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The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(2): 13-15

www.thepharmajournal.com
Received: 12-02-2015
Accepted: 15-03-2015

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Use of plant test system for the evaluation of cytogenetic effect of cadmium

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Abstract
The cadmium effect on the mitotic cycle, frequency and spectrum of chromosomal aberrations in root tip cells of *Allium cepa* was investigated to establish the cytogenetic effects of cadmium on hereditary apparatus. A research object was a cadmium chloride ($CdCl_2$) in the concentration 0.5 µM. Cytological analysis of *Allium cepa* samples showed that cadmium chloride (0.5 µM) inhibited significantly the germination of *Allium cepa* roots as compared to control at 18%, $p<0.05$. Mitotic index (MI) in cadmium chloride solution was not significantly different as compared to control and showed the result in 5.53 %, ($p<0.05$). Analysis of cells ratio in the mitotic cycle stages showed that cadmium chloride inhibited the proliferative activity of root tip cells and changed the course of metabolic processes that were expressed in the formation of the mitotic apparatus violations. The number of chromosomal aberrations in a cadmium chloride solution increased in 5.16 times compared to the control.
Chromosome aberrations (damage of chromosome components and mitotic ap-paratus) caused by cadmium chloride were established by means of cytogenetic analy-sis.
So, cadmium chloride has the inhibitory effect on the mitotic cycle and on the growth of root tip cells of *Allium cepa*. Cadmium chloride has expressed phyto- and genotoxic activity. Cadmium chloride at concentration (0.5 µM) makes credible mitosis and cytokinesis violation in *Allium cepa* apical meristem. The frequency of chromosomal aberrations in cadmium chloride is significantly higher (12.54 ± 0.10)% than control (2.4 ± 0.14)%.

Keywords: *Allium cepa* test, mitotical index, chromosomal aberrations, cytotoxicity.

1. Introduction
Nowadays, the imbalance in the biosphere as a result of anthropogenic pollution of nature is a significant problem. During the last decades the level of pollution compounds of heavy metals, including cadmium has significantly increased. This can be explained by the fact that cadmium is used extensively in various industries and is accumulated in the objects of the environment [1, 2, 3, 4, 5, 6].
Therefore, the study of cyto- and genotoxicity of cadmium as the potential mutagen, which has the ability to show cumulative effects, to cause inflammation, to induce mutagenesis and carcinogenesis [3, 4, 5, 7] is an important issue today.
Allium cepa-test as recommended by international experts of WHO and UN can be used for installation of cadmium chloride cytogenetic effects. *Allium cepa*-test gives a possibility to study the general toxicity (or phytotoxicity) based on *Allium cepa* root growth inhibition. Cytotoxicity that is documented by microscopic studies of chromosomal aberrations and other mitotic anomalies in root tip cells of *Allium cepa* can be investigated due to the *Allium cepa*-test. By the Gene-Tox program result *Allium cepa*-test was considered as" test № 1", as valid, adequate and sufficient for prognostic studies on genotoxicity in different conditions (*in vivo*, in situ, *in vitro*) [8, 9, 10, 11].
The **aim** of our study was to establish the cytogenetic effects of cadmium chloride on hereditary apparatus.

2. Materials and Methods
The cadmium chloride ($CdCl_2$) at concentration 0.5 µM was the object of the study. Distilled water served as control. *Allium cepa* test is used to determine the cytogenetic activity of cadmium chloride. Onion seeds (aged 11 months) were seeded in Petri dishes with experimental and control samples of solutions in the amount of 200 seeds per cup. Seeds were germinated in an incubator at 21 °C. The selection of roots was performed in 48–72 hours, that are corresponding to the first and second mitotic cycle. The roots that grew to a length of 10 – 15 mm were taken for the study.
Fixation, painting and preparing of slides were carried out according to the standard technique

[12]. Mitotic activity was determined by a calculation of prophase, metaphase, anaphase, telophase, and mitotic index (MI). 7 - 10 slides for each sample were analyzed. Chromosomal aberrations were determined by analysis of 100 ana - and telophase on the slide, for 5 specimens per sample. Number of bridges, fragments, c-mitoses, gaps, vagrants and laggards was counted. Mutagenic effect was determined by the increase of the modified ana -, telophase number, the frequency of chromosomal aberrations. The method of variation statistic using specialized computer softwares STATISTICA for Windows 5.0., MSOffice Excel was applied for statistical processing of results. The statistical reliability of results was assessed by Student's *t*-tests.

3. Results and Discussion

During the seeds germination of *A. cepa* in cadmium chloride solution the length of the roots were analyzed primarily. This rate depends on the concentration of active substances [4], because it is based on the mitotic activity of the apical meristem cells. The results of studies of the roots growth rate are presented in table 1. Results showed that cadmium chloride (0.5 μM) inhibited the germination of *Allium cepa* roots in comparison to control at 18%, $p<0.05$, significantly.

Table 1: The effect of cadmium chloride (0.5 μM) to the intensity of the growth and division of root tip cells of *Allium cepa*

No	Object of investigation	Length, mm	Mitotic index, %
1	Control (distilled water)	9.80±0.11	5.90±0.19
2	$CdCl_2$	8.07±0.25*	5.53±0.14*

- * $p < 0.05$ *versus* control (distilled water).

After seeds germination of *Allium cepa* in the cadmium chloride solution, darkening of the tips roots was observed along with growth inhibition. Necrosis of apical meristem of cells may indicate it [13].

Roots growth is determined by division of the primary meristem and the intensity of cells metabolism in the elongation zone. So, study of cadmium chloride effect on mitotic index (MI) was the logical continuation of this work. Mitotic index (MI) in cadmium chloride solution was not significantly different compared to control and showed the result in 5.53%, ($p<0.05$). It represents a slight slowdown in the completion of mitosis compared with the control.

Despite a significant decrease in the roots length (18)%, $p <0.05$, the value of MI decreased unreliable (6.3) %, $p> 0.05$. It may indicate a slight slowdown of mitosis passing compared to control.

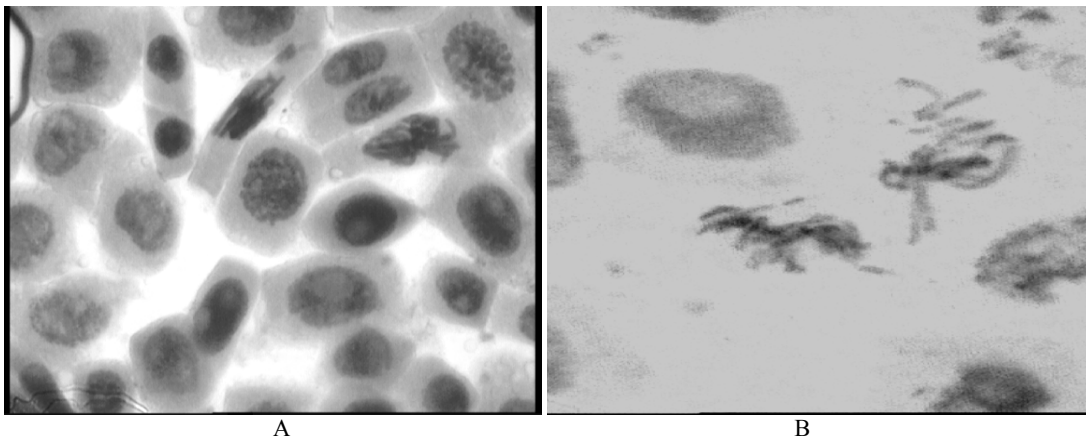
To study the mechanism of the cadmium chloride effect on the proliferative ability of root tip cells of *Allium cepa* the dynamic of mitotic phases were determined (Table. 2).

Table 2: The dynamics of mitotic phases of root tip cells of *Allium cepa* under the cadmium chloride influence

No	Object of investigation	Prophase index, %	Metaphase index, %	Anaphase index, %	Telophase index, %
1	Control (distilled water)	41.66±0.56	21.58±0.11	17.12±0.31	19.64±0.17
2	$CdCl_2$	42.17±0.45	26.10±0.03*	14.18±0.28*	17.55±0.19*

- * $p < 0.05$ *versus* control (distilled water).

The obtained values of mitotic index that are shown in table 2, allow to analyze the cells ratio in the different mitotic phases. Prophase index increases slightly in comparison with the control. It contributes to the accumulation of a small amount of prophase cells. It may be evidence of the metaphase block formation, which is associated with the spindle formation. Proliferative activity inhibition of root tip cells of *Allium cepa* under the $CdCl_2$ influence is confirmed by quantitative values of ana-, telophase indexes. In our opinion, cells distribution in mitotic phases is connected with the strong chromosomes condensation during cell division (Fig. 1 a, b), which determines the impossibility of a normal course of mitosis.



A B

Fig 1: Strengthening of chromosomes spiralization of root tip cells of *Allium cepa* in varies mitotic phases under the $CdCl_2$ influence: a) prophase, b) metaphase. Magnification 600^x

Studies of chromosomal aberrations in root tip cells of *Allium cepa* gave the opportunity to evaluate the cytogenetic effects of cadmium chloride. An increase of aberrant cells in 5.16 times, compared with the control was registered on the

microslides of roots, germinated in cadmium chloride solution (Table. 3).

Table 3: Effect of cadmium chloride on the frequency and range of chromatine and chromosomal aberrations in root tip cells of *Allium cepa*

Object of investigation	Abberation number, %	Chromatide abberation, %		Chromosome abberation, %		c-mitose, %	Lag-gings %
		bridges	frag-ments	bridges	frag-ments		
Control (distilled water)	2.4±0.14	2.43	-	-	-	-	-
$CdCl_2$	12.54±0.10*	4.68	1.67	2.55		2.01	1.63

- * $p < 0.05$ *versus* control (distilled water).
"-" - chromosome aberrations of this type were not induced.

To assess the extent of violations of the mitotic cycle the spectrum of chromosomal aberrations was analysed. Among the numerous pathologies caused by cadmium chloride, bridges, single and paired fragments (chromatine and chromosome aberrations) were registered. Such anomalies are explained by the fact that heavy metals induced the damages of different chromosomes components. Other pathologies (laggins, c-mitosis), connected with the damages of mitotic apparatus are also identified. According to published data [14], laggings and vagrant chromosomes show that specific test compound can damage the cell cytoskeleton. It is indicated by the metaphase block too.

So, the results, proposed in this paper, indicate the possibility of using *Allium cepa* as a common highly sensitive test system for establishment of cyto- and genotoxic effects of different substances in preclinical toxicological characteristics.

4. Conclusions

1. The effect of cadmium chloride on mitotic activity, the state of the hereditary apparatus in root tip cells of *Allium cepa* was established.
2. It is proved, that the cadmium chloride inhibited significantly the cell growth of test object compared with the control.
3. The inhibitory effect of cadmium chloride on the mitotic cycle of root tip cells of *Allium cepa* was established.
4. Cadmium chloride has expressed phyto - and genotoxic activity. Cadmium chloride at concentration 0.5 μM makes credible mitosis and cytokinesis violation in *Allium cepa* apical meristem. The frequency of chromosomal aberrations in cadmium chloride is significantly higher (12.54 ± 0.10) % than control (2.4 ± 0.14) %.

Prospects for further research in this direction – to study the molecular-genetic mechanisms of cytotoxic action of metal ions, comparative characteristic of chromosomal aberrations frequency and number of aberrations per aberrant cell.

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