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## Genotoxicity Assessment of Refined Petroleum Products and Popular Local Soft drink (Zobo) in Daily Use in Nigeria

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### ABSTRACT

The genotoxic effects of refined petroleum products (Petrol, Kerosene) and locally made soft drink (Zobo) were assessed with the *Allium* test. The roots were treated with 10, 25 and 50% of these chemicals for 1, 3 and 6 h. These substances exerted depressive effects on mitosis, including reduced mitotic indices and accumulation of mitotic phases. Chromosomal aberrations which include anaphase bridges were observed in petrol and zobo, laggards and vagrant chromosomes observed in zobo while sticky chromosomes were observed in petrol, kerosene and zobo. C-metaphases were observed in kerosene and zobo whereas binucleate cells were observed in petrol and kerosene. Micronuclei were observed in zobo and petrol while ghost cells were observed in root tips treated with the various concentrations of the zobo. These chromosomal aberrations occurred at various duration of treatment time. This suggests that these chemicals are clastogens depending on the exposure time. Therefore, minimal consumption, contact and reduction in the duration of exposure is advised.

**Key words:** *Allium* test, chromosomal aberrations, clastogens, mitotic inhibition, zobo, petrol, kerosene

### INTRODUCTION

The *Allium* test is a simple protocol and could be used to monitor pollutants in the environment; several modifications have become standardized as a tool for environmental monitoring (Fiskesjo, 1985; Levan, 1938). Its sensitivity to the toxicity and teratogenicity of several chemicals has been reported (Bellani *et al.*, 1991). The environmental effects of human activities on the associated genotoxic effects have been tested and reported by different authors (Timothy *et al.*, 2014; Yuzbasioglu *et al.*, 2009; Sudhakar *et al.*, 2001).

Studies on cytological effects of some aliphatic alcohols have shown that they have strong antimitotic effect with many anomalies ranging from stickiness, disturbed metaphase, multipolar nuclei, lagging chromosomes, bridges, somatic reduction and non-oriented chromosomes (Singh, 2003). Oil exploration, refining and transportation are obvious sources of environmental pollution (Imevbore and Odu, 1985). These toxic substances are released into the environment during refining (Boesch *et al.*, 1974). These chemical substances produce varying anomalies in mitosis, germination and growth of plants (Sarbhoy, 1980), could give rise to allergy at early ages, respiratory disorders, coronary problems and even cancer in middle ages (Carita and Marin-Morales, 2008) and *Allium* test could be used to evaluate the effects of toxic and mutagenic substances in the natural environment (Matsumoto, 2004; Matsumoto *et al.*, 2006; Tanti *et al.*, 2009). In order to determine the genotoxic and cytotoxic effects of these chemicals to the environment and possibly man, many authors have analysed their toxicity on *Allium cepa* roots.

Some of these studies include genotoxic effects of herbicide Illoxan (Yuzbasioglu *et al.*, 2009), fungicide tilt (Pulate and Tarar, 2014), mitotic effects of Procion (Tripathy and Patel, 2014) and an artificial food dye Indigo carmine (Bhattacharjee, 2014). Also, benzene, toluene, ethylbenzene and xylene (BTEX), components of hydrocarbons have been noted to cause different chromosomal aberrations (Bianchi *et al.*, 2015). Refined petroleum products (Petrol and Kerosene) from anthropogenic activities (mechanic workshops, spills, etc.) find their way into the environment and cause adverse effects to the environment (Imevbore and Odu, 1985).

In Nigeria, locally made soft drink (zobo) is consumed for its high levels of antioxidants as well as vitamins C, B1, B2 and minerals calcium, potassium, phosphorus, iron and magnesium (Sidibe *et al.*, 2002; Okereke *et al.*, 2015) or serve as cool and hot drinks or used as sauce for food, agent in local brewing or substitute for cream of tartar in baking (Becker, 1983). The *in vitro* and *in vivo* pharmacological abilities of the calyx extracts (which is used in zobo making) include strong antioxidant activity, antihypercholesterolaemic, antinociceptive and antipyretic, strong antihypertensive. It mostly inhibits the tone of the isolated muscles, significantly decreases the urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium and phosphate, but not oxalate and inhibition of some bacteria and fungi *in vitro* (Ali *et al.*, 2005; Mahadevan *et al.*, 2009; Okereke *et al.*, 2015). These pharmacological effects have made people to indiscriminately consume the locally made soft drink from this plant in Nigeria without considering the adverse potential health effects that could arise from excessive consumption.

The present study presents the sensitivity and effect of these substances on the root tips of *Allium cepa* and the potential cytotoxic effects on humans and other organisms.

## MATERIALS AND METHODS

**Source of materials:** The bulbs of the common onion (*Allium cepa*), of similar sizes and weights were purchased from Choba market in Port Harcourt, Rivers State. Twenty five 100 mL beakers, 25 sterile bottles, cover slips, measuring cylinder (100 mL), razor and slide were purchased. Also test chemicals which include locally made soft drink (zobo) purchased from Choba market and petroleum products (petrol and kerosene) purchased from African Petroleum filling station East-West road Choba were used for the experiment.

**Methods:** Washed bulbs of *Allium cepa* were grown in 100 mL beakers containing distill water to generate roots and the distilled water was changed daily. After 5 days of growth, serial concentrations of 50, 25 and 10% (volume per volume stocks/distilled water v/v) of refined petroleum products (petrol and kerosene) and locally made soft drink (zobo) prepared.

The onion bulbs grown in the distill water-filled beakers were transferred into the beakers containing the different concentrations of the test chemicals and allowed for 1, 3 and 6 h, respectively while the control was transferred to only distill water (these were done in 5 sets). At the expiration of each time (1, 3 or 6 h), some of the roots were excised, washed and the remaining placed back in distilled water to recover for 12-18 h at room temperature (28°C).

The harvested root tips from the treatments and the control were fixed in bottles containing acetic acid alcohol (glacial acetic acid 1 part and absolute alcohol 3 parts) for 12-24 h and stored in 70% ethanol in a refrigerator till required for slide preparation. The fixed root tips were hydrolyzed with 18% HCl acid for 10 min, rinsed in 70% ethanol, stained with carmine acetic acid solution, for 10 min squashed and observed for cytotoxic effects. The mitotic indices and chromosome aberrations were scored using Trinocular microscope Model at X40 objective. The frequencies of

chromosomal aberrations were scored for each treatment and plates with the best display of abnormalities were micro-photographed using the DC camera fitted a laptop.

## RESULTS

**Cell inhibition and indices:** The root growth inhibition assay indicated that there was an increase in the inhibition of the growth of the onion roots as the concentrations of the test materials increased. There was no growth inhibition in the control which had the highest root length of 5.8 cm. There was visible macro-morphological effects observed on the roots treated with refined petroleum products (petrol and kerosene) and locally made soft drink (zobo). When placed in distilled water for 18 h to recover, the roots treated with locally made soft drink (zobo) recovered while those treated with petroleum products did not recover.

The mitotic index values recorded varied from 2.86% in 50% zobo to 7.40% in the control. The values for the treatments were all below the control (Table 1). The value of the mitotic indices recorded decreased with increase in the concentration of the test chemicals (Table 1). The least mitotic indices were observed in the roots treated with different concentration of zobo (4.39 in 10%, 3.19 in 25% and 2.86 in 50%). This was followed by root tips treated with petrol (6.41 in 10%, 5.47 in 25% and 4.58 in 50%) and kerosene (6.18 in 10%, 6.83 in 25% and 7.31 in 50%). The mitotic inhibition was observed to be inversely proportional to the mitotic index (Table 1).

The differences in percentage phase indices are shown in Table 2. These values include prophase index (7.14-21.62%), metaphase index (21.62-50.00%), anaphase index (15.00-33.33%) and telophase index (25.00-57.14%). The frequency of prophase increased with increase in concentration of petrol and kerosene but decreases with increasing concentration of the locally made soft drink (zobo). Metaphase stage was accumulated at 50% of petrol but not much difference was observed

Table 1: Frequencies of mitotic phases, mitotic indices and mitotic inhibition in root tips of *Allium cepa* in the different concentrations of the test chemicals

Growth media	Conc. of product (%)	No. of cells	No. of dividing cells	Pro <sup>1</sup>	Met <sup>2</sup>	Ana <sup>3</sup>	Tel <sup>4</sup>	Mitotic index	Mitotic inhibition
Control	0	500	37	8	8	7	14	7.40	6.53
Petrol	10	480	25	4	6	5	7	6.41	6.53
	25	438	24	2	5	7	10	5.47	6.66
	50	390	22	3	9	6	7	4.58	6.78
Kerosene	10	485	30	1	10	10	9	6.18	6.56
	25	468	32	3	12	7	10	6.83	6.47
	50	410	30	4	9	7	10	7.31	6.41
Zobo	10	488	20	2	4	3	5	4.39	6.80
	25	470	15	1	5	4	5	3.19	6.96
	50	455	14	1	7	4	8	2.86	7.10

Pro<sup>1</sup>: Prophase, Met<sup>2</sup>: Metaphase, Ana<sup>3</sup>: Anaphase, Tel<sup>4</sup>: Telophase

Table 2: Percentage phase index of the different stages of mitosis at different concentrations of the test chemicals

Growth media	Conc (%) / time	No. of cells in division	Prophase	Metaphase	Anaphase	Telophase	PI (%)	MI (%)	AI (%)	TI (%)
Control	0	37	8	8	7	14	21.62	21.62	18.92	37.84
Petrol	10	25	4	6	5	7	16.00	24.00	20.00	28.00
	25	24	2	5	7	10	8.33	20.83	29.17	41.67
	50	22	3	9	6	7	13.64	40.91	27.27	31.82
Kerosene	10	30	1	10	10	9	3.33	33.33	33.33	30.00
	25	32	3	12	7	10	9.38	37.50	21.88	31.25
	50	30	4	9	7	10	13.33	30.00	23.33	33.33
Zobo	10	20	2	4	3	5	10.00	20.00	15.00	25.00
	25	15	1	5	4	5	6.67	33.33	26.67	33.33
	50	14	1	7	4	8	7.14	50.00	28.57	57.14

PI: Prophase index, MI: Metaphase index, AI: Anaphase index, TI: Telophase index

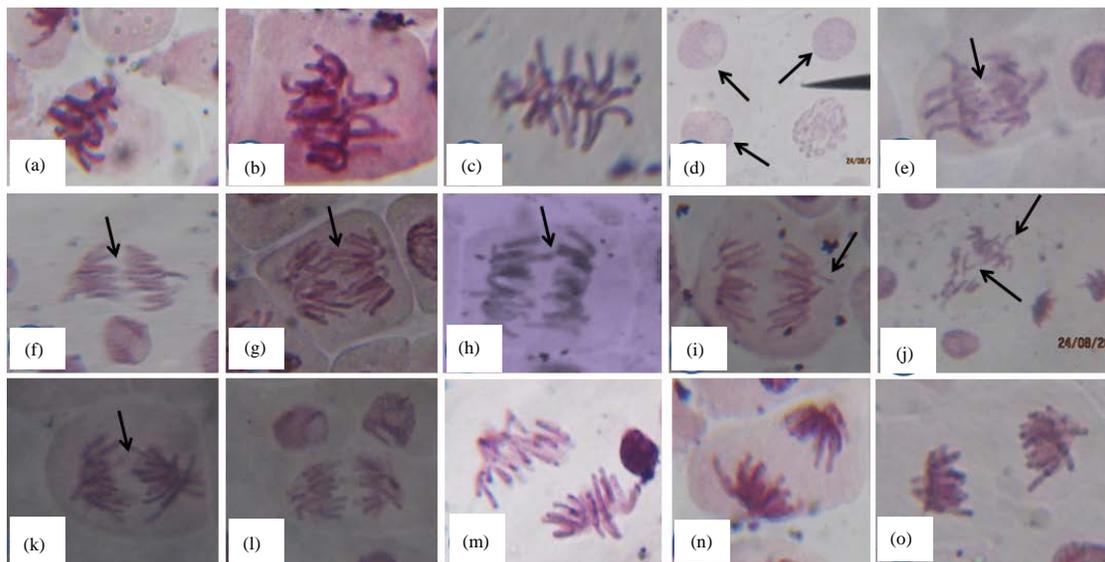


Fig. 1(a-o): Chromosomal aberrations (a-b) Sticky metaphase, (c) C-metaphase, (d) Ghost cells, (e-h) Bridges, (i-k) Laggards and (l-o) Normal anaphase

in 25 and 10% of petrol. After an initial depression in 10% of zobo, Anaphase stage was also accumulated at levels higher than that of control. There was a drastic depression of telophase at 50% of petrol and a mild one at 25 and 10% of petrol. Kerosene treatment on the other hand accumulated metaphase stages. But the effect of kerosene was not so different at the different concentrations (Table 2). Locally made soft drink (zobo) similarly accumulated metaphase over and above the figures obtained from the control.

**Chromosomal aberrations:** The three treatments showed one form of aberration or another on *Allium cepa* root tip chromosome integrity. These include sticky chromosomes (Fig. 1a-b), C-metaphase (Fig. 1c), ghost cells (Fig. 1d), bridges (Fig. 1e-h), lagging chromosomes (Fig. 1i-k) and normal anaphase (Fig. 1l-o). Other chromosomal aberrations observed in this study are binucleate, micronuclei and vagrant cells. Sticky chromosomes and binucleate cells were observed among the root tips treated with 10, 25 and 50% of petrol and kerosene and 25% of zobo (Table 3). C-metaphase was observed in root tips treated with 25% of kerosene and 10 and 50% of zobo drink while bridges were found in 25% petrol and 50% zobo. Ghost cells were observed in all the cytotoxic chemicals used (10 and 50% petrol), (50% kerosene) and all concentrations of zobo. Laggards and vagrants were observed only in root tips treated with different concentrations of zobo drink while Micronuclei were observed in root tips treated with 10, 25 and 50% of petrol and 25% of zobo (Table 3).

Furthermore, chromosomal aberrations observed varied with exposure time. For instance, anaphase bridges were observed in 25% of Petrol after 3 h and in 10% of zobo after 6 h. Laggards were observed in 50% after 1 h and 10% of zobo after 6 h. Vagrant chromosomes were observed in all concentrations of zobo while sticky chromosomes were observed in all concentrations of petrol, kerosene and in 25% zobo at 3 h. C-metaphases were observed in 25% of kerosene at 3 h, 50% at 1 h and 10% of zobo at 6 h whereas binucleate cells were observed in all concentrations of petrol

Table 3: Frequencies of chromosomal aberrations observed in *Allium cepa* root tips treated with different concentrations test chemicals

Media	Conc. of product (%)	Sticky	C-met <sup>1</sup>	Ghost cell	Bridges	Lag <sup>2</sup>	BN <sup>3</sup>	Vag <sup>3</sup>	MN <sup>4</sup>	Aberration (%)
Control	0	-	-	-	-	-	-	-	-	0
Petrol	10	45	-	10	-	-	10	-	6	14.7
	25	13	-	-	30	-	15	-	8	15.0
	50	50	-	25	-	-	20	-	10	26.9
Kerosene	10	16	-	-	-	-	9	-	-	5.15
	25	15	20	-	-	-	12	-	-	10.0
	50	20	-	20	-	-	10	-	-	12.6
Zobo	10	-	15	10	-	25	-	6	-	14.5
	25	10	-	21	-	-	-	9	10	10.6
	50	-	35	20	15	37	-	10	-	22.4

C-met<sup>1</sup>: C-metaphase, Lag<sup>2</sup>: Laggards., BN<sup>3</sup>: Binucleate cells, Vag<sup>3</sup>: Vagrant cells, MN<sup>4</sup>: Micronuclei

and kerosene. Micronuclei were observed in 25% of zobo at 3 h and in all concentrations of petrol. Ghost cells were also seen in root tips treated with the various concentrations of the zobo.

## DISCUSSION

In this study, genotoxic effects of different concentrations of refined petroleum products (petrol and kerosene) and locally made soft drink (zobo) were evaluated by analyzing root tips of *Allium cepa* for chromosomal aberration. Higher concentration caused inhibition on cell division and normal morphological characterization of the different stages of cell division. There was a significant difference between the treatments when compared with the control. The normal cell cycle was disrupted in root tips treated with petroleum products and the effects varied within the treatments. Such observation is not different from results obtained elsewhere using the *Allium* test. The potential cytotoxic and genotoxic effects were estimated by observing cytological parameters such as mitotic index and number of chromosomes abnormalities, including bridges in anaphase, sticky chromosomes (metaphase), micronuclei, laggard, ghost cell and vagrant. These chromosome abnormalities have been reported to be associated with toxicity of chemicals. For instance, Yuzbasioglu *et al.* (2009), Pulate and Tarar (2014) and Tripathy and Patel (2014) have investigated the cytotoxic effects of herbicide Illaxan, fungicide tilt and Procion, respectively and observed that the presence of C-metaphase, sticky metaphase, multipolar anaphase, bridges and laggards in root tips of *Allium cepa* is as result of the cytotoxic effect of these chemicals. In the same vein, these present test chemicals when evaluated showed decreased mitotic indices at different concentrations (50, 25 and 10%) as shown in Table 3. Comparatively, petroleum products have more inhibitory and mito-depressive effects than locally made soft drink (zobo). This is evident because the cytotoxic effects observed in zobo gradually disappeared when the bulbs were allowed to recover in distill water but those placed in petrol and kerosene were not able to recover. This suggests that consumption of the locally made drink should be minimized to avoid the effects that may result from regular or constant intake.

The decline of mitotic index below 20% in comparison to control can have lethal effect on the organism (Antosiewicz, 1990; Tripathy and Patel, 2014). While a decrease below 50% usually has sub-lethal effect and called cytotoxic limit value (Panda and Sahu, 1985; Pulate and Tarar, 2014). 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 (Rojas *et al.*, 1993). Reduction in the mitotic activity could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis (Sudhakar *et al.*, 2001). The result here suggests that the test chemical possess inhibitory and mitodepression effect on cell division and chromosomes behaviour of *Allium cepa* and it can prevent DNA synthesis and the

reduction in number of the dividing cell in roots. The petroleum products showed the strongest genotoxic effect in the root meristem cells. The presence of structural aberrations of the breaks type also suggested that the treatments acted as toxic agents on the formation of the mitotic spindle, on DNA or on DNA-protein complex. The findings of this study showed that these substances exerted depressive effects on mitosis, including reduced mitotic indices and accumulation of mitotic phases. This could be attributed to the mitotic inhibition and is associated with blocking of DNA synthesis at G1 or S phase (Schneiderman *et al.*, 1971; Sudhakar *et al.*, 2001; Pulate and Tarar, 2014) or blocking of G2 phase (Van't Hof, 1968).

Chromosomal aberrations which include anaphase bridges were observed in 25% of petrol after 3 h and 10% of zobo after 6 h. Laggards were observed in 50% after 1 h and 10% of zobo after 6 h. Vagrant chromosomes were observed in all concentrations of zobo while micronuclei were observed in 25% of zobo at 3 h and in all concentrations of petrol. Sticky chromosomes were observed in all concentrations of petrol, kerosene and in 25% zobo at 3 h. The sticky chromosomes have resulted in abnormal uncoiling of chromosomes during anaphase to telophase (Qian *et al.*, 2006; Pulate and Tarar, 2014; Bianchi *et al.*, 2015). Bridge formation can be due to the general stickiness of the chromosome at metaphase stage or breakage and reunion of chromosomes. Similar type of abnormality has been reported in the mitosis of *Vicia faba* after treatment with the organophosphorus insecticide (Amer and Farah, 1976; Pulate and Tarar, 2014). Lagging chromosomes arise mainly due to abnormal spindle formation and as a result spindle fibre failed to pull the respective chromosomes to the polar regions of the cells and resultantly lagging chromosomes appeared (Tarar and Dyansagar, 1980; Tripathy and Patel, 2014; Pulate and Tarar, 2014). According to Sudhakar *et al.* (2001), micronuclei may originate from acentric fragments (clastogenic response), chromosome laggards during anaphases, or even from malfunctioning of the spindle (aneugenic response). Thus chemical agents may induce the formation of micronuclei as a result of spindle disturbance as well as chromosome breaks. Fiskesjo (1993) and Tripathy and Patel (2014) suggest that chromosome breaks are associated with the formation of chromosome fragments and micronucleated cells. This also suggests the studied chemicals have the ability to disrupt cellular activities and therefore clastogenic.

C-metaphases were observed in 25% of kerosene at 3 h, 50% at 1 h and 10% of zobo at 6 h. Bhattacharjee (2014) noted that the additive Indigocarmine has mitodepressive effect. Also, Odeigah *et al.* (1997) describes the presence of C-mitoses as a possibly reversible effect (weak toxic effect). However, these changes may induce the formation of polyploid cells when not reversed. The presence of C-metaphase cells indicated that kerosene and zobo drink had cytotoxic effect on the mitotic spindle (Matsumoto *et al.*, 2006; Pulate and Tarar, 2014; Bhattacharjee, 2014).

Ghost cell was observed in groups in roots treated with different concentrations of locally made soft drink (zobo). This could be as a result of nucleus damage and prevention of cytoplasmic structures. It has been reported that if ATP level in the cell decreases by 50%, mitosis can completely be prevented (Kara *et al.*, 1994). Therefore, zobo drink has the potential to displace the chemical substances enhancing ATP synthesis thereby inhibit or delay metaphase. These ghost cells were observed in root tips treated with the various concentrations of the zobo while binucleate cells were observed in all concentrations of petrol and kerosene. This result therefore suggests that these chemicals are clastogens. Numerous potentially mutagenic chemicals have been reported to cause damage and heritable changes in the genetic material, without being immediately expressed (Vogel, 1982; Yuzbasioglu *et al.*, 2009). The antimitotic influence of these treatments is exemplified in the chromosomal aberrations they induced (Bellani *et al.*, 1991) and demonstrated that such

aberrations monitored by the *Allium* test could be translated to toxicity and or teratogenicity in animals/man. The mitodepressive and caryoclastic effects of these products probably reflect what goes on in the bodies of people who are occupationally and constantly exposed directly to these products. The results suggest that even at low concentrations petroleum products have genotoxic effects on DNA of plant and could be aggravated with increase in exposure time (Singh, 2003; Pulate and Tarar, 2014).

## CONCLUSION

The treatment of *Allium cepa* roots with refined petroleum product (petrol and kerosene) and locally made soft drink (zobo) respectively resulted in various forms of chromosomal aberrations. These chemicals are however, currently used by majority of Nigerians. Therefore, minimal daily consumption of Zobo drink, minimal contact with the petroleum products and reduction in the duration of exposure is advised.

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