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Full Length Research Paper

Evaluation of the cytotoxicity and genotoxicity of aqueous leaf extracts of *Azadirachta indica* A. Juss using the Allium test

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The cytotoxicity and genotoxicity of aqueous leaf extracts of *Azadirachta indica* A. Juss were evaluated with the aid of the Allium test. The extract was found to exhibit mitoclassic and chromatoclassic effects. The abnormalities observed included: nuclear lesions, binucleate cells, prophase accumulation otherwise called prophase poison, sticky metaphase, star metaphase, metaphase c-banding, precocious chromosomes, lagging chromosomes, anaphase bridge, star anaphase, tripolar anaphase and sticky late anaphase. No abnormal cells were observed at telophase. The implications of these anomalies were also discussed. The results lend more support to the reports of several investigators on the urgent need to standardize the dosages of medicinal plant extracts for the treatment of various ailments.

Key words: Cytotoxicity, genotoxicity, leaf extract, *Azadirachta indica*.

INTRODUCTION

Azadirachta indica belongs to the family Meliaceae. The common names are neem, Nimm, Margosa tree, Holy tree, Indian lilac tree among others. In Nigeria, it is known as “Ogwu-akom”, “Dogo yaro”, or “Darbeji ja”, and “Aforo-oyinbo”, by the Igbos, Hausas and Yorubas, respectively (Iwu, 1993). It is a native to India, where it is considered to be the “village pharmacy” (Gbotolorun et al., 2008) but has now been naturalized in Africa, especially in West and Central Africa where is widely cultivated as an ornamental plant (Iwu, 1993). The plant is widely used for the treatment of malaria. A decoction of the leaves or of leaves and stem-bark is used in the treatment of fever by water therapy or inhalation (Sofowora, 1982; Adegbite et al., 2009). Iwu (1993) observed that it is such a malaria remedy that it is uncommon to see an intact tree near homes without scars of human disturbance. The World Health organization (2003) according to Amadi et al. (2011) reported that the first line of treatment for 60% of Nigerian children with malaria associated fever is the use of herbal medicines at home. The later authors also noted that the use of herbs in adults may even be higher with the high growth of herbal market and its great commercial benefit.

Neem has been used in many countries as an insecticide, insect repellent, oral dentifrice, and in traditional medicine to treat malaria, diabetes, worms, and cardiovascular and skin diseases (www.health-care-clinic.org, 2009). According to the National Academy of Sciences Washington DC (2009), neem products are not outright insect killers, instead, they alter an insect’s behavior or life processes in such a way that it can no longer feed, breed or metamorphose and can therefore cause no further damage. One outstanding neem component, azadirachtin, according to the Academy is believed to disrupt the metamorphosis of insect larvae. It

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keeps the larvae from developing into pupae and they die without producing a new generation. The commission also reported that more than 200 insect species have been shown to be influenced in several other ways by neem products. In Germany and India, neem has been reported to be incorporated as the active ingredient in certain popular toothpastes.

Meanwhile, Amadi et al. (2011) observed that in Nigeria and many other developing countries, herbal preparations are sold over the counter in general stores and not in pharmaceutical stores. Srivastava et al. (2005) had earlier observed that the indiscriminate use of such preparations might adversely affect fertility/reproductive health when used over a long time or when used in high doses for the treatment of other human diseases. People generally consider herbal medicines to be safe because they are 'natural' in spite of the fact that there is dearth of information on the precise nature of constituents and on the likely side effects (Tyler, 1999; Srivastava et al., 2005; Amadi et al., 2011). Birdi et al. (2010) also stated that "toxicological evaluation of medicinal plants has often been neglected since prolonged and apparently uneventful use is usually considered as a testimony of its safety". However, recently, concerns over the safety of herbal medicines have arisen in many countries. Many authors have in fact reported on the potential genotoxicity and mutagenicity of some medicinal herbs (Soliman, 2001; Konan et al., 2007; Sowemimo et al., 2007; Saleh et al., 2009).

Moreover, Shaikh et al. (1993); Aladakatti and Ahmed (2005) noted that the leaves of *A. indica* possess emmanagogue, antiimplantational, spermicidal, anti-fertility and antispermatic activities. Khan and Awasthy (2003) reported that leaf extract of neem (*A. indica*) induced structural and numerical changes in the spermatocyte chromosomes as well as synaptic disturbances in murine germ cells at their first metaphase. They also observed a significant increase in the frequency of sperms with abnormal head morphology and a decrease in mean sperm count. The National Academy of Sciences Washington D.C. (2009) wrote that the neem seed oil is a strong spermicide and that when used intravaginally proved effective in reducing the birth rate in laboratory animals. The oil has also been reported to be toxic in large doses in human infants (www.health-care-clinic.org). Thirteen infants who received 5 to 30 ml of the oil were reported to have been severely poisoned. Symptoms of the toxicity included metabolic acidosis, drowsiness, seizures, loss of consciousness, coma and death in two infants. The infants exhibited Reye's syndrome-like symptoms, with death from hepato-encephalopathy.

Rosencrantz and Klopman (1995) analyzed the structure of Azadirachtin, the main active ingredient of neem and discovered that it has the potential for acting as a genotoxic carcinogen. Furthermore, they noted that since genotoxic carcinogens are regarded as presenting a potential carcinogenic risk to humans, they suggested that the possible metabolism of azadirachtin to DNA-reactive products be evaluated experimentally. Oyedare et al. (2009) were of the opinion that it is very necessary to evaluate the potential cytotoxic and genotoxic effects of herb extracts that are being used in Nigeria to obtain information for standardization and to understand the mechanism and broad range of action of these herbs. In addition, Birdi et al. (2011) strongly advocated for the toxicological evaluation of medicinal plants both on short and long term since Ernst (1998) noted that traditional medical practitioners cannot detect or monitor delayed effects (for example, mutagenicity), rare adverse effects and adverse effects arising from long-term use such as for food supplements and nutraceuticals. Remirez (2006) also suggested that toxicological studies should include tests such as acute, subchronic and special toxicology that are impossible to detect clinically such as immunotoxicity, genotoxicity, carcinogenicity and reproductive toxicity.

The *Allium cepa* root meristem assay is considered widely as a practical reliable system for the screening of environmental mutagens and carcinogens (Fiskesjo, 1985; Fiskesjo and Levan, 1993). Thus in this study, bulbs of *A. cepa* were used to assess the cytotoxicity and genotoxicity of aqueous leaf extract of *A. indica.*

MATERIALS AND METHODS

Fresh leaves of *A. indica* were collected from the botanical garden of the University of Nigeria, Nsukka. They were dried at room temperature and reduced into powder using a grinder. Two hundred grams of the powdered leaves was extracted by macerating in 1.5 L of water for 24 h. Following filtration with the aid of Whatman filter paper, perforation funnel and suction pump, the filtrate was evaporated to dryness under reduced pressure. A residue of 1 g was obtained and was used to prepare the stock solution. The extract was reconstituted in distilled water in appropriate concentration before administration. The one gram of the leaf aqueous extract was added to 100 ml of distilled water to serve as the stock. The dilution of the 100 ml stock to its lower concentration required for treatments, were made by pipetting the required milliliters of the stock solution into a beaker and making it up to 100 ml with distilled water. For example, for 10% concentration, 10 ml of the stock was added to 90 ml of distilled water, while for 15 and 20% concentrations, 15 and 20 ml of the stock were added to 85 and 80 ml of distilled water, respectively.

Fresh bulbs of *Allium cepa* were purchased from Nsukka main market. They were grown in sawdust for about 6 days to ensure proper root formation. On the 6^{th} day, the bulbs were harvested and washed thrice in tap water. Subsequently, they were transferred into beakers containing tap water and left to stand for 24 h. The bulbs of *A. cepa* were transferred from the beakers containing tap water to those containing the different concentrations of the extracts. One of the bulbs was left in the tap water to serve as the control. The durations of treatment ranged from 7.00 am - 11.00 am (4 h), 7.00 am - 3.00 pm (8 h) and 7.00 am - 7.00 am (24 h). At the

Table 1. The design of the experiment (3 replications per treatment combination)

Concentrations (%)(A)	Durations (h) (B)		
	4	8	24
A1 control	A1 4	A1 8	A1 24
A2 10	A2 4	A2 8	A2 24
A3 15	A3 4	A3 8	A3 24
A4 20	A4 4	A4 8	A4 24

A total of 1,500 cells were examined for each treatment combination.

Table 2. Analysis of variance of the mitotic stages and dividing cells with abnormalities.

Source of variation	DF	Prophase			Metaphase			Anaphase			Telophase			DCA		
		SS	MS	VR	SS	MS	VR	SS	MS	VR	SS	MS	VR	SS	MS	VR
Conc	3	1069.00	356.33	8.29***	397.861	132.620	20.14***	249.42	83.14	7.98***	19.33	6.444	1.47***	2302.00	767.33	14.56***
Duration	2	45.17	22.58	0.53^{ns}	35.389	17.694	2.69^{ns}	30.17	15.08	1.45^{ns}	2.389	1.194	0.27^{ns}	265.17	132.58	2.52^{ns}
Conc. × duration	6	67.50	11.25	0.26^{ns}	30.389	5.065	0.77^{ns}	85.17	14.19	1.36^{ns}	23.833	3.972	0.91^{ns}	534.17	89.03	1.69^{ns}
Residual	24	1031.33	24.97		158.00	6.583		250.00	10.42		105.333	4.389		1264.67	52.69	
Total	35		2213.00			621.639			614.75		150.889				4366.00	

DCA, Dividing cells with abnormalities, ns: not significant, ***significant at 0.1% level of probability; DF, degree of freedom; SS, sum of squares; MS, mean squares; VR, variance ratio.

expiration of each of the treatment durations, 4 to 6 roots were chopped off from each treated bulb, including the control. They were washed three times in tap water and fixed in Carnoy's solution (a mixture of acetic acid and absolute alcohol) in a ratio of 1: 3. The fixed materials were stored in the refrigerator at least for 24 h. Furthermore, the roots were hydrolyzed in 0.1 N hydrochloric acid for about 7 min at 60°C. The milky portion of the root tips were subsequently squashed and stained with 2% lacto-propionic orcein.

The prepared slides were examined under the microscope. The good ones were sealed off with nail varnish and photomicrographs of the important stages of interest were taken. The design of the experiment was a 4x3 factorial in CRD with 3 replications each (Table 1). Various types of aberrations induced by each treatment at various stages were recorded. Analysis of variance (ANOVA) was used to analyze the data obtained on the number of dividing cells at different mitotic phases and those with abnormalities.

RESULTS

Table 2 shows the analysis of variance of the different mitotic stages as well as dividing cells with abnormalities. It could be observed that the number of cells at the different mitotic stages with the exception of telophase, differed among the concentrations ($P<0.001$). The number of dividing cells with abnormalities also differed among the concentrations ($P<0.001$). The durations of treatments and the interactions between concentrations and durations did not induce significant differences in the number of cells at the various mitotic stages as well as on the number of dividing cells with abnormalities. Moreover, from Table 3, it could be observed that the treated root tips had more prophase cells than the control root tips. There appeared to be accumulation of prophase at the expense of other stages. The highest number of prophase cells was induced by 20% concentration of the water extract. However, this number was not significantly different from that of 15 and10% concentrations ($P<0.05$). Table 3 also revealed that the control root tips had more metaphase and anaphase cells than the treated root tips. Also, the numbers of these mitotic stages were not significantly different ($P<0.05$)

Table 3. The main effects of concentrations of treatments on the number of mitotic stages and dividing cells with abnormalities.

Concentration	Prophase	Metaphase	Anaphase	Telophase	DCA
Control	450.00 ± 2.31^{c}	21.33 ± 0.88^{a}	17.00 ± 1.528^{a}	11.67 ± 0.33^{ns}	1.33 ± 1.33^{c}
10%	459.89 ± 2.176^{ab}	14.78 ± 1.12^{b}	12.56 ± 1.26^{b}	11.78 ± 0.80^{ns}	7.22 ± 1.97^{bc}
15%	460.56 ± 1.76^{ab}	14.67 ± 0.78^{b}	11.56 ± 1.23^{b}	12.89 ± 0.82^{ns}	15.89 ± 3.31^{a}
20%	464.89 ± 2.60^{a}	12.44 ± 1.03^{b}	9.89 ± 1.18^{b}	13.33 ± 0.71^{ns}	22.22 ± 3.66^{a}
LSD (0.05)	6.39***	2.50***	3.14***	2.04^{NS}	7.06***

Means (vertical) followed by the same lower case letters are not significantly different at 5% level of probability.

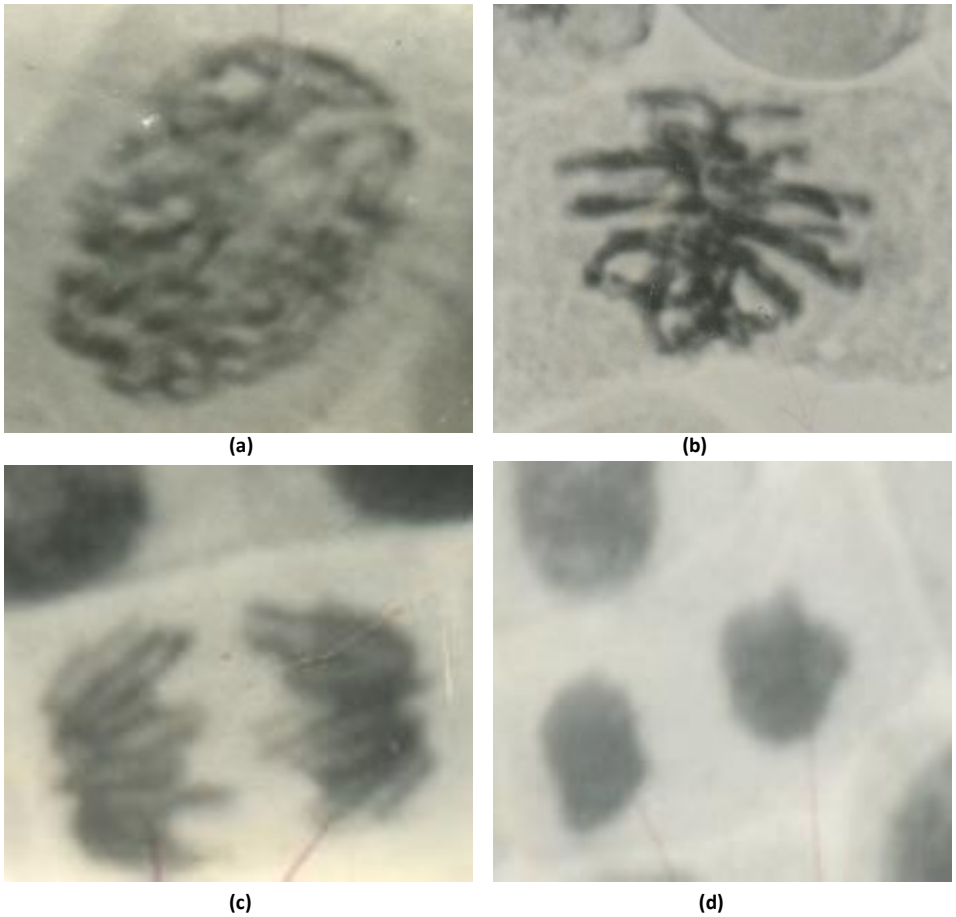

Figure 1. Normal mitotic stages in *Allium cepa*. (a) Prophase; (b) metaphase; (c) anaphase and (d) telophase.

among the root tips treated with the 3 different concentrations of the extract.

The number of dividing cells with abnormalities (Table 3) was more in the treated root tips than in the control root tips. Twenty percent (20%) concentration of the water extract induced the greatest number of abnormal cells; however, this number was not significantly different from that of 15% concentration. Among the three different concentrations of water extract, 10% induced the least number of abnormal cells. In fact, the number of dividing cells with abnormalities increased with increase in concentration. Figure 1 shows the normal mitotic stages in *A. cepa*. The different types of abnormal cells induced were as follows (Figures 2 to 4):

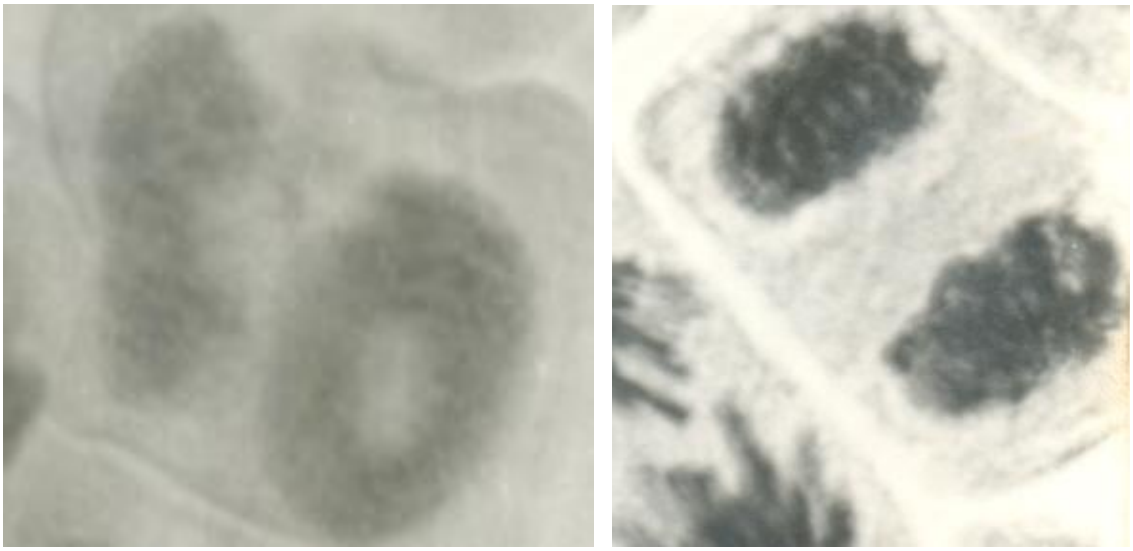

Figure 2. Abnormalities observed in *Allium cepa* treated with aqueous leaf extracts of *Azadirachta indica.* Abnormal Interphase cells showing: a, nuclear lesions; b, binucleate cell.

(i) At interphase: Nuclear lesions, binucleate cells,
(ii) At prophase: Prophase accumulation otherwise called prophase poison, binucleate cells,
(iii) At metaphase: Sticky metaphase, star metaphase, metaphase c-banding,
(iv) At anaphase: Precocious chromosomes, lagging chromosomes, anaphase bridge, star anaphase, tripolar anaphase and sticky late anaphase.

No abnormal cells were, however, observed at telophase.

DISCUSSION

Neem extract in this study have been shown to affect the mitotic apparatus (spindle) and chromosome structure. These aberrations were termed mitoclassic and chromato -classic effects, respectively by Deysson (1968). The observed aberrations have also been reported by Soliman (2001), Akinboro and Bakare (2007), Konuk et al. (2007), Yildiz and Evrim (2008), Saleh et al. (2009), Promkaew et al. (2010) and Abu and Mba (2011). The accumulation of prophase or prophase poison reaction occurred at the expense of other phases. Similar effect was observed by Shehab (1980) after treatments of alcoholic extract of *Pulicaria crispa* and water extract of *Teucrium pilosum* on *Allium cepa* and he attributed it to a disturbance or breakdown of spindle apparatus.

Ene-Obong (1991) reported that there is a consensus that many of the components of medicinal plants bind onto tubulin and either inhibit tubulin assembly or cause the depolymerization of already assembled microtubules. The spindle so disturbed according to him, is reduced and may eventually disappear, resulting in the blockage of cell division at prophase or even metaphase. He also speculated that there may be a possible amplification of anti-spindular effects where two or more anti-spindle compounds may be present in the same medicinal plant since different binding sites in tubulin may exist for different anti-spindle compounds. Binucleate cell formation is accepted to be due to inhibition of cytokinesis (Ateeq et al., 2002). Iwalokun et al. (2011) reported that Majewska et al. (2003) attributed such inhibition to phlamogram inhibition at the early stage of telophase. The presence of nuclear lesions and nuclear dissolution in cells (Mercykutty and Stephen, 1980) offer cytological evidence for the inhibitory action of chemicals on DNA biosynthesis. Inhibition of DNA synthesis (Ene-Obong, 1991) could occur in two ways: 1) either directly by affecting the incorporation of precursors into DNA or 2) through an influence on the biosynthesis of DNA precursors. The inhibition results in the total failure of mitosis.

Stickiness has been interpreted to be due to several causes viz: depolymerization of DNA (Abraham and Kashy, 1979); DNA condensation (Osterberg et al., 1984); physical adhesion of chromosomal proteins (Patil and Bhat, 1992). There is an agreement that stickiness reflects highly toxic and usually irreversible effect that probably leads to cell death (Liu et al., 1992; El-Ghamery

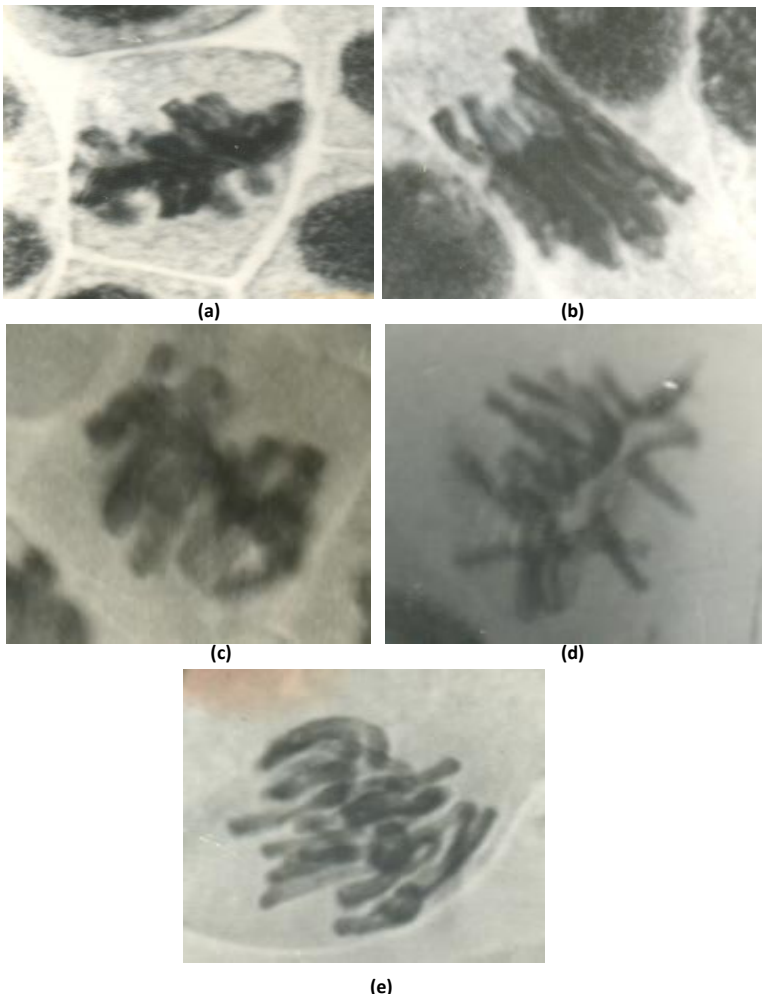

Figure 3. Abnormal Metaphase cells showing: a to c, sticky metaphase; d, star metaphase; e, metaphase c-banding.

et al., 2000; Tipirdamaz et al., 2003). According to Evandri et al. (2000), chromosome bridges and fragments are signs of clastogenic effects resulting from chromosome and chromatid breaks. The formation of bridges has been attributed to several causes which include:

(i) Breaks that may occur in both chromatids of the same chromosome and incorrect rejoining of the sticky ends to form a sister union (Badr et al., 1992; Hall and Garcia, 2006),
(ii) Incomplete replication of chromosomes by defective or less active replication enzymes (Sinha, 1979) or through breaks that may occur late in the cell cycle (in G2) after the chromosomes have replicated (Hall and Garcia, 2006)
(iii) Late replicating DNA sequences of the telomeric heterochromatin (Bennet, 1977). Kaltsikes (1984) according to Yildiz and Evrim (2008) reported that chromosome bridges could occur if heterochromatic DNA sequences do not complete DNA replication when the nucleus is ready to divide.

According to Chandra and Khuda-Bukhsh (2004) and Klopman et al. (1985) suggested that during the metabolism of Azadirachta, electrophilic ions and radicals are produced and that these interact with the nucleophilic sites in DNA, leading to breaks and other related damage in DNA. Hall and Giaccia (2006) also noted that anaphase bridge is one of the 3 types of aberrations that are lethal to the cell, the other two being dicentric and the ring chromosomes. Bridges cause structural chromosome mutations (duplications or deletions in DNA double-strand) (Evandri et al., 2000; El-Ghamery et al., 2000).

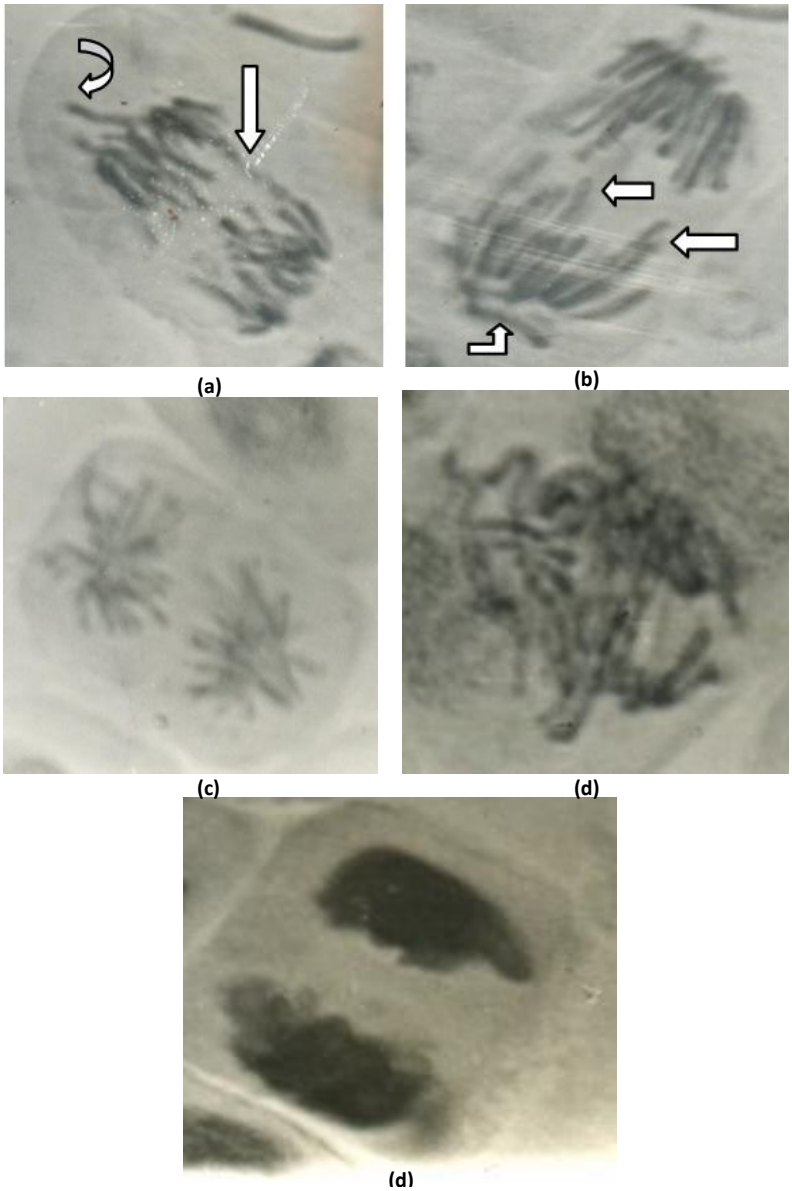

Figure 4. Abnormal anaphase cells showing: (a) precocious chromosome (shown with a curved arrow) and anaphase bridge depicted with a straight arrow; (b) precocious chromosome (shown with a curved arrow) and lagging chromosomes shown with straight arrows; (c) star anaphase; (d) tripolar anaphase; (e) sticky late anaphase.

Star metaphase, star anaphase and tripolar anaphase were also reported by Amer (1965) and Mercykutty and Stephen (1980) and they considered it to be a fore-step of the complete disturbance of the spindle. Deyson (1968) observed that the existence of star metaphases and of monocentric or pluricentric chromosome groups in spindleless anaphases points out the part played by centers during plant cell mitosis. He noted that these attractive centers can act irregularly, the spindle fibers being absent or they can remain together in the middle of the cell and attract all the chromosomes. Metaphase c-banding were also observed by Vicentini-Dias and Takahashi (1993) and Abderrahman (1998). Ronne (1977) explained that metaphase c-banding (that is re-duction in stainability in certain regions of chromosomes), results from the inhibition of non-histone protein synthesis. The presence of precocious chromosomes is thought to be due to unequal spindle movement where

some chromosome arms are pulled towards the extremity of the pole (Staykova et al., 2005; Bhatta and Sakya, 2008).

Lagging chromosomes have been a regular feature of many cytotoxicity/genotoxicity studies with medicinal plant extracts (Ozlem et al., 2008; Soliman, 2010; Sousa and Viccini, 2011). Sousa et al. (2010) explained that such chromosomes have the potentials to form micronuclei. The presence of such nuclei according to Sousa and Viccini (2011) is a manifestation of the efforts of a main nucleus to eliminate excess DNA in an attempt to restore the normal ploidy condition. To Iwalokun et al. (2011), micronuclei are associated with aneuploidy or polyploidy formation in the daughter cells after mitosis. However, El-Ghamery et al. (2003) was of the opinion that lagging chromosomes or fragments sometimes could dissolve in the cytoplasm and thus be lost. This type of aberration (chromosome deletion) is always lethal to humans (Hall and Garcia, 2006). A structure-based toxicity relationship for Azadirachtin (Aza), the major active principle in *A. indica* had been proposed (Rosenkranz and Klopman, 1995). They identified the presence of at least 5 copies of biophores in Aza and predicted it to be a potent carcinogen. In addition, Aza was found to also contain a furan moiety which the investigators speculated may undergo epoxidation during biotransformation. They concluded that the electro-negativity of Aza was of the same order of magnitude as that for DNA-reactive molecules. All these, according to the authors, point to the fact that Aza has the features of a potential mutagen that has the capability of inducing damage in genetic material including some clastogenic changes.

Fiskesjo (1995) wrote that ‘a positive result in Allium test system should be taken to indicate a potential biological hazard and that false negatives have been shown to rarely occur in either the Allium test or other similar plant tests”. Thus the occurrence of the above variety of abnormalities in this study is an indication of the high mutagenic potentials of leaf extracts of *Azadirachta indica.* The above assertion is perhaps corroborated by results obtained by several investigators on the neem plant. Various test organisms have been used by these investigators to evaluate the cytotoxicity, genotoxicity and antifertility effects of the neem plant. These include *A. cepa* (Soliman, 2001; Akintonwa et al., 2009; Adegbite et al., 2009); mice (Awasthy et al., 1995, 1999); rat (Roop et al., 2005; Gbotolorun et al., 2008); desert locust, *Schistocerca gregaria* (Linton et al., 1997); Murine germ cell (Khan and Awasthy, 2003) and fish, *Oreochromis mossambicus*, (Chandra and Khuda-Bukhsh, 2004).

These results from the plant assays and *in vivo* assays on rodents provide strong evidence for *A, indica* to be regarded as having a strong mutagenic or carcinogenic potential for man. The International Commission for Protection against Environmental Mutagens and Carcinogens had earlier written in 1985, that once plant data had been confirmed in a mammalian cell assay or from other eukaryotes, it may be prudent to designate the chemical in question as being potentially hazardous and that this may indicate that distribution and human exposure will be restricted. The Commission, however, suggested that it may be worthwhile to design further tests to either confirm this assessment or to determine the extent of the potential hazard if the chemical has huge positive potentials.

REFERENCES

Abderrahman SM (1998). Cytogenetic effects of *Peganum harmala* extract on maize root tips. Cytology, 63: 283–291.

Abraham S, Kashy MP (1979). Mutagenic potentials of green chillies in *Allium cepa* root meristems. Cytology, 44: 221–225.

Abu NE, Mba KC (2011). Mutagenicity testing of pharmaceutical effluents on Allium cepa root tip meristems, J. Toxicol. Environ. Health Sci., 3(2): 44–51.

Adegbite AE, Ayodele MS, Odunbaku KR, Idehen EO (2009). Mutagenic effect of neem leaf extract used in traditional medicine on Allium cepa (L.). Sci. Res. Essays, 4(11): 1315–1321.

Akinboro A, Bakare AA (2007). Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa Linn*. J. Ethnopharmacol., 112(3): 470-475.

Akintonwa A, Awodele O, Afolayan G, Coker HAB (2009). Mutagenic screening of some commonly used medicinal plants in Nigeria, J. Ethnopharmacol., 125(3): 461- 470.

Aladakatti RH, Ahmed RN (2005). Ultrastructural changes in Leydig cells and cauda epididymal spermatozoa induced by *Azadirachta indica* leaves in albino rats. Phytother. Res., 19: 756 766.

Amadi CN, Siminialayi IM, Orisakwe OE (2011). Male Infertility and Herbal Supplements: An Update. Pharmacology, 2(11): 323–348.

Amer SM (1965). Cytological effects of pesticides 1. Mitotic effects of N-methyl-1-naphthylcarbamate. “Sevin” Cytology, 30: 175–181.

Ateeq B, Farah MA, Ali MN, Ahmad W (2002). Clastogenicity of pentachlorophenol, 2, 4D and butachlor evaluated by Allium root tip test. Mutat. Res., 514: 105–113.

Awasthy KS, Chaurasia OP, Sinha SP (1995). Genotoxic effects of crude extract of neem (*Azadirachta indica*) in bone marrow cells of mice. Cytology, 60: 189–193.

Awasthy KS, Chaurasia OP, Sinha SP (1999). Prolonged murine genotoxic effects of crude extracted from neem. Phytother. Res., 13(1): 81-83.

Badr A, Ghareeb A, El-Din HM (1992). Cytotoxicity of some pesticides in mitotic cells of *Vicia faba* roots. Egypt J. Appl. Sci., 7: 457–468.

Bennet MD (1977).Heterochromatin, aberrant endosperm nuclei and grain shriveling in wheat-rye genotypes. Hereditory, 39: 411–419.

Bhatta P, Sakya SR (2008). Study of mitotic activity and chromosomal behavior in root meristem of *Allium cepa* L. treated with magnesium sulphate. Ecoprint, 15: 83–88.

Birdi TJ, Brijesh S, Daswani PG (2010). Approaches towards the preclinical testing and standardization of medicinal plants. http://www.aifo.it/english/resources/online/books/other/tradmedicine06/TradMedicine-birdi.pdf . Retrieved on 10th January, 2012

Chandra P, Khuda-Bukhsh AR (2004). Genotoxic effects of cadmium chloride and azadirachtin treated singly and in combination in fish. Ecotoxicol. Environ., 58(2): 194 – 201.

Deysson G (1968). “Antimitotic substances” In: International Review of Cytology 24: 99–145, Bourne, G. H and Danielli, J. F (eds.), Academic Press Inc. New York.

El-Ghamery AA, Elkholy MA, Elyousser A (2003). Evaluation of

cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L., Mutat. Res., 537: 29–41.
El-Ghamery AA, Elnahas AI, Mansour MM (2000). The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acid content in root meristems of *Allium cepa* and *Vicia faba*. Cytology, 55: 209–215.
Ene-Obong EE (1991). Anti-DNA and Anti-Spindle effects of tropical medicinal plants. In: Cheremisinoff PN, Ferrante LM (eds) Biotechnology Current Progress, Vol.1 Technomic Publishing Co. USA, pp. 295–310.
Ernst E (1998) Harmless herbs?. A review of the recent literature. Am. J. Med., 104: 170 – 178.
Evandri MG, Tucci P, Bolle P (2000). Toxicological evaluation of commercial mineral water bottled in polyethylene terephthalate: a cytogenetic approach with Allium cepa. Food Addit. Contam., 17(1):1037–1045.
Fiskesjo G (1985). The Allium test as a standard in environmental monitoring. Hereditas, 102: 99–112.
Fiskesjo G (1995). Allium test In: In vitro Toxicity Testing Protocols – Methods in Molecular Biology, Vol 43, (S, O' Hare and C. K, Alterwill, Eds.), Humana Press Inc., Totowa NJ, pp. 119 – 127.
Fiskesjo G, Levan A (1993) Evaluation of the first ten MEIC chemicals in the Allium test. ATLA, 21: 139 – 149.
Gbotolorun SC, Osinubi AA, Noronha CC, Okanlawon AO (2008). Antifertility potential of neem flower extract on adult female Sprague-Dawley rats. Afr. Health Sci., 8(3): 168–173.
Hall EJ, Garcia AJ (2006). Radiobiology for the Radiologist, 6th Ed, Lippincott Williams & Wilkins, Philadelphia, p. 656.
International Commission for Protection Against Environmental Mutagens and Carcinogens (1985). "Guide to short-term tests for detecting mutagenic and carcinogenic chemicals". http://www.inchem.org/documents/ehc/ehc/ehc51.htm#PartNumber:4 , Retrieved on 11th January, 2012.
Iwalokun BA, Oyenuga AO, Saibu GM, Ayorinde J (2011). Analyses of cytotoxic and genotoxic potentials of *Loranthus micranthus* using the Allium cepa test. Curr. Res. Biol. Sci., 3(5): 459–467.
Iwu M (1993). Handbook of African Medicinal Plants. CRC Press Inc. London. p. 434.
Kaltsikes PJ (1984). Breeding vegetable varieties resistant to diseases. Proc. 3rd Meeting on protected vegetables and flowers, May 911, Heraklion, Crete, p.60.
Khan PK, Awasthy KS (2003). Cytogenetic toxicity of neem. Food Chem. Toxicol., 41(10): 1325–1328.
Klopman G, Conttreras R, Rosenkranz HS, Waters MD (1985). Structure-genotoxic activity relationships of pesticides: comparison of the results from several short-term assays. Mutat. Res., 147: 343–356.
Konan NA, Bacchia EM, Lincopan N, Varelac SD, Varandac EA (2007). Acute, subacute toxicity and genotoxic effects of a hydroethanolic extract of the Cashew (*Anacardium occidentale* L.). J. Ethnopharmacol., 110: 30-38.
Konuk M, Liman R, Ciğerci IH (2007). Determination of genotoxic effect of Boron on *Allium cepa* root meristematic cells, Pak. J. Bot., 39(1): 73–79.
Linton YM, Nisbet AJ, Mordue AJ (1997). The effects of azadirachtin on the testes of the desert locust, *Schistocerca gregaria* (Forskal). J. Insect Physiol., 43(11): 1077–1084.
Liu D, Jiang W, Li M (1992) Effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa*. Hereditas, 117: 23-29.
Majewska AE, Wolska E, Sliwinska M, Furmanowa N, Urbanska A, Pietrosiuk A, Zobel A, Kuras M (2003). Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhadiola rosea* roots. Caryologia, 56: 337 – 351.
Mercykutty VC, Stephen J (1980). Adriamycin induced genetic toxicity as demonstrated by the Allium test. Cytology, 45: 769–777.
National Academy of Sciences Washington D. C (2009) "Neem: A tree for solving global problems," http://books.nap.edu/openbook.php?record_id=192&page, Retrieved on 12th September, 2011.
Osterberg R, Persson D, Bjursell G (1984). The condensation of DNA by chromium(111) ions. J. Biomol. Structure Dynam., 2: 285–290.
Oyedare BM, Bakare AA, Akinboro A (2009). Genotoxicity assessment of water extracts of *Ocimum gratissimum*, *Morinda lucida* and *Citrus medica* using the *Alliumcepa assay.* Bol Latino am Caribe Plant Med. Aromat., 8: 97-103.
Ozlem A, Nuran E, Feruzan D (2008). Mitotic changes in root meristems of lens culinaris treated with fusilade (fluazifop-p-butyl). Asian J. Cell Biol., 3(1): 34–40.
Patil BC, Bhat TGI (1992). A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in *Clitoria termata* L. Cytology, 57: 259–264.
Promkaew N, Soontornchainaksaeng P, Jampatong S, Rojanavipart P (2010) Toxicity and Genotoxicity of Pendimethalin in maize and onion, Kasetsart J. Nat. Sci., 44: 1010–1015.
Remirez DC (2006) 'Update in Pre-Clinical Regulatory Requirements for Phytomedicines in Latin America' : In: Birdi TJ, Brijesh S, Daswani, PG (2010) Approaches towards the preclinical testing and standardization of medicinal plants. http://www.aifo.it/english/resources/online/books/other/tradmedicine06/TradMedicine-birdi.pdf . Retrieved on 10th January, 2012
Ronne M (1977). '*In vitro* induction of G-bands with cycloheximide, In: Abderrahman SM (1998) Cytogenetic effects of *Peganum harmala* extract on maize root tips. Cytologia, 63: 283–291.
Roop JK, Dhaliwal PK, Guraya SS (2005) Extracts of *Azadirachta indica* and *Melia azedarach* inhibit folliculogenesis in albino rats. Braz. J. Med. Biol. Res., 38: 943–947.
Rosenkranz HS, Klopman G (1995). 'An examination of the potential genotoxic carcinogenicity of a biopesticide derived from the neem plant' In: Chandra P, Khuda-Bukhsh AR (2004) Genotoxic effects of cadmium chloride and azadirachtin treated singly and in combination in fish. Ecotoxicol. Environ., 58(2): 194 – 201.
Saleh K, Khanfar A, Mosaad I (2009). Detection of abnormal and micronucleus in root tips of Allium cepa cultivated in water wells, http://www.thefreelibrary.com/Detection+of+abnormal+and+micronucleus+in+root+tips+of+Allium+cepa...-a0216041401, Retrieved on 11th January, 2012.
Shaikh PD, Manivannan B, Pathan KM, Kasturi M, Ahamed NR (1993). Antispermatic activity of *Azadirachta indica* leaves in albino rats. Curr. Sci., 64: 688 – 689.
Shehab AS (1980). Cytological effects of medicinal plants in Qatar 11.Mitotic effect of water extract of *Teucrium pilosum* on *Allium cepa*. Cytology, 45: 57–64.
Sinha U (1979). Cytomorphological and macromolecular changes induced by p-flurophenylalanine in *Allium cepa* and Triticale. J. Cytol. Genet., 14: 198.
Sofowora A (1982). Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd. Chichester, UK, pp. 142-145.
Soliman AH (2010). Mutagenic effects of *Kochia indica* extract on *Vicia faba* L. J. Am. Sci., 6(7): 292 – 297.
Soliman MI (2001). Genotoxicity testing of Neem Plant (*Azadirachta indica* A. Juss.) using the *Allium cepa* chromosome aberration assay. J. Biol. Sci., 1(11): 1021- 1027.
Sousa SM, Viccini LF (2011). Cytotoxic and genotoxic activity of *Achillea millefolium* L., Asteraceae, aqueous extracts. Rev. Bras. Farmacogn., 21(1): 98-104.
Sousa SM, Silva PS, Viccini LF (2010). Cytogenotoxicity of *Cymbopogon citrates* (DC) Stapf (lemon grass) aqueous extracts in vegetal test systems. Anal. Acad. Bras. Sci., 82(2): 305–311.
Sowemimo AA, Fakoya FA, Awopetu I, Omobuwajo OR, Adesanya SA (2007). Toxicity and mutagenic activity of some selected Nigerian plants. J. Ethnopharmacol., 113: 427-432.
Srivastava SR, Kesarwani S, Keshri G, Singh MM (2005). Evaluation of contraceptive activity of a mineralo-herbal preparation in Sprague-Dawley rats. Contraception, 72: 454 – 458.

Staykova TA, Ivanova EN, Velcheva IG (2005). Cytogenetic effect of heavy-metal and cyanide in contaminated waters from the region of Southwest Bulgaria. J. Cell Mol. Biol., 4: 41-46.

Tipirdamaz R, Gömürgen AN, Kolankaya D, Doğan M (2003). Determination of toxicity of pulp-mill effluents by using Allium test. Tarim Bilimleri Dergisi, 9(1): 93–97.

Tyler VE (1999) Herbs of Choice – The therapeutic use of phytomedicinals, Pharmaceutical Products Press, New York. pp. 287.

Vicentini-Dias VEP, Takahashi CS (1993). Action of extract of *Stryphnodendron obovatum* Benth seed on rat bone marrow and on human lymphocytes. Rev. Brasil. Genet., 16(1): 175 – 185.

WHO (2003). Traditional Medicine. Fact Sheet No. 134. WHO, Geneva, Switzerland. Available at : http://www.who.int/www.health-care-clinic.org/alternative-medicine/neem.html, “Neem Tree/Neem oil”, Retrieved on 16th September 2009.

Yildiz M, Evrim SA (2008). Genotoxicity testing of quizalofop-P-ethyl herbicide using the Allium cepa anaphase-telophase chromosome aberration assay, Caryology, 61(1): 45–52.