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Prevention of Aluminium Chloride-Induced Mitodepression with Myrobalan (Fruit of *Terminalia chebula*, Retz, Combretaceae) in *Allium cepa* Model

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ABSTRACT

Allium cepa bulbs were grown in pure tap water (Group I), in five concentrations (10^{-1}M to 10^{-5}M) of aluminium chloride in the absence (Group II) and in the presence (Group III) of myrobalan (fruit of *Terminalia chebula*) at a fix concentration of 0.10 mg/ml. Parameters of study were mean root length (after 72 hr) and mitotic index, abnormal mistosis, chromosomal aberrations and nucleolar morphology (after 48 hr). $AlCl_3$ at all concentrations except at 10^{-1}M where roots did not grew at all, significantly lowered root growth and mitotic index, effect appeared concentration dependent (Group II). In the presence of myrobalan (Group III) $AlCl_3$ induced mitodepression could be checked significantly only at 10^{-4}M and 10^{-5}M. No morphological i.e. shape and colour changes, abnormal mitosis and any type of chromosomal aberrations could be detected in any group. $AlCl_3$ induced hypertrophy of nucleoli at 10^{-2}M-10^{-5}M which could be remedied at 10^{-4}M and 10^{-5}M in (Group III). Probable toxic action of $AlCl_3$ and possible protective role of myrobalan are discussed.

Key words: *Allium cepa*, mitodepression, $AlCl_3$, tannins antioxidant, myrobalan, *T. chebula*).

INTRODUCTION

Aluminium is a ubiquitous element found in almost every food [1] and is debatable and suspected etiological factor in neurodegenerative disorder like Alzheimer's brain as it modulates DNA topology [2]. Aluminium induced DNA damage, inhibition of DNA repair, micronuclei formation and apoptosis in human peripheral blood lymphocytes are on record [3, 4]. Efforts are being made for the synthesis of superior chelating agents to improve efficacy of treatment for Al-toxicity over desferrioxamine (DFO), a classical agent used in modern system of medicine [5]. On the contrary studies were not conducted to find ability of any herbal compound which can combat Al-toxicity except two attempts which claimed that extract of *Phyllanthus emblica* could antagonise Al-induced clastogenicity and sister chromatid exchange in mice bone marrow cells [6,7]. Experimental studies from present laboratory provided evidence that myrobalan could reduce lead toxicity in mice [8] and in *Allium* model [9]. Aluminium is also known to disturb mitosis in *Allium Cepa* root tip cells [10, 11] hence present study was planned to find out whether myrobalan can also counteract Al-toxicity in *Allium* test which is now internationally accepted model for such studies [12].

EXPERIMENTAL

Allium cepa

Dry healthy onion bulbs 1.5 to 2.00 cm in diameter were obtained from local market.

Test herbal drug

Myrobalan, dried young nuts of *Terminalia chebula* was procured from local herbal medicine shop and were gently backed for few minutes and cooled. Swollen nuts were grinded to fine powder. A recent study [13] revealed lack of any adverse effect of myrobalan in *Allium* test at 0.10 mg/ml, therefore, this concentration is selected for the present study.

Aluminium compound

Aluminium chloride hydrated: $AlCl_3.6H_2O$ made by Sarabhai Chemicals, India was used. Its molecular weight was 241.43 and purity was 96%. This salt was dissolved in tap water to prepare different concentration ranging from 10^{-1}M to 10^{-5}M molarity.

Administration of Drug

Very fine powder of myrobalan was added to each solution of each concentration of aluminium chloride to prepare a suspension of 0.10 mg/ml.

Experimental design

Experiments were planned as per protocol of Fiskesjo [12] for *Allium* test. For each set, twelve test tubes were filled with pure tap water (Group I, controls). Another series of 12 test tubes were filled with each concentration of aluminium chloride (Group II, aluminium exposed). Third series of test tubes (12) were also filled with different concentration of aluminium chloride but each one contained myrobalan powder in it (0.10 mg/ml).

All solutions were changed every 24 hr. After 48 hr two onions out of twelve in each series with most poorly growing roots were removed. Same day i.e. after 48 hr. distal 2 mm of five roots was cut off from five individual bulbs from each series and fixed in aceto-alcohol (1:3 v/v acetic acid and absolute alcohol) for chromosomal study. Every time fixation was done at a fixed time, 11.00 A.M.

After 72 hr total length of the 05 root bundle in each series of each onion was measured to record mean root length.

Squashing of root tips and observation of slides

Root tips were squashed in N-HCl and 2% acetocarmin (BDH) stain. Four fields from each slide was observed to cover 50 cells in each i.e. total 200 cells per slides and 3000-4000 cells were observed for each group of onion. Mitotic index was calculated as total number of dividing cells per 100 observed cells. Slides were also observed to find out mitotic arrest, chromosome fragments, abnormal orientation, lagging chromosomes, nucleolar disorganization, polyploidy and apoptosis etc.

Statistics

Experiments were done trice. Student t-test was applied at 5% level of significance.

RESULTS

1. Mean Root Length (MRL, Table - 1)

Root did not grew at 10^{-1}M and very poorly at 10^{-2}M. Root grew in 10^{-3}M to 10^{-5}M aluminium solutions but MRL remained significantly lower than controls. Drug could not revert aluminium induced root inhibition at 10^{-2}M and 10^{-3}M however, at last two lower concentrations (10^{-4}M and 10^{-5}M) drug could significantly reduce aluminium induced root growth inhibition.

Table 1. Mean root length (MRL as mm) of *Allium cepa* after 72 hrs of cultivation in different concentration of $AlCl_3$ alone or in combination with myrobalan (mean ± SEM).

S. No.	Concentration	Group of Onion Bulbs					
	Molarity	Gr-I Control	Gr-II $AlCl_3$ exposed	Gr-III $AlCl_3$ + Myrobalan Exposed	% Inhibition Gr-I Vs Gr-II	% Inhibition Gr-I Vs Gr-III	% Inhibition Gr-II Vs Gr-III
1	0.00	59.72 ± 0.86					
2	10^{-5}M		44.90^{a} ± 0.82	49.10^{bc} ± 0.69	24.81	17.78	7.03 S
3	10^{-4}M		35.60^{a} ± 0.44	40.52^{bc} ± 0.84	40.38	32.15	7.23 S
4	10^{-3}M		7.97^{a} ± 0.76	9.00^{b} ± 0.56	86.65	84.92	1.73 NS
5	10^{-2}M		2.71^{a} ± 0.59	2.53^{b} ± 0.52	95.46	95.76	0.30 NS
6	10^{-1}M		-	-	-	-	-

Statistically significant based on t-test at 5% level of significance. 'a' = Control Vs Gr. II, 'b' = Control Vs Gr. III, 'c' = Gr. II Vs Gr. III, NS = Non significant, M = Molarity, S = Significant, p = 1.96, n = 100, (-) = No growth

2. Morphology: colour and shape of root tips

Morphology i.e. colour and shape of *Allium cepa* tips cultivated in all test concentrations of aluminium chloride (Group II) and in the presence of drug (Group III) did not reveal any change from controls (Group I).

3. Mitotic Index (MI, Table - 2)

Significant low MI is found at 10^{-2}M to 10^{-5}M. Presence of drug could not check Al-induced mitodepression at 10^{-2}M and 10^{-3}M but drug could significantly reduce Al-induced mitodepression at only at 10^{-4}M and at 10^{-5}M.

Table 2. Mitotic Index (MI) of *Allium cepa* root tip cells following 48 hrs cultivation in $AlCl_3$ alone or in combination with myrobalan (mean ± SEM).

S. No.	Concentrations	Groups of Onion Bulbs					
	Molarity	Gr-I Control	Gr-II $AlCl_3$ exposed	Gr-III $AlCl_3$ + Myrobalan + Exposed	% Inhibition Gr-I Vs Gr-II	% Inhibition Gr-I Vs Gr-III	% Inhibition Gr-II Vs Gr-III
1	0.00	41.79 ± 1.33					
2	10^{-5}M		29.12^{a} ± 0.71	36.81^{bc} ± 0.91	30.31	11.91	18.40 $_{S}$
3	10^{-4}M		23.00^{a} ± 1.31	32.54^{bc} ± 0.90	44.96	22.13	22.83 $_{S}$
4	10^{-3}M		12.31^{a} ± 1.14	12.54^{b} ± 1.15	70.54	69.99	0.55 $_{NS}$
5	10^{-2}M		10.18^{a} ± 1.09	9.37^{b} ± 0.76	75.63	77.57	1.94 $_{NS}$
6	10^{-1}M		-	-	-	-	-

Statistics and other symbols are same as detailed below Table 1.

4. Cytological Effects

No chromosomal aberrations and any type of abnormal mitosis could be seen in the root tip cells after any treatment with $AlCl_3$, $AlCl_3$ + myrobalan or in controls.

5. Morphology of Nucleoli (Table - 3)

Aluminium chloride exposure at 10^{-2}M to 10^{-5}M caused hypertrophy of nucleoli in all the nuclei of root tip cells but disorganisation as reported by Fiskesjo [11] could not be observed. Drug could maintain usual means i.e. control like nucleoli at 10^{-4}M and 10^{-5}M.

Table 3. Nucleolar morphology in the nuclei of *Allium cepa* root tip cells after 48 hr cultivation in $AlCl_3$ or $AlCl_3$+myrobalan.

S.No.	Concentration of $AlCl_3$	Observations on nucleoli
1	0.00	Distinct two nucleoli per nucleus
2	10^{-5}M alone	Hypertrophy of both nucleoli
	10^{-5}M + drug	Usual, *control like*
3	10^{-4}M alone	Hypertrophy of both nucleoli
	10^{-4}M + drug	Usual, *control like*
4	10^{-3}M alone	Hypertrophy of both nucleoli
	10^{-3}M + drug	Hypertrophy of both nucleoli
5	10^{-2}M alone	Hypertrophy of both nucleoli
	10^{-2}M + drug	Hypertrophy of both nucleoli

6	10^{-1}M alone	NG
	10^{-1}M + drug	NG

NG = No growth (n = 100 - 200)

DISCUSSION

Earlier reports have shown both i.e. aluminium induced declined in mitosis and chromosomal aberrations in plants [10, 14] and DNA damage and inhibition of its repair [3] and apoptosis in human peripheral blood lymphocytes [4] however, present results have shown only mitodepression to mitostatic effect (low mitosis to no mitosis at all) with increasing concentrations of aluminium chloride. This discrepancy i.e. no chromosomal effect in the present study might be due to low purity (96%) of $AlCl_3$ used in the present study or differences in the physicochemical properties of water if any, and room temperatures.

A perusal of results indicate that two issues emerge out which deserve discussion, first one is to understand probable mechanism of action of aluminium chloride in root tip cells for lowering mitosis and second one is for explaining probable protective role played by myrobalan against Al-toxicity.

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Probable action of $AlCl_3$ induced mitodepression

Aluminium chloride induced inhibition of root growth and low mitosis is not unexpected findings as several earlier similar reports do exist in the literature.

Aluminium chloride induced progressive root growth inhibition from 10^{-5}M to 10^{-1}M [10] and later on nucleolar dissolution was also became evident [11]. In plants aluminium accumulate in the root cap [15] and binds to the chromatin [16] inhibits cell division [17,18] and repress template activity of both DNA [19] and RNA [20]. Furthermore, a mutagenic effect (chromosomal breakage) has been observed in plants [21]. In *Allium sativum* aluminium sulphate caused mitotic depression, aberrant cells and micronuclei formation [14]. It is suggested that strong interaction of Al^{3+}, the main toxic form with protein, nucleic acids and polysaccharides result in the inhibition of cell division, cell extension and transport [22].

In plants, aluminium causes increased production of reactive oxygen species i.e. ROS [23] which was a potential cause of root growth inhibition by exposure to aluminium. Above cited ill effects of aluminium can be held responsible for no root growth at highest concentration and low growth at lower concentrations.

Probable protective role of myrobalan against aluminium toxicity

a) Based on antioxidant property of myrobalan

The common feature of Al-toxicity in plants and animals/human cells is increased production of reactive oxygen species (ROS) resulting in the oxidative stress. In mammalian cells Al can potentiate Fe-induced oxidative stress through increased production of ROS [24], which have been implicated in neurological disorders [25,26]. In plants, Al also causes increased production of ROS [27] as well as lipid peroxidation [23] with the former being a potential cause of root growth inhibition upon exposure to Al [23].

Present results show that at lower concentrations of $AlCl_3$ (10^{-4}M and 10^{-5}M) drug could significantly counteract Al-induced mitodepression effect. This is possible only if myrobalan possesses antioxidant properties and indeed myrobalan has already been shown to exert such action. Fu etal [28] reported antioxidant action of *T. chebula* and found preventive effects on DNA breaks in human white cells induced by TPA, cigarette smoke condensate and lipid peroxidation in mice liver, lung and red blood cells. Antioxidant-property of myrobalan was confirmed in DPPH radical scavenging assay [29] and in electron spin resonance spectroscopy [30]. Myrobalan was also found to be a potent antioxidant and probable radioprotector against gamma radiations in rat liver mitochondria; it also prevented DNA breaks [31].

b) Based on probability of formation of an inert Al-drug complex

In Al-accumulators, Al is usually complexed with organic acids or other organic compounds to make it non toxic. The predominant Al form is Al-catechins in the leaves of tea plant [32], Al-citrate in *Hudrangea* leaves [33] and Al-oxalate in buckwheat [34] rendering the high total tissue concentrations non-phytotoxic to the cell cytosol. In addition, the cytosol is protected by Al-accumulating predominantly in the cell wall or vacuoles [34]. Binding of Al in the cell wall is mainly to pectic substances as shown in *Melastoma malabatrichum* [35]. In black tea infusions, 10-19% of total Al was present as cations species, whereas 28-33% was present as hydrolysable polyphenol complex [36]. Myrobalan also possesses polyphenols which can bind with Al thereby reducing toxicity. Myrobalan possesses large number of components some of which can bind with Al making it inert. Further research is needed to pin point exact role played by myrobalan against Al-toxicity in *Allium cepa* root tip cells.

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