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Cytotoxic effects of *Aloe vera* leaf extract on *Allium sativum* root tips

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ABSTRACT

The cytotoxic effects of Aloe vera leaf extract at different levels of concentrations on the mitotic cell division of Allium sativum root tip cells was investigated. The root tips of Allium sativum from the same clove were grown in different concentrations of Aloe vera gel extracts (0%, 25%, 50%, 75% and 100%) and harvested between 7:30am and 8:30am for cytological studies. Pretreatment, fixation, hydrolysis, squashing and staining of the cells for mitotic study were carried out and chromosome counts were done under X400 magnification of the light microscope. Observation in this study shows that the addition of the gel extracts induces two major chromosomal aberrations which include binucleate cells and C-mitotic cells. The result also revealed that no dosage of Aloe vera gel extract is completely safe from inducing mutagenic effect on the root tips of Allium sativum. Further investigations are recommended to ascertain the mutagenic effect of the consumption of Aloe vera gel on man.

Key words: Cytotoxic, *Aloe vera* , mutagenic, binucleate cells, C-mitotic cells.

INTRODUCTION

Aloe vera according to [10] is without doubt the most widely known medicinal herb highly valued for its health benefits and at the same time used in the cosmetic industry. It is well known that the use of plants as therapeutic material is due to the presence of chemical substances of medicinal value [14]. *Aloe vera* has been used for its curative and therapeutic properties on ailments ranging from dermatitis to cancer for centuries. It continues to be used even though its therapeutic effects have not been correlated well with individual components of *Aloe vera* [7], [9], [12].

The heterogeneous compositions of *Aloe vera* pulp according to [15] may contribute to the diverse pharmacological and therapeutic activities that have been observed in *Aloe vera* L. Different studies have indicated the anti-tumour activity of *Aloe vera* gel in terms of its ability to reduce tumour burden, tumour shrinkage, tumour necrosis and prolong survival rates. In addition to these effects, *Aloe vera* gel was also shown to have chemo-preventive and anti-genotoxic effects [6].

Herbal preparations in general contain active ingredients which act on the ailments as well as those that have no activity on the ailments. Since the herbal preparations are not refined as the western medicine, the non-essential ingredient in the herbal preparations which are consumed along with the essential ones, are accumulated in the human system [8]. The lack of standard prescriptions and the unrefined nature of the herbal preparations sometimes lead to over dosage and bioaccumulation of both essential and non-essential plants metabolites in human system

[1].This bioaccumulation if continued for a long time can become cytotoxic and can offset the biochemical equilibrium of the delicate human system or cause some genomic disruptions in the cells of the human system. The genomic disruptions which are damaging to the DNA could range from point mutation to chromosomal mutations [5]. This suggestive biochemical and genetic risks involved in the indiscriminate consumption of herbal preparations therefore calls for caution in adopting herbal preparation for Medicare.

Allium sativum (Garlic) is commonly used in this type of cytological work because it contains few chromosomes suitable for cytological studies and presents clear elucidation of aberrations and also it contains genetically uniform cloves. [1] opined that the defects occurring on the treated *Allium* bulbs and cloves can also be expressed in human cells since both *Allium* and human genomes are living systems containing DNA in the cells. Published information is very scarce on this study to the best of my knowledge, it is therefore necessary to study the effect of different concentrations of *Aloe vera* gel extract on the genetic system using *Allium sativum* as the test organism.

The aim of this research work is to examine the effect of leaf gel extracts of *Aloe vera* on the mitotic cell division of the root tip cells of *Allium sativum*.

MATERIALS AND METHODS

Collection of Material

Fresh leaves of *Aloe vera* were collected from Kogi State University Staff Quarters in Dekina Local Government Area of Kogi State, Nigeria. The leaves collected were thoroughly washed with tap water. Garlic (*Allium sativum)* used were purchased from Anyigba market and these were also washed thoroughly with water.

Preparation of Test Materials

The gel extracts of *Aloe vera* were pressed out of the leaves into a beaker by manually squeezing the leaves and the following concentrations of *Aloe vera* gel extracts were prepared: 100%, 75%, 50% and 25%. *Allium sativum* with 20 cloves was considered for this study. Each clove was allowed to sprout roots by placing them in beakers containing water at room temperature. After 72 hours, the roots were transferred to beakers containing different concentrations of *Aloe vera* extracts and the Garlic roots were left in the solutions for 24 hours. Root tips of the Garlic sample measuring about 1cm in length were harvested into vials between 7:30am – 8:30am. Pretreatment, fixation, hydrolysis, squashing, staining of cells and preparation of slides were carried out according to the method outlined by [2].

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 different mitotic stages and chromosome abnormalities observed were taken using photomicrograph. Counts of different mitotic stages were also recorded.

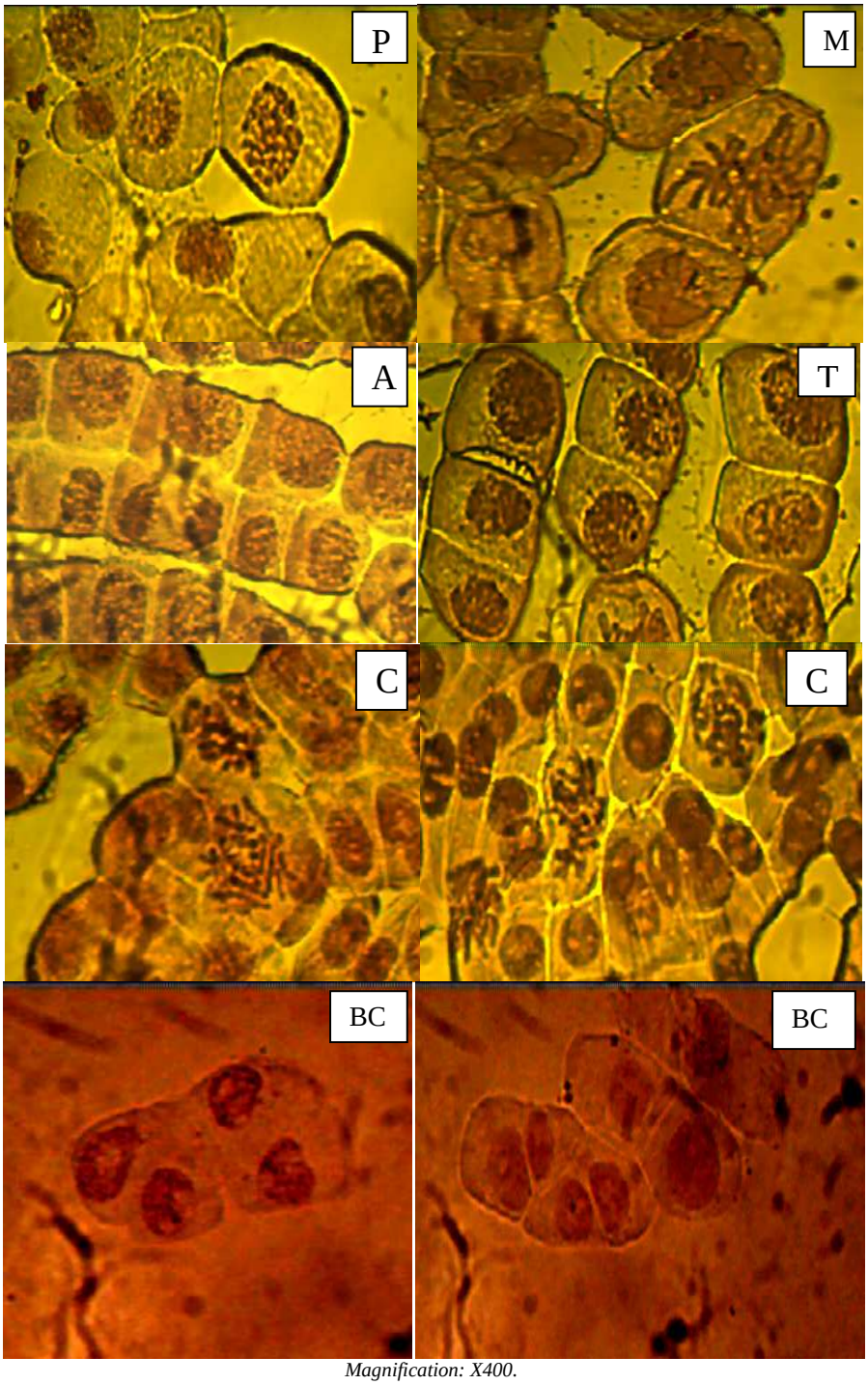
Data Analysis

Ten different counts each were taken for total number of cells, number of dividing cells, number of binucleate cells and number of C-mitotic cells for all the different concentrations of *Aloe vera* gel extracts considered. Data pooled for each attribute were subjected to Analysis of Variance (ANOVA) and means with significant differences were separated using Duncan Multiple Range Test (DMRT).

The Mitotic Index (MI) for the cells treated with different concentration of *Aloe vera* gel extracts was calculated using the formula proposed by [3] as given below:

$$\%\text{Mitotic Index (MI)} = \frac{\text{Total Number of dividing cell}}{\text{Total number of cell examined}} \times 100$$

RESULTS



Magnification: X400.

Plates 1: Photomicrographs of the treated *Allium sativum* root tip cells showing different mitotic stages and aberrations

Key: P-Prophase, M: Metaphase, A: Anaphase, T: Telophase, C: C-Mitosis, BC: Binucleate cell.

The different mitotic stages and the two major aberrations induced by the addition of different concentrations of *Aloe vera* gel extracts are shown in plate 1. The aberrations observed in this study are binucleate cell and C-mitosis. The effect of different concentrations of the gel extracts on mitotic stages of *Allium sativum* is shown in table 1. The

result shows that more cells are under division with the introduction of *Aloe vera* gel extract where a total of 8 cells were observed to be dividing in control while 108, 103, 104 and 104 cells were dividing in 25%, 50%, 75% and 100% concentration of *Aloe vera* gel extract respectively.

Table 1: Effect of different concentrations of *Aleo vera* gel extracts on Mitotic Stages of Garlic Root Tips

Treatments	Prophase	Metaphase	Anaphase	Telophase	Total No. of dividing cells	Mitotic Index (MI) (%)
0 % (Control)	2	1	3	2	8	2.79
25%	28	31	39	8	108	40.01
50%	33	26	27	17	103	38.76
75%	36	43	14	11	104	36.38
100%	22	37	35	10	104	36.37

The Mitotic Index (MI) was recorded to be very low in control (untreated Garlic root tips) with 2.79% while higher values of 40.01%, 38.76%, 36.38% and 36.37% were recorded for 25%, 50%, 75% and 100% concentrations of *Aloe vera* extracts respectively.

Table 2: Mean Effect of different concentrations of *Aleo vera* gel extracts on Cytological Parameters of Garlic Root Tips

Treatments	Total number of cells	Numbers of dividing cells	Numbers of binucleate cell	Numbers C- Mitosis
0 % (Control)	265.20	7.40^{a}	0.00	0.00^{a}
25%	265.10	105.00^{b}	3.55	15.56^{b}
50%	263.70	102.20^{b}	1.82	11.89^{b}
75%	283.40	103.40^{b}	3.36	13.44^{b}
100%	283.40	103.10^{b}	5.68	9.78^{b}
LSD Value	NS	10.41	NS	8.15

Means with the same alphabets are not significantly different from each other.
*Key: NS = Not Significant ; + =Highest Value; * =Lowest Value*

The effect of different concentrations of *Aloe vera* extracts on cytological parameters of *Allium sativum* root tips is shown in table 2. Three out of the five cytological parameters showed significant difference among the treatments (control, 25%, 50%, 75% and 100%). These are numbers of dividing cells, number of C-mitotic cells and mitotic index. The total number of cells and number of binucleate cells did not show significant differences among the treatments (Table 2).

Number of dividing cells in treated root cells was recorded to be highest in 25% (105.00) while control (untreated root) had the least number of dividing cells (7.40).

Number of C-mitotic cells was recorded to be highest in roots treated with 25% concentration (15.56) while 50%, 75% and 100% concentrations had 11.89, 13.44 and 9.78 respectively. No binucleate and C-mitotic aberrant cell was recorded in the untreated (control) root tip cells (Table 2).

It was also observed that roots treated with different concentrations of *Aloe vera* extracts (25%, 50%, 75% and 100%) showed no significant difference among themselves for all the five cytological parameters considered. However, some levels of differences were recorded between the untreated roots and each of the concentrations for all the cytological parameters considered except the total number of available cells.

DISCUSSION

In this study, *Allium sativum* was used as the experimental material because chromosomal aberration observed in plants according to [3] shows resemblance with the aberration produced in mammalian cells. Also [13] reported that positive correlation existed between the aberrations induced by omnacortil in plant root tip cells and in cultured mammalian cells which indicates that plant root tip system can be recognized as an appropriate first-tier assay system for this type of study.

Cell division is one of the most important phenomenons, which controls the growth of an organism and the behaviour of chromosomes during cell division is one of the unique features of cell division.

In this study, the mitotic index values (table 1) in the treated root cells were higher than the values calculated for control. Also from the same table 1, more cells were observed to undergo different stages of cell divisions (prophase, metaphase, anaphase and telophase) compared with the untreated root tips (control). This suggests that *Aloe vera* gel extracts enhance the mitotic activities of *Allium sativum* root tip cells. The mitotic index was observed to slightly reduce with increase in concentrations of *Aloe vera* extracts applied. This indicates that the higher the concentration the lower the mitotic activities of the cells. This observation contradicts the finding of [1] that the mitotic index values in the treated onion root cells were lower than the value calculated for control as a result of the treatment with neem leaf extract. But this finding agrees with the report of [4, 16] who reported reduction in mitotic indices with an increase in the concentration of leachate wastes.

The treatment of *Allium sativum* root tip cells with different concentrations of *Aloe vera* gel extracts induced two major types of chromosomal aberrations, viz: binucleate cells and C-mitosis (plates 1g, 1h, 1d and 1e and table 2). From table 2, it could be observed that there are no statistical significant differences among the different concentrations of *Aloe vera* gel extracts and the control in terms of the total number of cells and the number of binucleate cells observed in the root of *Allium sativum*. This indicates that the introduction of *Aloe vera* gel extract does not affect the total number of cell available in the roots and the induction of binucleate cells aberration. This finding contradicts the report of [3] on the effect of omnacortil on *Allium cepa* root tips where the total number of cells was reported to be higher in control than the cells in treated root tips. Binucleate cell arises as a result of the inhibition of cell plate formation i.e arrest of cytokinesis.

A statistical significant difference was observed in this study in terms of the total number of dividing cells and C-mitosis (table 2). This is an indication that the addition of *Aloe vera* gel extracts affects the total number of dividing cells and number of C-mitotic cells. This suggested that every concentration of *Aloe vera* gel extract can affect the rate of spindle formation which probably induced cells that were not initially dividing to start the division process. From the observation in this study, the consumption of *Aloe vera* extracts as a raw product calls for caution since it has been indicated to be mutagenic though not as mutagenic as other plant extracts previously reported by researchers. [1] reported the induction of polyploidy cells, laggards, anaphase bridge, scattered chromosomes and chromosome breakage at anaphase when onion root tip cells were treated with neem leaf extract. [13] also reported stickiness of chromosomes, anaphase bridge, C-mitosis and chromosome vagrant as a result of treatment of *Allium cepa* (L.) with different concentrations of *Euphobia hirta*. *Boerhaavia diffusa* root extracts according to [11] induced stickiness of chromosomes, C-mitosis, chromosome variant, bridges fragment and multi-polar anaphase on *Crinum jagus* root tips. According to [1] the chromosomal aberration associated with herbal medication were traced to the presence of chemical such as alkaloids, flavonoids, terpenoids, tannins, carcinogens, etc, contained in the plant. They attributed the induction of C-mitosis by neem leaf extracts to the arrest of the activities of the spindle fibres which are supposed to move the separated sister chromatids to the poles.

CONCLUSION

This study revealed that generally the addition of *Aloe vera* gel extract affects the mitotic activities of the root tip cells of garlic *(Allium sativum)*. Therefore, it can be concluded that the consumption of *Aloe vera* extracts as a raw product should be with caution since it has been indicated to be mutagenic on the root tip of *Allium sativum* although it is not as mutagenic as other plant extracts previously reported by researchers. However, the findings in this study cannot be overlooked because the toxicological target is the DNA, which exists in all cellular forms including humans.

Further studies should therefore be directed towards considering the application of *Aloe vera* extracts for different durations and its cytological effects on man.

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