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Assessing the Genotoxic Effects of Aqueous Leaf Extract of Neem (*Azadirachta indica* A. Juss), Cosmetics and Alomo Bitters (Alcoholic Drink) Using *Allium* Test

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors participated in this research work while authors GCO and CE read and approved the final manuscript.

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ABSTRACT

Genotoxic effect of aqueous leaf extract of Neem (*Azadirachta indica*) NZI, Alomo bitters alcoholic drink (ABAD) and cosmetics (Baby-face facial cleanser, BFFC; Peau clair body lotion, PCBL and Vanilla essence roll-on, VERO) were assessed using *Allium* test. The root tips were treated with serial dilutions of the test chemicals at 20%, 40%, 60% and 100% for 3 hours along with the control, hydrolyzed in 18% HCl, squashed and stained with FLP orcein. Aberrations such as C-metaphase, precocious, stickiness (sticky metaphase), ghost cells, nuclear lesions, chromosome erosion, laggards, binucleated cells, anaphase bridges and vagrant chromosomes were observed in the root tips studied. These products are used widely by humans around the world and Nigeria in particular. Although, the products elicited varying chromosomal abnormalities, they also have beneficial effects. Also, the products showed genotoxic effects even at low concentrations on *Allium cepa* root

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cells. In a long run, the use of these products may cause serious damages on the chromosome of plants, animals and humans. More effort is required to actually ascertain the active constituents of these products that elicited the genotoxic effects. Constant use and consumption of these products is therefore discouraged.

Keywords: Allium test; cosmetics; genotoxicity; chromosome aberration.

1. INTRODUCTION

Allium cepa (2n =16), constitutes a very convenient test system for estimating the harmful effect of chemicals on biological materials. *Allium* test is distinctive in regards to its efficiency in detecting genetic damage while investigating the effect of colchicine Levan [1]. The *Allium* test is an efficient test for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants [2-6]. This has been used to evaluate the genotoxic effects of the plant extracts on mammalian test system and more accurate evaluation of environmental risks, as well as the extrapolation of data to other organisms such as humans [7-8]. For cytogenetic testing of various genotoxic substances, *Allium* test, a classical, low budget and short term biological assay has been proposed as a standard method [9-11]. Relevant studies [2] showed the importance of the *Allium test* system for evaluating genotoxicity, demonstrating that *A. cepa* cells contain an oxidase enzyme system capable of metabolizing polycyclic hydrocarbonates. Vincentini [12] reported that the *Allium* test system is well accepted for the analysis of cytotoxicity and genotoxicity because the roots are in direct contact with the tested substance, allowing evaluation of different concentrations and time. It sensitivity to the toxicity and tetratogenicity of several chemicals has been reported [13].

Today, there is extensive consumption of herbal preparation such as aqueous leaf extract of Neem (*A. indica)* and Alomo bitters alcoholic drink [14]. In the same vein, several chemicals are used as cosmetics without a thought to their harmful effects on genetic makeup of humans. The need to understand the interaction of these substances in relation with the human chromosome made this research essential. The aim of this research work is to determine the genotoxic and cytotoxic effects of aqueous leaf extract of Neem (*A. indica*), Alomo bitters alcoholic drink, Cosmetics (Babyface facial cleanser, Vanilla essence roll on and Peau clair body lotion) on *Allium cepa* root meristem.

2. MATERIALS AND METHODS

2.1 Sources of Materials

Commercial onion bulbs (*Allium cepa*) of similar size were obtained from Choba market in Port Harcourt, Rivers state in April, 2015. Also, the test chemicals (Alomo bitters alcoholic drink, Babyface facial cleanser, Peau clair body lotion and Vanilla essence roll on were also obtained from Choba market and the Neem leaves harvested from Neem trees growing in the Abuja Park, University of Port Harcourt.

2.2 Genotoxicity Test

A discontinuous treatment protocol similar to the method of [15] was employed. Twenty-four healthy onion bulbs were used. The loose outer scales were carefully removed. The dry bottom plates were scraped off with sharp razor blade without destroying the root primordia and were washed in clean tap water. The washed onion bulbs were grown in 100ml beakers for 2 days (48 hours) in distilled water to induce new root formation and maintained the roots attained a length of 1-2 cm (Fig. 1).

2.2.1 Preparation of aqueous leaf extract of *A. indica* (Neem)

Fresh leaves were collected from different stands of *A. indica* growing the Abuja Park, University of Port Harcourt in April, 2015. The leaves were rinsed and air-dried at room temperature by spreading them on laboratory table for 24 hours. 560g of the fresh leaves were weighed, blended and soaked in 50 ml of distilled water for 24 hours and filtered. The filtrate is now used as the stock aqueous leaf extract of Neem.

2.2.2 Dilution of the stock (Test chemical)

Serial dilutions of 20%, 40%, 60%, and 100% were made (using the volume per volume stocks / distilled water v/v) method. The respective diluted concentrations were aqueous leaf extract of Neem (*A. indica*), Alomo bitters alcoholic

Fig. 1. Experimental setup for *A. cepa* root induction
(NZI=Aqueous leaf extract of Neem; ABAD = Alomo bitters Alcoholic drink; BFFC = Baby face facial cleanser; PCBL = Peau clair body lotion and VERO = Vanilla essence roll on)

drink, locally made soft drink (Zobo), cosmetics (Babyface facial cleanser, Vanilla essence roll on and Peau clair body lotion), which were used as treatments for each products test. The measurement was carried out with the aid of measuring cylinder and the required volume concentration was made up with distilled water. Agitation of the stock ensures proper dispersion of the concentration. Also, four beakers were setup as controls as shown in Fig. 1.

2.2.3 Pretreatment and harvesting of roots

The onion bulbs grown in distilled water were raised up, transferred to beakers containing the different test solution and allowed for 3 hours. The control was allowed in distilled water for this same period of time. Thereafter, 40 roots from four onion bulbs were excised and immediately introduce in specimen bottles containing Carnoy's fluid: 1:3 (v/v) Glacial acetic acid and alcohol (Glacial acetic acid 1 part and absolute alcohol 3 parts) for 24 hours and stored in 70% ethanol in refrigerator until required for slide preparation [9].

2.2.4 Slide preparation

The fixed roots tips were hydrolyzed with 18% HCl for 10 minutes, rinsed in 70% ethanol and about 0.5 mm of the root tip was excised, stained with FLP orcein for 5 minutes and squashed on a slide [16]. The slides were left to stand for 10 minutes and sealed with the nail polish and photomicrographs of the different chromosomal aberrations were captured at ×40 objective.

2.2.4.1 Calculations

Ten of the prepared slides were selected and the values of mitotic index, mitotic inhibition, phase index and percentage chromosomal aberration were calculated [16-17] and the formulae are as follows:

$$\text{a). Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

$$\text{b). Mitotic inhibition} = \frac{\text{Mitotic index in control - Mitotic index in treatment}}{\text{Mitotic index in control}} \times 100$$

$$\text{c). Phase index} = \frac{\text{Number of cells on each mitotic phase}}{\text{Number of dividing cells}} \times 100$$

$$\text{d). Percentage aberration} = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells counted}} \times 100$$

2.2.5 Statistical calculation

The data from this study was subjected to statistical analysis to determine the mean and standard deviation using Microsoft excel 2010.

3. RESULTS

3.1 Chromosomal Aberrations and Normal Mitotic Cells

All the test substances used for this study showed one form of aberration or another on the chromosomal integrity of *A. cepa* but we also observed other stages of mitotic cell division (Tables 1-4). These aberrations and normal mitotic cells include; nuclear lesion (Fig. 2a), precocious chromosome and laggard (Fig. 2b), normal anaphase (Fig. 2c), sticky metaphase (Fig. 2d), normal metaphase (Fig. 2e), C-metaphase (Figs. 2f and k), precocious chromosomes (Fig. g-h), normal anaphase (Fig. 2i), Anaphase bridge (Fig. 2j) and ghost cells (Fig. 2l). Other chromosomal aberrations observed are vagrant and binucleated cells.

Aberrations such as C-metaphase and precocious chromosomes were observed in all the concentrations of the test substances used while stickiness (sticky metaphase) was observed in all the concentrations of the test chemicals except 60% NZI and 20% VERO. Ghost cells were not observed in 100% NZI, 60% and 100% BFFC and all concentrations PCBL but Nuclear lesions was found only in all concentrations of PCBL (Table 1). Chromosome erosion was not observed in all the concentrations of PCBL and 20% ABAD but was found in all other concentrations of the test chemical. All concentrations of ABAD and VERO did not have laggards and binucleated cells in the vein, in BFFC, 20%, 60% and 100% did not have laggards and the 60% and 100% concentrations did not have binucleated cells. Similarly, binucleated cells were not observed in 60% and 100% concentrations of NZI and BFFC (Table 1). Bridges was observed in all the products but more in NZI, PCBL and VERO however, they were observed in 100% ABAD; 20% and 100% BFFC. No vagrant chromosome was observed in all the concentrations of VERO but some were observed NZI (20% and 60%), ABAD (20%, 40% and 100%), BFFC (40% and 100%) and BCBL (20%, 40% and 100%).

3.2 Mitotic Index

The values recorded in the mitotic index were lower in the treatments when compared to the control which had the highest mitotic index of 9.40% (Table 3). The range of mitotic indices in the other test chemicals are as follows; NZI (4.37 – 5.85), ABAD (4.47 – 4.82), BFFC (4.19 – 5.90), PCBL (4.62 – 6.57) and VERO (5.4 – 5.8). The least mitotic index (4.37) was observed in 100% NZI (Table 3).

3.3 Mitotic Inhibition

Table 3 shows the mitotic inhibition effects of aqueous leaf extract of Neem (NZI), Alomo bitters alcoholic drink (ABAD), Baby-face facial cleanser (BFFC), Peau clair body lotion (PCBL) and Vanilla essence roll-on (VERO). The mitotic inhibition increased with an increase in the concentrations of NZI, ABAD, BFFC and VERO while in PCBL, it increases and decreases at different concentrations. The percentage inhibition in the test chemicals varied from 30.1% in PCBL (40%) concentration to 55.4% in BFFC (100%) concentration. The percentage inhibition in the test chemicals include: NZI (37.8% - 53.5%); ABAD (48.7% - 52.5%); BFFC (37.2% - 55.4%); PCBL (30.1% - 50.9%) and VERO (38.3% - 42.6%). In the control, the percentage inhibition is zero (0) indicating that the distilled water does not have any inhibition effect (Table 3).

3.4 Percentage Phase Index (PPI)

This is the percentage of the cells in several phases of the cell division (Prophase, Metaphase, Anaphase and Telophase). There was an increase in the prophase index with an increase in the concentration of NZI (Table 4). The highest prophase index was observed in VERO at 20% concentration with the value of 60.0%. This implies that the product induces prophase formation at 20% concentration of VERO. The maximum metaphase index was observed in 20% concentration of PCBL (Table 4). Similarly, the maximum anaphase index (30.4%) was observed at 60% concentration of PCBL and 100% concentration of VERO while the highest telophase index (31.2%) was observed in 100% concentration of BFFL (Table 4).

Table 1. Frequencies of chromosomal aberrations observed in *Allium cepa* root tips treated with the different test chemicals

Test chemicals	Conc. (%)	Number of cells										Total	% aberration
		SM	CM	GC	NL	CE	L	B	PC	V	BN		
Control	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.0**
Aqueous	20	3	10	3	2	1	1	6	2	-	5	33	6.9
leaf extract	40	8	14	7	15	13	7	10	3	1	5	83	18.8
of Neem	60	-	15	18	25	10	-	12	2	-	-	77	18.2
(NZI)	100	6	18	-	28	13	-	14	5	1	-	85	20.6
Alomo	20	5	8	22	19	-	-	5	1	-	-	60	12.6
bitters	40	3	10	26	20	15	-	7	3	-	-	84	17.9
(ABAD)	60	4	17	25	20	9	-	8	4	1	-	88	20.2
	100	2	11	27	22	3	-	-	6	-	-	71	16.7
Baby-face	20	8	18	10	-	9	-	-	1	2	6	54	12.3
facial	40	9	13	20	10	3	2	9	3	-	1	70	16.5
cleanser	60	2	20	-	15	5	-	7	2	2	-	53	12.9
(BFFC)	100	7	15	-	23	9	-	-	6	-	-	60	15.7
Peau clair	20	3	2	-	-	-	5	4	1	-	2	17	4.1
body lotion	40	3	4	-	-	-	15	5	1	-	4	32	9.1
(PCBL)	60	5	11	-	-	-	7	4	2	2	5	36	9.3
	100	7	22	-	-	-	2	5	2	-	1	39	12.1
Vanilla	20	-	4	21	18	2	-	6	1	-	-	52	11.6
essence	40	3	5	12	20	6	-	2	3	-	-	51	11.7
Roll-on	60	2	10	20	23	8	-	4	2	-	-	69	16.2
(VERO)	100	2	12	25	20	5	-	2	4	-	-	70	16.6

Note: CM = C-metaphase; SM = Sticky metaphase; GC = Ghost cell; NL = Nuclear lesions; CE = Chromosome erosion; L = Laggard; BR = Bridges; PE = Precocious chromosome; BN =Binucleated cells, V = Vagrant

Table 2. Mean and standard deviation of chromosomal aberrations observed in *A. cepa* root tips treated with five different test chemicals

Chromosomal aberrations	Test chemicals				
	NZI	ABAB	BFFC	PCBL	VERO
Sticky	4.25 ± 3.50	3.5 ± 1.29	6.5 ± 3.11	4.5 ± 1.91	1.75 ± 1.3
C-Metaphase	14.25 ± 3.30	11.5 ± 3.9	16.5 ± 3.11	9.75 ± 9.03	7.75 ± 3.9
Ghost cell	5.75 ± 5.62	25 ± 2.16	7.5 ± 9.57	0.0 ± 0 .0	19.5 ± 5.4
Nuclear lesions	17.5 ± 11.7	20.3 ± 1.26	12 .0 ± 9.63	0.0 ± 0.0	20.25 ±2.1
Chromosome erosion	9.25 ± 5.68	6.75 ± 6.70	6.5 ± 3. 00	0.0 ± 0.0	5.25 ± 2.5
Laggards	2.0 ± 3.37	0.0 ± 0.00	0.5 ± 1.00	7.25 ± 5. 6	0.0 ± 0.0
Bridges	9.75± 3.90	5.0 ± 3.56	4.0 ± 4.69	4.5 ±0.58	3.5 ± 1.91
Precocious chromosome	3.0 ±1.41	3.5 ± 2.10	3.0 ± 2. 16	1.5 ± 0.58	2.5 ± 1.29
Vagrant chromosome	0.5 ± 0.58	0.25 ± 0.50	1.0 ± 1.15	0.5 ± 1.00	0.0 ± 0.0
Binucleated cells	2.5 ± 2.89	1.5 ± 3.00	0.75 ± 0.96	3 ± 1.83	0.0 ±0.0

Note: Aqueous leaf extract of Neem (NZI); Alomo bitters Alcoholic drink (ABAD); Baby face facial cleanser (BFFC); Peau clair body lotion (PCBL); Vanilla essence roll on (VERO)

Table 3. Mitotic indices, Mitotic inhibition and frequencies of mitotic phases of root tips of *A. cepa* treated with five different test chemicals

Test chemicals	Conc. (%)	NCC	Number of dividing cells				NDC	MI (%)	MIH (%)	AMIH (%)
			P	M	A	T				
Control	**0**	**500**	**18**	**12**	**8**	**9**	**47**	**9.40**	**0**	
Aqueous leaf extract of Neem (NZI)	20	478	8	5	7	8	28	5.85	37.8	45.20
	40	441	7	6	5	7	25	5.66	39.8	
	60	422	8	5	5	2	20	4.73	49.7	
	100	411	9	2	4	3	10	4.37	53.5	
Alomo bitters alcoholic drink (ABAD)	20	477	13	4	3	3	23	4.82	48.7	50.63
	40	468	11	2	4	5	22	4.70	50.0	
	60	436	7	5	3	5	20	4.58	51.3	
	100	425	10	2	3	4	19	4.47	52.5	
Baby-face facial cleanser (BFFC)	20	440	11	4	5	6	26	5.90	37.2	45.20
	40	424	10	6	3	5	25	5.89	37.3	
	60	411	9	3	5	2	19	4.62	50.9	
	100	381	6	4	1	5	16	4.19	55.4	
Peau clair body lotion (PCBL)	20	411	5	6	3	5	19	4.62	50.9	39.95
	40	350	8	5	7	3	23	6.57	30.1	
	60	383	10	3	2	6	21	5.48	41.7	
	100	321	9	5	2	3	19	5.91	37.1	
Vanilla essence roll-on (VERO)	20	447	15	5	5	1	26	5.80	38.3	40.08
	40	436	8	8	4	5	25	5.70	39.4	
	60	426	9	6	5	4	24	5.60	40.4	
	100	421	8	5	7	3	23	5.40	42.6	

Note: P = Prophase; M = Metaphase; A = Anaphase; T = Telophase; NCC = Number of cells counted; NDC = Number of dividing cells; MI = Mitotic index; MIH = Mitotic inhibition and AMIH = average mitotic inhibition

4. DISCUSSION

There is evidence that the five products i.e. aqueous leaf extract of Neem (NZI), Alomo bitters alcoholic drink (ABAD), cosmetics [Peau clair body lotion (PCBL) Babyface facial cleanser (BFFC) and Vanilla essence roll on (VERO)] used in this study excited chromosomal aberrations in *Allium cepa* root cells. These aberrations are termed mitodepressive, mitoclassic and chromatoclassic effects [18]. The observed aberrations in Table 3 such as nuclear lesions, binucleated cell, C-metaphase, precocious chromosome, bridges and laggards have also been reported by [19-25]. The higher concentrations caused an inhibition of the normal

mitotic phases and there was a significant difference between the treatment concentration and control group. The normal cell cycle was disrupted by the five products evaluated and the effect varied with the products. Such observation is not different from results obtained elsewhere using *Allium* test. The potential cytotoxic and genotoxic effects were estimated by observing cytological parameters such as mitotic index, number and type of chromosomal aberration which include sticky chromosomes in metaphase, bridges in anaphase, C-metaphase, ghost cell, laggards, vagrant chromosomes, precocious chromosomes, nuclear lesions and binucleated cells. When the products were tested on the *Allium cepa* cells to evaluate their action on the kinetics of the cell cycle, a decrease in their mitotic indices were observed which was more marked at 100%, 60%, 40% and 20% of the chemicals used as shown in Table 1. Comparatively, some named concentrations have more mitodepressive effects than other concentrations. Mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death or a delay in the cell proliferation kinetics [26]. Reduction in the mitotic activity could be due to inhibition of DNA synthesis or a blocking in the G_2 phase of the cell cycle, preventing the cell from entering mitosis [27]. Mitodepressive effect of some plant extracts, including the ability to block DNA synthesis and nucleus proteins were reported earlier [28-29]. They may not even allow the inhibition of their biosynthesis and such action occurring in the interphase nucleus apart from influencing the ultimate structure of the chromosome during cell division could cause reduction of number of other stages. Several other herbal extracts have been reported to inhibit mitosis [21,30-31]. The result here in, suggests that the evaluated substances have inhibitory, mitodepressive, mitoclassic and chromatoclassic effects on cell division of *A. cepa* and it can prevent DNA synthesis and the reduction in number of the dividing cells in roots produced by the cytotoxic effect of compounds found in these chemicals. ABAD and NZI showed the strongest genotoxic effect in *A.cepa* root meristem cells. The observation of the sticky metaphase reinforces the hypothesis of the toxic effects of the test chemicals. Metaphase with sticky chromosome loses their normal appearance and they are seen with sticky "surface", causing chromosome agglomeration [32]. Chromosome stickiness is reported to be due to DNA depolymerisation [33], partial dissolution of nucleo-proteins [34] and stripping of the protein covering of DNA [35]. Since this products elicited similar effects, it clearly indicated that they interacted with the DNA molecules to produce these aberrations also. Inhibition of the mitotic apparatus that leads to spindle formation most likely led to the C-metaphase stages as seen in other plants like *Vigna unguiculata* [36]. C-metaphase was also observed by [37-38]. Ronne [39] explained the C-metaphase is as a result of reduction in stainability in certain regions of chromosomes which result from the inhibition of non-histone protein synthesis. Chromosome aberrations are changes in chromosome structure resulting from break or exchange of chromosomal material. Most of the chromosomal aberrations observed in cell are lethal, but there are many related aberrations that are viable and that can cause genetic effects, either somatic or inherited [40]. The presents of vagrant chromosome is an indication of spindle poisoning [41]. The effect increases the risk of aneuploidy because during chromosome movement and subsequent cell division, the chromosome may be too slow to meet up with the other chromosomes and is eventually lost [2]. Somehow, the treatment not only interferes with the cell cycle, but also affects chromatin organization or DNA replication, causing chromosome breaks. Frequencies of total chromosomal aberrations increase significantly upon exposure to the five chemicals which indicate clastogenic activity (Table 1). This result is in line with the results of many research groups that examined the effects of different medicinal herbs [20-21,42]. This result showed induction of chromosome or chromatid type of aberration in the treated cell. Chromosome bridges are signs of clastogenic effects resulting from chromosomes and chromatids breaks. The formation of bridges has been attributed to several causes which include:

i. Breaks that may occur in both chromatids of the same chromosome and incorrect rejoining of the sticky ends to form a sister union [43-44].
ii. Incomplete replication of chromosomes by defective or less active replication enzymes [45] or through breaks that may occur late in the cell cycle (in G_2) after the chromosomes have replicated [44].
iii. Late replication DNA sequences of the telomeric heterochromatin [46-47]. According to [22] reported that chromosome bridges could occur if heterochromatic DNA sequencies do not complete DNA replication when the

nucleus is ready to divide. According to [48-49] suggested that during the metabolism of (*Azadirachta indica* (Neem), electrophilic ions and radicals are produced and that these interact with the nucleophilic sites in DNA. Hall and Garcia [44] also noted that Anaphase bridge is one of the 3 types of aberrations that are lethal to the cell. Bridges cause structural chromosome mutations (duplications or deletions in DNA double -strand) [50-51]. The presence of precocious chromosomes is thought to be due to unequal spindle movement where some chromosome arms are pulled towards the extremity of the pole [52-53]. Lagging chromosomes have been a regular feature of many cytotoxicity/genotoxicity studies with medicinal plant extracts [54-56]. Sousa [6] explained that such chromosome have the potentials to form micronuclei. The presence of such nuclei according to [56] is a manifestation of the efforts of a main nucleus to eliminate excess DNA in an attempt to restore the normal ploidy condition.

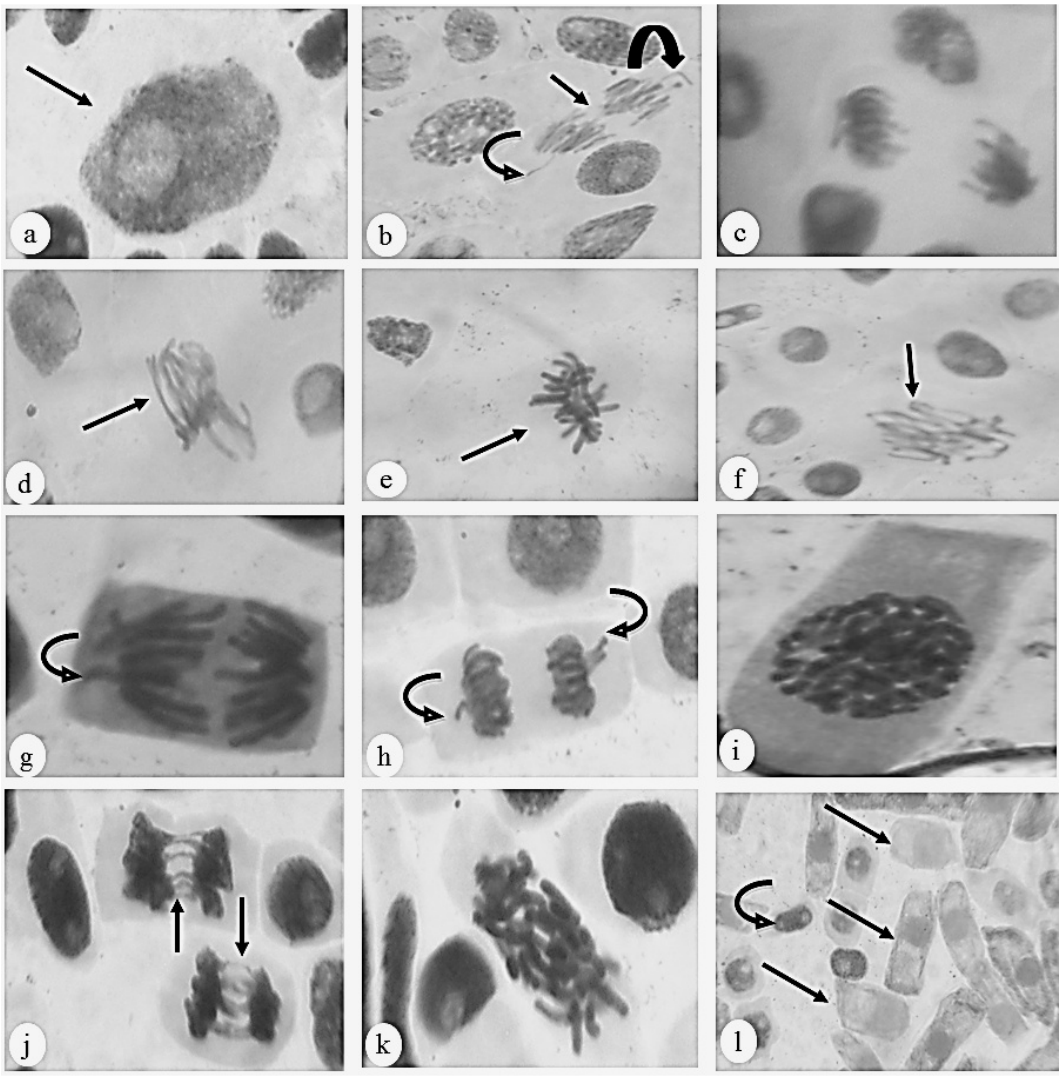

Fig. 2. Normal cells and different chromosomal aberrations (a) Nuclear lesion; (b) curved arrows show precocious chromosomes; straight arrow shows Laggards; (c) Normal anaphase; (d) Sticky metaphase; (e) Normal metaphase; (f) C-metaphase; (g-h); Precocious chromosomes (i) Normal prophase; (j) Anaphase bridge; (k) C-metaphase and (l) Ghost cells

Table 4. The percentage phase index of the different stages of mitosis at different concentrations of the treatment

Test chemicals	Conc. (%)	Number of dividing cells	No. of dividing cells				Phase index			
			P	M	A	T	PI (%)	MI (%)	AI (%)	TI (%)
Control	**0**	**47**	**18**	**12**	**8**	**9**	**38.2**	**25.5**	**17.0**	**19.1**
Aqueous leaf extract of Neem	20	28	8	5	7	8	28.5	17.8	25.0	28.5
	40	25	7	6	5	7	28.0	24.0	20.0	28.0
	60	20	8	5	5	2	40.0	25.0	25.0	10.0
	100	18	9	2	4	3	50.0	11.1	22.2	16.6
Alomo bitters alcoholic drink	20	23	13	4	3	3	56.5	17.3	13.0	13.0
	40	22	11	2	4	5	50.0	9.09	18.1	22.7
	60	20	7	5	3	5	35.0	25.0	15.0	25.0
	100	19	10	2	3	4	52.6	10.5	15.7	21.0
Baby-face facial cleanser	20	26	11	4	5	6	42.3	15.3	19.2	23.0
	40	25	10	6	3	5	40.0	23.0	11.5	19.0
	60	19	9	3	5	2	47.3	15.7	26.3	10.5
	100	16	6	4	1	5	37.5	25.0	6.25	31.2
Peau clair body lotion	20	19	5	6	3	5	26.3	31.5	15.7	26.3
	40	23	8	5	7	3	34.7	21.7	30.4	13.0
	60	21	10	3	2	6	47.6	14.2	9.52	28.5
	100	19	9	5	2	3	47.3	26.3	10.5	15.7
Vanilla essence roll-on	20	25	15	5	5	1	60.0	20.0	20.0	4.0
	40	26	8	8	4	5	30.7	30.7	15.3	19.2
	60	24	9	6	5	4	37.5	25.0	20.8	16.6
	100	23	8	5	7	3	34.7	21.7	30.4	13.0

Note: P = Prophase; M = metaphase; A = Anaphase; T = Telophase; PI = Prophase index; MI = Metaphase index; AI = Anaphase index; TI = Telophase index

However, El-Ghamery [10] was of the opinion that lagging chromosomes sometimes could dissolve in the cytoplasm and thus be lost. This type of aberration (chromosome deletion) is always lethal to humans [44]. A structure-based toxicity relationship for Azadirachtin (Aza), the major active principle in *A. indica* had been proposed [57]. They identified the presence of at least 5 copies of biophores in *A. indica* (Neem) and predicted it to be a potent carcinogen. In addition, Aza was found to be also contain a furan moiety which the investigators speculated may undergo epoxidation during bio-transformation. They concluded that the electronegativity of Aza was of the same order of magnitude as that for DNA-reactive molecules. All these according to authors, point to the fact that Aza has the potential mutagen that has the capability of inducing damage in genetic materials including some clastogenic changes. Thus, the occurrence of the above variety of abnormality in this study is an indication of high mutagenic potential of aqueous NZI. Binucleated cell formation is accepted to be due to inhibition of cytokinesis [58]. The presence of nuclear lesions in cells [28] offers cytological evidence for the inhibitory action of chemicals on DNA biosynthesis. Inhibition of DNA synthesis [59] 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.

Ghost cells observed in this study are dead cells in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable. Ghost cells were observed in groups in samples treated with NZI, BFFC, VERO and ABAD. The treatments lead to nucleus damage and prevention of cytoplasmic structures resulted in ghost cell. The most distinctive chromosomal aberrations and those having the highest observation frequency are stickiness, C-metaphase, anaphase bridges, precocious chromosomes, nuclear lesions and chromosome erosion. The mitodepressive, mitoclassic and chromotoclassic effects of these products probably reflect what goes on in the bodies of people who occupationally come in contact with them directly. Even so, nature has provided away by which such assaults on DNA are repaired thus keeping the rate of mutation /evolution very slow.

However, it is speculated that continuous exposure to these products places this section of the population under an artificial selection pressure. Since this pressure is skewed, it potentially can overwhelm the repair mechanism, induce mutation and cancer and eventually cause mortalities [60]. Eventually, these aberrations can be monitored as shown in this study using the *Allium* test. It is even more desirable since the concentrations of the treatment at which the level of contact by the end users are rather high. A good correlation has also been shown between these aberrations in plant system and those in animal systems [61]. Results here presented, suggest that even at low concentration of the treatment, have genotoxic effects on DNA of plant origin. Since low levels would elicit the same effects on DNA from animal or humans sources [61].

5. CONCLUSION

From this study, it is clear that the treatment of *A. cepa* root tips with aqueous leaf extract of Neem (*A. indica*), cosmetics (Babyface facial cleanser, Peau clair body lotion and Vanilla essence roll on) and Alomo bitters alcoholic drink respectively, resulted in various chromosomal aberrations. We noted that the lowest and highest mitotic indices were recorded in BFFC and PCBL respectively. The reverse was the case in the highest and lowest mitotic inhibition values. The average maximum and minimum mitotic inhibition effects were observed in ABAD and PCBL respectively. These products are used widely by all humans around the globe and Nigeria. Although, these products elicited varying chromosomal abnormalities, they also have beneficial effects i.e. aqueous leaf extract of Neem is used traditionally for the treatment of malaria. These products have shown genotoxic abilities even at low concentrations on *Allium cepa* root cells. In a long run, the use of these products may cause serious damages on the chromosome level of plants, animals and human life. More effort is required to ascertain the active components of these products that elicited these genotoxic effects. Constant use and consumption of these products is therefore discouraged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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