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RESEARCH ARTICLE

ANTIGENOTOXIC POTENTIAL OF *MORINGA OLEIFERA*, LAM. LEAVES EXTRACT AGAINST CADMIUM AND NICKEL INDUCED MITOTIC ABERRATIONS IN *ALLIUM CEPA*, L.

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ABSTRACT

The study assessed the cytotoxic effect of two heavy metals, Cadmium and nickel in *Allium cepa*, L and antigenotoxic potential of *Moringa oleifera*, Lam. leaves extract against these heavy metals. Bulbs of *A. cepa* were exposed to 20, 40, 60, 80 and 100 ppm of Cadmium and Nickel supplied in the form of CdCl₂ and NiCl₂. A gradual decrease in the germination percentage, mitotic index and increase in the frequency of aberration were observed with increasing concentrations of the heavy metals. Chromosomal stickiness and c-mitosis were found to be common aberrations due to metal exposure. Of the two heavy metals used in the study, Cd was found to be more toxic to *A. cepa* compared to Ni. The investigation also establishes the antigenotoxic potential of *M. oleifera* leaves, as there was an increase in germination percentage and mitotic index and a decrease in the frequency of aberrations in both the metal treated cells on exposure to 0.5% aqueous extract of *M. oleifera* leaves. The recovery action was more pronounced in Cd exposed cells compared to Ni.

INTRODUCTION

A heavy metal is a member of loosely defined subset of elements that exhibit metallic properties. Heavy metals are considered as the stimulators or inhibitors of life processes depending on their concentration, ability to form complexes and degree of oxidation (Szyzewski et al., 2009). Plants absorb these metals easily from soil and accumulate them in indifferent parts such as root, stem and leaf causing significant alternations in the plant cell (Suzuki, 2005). Cadmium and Nickel are the most important heavy metals found in the environment after the rapid industrialization and urbanization during the recent past. In humans, long-term exposure to Cd is associated with renal dysfunction, lung disease, lung cancer, osteomalacia and osteoporosis in humans. Long-term exposure to Ni can cause decreased body weight, heart and liver damage, and skin irritation. The current study was conducted to find out the genotoxic effect of these chemical elements in onion root tip, so as to assess their mode of action in human beings and also to evaluate the antigenotoxicity of *Moringa oleifera* leaf extract against these heavy metals. Ample literature is available regarding the usage of Onion root tip as the reliable assay system to evaluate the

genotoxic potential of the chemicals present in the environment (Sudipa Nag et al., 2013; Komal Arora, 2013; Rekha and Anusree, 2011)

MATERIALS AND METHODS

The experiment was conducted in two stages

Test for mitotic aberrations

For this healthy bulbs of *Allium cepa* (onion) were placed in petridishes lined with cotton moistened with respective concentrations of test solutions. Ni and Cd were supplied in the form of their chlorides. From the results of range finding test, test solutions containing 20ppm, 40ppm, 60ppm, 80ppm and 100ppm heavy metals were selected for the experiment. Control plates were raised in distilled water. All the experiments were conducted in triplicate. Roots were observed for mitotic aberrations after 48hrs of exposure. Chromosome squash technique was adopted for the preparation of root tip for cytological studies.

Test for antigenotoxicity

From the range finding test, 0.5% of *Moringa* leaf extract was selected for the antigenotoxic studies. A separate set of control and variants were raised for recovery test. Equal volume of

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leaf extract was supplied to each petriplate. The microscopic preparations of the root tip were made after 24 and 48 hours of exposure time.

Parameters studied were

a. Germination percentage

Germination percentage was determined using the equation:

$$GP = \frac{\text{No. of bulbs germinated}}{\text{Total no. of bulbs}} \times 100$$

b. Mitotic index (MI)

Mitotic index is a measure for the proliferation status of a cell population. It was calculated using the formula:

$$MI = \frac{\text{Number of dividing cells}}{\text{Total number of cells}}$$

c. Frequency of aberrations (FA)

It is the number of occurrence of mitotic aberrations in the field view. It was estimated as:

$$FA = \frac{\text{Number of cells showing aberration} \times 100}{\text{Total number of cells}}$$

RESULTS AND DISCUSSION

The onion bulbs placed in the cadmium and Nickel treated plates showed reduced germination rate, lesser mitotic index and incidence of mitotic aberrations. Percentage reduction in germination increased with the increase in the concentration of test metals. Minimum germination percentage of 55% and 37% was noticed in the bulbs exposed to 100ppm Cd and Ni respectively.

In control, onion bulbs exhibited 100% germination on second day of exposure. Mitotic index was maximum (0.51) in the onion root tips grown in distilled water. Mitotic index in 20 ppm Cd was 0.30 and it decreased to 0.19 in 100ppm. Mitotic index registered a 40% decrease over the control in 100 ppm Ni treated cells with a value of 0.17. The maximum frequency of aberration (80%) was noticed in 100ppm Cd exposed cells and in 20ppm Cd treated cells frequency of aberrations noticed was 66% (Table 1). Various anomalies recorded were Ball metaphase, Nuclear lesions, Telophase bridges, Dislocation of chromosomes, Stickiness at metaphase, Chromosome breaks and strap nucleus (Fig.1). Mitotic aberrations were not observed in the cells kept as control. The frequency of aberration was much lower in Ni treated cells compared to those treated with Cd. Minimum aberration was observed in 20ppm (37%) and the maximum aberration observed was 66% in 100ppm (Table 1). Nuclear lesions, polar deviation, chromosome breaks, strap nucleus, star metaphase, fragmented anaphase and c- mitosis were the various aberrations occurred in Nickel treated cells (Fig.2). Of the two heavy metals used in the study, Cd was found to be more toxic to *A. cepa* compared to Ni.

Treatment with *Moringa oleifera* leaves extract significantly reduced cytotoxic effect of heavy metals. On the 2nd day of exposure to 0.5% extract, mitotic index was 0.61 in 100ppm Cd with a recovery effect of 69%. Frequency of aberrations was reduced to 41% from 80% in 100ppm Cd exposed cells (Table 2). 54% increase in mitotic index and 34% decrease in frequency of aberrations was obtained in 100ppm Ni treated cells on exposure to *M.oleifera* leaves extract (Table 3). The results obtained revealed the inhibitory action of cadmium and nickel on mitotic index and their inducing action on mitotic aberrations in *A. cepa* root tip cells.

Table 1. Effect of Cadmium and Nickel on cell division of Allium cepa.

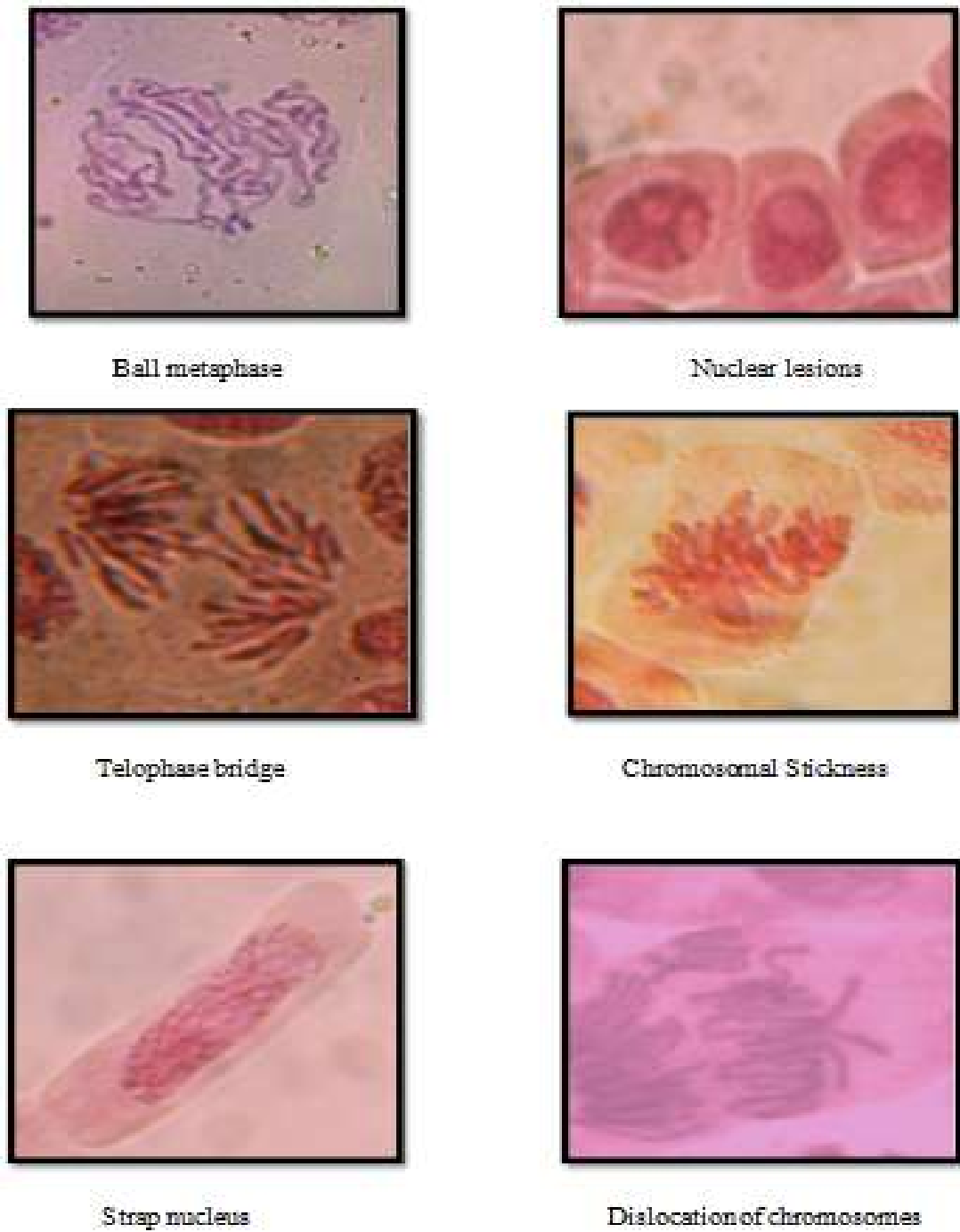
Concentration	Germination (%)		Mitotic index		Frequency of aberrations	
	Cd	Ni	Cd	Ni	Cd	Ni
Control	100	100	0.51	0.51	0	0
20	64	72	0.3	0.17	67	37
40	63	70	0.25	0.21	72	45
60	62	65	0.25	0.22	78	45
80	60	51	0.20	0.24	79	50
100	55	37	0.19	0.28	80	66

Table 2. Antigenotoxicity of M. Oleifera leaves extract against Cadmium

Concentration	1 st day of exposure			2 nd day of exposure		
	GP	MI	FA	GP	MI	FA
Control	100	0.63	0	100	0.68	0
20	77	0.60	34	78	0.66	32
40	72	0.60	43	78	0.65	33
60	60	0.58	45	70	0.64	37
80	45	0.47	47	50	0.64	39
100	40	0.40	59	45	0.61	41

Table 3. Antigenotoxicity of *M.Oleifera* leaves extract against Nickel

Concentration	1 st day of exposure			2 nd day of exposure		
	GP	MI	FA	GP	MI	FA
Control	100	0.63	0	100	0.87	0
20	73	0.56	34	85	0.81	17
40	70	0.55	47	70	0.78	28
60	50	0.50	50	62	0.77	33
80	48	0.47	55	58	0.65	35
100	45	0.34	56	53	0.61	43

Fig.1. Cadmium induced mitotic aberrations in *Allium cepa*

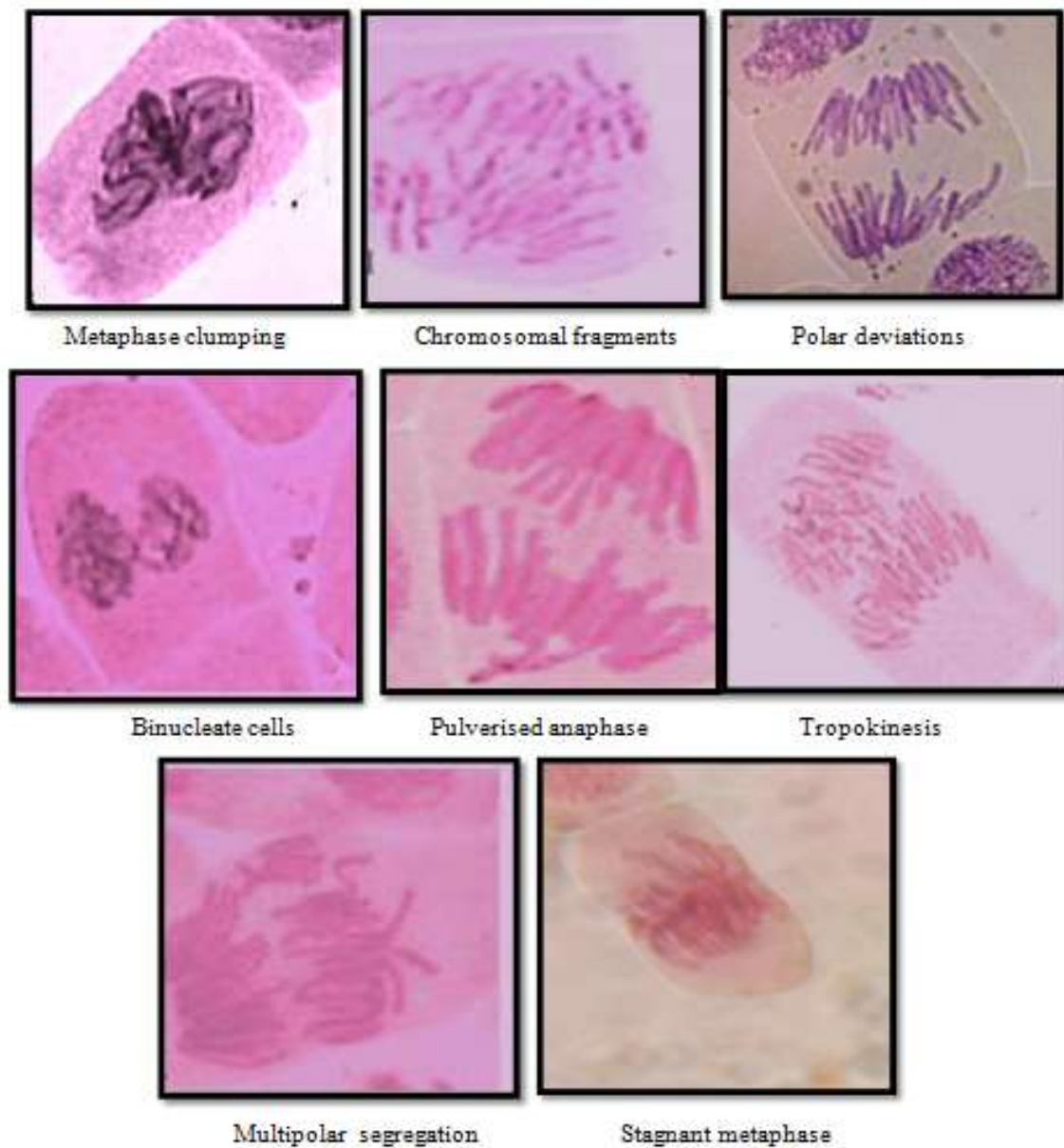


Fig. 2. Nickel induced mitotic aberrations in *Allium cepa*

Similar findings were observed in safflower seed germination and seedling growth treated with mixed cadmium, calcium, nickel and zinc (Alireza and Farhang, 2011) and also in *Allium sativum* and *Vicia faba* root cell treated with cadmium (Unyayar, et al., 2006). The frequency of abnormal cells had a positive relation with the concentration of treatment solution. Similar results have reported after treatment of *Allium cepa* root tip cells with various heavy metal elements by Szyzewski et al. (2009) and Siroka et al. (2004).

Of various mitotic aberrations, chromosomal stickiness and c- mitosis were found to be common in both the metals treated cells. Chromosome stickiness reflects highly toxic effects, usually of an irreversible type probably leading to death. c - Mitosis indicates the chemical inhibited spindle formation similar to the effect of colchicine (Badr and Ibrahim, 1983) and mainly associated with spindle poisoning (Shahin and El-Amoodi., 1991). There are many reports showing the raising

trends of antimutagenicity studies with extracts of plants like aqueous extracts of fermented and non-fermented tea (Marnewick et al., 2000), *Embllica officinale* against CdCl₂ induced genotoxicity in bone marrow cells swiss albino mice (Singh et al., 2007) and the protective effect of anthocyanin rich extract against heavy metals toxicity in *Allium cepa* (Glinska et al., 2007). The current study reveals the antigenotoxic effect of *M. oleifera* leaves extract against Cd and Ni. The recovery action of *M.oleifera* extract was more pronounced in Cd exposed cells compared to Ni.

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