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Original Article

STUDIES ON CYTOTOXICITY INDUCED BY AQUEOUS EXTRACTS OF *Cola nitida* (KOLANUT) ON *Allium cepa* (ONION)

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ABSTRACT

A study was carried out to evaluate the cytotoxic effects of *Cola nitida* water extract on root meristems of *Allium cepa.*The stock water extract of *Cola nitida* at 100% was serially diluted with distilled water to 25%, 50% and 75% while distilled water served as control for the study. Root tips of *Allium cepa* grown in the different concentrations of the extract and control were harvested around 7:30am to 8:30am for this study. Processes like pretreatment, fixation, hydrolysis, squashing of harvested root tips and staining of cells were done while data were taken on cytological parameters under X400 magnification of the light microscope. Analysis of Variance (ANOVA) of the results showed that seven out of the twelve cytological parameters considered were significantly different in relation to the concentrations of *Cola nitida*extract. The chromosomal aberrations associated with different concentrations of *Cola nitida* extract were C-mitosis, binucleate cells, sticky chromosomes, variant chromosomes and vacuolated cells. The results obtained in this study revealed that *Cola nitida* extract has stimulatory and mutagenic effects at high concentration while low to intermediate concentrations have inhibitory effect on dividing cells.

Keywords: Cytotoxic, *Cola nitida*, *Allium cepa*, aberrations, anti-mitotic.

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INTRODUCTION

Kola nut (*Cola* spp), a native plant of the tropical West African rainforest, is represented by over 20 tree species which belong to the family Sterculiaceae (Tende *et al.*, 2011).*Cola nitida* and *Cola acuminata* are particularly very common because of their great socio-economic importance in West African countries (Ajai *et al.*, 2012). Dewole *et al.* (2013) reported that *Cola nitida* and *Cola acuminata* are rich in proximate and novel bioactive compounds such as protein, crude fibre, tannin, saponin and alkaloid. The greatest concentration of *Cola nitida* is found in the forest area of

Ivory Coast and Ghana while *Cola acuminata* stretches from Nigeria to Gabon. *Cola nitida* according to Ibu *et al.* (1986) has great social and traditional significance in many countries in West Africa.

Kola nut, according to Ajai *et al.* (2012) has wide applications in the food and pharmaceutical industries where it is used as source of caffeine in their products. According to Chavarro *et al.* (2009) many people chew kolanut as stimulant because of its caffeine content. Kola nut is also used in the manufacture of cola beverages like coca-cola, pepsi cola and other kola drinks (Javies, 2002). The consumption of cola soft drinks has increased considerably all over the world during the last decades (Mahboub, 2013). Because of the high consumption of cola drinks, the health effect is of research and public health interest. Jensdottir *et al.* (2004) and McGartland *et al*. (2003) reported that enamel softening, hypokalemic myopathy and development of metabolic syndrome, diabetes mellitus and chronic kidney diseases are associated with high consumption of kola. Kola nuts are commonly used to counteract hunger and thirst. In some cases, they are used to help pregnant women to control vomit. Kola nuts are also used by students and menial workers as stimulanting agents to resist fatigue and keep awake (Chukwu *et al*., 2006; Esimone *et al*., 2007). *Cola acuminata* is more popular among the Igbo and Igedde people of Eastern and middle belt regions of Nigeria, whereas *Cola nitida* is highly consumed among the Hausa and Fulani in the Northern part of Nigeria (Ibu *et al*., 1986). The plant according to Akerele (1988), has been used since antiquity for medicinal purposes by diverse people and cultures throughout the world. Newall *et al*. (1996) reported that its consumption should be discouraged for individuals with stomach ulcers because of its caffeine and tannin content.

Allium test for monitoring and toxicity screening of the environment has been recommended (Cuyacot *et al.,* 2014). The *Allium* test according to Alege and Egwuda (2014) could detect the presence of cytotoxic or mutagenic substances in the environment and in food substances, which according to Pulate and Tarar (2014) represents direct or indirect risks for all living organisms. *Allium* test which is relatively inexpensive and easy to handle can be used for the determination of cytotoxic and genotoxic effects of complex molecules (Abu and Nwere, 2013; Jayasree *et al*., 2014). IIbas *et al.* (2012) reported that the defects occurring on the treated onion root meristems can also be expressed in human cells since both *Allium* and human genomes are living systems containing DNA in the cells. Recently Nwakanma *et al.* (2014) reported the use of *Crinum jagus* as a reliable alternative to the popular *Allium* spp for cytotoxic studies. Therefore members of the Amaryllidaceae family are the best materials for cytotoxic and genotoxic researches.

Based on this report, it is of interest to test the cytotoxic effect of *Cola nitida* and safety of consuming it since it is a component of some pharmaceutical and beverage products. This study therefore aimed at evaluating the cytotoxic potentials of aqeous extracts of *Cola nitida* using *Allium cepa* root meristem.

MATERIALS AND METHODS

Collection of Materials

Kolanut (*Cola nitida*) fruits and bulbs of *Allium cepa* used for the study were obtained from the market in Anyigba, Kogi State, Nigeria.

Preparation of Kola nut extract

The kolanuts were air-dried at room temperature and ground to powder using pestle and mortar. The powder was sieved through an 8.0mm aperture size wire mesh. Fifty grammes (50g) of the sieved powder was added to 1000ml of distilled water and left for 24hours following the method outlined by Akinboro *et al.* (2013). The mixture was filtered into 100ml beakers using Whatman filter paper number 2 and kept at 4^{o}C for 24hours. The stock (aqueous extract of *Cola nitida*) was serially diluted to 75%, 50% and 25% while distilled water served as Control for the study. The treatments were replicated five times and arranged in Complete Randomized Design (CRD).

Allium cepa bulbs having diameter ranging between 1.5 and 2.2cm were properly washed while outer scales and old roots reminants were carefully removed. These healthy onion bulbs were dissected transversely and the reduced stems were allowed to make contact with water in beakers for root development while the upper parts were discarded. The dissected onions were allowed to sprout shoots and only those that sprouted up to 5 small onion sets were selected and separated for the study, to maintain uniform genotypes. Each of the onion sets was transferred into beakers containing the different concentrations of *Cola nitida* extract. The onion roots were left to grow in each solution for 24 hours. Root tips of the onion sample were harvested between 7:30am –8:30am into vials and the study proceeded in a series of stages according to the method outlined by Akinyele (2007) as follows:

Pretreatment

The harvested onion root tips from each treatment were transferred into specimen vials containing paradichlorobenzene and left in the pretreatment solution for 3hours.

Fixation

The pretreated root tips were then removed from paradichlorobenzene, rinsed in distilled water three times to wash away the paradichlorobenzene and then fixed in glacial acetic and absolute ethanol in a ratio of 1:3. The fixative was to kill the root cells and keep the cells in their natural condition such that cell contents were not leached out. Appropriate labeling of the specimen was done and all the vials were kept in the refrigerator at 4^{o}C for 24 hours.

Hydrolysis

The root tips were removed from the fixative after 24 hours and washed thoroughly in distilled water for 3 minutes before hydrolysis. The washed root tips were, therefore, dropped in 10% HCl for 10 minutes at room temperature. Hydrolysis was carried out to soften the root tips.

Squashing and staining

The hydrolyzed root tips were washed thoroughly in distilled water and then each tip was placed on a clean glass slide. Two drops of Aceto-carmine stain were put on the root tips and then squashed using the broader flat end of a cylindrical search needle until a turbid suspension was seen. The turbid suspension on the slide was then covered with cover slip.

The slides were then gently warmed using the flame of a spirit lamp. Excess stain was drained off by placing the slides in between folded filter paper and gently applying pressure. Transparent finger nail polish was applied to the edge of the cover slip to prevent air from entering. Five slides were prepared in this manner for each treatment and labeled accordingly.

Chromosome Observation

The slides were mounted and observed under the light microscope. The X4, X10 and X40 objectives were used for viewing the slides. Photographs of normal mitotic stages and aberrant cells were snapped using photomicrograph at X40 objectives.

Data analysis

Ten counts each were taken from ten different slides from each concentration and the following parameters were taken: total number of cells, number of interphase cells, prophase cells, metaphase cells, anaphase cells, telophase cells and aberrant cells observed in the study. The percentage of Aberrant Cells (AC) Active Mitotic Index (AMI) and Mitotic Index (MI) for the cells treated with different concentrations of kolanut and control were calculated using the formula of Malode *et al.* (2012) as given below:

% aberrant Cells (AC) =

Active Mitotic Index (AMI) =

Mitotic Index (MI) =

The number of dividing cells was taken as the number of prophase, metaphase, anaphase and telophase. Data pooled for each attribute were subjected to Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to separate significant means.

RESULTS

The photomichrographs of *Allium cepa* root tip cells showing different stages of cell division and aberrant cells induced by the treatment with different concentrations of *Cola nitida* are shown in Plate A to K. The aberrations observed in this study were C-mitosis (Plate F), binucleate cells (Plate G), sticky chromosomes (Plate I), variant chromosomes (Plate J) and vacuolated cells (Plate K). Analysis of variance result (Table1) showed that seven out of the twelve cytological parameters considered were significantly different in relation to the different concentrations of *Cola nitida*. Those cytological attributes with statistical significant differences at $P<0.05$ were, total number of cells, number of metaphase cells, number of binucleate cells, number of sticky chromosome, number of variant chromosome, number of vacuolated cells and total number of aberrant cells.

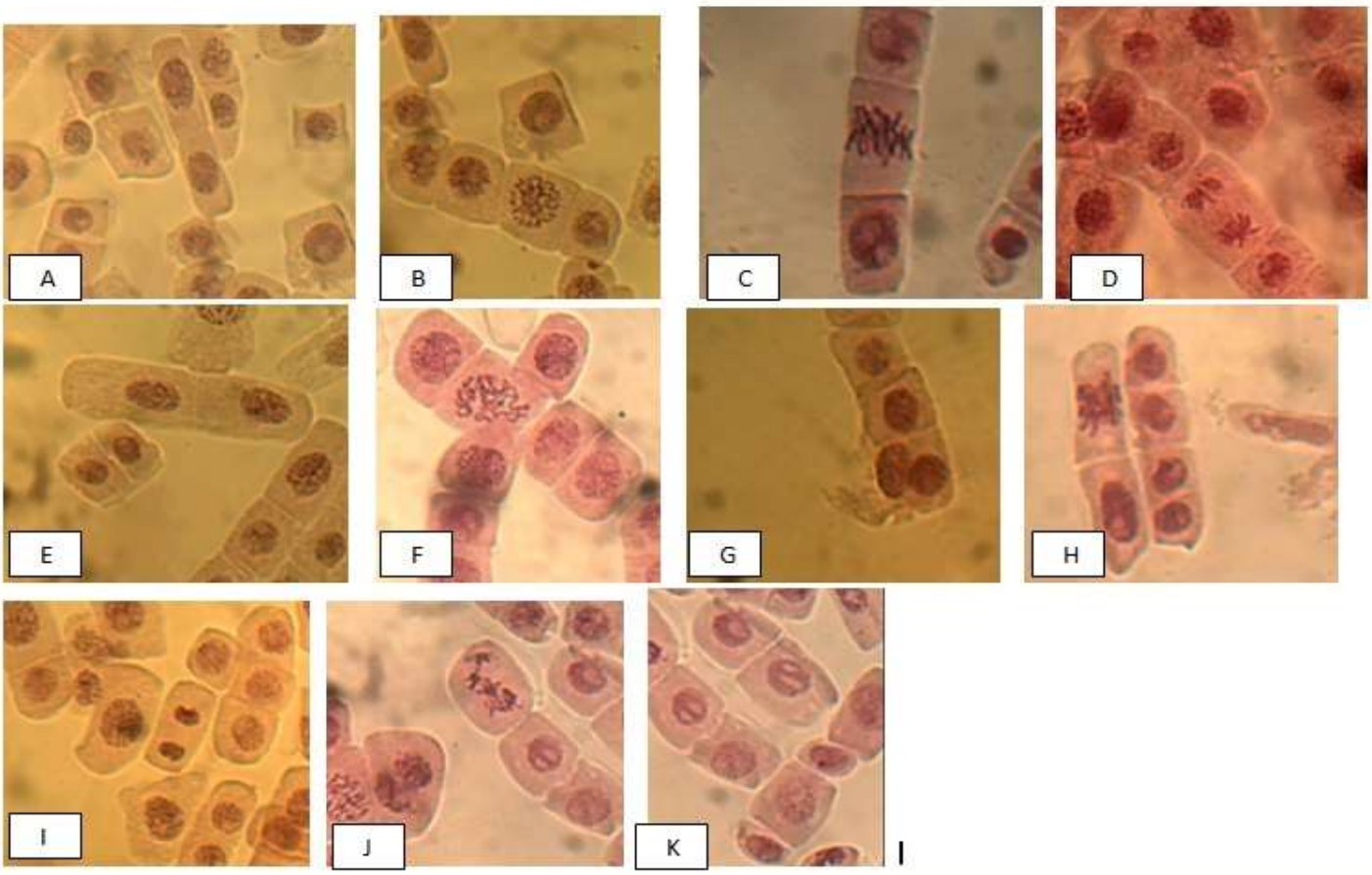

Plate I: Photographs of *Allium cepa* root tip cells showing normal stages of mitotic division (plates A to E) and aberrant cells from treated root tips (plates F to K).

A: Normal interphase cell, B: Normal prophase cell, C: Normal metaphase, D: Normal anaphase, E: Normal telophase, F: C-mitosis, G: Binucleate cells, H: Sticky chromosome at metaphase stage, I: Sticky chromosome at anaphase stage, J: Variant chromosomes, K: Vacuolated cells (Magnification ×400).

Table 1: Effects of water extracts of *Cola nitida* on the Cytology of *Allium cepa*.

Treatments (%)	TNC	NOI	NOP	NOM	NOA	NOT	NOC	NOB	NSC	NVC	NVA	TNA
Control	126.80^{ab}	113.10	3.10	3.30^{b}	3.80	3.50	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
25	123.60^{ab}	113.00	3.40	1.50^{a}	2.20	2.60	0.40	0.30^{a}	0.30^{ab}	0.00^{a}	0.00^{a}	1.00^{ab}
50	122.30^{a}	111.90	2.10	2.60^{ab}	2.40	2.30	0.40	0.30^{a}	0.30^{ab}	0.20^{ab}	0.00^{a}	1.20^{ab}
75	126.70^{ab}	111.80	2.70	4.70^{bc}	2.80	3.60	0.20	0.60^{ab}	0.50^{b}	0.60^{b}	0.00^{a}	1.90^{bc}
100	127.80^{b}	112.30	2.40	4.30^{bc}	2.60	3.80	0.30	1.20^{b}	0.50^{b}	0.50^{b}	0.40^{b}	2.90^{c}
LSD (P<0.05)	2.42	NS	NS	0.83	NS	NS	NS	0.35	0.22	0.21	0.10	0.61

Means with the same alphabets in the same column are not significantly different at P<0.05

KEY
NS- Not Significant
TNC- Total number of cells
NOI- Number of cells at interphase stage
NOP- Number of cells at prophase stage
NOM- Number of cells at metaphase stage
NOA- Number of cells at anaphase stage
NOT- Number of cells at telophase stage
NOC- Number of cells with C-mitosis
NOB- Number of binucleate cells
NSC- Number of cells with Sticky chromosomes
NVC- Number of cells with variant chromosomes
NVA- Number of cells with vacuolated cells
TNA- Total number of aberrant cells

Table 2: Effect of water extracts of *Cola nitida* on cell division of *Allium cepa* root cells.

Treatments (%)	% of aberrant cells	Active Mitosis Index (%)	Mitotic Index (%)	Total Number of Dividing Cells
Control	0.00	5.60	10.80	13.70
25	9.35	3.00	8.66	10.70
50	11.32	4.09	8.67	10.60
75	12.10	5.92	12.46	15.70
100	18.13	5.40	12.52	16.00

DISCUSSION

This study showed a clear effect of kola nut extract on the cytology of *Allium cepa* root tip cells. The observation of C-mitosis, binucleate cells, sticky chromosomes, variant chromosomes and vacuolated cells in this study is an indication that *Cola nitida* is mutagenic.

Ping *et al.* (2012) reported C-mitosis, binucleate cell, sticky and variant chromosomes in *Allium cepa* root tip cells

treated with methanolic extracts of *Euphorbia hirta.* The significant difference observed in total number of cells among the different extract concentrations and the control is similar to the earlier report of Auti *et al.* (2010). High number of interphase cells in relation to other stages observed in the study is similar to the reports of several workers like Abu and Nwere (2013), Alege and Ojomah (2014), Malode *et al.* (2012) and Nwakanma *et al.* (2014). Nwakanma *et al.* (2014) reported high frequency of interphase stage because the stage lasts much longer than the other stages of mitosis.

The effect of the extract was most noticeable at metaphase stage when the cells are preparing for spindle fibre formation which could lead to the subsequent movement of chromosomes to the poles in the later stages. The fact that most of the aberrations such as C-mitosis, sticky chromosomes and variant chromosomes observed in this study are related to inhibition of spindle fibre synthesis and poor movement of chromosomes to the poles of the cell further supported the effect of the extract at metaphase stage. It was also observed that higher numbers of metaphase cells were recorded in 75% and 100% concentrations while the reverse was the case for 25% and 50%. This showed that at low concentration the extract exhibits anti-mitotic properties by inactivating spindle fibre formation. This finding is similar to the earlier report of Auti *et al.* (2010) on Omnacortil drug.

The high Mitotic Index (MI), Active Mitotic Index (AMI) and percentage aberration rates at 75% and 100% concentrations showed that high concentration of *Cola nitida* extract exhibits both stimulatory and mutagenic effects on genetic systems. This is in line with the earlier report of Ajai *et al.* (2012) who reported that excessive consumption of kolanut have adverse effects on the health of the consumer. In contrary, the observed low Mitotic Index (MI) and Active Mitotic Index (AMI) at 25% and 50% supported the inhibitory effects of lower concentration of *Cola nitida* extract on cell division. The reduction in mitotic may result from a block in G1 stage leading to suppression of DNA synthesis (Jayasree *et al.*, 2014). According to Udo and Akpan (2014), the retardation of the process of cell division is due to the inhibitory action of the extract applied. In this study, low to intermediate concentrations of *Cola nitida* extract has anti-mitotic effects which could be a very good source of anticancer drugs if well utilized.

CONCLUSION

This study revealed that *Cola nitida* extract had stimulatory and mutagenic effect at high concentrations while its low to intermediate concentrations had inhibitory effect on dividing cells. This suggests that *Cola nitida* contains some anti-mitotic properties. Low amount of kola in beverage and pharmaceutical products will be beneficial to the consumers because of its low mutation frequency and mitotic inhibitory effects.

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