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Cytogenetic and Mutagenesis evalution of *Morinda citrifolia* (NONI) aqueous Leaf Extracts using *Allium cepa* assay

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ABSTRACT

The widespread use of medicinal herbs has raised serious concerns over its quality, safety, and efficacy. Therefore, precise scientific evaluation has become a precondition for acceptance of herbal health claims. In this study the cytogenetic and mutagenesis of *morinda citrifolia* (noni) aqueous leaves extract on the root tips of *Allium cepa* was evaluated using *Allium cepa* (L.) assay, Onion bulb was sun dried for 4 days, the dried outer scales were carefully removed and the root was scraped leaving the ring of the primordial root intact to promote the emergence of new roots. For the root growth inhibition, four concentrations of each extract, viz: 10 g, 15g, 20 g, and 25 g, was considered, with the positive control group treated with glyphosate and the negative control group treated with tap water respectively. The effective concentration (EC_{50}) values of the leaves was determined. The plant extracts induced a dose-dependent root growth inhibitory, mitotic index and mitodepressant effects at 24, 48, 72 and 96 hours. The extracts used induced a relatively low % of chromosomal aberration in the *A. cepa* root tips cells. *In vivo* testing of the plants extracts on *Allium cepa* root-tips revealed that the extract do not induced cytogenetic and mutagenic effect at 10 % concentration, but as the concentration of the plants extracts increases to 15 %, 20 %, 25 %, the leave extracts showed the potency of induce genetic damage at chromosomal level and it spindle apparatus on *Allium cepa* root-tips. The Aberrations observed were Binucleate, Laggard, Nuclear lesion, Fragmentation, Polyploidy, Dissolution, Vagrant metaphase, Chromosome gap Anaphase Bridge, Micronuclei (MN), and unequal separation. It is therefore suggested that users of *morinda citrifolia* plants extracts as traditional medicine be advised to always use definite measurement and at lower concentration of 10g, because it has cytogenetic and mutagenesis effect at higher concentration.

Keywords: *morinda citrifolia*, cytogenetic, mutagenesis, chromosome, aberration, *Allium cepa*

INTRODUCTION

Plants are believed to be the foundation of alternative medicine because of their antimutagenic properties in the presence of both chemicals and environmental variables. However, plants extract may potentially have cytotogenetic and mutagenic effects on many organisms. Drug-producing plants have been the source of biomolecules with great potential for biotechnology use being among others, an important source for agro-pharmaceutical industries [1]. Alternative treatment mainly with medicinal plants, are part of human culture and in many countries are of regular use especially because of its affordability and availability [2]. Medicinal Plants are a source of innovative drugs, including those used in pharmaceuticals, nutraceuticals, food supplements, folk remedies, modern medicine, pharmaceutical intermediate bioactive principles, and lead molecules in synthetic drugs [3], [4]. Medical herbs have also been demonstrated to have

negative effects or the ability to interact with other drugs, to alter the mutagenic and carcinogenic effects of environmental pollutants, and to generate both mutagenic and antimutagenic effects on recognized mutagens in various test systems [5]. These findings raise questions regarding the potential mutagenic or cytogenetic risks associated with the prolonged use of these plants as food or medication, especially given the lack of information on the potential health risks associated with many of these plants [6]. Plants material such as leaves, fruits extract in general are rich in antioxidants such as flavonoids and phenolic compounds [7]. These phytochemicals were proven to be effective against various mutagens either through scavenging free radicals or by their reducing power, thus decrease the dangers present by ROS to genome [8], [9]. Thus, it is believed that the healing property of phytochemicals was the implicit success of the use of plant based raw materials in the well-known Indian medicinal systems, namely, the Ayurveda, Unani and Siddha and most Chinese folk medicines [10], [11]. The Allium test is regarded as a good bio-indicator test system due to its simplicity, affordability, ease of use, and good correlation with other test systems, according to the International Program on Plant Bioassay, US Environmental Protection Agency (EPA), World Health Organization (WHO), International Program on Chemical Safety, and United Nations Environmental Program. Allium test may assess a variety of parameters, including root growth, membrane integrity, antioxidant enzyme activities, mitotic index, and chromosomal aberrations because of its quickly growing roots and its large and reduced [12] [13]. Morinda *citrifolia* is a fruit-bearing tree in the coffee family, Rubiaceae, native to Southeast Asia and Australasia, and was spread across the Pacific by Polynesian sailors [14]. The species is now cultivated throughout the tropics and widely naturalized [15].There are over 100 names for this fruit across different regions. Common English names include great morinda, Indian mulberry, noni, beach mulberry, vomit fruit, awl tree, and cheese fruit. The pungent odor of the fresh fruit has made it a famine food in most regions, but it remains a staple food among some cultures and is used in traditional medicine. In the consumer market, dietary supplements are sold in various formats, such as capsules and juices [15]. In Nigeria, traditional healers use ancestral knowledge to prescribe, prepared the correct product to patients. There is no documentation showing standardization methods of prescription and the dosage of the drugs, and also there is a lack of knowledge of a broad variety of regionally important medicinal plants and their significance in the treatment of illnesses or negative consequences, Although various studies have been conducted to assess the cytotoxicity and mutagenic properties of medicinal plants species, there is still lack of information concerning the cytogenetic and mutagenesis potentials of *M. citrifolia*. The lack of standard prescription and the unrefined nature of the herbal preparation sometimes led to over dosage and bio-accumulation of both essential and non-essential of metabolites [16]. The accumulation, if it persists for a long period, can become cytotoxic and disturb the delicate human system's metabolic equilibrium. Genomic disruptions that harm DNA can range from point mutations to chromosomal mutations). However, it is well known that consumption of plants and plant products of which the content and the toxicity profile safe dose were not known may cause severe toxicity problem [17]. This research is aim at evaluating the possibility that the leaves extracts of *Morinda citrifolia* plants, whether or not have some cytotogenetic and mutagenesis potential on the mitotic chromosomes of *Allium cepa* (L) root- tips cells. The Specific Objective include**;** to determine the minimum and maximum effective concentration (EC_{50}) of *Morinda citrifolia* leave extracts on the root growth of *Allium cepa* bulb, to determine the cytotogenetic and mutagenesis potentials of *Morinda citrifolia* plant extracts, to determine the concentration of the plant extract that has significant effect on chromosome aberrations on root tip cells of *A. cepa* seed and to determine the types of chromosomal aberration that may be caused by these plant extracts.

MATERIALS AND METHODS

Research Site

The research was carried out in the laboratory of the Department of Biological Science Technology Federal Polytechnic Mubi, Adamawa State

Sources of Sample for the Medicinal Plants (Collection and Authentication of Plant Sample)

Leaves extract of *Morinda citrifolia* were collected around Mubi-north and Mubi-south Local Government Area of Adamawa State, purplish-red skin onion bulbs and seeds (*Allium cepa* L.) were obtained commercially at Mubi main market. All the collected plants specimen were move to the laboratory of the Department of Biological Science Technology Federal Polytechnic Mubi, Adamawa State for identification using the preserved specimen voucher. Each harvested plant material was given a code. The leaves of the collected medicinal plants were washed, dried and store under shade in a greenhouse at the Department of Biological Science Technology Federal Polytechnic Mubi, Adamawa State.

Preparation of Medicinal Plants Extract

Pretreatment of plants specimen

The leaves of *Morinda citrifolia* were shade dry and pounded into power using pestle and motor

Allium cepa Assay (Determination of cytogenetic and mutagenesis potential)

Onion bulb was sun dried for 4 days and the dried outer scales was carefully removed and the root were scraped leaving the ring of the primordial root intact to promote the emergence of new roots. These was used for the bioassay according to standard procedures [18]. For the root growth inhibition, four concentrations of each extract, viz: 10 g, 15g, 20 g, and 25 g, were considered, six onion bulbs was used of each with the positive control group treated with different concentrations of glyphosate and the negative control group treated with tap water. The base of each of the bulbs was suspended on the extract inside 100 mL beakers in the laboratory for 96 hours. The test extracts were changed daily and the root length was measured at the end of the exposure period, the length of the roots of five onion bulbs with the best growth at each concentration was measured (in cm) with a ruler. Average length for each concentration and the control was obtained, the percentage root growth inhibition in relation to the negative and positive control and the EC_{50} (the effective concentration where root growth amounts to 50 % of the control) for the leave extract was also determined. The effect of each sample on the morphology of growing roots cells was also examined according to the protocol described by [19].

Laboratory germination

To obtain root tip for cytological analysis, seeds were germinated in perforated plastic containers with very fine loam soil. In order to break dormancy in the seeds and allow for quick germination Seeds were pre-soaked in 1% potassium hydroxide for 12 hours; watering was done on daily basis until germination.

Pretreatment and fixation

When the germinating roots reached 2.5cm long, they were excised using the pointed end of a pair of forceps. This excised was done between 7.30 – 8.30am when cells of the growing roots are known to be in active division. The excised root tip was immediately drop into a solution of 0.05 % colchicine ($C_{22}H_{35}O_4$) for pretreatment. Pretreatment in colchicine last for 5 hours, at the end of which the root tips were washed thorough in running tape water and placed in glacial acetic alcohol 1:3 v/v for 24 hours for fixation. After fixation, the root tips was hardened in absolute ethanol for one hour and washed in 30 % ethanol for 3 hours before storing in 70 % ethanol in the refrigerator at 4^oC until required for use.

Hydrolysis, staining and squashing

The root tips was picked from the 70 % ethanol using a clean pair of forceps into a test tube containing distilled water. After 15 minutes of repeated changes in distilled water, the root tips was rinsed in two quick changes 1 NHCL; first in cool, followed by warm 1 NHCL. The root tip was then hydrolyze in 1 NHCL for 8-10 minutes in a water bath regulated at 60^oC [20]. On removal from the water bath, the root was rinsed again in cold 1NHCL in order to arrest the process of hydrolysis, and again washed in distilled water for another 15 minutes. At the end of the last washing time, the root tip was sufficiently softened for squashing. Squashing was done by placing one root tip at a time on a clean dry glass slide using a pair of pointed forceps. The young meristematic portion was cut out to allow for discarding of the older portion. One drop of 2% acetic orcein ($C_{28}H_{24}O_7N_2$) was added to the meristematic root tip and allow for 60 seconds for proper stain absorption. The stain root tip was carefully covered with a clean cover slip. Gentle taps on the cover slip using the blunt end of a mounted pin ensured proper squashing of the root tips. The whole set-up was allowed to stand for five minutes in order for deep staining of the chromosomes to take place. The slide was mounted and viewed, first with the X10 objective of an optical binocular research microscope, slides with good metaphase stages containing well spread chromosomes was first be drained of excess stain and then sealed using Ladies white vanish. When the seal dried, light microscope couple with digital camera will be used for observation, 200 cells was scored per slide and photographs of the cell were made.

STATISTICAL ANALYSIS

The computer software motic images plus 2.0 ml was used to capture, organize and analyzed data from the microscope. Descriptive statistical function was employed in preliminary analysis of data. The percentage of aberrations was analyzed using analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Maximum and Minimum Inhibitory Effects (EC50) on *Alllium cepa* Assay

The inhibitory effect of the concentrations of *M. citrfolia* leave extract on the root lengths of exposed *A. cepa* seedlings. The EC50 (Effective concentration resulting in the inhibition by 50 % of the root length of the untreated *A. cepa* seedlings) was determined for the extract using a graph to indicate the maximum and minimum EC50 (Effective concentration) The EC ranged from 10 g/ml with root length of 2 cm. As the concentrations of the extracts rose, meristematic growth decreased in comparison to the control. The extracts from *M. citrfolia* leaves, significantly inhibited growth. As showed in Figure 1

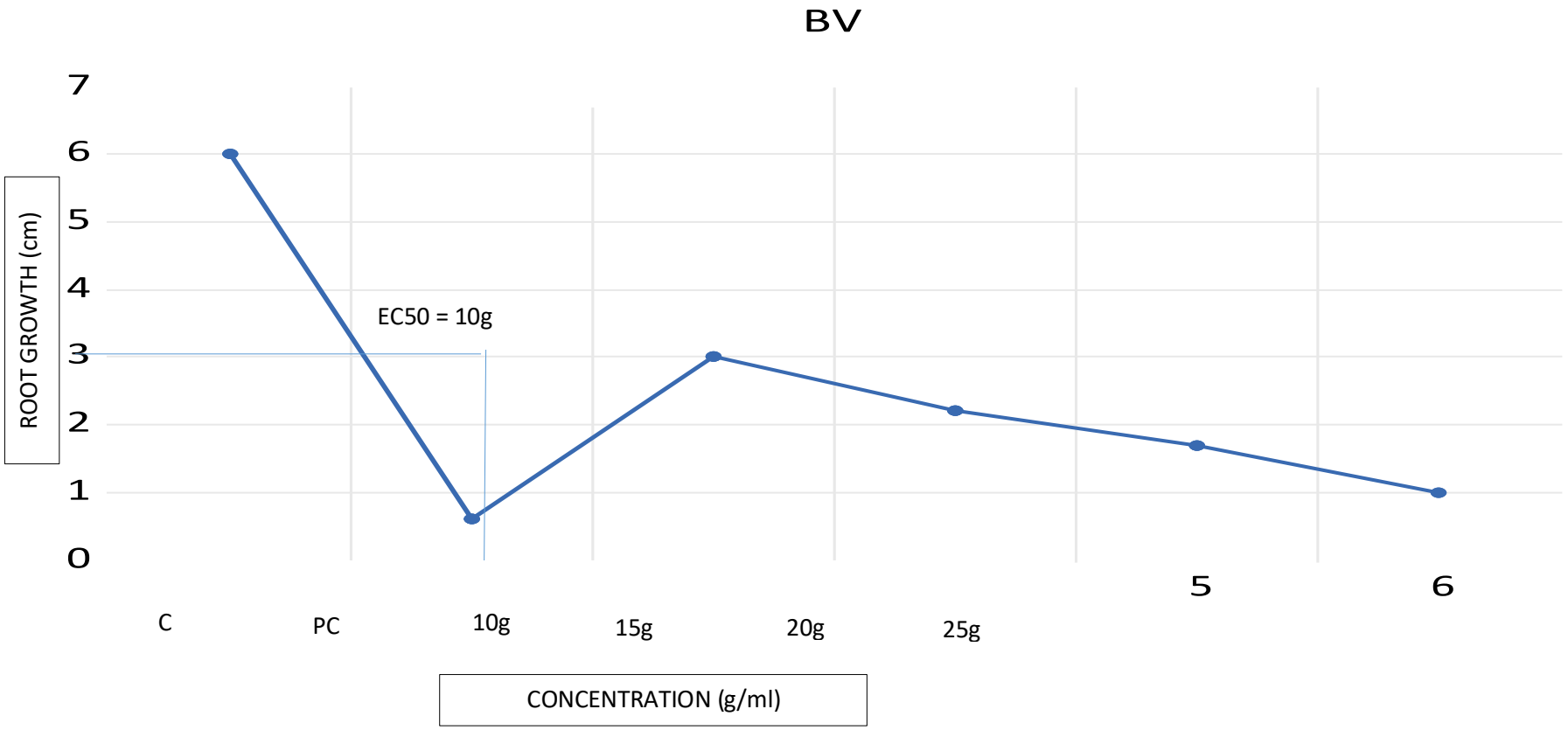


Figure 1: Inhibitory effect of different concentrations *Morinda citifolia* leaves extract on *Allium cepa* root growth

Photograph showing the Effects of *Morinda citrifolia* leave Extracts on *Allium cepa* Root Growth Morphology

Morphologically, the roots of onion bulbs were growing well hydroponically in negative control. The roots are healthy, longer, shiny and growing very fast indicating high cell division which helps growth and development of the root. The root morphology showed obvious differences in its appearance as it became, short not dense stiff, turned to yellow and change in texture in positive control and the treatment group as shown on (plate i-vi) below.


Plate i


plate ii


plate iii


plate iv


Plate v


plate vi

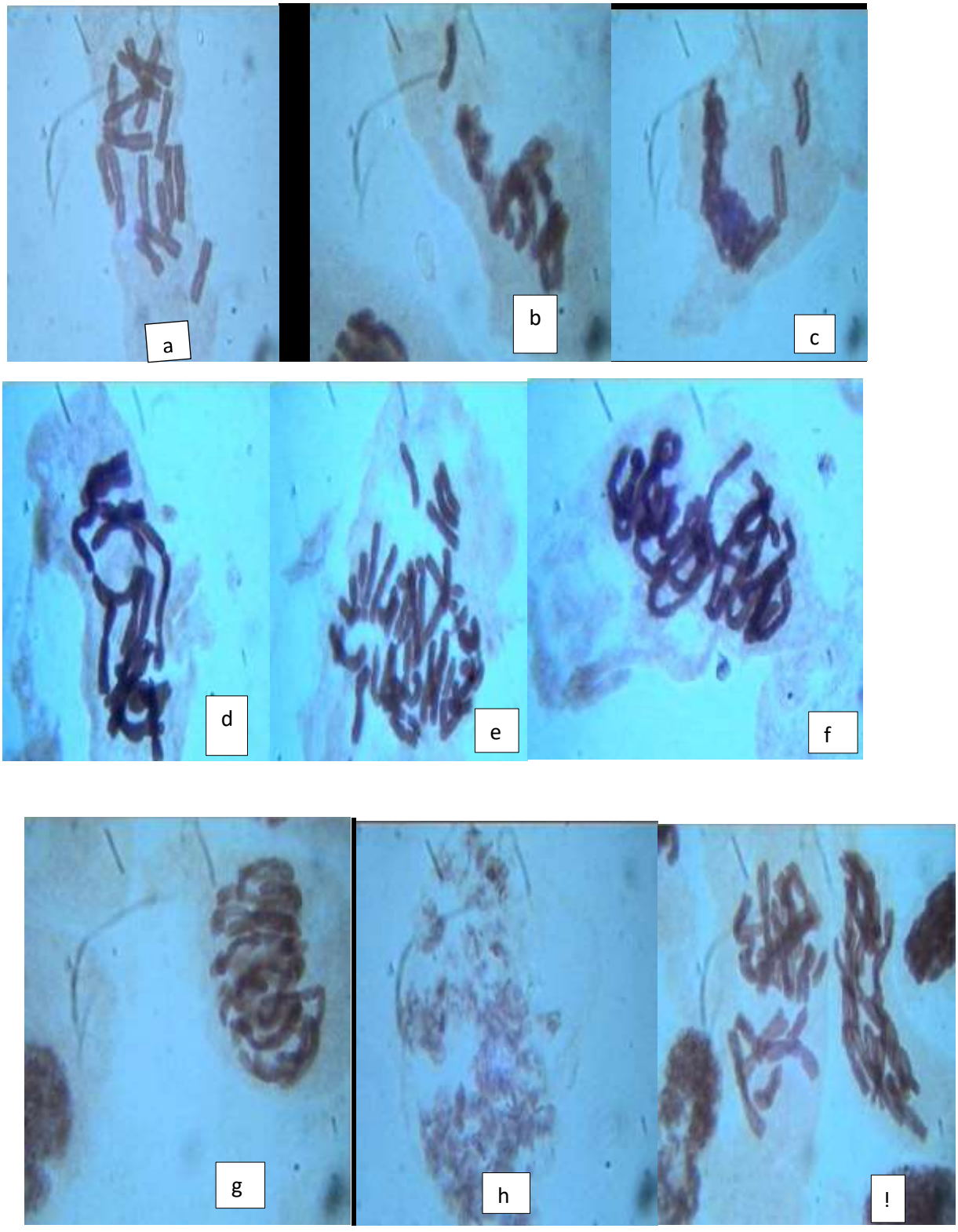
Table 1: Chromosomal Aberration observed in the Root Cells of *A. cepa* treated with *Morinda citrifolia* Leave Extracts

Conc. g/ml	Total cell scored	Chromosomal aberration													% of aberration cells
		TNCD	PP	L	AB	D	VM	BN	NL	F	CG	MN	US	TNAC	
NC	200	180	0	0	0	0	0	0	0	0	0	0	0	0	0[a]
PC	200	154	7	3	2	3	4	0	5	6	2	3	10	45	22.5[b]
10	200	166	0	0	0	0	0	0	0	0	0	0	0	0	0[a]
15	200	163	6	3	0	3	4	4	0	3	1	4	4	32	16[b]
20	200	158	10	6	3	0	0	7	3	6	4	3	6	48	24[b]
25	200	156	11	8	4	3	2	4	0	7	4	0	8	51	25.5[b]

NC= Negative Control, PC= Positive Control, PP = Polyploidy, D= Dissolution, V= Vagrant metaphase, BN= Binucleate, L = Laggard, NL = Nuclear lesion, F= Fragmentation, CG= Chromosome gap, AB = Anaphase bridge, MN= Micronuclei, US= Unequal separation, TNCD= Total number of dividing cell, TNAC= Total number of aberrant cell.

Table 1 above shows the percentage of different types of chromosomes aberrations caused by *M. citrifolia* leave extract on the root tip cells of Allium cepa, although the percentage of aberrations at 20 g is higher, but not statistically different from 25 g, 10 g and positive control (P=0.117), as the concentration of the extract increased, the percentage of aberrations increases, while the number of normal cells decreased.

Micrograph showing different Types of Chromosomal Aberration Induced by *Morinda citrifolia leaf* Extracts on the Root Tips of *Allium cepa*



(a) showing normal chromosome with 2n= 24,(b) showing chromosome stickiness, (c) lagging chromosome (d) chromosome gap (e) polyploidy (f) vagrant metaphase (g) nuclear lesion (h) dissolution (i) unequal seperation

Mutagenesis effect of *Morinda citrifolia* leaf extract on *Allium cepa* Root Tips

This assay demonstrates alterations in all phases of the cell cycle, which are considered evidence for mutagenic effects induced by clastogenic or aneugenic agents (classified according to the type of alteration induced). Some of these alterations, such as chromosomal breaks and micronuclei (MN), are chromosomal aberrations used to evaluate mutagenicity [19]. The increased frequency of MN and Chromosomal aberrations in the *A. cepa* are strong evidence for mutagenicity of the substance evaluated [21], It is also known that DNA damage caused by mutagenic compounds depends on the intensity, duration of exposure and the efficiency of DNA repair activated by the exposure [22]. Some of the cellular changes found during the analysis of *Morinda citrifolia* leave extracts were polyploidy, dissolution, Vagrant metaphase, stickiness, Binucleate, laggard, nuclear lesion, fragmentation, Chromosome gap, Anaphase Bridge, and unequal separation. It is known that anaphase bridges are formed during an unequal exchange of chromatids or by breakage and fusion of chromosomes and chromatids, and these bridges can cause structural chromosomal mutations [23]. *MN* Formation is the result of chromosome breaks and lagging chromosome. It is reported that chromosomal bridges results from chromosome break- fusion cycle [24] and are initiator of aneuploids and polyploids. Lagging chromosome results from spindle malfunction, which is known to be induced by heavy metals including *Pb*. [25], also reported that, the Clastogenic effect of metals, are mostly due to inhibition of activity of genome repairing enzymes leading to cellular death which cannot recover from the damage and thus increase the frequency of aberrations as new cycle begins. The chromosomal aberrations observed in the root tip of *Allium cepa* suggest that the *Morinda citrifolia* exert a cytotoxic/mutagenic effect on the mitotic chromosome of *Allium cepa*. Supporting the view that this bioassay works well for evaluating the mutagenicity of medicinal plants extracts on the root tip of *Allium cepa*. In addition to the chromosome fragments, stickiness was also observed and In general, it was possible to observe an increase of different abnormalities as the plants extracts concentration increased and a decrease in normal cells. A strong cytotoxic and mutagenic effect of plants extracts was observed, supported by great occurrence of sticky metaphases, leading to cellular death. These is in agreement with [26], which reported that the cytotoxicity levels of an agent can be determined by increases or decreases in the percentage of aberrations. Therefore, with the progressive increased in the number of aberrant cells as the concentrations increases significantly compare to negative control indicates inhibition in cell proliferation (by the action of plant extracts or chemical substance) [26]. However, this study observed increased in chromosomal aberration percentage with a concomitant increased in concentrations of plants extracts. This may be due to presence of mitogenic agents which could act to overcome intracellular braking mechanisms that block cell cycle progression (mitostimulatory) or may be due to antioxidants present in plants extracts which have been reported by other research works to be present in the leave extracts [27]. Antioxidants have been shown to improve DNA repair as well as modulate toxicity in different independent studies [28].

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