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Prevention of Cadmium induced genotoxicitywith *Emblicaofficinalis* L. (Amla) in Allium Test.

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ABSTRACT

Cadmium is a potent environmental pollutant and human exposures to cadmium cause both genotoxic and carcinogenic effects. Occupationally cadmium exposed people are also at potentially high health risk. Few reports have been observed on the use of herbal compounds to reduce cadmium toxicity. So, the experiment is designed in such a way that the adverse effect of cadmium on human health and recovery of the toxicity can be studied. In our experiment bulbs of *Allium cepa*were grown in tap water (Group I), in five concentrations (10^{-1}M to 10^{-5}M) of cadmium chloride in the absence (Group II) and in the presence (Group III) of amla (fruit of *Emblicaofficinalis*) at a fix concentration of 0.10 mg/ml. After 72 hours, the different parameters such as mean root length, mitotic index, chromosomal aberrations and nucleolar morphology were studied. Cadmium chloride at all concentrations significantly inhibited root growth, declined mitotic index caused abnormal mitosis and chromosomal aberrations (Group II). In the presence of amla (Group III) cadmium chloride induced genotoxicity could be checked significantly at 10^{-3}M to 10^{-5}M. No morphological i.e. shape and colour changes and any type of chromosomal aberrations have been detected in Group III. Hypertrophy of nucleoli in cadmium chloride induced root tip cells were appreciably reduced at 10^{-4}M and 10^{-5}M in Group III. From the above study, it can be concluded that cadmium chlorideproduces genotoxic effects on human health and amla can provide some protective role against cadmium chloride mediated toxicity.

Key words:*Allium cepa*, genotoxicity, cadmium chloride, toxicity, amla.

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INTRODUCTION

Cadmium is a non-essential element that negatively affects plant growth and development. It is released into the environment by power stations, heating systems, metal-working industries or urban traffic. It is widely used in electroplating, pigments, plastic stabilizers and nickel-cadmium batteries [1]. Soil solutions which have a Cadmium concentration varying from 0.32 to about 1 mM can be regarded as polluted to a moderate level [1]. Cadmium is potentially genotoxic and carcinogenic to most organisms [2-4], which, at low concentrations, can act as an essential micronutrient for plant and microbial growth [5]. In developed agricultural systems, inorganic fertilizers are applied to the soil to supply the essential nutrients required for the growth of plants. However, the accumulation in the environment of heavy metals as a result of current agricultural systems is steadily increasing. Many cytological studies have been carried out to detect the harmful effects of various heavy metals on different plants [6-8]. In addition, in developed industrial systems, industrial wastes affect genetic systems by producing various types of chromosomal abnormalities.

Keeping pace with the above observation, our aim was to determine the effects of cadmium chloride on cell division and the somatic chromosomes in *Allium* model and to find out whether amla can also antagonize Cadmium-genotoxicity in *Allium* model.

MATERIALS AND METHODS

Allium cepa

Equal sized healthy dry brown pink bulbs of onions (2n=16) were obtained from the local market.

Test Chemical

Cadmium chloride monohydrate as $CdCl_2.H_2O$, MW 201.32, and purity 99% of E.Merck was used. Salt was dissolved in tap water to prepare solutions of different concentrations ranging from M^{-1} to M^{-5}. Experimental design was planned as per internationally accepted protocol [9].

Methods

The pink brown dry outer scales and some of the brownish bottom plate of each bulb were removed carefully leaving root primordial intact. For each concentration of test compound i.e. $CdCl_2$, a series of 5 test tubes were arranged in a test tube rack. Five series of the test tubes were filled with the different molar concentrations (10^{-1} to 10^{-5} M) of solutions of $CdCl_2$ in tap water (Gr II). Five tubes were filled with only pure tap water and maintained to provide control (Gr I). 5 tubes were filled with five concentration of cadmium chloride solution as in Gr II but having amla in it at 0.10 mg/ml concentration (Gr III). Each descaled onion was placed on the top of each tube with root primordial downward in the liquid. After 24 hours test

suspension in (Gr III) and test solutions in (Gr II) and tap water in (Gr I) were changed. Change of liquid was repeated after 48 hours. After 72 hours length of the 05 root bundles in each series of each onion was measured using a ruler. Mean length of roots for each series was calculated and recorded for further statistical analysis. Morphology (shape and colour) of root tips were also recorded after 72 hours. Squashing of Root tips was done in 2% acetocarmine (BDH) + 1N HCl (9:1 v/v) after gently warming.

Statistics

Student's t-test (at 5% level of significance) of the data was performed with SPSS. The statistical analysis presented in Table 1 indicates significant variation (P = 0.05) in mitotic cells comparing the number of normal and aberrant cells at each concentration with the control sample.

RESULTS

The effects on the mitotic index and the frequency of the mitotic phase are given in Table 1. Cadmium chloride caused a decrease in mitotic index at all concentrations. When the phase frequencies were examined, it was observed that cadmium inhibited mitosis and also blocks it at the metaphase. This indicates that drug could partially prevent Cadmium-induced mitodepression at 10^{-4} M and 10^{-5} M. Cadmium affected the spindle and decreased anaphase and telophase stages while the metaphase stage was increased. In addition, in most cases the percentages of abnormal mitotic phases were seen to increase with increasing concentration (Table 1).

Table 1.Mitotic Index (MI) of *Allium cepa* root tip cells following 48 hrs exposure in $CdCl_2$ alone or in combination with amla (mean ± SEM).

S.No	Concentration	Group of onion bulbs		
	Molarity	Group I Control	Group II $CdCl_2$ exposed	Group III $CdCl_2$ + Amla
1	Control	36.92 ± 1.07		
2	10^{-5}		19.12 ± 0.69	23.57 ± 0.50
3	10^{-4}		17.76± 1.15	22.63 ± 1.21
4	10^{-3}		15.93 ± 0.14	16.51 ± 1.02
5	10^{-2}		(-)	(-)
6	10^{-1}		(-)	(-)

(Statistically significant on based on t-test at 5% level of significance.)

All test concentrations of cadmium chloride (except at 10^{-1} M and 10^{-2} M where roots did not grew at all) caused significant inhibition in the growth of roots (Gr. II) in comparison to controls (Gr. I). A comparison between Gr. II and Gr. III ($CdCl_2$ + amla) revealed that amla could partially check cadmium induced root growth inhibition at 10^{-4} M and 10^{-5} M as mean values could not reach up to controls MRL value (Table-2).

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Table 2.Mean root length (MRL as mm) of *Allium cepa* after 72 hr exposure in different concentrations of $CdCl_2$ alone or in combination with amla (mean ± SEM).

S.No	Concentration	Group of onion bulbs		
	Molarity	Group I Control	Group II $CdCl_2$ exposed	Group III $CdCl_2$ + Amla
1	Control	65.66 ± 1.02		
2	10^{-5}		49.33 ± 0.91	55.22 ± 1.16
3	10^{-4}		16.21± 1.62	23.34 ± 2.41
4	10^{-3}		3.13 ± 1.14	4.85 ± 1.02
5	10^{-2}		(-)	(-)
6	10^{-1}		(-)	(-)

(Statistically significant on based on t-test at 5% level of significance)

Morphology i.e. colour and shape of *Allium cepa* root tips exposed in all test concentrations of cadmium chloride alone (Gr. II) or cadmium chloride plus amla (Gr. III) did not reveal any change from controls (Gr. I). (Table-3)

Table 3.Morphology of *Allium cepa* root tip following 72 hrs. exposure in $CdCl_2$ alone or in combination with amla.

S.No	Group	Colour of root tip			Shape of root tip			
		Normal	Abnormal		Normal	Abnormal		
		White	Pale	Dark brown/Black	Straight	Bulb	Broken tips	Crochet hooks
	Group I Control	Yes			Yes	No	No	No
	Group II $CdCl_2$							
1	10^{-5}	Yes	No	No	Yes	No	No	No
2	10^{-4}	Yes	No	No	Yes	No	No	No
3	10^{-3}	Yes	No	No	Yes	No	No	No
4	10^{-2}	(-)	(-)	(-)	(-)	(-)	(-)	(-)
5	10^{-1}	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Group III $CdCl_2$ + Amla							
1	10^{-5}	Yes	No	No	Yes	No	No	No
2	10^{-4}	Yes	No	No	Yes	No	No	No
3	10^{-3}	Yes	No	No	Yes	No	No	No
4	10^{-2}	(-)	(-)	(-)	(-)	(-)	(-)	(-)
5	10^{-1}	(-)	(-)	(-)	(-)	(-)	(-)	(-)

In controls no abnormal mitosis or chromosomal aberrations could be observed however, cultivation of *Allium* bulbs at 10^{-3} M to 10^{-5} M cadmium chloride caused chromosome stickiness and scattered chromosomes at metaphase and chromosome fragmentation at anaphase at 10^{-3} M to 10^{-5} M (Gr. II). All these effects were found fully

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prevented at 10^{-5} M and significantly less pronounced in the presence of amla at 10^{-4} M but drug could not act at 10^{-3} M (Gr. III). (Table-4).

Table 4.Cytological effects of *Allium cepa* root tip cells following 48 hr exposure in different concentrations of cadmium chloride alone or in combination with amla (mean shown as percentage, 2000 cells observed in each group).

S.No	Group	Treatments	Observed stages of mitosis					
			Metaphase		Anaphase			
			N	SC and STC	N	FRG	MPA	CB
1	Gr I	Control (Tap water)	100	-	100	-	-	-
2	Gr I	10^{-5} $CdCl_2$ exposed	84.33 ± 0.26	10.16 ± 1.03	91.23 ± 1.03	9.36 ± 1.03	-	-
	GR II	10^{-5} $CdCl_2$ exposed + Amla	91.03 ± 1.24	2.16 ± 2.03	97.40 ± 1.15	1.13 ± 2.03	-	-
3	Gr I	10^{-4} $CdCl_2$ exposed	81.52 ± 1.52	21.13 ± 1.23	84.45 ± 1.42	16.23 ± 1.46	-	12.16±1.86
	Gr II	10^{-4} $CdCl_2$ exposed + Amla	92.16 ± 1.72	6.11 ± 1.22	93.11 ± 1.06	4.33 ± 1.75	-	-
4	Gr I	10^{-3} $CdCl_2$ exposed	75.52 ± 1.09	28.85 ± 1.85	86.63 ± 1.56	16.15 ± 1.52	-	9.65±2.12
	Gr II	10^{-3} $CdCl_2$ exposed + Amla	76.32 ± 1.22	27.13 ± 1.11	85.12 ± 1.86	15.13 ± 1.46	-	-
5	Gr I	10^{-2} $CdCl_2$ exposed	NG	-	-	-	-	-
	Gr II	10^{-2} $CdCl_2$ exposed + Amla	NG	-	-	-	-	-
6	Gr I	10^{-1} $CdCl_2$ exposed	NG	-	-	-	-	-
	Gr II	10^{-1} $CdCl_2$ exposed + Amla	NG	-	-	-	-	-

NG = No Growth
STC = Scattered Chromosome
FRG = Fragmented Chromosome
STC = Prokaryotes
(-) = Nil
MPA = Multipolar Anaphase
SC = Sticky Chromosome
CB = Chromosome Bridge

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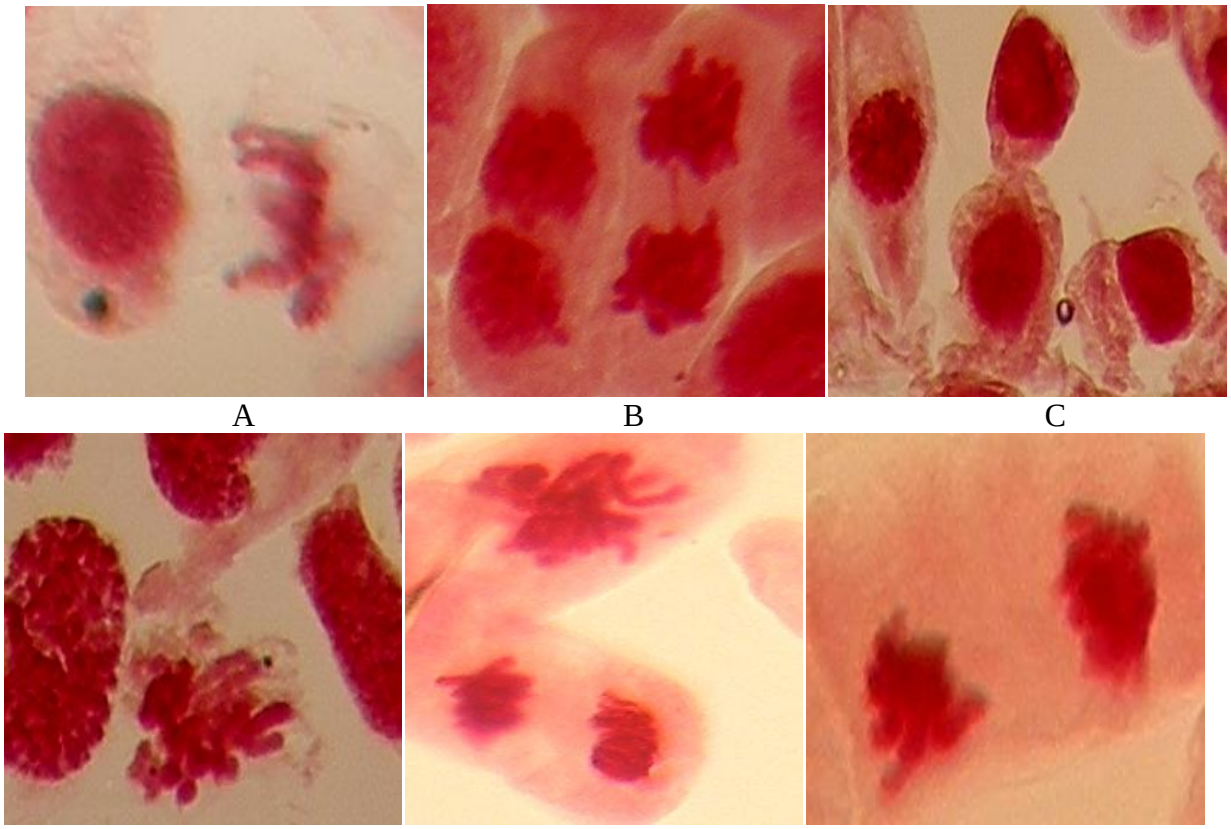

Figure 1 – Chromosomal aberration induced by cadmium chloride in root tip of *Allium cepa*.
a. Sticky chromosome. b. Chromatin Bridge. c. Ruptured cells. d. Fragmented chromosome
e. Condensed chromosome. f. Sticky chromosome and condensed chromosome.

DISCUSSION

The different cytotoxic effects of various $CdCl_2$ treatments or mitotic division in root tip cells of *Allium cepa* showed a higher degree of chromosomal aberrations. Earliest studies also showed cadmium induced cytogenetic effects, such as c-mitosis, chromosome fragmentations, laggard chromosomes, low mitotic index etc in *Allium cepa* bulb root and seed roots. [10-11]. In our present study we also observed the same type of chromosomal abnormalities. From table 1 it was observed that the same type of chromosomal abnormalities. The mitotic index decrease with increasing concentration of $CdCl_2$. Similar results were obtained after treating *Allium* root cells with insecticides, herbicides and chemical mutagens [12-15]. Such decrease in mitotic index could be due to inhibition of DNA replication [16]. Hence, it can be suggested that $Cdcl_2$ interferes with DNA replication. A study on *Terminaliachebula* showed that $CdCl_2$ lowered cell population at G0/G1 and G2/M stages [17]. Cadmium modulates signal transduction pathway and also affects both transcription and translation [18]. The above effects of $CdCl_2$ can be held responsible for mitodepression in *Allium* root tips cells as observed in our present study.

It has been observed from table 2 that complete inhibition of root growth was noticed at 10^{-1}M and 10^{-2}M concentration of $CdCl_2$ and this inhibition might be due to death of root primordial cells in G0 stage of cell cycle. This explanation was confirmed from a study with *Allium sativum* that had shown cadmium induced disintegration of organelles and cell death.

In *Viciafaba* an increase in antioxidant stress enzymes (Super oxide dismutase, glutathione reductase and catalase), in response to cadmium was evident for enhanced detoxification towards reactive oxygen species. Also, micronuclei induction was interpreted as a result of oxidative stress and authors were assumed that cadmium-induced damage up to certain extent was via generation of ROS i.e. reactive oxygen species [19].

Pluchealanceolata could reduce Cd-induced oxidative stress and genotoxicity in mice [20]. It is likely that cadmium-induced peroxidative damage declined mitosis in *Allium* root tip cells but if amla possesses antioxidant properties it can reduce Cd-toxicity. In fact amla has been shown to contain antioxidant and free radical scavenging activities [21-23].

Individual plant components like sulfhydryl and flavonoid compounds, gallic acid, ellagic acid, mucic acid, citric acid, reducing sugars and tannins can modulate effect of many genotoxicant [24]. Amla possesses many of such compounds [25] especially flavonoids which are ideal antioxidants [26] hence can be held responsible for reducing Cd-genotoxicity in *Allium* root cells. Infact polyphenols (tannins, gallic acid and tannic acid) were found to detoxify cadmium toxicity in water lily [27].Hence; the presence of some phytochelatin in amla can also contribute towards antagonizing Cadmium-toxicity [28].

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REFERENCES

[1] Sanitá di Toppi L, Gabbrielli R. Environ Exp Bot 1999; 41:105–130.
[2] Fogu G, Congiu AM, Campus PM, Ladu R, Sanna R, Sini MC and Soro G. Ann chim 2000; 90(11-12): 709-714.
[3] Rozgaj R,Kasuba V and Fucic AJ. Trace Ele Med Biol 2002; 16(3): 187-192.
[4] Lutzen A, Liberti SE and Rasmussen LJ.BiochemBiophys Res Commun 2004; 321(1): 21-25.
[5] Samiullah Khan and N Nazar Khan. Plant and Soil 1983; 387-394.
[6] Nandi S. Cytologia 1985; 50: 921-926.
[7] Lerda D. Mutat Res 1992; 281: 89-92.
[8] Inceer H, Beyazoglu O. Turk J Biol 2000; 24: 553-559.
[9] FiskesjoGeirid. Humana Press Inc. Totowa NJ 1995; 43: 119-127.
[10] Dovgaliuk AI, Kaliniak TB and BliumIaB. Tsitol Genet 2001; 35(2): 3-10.

[11] Evseeva TI, Geras'kin SA and Khramova ES. Tsitologiia 2001; 43(8): 803-808.
[12] Ajay KJ, Sarbhoy RK. Cytologia 1987; 52: 47-53.
[13] Rao BV, Sharma CBSR,Rao BGS. Cytologia 1987; 52: 365-371.
[14] Shanker R, Chauhan LKS, PrahladKS.Cytologia 1987; 52: 895- 899.
[15] El-Khodary S, Habib A, Haliem A. Cytologia 1989; 54: 465-472.
[16] Beu SL, Schwarz OJ, Hughes KW. Can J Genet Cytol 1976; 18(1): 93-99.
[17] Yew Cheng Huae, Lin Tachen, Yu Kuohua. Biol Pharm Bull 2003; 26: 1331-1335.
[18] Waisberg M, Joseph P, Hale B and Beyersmann D. Toxicology 2003; 192(2-3): 95-117.
[19] Rosa EV, Valgas C, Souza-Sierra MM, Correa AX and Radetski CM. Environ ToxicolChem 2003; 22(3): 645-649.
[20] Jahangir T, Khan TH, Prasad L and Sultana SJ. Pharm Pharmacol 2005; 57(9): 1199-1204.
[21] Yew Cheng Huae, Lin Tachen, Yu Kuohua. Biol Pharm Bull 2003; 26: 1331-1335.
[22] Fu Naiwu, Lanping Q, Huang L. Chinese Traditional and Herbal Drugs 1992; 23(1): 26-29.
[23] NaikGH, Priyadarshini KI, Naik DB, Gangabhagirathi R and Mohan H. Phytomedicine 2004; 11(6): 530-538.
[24] Sarkar D and Sharma A. Bot Rev 1996; 6:275-300.
[25] The Wealth of India. Raw Materials Vol. X; S-W, New Delhi: PID-CSIR 1960, pp. 171-177.
[26] Blokhina Olga, VirolainenEija, Fragersted Kurt V. Annals of Botany 2003; 91: 179-194.
[27] Lavid N, Schwartz A, Yarden O and Tel-Or E. Planta 2001; 212(3): 323-331.
[28] Cobett S Christopher. Plant Physiol 2000; 123: 825-832.