International Journal of Advances in Scientific Research
ISSN: 2395-3616 (Online)
Journal DOI: https://doi.org/10.7439/ijasr **Research Article**

Genotoxicity effects of selected alcoholic polyherbal products using *Allium cepa*

Oluwaseyi Adegoke Adetunji*, Ifeoluwa Hannah Oladipo, Modupe Olusola Adetayo, Gogonte Hezekiah Amah and Adesola Moyosore Kuti

Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria

Abstract

Alcoholic herbal products have been used for years in folk medicine for its believed increase in sexual desire and pleasure. This study is due to the current upsurge in the use of polyherbal products and coupled with loose regulation on public access to these products, to evaluate their biochemical effect, noting also that many of the polyherbal products lack scientific evidence to support their medicinal claims. The objective is to investigate the potential genotoxicity of some commonly consumed polyherbal formulations (Action bitters, Black wood, Jedi-jedi, Agbara, Orijin bitters), *using allium cepa*. The method involves onion bulbs that were exposed to varied concentrations of herbal alcoholic productsfor microscopic analysis. Tap water has been used as a negative control. All the tested products have been observed to have genotoxic effects on cell division in *Allium cepa*, with the highest mitotic index as 59% (orijin bitters 25%) and the lowest being 23% (action bitters 75%). The results showed that all tested concentrations of alcoholic herbal products caused decreased mitotic index value and increased chromosomal aberration of *Allium cepa* root cells. From the findings, alcoholic polyherbal formulations may possess genotoxic effects.

Keywords: Genotoxicity, meristematic cells, *Allium cepa,* alcoholic herbal products, cell division.

***Correspondence Info:**
Oluwaseyi Adegoke Adetunji
Department of Biochemistry,
School of Basic Medical Sciences,
Babcock University, Ilishan-Remo,
Ogun State, Nigeria

***Article History:**
Received: 25/07/2020
Revised: 23/08/2020
Accepted: 28/08/2020
DOI: https://doi.org/10.7439/ijasr.v6i8.5509

QR Code

How to cite: Adetunji OA, Oladipo IH, Adetayo MO, Amah GH and Kuti AM. Genotoxicity effects of selected alcoholic polyherbal products using *Allium cepa*. *International Journal of Advances in Scientific Research* 2020; 6(8): e5509. Doi: 10.7439/ijasr.v6i8.5509 Available from: https://ssjournals.com/index.php/ijasr/article/view/5509

1. Introduction

Genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic. Alcohol has been reported to be harmful to man owing to its mutagenic and carcinogenic effects [1]. Herbal bitters are blended herbs, spices, roots, seeds, and bark with a characteristic bitter taste. 'Jedi' is basically an umbrella name for herbal mixtures like 'ale', 'afato, 'opaeyin' and other types of strong herbal mixtures that seriously impact the human body. The origin of 'Jedi' is rooted in the African belief in herbs and herbal mixtures as a remedy for any ailments, illnesses or sicknesses. Africans historically believe that in the potency of herbal mixtures that Yoruba People call, 'agbo', is far greater than anything modern medicine would guarantee [2].

The serial consumption of 'Jedi' then became premised on this belief, but more as a prevention or management mechanism, or for a cure to a certain major, but mostly minor ailments in the body.'Jedi' is consumed on the pretext of cleaning the bile, strengthening the spine or increasing sexual potency. In addition to herbal extracts, reports have shown the presence of heavy metals in many products [3]. Issues with the consumption of herbal alcoholic products include it is being sold without any regulation, no known dosage, alcoholic interference with the brain's communication pathways. Examples of alcoholic herbal products include: orijin bitters, action bitters, alomo bitters, jedijedi, blackwood, agbara, aleko.

Allium cepa is one of the many methods for detecting and measuring the degree of alterations in the system subjected to carcinogens/mutagens or chemical causing damage and allow to describe the effects of these damages by observing chromosomal aberrations [4]. Hence, this study will evaluate the genotoxicity effect of herbal alcoholic products at the level of the chromosome using *Allium cepa* tips cells.

2. Materials and Methodology

2.1 *Collection of onions and alcoholic herbal products*

Onion bulbs and alcohol herbal products were readily found at Ilishan market, Ogun State, Nigeria. Small onion bulbs were preferably selected for this test. Five different alcoholic herbal products were selected for this study. These materials were bought and used to carry out *Allium cepa* test.

2.2 *Materials*

Forty-eight (48) onion bulbs, alcoholic products (orijin bitters, agbara, blackwood, jedi-jedi and action bitters), microscope, distilled and tap water, refrigerator, 1N HCl, acetic orcein, carnoy's fixative (ethanol-acetic acid 3:1), petri dish, glass cups, microscope slides, nail polish, holder (toothpick), hand gloves, nose masks, razor blade, glass rod.

2.3 *Component of alcoholic products used*

- ***Action Bitters***

Ingredients: Herb Extracts (Symphonia Globulifera, Garcinia Kola, Tetrapleura Tetraptera, Lannea Welwitschii), Demin Water, Ethyl Alcohol, Colours: E150 and Brandt Flavour. % alcohol content = 40% in 100ml.

- ***Black Wood***

Ingredients: Water, Ethyl Alcohol, Plant Extract (Quaseinoids), Glyceine, Caramel. % alcohol content = 40% in 120ml.

- ***Agbara***

Ingredients: Ethyl Alcohol, Bitter Extract, Caramel, Flavor, Water. % alcohol content = 33% in 125ml.

- ***Jedi-Jedi***

Ingredients: Water, Ethyl Alcohol, Plant extracts, Natural Flavor. % alcohol content = 33% in 125ml.

- ***Orijin Bitters***

Ingredients: Neutral Spirit, Sugar, Citric Acid, Trisodium Citrate, Caramel, Extracts (Naartjie, Chamomile, Thyme, Cinnamon, Orange). % alcohol content = 30% in 200ml.

2.4 *Growing of rootlets*

The onion bulbs were sun-dried for two weeks and the dried roots present at the base of the onion bulbs were carefully shaved off with a razor blade to expose the fresh meristematic tissues. The experiment was set up by allowing rootlets to grow by placing all bulbs in a small 12 mL glass cup containing tap water, for rootlets emerge.

2.5 *Treatment of A. cepa roots*

The treatments were grouped into 25%, 50% and 75% concentrations for each alcohol herbal product, using five (5) onion bulbs per group. The *A. cepa* roots were exposed to treatment for 12 hours. After 112 hours, the root tips were washed thoroughly in distilled water.

2.6 *Fixing of A. cepa roots*

The root tips of *A. cepa* were cut into a properly labeled disposable cups and fixed in carnoy's fixative (ethanol-acetic acid 3:1) for 24 hours in a refrigerator. The fixed rootlets were then transferred to 70% ethanol and be kept under refrigeration.

2.7 *Microscopic staining of A. cepa roots.*

The *A.cepa* root tips were hydrolyzed with 1N HCl and incubated inside a water bath at 60°C for 5 minutes. The roots tips were washed after hydrolysis with distilled water. Microscopic slides were prepared by squashing (using a glass rod) the *A. cepa* meristem on the slide and adding a drop of 2% aceto-orcein stain for two minutes. Three (3) slides were prepared per treatment group. A cover slip was placed over the slide carefully to avoid air bubbles. The slide covers were then sealed with nail polish on the sides of the slide. Each slide was examined under a microscope at X400 magnification.

2.8 *Parameters used for determination of genotoxicity*

(i) The mitotic index (MI) was calculated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage and

(ii) Chromatin aberrations (stickiness, breaks and polar deviation) were used as endpoints for determination of cytogenetic effects and micronuclei (MNC) were scored in interphase cells per 1000 cells (‰ MNC). The most frequent abnormalities are shown in microphotographs.

3. Results

3.1 Mitotic Index (MI) and Mitotic Phases of *Alium Cepa* roots with Different Concentrations of Alcoholic Herbal Products

The mitotic index is a measure of cellular proliferation. It is defined as the percentage of cells undergoing mitosis (prophase, metaphase, anaphase and telophase) in a given population of cells. Mitosis is the division of somatic cells into two daughter cells. An elevated mitotic index indicates more cells are dividing. It is used to identify the sites of growth.

The result as seen in Table 1, patterned a decreased *mitotic index* as the *concentrations* of the selected alcoholic herbal products increased. Low mitotic index reflects a direct genotoxic effect of alcoholic herbal products. Orijin bitters treatment group had the highest mitotic index (more dividing cells) with respect to the various concentration (25%, 50% and 75%).

Table 1: Mean mitotic index (%) of *allium cepa* cells at 25% alcoholic polyherbal concentration

Alcoholic Herbal Products						
	P	**M**	**A**	**T**	**CA**	**MI ± SEM (%)**
Control	14	5	4	3	11	17.33 ± 1.09
Orijin bitters	45	35	16	8	8	56.33 ± **1.19**
Blackwood	34	18	18	12	13	41.33 ± **0.54**
Action bitters	32	16	11	2	23	30.66 ± **0.54**
Jedi-Jedi	41	16	26	10	13	46.33 ± **0.27**
Agbara	26	28	10	9	14	46. 33 ± **0.27**

MI = mitotic index; CA = chromosomal aberration; P= prophase; M = metaphase; A = anaphase; T = telophase
200 cells (three slides) for 25% alcoholic polyherbal concentration. MI = mitotic index; SEM = standard error of mean. The results are expressed as the mean ± SEM; * significant at $p \leq 0.05$ as compared to control.

Table 2: Mean mitotic index (%) of *allium cepa* cells at 50% alcoholic polyherbal concentration

Alcoholic Herbal Products						
	P	**M**	**A**	**T**	**CA**	**MI ± SEM (%)**
Control	14	5	4	3	11	17.33 ± 1.09
Orijin bitters	46	28	20	10	14	51.66 ± **0.72**
Blackwood	32	19	15	8	17	37.00 ± **0.47**
Action bitters	27	14	10	2	19	26.66 ± **0.54**
Jedi-Jedi	38	19	19	9	12	42.33 ± **0.27**
Agbara	39	22	18	11	21	45.00 ± **0.47**

MI = mitotic index; CA = chromosomal aberration; P= prophase; M = metaphase; A = anaphase; T = telophase
MI = mitotic index; P= prophase; M = metaphase; A = anaphase; T = telophase
200 cells (three slides) for 25% alcoholic polyherbal concentration. MI = mitotic index; SEM = standard error of mean. The results are expressed as the mean ± SEM; * significant at $p \leq 0.05$ as compared to control.

Table 3: Mean mitotic index (%) of *allium cepa* cells at 75% alcoholic polyherbal concentration

Alcoholic Herbal Products						
	P	**M**	**A**	**T**	**CA**	**MI (%) ± SEM**
Control	14	5	4	3	11	17.33 ± 1.09
Orijin bitters	41	26	24	8	19	50.00 ± 0.94
Blackwood	31	14	17	8	17	35.00 ± 0.47
Action bitters	27	6	12	3	25	24.44 ± 0.72
Jedi-Jedi	39	24	13	6	21	41.00 ± 0.47
Agbara	36	26	15	2	21	39.66 ± 0.27

MI = mitotic index; CA = chromosomal aberration; P= prophase; M = metaphase; A = anaphase; T = telophase
200 cells (three slides) for 25% alcoholic polyherbal concentration. MI = mitotic index; SEM = standard error of mean. The results are expressed as the mean ± SEM; * significant at $p \leq 0.05$ as compared to control using one way analysis of variance (ANOVA)

3.2 Photomicrograph of mitotic stages

The photomicrograph of the stages of mitotic division exhibited in the *Allium cepa* cells treated with various concentrations of the alcoholic herbal products are shown in figure 1. The microscopic slides were scored at X400 magnification. The photomicrograph results showed the cells dividing at an enlarged view, thereby enabling proper counting of cells.

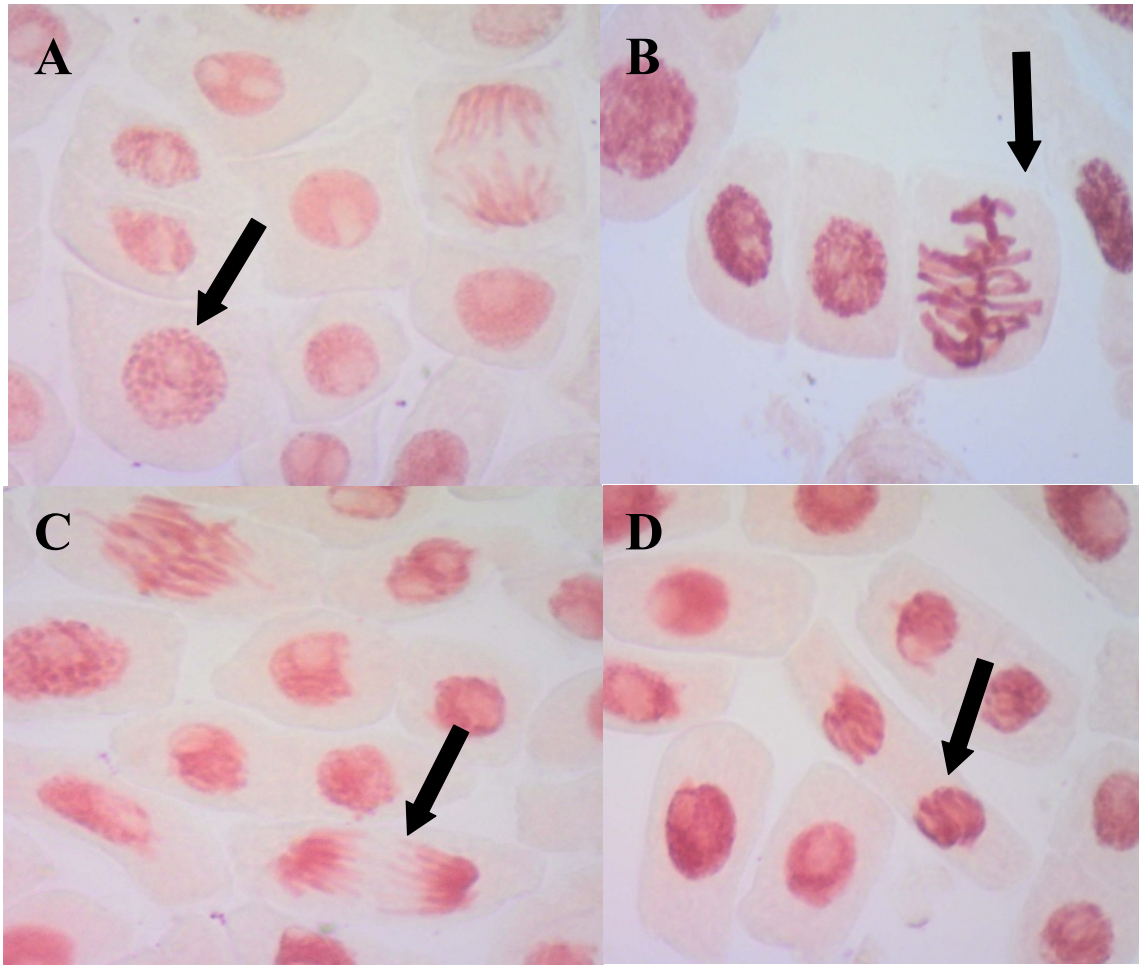

Figure 1: Stages of normal mitotic division in root cells of *Allium cepa* on exposure to distilled water (control (A) Prophase (B) Metaphase (C) Anaphase (D) Telophase. Magnification at X400

3.3 Genotoxic Analysis

The polyherbal *alcoholic* products provoked strong chromosomal abberation (Figure 2) in the treatment group when compared with the control (Figure 1). The decrease in the mitotic index and cytogenic alteration is positively correlated with increasing concentration of the alcohol concentration, as shown in Table 1, 2 and 3. The polyherbal alcoholic products induced chromosomal and cytological alterations in treatment groups as indicated in figure 2. The observed chromosomal break implies the clastogenic effect of the polyherbal alcoholic products on *Allium cepa* root cells. In addition to the chromosome fragments, sticky metaphase and polar deviations (wrong directions of chromosome movement) were also observed. Significant aberrant chromosomes as seen in the groups treated with polyherbal alcoholic product (Figure 2) when compared with the control group (Figure 1) that is treated with distilled water. The photomicrograph shows the genotoxic activities of the polyherbal alcoholic products as seen in cells with lobulated nucleus (Figure 2E).

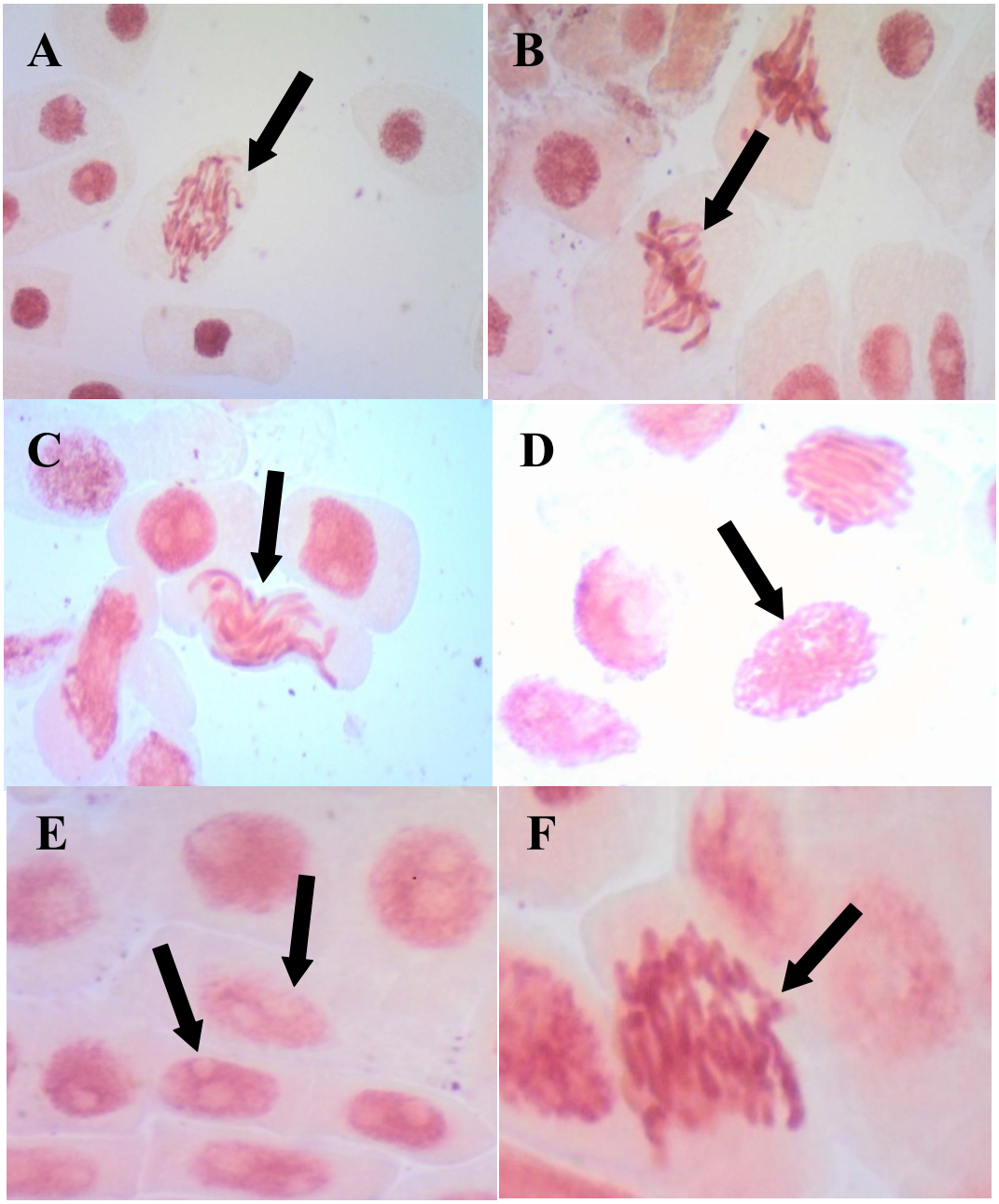

Figure 2: Meristematic cells of allium cepa showing different chromosomal aberrations after treatment with polyherbal alcoholic products. Magnification at x400.

(A) Irregular anaphase, indicating chromosomal breakage as seen in 75% orijin polyherbal alcoholic treatment (B) Numerical alteration, due to duplication of the number of chromosomes at metaphase as seen in 50% action bitters polyherbal alcoholic treatment (C) Irregular metaphase, with unorganized chromosomes with no orientation on the equatorial plate as seen in 75% blackwood polyherbal alcoholic treatment (D) Irregular prophase showing decompressed chromosomes as seen 75% in jedi-jedi polyherbal alcoholic treatment (E) Cells with lobulated nucleus as seen in 75% agbara polyherbal alcoholic treatment (F) Fragmented anaphase as seen in 75% jedi jedi polyherbal alcoholic treatment.

4. Discussion

The genotoxic effects of polyherbal alcoholic products (origin bitters, blackwoods, action bitters, jedi jedi and agbara) on *Allium cepa* root tips were evaluated. Genotoxicity was estimated by observing cytological parameters such as the mitotic index and number of chromosome abnormalities, including chromosome breaks, stickiness, and polar deviations. The mitotic index are presented in Table 1, 2 and 3, while Figure 2 shows the various chromosomal aberrations resulting from the effects of polyherbal products on *Allium cepa* root tips. Frequent consumption of polyherbal alcoholic products have been reported to show significant increase in chromosomal aberrations and sister-chromatid exchanges (SCE) two

cytogenetic endpoints that are considered to be an expression of genetic damage induced by physical or chemical agents [5]. To this effect, plant chromosomes have also been shown to be sensitive to ethanol [3].

The observation of altered metaphase (Figure 2B) reinforces the hypothesis of the toxic effect of alcoholic herbal products. Chromosomal aberrations (CA) are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the CA observed in cells is lethal, but there are many related aberrations that are viable and that can cause genetic effects, either somatic or inherited. The presence of chromosomal fragments (Figure 2F) is an indication of chromosome breaks, and can be a consequence of anaphase bridges. The mitotic index decreases with increasing concentration of polyherbal alcoholic products as seen in Table 1, 2 and; also, the frequencies of chromosome aberrations increases as the concentrations of the polyherbal alcoholic products increases (Figure 2). This is an indication of clastogenic activity. These results are in line with the results of many research groups that examined the effects of different medicinal herbs] [8].

Several other herbal extracts have been reported to inhibit mitosis [1,2]. The decreased mitotic index in *Alium cepa* roots treated with alcoholic herbal productsvis probably due to either disturbances in the cell cycle or chromatin dysfunction induced by an external factor. The results herein suggest that the tested *alcoholic product* concentrations have inhibitory, mito-depressive effects on root growth and cell division of *Allium cepa* and it can prevent DNA synthesis and the reduction in number of the dividing cells in roots produced by the genotoxic effects of compounds found in *alcoholic* products.

5. Conclusion

This study evaluated the geotoxicity effect of selected alcoholic polyherbal products using *Allium cepa* test. Mitotic index value was generally dependent on the concentration of the alcoholic polyherbal products; the higher the concentration of the alcoholic polyherbal product, the higher the mitotic-suppression in the *Allium cepa* cells. It was also observed that the alcohol percentage played a role in the inhibition of mitotic index.

Chromosomal aberrations (CA) were also observed in this study, with the highest CA being 25%, which was found in 'action bitters' 75% concentration and the lowest chromosomal aberration being 8%, which was found in 'orijin bitters' 25% concentration. This shows that polyherbal alcohol products possess potential genotoxic effect when consumed by humans as depicted in the *Allium cepa* test. Hence, adequate regulatory measure should be enforced on the production and consumption of alcoholic polyherbal products.

Acknowledgement

I duly appreciate the Department of Biochemistry for providing the enabling laboratory space to successfully carry out this research work.

References

[1]. Akinboro, A., & Bakare, A. A. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa Linn. *Journal of Ethnopharmacology*, 2007; 112(3): 470–475.

[2]. Aşkin Çelik, T., & Aslantürk, Ö. Anti-mitotic and anti-genotoxic effects of Plantagolanceolata aqueous extract on *Allium cepa* root tip meristem cells. *Biologia*, 2006; 61(6).

[3]. Jansson, T. The frequency of sister chromatid exchanges in human lymphocytes treated with ethanol and acetaldehyde. *Hereditas*, 2008; 97(2); 301–303.

[4]. Maduabuchi, J.-M. U., Nzegwu, C. N., Adigba, E. O., Oragwu, C. I., Agbo, F. N., Agbata, C. A., Ani, G. C., &Orisakwe, O. E. Iron, Manganese and Nickel Exposure from Beverages in Nigeria: A Public Health Concern? *Journal of Health Science*, 2008; 54(3): 335–338.

[5]. Maffei, F. Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. *Mutation* 2004

[6]. Martin, J. Global institutions: the World Health Organization (WHO). *Bulletin of the World Health Organization*, 2009; 87(6); 484–484.

[7]. Okareh, O., Oyelakin, T., & Ariyo, O. Phytochemical Properties and Heavy Metal Contents of Commonly Consumed Alcoholic Beverages Flavouredwith Herbal Extract in Nigeria. *Beverages*, 2018; 4(3): 60.

[8]. Rojas, E., Herrera, L. A., Sordo, M., Gonsebatt, M. E., Montero, R., Rodriguez, R., & Ostrosky-Wegman, P. Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. *Anti-Cancer Drugs*, 1993; 4(6): 637–640.