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Cytogenotoxicity of the Aqueous Extract of Bitter Kola (*Garcinia kola:* Clusiaceae) Using *Allium cepa* Assay

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Abstract: Bitter kola (*Garcinia kola*) is the African wonder nut that is widely eaten for its medicinal properties. The aim of this study was to investigate the potential genotoxic effects of the aqueous extract of *G. kola* nuts using the *Allium cepa* test system. Roots of *A. cepa* were treated to a series of concentrations, 0 µg/ml, 5.0 µg/ml, 10.0 µg/ml, 25.0 µg/ml and 40.0 µg/ml for a period of 72 hours. The results indicated that percentage growth of roots and mitotic chromosome behaviour was inversely proportional to the concentration of extract. Increase in concentration of extract significantly reduced the number of roots and the length of roots recorded indicating a progressive inhibition of the mitotic activity of the meristematic cells. The mean number of roots (3.2±5.97) and mean length of roots (1.3±0.53cm) were minimum at the highest concentration of extract (40µg/ml). Mitotic index was also minimum (0.44±0.05%) at the highest concentration (40µg/ml) of the *G. kola* extract. The genotoxicity of the extract was measured using the frequency of chromosomal aberrations which revealed a high frequency of Anaphase chromosomal bridges, Anaphase laggards, sticky chromosomes and nuclear vacuoles. The highest percentage of abnormal cells (3.65±3.49%) was determined for the highest concentration (40µg/ml) of extract. The chromosomal abnormalities were evidences of the action of the aqueous extract on the mitotic spindle and the coiling of chromosomes during anaphase to telophase These results are therefore enough to conclude that *G. kola* extract possesses cytotoxic and cytogenotoxic properties.

Keywords: *Garcinia kola*, *Allium cepa*, Root Growth, Mitotic Index, Chromosome Aberrations

1. Introduction

Garcinia kola Heckel, the African wonder nut is called bitter kola in Cameroon. The seed has a bitter taste hence the name "bitter kola". *G. kola* is a member of the family Clusiaceae / Guttiferae [1]. It is a common Tropical evergreen tree that grows up to 30m tall. It is found in the rain forests throughout West and Central Africa from Sierra Leone through Southern Nigeria into Zaire and Angola [2]. In Cameroon, this species is indigenous to the tropical forests of the South-West and Littoral Regions and is a highly valued multipurpose tree for its fruits, seeds, stems and bark [3]. This species has proven to be one of the many non-timber forest products that are of high socio-economic importance in Tropical Africa and Cameroon in particular [4]. The nuts have economic and cultural values across West and Central African countries where they are commonly chewed and used for traditional ceremonies [5].

Bitter kola, *G. kola,* has been shown to contain significant amounts of some bioactive constituents such as saponins, tannins, flavonoids, sterols, triterpenoids, alkaloid, and phenol. The plant also has a rich content of carbohydrate,

crude protein, crude fibre, minerals and vitamins that provide this species the potentials for good source of food and traditional medicinal properties [6-11]. Hence its uses in the treatment of laryngitis, cough, and liver diseases [1, 12-14]. In Cameroon, the seeds and the bark of *G. kola* are the most commonly used parts for food and traditional medicinal purposes [15-17]. The seeds are smooth, elliptically shaped, with whitish to yellowish pulp and covered with brown papery seed coat.

Despite its popularity and usage, there is no information on the cytotoxicity and genotoxicity of bitter cola, *G. kola* in available literature. The present study was therefore aimed to evaluate the cytotoxic and genotoxic potentials of bitter cola, *G. kola*, using the onion, *Allium cepa* assay.

2. Materials and Methods

2.1. Preparation of Extract

Medium sized bitter kola (*Garcinia kola*) seeds (Figure 1a) originating from the South West Region of Cameroon were used for this study. The seeds were cleaned of dust and other debris by washing with several exchanges of clean tap water. The seeds including the outer brownish papery coverings were next cut into small pieces, dried in an oven at 60°C and ground into powder using a 500 watts blender. Two hundred grams of the powder was extracted with distill water using the method of Seino et al [18]. The extract was concentrated in an oven at 60°C for 72hrs and 28.46gms of white crystals were obtained and used to prepare different concentrations (5.0 µg/ml, 10.0 µg/ml, 25.0 µg/ml and 40.0 µg/ml) of aqueous solutions.

2.2. Cytotoxic Evaluation

This was evaluated by examining the inhibition effect of extracts on the root growth of *A. cepa*. Viable red variety of *Allium cepa* (onion) obtained from the local Bamenda market, were used for this study. However, onion bulbs that were dry, mouldy and with shooting green leaves were discarded. Those that sprouted roots in tap water during 48 hours of incubation at our laboratory temperature (28°C) were considered to be viable and were used for this study. The onion bulbs were cleaned by removal of the outer scales as well as both the dry and sprouted roots at the base of the bulbs. This helped to expose the fresh meristematic tissues to the various treatments. Five groups of onion bulbs consisting each of five bulbs (hence five replicate bulbs per treatment), were respectively treated with distilled water, and *G. kola* aqueous extracts of concentration 5.0 µg/ml, 10.0 µg/ml, 25.0 µg/ml and 40.0 µg/ml) for 72 hours. Distilled water was used as the control.

At the end of the treatment period of 72 hours, the roots of five onion bulbs with the best growth at each concentration were counted and removed with a pair of forceps and their lengths measured (in cm) with a metre rule.

2.3. Cytogenetic Evaluation

For the evaluation of induction of chromosomal aberration, root tips from these bulbs were cut and used to prepare mitotic chromosome smears. The mitotic chromosomes smears were prepared using the method of Akwu et al, [19]. Two root tips from each treatment were hydrolyzed in 1N HCl at 60°C for five minutes after which they were washed in distilled water. Two root tips were then squashed on clean glass microscope slides, stained with aceto-orcein for 10 min and cover slips carefully lowered on them to exclude air bubbles. Holding the cover slip in position with the thumb and first finger, the preparation was gently tapped with the blunt end of a dissecting needle to spread the cells evenly to form a monolayer. The edges of the coverslips were carefully sealed with colourless nail polish as suggested by Seino and Akongnui [20], to prevent drying out of the preparation. A total of 115 slides were prepared and examined.

The slides thus prepared were examined for mitotic stages and chromosome aberrations using the 10X and 40X objective lenses of the Fisher compound light microscope. For each slide, at least 200 cells were scored for chromosomal aberrations using various aberrant templates as previously reported [18, 21, 22]. A total of 1000 cells were scored per concentration in each treatment and the control.

2.4. Statistical Analysis

The data obtained from cell division and mitotic indices as well as number of roots and root length of the treated *A. cepa* from different concentrations and control were compared using analysis of variance (ANOVA). The mitotic index and percentage of chromosome aberrations recorded were calculated. Mitotic index was determined using the formular:

$$\text{Meiotic index (\%)} = \frac{Number\ of\ dividing\ cells\ recorded}{Total\ number\ of\ cells\ examined} X\ 100$$

3. Results and Discussion

3.1. Cytotoxic Analysis

The cytotoxic parameters, root number and root length, obtained for the various treatments during this study are shown in Figure 1(b–e) and Table 1. The results obtained revealed that all the concentrations of the aqueous extracts of bitter cola (*G kola*) caused significant inhibition in the growth of the roots of *A. cepa* in comparison to the control distill water. The decrease in the number of roots and root length was greater with increasing concentration of the extract. In addition, increase in concentration of the aqueous extract was accompanied with increase in yellowish rotting at the base of the roots of *A. cepa.*

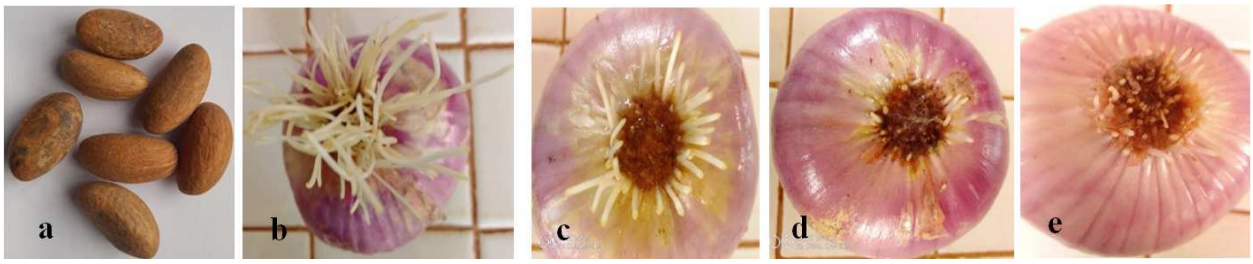

Figure 1. *Bitter kola nuts and pictures to show cytotoxic effect of aqueous extracts of G. kola in the A. cepa assay. a) Bitter kola nuts; b) Normal root growth in control group; c) Reduced length of roots with yellowish rotting bases; d) Scanty and short roots with severely rotting bases; e) Scanty and very short roots with pronounced rotting at the base.*

Table 1. *The average root number and root lengths of A. cepa in control and treatment concentrations of G. kola.*

Treatment groups	Concentrations	Average root number ±SD	Average root Length (cm) ±SD
Control	Distilled water	57.3±4.03[a]	9.8±0.61[a]
1	5µg/ml	26.4±4.72[b]	5.7±0.42[b]
2	10µg/ml	13.7±5.52[c]	3.8±0.52[c]
3	25µg/ml	8.3±2.30[d]	2.2±0.33[d]
4	40µg/ml	3.2±5.97[e]	1.3±0.53[e]

[a] Significant at $p<0.05$; [b] Significant at $p<0.01$; [c] Significant at $p<0.0001$ as compared to their respective control analyzed with ANOVA one Way performed.

3.2. Cytogenetic Analysis

Genotoxicity assessment of test compounds in both invitro and in vivo conditions have been widely used for cytogenetic assays. These tests are used to identify the damaging effects of substances on living organisms [23]. The *Allium cepa* test has been used to evaluate the genotoxic potential of several plant extracts [24-26], because this test uses a model that is adequately sensitive to detect numerous substances that cause chromosomal alterations. The *A. cepa* test has been previously used for mutagenicity and cytotoxicity determination of plant extracts and products [18, 27-33].

The cytogenotoxic parameters often evaluated and used as endpoints in such studies have included mitotic index, micronuclei formation and chromosome aberrations [34]. In the present study we used mitotic index and chromosome aberrations. Results obtained revealed normal mitotic divisions in the root tips of the *A. cepa* bulbs in the control. Treatment with *G. kola* extracts showed cytogenotoxic effects that were recorded as significant decreases of mitotic index and many chromosome aberrations. The per cent proportion of cells in prophase was the most abundant mitotic stage for the control and all the treatments. Reduction was observed for cells in anaphase and telophase in all treatments as compared to the control. A dose-dependent decrease in cumulative dividing cells was observed with the value obtained for the control higher than all concentrations of the treatments. This mitotic index values were found to be less than half that of the control at all concentrations (Table 2).

Table 2. *Dividing and total cells counted in microscopic observations and mitotic values in control and in G. kola aqueous extract treatment concentrations.*

Treatment groups	Concentrations	Total cells	Dividing cells	MI (%) ± SD
Control	Distilled water	500	387	7.74±1.17[a]
1	5µg/ml	500	126	2.52±0.91[b]
2	10µg/ml	500	82	1.64±0.60[c]
3	25µg/ml	500	58	1.16±0.75[c]
4	40µg/ml	500	22	0.44±0.05[d]

MI = Mitotic Index

[a] Significant at $p<0.05$; [b] Significant at $p<0.01$; [c] Significant at $p<0.0001$ as compared to their respective control analyzed with ANOVA one Way performed.

The reduction of the mitotic index as recorded in this study could be the result of the arrest of the division of the interphasic nucleus or the death of interphasic nucleus, which hinders the onset of prophase and therefore, the division of the cells [21].

In addition, cytogenotoxic evaluation also revealed the occurrence of chromosome aberrations of different proportions for each of the concentrations in the treatment groups except the control. A great percentage of Anaphase lagging migration of chromosomes, Anaphase chromosome bridges, sticky chromosomes and double nuclei were recorded (Table 3, Figure 2). The highest frequency of chromosome aberrations was observed in 40µg/ml and was found to be significantly higher from the control as well as aberrant cells examined at all concentrations of *G. kola* extracts.

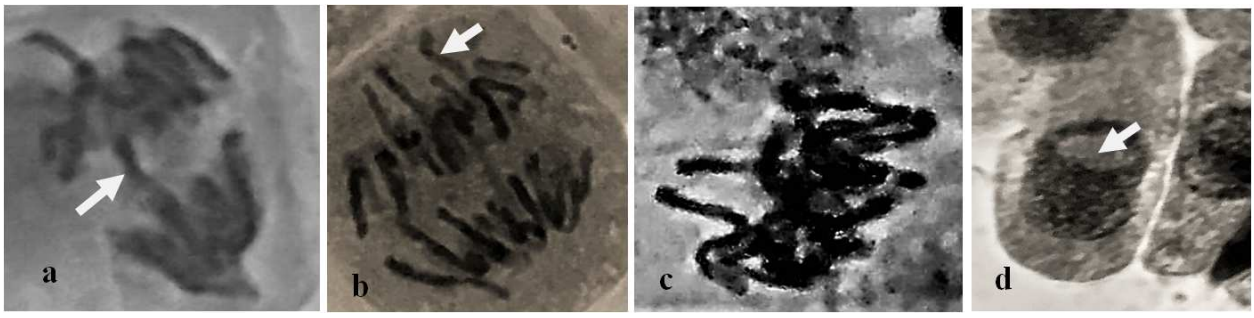

Figure 2. *Chromosomal abnormalities induced in A. cepa root cells by various concentration of Bitter kola (G. kola) aqueous extracts. a) Anaphase bridge (arrow); b) Anaphase laggard (arrow); c) Sticky Metaphase chromosomes; d) Nuclear vacuoles (arrow).*

During this study, Anaphase bridges and laggards were recorded for all concentrations of *G. kola* aqueous extracts. The number of bridges and laggards significantly increased with increase concentration of the extracts (Table 3). This indicated that the formation of anaphase bridges and laggards was concentration dependent. Anaphase bridges and laggards were not recorded in the control of this study. This was not strange since Finardi et al [35] reported that anaphase chromosome bridges have been observed in both cells not experiencing stress as well as cells experiencing stress. The appearance of bridges in treated cells could be the result of the induction of some replicative stress which is known to enhance the formation of Anaphase bridges [36, 37]. The formation of bridges and laggards often results from improper separation of chromatids because the spindle is not well formed. It is widely accepted that Anaphase chromosome bridges and laggards generate breakages in subsequent generations of the population [38]. In consequence, breakages and poor anaphase migration result in the loss of genetic material. Anaphase chromosome bridges and laggards are particular errors likely to cause genome instability [39, 40]. Survival of the cell depends on the genetic content of the genetic material lost with respect to the genetic background of the cell [41].

Table 3. *Chromosome and mitotic aberrations in the root meristem cells of Allium cepa after extract treatment with various concentrations of aqueous extract of G. kola.*

Treatment groups	Concentration	Chromosome bridges (%) ±SD	Chromosome laggards (%) ±SD	Sticky chromosomes (%) ±SD	Nuclear vacuoles (%) ±SD	Total chromosome aberrations (%) ±SD
Control	Distilled H_2O	-	-	-	-	-
1	5µg/ml	-	0.15±2.05	0.29±1.17	-	0.44±2.64[a]
2	10µg/ml	0.12±1.23	0.37±6.12	0.85±0.88	-	1.34±7.64[b]
3	25µg/ml	0.21±3.12	0.64±4.32	0.21±3.52	0.21±3.28	1.27±3.56[b]
4	40µg/ml	0.32±3.33	1.11±5.66	2.06±4.05	0.16±0.91	3.65±3.49[c]

[a] Significant at $p<0.05$; [b] Significant at $p<0.01$; [c] Significant at $p<0.0001$ as compared to their respective control analyzed with ANOVA one Way performed.

Nuclear vacuoles were recorded in this study (Figure 2d). These nuclear vacuoles appeared as clear and unstained portions of the Prophase nucleus. They were surrounded by chromatin material. In some of the cells, one or two nuclear vacuoles were evident. This type of aberration was present only in the higher concentrations of 25µg/ml and 40µg/ml of the *G. kola* extract. Nuclear vacuolization is a rare phenomenon that occurs in the nucleus [42]. It has been variously recorded in hepatocytes [43] and *A. cepa* cells treated with various concentrations of plant extract and water collected from contaminated sources [44-47]. Environmental toxicity is one of the main sources of nuclear vacuoles [48]. It therefore follows that nuclear vacuoles were recorded in this study because the aqueous extracts of *G. kola* made the micro environment toxic. Evidence abound to the fact that toxic environments affect chromosome coiling and mitotic spindle activities and hence, the mitotic process [49].

4. Conclusion

This study revealed that aqueous extracts of *G. kola* inhibited onion (*A. cepa*) root germination and growth, reduced mitotic index, induced chromosomal aberrations such as anaphase chromosome bridges, anaphase chromosome laggards, chromosome stickiness, and nuclear vacuoles. These results indicated that the aqueous extract of *G. kola* contains some cytogenotoxic agents. It is advised that in spite of health benefits, bitter cola should be eaten with moderation.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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