Studies on the reversal of lead induced mitostatic effect in Allium Cepa root tip cells with myrobalan (fruit of Terminalia Chebula, Retz, Combretaceae)

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Citation

Abstract
The present study was undertaken to find the protective role of myrobalan against lead (Pb) induced cytogenetic effects on mitosis in Allium-cepa root tip cells. Onions were initially cultivated in deionized water for 3 days and were then exposed at 100, 1000, 3000 and 10,000 ppm of lead nitrate solution for 1 hr. After exposure to lead, the onions were allowed to recover naturally or in myrobalan suspension (0.01 mg/ml) for 72 hrs. The root color, mean root length (MRL) and mitotic index (MI) were recorded and the metaphases and anaphases were scored for chromosomal aberrations. During the natural recovery (NR), roots did not grow following 1000, 3000 and 10,000 ppm Pb exposure but myrobalan treated drug recovery (DR) showed root growth following 1000 and 3000 ppm Pb exposure. The root growth was observed in both NR and DR, more in DR in 100 ppm Pb exposure. Among the control, root growth during these periods and MI did not change throughout the experimentation. Pb exposure at all concentrations, lowered MI. NR was ineffective in Pb treated root tip cells as these were seen in interphase with hypertrophied nucleoli showing mitostatic effect. DR could not revert the mitostatic effect in root tip cells exposed at 10,000 ppm Pb, however, drug could do so in 100, 1000 ppm and 3000 ppm Pb exposure. Both, NR and DR reverted mitostatic effect after 100 ppm Pb exposure and the effect was observed earlier in DR. No chromosomal aberrations could be seen at 1 hr, the cells failed to show typical metaphase arrangement. The effect appeared dose dependent. DR reverted mitostatic effect from 1 hr onwards and completed at 72 hrs in 1000 and 3000 ppm Pb exposure. At 100 ppm Pb exposure, mitostatic effect disappeared at 48 hr in NR while in DR, it reverted after 24 hr. Control root tip cells showed no mitostatic effect.

INTRODUCTION
Lead is known to be toxic, mutagenic and carcinogenic in human beings [1]. The modern system of medicine uses chelation therapy to cure Pb toxicity [1]. However, Ayurveda (Indian herbal medicine system) in Pb toxicity suggest the use of myrobalan (fruit of Terminalia chebula) [1]. Our earlier study, showed the protective role of myrobalan towards Pb toxicity in mice [1]. The present study, was undertaken to find out whether myrobalan can lower the cytogenetic effects of inorganic Pb in Allium test. The short term Pb exposure for 1 hr was used as Pb is known to disturb mitosis in Allium root tip cells [1][2][3][4]

MATERIALS AND METHODS
ALLUM CEPA
Dry healthy onion bulbs 1.5-2.0 cm in diameter were obtained from the local market.

DRUG
Myrobalan, dried young nuts of Terminalia chebula were procured locally, gently baked for few minutes and cooled. The swollen nuts were grinded to a fine powder. The recommended dose of myrobalan for adults is 3-9 gm/day [1]. However, low dose (0.01 mg/ml) of myrobalan was used in the present study to test the recovery of the mitostatic effects of Pb in Allium test.

Lead nitrate (Hi Media A.R) was dissolved in deionized water to prepare solutions of 100, 1000, 3000 and 10,000 ppm concentrations.

CULTIVATION OF ONIONS
Descaled healthy onions (Allium cepa L.) bulbs (90) were grown initially in deionized water for 3 days. Mean root length (MRL) was taken as baseline and were divided into 5 groups of 18 bulbs in each group. The onion bulbs with growing roots were exposed to Pb solution at 100, 1000,
3000 and 10,000 ppm (group 2-5) and in deionized water as control (group 1) for 1 hr. After Pb exposure few of the root tips from each group were fixed for cytogenetic study.

After Pb exposure to onion bulbs for 1 hr, each group was subdivided into two and was allowed to recover naturally (NR) or in the presence of the drug (DR). Roots were exposed to myrobalan at a concentration of 0.01 mg/ml. The recovery in NR and DR was allowed for the next 72 hr. MRL was recorded at 24, 48 and 72 hr of myrobalan treatment. Five root tips from each onion were cut and fixed in acetoalcohol (1:3 v/v) stained in N-HCl-acetocarmine (1.9 v/v) and squashed in 45% acetic acid. The squashed preparations were stored in a refrigerator. The mitotic index and the movement and arrangement of chromosomes at metaphase and anaphase after 24, 48 and 72 hr was observed.

The statistical analysis was performed by student t-test and values less than 5% were considered significant.

RESULTS

No morphological i.e., shape and color changes were noticed in the tips of roots of any group of bulbs. The root tips under NR for 72 hr did not grow following 1000, 3000 and 10,000 ppm Pb exposure for 1 hr (Table 1). DR for 72 hr also remained ineffective following 1 hr Pb exposure at 10,000 ppm, however, roots grew following Pb exposure at 1000 and 3000 ppm. The roots grew in both, NR and DR, more in DR following 1 hr Pb exposure at 100 ppm. The control roots grew during the corresponding recovery period.

Among the control root tips, MI did not change throughout the experiment (Table 2). Pb exposure for 1 hr to 1000, 3000 and 10,000 ppm Pb exposure as cells of root tips appeared in interphase with hypertrophied nucleoli and did not resume mitotic course. DR could not revert mitostatic effect of lead at 10,000 ppm Pb exposure, however it was able to do so in roots expose to 1000 and 3000 ppm Pb during the 72 hr period of recovery.

Following, 100 ppm Pb exposure, both NR and DR could overcome the mitostatic effect, more earlier in DR. Control root tips revealed no any mitostatic effect.

The observation of metaphases and anaphases after 1 hr exposure to growing roots indicates probable interference during transition of cells from prophase into metaphase (Table 3). The effect appeared dose dependent as following 100, 1000, 3000 and 10,000 ppm Pb exposure, 51%, 55%, 67% and 88% of the cells failed to show typical metaphase arrangement. No aberrations were noticed. Control root tips did not revealed any disturbed pro-metaphase transition.

**Table 1:** Mean root length (MRL) of onion bulbs grown following lead exposure at various concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>NR</th>
<th>DR</th>
<th>Control</th>
<th>NR</th>
<th>DR</th>
<th>Control</th>
<th>NR</th>
<th>DR</th>
<th>Control</th>
<th>NR</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL at 24 hr</td>
<td>56.39±0.98</td>
<td>56.79±0.54</td>
<td>62.49±0.19</td>
<td>62.83±0.19</td>
<td>64.70±0.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) change</td>
<td>32.2%</td>
<td>3.2%</td>
<td>20.2%</td>
<td>27.5%</td>
<td>26.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRL at 48 hr</td>
<td>56.39±0.60</td>
<td>59.22±0.45</td>
<td>56.09±0.60</td>
<td>51.62±0.32</td>
<td>52.83±0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) change</td>
<td>41.50%</td>
<td>8.2%</td>
<td>3.2%</td>
<td>27.5%</td>
<td>14.35%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRL at 72 hr</td>
<td>56.39±0.60</td>
<td>59.22±0.59</td>
<td>56.31±0.23</td>
<td>62.39±0.40</td>
<td>58.10±0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) change</td>
<td>53.95%</td>
<td>12.8%</td>
<td>4.9%</td>
<td>36.50%</td>
<td>35.15%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Root tips were initially grown in deionized water for 3 days and were then exposed at 0, 100, 1000, 3000 and 10,000 ppm lead nitrate for 1 hr. After lead exposure the root tips were washed with deionized water and were allowed to recover naturally (NR) or in presence of drug (DR) for 24, 48 and 72 hr. MRL is given in mm.

Values are shown as mean ± SEM of 20 roots in each group.

p > 0.05 a,b,c. MRL at 24, 48 and 72 hr vs MRL at 3 days of initial growth. d,e MRL at 48 and 72 hr vs 24 hr. (—) is nil.
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Figure 2
Table 2: Mitotic Index (MI) of onion roots following lead exposure at various concentrations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Groups</th>
<th>MI</th>
<th>% change</th>
<th>MI</th>
<th>% change</th>
<th>MI</th>
<th>% change</th>
<th>MI</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MI (at 3 hr)</td>
<td>1</td>
<td>48.56 ± 1.30</td>
<td>23.44 ± 0.48</td>
<td>22.69 ± 0.40</td>
<td>21.64 ± 1.30</td>
<td>15.64 ± 1.30</td>
<td>1.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>48.41 ± 1.30</td>
<td>25.05 ± 1.05</td>
<td>23.44 ± 0.22</td>
<td>22.77 ± 0.78</td>
<td>15.64 ± 0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>48.44 ± 1.30</td>
<td>25.05 ± 1.05</td>
<td>23.44 ± 0.22</td>
<td>22.77 ± 0.78</td>
<td>15.64 ± 0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>48.44 ± 1.30</td>
<td>25.05 ± 1.05</td>
<td>23.44 ± 0.22</td>
<td>22.77 ± 0.78</td>
<td>15.64 ± 0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>48.44 ± 1.30</td>
<td>25.05 ± 1.05</td>
<td>23.44 ± 0.22</td>
<td>22.77 ± 0.78</td>
<td>15.64 ± 0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

72 hr recovery period after 1 hr lead exposure

After 3 days of initial growth, MI was 48.97 ± 1.15.

p > 0.05. * MI after 1 hr of Pb exposure vs MI after 3 days of initial growth. b, c, d MI after 24, 48 and 72 hr of recovery vs MI after 1 hr Pb exposure.

e DR vs NR at 24 hr, 48 and 72 hr

f MI at 72 hr vs MI after 3 days of initial growth.

Figure 3
Table 3: Metaphases, anaphases and chromosomal aberrations following 1 hr lead exposures

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>MI</th>
<th>Aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>48 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>72 hr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After 3 days of initial growth, MI was 48.07 ± 1.15.

p > 0.05. * MI after 1 hr of Pb exposure vs MI after 3 days of initial growth. b, c, d MI after 24, 48 and 72 hr of recovery vs MI after 1 hr Pb exposure.

DR vs NR at 24 hr, 48 and 72 hr

MI at 72 hr vs MI after 3 days of initial growth.

DISCUSSION
In Allium test, the important effect exhibited by Pb at a lower concentration was mitodepression and with higher concentrations, Pb exerted inhibition of prophase and finally...
resulted in the disappearance of mitosis. The mitostatic effect did not revert to normal condition i.e., cells did not reentered into mitosis from G1 during NR except among 100 ppm Pb exposed roots, however, recovery with DR could remove the mitostatic effect following 1000 and 3000 ppm Pb exposed roots. The effect of 10,000 ppm Pb exposure was not reverted during drug or in natural recovery process.

The earlier workers observed inhibition of root growth accompanied with spindle disturbing effect along with chromosomal aberrations following Pb exposures [5,7]. In the present study, no chromosomal effects could be noticed. This may be due to the short Pb exposure time of 1 hr in the present study, while it was 24 hr or more in other studies. However, 1 hr exposure time was enough to find out early effects of Pb as within 1 hr Pb gets accumulated in the onion root cells [1]. A temporary effect of Pb i.e., incompletely organised metaphase were seen after 1 hr Pb exposure, however, no abnormal anaphase was observed.

Pb has been shown to have varied effects. Pb may inhibit DNA repair processes [14-15], denature proteins [16] and disturb the transcription process [17]. Pb has been shown to induce lipid peroxidation [18], alter membrane permeability [19] and bind to SH-groups of tubulins in cell, disturbing typical arrangement of chromosomes at metaphase [20]. Pb inhibits cell elongation, irrespective of mitotic activity due to changes in cell wall characteristics [21]. Pb has been shown to compete with calcium ions, altering certain signal transduction processes [22]. During the late G1 phase, restriction point gate opens in the presence of complex molecules at promoters for essential cell cycle genes [23]. It appears that Pb may interfere with some of these processes, resulting in the mitostatic effect in onion root top cells and myrobalan may be able to counteract some of these activities.

Pb is removed from the cytoplasm of the cells in the process of detoxification [1]. Root cell cortex wall is main storage site of lead [24]. The ability of plants to bind heavy metals in cell wall and external polysaccharides has been shown [25]. The detoxication takes place in the cytoplasm and cell wall within 12-24 hr, which may be responsible for the mitotic activity in root tips after low Pb intoxication.

Myrobalan may bind with the root tips as color changes were noticed from very pale to dark brown at higher concentration. It is suggested that myrobalan bind to Pb, making it unavailable, thereby reducing Pb toxicity. It is likely that Pb induced peroxidative damage may result in the mitostatic effect. Myrobalan has been shown to exert antioxidant and free radical scavenging activities [26-27], which may counteract the mitostatic effect of Pb.

The unrepaird DNA does not allow cells to go beyond G1 stage [28]. The Pb induced DNA damage may be corrected by myrobalan as it has been shown to exert antimutagenic activity in Salmonella typhimurium against direct acting mutagens such as sodium azide and 4 nitro-o-diphenylenediamine [29]. The antimutagenic activity of myrobalan was suggested to be due to hydrolysable tannins [30]. The various plant components present in the extracts such as sulphahydryl and flavonoid compounds, gallic acid, ellagic acid, mucic acid, citric acid, reducing sugars and tannins etc. can modulate effects of environmental genotoxictants [31]. As myrobalan possess many of these compounds, it may be able to reduce the cytotoxic effect of Pb.

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