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Full Length research Paper

Mutagenic effect of neem leaf extract used in traditional medicine on *Allium cepa* (L.)

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Allium **test carried out on different concentrations of raw** ***Azadirachta indica*** **(neem) leaf extract revealed induction of chromosomal aberrations in the genome of** ***Allium cepa.*** **Chromosomal aberrations such as scattering of chromosomes at anaphase, anaphase bridge, chromosome fragmentation, laggards and endopolyploidization were observed in onion roots treated with 0.1, 0.2, 0.4 and 0.5 kg/L concentrations of the neem leaf extract. Highest percentages (39.41%) of chromosomal aberrations were recorded for 0.5 kg/L of the neem leaf extract treatment, while 0.1 kg/L of it gave the lowest percentage (5.56%). No chromosomal aberration was observed in the control. Observations made in this study call for caution in the consumption of raw leaf extract of neem for treatment of ailment. Low concentration and wide spacing of dosage are therefore suggested for the use of** ***A. indica*** **(neem) leaf extract in traditional medicine in order to prevent the risk of genetic accidents.**

Key words: *Azadirachta indica* (neem), Allium cepa (L), chromosomal aberrations, traditional medicine.

INTRODUCTION

In most countries of the world, there exists traditional knowledge about the health of human and animals. In recent times, attention is being drawn to the adoption of traditional medicine, which is largely due to the current resurgence of interest in the use of naturals in developed countries and the search for new phytophamaceuticals for the prevention and cure of deadly diseases (De Silva, 1997).

In Africa generally, and West Africa in particular, medicinal plants are indispensable constituent of human diets (Dalziel, 1937). Traditional healings and remedies made from plants play important roles in the health care of millions of people in the developing and underdeveloped countries of the world.

The neem plant (*Azadirachta indica* A. Juss) is one of the most commonly used and well known medicinal plants used in traditional medicine. A large number of medicinals, cosmetics, toiletries and pharmaceuticals are now based on neem products (Vanna, 1976; Krans, 1995). The anti-malarial activity of neem is attributed to gedunin, which is a limonoid (Krans, 1995).

Neem plant parts can be used alone either in the decoction form or by squeezing to get the plant extract which is usually consumed raw for treatment of fever. It can also be combined with parts of other plant species or mixed with black soap (local soap) for the treatment of all kinds of fever and skin diseases when used for bathing (Jolaoso, personal communication).

There has not been any formal report on dosage problems, side effects or known reactions traced to the excessive use of herbal preparations of neem in Nigerian traditional medicine. The major discrimination against the use of neem herbal preparations is the bitterness, which has reduced its acceptability and utilization by the people, especially those having dislike for bitter products. This problem can however be solved by making the neem products into sugar coated capsules.

In places where herbal preparations are popular and widely used, people tend to consume them indiscriminately. The improper uses of herbal preparations arise from the fact that there is no standard prescription of dosage for most of the preparations, hence people tend to consume them depending on the severity or magnitude of the ailments or infections.

Herbal preparations in general, contain active ingredients which act on the ailments, as well as those that have no activity or bearing on the ailments. Since the herbal preparations are not refined as the western medi-

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cines, the non-essential ingredients in the herbal preparations, which are consumed along with the essential ones, are accumulated in the human system. 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 plant ingredients in human system.

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 damages to the DNA, could range from point mutations to chromosomal mutations (Bakare, 2001). This suggestive biochemical and genetic risks involved in the indiscriminate consumption of herbal preparations therefore, calls for caution in adopting herbal preparations for medicare.

Allium test (Fiskesjo, 1985) involves the direct treatment of onion bulbs with plant extracts or such substances suspected of being capable of causing cytological defects. Such defects are damages to DNA. It is therefore, believed that the defects occurring on treated onion bulbs can also be expressed in human cells since both *Allium* and human genomes are living systems containing DNA in the cells.

This study was undertaken to monitor and assess any cytotoxic effect of extracts of some medicinal plants (e.g. neem) used in tradomedical practices. The study was designed to carry out *Allium* test on different concentrations of herbal preparations made from neem leaves. The effect of these on chromosome activities in treated onion root cells were closely monitored for any mutageneic effects due to treatment with the different concentrations of the neem herbal preparations. This is with a view to identifying and recommending a mutagenically safe concentration for consumption of such herbal preparations.

MATERIALS AND METHODS

Fresh leaves of neem were collected from a tree growing in the compound garden/yard of a building at Obantoko, Abeokuta, Odeda Local Government area of Ogun State, Nigeria. The tree which is over ten years old is known to serve a lot of people living in the area, who collect leaves and stem bark from it to make herbal preparations to cure fever. Leaves collected from the middle region of the tree canopy were washed and air- dried by spreading them on a clean laboratory table for 24 h. Leaf samples of 100, 200, 400 and 500 g were taken from the lot and kept in separate bags with labels. The leaf samples were weighed to prepare low concentration extract with 100 and 200 g samples and high concentration extract with 400 and 500 g samples which are the concentrations used by local herbalists for curing mild and acute fever.

Preparation of neem leaf extract

Each of the weighed neem leaf samples was pounded in a mortar with pestle after which 1 litre of clean ordinary tap water was added to each sample to squeeze out the extract. By this extraction, the four different concentrations of the extract obtained are 0.1, 0.2, 0.4 and 0.5 kg/L. Traditionally, neem leaf extracts are obtained either by pounding the leaves as described above or by squeezing the leaves with bare hands in water. However, accurate measurements of leaf sample weights and volume of water are not usually taken by the traditionalists.

Mitotic studies

Onion roots used were the new roots initiated from washed onion bulbs by sitting the bulbs on beakers containing the prepared extracts. Five onion bulbs were set up for each treatment. The initiated roots were collected when they were about 1 - 2 cm long, between the hours of 9.00 am and 12.00 noon when mitotic activities are believed to be high. The roots were fixed in 1:3 (v/v) acetic acid/ethanol fixative for 24 h before using them for mitotic studies. Roots initiated by sitting onion bulbs on beaker containing clean water were used as control.

Squashing techniques described by Olorode (1974) and modified by Adegbite and Olorode (2002) were used in preparing slides from which photomicrographs of good dividing cells were taken at X1000 magnification under oil immersion, using a Leica 2000 phase-contrast microscope.

Mitotic data were taken from 100 microscope fields on at least 20 slides prepared for each treatment and control. The cells were scored for the different cell division stages, total dividing cells, total undividing cells and number of cells carrying chromosomal aberrations in the microscope fields covered.

Mitotic index and percentage chromosome aberration were calculated for each treatment and the control, using the following formulae:

Mitotic Index = (Number of dividing cells/Total number of cells counted) x 100

% Chromosomal Aberration = (Number of aberrant cells/Number of dividing cells counted) x 100

Data analysis

The data on mitotic index and incidence of chromosomal aberrations were subjected to analysis of variance (ANOVA) to examine the relationship between the concentrations of the neem leaf extract used and mitotic index of the *A. cepa* cells on one hand and incidence of chromosomal aberrations in *A. cepa* on the other hand.

RESULTS

The mitotic activities and the chromosomal aberrations observed in the cells of the treated onion roots and the control are shown in Tables 1 and 2, respectively. The mitotic index values estimated for onion root cells were found to be lower in all the concentrations used than that of the control root cells. The highest mitotic index value of 9.14% was recorded for roots treated with 0.5 kg/L of the neem leaf extract, while was recorded for roots treated with 0.1 kg/L of the extract (Figure 1). The control gave a mitotic index value of 9.93%. Mitosis was observed to be normal in the cells of the control. Somatic chromosome counts of 2n = 16 were made at metaphase, and subsequent stages of mitosis were normal (without any aberration). Plates 1A - D show normal prophase, metaphase,

Table 1. Mitotic activities of root cells of *A. cepa* treated with neem leaf extract.

Concentration of neem leaf extract (kg/L)	Prophase cells	Metaphase cells	Anaphase cells	Telophase cells	Cytokinesis cells	Total dividing cells	Total cells counted	Mitotic Index (%)
Control	67	76	90	85	81	399	4020	9.93
0.1	24	57	80	68	41	270	4012	6.72
0.2	30	67	41	57	48	243	3085	7.88
0.4	20	62	40	84	54	260	2984	8.71
0.5	22	54	73	72	48	269	2942	9.14
Mean	32.60	63.20	64.80	73.20	54.40	288.20	3408.60	8.47
SE	8.76	3.89	10.28	5.23	6.96	28.12	249.06	0.55

Table 2. Chromosomal aberrations observed in the root cells of *A. cepa* treated with neem leaf extract.

Conc. of Neem leaf extract (kg/L)	Total cells counted	Total dividing cells counted	Chromosomal aberrations					Total aberrant cells counted	Incidence Of aberrant cells (%)
			Polyploid cells	Cells with laggards	Anaphase bridge	Scattered chromosomes at anaphase	Chromosome breakage at anaphase		
Control	4020	399	0	0	0	0	0	0	0
0.1	4012	270	3	0	0	8	4	15	5.56
0.2	3085	243	5	4	4	11	10	34	13.99
0.4	2984	260	8	7	12	18	16	61	23.46
0.5	2942	269	12	9	22	28	35	106	39.41
Mean	3408.60	288.20	5.60	4.00	7.60	13.00	13.00	43.20	16.48
SE	249.06	28.12	2.06	1.82	4.21	4.73	6.13	18.72	6.97

anaphase and telophase stages in the cells of the control. Chromosomal aberrations were observed in the cells of the treated roots for all the concentrations used, though the incidence of aberrant cells increased with the concentration of the leaf extract. The incidence of aberrant cells was observed to be lowest (5.56%) in cells of roots treated with 0.1 kg/L of neem leaf extract and highest (39.41%) in cells of roots treated with 0.5 kg/L of the extract (Figure 2). Plates 2A - F show the various chromosomal aberrations observed in the treated root cells. Plates 2A and B are polyploidized prophase and anaphase cells of roots treated with 0.5 kg/L of neem leaf extract. Plate 2C shows chromosome breakage and scattering of chromosomes at anaphase in cells of roots treated with 0.2 kg/L of neem leaf extract. Plate 2D is an anaphase cell of roots treated with 0.1 kg/L of neem leaf extract showing laggards and broken chromosome fragments. Plate 2E shows anaphase bridge, while plate 2F shows telophase with laggards in cells of roots treated with 0.4 kg/L of neem leaf extract.

The statistical analysis on the relationship of the different concentrations of the neem leaf extracts with mitotic index and chromosomal aberration data revealed a strong positive relationship (Tables 3 and 4). The analysis showed significant relationship between the treatments and the different values of mitotic index and chromosomal aberration percentages, with the relation-ship accounting for 95% of the total variation observed.

DISCUSSION

The observations made in this study provide

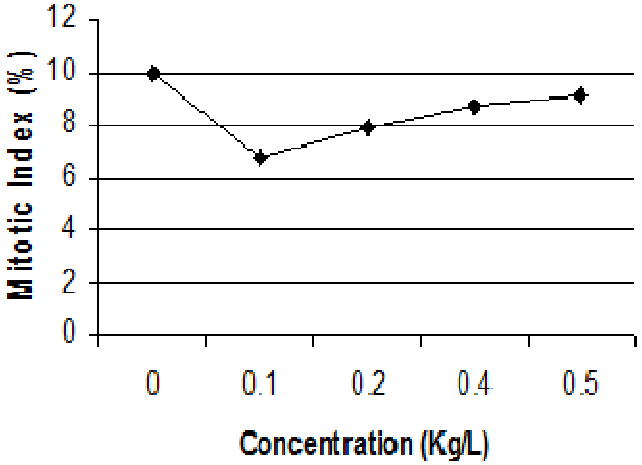
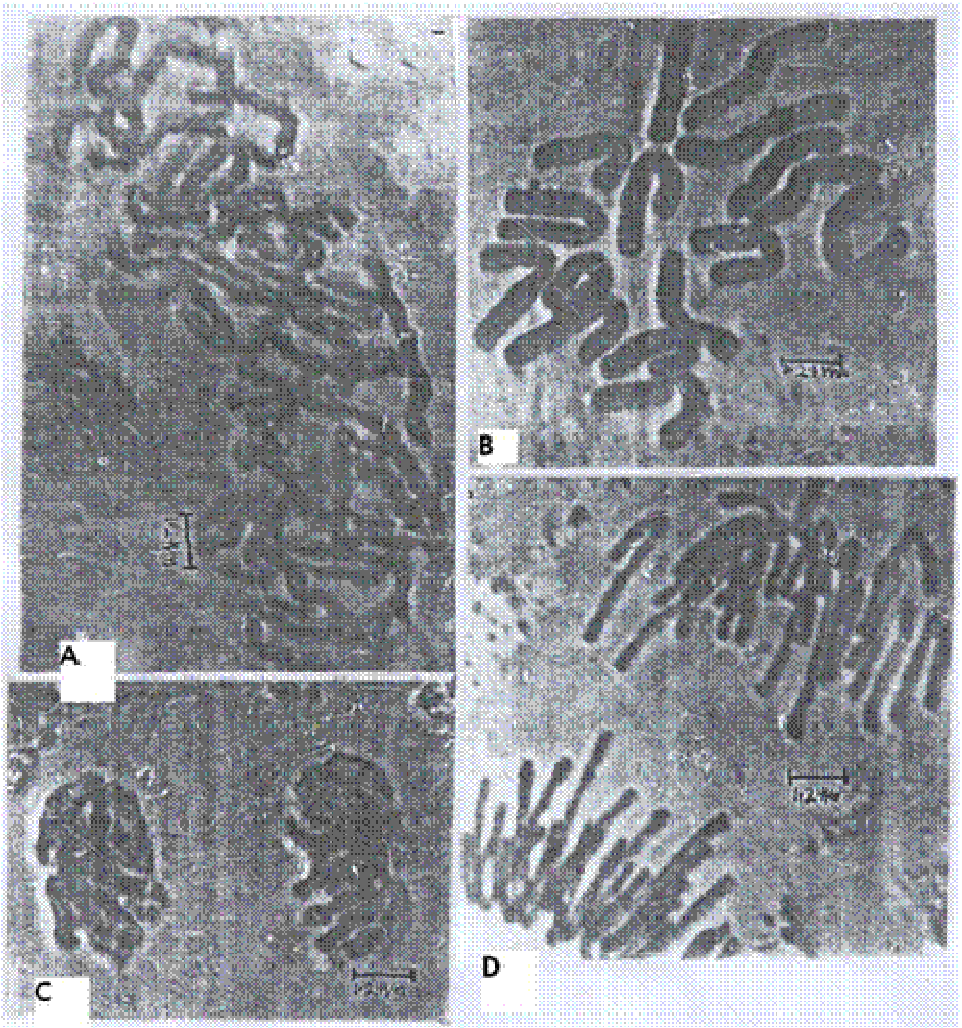
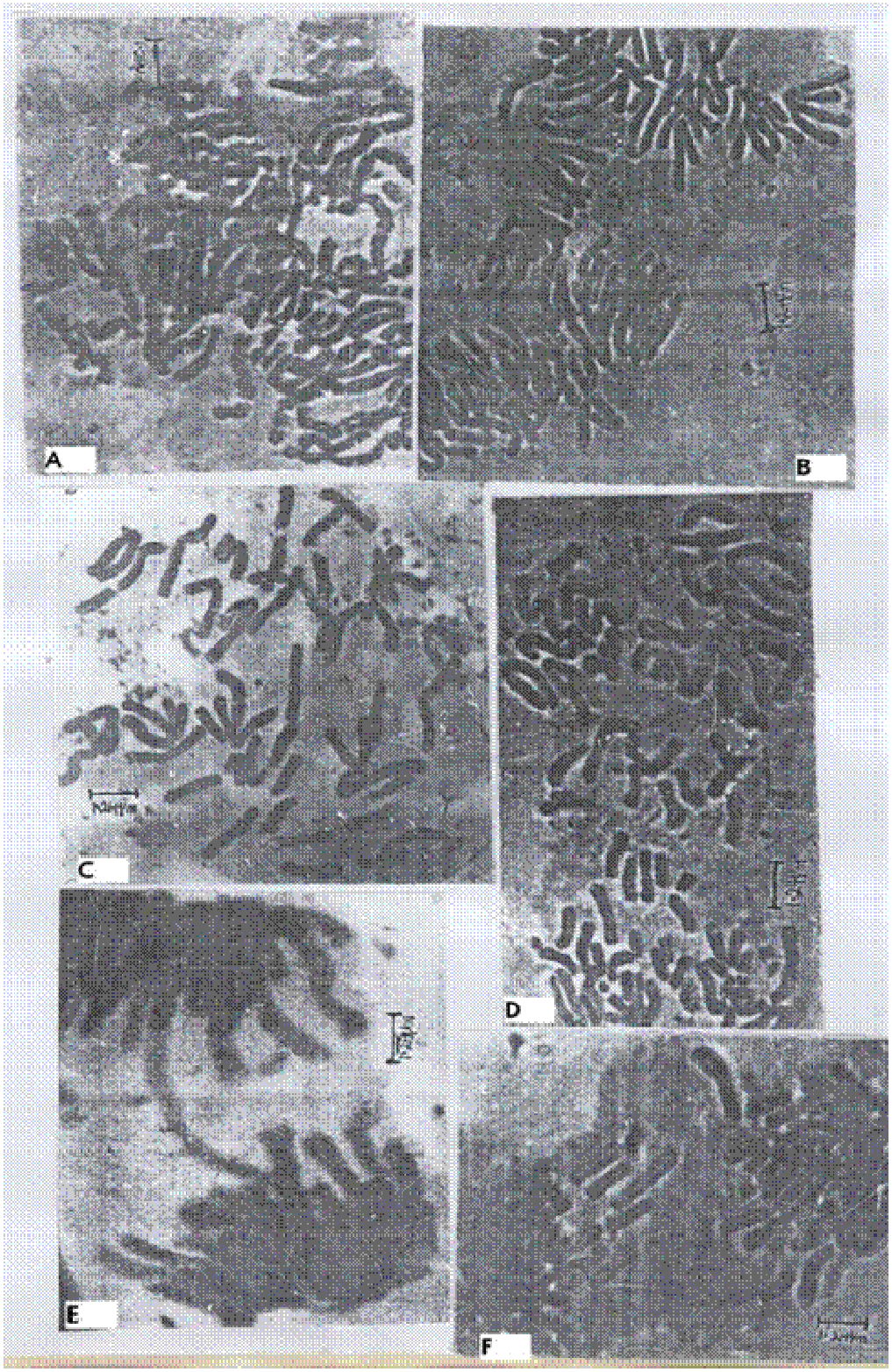


Figure 1. Effect of varying concentration of neem leaf extract on mitotic index of *Allium cepa*.



Plates 1A-D. Normal mitotic division of *Alliu cepa* (control). A = Regular prophase, B = regular metaphase (2n = 116), C = regular anaphase, and D = regular telophase. Scale line represents 1.25 µm.



Plates 2A-F. Chromosome aberrations in *Allium cepa* treated with neem leaf extracts. A = endopolyploid prophase cell of *Allium cepa* treated with 0.5kg/L extract, B = endopolyploid anaphase cell with *Allium cepa* treated with 0.5 kg/L of extract, C = anaphase with broken and scattered chromosomes in *Allium cepa* treated with 0.2 kg/L of extract, D = anaphase with laggards and broken chromosome fragments in *Allium cepa* treated with 0.1 kg/L of extract, E = anaphase bridge in *Allium cepa* treated with 0.4kg/L of extract, and F = telophase with laggards in *Allium cepa* treated with 0.4 kg/L of extract. Scale line represents 1.25 µm.

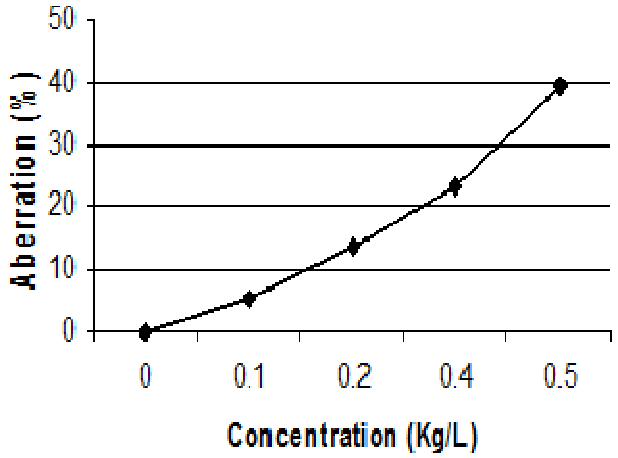


Figure 2. Effect of varying concentration of neem leaf extract on chromosomal aberration in *Allium cepa*.

Table 3. Analysis of variance (ANOVA) for mitotic index.

Source of variation	SS	df	MS	F-ratio
Regression	6.32	1	6.32	176.9
Residual	0.50	14	0.036	
Total	6.82	15		

$F_{0.05}$ (1,14) Reject Ho at P <= 0.0005.
r^2 = 0.93.

cytological evidences that ordinary clean water did not induce chromosomal aberrations in *A. cepa* cells' genomes, but neem leaf extract did. Similar observations have been reported by Bakare et al. (2003) on the induction of chromosomal aberration in *A. cepa* chromosomes by raw and simulated leachate from a refuse dump. The mitotic index values in the treated onion root cells were lower than the value calculated for the control. This suggests the suppression of mitotic activities in *A. cepa* by the neem leaf extract, since mitotic index is a quantitative estimation of the mitotic activities in an organisms or a particular organ of an organism. This observation corroborates the findings of Bakare et al. (1999a, 1999b, 2000), who recorded lower mitotic index values in the treated root cells of *A. cepa* when compared with the control root cells. The mitotic indices estimated for the treated roots increased with the concentration of The increase in the mitotic index values with the concentration of the neem leaf extract observed in the treated root cells could be an adaptive response by the *A.* the neem leaf extract. This observation is contrary to the findings of Bakare et al. (2003), who reported reduction in the mitotic indices obtained in the treated roots as the concentration of treatment given increased. *cepa* genome in compensating for the aberrant cells that were produced in the root cells treated with high concentrations of the

Table 4. Analysis of variance (ANOVA) for chromosome aberration.

Source of variation	SS	df	MS	F-ratio
Regression	3.208	1	3.208	32.24
Residual	0.199	2	0.10	
Total	3.407	3		

$F_{0.05}$ (1,2)= 18.51 Reject Ho at P <= 0.0005.
r^2 = 0.94.

neem leaf extract. The need to get rid of the aberrant cells and replace them with new normal cells accounts for the increase in the mitotic index values with the concentration of the extract.

The observed chromosomal aberrations in the treated root cells were definitely induced by the ingredients contained in the neem leaf extract since such aberrations were not observed in the control. This suggests that the neem leaf extract could be genotoxic at the chromosomal level, especially at high concentrations. The chromosomal aberrations are parts of the hazards associated with herbal medications due to effects of the chemicals such as alkaloids, flavonoids, terpenoids, tannins, carcinogens, etc, contained in the plants (Kurnkum, 1993).

The induction of polyploidy by the neem leaf extract could be due to the arrest of the activities of the spindle fibres which are supposed to move the separated chromatids to the different poles or the prevention of the 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 duplicated chromosomes.

The observation of laggards, chromosomes' fragments, anaphase bridge and scattering of chromosomes at anaphase is an indication of the capability of neem leaf extract in causing chromosome breakage, resulting in genetic imbalance in the genome.

The actual ingredients in the extract responsible for these observations are not known and their determination is beyond the scope of this study. However, they are all consumed along with the essential ingredients (Limonoids e.g. gedunin) that act on the ailments that neem plant is used for curing. Since most herbal preparations are not refined and are mostly consumed raw, this suggests possible exposures of the cells of the consumers to such genetic defects, as occurred in the chromosomes of *A. cepa* genome.

Allium test is a widely adopted method in mutagenic studies for ascertaining agents of mutations that can also affect human genome (Fiskesjo, 1985, 1997). The observation of chromosomal aberrations in *A. cepa* roots treated with high concentrations of neem leaf extract is an indication that the leaf extract could be cytotoxic to human genome, especially at high concentrations. Though the chromosomal aberrations were observed in plant system

plant system, the findings cannot be overlooked as results from genetic bioassay are relevant to human health because the toxicological target is the DNA, which exists in all cellular forms (Houk, 1992). This observation therefore calls for caution in the consumption of raw herbal products prepared from neem. Low concentration of the neem extract at long intervals is suggested, if neem extract is to be used in traditional medicare. Further studies should be directed towards considering concentrations lower than the ones used in this study and using leaf samples from trees of different ages from different localities and for different seasons.

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