

Mutagenic Effects of *Borassus Aethiopum* (Mart) Placenta Ash Extracts on the Root Tips of *Allium Cepa* (L)

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Abstract

This study investigates the Mutagenic Effects of *Borassus aethiopum* (Mart) Placenta Ash Extracts on the Root Tips of *Allium cepa* (L). The aim of the study was to determine the mutagenic effects of *Borassus aethiopum* placenta ash water extracts on onion (*Allium cepa*) root tips. The seeds of *A. cepa* were obtained locally from Jimeta main market. The Seeds were dried in the laboratory. Ash extraction was prepared using the traditional method practiced by the Kilba, Marghi and Bura people, with some modification. The seeds selected were soaked for 48 hours. Root tips were harvested when the roots are about 2.5 cm long. The harvested root tips were immediately dropped into a solution of 0.05% colchicine for 5 hours, at the end of which the root tips were washed thoroughly in running tap water and placed in glacial acetic alcohol 1:3 v/v for 24 hours for fixation. The root tips were then hydrolysed in a water bath at 60 degrees. *Allium* test carried out on different concentrations of raw *Borassus aethiopum* Placenta Ash water extract revealed induction of chromosomal aberrations in the genome of *Allium cepa*. Chromosomal aberrations such as sickness, fragmentation, break, laggards, dissolution, Chromosome gap, Vagrant metaphase and Agglutination were observed in onion roots tips treated with 0.5, 0.75, 1.0 and 1.25 g/ml concentrations of the *Borassus aethiopum* Placenta Ash water extract. Highest percentages (16.6%) of chromosomal aberrations were recorded for 1.25 g/ml extract, while 0.75g/ml of it gave the lowest percentage (7.3%). No chromosomal aberration was observed in the control and 0.5g/mls concentration. Observations made in this study call for caution in the consumption of raw *Borassus aethiopum* Placenta Ash water for treatment of ailment. Low concentration (0.5g/ml) and wide spacing of dosage are therefore suggested for the use of *Borassus aethiopum* Placenta Ash water extract in traditional medicine and soup preparation in order to prevent the risk of genetic accidents (mutation).

Keywords: *Borassus Aethiopum*, Mutagenic, *Allium Cepa*, Aberration, Ash Extracts.

Introduction

Since the middle of the twentieth century and until today, the use of food additives has become more widespread. A food additive is any substance or combination of chemicals, excluding basic food components, added to food in a scientifically controlled amount (Mpountoukas *et al.*, 2008). These additives are used mainly to maintain or improve nutritional quality and to maintain the freshness and quality of the product. to aid in the processing or preparation of food, and finally to make the food more appealing.

Manufacturers use them in various food products, medicines, and cosmetics to raise their use value, give them more flavor, give them the appropriate organoleptic qualities, and give them a longer shelf life (Leathwood *et al.*, 2007; Carochio *et al.*, 2014). Unfortunately, however, these additives can have adverse health effects (Jarskog, 2006). The use of food additives regulated in Codex Alimentarius is justified only when it does not pose a risk to the health of consumers, does not mislead the consumer, and gives the affected product a benefit that cannot be obtained through alternative economic and technological means (FAO and WHO, 2019). Food additives are essential to the modern food supply.

However, because it is carbon-neutral, renewable, and a byproduct of burning wood, ash is categorized as a green energy source (Auta *et al.*, 2015). According to reports, among other things, it is used for medicinal purposes, as a food additive, to make soap, to ripen crops, in agriculture, and for insect control. Kyarisiima *et al.* (2004) reported that several residents of the Nigerian middle belt have reportedly used the ash extract as a food ingredient and medicine (Auta *et al.*, 2015). Some food additives (FA) have genotoxic and carcinogenic effects, and some of them have been found to play a role in the formation of neurodegenerative diseases, hyperactivity, allergies, diabetes, obesity, and reproductive and gastrointestinal system disorders. During food production, preparation, processing, packaging, transportation, and storage, which are not consumed as food with or without nutritive value, selected technologies that are allowed to be used to prevent adverse effects are defined as food additives. Therefore, it is not an exception (Dosay-Akbulut, 2020).

Additionally, it has been noted that the aqueous wood-ash extract of plants impairs male fertility in male albino mice by negatively affecting sperm motility, count, morphology, and pathological abnormalities of the testes (Auta and Hassan, 2016). The oestrus cycle and implantation in female *Mus musculus* have been found to be altered in females by the aqueous wood-ash extract of *Azadirachta indica* (Auta and Hassan, 2016). High amounts of polycyclic aromatic hydrocarbons and phytochemicals have been found in the aqueous wood-ash extract of *Parkia biglobosa*, which are thought to have negative effects on animals, including humans (Auta *et al.*, 2015). In 2018, it was reported that exposure to an aqueous wood-ash extract of *P. biglobosa* caused dose-dependent depletion of spermatogenic cells in the testes of male Swiss albino mice (Auta and Hassan, 2018).

The Gbagyi, Nupe, Ebira, Marghi, Bura, Kilba, and other significant ethnic groups in Nigeria's north-central, middle-belt, and northeastern regions use ash extract to make soups and as a stomach-ache remedy. With the exception of nitrogen, which is evaporated after fire, wood ash contains all the necessary plant nutrients (Karlton *et al.*, 2008). Wood ash contains heavy metals, including lead, zinc, and cadmium, in addition to beneficial mineral nutrients. Wood ash generally contains amounts of cadmium, lead, and zinc that are high enough for it to be considered a "toxic waste." Rodriguez *et al.*, 2019).

The plant *Borassus aethiopum* Mart is a member of the Aracaceae family, also referred to as the African Fan Palm. The plant's shoot is widely accessible, known as "Muruchi" in Hausa, and it is edible (Tata *et al.*, 2021). Locally, it is referred to as Giginya by the Hausa,

Dubbe by the Fulani, Agbon Oludu by the Yoruba, and Kemelutu by the Kanuri. Only in Northern Nigeria is the young sprouting stalk of the plant known as Muruchi in Hausa, and it is consumed there both as food and as an aphrodisiac. The stated benefits of the plant's various sections (Akinniyi *et al.*, 2000) In northern Nigeria, *Borassus aethiopum* shoots are grown and eaten. Although there has been little research on its toxicity, its starch has been studied for application in pharmaceutical formulation. The degree to which a chemical (a toxin or poison) can harm people or animals is known as its toxicity. The potential result of genetic harm that a cell may sustain is referred to as mutagenicity in a cell. All cells and organisms have a damage response mechanism. Mutagenicity agents, or mutagens, are substances that cause mutagenicity in a cell (Committee on Mutagenicity, 2018, Alessandro 2013).

The consumption of ash extracts as a food additive by some ethnic groups in Adamawa State, Nigeria, without any or adequate knowledge of their possible cytological effects on their health, has been a continuous practice from time immemorial. Earlier studies on plant ashes have focused mainly on their uses and environmental impacts; there is no adequate information on mutagenic effects on ash, especially that of *Borassus aethiopum* placenta ash, which is mostly used as food additives, medicinal products, and other essential purposes. Different ethnic groups in Nigeria are using aqueous ash extracts as food additives and for medical purposes, which is causing worry about their harmful reproductive consequences (Auta *et al.*, 2015). There is currently a dearth of knowledge on the potential harmful effects of *Borassus aethiopum* placenta ash extracts; therefore, this research aims at determining the mutagenic potentials of *Borassus aethiopum* placenta ash extract on the root tip cell of *Allium cepa*.

Materials and Method

Procuring the Plant Material

The seeds were obtained locally from Jimeta main market. The Seeds were dried in the laboratory. Those seeds with full endosperm devoid of black spots were selected as being probably viable.

Procuring the *Borassus Aethiopum* Placenta Ash Water Extract

Ash extraction was prepared using the traditional method practiced by the Kilba, Marghi and Bura people, with some modification. The ashes collected from the burned placenta of *Borassus aethiopum* were placed into stainless steel bowls and wetted with pump water. The bowl, containing the wet ash was heated on hotplate for 120 minutes (2 hours) at a moderate temperature of 40 °C to remove all moisture and volatiles (as vapor) and organics (as carbon dioxide and nitrogen) . The ash was later transferred to a perforated bowl, placed on a clean empty container (bowl) that served for collection of extracts. The perforated bowl was underplayed with cloth sieve. The ash in the perforated bowl was stayed for an hour to settle and tap water was poured into the bowl and allowed to percolate into the

container. This process was carried out repeatedly until enough extract was collected. The collected aqueous extracts were further processed to its solidification for 1-2 hours. Considering the quantity being used by house hold in soup preparation which was, 0.5g, 0.75g, 1.0 g and 1.25g of the extract measured was mixed in 100 mls of each distilled water as adopted from (Auta *et al.*, 2015).

Planting of the Seed (*A. Cepa*)

The seeds selected were soaked for 48 hours. Seeds were also soaked in different concentration of the *Borassus aethiopium* placenta ash water extract for 48 hours before planting on a filter paper in a petri dish at room temperature. Watering of the plant was done ones daily in the morning and evening using tap water.

Harvesting of Root Tip

Root tips were harvested when the roots are about 2.5 cm long. Harvesting of the root tip was done between the hours of 7 am-9 am in which the metaphase spread of meristematic cells of the growing root tip was assumed to be very active. Before root harvesting, the setup was not watered for 24 hours.

Pre-treatment and Fixation

The harvested root tips were immediately dropped into a solution of 0.05 % colchicine ($C_{22}H_{35}O_4$) for pretreatment. Pretreatment in colchicine was for 5 hours, at the end of which the root tips were washed thoroughly in running tape water and placed in glacial acetic alcohol 1:3 v/v for 24 hours for fixation. After fixation, the root tips were hardened in absolute ethanol for one hour and washed in 30 % ethanol for 3 hours before storing in 70 % ethanol in the refrigerator at 4°C until required for use as adopted (Yildiz *et al.*, 2009).

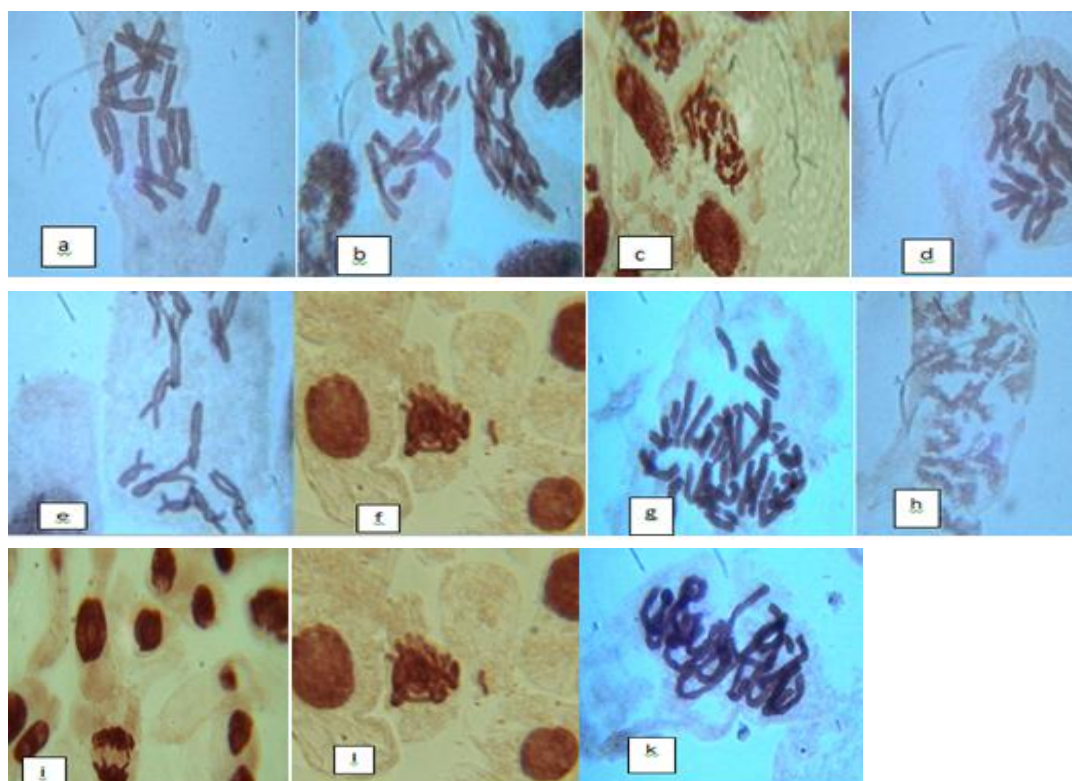
Hydrolysis and Staining

The root tips were picked from the 70 % ethanol using a clean pair of forceps into a test tube containing distilled water. After 15 minutes of repeated changes in distilled water, the root tips were rinsed in two quick changes 1NHCL; first in cooled, followed by warm one normal hydrochloric acid (NHCL). The root tips were then hydrolyzed in 1 NHCL for 10 minutes in a water bath regulated at 60°C (Syakhril and Kuswanto, 2019). On removal from the water bath, the root tips were rinsed again in cold 1NHCL to arrest the process of hydrolysis, and again washed in distilled water for another 15 minutes. At the end of the last washing time, the root tips were sufficiently softened for squashing. Squashing was done by placing one root tip at a time on a clean dry glass slide using a pair of pointed forceps. The young meristematic portion was cut out to allow for the discarding of the older portion. One drop of 2 % acetic orcein ($C_{28}H_{24}O_7N_2$) was added to the meristematic root tip and allowed for 60 seconds for proper stain absorption. The stained root tip was carefully covered with a clean cover slip. Gentle taps on the coverslip using the blunt end of a mounted pin ensured proper

squashing of the root tips as prescribed by Östergren and Heneen (2009). The whole set-up was allowed to stand for five minutes for deep staining of the chromosomes to take place. The slide was mounted and viewed, first with the X10 objective of an optical binocular research microscope, slides with good metaphase stages containing well-spread chromosomes were first drained of excess stain and then sealed using Ladines white vanish.

Result and Discussion

Micrograph showing normal cell and different types of aberration induced by *Borassus aethiopium* placenta ash water extract.



(a) Normal cell (b) chromosome stickiness (c) fragment (d) c-mitosis (e) laggard (f) micronuclei (g) chromosome break (h) dissolution (i) chromosome bridge (j) vagrant metaphase (k) agglutination

In vivo testing of the mutagenic effects of *Borassus aethiopium* placenta ash extract on the mitotic chromosome of *A. cepa* root tip cells revealed that plant extracts are not mutagenic at the recommended dose of 0.5g or 0.5% concentration. However, at higher concentrations, the plant ash extracts showed the potency to induce genetic damage at the levels of the chromosomes and their spindle apparatus in *Allium cepa*, thus causing both chromosomes and chromatid-type aberrations. The specific types of aberration observed were as variable as the plant ash extracts employed. The aberrations observed were: chromatid gap, agglutination, dissolution, fragmentation, stickiness, vagrant metaphase,

laggards, and chromosome polyploidy (endomitosis). However, the levels at which any of the chemical metabolites present in the ash extracts could have been responsible for the aberrations observed on the micrograph above appear to be beyond the scope of the present investigation. These investigations, however, provided cytological evidence, that ordinary clean water does not induce chromosomal aberrations in cell genomes, but *Borassus aethiopum* placenta ash extract did. Similar observations have been reported by Bakare *et al.* (2003) on the induction of chromosomal aberration in *Allium cepa* chromosomes by raw and simulated leachate from a refuse dump.

Table 1: Types and Percentage of Aberrations Observed on the Root Tip of *A. Cepa* Treated with *Borus athiopum* Placenta Ash Extracts

Conc. g/ml	Chromosomal aberration													
	TCS	TDC	ST	F	CM	LG	MN	BK	DS	CG	VM	AG	TNAC	%AC
Control	150	142	-	-	-	-	-	-	-	-	-	-	0	0
0.5	150	140	-	-	-	-	-	-	-	-	-	-	0	0
0.75	150	135	1	1	-	1	-	-	4	0	2	2	11	7.3
1.0	150	127	6	1	6	3	3	4	3	2	1	2	28	16.6
1.25	150	126	6	1	6	3	3	4	3	2	1	2	28	16.6

TCS (total cell score), **TDC** (total number of dividing cells), **ST** (stickiness), **F** (fragmentation), **CM** (c-mitotic), **LG** (laggard), **MN** (micronucleic), **BK** (break), **DS** (dissolution), **CG** (chromosome gap), **VM** (vibrant metaphase), **AG** (agglutination), **TNAC** (total number of aberrant cells), **%AC** (percentage of aberrant cells)

Table 1 above shows the effects of ash extracts at different concentrations. The results show significant inhibition of the mitotic process obtained during cell division and the progressive damage of chromosomes through agglutinations, induction of chromatid gaps, bivalent chromosome dissolution, fragmentation, stickiness, lagging, and non-orientation of bivalents, including endomitosis recorded at the 0.75 %, 1.0 %, and 1.25 % concentrations of *Borassus aethiopum* placenta. Ash extracts suggest the ability of the extracts to interfere with the organism's mitotic as well as its meiotic processes. The same effect has earlier been reported by Gomez-Arroyo *et al.* (1985) in *Vicia faba*. Indications from the results of the present study also suggest that the ability of these ash extracts at high concentrations to cause chromosome fragmentation permits their characterization as clastogens. This is also supported by Amer and Ali (1969), who found that penta-chlorophenol induced fragmentation of both mitotic and meiotic chromosomes in *Vicia faba*. They opined that most chemical clastogens can decrease the rate of cell division in addition to their role as chromosome-breaking agents.

At a higher concentration (1.25%) of treatment with *Borassus aethiopum* placenta ash extracts, polyploidy (C-mitosis) was induced in a situation in which the number of

chromosomes doubled. The induction of polyploidy by the ash extracts could be due to the arrest of the activities of the spindle fibers, which are supposed to move the separated chromatids to the different poles, or the prevention of the formation of the cell wall to separate the divided chromosomes during cytokinesis. Both events result in a genome consisting of duplicate chromosomes. This agrees with the results obtained by Gomez-Arrayo *et al.* (1985), The polyploidy (C-mitosis)-inducing effect of *Borassus aethiopum* placenta Ash extracts, therefore, poses the likely danger of the said abortion in pregnant women when *Borassus aethiopum* placenta Ash extracts are taken above the recommended concentration. This anti-spindle action of ash extracts indicated in this study probably involves the same mechanism as that of other inhibitors such as colchicine. The potential for *Borassus aethiopum* placenta ash extracts to cause mutation, as exemplified, might also alter the genetic material of their patients so that these changes can be transmitted during cell division. Alterations and deficiencies as seen in the present investigation during fertilization could lead to early embryonic deaths or later result in abortion or serious congenital abnormalities. The observation of chromosomal aberrations in *Borassus aethiopum* placenta ash extracts is an indication that ash extracts could be mutagenic to the human genome, especially at high concentrations. Though the chromosomal aberrations were observed in the plant system, the findings cannot be overlooked because the nucleus of a plant cell, as in man and other animals, contains the same complete blueprint for inheritance. All information, as in man, also resides on the chromosomes of typical plants. (M. M. Malgwi, 2016 Inaugural Lecture).

Conclusion

The study of mutagenic effects of *Borassus aethiopum* placenta ash water extracts on onion (*Allium cepa*) root tips whose seeds were obtained locally from Jimeta main market was successfully carried out and the effect of the ash extracts on the mitotic chromosome of *Allium cepa* root tips was also determined. *In vivo* testing of the ash extracts on *Allium cepa* root-tips revealed that the extracts were not mutagenic at 0.5 % concentration, but as the concentration of the ash extracts increases to 0.75 %, 1.0%, and 1.25%, the *Borassus aethiopum* placenta Ash extract showed the potency of induce genetic damage at the level of chromosome and its spindle apparatus on *Allium cepa* root-tips. The Aberrations observed were stickiness, Fragmentation, C-mitosis (polyploidy), Lagard, Micronucleic, Break, Disolution, Chromosome gap, Vagrant metaphase, and, Agglutination. And also as the concentrations of the ash extracts is increasing there is decrease in the number of normal cell and the percentage of mitotic index (MI) respectively.

Recommendations

- Since it has been indicated as mutagenic on the root tip of *Allium cepa*, a low concentration of the ash extract is suggested.

- If *Borassus aethiopum* placenta ash extracts are to be used as food additives or for traditional medicine, further studies should be directed towards considering concentrations lower than the ones used in this study and using ash extract samples from different localities.
- More research at the molecular level should be encouraged in this area so as to reveal the mutagenic potentials of plants materials used as food or medicinal purposes ensuring the revelation of the effects at the cellular level.
- The potential *Borassus aethiopum* placenta Ash extracts to cause mutation as exemplified, might also alter the genetic material of their patients, so that these changes can be transmitted during cell division therefore, user of ash extracts should be advised to always use definite measurement in their day to day meals or prescribing dose to their patients.
- Alterations and deficiencies as seen in the present investigation during fertilization could lead to early embryonic deaths or later result in abortion or serious congenital abnormalities. As a result of that, appropriate quantity of chemicals should be use during the preparatory of locally made food additives industries.

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Conflict of Interest

Authors declared that there is conflict of interest.

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