


Fig. 5: Bridge Anaphase and fragment (1600 mg)

and one attached chromosome (Table 3a, b) were observed. Chromosome aberrations increased with higher concentrations. The highest concentration (1600 mg) produced significant aberration of $1.8\pm0.4^{**}$ compared to the control (0.2 ± 0.1). The decoction at 400 mg concentrations had a minimal effect on the Mitotic Index (MI) in root-tip cells, but 800 and 1600 mg concentrations significantly reduced the MI (Table 3b).

DISCUSSION

Acute toxicity study is a useful parameter in estimating the Therapeutic Index (i.e., LD_{50}/EC_{50}) of drugs and xenobiotics (Rang *et al.*, 2001). The oral LD_{50} value of the decoction in mice was 2000 mg kg^{-1} as observed in this study.

The LD_{50} value of 400 mg kg^{-1} observed among the intraperitoneally treated mice is indicative that the decoction is toxic when given via this route. Gathumbi *et al.* (2002) stated that the mode of administration of an herbal preparation is important in assessing the suitability of such preparation for therapeutic use. The high mortality recorded among the intraperitoneally treated mice in comparison with the low rate observed within the set that were orally treated, could be attributed to the purity of the decoction. Since, the decoction is a crude extract, prepared by infusion, some particles in the extract may have entered directly into the bloodstream and this may have raised the mortality rate via intraperitoneal route unlike the oral route where several barriers had to be crossed in the gastrointestinal tract before its absorption as suggested by Etuk *et al.* (2006). We observed that our decoction induced a statistically significant ($p<0.05$) number of chromosome aberrations at high concentrations of 800 and 1600 mg and the abnormal chromosome types observed were vagrant, bridges, laggards and fragments. Rathore *et al.* (2006) showed that the extract from Myrobalan reduced mitotic index of *Allium* root tip cells but did not cause any chromosomal aberrations at the highest concentration.

There was a dose response correlation in the growth of *Allium cepa* root in the decoction. This gradual and significant ($p < 0.05$) reduction in root growth at increasing concentration may suggest toxicity which caused the significant chromosomal aberrations and consequently resulting in a gradual and significant decline in mitotic index value. The decline in mitotic index value shows interference in the cell cycle. This interference occurred mostly during metaphase/anaphase. It has been observed that when the separation of chromosomes occurs during cell division, any chemical or toxic substance introduced into the system affects the structural integrity of the cell. Li *et al.* (2005) observed that a microtubule drug benomyl, may lead to chromosome loss as a result of a defect in kinetochore-to-microtubule attachment during prometaphase, metaphase, or anaphase, if such a defect is not caught by the spindle assembly checkpoint and corrected.

The ability of the decoction to reduce MI by inhibiting cell proliferation particularly at the metaphase and anaphase stage may be adjudged similar to the action of plant derived drugs such as Taxol, vinblastine and vincristine. Vinblastine and vincristine block the process of microtubule assembly whereas Taxol stabilizes microtubule and promotes the formation of abnormal microtubule bundles (Lewis, 2006). In either case, the mitotic spindle will be disrupted and cells cannot divide. It is also possible that the decoction will have phytochemicals that may bind to proteins of the cell cycle, thereby influencing their activities and this would obviously affect cell division.

The overall result of this investigation is that this decoction exhibits cytotoxic properties, which may be an indication that it may be inhibiting the activity of one or more components of the cell cycle. Thus, this decoction may indeed, prove valuable for managing tumors which corroborate its use in ethno medicine.

REFERENCES

- Badria, F.A.R., W.E. Houssein, M.G. Zaghloul and A.F. Halim, 2001. Antimitotic activity of gossypol and gossypolone. *Pharm. Biol.*, 39: 120-126.
- Bishayee, A. and M. Chatterjee, 1994. Protective effect of *Mikania cordata* root extract against physical and chemical factors induced gastric erosions in experimental animals. *Planta Med.*, 60: 110-113.
- Etuk, E.U., B.M. Agaie, P.A. Onyeyili and C.U. Ottah, 2006. Toxicological studies of aqueous stem bark extract of *Boswellia dalzielii* in albino rats. *Indian J. Pharmacol.*, 38: 359-360.
- Fiskesjo, G., 1988. The *Allium* test-an alternative in environmental studies: The relative toxicity of metal ions. *Mutat. Res.*, 197: 243-260.
- Gathumbi, P.K., J.W. Mwangi, G.M. Mugeru and S.M. Njiro, 2002. Toxicity of chloroform extract of *Prunus africana* stem bark on rats: Gross and histological lesions. *Phytother. Res.*, 16: 244-247.
- Lewis, J.K., 2006. Principles of Cancer Biology: International Edition. Pearson Higher Education, Benjamin Cummings, ISBN: 9780321432841, pp: 312.
- Li, S., C.E. Oakley, G. Chen, X. Han, B.R. Oakley and X. Xiang, 2005. Cytoplasmic dynein's mitotic spindle pole localization requires a functional anaphase-promoting complex, gamma-tubulin and NUDF/LIS1 in *Aspergillus nidulans*. *Mol. Biol. Cell*, 16: 3591-3605.
- Nwanjo, H.U., 2005. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. *Nig. J. Physiological Sci.*, 20: 39-42.
- Odeigah, P.G., J. Ijimakinwa, B. Lawal and R. Oyeniyi, 1997. Genotoxicity screening of leachates from solid industrial wastes evaluated with the *Allium* test. *Alternatives Lab. Anim.*, 25: 311-321.
- Odugbemi, T., 2006. Outlines and Pictures of Medicinal Plants from Nigeria. University of Lagos Press, Nigeria, ISBN: 978-38235-9-0, pp: 283.
- Okochi, V.I., J. Okpuzor and L.A. Alli, 2003. Comparison of an African herbal formula with commercially available haematinics. *Afr. J. Biotechnol.*, 2: 237-240.
- Oloyede, A.M., J. Okpuzor, O. Omidiji and H.O.C. Mbagwu, 2008. A pharmacological evaluation of herbal cocktail. *Int. J. Pharmacol.*, 4: 196-201.
- Rang, H.P., M. Dale and J. Ritter, 2001. Pharmacology. 4th Edn., Churchill Livingstone, New York, ISBN-13: 9780443059742.
- Rank, J., 2003. The method of *Allium* anaphase-telophase chromosome aberration assay. *Ekologika*, 1: 38-42.
- Rathore, H.S., K. Amit, S. Anjiali, S. Sharad and D. Bhatnagar, 2006. A study on the cytological effects of myrobalan (Fruit of *Terminalia chebula*) in *Allium* tests. *Ethnobotanical Leaflets*, 10: 92-97.
- Sehgal, R., S. Roy and V.L. Kumar, 2006. Evaluation of cytotoxic potential of latex of *Calotropis procera* and Podophyllotoxin in *Allium cepa* root model. *Biocell*, 30: 9-13.
- Zhu, M., K.T. Lew and P. Leung, 2002. Protective effect of plant formula on ethanol induced gastric lesions on rats. *Phytother. Res.*, 16: 276-280.