INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

INDUCTION OF NUCLEAR BUDS AND MICRONUCLEI IN *ALLIUM CEPA* ROOT MERISTEM BY THE LEAF EXTRACT OF *DIEFFENBACHIA MACULATA* (LODD.) SWEET

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Article Received on: 25/11/18 Approved for publication: 12/01/19

DOI: 10.7897/2230-8407.100262

ABSTRACT

The plant selected for the present study, *Dieffenbachia maculata* (Lodd.) Sweet is an ornamental plant that belongs to the family Araceae. It is an ornamental and perennial monocot plant. Present investigation is mainly focused to analyze the induction of cytological aberrations in the root tip meristem of *Allium cepa*, after treatment with the aqueous leaf extract of *D. maculata*. The result obtained revealed considerable number of nuclear budding and micronuclei along with other aberrations. Different concentrations of the leaf extract *viz*., 0.005, 0.01, 0.05 and 0.1% (w/v) were used. The occurrence of nuclear buds and micronuclei was found to be increased with increase in the concentrations used. The aberration percentages of the nuclear buds are observed in between the range of 36.46±0.33 to 61.69±0.50.

KEYWORDS: *Dieffenbachia maculata*; Cytotoxicity; Nuclear buds; Micronucleus

INTRODUCTION

Ornamental plants are grown for decorative purposes in gardens and landscape design projects, as houseplants, for cut flowers and specimen display. The cultivation of these, called floriculture, forms a major branch of horticulture. Commonly, ornamental (garden) plants are grown for the display of aesthetic features including: flowers, leaves, scent, overall foliage texture, fruit, stem and bark, and mixed aesthetic forms.

Genus *Dieffenbachia* includes about 30 species and over 100 cultivars with spotted, stripped or speckled with cream, white, yellow, gold, silver, or a combination of these colored leaves. Leaves are 15 to 40cm in length[1, 2]. They are native to tropical America, belonging to the family Araceae [3]. All parts of *Dieffenbachia* show poisonous content and it has a great influence in antimicrobial activities[4]. Conventionally *Dieffenbachia* is propagated by seed, tip or cane cuttings, division and air layering[5, 3]. *In vitro* micropropagation contributed very much in the propagation of *Dieffenbachia* [6]. Flowers are unisexual, dichogamous in nature, and the chromosome number of most species is 2n = 34 [7]. The present study is mainly focused on the cytotoxic potential of the aqueous extract of *Dieffenbachia maculata*.

Micronuclei and other nuclear anomalies such as nucleoplasmic bridges and nuclear buds are biomarkers of genotoxic events and chromosomal instability. Nuclear buds represent the process of elimination of amplified DNA, DNA repair complexes and possibly excess chromosomes from aneuploid cells.

Micronuclei are generated not only from chromosomal materials, but also from extrachromosomal elements called double minutes (DMs). They are mainly detected in many human cancer cells. Elimination of DMs from cancer cells results in the loss of malignant phenotypes. It is mainly due to the amplified genes on DMs. The malignant phenotype of cancer cells was determined by these DMs [8, 9, 10]. The present study envisages the aspects of nuclear budding and micronuclei, which were formed by the extracts of *Dieffenbachia maculata* using *A. cepa* assay.

Micronuclei are formed as a consequence of chromosome breakage (clastogenic agent) or whole chromosomes (aneugenic agent) that were not incorporated to the main nucleus during the cell division cycle[11, 12] .

Micronuclei can be a result of acentric fragments or entire chromosomes that were not incorporated to the main nucleus during the cell cycle[13]. Nuclear budding process is the mechanism by which cells remove amplified DNA and is therefore a marker of gene amplification. The strong correlation between micronucleus formation, nuclear budding and nucleoplasmic bridges of the hypothesis that folic acid deficiency caused by genomic instability and gene amplification by the initiation of breakage-fusion-bridge (BFB) cycles is reported [13]. There are several reports on the accumulation of p^{53} protein in micronuclei [14, 15]. Presence of lamina around the micronuclei had important implications because transcription or DNA replication was detected only in the micronuclei with lamin B protein [16, 17].

The generation of micronuclei through a budding process in the mammalian nucleus, which is reinforced by the nuclear lamina, is surprising. Prior work based on the simultaneous visualization of DMs and the lamin protein did not support the protrusion of a portion of the nucleus with lamina as a mechanism for the generation of buds/micronuclei [18].

Micronuclei are formed from chromosomes and chromosomal fragments that lag behind in anaphase and are left outside daughter nuclei in telophase. They may also be derived from broken anaphase bridges. Nuclear buds, micronucleus-like bodies attached to the nucleus by a thin nucleoplasmic connection, have

been proposed to be generated similarly to micronuclei during nuclear division or in S-phase as a stage in the extrusion of extra DNA, possibly giving rise to micronuclei. Most nuclear buds, however, are suggested to originate from interstitial or terminal acentric fragments, possibly representing nuclear membrane entrapment of DNA that has been left in cytoplasm after nuclear division or excess DNA that is being extruded from the nucleus [19].

MATERIALS AND METHODS

Preparation of extracts

The leaves of the plant *D. maculata* were collected from the Botanical Garden, University of Calicut. Taxonomic authentication of the plant was done by Dr A. K. Pradeep, Angiosperm Taxonomy Division, Department of Botany, University of Calicut. The collected materials were shade dried and powdered using a blender and stored in a moisture free atmosphere. Fresh aqueous extract was prepared from the leaves of the plant with the help of mortar and pestle. 0.1g of the powdered material was dissolved in 100 ml distilled water to prepare the stock solution. Different concentrations of the extract, *viz*, 0.005, 0.01, 0.05 and 0.1% (w/v) were prepared in distilled water.

Treatment of root meristem with the extract

Chemically untreated *Allium cepa* bulbs were used for the present study. This was grown in autoclaved sand until the root acquires the length of approximately 10 mm. The bulbs were treated with the various concentrations of leaf extracts of *D. maculata.* In order to find out the time of peak mitotic activity, the untreated root tips of *Allium cepa* were fixed in acetic alcohol (1:2) mixture at different times from 8.30 am to 11.00 am. After many trials, it was found that maximum dividing cells (peak mitotic activity) occurred between 9.00 am-10.00 am under normal sunshine conditions. For the effective comparison, both negative and positive controls were used. Distilled water was taken as the negative control and 0.01% of methyl parathion was taken as the positive control. The washed root bulbs were treated with different concentrations of the extract taken in bottles, in such a manner that only the roots were immersed in the solution. Root tips were cut from the samples of each concentration at different time intervals such as 1h, 2h, 3h and 24h, washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (1 acetic acid : 2 alcohol) for 1hour. The root tips were washed with distilled water and hydrolysed in 1N HCl for 5-10 minutes to separate the cells during squashing. The root tips were then washed thoroughly with distilled water and stained with 2% acetocarmine for 4 hours. After staining, the root tips were destained with 45% acetic acid, squashed and mounted on clean microslides.

Mounted slides were observed for aberrations under microscope (Olympus CX21 FSI, Japan) and photographs were taken using Amscope Mu 1000 digital camera- FMA050 attached to the microscope.

Calculation of Mitotic index and abnormality percentage

Mitotic index (%) and abnormality percentage were calculated using the following formula.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Abnormality Percentage} = \frac{\text{Number of abnormal cells}}{\text{Total number of cells}} \times 100$$

RESULTS AND DISCUSSION

The results revealed the severe cytotoxic potential of *D. maculata* (Lodd.) Sweet using *Allium cepa* assay. Present study gave a large number of nuclear buds, micronuclei and other abnormalities induced by different concentrations of plant extract. Mitotic indices of the root tip meristems of *A. cepa* treated with the aqueous extract of *D. maculata* were found to be decreasing with increasing concentration of the extract (Table 2). The mitotic indices of treated roots were lower than the roots treated with negative control and greater than the roots treated with positive control. The roots treated with the aqueous extract gave considerable extend of abnormalities. The abnormality percentage of the treated roots was found to be higher than that of negative control (Table 1). Thus it was clearly observed that the percentage of abnormalities of the treated roots lies in between the positive and negative control. From these observations, it was clear that the percentage of abnormality gradually increases with increase in the concentration of the extract. Lowering of mitotic index is generally considered as the result of cytotoxic activity. Cell division was found to be normal in the root tips treated with negative control.

Formation of nuclear buds and micronuclei was the most frequent abnormality observed in the various treatments (fig. 1). It may be due to the interference of several phytochemical compounds present in it, with the normal course of mitosis. The number of nuclear buds and micronuclei were also observed to be increasing with the concentration and duration of treatment (Table 2; fig. 2). Together with the nuclear buds and micronuclei, several other important abnormalities are also found. Such as nuclear lesion, binucleate cell, hyperchromasia, multiple nuclear lesions *etc*.

Cytogenetic assay revealed many apoptosis inducing cytogenetic aberrations viz., cytoplasmic breakage, cytoplasmic disintegration, cytoplasmic shrinkage, receding of cytoplasm, cytoplasmic vacuolation, enucleated cell, ghost cell, nuclear vacuolation, nuclear fragmentation and nuclear disintegration[20].

Roots of *A. cepa* treated with *D. maculata* extract, resulted in the nuclear appendages or nuclear budding. Naturally occurring nuclear budding, apart from the induced bud or appendage formation occurs as a result of the selective entrapment of extra chromosomal amplified DNA by the nucleus and which can probably end in micronucleation during S-phase [21]. Nuclear buds and vesicles resembling protuberances were observed to originate from the outer membrane of the nuclear envelope *in situ* at certain regions of the nuclear envelope. All mitotic phases show considerable induction of giant cells and the interphase stage shows higher percentage of giant cells, whereas in cytokinesis it was negligible. In this study also nuclear buds and micronuclei are prominently resulted at interphase stage [22].

Hellgren and Morre [23] reported that, probably these nuclear buds arise as a result of the excessive production of nucleic acids and proteins, induced by the cytotoxicants. This may be the probable reason for scoring nuclear appendage or nuclear buds in *A. cepa* after treatment with *D. maculata* extract in the present investigation. Treatment of *A. cepa* root tips with the extract of *D. maculata* resulted in interphase cells with micronucleus. It may be formed due to the action of the extract on the spindle apparatus, leading to unequal separation of chromosomes at anaphase. The larger group of daughter chromosomes forms a larger nucleus and the smaller group forms a micronucleus. According to Sparrow and Singleton [24], micronuclei are a fair index of chromosome fragment production. Micronucleus may originate from a lagging chromosome at anaphase or from a chromosome fragment [25]. Micronuclei are true mutagenic aspects of Auerbach [26] which may lead to a loss of genetic material and

have been regarded as an indication of mutagenicity of their inducers [27].

CONCLUSION

Allium cepa roots treated with the leaf extract of *D. maculata* showed the appearance of nuclear buds and micronuclei. Present study gave important information about the toxicity of *Dieffenbachia maculata* due to the high percentage of the anomalies observed by *A. cepa* assay, which may be utilized for the development of chemotherapeutic drugs.

Table 1: Mitotic index and abnormality percentage

Concentration	Time duration	Total cells	Mitotic index±SE (%)	Abnormality±SE (%)
Negative control	1	486	80.39±0.90^{e}	0.00
	2	428	72.33±1.78^{e}	0.00
	3	455	72.34±0.99^{e}	0.00
	24	481	75.88±2.68^{e}	0.00
Positive control	1	436	34.54±2.10^{a}	59.16±1.42^{e}
	2	460	27.01±1.74^{a}	58.92±0.74^{d}
	3	431	28.52±1.73^{a}	61.69±0.50^{e}
	24	407	23.32±1.32^{a}	40.77±6.88^{b}
0.005	1	384	65.88±0.91^{d}	36.46±0.33^{b}
	2	423	61.29±1.39^{d}	38.40±3.20^{b}
	3	470	60.51±1.95^{d}	44.36±1.49^{b}
	24	412	60.24±0.88^{d}	52.28±1.21^{b}
0.01	1	427	60.62±0.48^{c}	42.79±1.38^{c}
	2	388	57.57±2.58^{c}	45.06±0.79^{c}
	3	381	52.82±1.08^{c}	50.43±1.08^{c}
	24	382	59.69±0.59^{c}	53.60±4.10^{b}
0.05	1	420	56.52±2.49^{bc}	54.24±0.25^{d}
	2	406	50.29±1.06^{b}	55.17±0.44^{d}
	3	401	47.45±1.59^{b}	52.50±1.97^{cd}
	24	409	52.34±1.33^{c}	49.14±4.30^{b}
0.1	1	407	53.93±2.15^{b}	56.27±0.55^{d}
	2	390	52.21±2.58^{b}	57.71±3.71^{d}
	3	432	46.45±2.12^{b}	56.05±1.14^{d}
	24	460	42.89±0.78^{b}	45.36±2.03^{b}

SE, standard error. Means within a column followed by the same letters are not significantly different ($p<0.05$) as determined by DMRT

Table 2: Induction of nuclear buds and micronuclei in different concentrations including negative and positive control

Concentration	Time duration	Percentage of nuclear buds and micronuclei ± SE
Negative control	1	0.00
	2	0.00
	3	0.00
	24	0.00
Positive control	1	24.18±5.57^{b}
	2	36.43±4.23^{b}
	3	32.55±4.22^{b}
	24	40.77±6.88^{b}
0.005	1	31.23±0.33^{bc}
	2	45.26±8.27^{b}
	3	40.42b±5.47^{c}
	24	52.28±1.21^{b}
0.01	1	40.34±6.03^{cd}
	2	51.57±5.11^{b}
	3	50.35±2.78^{c}
	24	53.60±4.10 ^{b}
0.05	1	47.62±1.83^{d}
	2	50.02±4.71^{b}
	3	43.35±5.28^{bc}
	24	49.14±4.30^{b}
0.1	1	38.39±5.22^{cd}
	2	46.88±7.23^{b}
	3	46.07±3.62^{c}
	24	45.36±2.03^{b}

SE, standard error. Means within a column followed by the same letters are not significantly different ($p<0.05$) as determined by DMRT

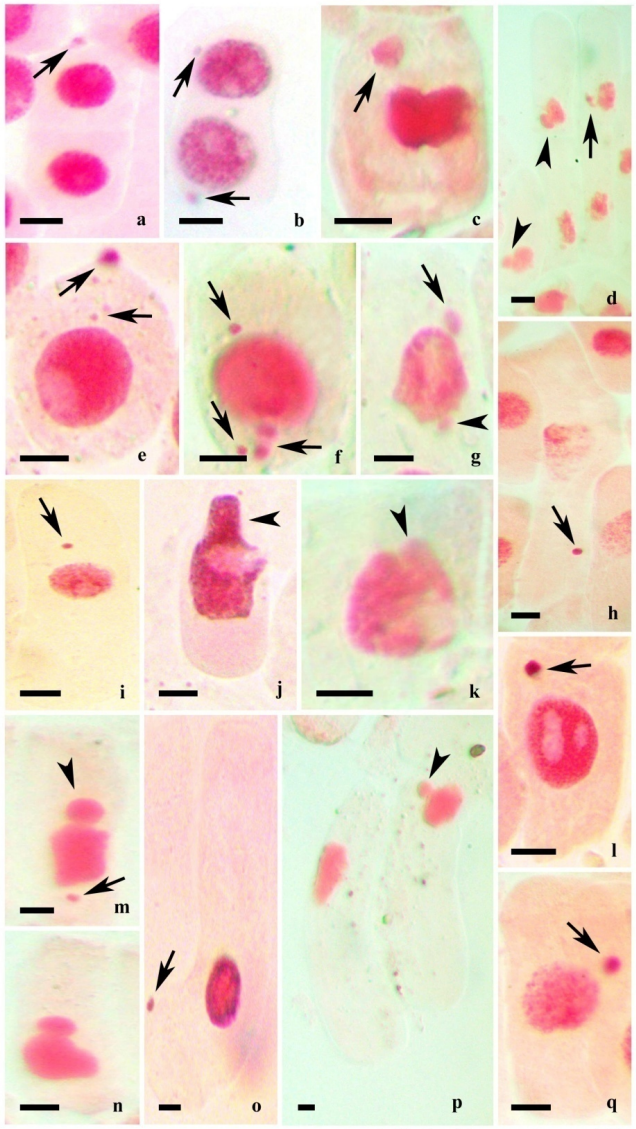

Fig. 1 Nuclear buds and micronuclei induced by *Dieffenbachia maculata* in *Allium cepa* root tip meristem. Arrows indicate micronuclei and arrowheads nuclear buds. Figs a-q: a Binucleate cell showing budding with comet micronucleus, b Binucleate cell with two micronuclei, c Cell showing bilobed nucleus and micronucleus, d Different stages of nucl ear budding, e Extrusion of micronucleus at interphase, f Formation of micronuclei after budding at interphase, g Formation of multiple nuclear buds and micronucleus, h Giant ghost cell formation and a micronucleus, i Micronucleus at interphase, j Nuclear budding and chromatin extrusion at interphase, k Nuclear budding and lesion, l Nuclear lesions and micronucleus, m Nuclear budding and micronucleus, n Nuclear budding, o Nuclear diminu tion and micronucleus, p Nuclear budding in a giant cell, q Pulverized macronucleus and micronucleus. Scale bar 10 µm

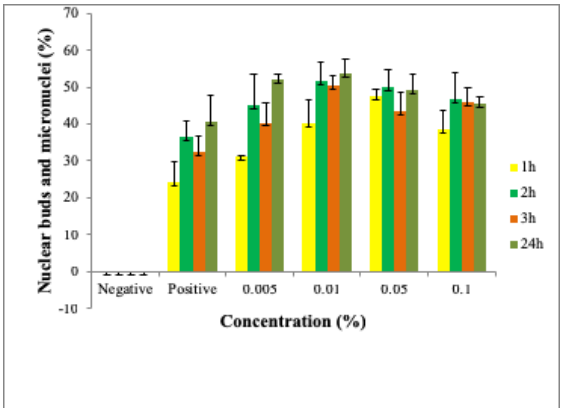

Fig. 2: Induction of nuclear buds and micronuclei at different concentrations of *Dieffenbachia maculata*

ACKNOWLEDGEMENT

The first author is grateful to the University of Calicut for the financial support.

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Cite this article as:

Pokkadath Aswathi and John E. Thoppil. Induction of nuclear buds and micronuclei in *Allium cepa* root meristem by the leaf extract of *Dieffenbachia maculata* (Lodd.) sweet. Int. Res. J. Pharm. 2019;10(2):166-170 http://dx.doi.org/10.7897/2230-8407.100262

Source of support: University of Calicut, Conflict of interest: None Declared