



GENOTOXICITY EVALUATION OF AQUEOUS LEAF EXTRACT OF BAMBOO LEAF (*Bambusa vulgaris*) USING *Allium cepa* ASSAY

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Abstract

The genotoxicity of aqueous leaf extract of *Bambusa vulgaris* was evaluated using *Allium cepa* assay. To evaluate the induction of chromosomal aberration, six onion bulbs were suspended on 2%, 10%, 20%, 50% and 100% concentrations of each of the extract and the control (tap water) for 48 hours. The abnormalities observed include stickiness, c-mitosis, vagrant, bridges fragment, multiple anaphases, and attached. The result is in line with several investigations on the urgent need to standardize the dosage of medicinal plant extract for the treatment of various ailments. Mitotic index was recorded to be high at control and decrease as concentration of extract increased. The result suggested that the tested extract possess inhibitory and mitotic depression effect on the root growth cell of division and chromosomes behavior of *Allium cepa*. It was recommended that genotoxicological effect of the tested extract in animal system (such as mice and rats) for human welfare as *Bambusa vulgaris* aqueous leaf extract has been consumed to cure many diseases.

Key words: Aqueous leaf; *Allium cepa*; *Bambusa vulgaris*; mitotic depressive

Introduction

Background to the study

The medical use of plant has always been part of human practice and it may be as old as human kind itself. It has been estimated that between 60-90% of the population of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002). The interest in the use of medicinal plants is based on the assertion that they contain natural substances that can promote health and alleviate illness. They are considered to be safe, relatively less expensive, globally competitive and patient tolerate them well (Partap *et al.*, 2012a). The evaluation of toxic, cytotoxic and genotoxic damage caused by plant compounds is of fundamental importance in minimizing the possible risks of these agents, especially when they are part of long term treatment (Rodeiro *et al.*, 2006). Nature has given us plant as a free gift for our own good, but the untapped benefits of this free gift of nature still remain a work to be done. Medicinal plants are used by the human population, especially in developing countries where the lack of financial resources hinders access to the treatment of disease by conventional medicine. However, the majority of the species uses in folk medicines have not been sufficiently studied,

especially when it comes to studies regarding mutagenic compound.

Ethnobotanical knowledge provides a wealth of information regarding both past and present relationship between plants and the traditional society. Investigation into traditional use and management of local flora demonstrated the existence of local knowledge of not only about the physical and chemical properties of many plant species but also the phonological and ecological futures in case of domesticated species. One of the most prominent contribution of ethnobotany to the society is natural medicine. In addition, the ethnobotany also contributed many beneficial things such as food, shelter, dyes, fibre and non-timber forest products (NTFPS). In India, about 400 larger and 277 smaller tribal community altogether make about 7.5% of total population. The tribal population of 70 million niche is to the extent of 0.075% of its total population. These tribal communities depend entirely on the forest and forest products they gather wide leaves, roots, tubers and fruits makes rooms for wide creepers and barks and collect honey bee wax for use or for exchange of agricultural produce with neighboring communities. In Nigeria, a large percentage of the population are dependant on herbal medicine because the international commercial medicine are becoming increasingly expensive and out of reach (Fasola and Egunyomi, 2005). Extracts from plants have been reported to be effective in treating illness, sleeping

sickness, wounds, diarrhea, reproductive and liver problem, circulatory and respiratory and parasite infections (Obi *et al.*, 2006). Despite the profound therapeutic advantages posed by some of the plants, some constituents of medicinal have shown to be potentially toxic, carcinogenic and tetratogenic (Gadeno *et al.*, 2006). However, Nigeria is indigenously rich in plant, biodiversity commonly used as medicine for the management and treatment of different form of ailments. The bamboo trees can be found in every continent mostly in Asia, America and Africa except Antarctica. Bamboo plant is distinguished by its oral leaves and hallow stem that have node in between the segment. Both bamboo leaves, branches and stems tends to remain greenish in colour during its lifetime except during fire outbreak or harsh sunlight. The leave of *Bambusa vulgaris* (bamboo leave) are used for treatment of fever and hypertension. Among the various species of medicinal plants used as treatment, *Bambusa vulgaris* is indicated due to its medicinal properties for a range of pathological signs and symptoms. The *Allium cepa* root meristem assay is considered widely as a practical reliable system for the screening of environmental mutagenic and casinogenic. Thus in this study, bulb of *Allium cepa* was used to access the genotoxicity of aqueous leave extract of *Bambusa vulgaris*. However, despite the profound therapeutic advantages possessed by some of the medicinal plant, some constituents of medicinal plants have been shown to be potentially toxic, mutagenic and carcinogenic. Hence, this present study is aimed at determine the genotoxicity evaluation of aqueous leaf extract of *Bambusa Vulgaris* using *Allium cepa* assay.

Materials and methods

Collection of leaf of *Bambusa vulgaris*

Fresh plants of *Bambusa vulgaris* (Bamboo leaf) were collected from Oyo State College of Agriculture and Technology area of Igbo-ora. The plant was identified taxonomically in the biology laboratory department of Science Laboratory Technology. For the *Allium cepa* assay, onion bulbs of the same size were purchased from Towobowo Market, Igbo-ora, Oyo State.

Preparation of leaf extract

Extractions were carried out as practice locally. Leaf of *Bambusa vulgaris* was weigh (320g) and boiled in one litre (1Ll) of tap water. The result of the decoction was kept. The decoction extract was filtered to remove the suspended particles at room temperature. This was considered as the stock solution.

Allium cepa assay

The onion bulbs were sundried for two weeks to prevent rotting as well as to encourage usability. Poorly preserved bulbs, moldy and those that have started shooting green leaves were all discarded.

Microscopic evaluation

The onions were exposed for 48 hours to test the sample on 2%, 10%, 20%, 50% and 100% concentrations of extract respectively. Tap water was used as control, the base of each bulbs was suspended on each test sample inside 100ml beaker in the dark cupboard for 48 hours. Each sample was setup in 5 replicates. The test samples were changed every 24 hours with fresh test samples. At the end of the exposure period, only four bulbs showing the best root growth were selected from each concentration of the test samples and control. For microscopic evaluation of induction of chromosomal aberration, four onion bulbs were suspended in each of the test samples and control for 48hrs. Afterward, the root tips from these bulbs were excised and fixed in ethanol; glacial acetic acid (3:1 vlv) farmer's fixative and kept in tooth pick containers to prevent the root from drying out and to maintain the genetic integrity of the roots cells. The root tips were stored in the freezer at 40⁰c. The already fixed roots tips were removed from the container and placed in petri dishes containing 1N hydrochloric acid then heated in an oven at 60⁰c for five minute to soften the tissue after which they were rinsed three times in distilled water (Odeigah *et al.*, 1997) The root tips were then gently collected with a spatula and each placed on clean microscopic slide and squashed with a clean glass rod. After through maceration, the root tips were stained with 2% acetocermine dye. They were allowed to stand for three minutes before carefully placing clean cover slips over them at an angle of 45⁰ to exclude air bubbles. Excess stains were removed with tissue paper acting as a blotter. The blotter was placed on the cover slip and pressed down to fix into position. Two fingers were used to press from left to right over the cover slip to remove superfluous stain and arrange the chromosome in one plane. Then the edges of the cover slips were sealed on the slides with nails varnish, this is to prevent dry out of the preparation under the heat of the microscope (Sharma, 1983). A total of about 500 cells from five scorable slides per sample (i.e 100 dividing cells per slide made from onion root tips excised from each concentration of extract in which they were planted) were observed at 100 x magnification for different mitotic stages and chromosomal aberration were calculated based on the number of aberrant cells per total cells scored at each concentration of each sample (Bakare *et al.*, 2000)

The mitotic inhibition was determined using the following formula:

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{ Aberrant Cells} = \frac{\text{Number of Aberrant cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{ root growth of control} = \frac{\text{Overall mean root Length of test solution}}{\text{Overall mean root Length of Control}} \times 100$$

Statistical analysis

Data were expressed as standard error of mean (SEM). Differences between the different concentrations of

the sample was analyzed using the student's unpaired t-test. All statistical analyses were carried out by SPSS14.0 statistical package.

Results

Table 1: Effects of Treatment with Different Concentrations of Aqueous of *Bambusa vulgaris*

Concentration %	Mitotic index	Number of cell	Number of dividing cell	Stickiness	C. mitosis	Chromosomes Vagrant	Aberration Bridges fragment	Multiple anaphase	Attached	% aberration
0	7	500	35	0	0	0	0	0	0	0
2	5.6	500	28	0	1	0	4	1	0	1.8
10	4.8	500	24	3	1	4	2	1	1	2.4
20	3.6	500	18	3	0	3	1	0	1	1.4
50	2	500	10	2	0	2	2	1	0	1.4
100	1.6	500	8	2	0	1	2	0	1	1.4

Table 1 shows the effect of treatment with different concentrations of aqueous leaf extract of *Bambusa vulgaris*. There was a decrease in the Mitotic Index (MI) with increasing concentration of aqueous leaf extract. Analysis of the chromosomes showed that the concentration of aqueous leaf extract of *Bambusa vulgaris*

induced chromosomal aberration significantly when compared to the control. No aberration was recorded in the chromosome of *Allium .cepa* exposed to the control, sickness, C-mitosis, vagrant, multiple anaphase and attached were observed in the chromosomes of *Allium .cepa* exposed to different concentrations.



Figure 1: Image of bamboo leaf (*Bambusa vulgaris*)

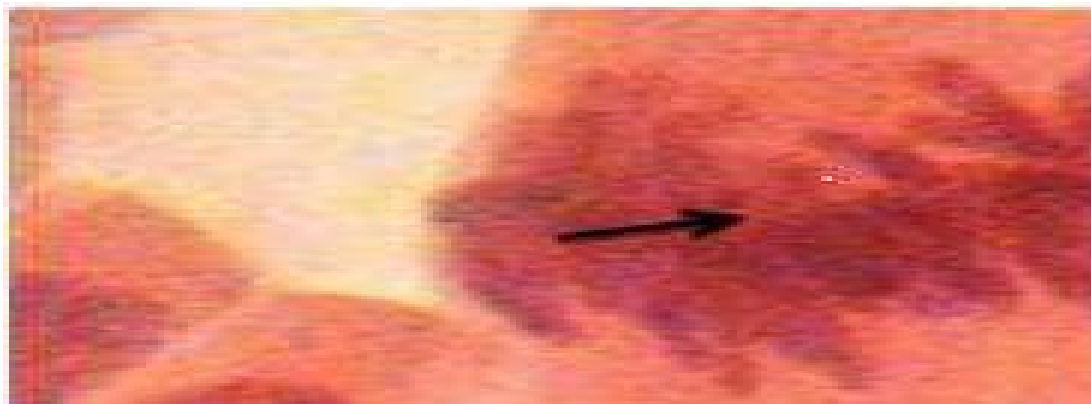


Figure 2: Sticky chromosomes induced by samples in root tips of *Allium cepa*

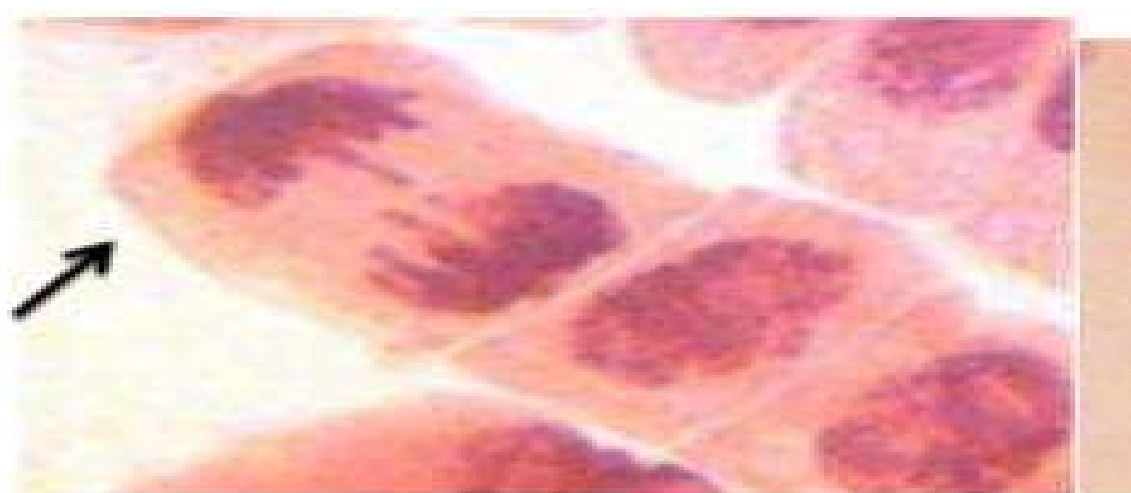


Figure 3: C-mitosis chromosomes induced by samples in root tips of *Allium cepa*. This indicates induced abortive nuclear division in which the chromosomes number is double.



Figure 4: Vagrant induced by samples in the root tips of *Allium cepa*. This shows when chromosomes moves ahead from its chromosomal group toward poles and lead to the unequal separation of number of chromosomes of daughter cell.

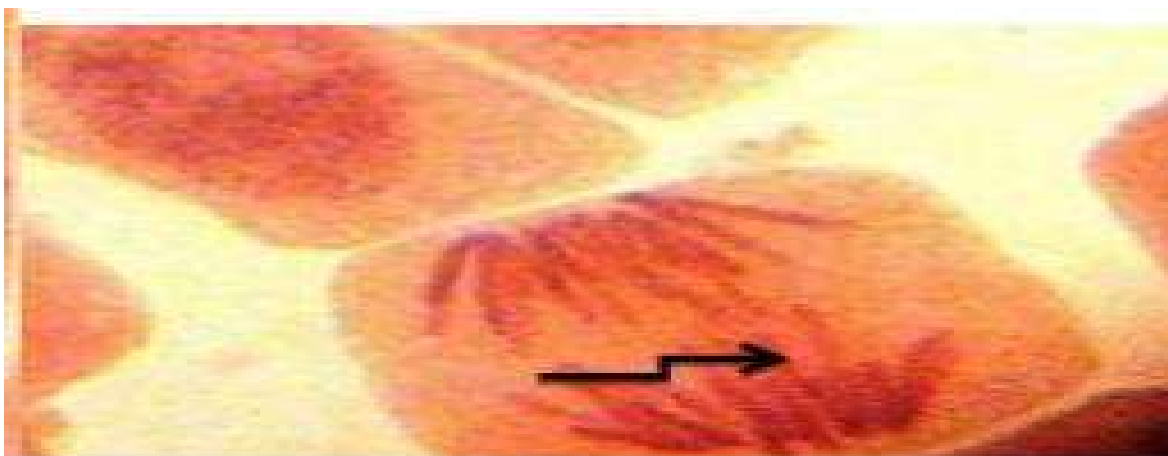


Figure 5: Bridges fragment induced by samples in root tips of *Allium cepa*.



Figure 6: Multiple anaphase induced by samples in root tips of *Allium cepa*.

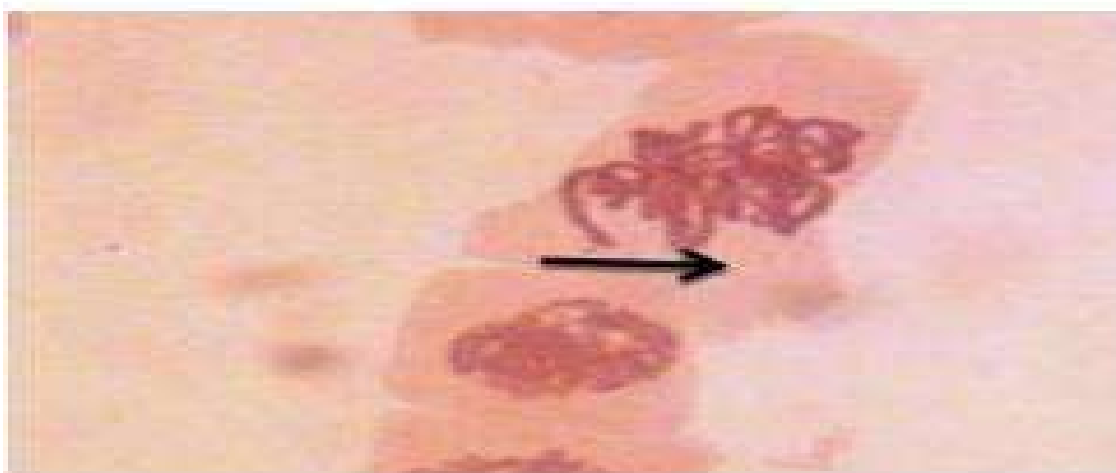


Figure 7: Attached induced by samples in root tips of *Allium cepa*.

Discussion

Research on medicinal plant with proven pharmacological activities and therapeutic benefits especially in the management of chronic disease for which their uses is prolonged now focuses on the investigation on their roles in human mutagenesis.

The potential genotoxic effect of aqueous leaf extract of *Bambusa vulgaris* on *Allium cepa* was evaluated. The tap water was used as a control which is of extremely good quality.

The *Allium* model has long been acknowledged to possess the ability to interact with mutagenic agent during its cell cycle as a result the test has often been

used to determine the genotoxic effects of harmful chemicals (Fiskesjo, 1997). Data obtained from the effect of the aqueous leaf extracted from *Bambusa vulgaris* on root growth of *Allium cepa* showed that there was a concentration suggesting toxicity, which corroborates with earlier reports on the cytotoxic and genotoxic of aqueous leaf extracts of some herbal preparations (Akinboro and Bakare, 2007). Numerous studies have shown that whenever there is root growth inhibition in the *Allium* test, there is always reduction in the number of dividing cell (Olorunfemi *et al*, 2011). The reduction in the number of dividing cell is a reflection of the mitodepressive effects of the cell division of *Allium cepa*. An aqueous extract of *Bambusa vulgaris* may possess ability to block the synthesis of DNA and nucleus-proteins. The mitotic index (MI) observed to decrease with increase in concentration of extract. This decline is attributed to interference in the cell cycle and the increase in the concentration of the extract as earlier reported by Adegbite and Sanyaolu (2009).. Chromosomal aberrations are change in chromosome structure resolution from a break of exchange of chromosomal material. The chromosomal aberration noticed in this study was majorly sticky chromosomes, bridges, C.mitosis, vagrant, multiple anaphases and attached. The altered functioning of this protein is caused by mutation in the structural genes coding for them or by the direct action of mutagens (Turkoglu, 2007). Stickiness reflects high toxicity of substances as well as irreversibility of the change, bridges and fragments have clastogenic effects, both resulting from chromosomal and chromatic breaks. C. mitosis otherwise known as colchicines mitosis is and inactivation of spindle followed by a random scattered of the chromosomes over the cell. A delayed centromere division may results in the chromosomes assuming the characteristic C pairs configuration in which sister chromatids, while the remaining attached at the centre mere, no longer remain adjacent to one another. The chromosomal aberration observed in the study is evidently caused by the chemical constituent in the extract since no aberration was observed in the control.

Conclusion

The result herein suggests that the tested extract possess inhibitory and mitodepressive effects on the root growth cell division and chromosome behavior of *Allium cepa*

Recommendation

It was recommended that genotoxicological effects of the tested extract in the animal system (such as mice

and rats) for human welfare as *Bambusa vulgaris* aqueous leaf has been consumed to cure many diseases.

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