

Full Length Research Paper

# Cytotoxicity testing of aqueous extract of bitter leaf (*Vernonia amygdalina* Del.) using the *Allium cepa* chromosome aberration assay

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**Mitotic studies were carried out on *Allium cepa* (onion) root cells treated with different concentrations of aqueous leaf extract of *Vernonia amygdalina* (bitter leaf). The onion root cells showed reduced mitotic indices with corresponding increase in concentration of the bitter leaf extract [200 g/L (C<sub>2</sub>), 400 g/L (C<sub>3</sub>) and 500 g/L (C<sub>4</sub>)]. Chromosomal aberrations, such as endopolyploidization, lagging of chromosomes and cells with giant chromosomes, were also observed in onion roots treated with same. No chromosomal aberration was observed in the control and in onion roots treated with 100 g/L (C<sub>1</sub>) of the leaf extract. These observations indicate that abnormal use of this medicinal herb could cause genetic damage. Low concentration and wide spacing of dosage are, therefore, suggested for its dietary intake or use in herbal medicine.**

**Key words:** *Vernonia amygdalina*, *Allium cepa*, chromosome aberration, cytotoxicity, herbal medicine.

## INTRODUCTION

Medicinal plants have been widely used by both ancient and modern man of all cultures for treating different ailments. A single plant processed in different formulations can be used to cure a wide range of diseases.

The universality and efficacy of traditional medicine/medicinal herbs is evident in their continued use and dependence up till the present day by a significant portion of the world's population (Mathews et al., 1999). However, the historic role of medicinal herbs in the treatment and prevention of diseases and in the development of pharmacology do not assume their safety for uncontrolled use by an uninformed public (Mathews et al., 1999). Injuries and even death resulting from misuse, contamination and/or adulteration of medicinal herbs have been reported (De Smet et al., 1997).

In spite of the efficacy of the medicinal herbs in the treatment of various kinds of ailment, the unrefined nature of the preparations and the lack of standard prescriptions on dosage constitute a major setback in the use of herbs in medicare. These two weak points on the medicinal herbs can lead to complications in human sy-

stem resulting from bioaccumulation of plant ingredients due to over consumption of the herbs (Okafor, 1987). Other causes of complications include uptake of toxic plant ingredients, and possible herb/herb and herb/drug interactions (Okafor, 1987; Mathews et al., 1999).

*Vernonia amygdalina* Del. belongs to the family Asteraceae, tribe Vernoniaeae and it is widely distributed in Nigeria (Hutchinson and Dalziel, 1963). The plant is a perennial shrub or small tree, usually cultivated for its leaf as vegetable, medicinal, traditional and domestic uses. The leaves have a very bitter taste, due to its chemical contents (Belitz and Grosch, 1987; Lasekan et al., 1998), which are responsible for its medicinal and anti-microbial properties (Rice et al., 1987; Okoh et al., 1995). Herbal preparations made from leaves of *V. amygdalina* are used in curing ailments such as malaria, measles, dysentery, onchocerciasis, yellow fever, constipation, stomach pain, etc, while slender roots and stem branches of the plant are used as chewing stick that are very effective in dental care (Oluwalana and Adegunle, 1998). Herbal preparations of various forms are prepared from different parts of bitter leaf plant, but the easiest form is the fresh leaf extract, which is prepared by squeezing the leaves in water. The leaf extract is usually taken raw by people at an unregulated rate, depending on the severity

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of the ailment (Eisenberg et al., 1993).

The unrefined nature of the herbal preparations, coupled with the apparent lack of specificity or precision in the application of the plant in traditional medicine could lead to over dosage of the herbal medicine, which can result in accumulation of essential and non-essential plant ingredients in the human system. The accumulation can reach a toxic level, especially in the systems of people who rely heavily on unrefined herbal products, with severe consequences on their biochemical and genetic systems (Adebowale, Personal Communication).

*Allium* test is a sensitive test that has often been used for the determination of cytotoxic and/or genotoxic effects of various substances (Grant, 1982; Smaka-Kinel et al., 1996). The test has been shown to have a good correlation with tests in other living systems; hence, results obtained from *Allium* test are usually handled with care, because it could serve as an indicator of toxicity of the test materials (Fiskesjo, 1997). The usefulness of root tips of *A. cepa* as a test system for monitoring the genotoxic effects of test materials was demonstrated by Fiskesjo (1985).

Different concentrations of aqueous leaf extract of *V. amygdalina* were subjected to *Allium* test to check their mutagenic effects on the chromosome activities of *A. cepa*.

## MATERIALS AND METHODS

### Preparation of *V. amygdalina* aqueous leaf extract

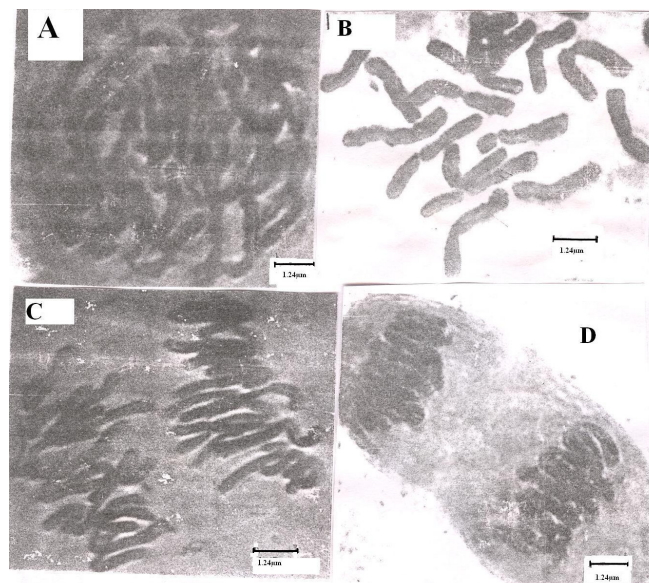
Leaves were collected from different stands of *V. amygdalina* growing in one location at the Research Farm of the University of Agriculture, Abeokuta, Nigeria. The leaves were rinsed and air-dried at room temperature by spreading them on laboratory table for 24 h. 100, 200, 400 and 500 g of leaf samples collected were weighed and blended in 1 L of water until homogenous formulations were obtained. The resultant formulations were filtered with a piece of muslin cloth and the filtrates were tagged C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub> respectively. The filtrates were preserved in the refrigerator (4°C) until they were used.

### Initiation and fixing of onion root-tips

The old roots and loose scales on onion bulbs purchased from the local market were carefully removed, after which the bulbs were rinsed in water. The onion bulbs were placed on medium-sized beakers filled with different concentrations of the *V. amygdalina* aqueous leaf extract, with the base of the onion bulbs touching the surface of the extract. Onion bulbs seated on beaker filled with clean water served as control. Bulbs that did not root directly on the extract were first seated on water to initiate rooting before transferring them on beaker containing the *V. amygdalina* aqueous leaf extract. The roots were allowed to elongate to about 1 - 2 cm before fixing them in 1:3 (v/v) acetic acid/ethanol between the hours of 9.00 a.m. and 12.00 noon for 24 h.

### Cytological studies

The fixed roots were hydrolyzed in 1 N HCl for 5 min and rinsed



**Figure 1.** Regular mitotic division in *A. cepa*. A = Prophase, B = metaphase, C = anaphase, and D = telophase. Scale line represents 1.24 µm.

with water. Slides were prepared using the squashing and staining techniques described by Adegbite and Olorode (2002). The slides were observed under the light microscope and data on total cells, total dividing cells and cells carrying chromosomal aberrations were taken from 100 microscope fields on at least 20 slides prepared for each of the different treatments and the control.

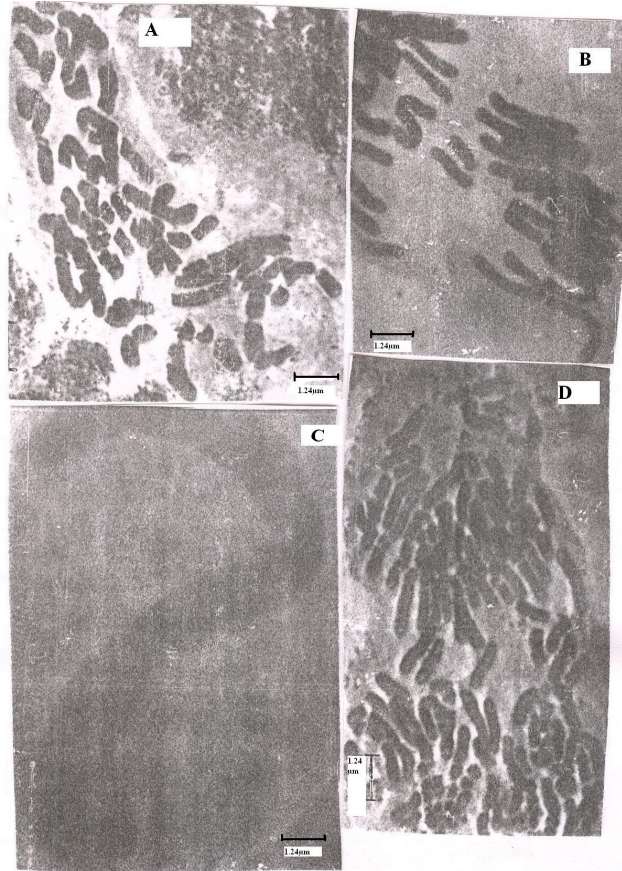
Incidence of chromosome aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment. Mitotic index was calculated by expressing the number of dividing cells as a percentage of total cells counted for each of the treatments and the control. Photomicrographs of normal and aberrant dividing cells were taken at X1000 magnification under oil immersion, using a Leica 2000 phase contrast microscope.

## RESULTS AND DISCUSSION

Cell division was normal in the control and onion root cells treated with C<sub>1</sub>, with somatic chromosome counts of  $2n = 16$  (Figure 1). Chromosome aberrations observed include anaphase with laggards, endopolyploid metaphase and anaphase cells, cells with giant chromosome and chromosome breakages (Figure 2).

Tables 1 and 2 show the data on the mitotic activities and chromosome aberrations observed in onion root cells treated with *V. amygdalina* aqueous leaf extract. The mitotic index values were lowered in the treated onion root cells when compared with the control, and the mitotic index values were observed to be decreasing with increasing concentrations of the *V. amygdalina* aqueous leaf extract. The number of aberrant cells was also observed to be increasing with the concentration of the *V. amygdalina* aqueous leaf extract.

The decreased mitotic index values in the treated onion roots is an indication of the presence of cytotoxic subs-



**Figure 2.** Mitotic irregularities observed in the treated *A. cepa*. A. Endopolyploid metaphase cell with broken chromosomes. B. Anaphase cell with laggards. C. Cell with giant chromosome. D. Endopolyploid Anaphase cell. Scale line represents 1.24  $\mu\text{m}$ .

**Table 1.** Mitotic activities in *Allium cepa* root cells treated with *V. amygdalina* aqueous leaf extract.

Formulation	Prophase cells	Metaphase cells	Anaphase cells	Telophase cells	Total dividing cells	Total cells counted	Mitotic index
Control	352	255	350	751	1708	5276	32.37
C <sub>1</sub>	69	83	59	461	672	2762	22.52
C <sub>2</sub>	56	74	58	324	517	2546	20.31
C <sub>3</sub>	45	41	53	428	567	3554	15.95
C <sub>4</sub>	35	54	35	200	324	2521	12.85

**Table 2.** Chromosomal aberrations in *Allium cepa* root cells treated with *V. amygdalina* aqueous leaf extract.

Formulation	Total cells counted	Total dividing cells	Anaphase laggards	Polyploid metaphase	Polyploid anaphase	Giant chromosome	Total aberrant cells	Aberration incidence (%)
Control	5276	1708	0	0	0	0	0	0
C <sub>1</sub>	2762	672	0	0	0	0	0	0
C <sub>2</sub>	2546	517	0	2	4	0	6	1.16
C <sub>3</sub>	3554	567	2	5	10	2	19	3.25
C <sub>4</sub>	2521	324	5	10	10	0	25	7.71

tances in the *V. amygdalina* aqueous leaf extract, which caused inhibition of mitotic activities, while the observation of aberrant cells in the treated onion root tip meristems indicates genotoxic effects of the leaf extract (El-Shahaby et al., 2003). *Allium* assay is a sensitive test, and it has been shown to have correlation with tests in other living systems and serve as an indicator of toxicity of the tested material (Fiskesjo, 1985). The observed decrease in the mitotic index values and the increase in the incidence of chromosomal aberrations with corresponding increase in the concentration of the extract conformed to the findings of Bakare et al. (1999, 2003). The chromosomal aberrations induced in the treated onion root cells were definitely caused by the chemical ingredients in the *V. amygdalina* aqueous leaf extract, since such aberrations were not observed in the control. No aberration was recorded for onion root cells treated with C<sub>1</sub>, but higher concentrations of the extract induced chromosomal aberrations. This indicates that this concentration, C<sub>1</sub>, is safe for consumption and for the treatment of ailments that *V. amygdalina* is known for. However, risk assessment investigations are required, as well as pharmacokinetics and toxicokinetics studies.

The induction of endopolyploidy could be attributed to either the arrest of the activities of the spindle fibers (which are supposed to move the separated chromatids to different poles) or chromosome duplication that was not followed by cytokinesis. Thus, resulting in a single genome consisting of duplicated chromosomes.

Cells with giant chromosome observed in this study were similar to those reported in *Crotalaria retusa* (L) and *Aspilia africana* (Pers) by Akpabio (1986) and Adegbite (2003), respectively. These workers speculated that the giant cells probably resulted from the coalescence of the metaphase elements, which then replicated many times to give such a large mass of chromatin material. The formation of polytene chromosomes has been attributed to multiple DNA replications without separation of the individual chromatin strands (Swanson et al., 1967; Mackean, 1977).

The observation of cells with laggards, chromosome breakage, giant cells and endopolyploid cells in the treated onion cells is an indication that the extract, especially at high concentrations, is capable of causing changes in chromosome number and structure.

The actual plant ingredients responsible for the observed chromosomal aberrations were not ascertained in this study, but they are consumed along with the ones acting on the ailments that *V. amygdalina* is known for. These observations, therefore, call for caution in the unguided use and consumption of herbal preparations in medicare, because of their potential cytotoxic and genotoxic effects on the genetic systems of individuals that rely so much on herbal preparations.

These observations did not rule out the efficacy of bitterleaf aqueous leaf extract in traditional medicare, and the study was not designed to jettison the use of bitter leaf or any other herb in traditional medicine, but it has

shown the need to apply caution in the indiscriminate consumption of herbal preparations. Low concentrations and long spacing of dosage are, therefore, suggested for the use of bitter leaf extract in traditional medicare.

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