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CYTOGENETIC EFFECT OF LEAD ACETATE EXPOSURE ON ROOT TIP CELLS OF *ALLIUM CEPA* L.

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Abstract

Environmental problems are one of the most important dangers threatening to human and animal health and the ecological balance. Lead (Pb) exists in many forms in natural sources throughout the world. The cytotoxic effect of lead acetate (10, 50 and 100 mgl^{-1}) was investigated in the toot tip cells of *Allium cepa* L. lead acetate causes a dose dependent decrease in the growth of the root tips when compared to the control group. At low doses lead found to interfere with the normal mitotic processes of the cells. Lead acetate is found to cause a dose dependent decrease of mitotic index (1.77 ± 1.64, 3.33 ± 1.52 and 4 ± 4.35) in the root tip of cells of *Allium cepa* L. when compared to the control groups (29 ± 1.73). An increase of cytoplasmic vacuolation was observed after 96 hours of treatment with 10 and 50 mgl^{-1} lead acetate, whereas a significant amount of cell death was observed in the high dose group (100 mgl^{-1}). A spectrum of morphological abnormalities such as bridge chromosome, improper metaphase, vacuolation and pyncnotic nuclei were observed in root meristems after exposure to lead acetate. The present study illustrated the toxic effect of lead acetate exposure in the root tip cells of *Allium cepa* L.

Key words: *Lead acetate, Allium cepa L., Cytotoxic, Chromosomal aberration*.

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INTRODUCTION

Environmental problems are one of the most important dangers threatening to human and animal health and the ecological balance. To meet the increasing food demands in relation to the rise in the population of the world and to enhance the quality of life brings about many environmental problems. Heavy metal deposition has increased dramatically over the last decades as a result of anthropogenic activities leading to heavily polluted areas worldwide. Anthropogenic activities have concentrated some of these metal elements in certain areas up to dangerous level for living organisms [1]. Activities such as mining and agriculture have polluted extensive areas throughout the world [2, 3].

Lead, the fifth most abundant metal in the earth's crust, is known to man since the beginning of the civilization. Due to its versatile properties like ductility, high resistance to erosion and corrosion, lead has been used by man since centuries for making tools, pots, statues, water pipes and other hardware. As a consequence, today it has become one of the most widely distributed pollutant in environment. Lead is non biodegradable and therefore persists in soil, air, drinking water and in homes. According to the USA Environmental Protection Agency, lead is one of the most common heavy metal contaminants in aquatic and terrestrial ecosystems and can have adverse effect on growth and metabolism of plants due to direct release into atmosphere [4]. There has been many reports of lead toxicity in plants [5] including disturbance and toxicity of mitosis and nuclei [6, 7, 8, 9], inhibition of root shoot growth [10], induction of leaf chlorosis [11], reduction in photosynthesis [12] and inhibition and activation of enzymatic activities [10, 13, 14]. It is well known that the root are the main route through which lead enters plants [15] and about 90% of lead is accumulated in roots of some plants [16]. Most lead in roots is localized in the insoluble fraction of cell walls and nuclei, which is connected with the detoxification mechanism of lead [15].

The use of short term bioassays, especially genetic toxicity bioassays, to assess potent environmental pollutants has gained special attention over the last decades. These assays are capable of predicting the genotoxic potential of the pollutant under investigation by measuring gene mutations and damage

to chromosomes and DNA. Plant assays are quite easy to conduct, inexpensive, rapid and good predicators of genotoxicity [17]. It is known that *Allium cepa* L. is favorable material in the chromosomal aberration tests in the environmental pollution of the meristematic cells. Dovgalyuk *et al*. (2001) comparatively investigated the cytogenetic effects caused by metal salts such as lead, zinc, aluminium, copper, nickel and cadmium on apical meristems cells of *Allium cepa* L [18].
During present study the cytogenetic effect of lead acetate exposure on the root tip cells of *Allium cepa* L. has been investigated.

MATERIALS AND METHODS

Chemical and plant material

Lead acetate trihydrate has been purchased from the Sigma-Aldrich. Good quality and free from any external injury *Allium cepa* L. bulb (n=60) has been purchased from the local market.

Experimental design

Different concentrations of lead acetate (0, 10, 50 and 100 mgl^{-1}) were prepared in tap water. *Allium cepa* L. bulb (n=15) were then cultured in wide mouthed bottle for five days. Three bulbs from each treatment group were then used for analysis of the root length, mitotic index and abnormal mitosis. The roots were cut and length were measured with standard scale and then fixed in Carnoy's fixative (Aceto-methanol, 3 part methanol: 1 part acetic acid) for 24 hours. The root tips were then passed through 100%, 90% and 70% alcohol and preserved in 70% absolute alcohol for mitotic index analysis. The root tips were then passed through 50% and 30% alcohol up to distilled water. The apical root tips were separated and stained in 2% aceto-carmine and squashed under a cover slip. Abnormal mitosis were recorded and photographed. Mitotic index were calculated as follows:

Mitotic index (MI) = No. of cell in mitotic stages/ total no. of cells counted X 100

Statistical analysis:

Data were presented as mean ± SD. Statistical significance between different groups were analyzed by t-test and a p□0.05 were considered as significant.

RESULTS AND DISCUSSION

The result of the present investigation clearly demonstrated that exposure to lead acetate causes a wide range of cytogenetical abnormalities in the root tip cells of *Allium cepa* L.
The exposure to lead acetate causes a significant delay in the growth of the root tips of *Allium cepa* L. as observed during present study (Fig. 1). The growths of root tips were not different between control and treated group after 48 and 72 hours of lead acetate exposure. However after 96 and 120 hours of exposure root growth of root was completely inhibited by lead acetate and it was significantly different from the control group (Fig. 1). Similar growth inhibitory activity of lead acetate was reported earlier [19].
Table 1 represents the mitotic index and percentage of abnormal mitosis in the root tip cells of *Allium cepa* L. were grown under different the concentration of lead acetate. Mitotic index is a measure for the proliferation status of a cell population. It is define as the ratio between the numbers of cells in mitosis and the total number of cells. The mitotic index is a well known parameter that reflects the frequency of the cell division and thus offers an indirect assessment of growth of cells or tissues. The present study reflects the exposure of the root tip cells to lead acetate causes a progressive decrease in the mitotic index as well as increases in the percentage of abnormal mitotic cells with increased lead concentration (Table 1). This is also reflects in the growth rate of the root tips exposed to lead acetate when compare to the control untreated groups [20].

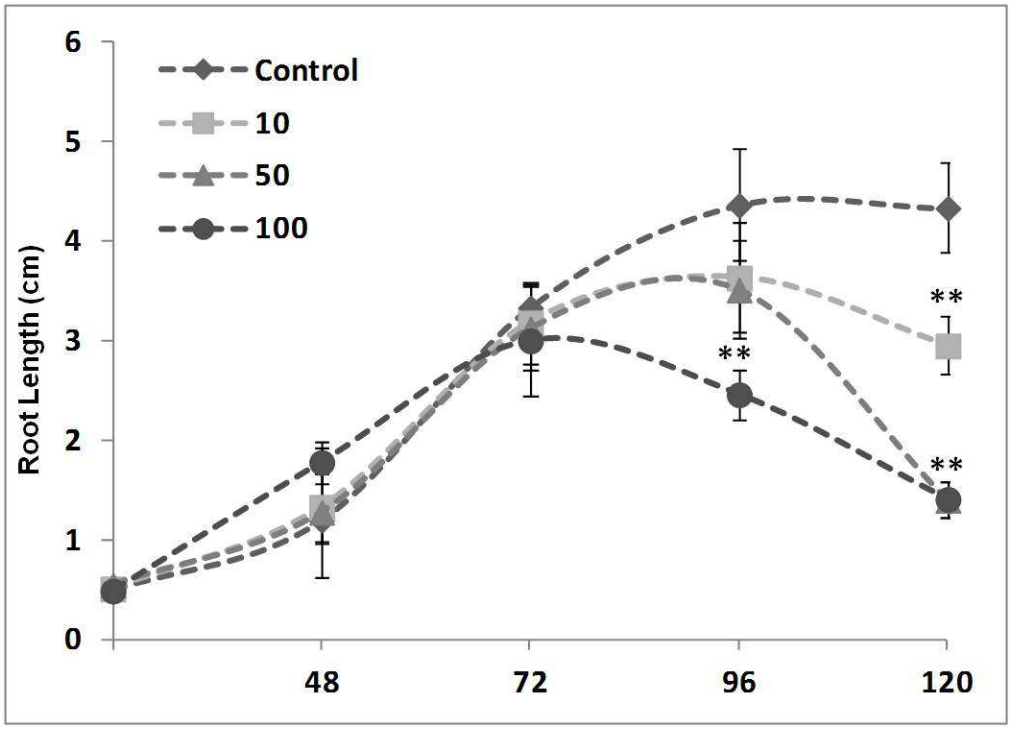

Figure 1: Effect of Lead acetate exposure on the growth of the root tips in *Allium cepa* L. at 48, 72, 96 and 120 hrs.

The cytomorphological study indicated the adverse effect of chemical on the root tip cells compared to the control untreated group (Fig. 2). At low doses of lead acetate causes the appearance of many chromosomal abnormalities (Fig. 2 A and B) and this supported by earlier reports also [6]. Lead causes development of extensive vacuoles in the root tip cells growing in 100 gml^{-1} (Fig. 2 C). The vacuoles of root tip cells are the major sites of metal sequestration under chronic exposure scenario and it is thought as a detoxification pathway for preventing cell damage and retaining metal in specific vacuoles [21]. Immobilization of metal in the cell wall and detoxification of its cytoplasmic pool by sequestration in vacuoles are important processes influencing plants tolerance to lead; both processes protect the cell metabolism from its toxic effects [22]. Up to 96% of the metal taken by plants can be accumulated in these cellular compartments [23]. The root tip cells of *Allium cepa* L. high dose exposed group after 120 hours exhibited nuclear pycnosis and condensation of the chromatin material, indicating apoptotic death of root tips cells (Fig 2 D). Similar finding of apoptotic death of root tips cells after aluminium exposure was reported in barley [24].
The result of the present study clearly defines the mitosis inhibitory and growth inhibitory activities of lead acetate on *Allium cepa* L. root tip cells.

Table 1: Mitotic index of abnormal mitosis in the root tip cells of *Allium cepa* L. after 72, 76 and 120 hrs exposure of lead acetate. Values are expressed as mean ±SD of the observed values. *p□0.05 and **p□0.001.

Exposure time (Hrs)	Groups	Total cell counted	Cell in mitotic stage	Mitotic Index (%)	Abnormal mitosis (%)
72 hrs	**Control**	3456	1071.36	31 ± 7.34	0.21
	10 mgl^{-1}	4256	1149.12	27 ± 3.60	1.34
	50 mgl^{-1}	3876	542.64	14 ± 6.67*	3.76
	100 mgl^{-1}	5256	93.03	1.77 ± 1.64**	5.32
96 hrs	**Control**	3723	1166.41	31.33 ± 1.15	0.78
	10 mgl^{-1}	4256	737.56	17.33 ± 3.21	1.45
	50 mgl^{-1}	3217	214.25	6.66 ± 1.52*	2.87
	100 mgl^{-1}	2356	78.45	3.33 ± 1.52**	1.67
120 hrs	**Control**	2256	654.24	29 ± 1.73	0.67
	10 mgl^{-1}	3256	857.30	26.33 ± 10.06	2.17
	50 mgl^{-1}	1353	216.48	16 ± 5.56*	1.05
	100 mgl^{-1}	1785	71.4	4 ± 4.35**	0.76

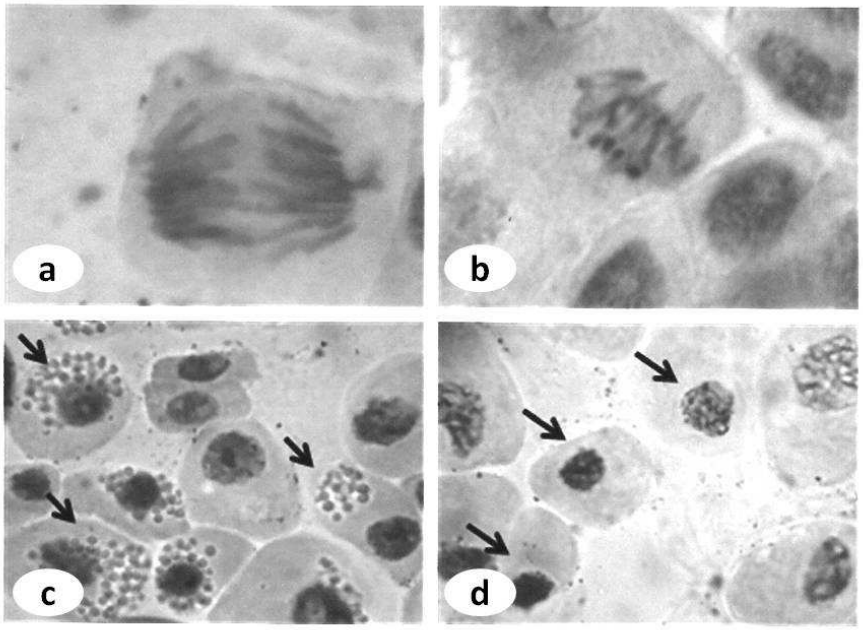

Figure 2: Effect of Lead acetate on the root tip cells of *Allium cepa* L. (A) Chromosome bridges. (B) Improper metaphase. (C) Vacuolation and (D) Nuclear condensation indication apoptotic death of root tip cells, aceto-orecin stain, a and b X100, while c and c X40 magnification

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