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Original Article

Evaluation of cytotoxicity of Atenolol in *Allium cepa.* L.

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Abstract

Atenolol, an adrenergic beta blocker, commonly employed in the management of cardiovascular diseases, has been evaluated for its cytotoxicity using *Allium cepa. L* assay system. Four different treatments, 2 spurt treatments (T_1 & T_3) of 2h duration each varying at time points of cell division cycle (6AM to 8AM and 11AM to 1PM), T_2 of 3h duration (8AM to 11AM), and T_4 of 17h duration (approximately for one cell cycle), were given with 10µg/ml Atenolol to the root meristems of *A.cepa* and were observed for their recovery for 72h with 24h intervals. All the treatments induced wide range of aberrations including disturbed meta-anaphases, chromosome breaks, bridges, and stickiness. The 0h of T_2, 48h of T_1, 72h of T_1 and T_4 showed significant decrease in cell division. Differential response to the drug toxicity and significant decrease in cell division indicates the possibility of Atenolol affecting mechanisms that ensure DNA stability and the cell cycle progression. In view of the wide spread use of the drug in the management of cardiovascular diseases and chronic hypertension, more intensive studies employing mammalian and submammalian assay systems are needed.

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Key words: Mitotic Index, Cell Division Cycle, Genotoxicity.

1. INTRODUCTION

Atenolol, a beta blocker is a replacement of propanolol, generally employed by the physicians for the management of hypertension, angina, tachycardia, and acute myocardial infraction [1]. The dose levels are between 50 to 100mg/day of oral administration with a half life of about 10h. It is rapidly absorbed from the gut and attains a peak concentration in the serum within 2-3h [2]. Its metabolism is minimal due to its hydrophilic nature and almost 50% of the drug is always available in the circulatory system [3]. Adverse side effects *viz*., hepatotoxicity, lupus erythematosus, septal panniculitis, memory impairment, breast pain and swelling are known to be induced by Atenolol [4-9]. It is also associated with fetal growth retardation when given in pregnancy [10, 11]**.** Moreover, chronic exposure to Atenolol resulted mainly in the induction of chromosome loss in *in vitro* and *in vivo* studies [2]. No teratogenicity of Atenolol is reported till date. However, some studies have shown mutagenic potential of other widely used beta blockers [1, 12-15].

The published data on cytotoxicity of Atenolol affecting the cell division and repair mechanisms is not available. In view of this, the present study was conducted to evaluate the effect of Atenolol on cell division cycle (CDC) and maintenance of the check points. It is assumed that in short time frame most cells will be in similar phase and consequently a similar number of cell divisions will takes place. The hypothesis evaluated is whether there is a difference between times of exposure to the drug *vis a vis* dynamics of cell cycle in inflicting genetic damage. The preliminary results are presented in this paper.

2. MATERIALS AND METHODS

Onion bulbs were purchased from a farmer's market (Rythu Bazar) situated at Mehadipatnam, Hyderabad. Uniform sized

Table S1: The effect of Atenolol on Percent frequency of chromosomal aberrations, Mitotic and Phase Indices

Time	System/ Treatment	No of Cells examined	No of dividing cells	Mean ± SE	Mitotic Index	Total Aberrant cells	% Frequency of Abnormal cell		Phase Index (%)			
							Total cells	Dividing cells	Prophase	Metaphase	Anaphase	Telophase
Treatment before the Cell Division maxima												
0 hrs	Control	3000	300	60± 7.2	10	0	0	0	93.97	5.33	5	5.33
	Treatment	3000	243	48.6± 14.1	8.1	68	2.28†	28.09	71.87	14.77'	12.32	1.23
24hrs	Control	3000	216	43.2± 9.4	7.2	3	0.1	1.39	74.23	15.77	6.49	3.71
	Treatment	3000	263	52.8± 7.4	8.8	56	1.87†	21.29	60.47	18.25	14.45	5.7
48hrs	Control	3000	254	50.8± 5.1	8.5	18	0.6	7.07	51.88	18.64	11.4	18.53
	Treatment	3000	422	84.4± 8.4	14.1 **	214‡	7.15†	50.86	46.96	21.21	31.18'	0.71'
72Hrs	Control	3000	111	22.2± 6.9	3.7	0	0	0	67.75	20.78	1.81	10.84
	Treatment	3000	22	4.4± 2.8	0.8 *	16	0.53†	66.67	33.33'	37.5'	37.5'	0.5'
Treatment at the Cell Division maxima												
0 hrs	Control	3000	445	89.00±11.1	14.8	11	0.36	2.45	72.79	16.1	7.76	3.35
	Treatment	3000	237	47.2± 9.7	07.9 *	79	2.63†	33.32	65.95	19.53	14.71	0
24 Hrs	Control	3000	448	89.8± 6.9	14.9	14	0.46	3.05	58.2	25.18	11.64	4.98
	Treatment	3000	593	118.4±17.3	19.8	271 ‡	9.05†	45.75	53.59	23.26	22.68	0.49
48hrs	Control	3000	624	125± 5.5	20.8	19	0.63	3.05	60.76	21.66	10.89	6.65
	Treatment	3000	621	124.5±12.3	20.7	179	5.90†	28.82	59.41	21.99	16.27	2.39
72Hrs	Control	3000	468	93.8± 9.2	15.6	15	0.52	3.31	57.27	19.75	13.35	9.73
	Treatment	3000	479	95.8± 6.02	16	116	3.80†	24.26	67.38	17.17	13.57	1.93'
Treatment after the Cell Division maxima												
0 hrs	Control	3000	194	38.8± 8.5	6.5	9	0.3	4.6	74.75	11.09	8.13	11.34
	Treatment	3000	368	73.6± 12.4	12.3	159 ‡	5.30†	43.27	54.15	14.29	19.46'	12
24 Hrs	Control	3000	184	36.5± 3.6	6.1	9	0.3	4.8	76.02	6.79	7.47	10.18
	Treatment	3000	149	30.0± 4.7	4.9	13	0.43†	8.72	51.4'	7.22	11.11	29.15'
48hrs	Control	3000	33	6.60± 5.2	1.1	6	0.2	18.18	42.42	24.24	24.24	9.09
	Treatment	3000	188	37.60±12.7	6.3	65‡	2.17†	34.57	64.22'	13.8	20.7	2.65
72Hrs	Control	3000	86	17.40± 7.1	2.9	0	0	0	99.68	0.5	0.5	0
	Treatment	3000	54	10.80± 5.8	1.8	14	0.47†	26.17	74.76	13.08'	13.08'	0
Treatment on one Cell Division Cycle												
0 hrs	Control	3000	247	49.40±7.0	8.2	5	0.17	2.02	50.61	20.6	15.63	14.07
	Treatment	3000	432	86.20±15.9	14.4	190‡	6.32†	43.94	55.84	24.64	19.17	0.64'
24 Hrs	Control	3000	288	57.60±18.8	9.6	5	0.17	1.74	54.51	23.69	11.81	11.15
	Treatment	3000	436	87.20±17.2	14.5	181	6.04†	41.54	56.88	22.01	21.22	0.33'
48hrs	Control	3000	315	63.00±10.7	10.5	7	0.23	2.24	56.81	15.5	16.24	11.4
	Treatment	3000	278	55.60±6.07	9.3	143	4.75†	51.35	47.92	25.84	26.32	0.5'
72Hrs	Control	3000	430	86.00±12.1	14.3	16	0.55	3.8	50.88	26.64	14.89	7.69
	Treatment	3000	103	20.80± 2.9	03.4***	60	2.01†	58.56	30.05	37.45	26.3	6.62

* $p < .05$,** $p < 0.01$, *** $p < 0.005$ in One Way ANOVA, ‡‡. $p < 0.01$ level (2-tailed) and ‡.$p < 0.05$,Correlation significant level (2-tailed),† Z test Significant, ' < 0.05 Chi sqare significant

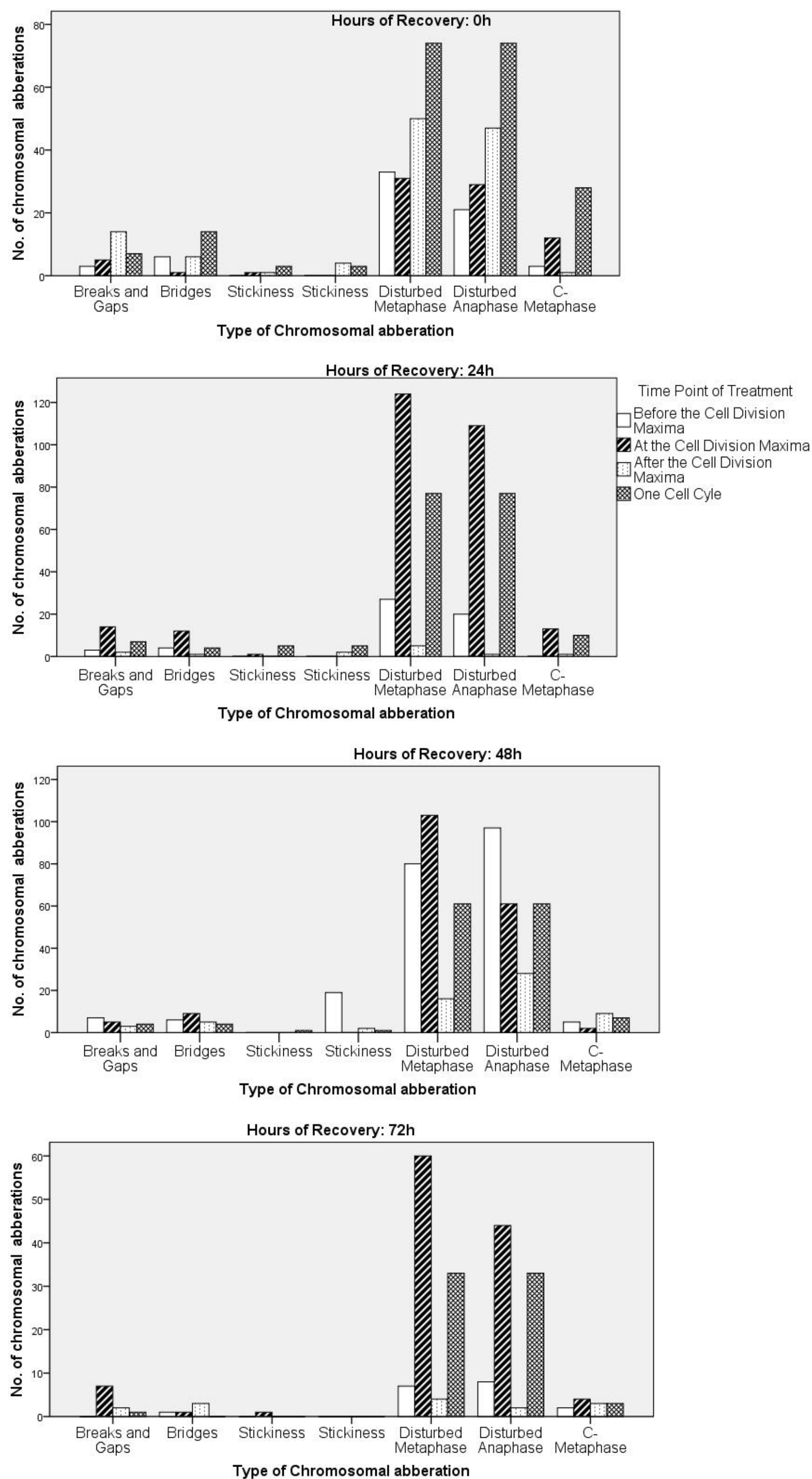

Figure 1a-d: Spectrum and frequency of chromosomal aberrations induced by Atenolol.

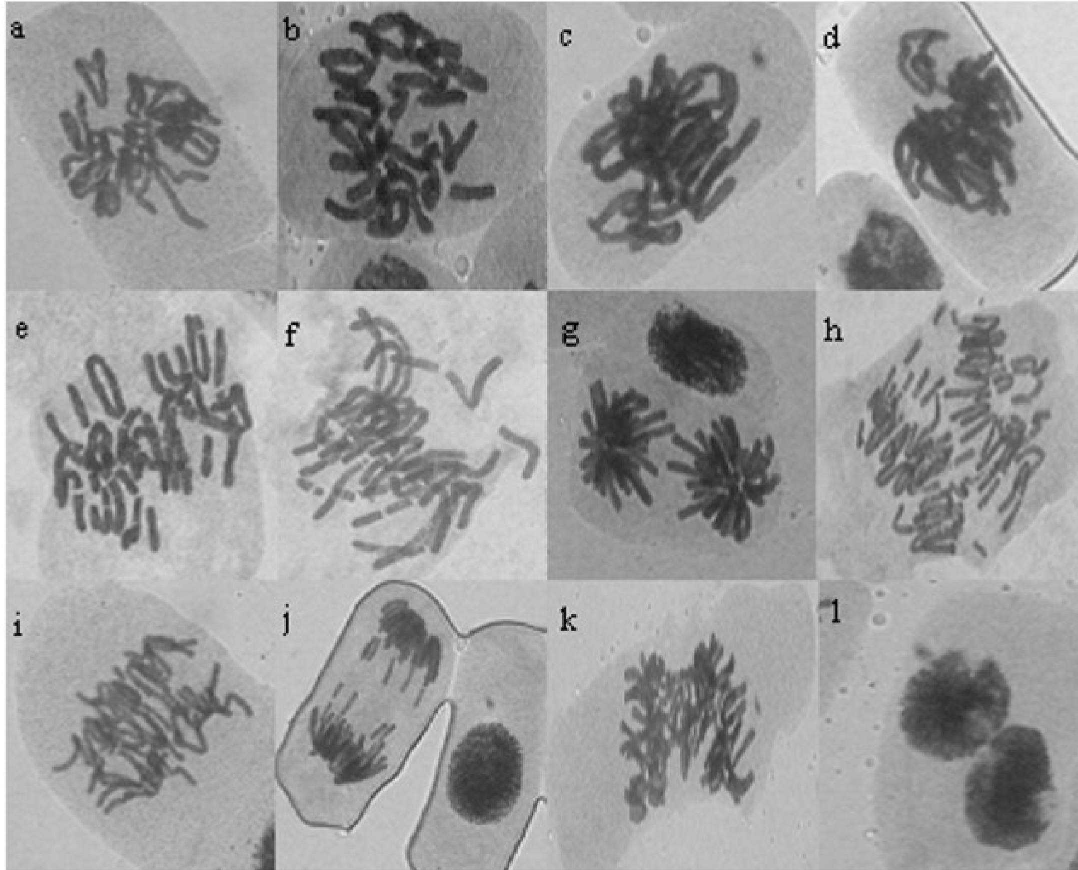

Figure 2: Different Chromosomal Aberrations induced by Atenolol in root tips of Allium cepa a) Late prophase with ring Chromosome b) Late Prophase with fragments c) Disturbed Metaphase with fragments d) Sticky Metaphase with ring chromosome e) Metaphase with ring chromosome and breaks and gaps f) Disturbed Metaphase with breaks and fragments g) Star Anaphase h) Anaphase with breaks and vagrants i) Anaphase with bridges j) Late Anaphase with breaks and adjacent cell with micronucleus k) Disturbed metaphase in a polyploid cell l) micronucleus in a bivalent cell

bulbs were selected and the yellow shallows of dry bottom plate covering the root primordial is carefully removed prior to the immersion of the root zone into the water. Healthy bulbs with growing roots of 1-2cm long were used in the experiment. Atenolol tablet were purchased from the local chemists and druggist shop and aqueous solution of 10µg/ml Atenolol was poured into 100ml corning glass beakers, over which onion bulbs were placed for the treatment in such a way that growing roots are well immersed in the solution. Four different treatments, $T_{2,}$ T_4 of 3h (8AM to 11AM) and 17 h duration respectively and two spurt treatment T_1 and T_3 of 2h duration at different time point (6AM to 8AM and 11AM to 1PM) were given. Appropriate controls were maintained. Each treatment was replicated 4-5 times and the recovery was observed at the end of 24h, 48h and 72h.

The root tips were fixed in a Carnoy's fixative (ethyl alcohol: acetic acid in 3:1 ratio) for 24h and further processed by Heamotoxylin squash technique of Subramanyam and Subramanyam (1970). Briefly, the root-tips were hydrolyzed in 1N HCl for eight minutes at 60^0C, rinsed with distilled water for 2-5 minutes and then transferred to 4% Ferric alum (mordent) for 20 minutes followed by rinsing with water. 1-2mm long root tips were stained using Haematoxylin and squashed in a drop of 45% acetic acid after smearing the cover slip with Meyer's albumin and flaming it. Observations were made under Olympus microscope for various cell division stages. Approximately, 500 cells of 6 meristems were screened to determine mitotic index, phase index and frequency of specific chromosomal aberration. The percent frequency of aberrations was computed based on the total number of cells scored and on the number of cells in division.

Statistical analyses were performed on mitotic index and chromosomal aberration frequency using SPSS vs 18 (Chicago).The one way ANOVA, Chi Square and Z test was performed to determine the significant differences between the cell divisions, mitotic phases and chromosomal

aberrations of treated and their respective controls. Pearson's Correlation was performed to find the correlation between cell division and chromosomal abnormalities.

3. RESULTS AND DISCUSSION

The cell division cycle can be delineated into major phases G_1, S, G_2 and M phase. Cell division manifests in a periodic manner during 24h period [26-31]. A double rhythm was found in roots of Allium cepa grown under room condition in moist sawdust[32]. In our study, the cell division was shown to be maximum between 8AM to 11AM (Table S1). Treatments before and after the cell division maxima are expected to be in G_2 phase and in G_1 phase respectively. By treating with drugs specifically for the duration of each phase and allowing the recovery period of three cycles of cell division (24, 48 and 72 hours) one can evaluate the effect of the drugs on each check point and their role in maintaining DNA and/or chromosomal stability during the cell division cycle.

DNA damage checkpoints are essential for the survival of cell and organism. Several genes control the ability of cells to arrest the cell cycle in response to DNA damage, providing chance to repair [16-18]. The genome data of Arabidopsis pointed out that the DNA repair is conserved highly between plants and mammals than within the animal kingdom[19, 20]. *Allium cepa* assay system was evaluated by several workers to assess genotoxicity [21-25]. In the present study, *Allium cepa* assay was employed to evaluate the cytotoxicity of Atenolol in relation to cell division cycle.

The specific aberration induced depends on the time at which the interphase nucleus is exposed to a clastogen [33]. Interesting results were obtained when mitotic index was considered as a function of damage afflicted on the genetic material. The data on mitotic index and different phase indices are presented in Table S1. The mitotic index results (one way ANOVA) showed significant difference between the treated and respective controls at 48h and 72h recovery of T_1, 0h recovery of T_2, and 72h of T_4 (Table S1). Moreover, significant differences in mitotic phase indices (chi-square) were found at different recovery periods when compared to their respective controls of different timepoint treatments.(Table S2)

When treatment was given before the cell division maxima (T_1), the MI was enhanced and declined subsequently, when compared to the control at the end of second and third recovery periods respectively. The G_2 checkpoint arrests damaged cells in G_2, delaying entry into mitosis until the damage gets repaired [34]. Possibly, the G_2 check point arrested the cells entering into the mitosis until 48h. The unrepaired cells entering into mitosis may account for the significant enhancement and decline of MI. Significant decrease in prophase (72h) and telophase (48h and 72h) and increase in metaphase (0h and 72h) and anaphase (48h and 72h) indicates accumulation of the cells at meta-anaphase.

Significant decline in MI was seen even at 72h recovery period of T_4 (one cell cycle treatment) indicating cell death due to damage. The phase index varied significantly at the telophase (0h, 24h and 48h). Furthermore, the fact that the chromosomal aberrations prevailed even after three cycles of recovery indicates that the damage caused by the chronic exposure to the drug failed to get repaired, and eventually resulting in the cell death.

When the treatment is given exclusively after the cell division maxima (T_3), there was cyclic increase and decrease in the MI and chromosomal aberrations as compared to the control. The Prophase, Anaphase and Telophase index differed significantly at different recovery periods. Exposure in the G_1 phase results in damage of the entire chromosome. Probably, the aberrations seen at the 48h recovery period is due to the lesions becoming homozygous. No significant difference could be seen in the mitotic index; however, chromosomal aberration decreased considerably at the end of 72h. This implies that the repair mechanisms after cell division maxima were efficiently correcting the errors.

T_2 treatment showed high sensitivity and the chromosomal aberrations persisted in all the recovery periods. Significant decrease in the MI immediately after the treatment indicates the arrest of the cells entering into the mitosis. The increase in chromosomal aberration frequency at the first recovery cycle possibly be due to the release of arrested cells or/and aberration caused earlier could have resulted in more aberration in the next generation. Further, significant correlation was observed between the cell division and chromosomal aberrations at the recovery periods of 48h of T_1, 24h of T_2, 0h and 48h of T_3, 0h of T_4 recovery periods (Table S1) indicating that the aberrations seen in these treatments are dependent, perhaps due to arrest at the mitotic division. However, no significant difference was seen in the phase indices suspecting that the arrest of the cells was before the cell division maxima probably at the G2 phase.

Frequency and the spectrum of chromosomal abnormalities elicited by Atenolol following the treatment of onion root meristems during different timepoints of cell cycle *viz*; T_1, T_2, T_3 and T_4 are given in Fig S1 and S2. The frequency of aberrant cells observed was significantly higher in the treatments than that of controls.

The data on the distribution of chromosomal aberrations (Fig S1 and S2), indicate that Atenolol provoked a wide spectrum of cytological abnormalities; disturbed meta and anaphases, chromosome breaks, stickiness, bridges, fragments at ana-telophase, chromosomes lagging and condensation, micronucleus and polyploidy. The metaphase and anaphase perturbations were observed more frequently. The clastogenic action of Atenolol was evident from the

appearance of breaks and gaps. Chromosome breakages are the result of unfinished repair or misrepair of DNA which can result in cell death or a wide variety of genetic alteration [35-37]. A sticky chromosome can also lead to death of the cells [38]. The presence of the micronucleus in different cells leads to permanent chromosomal loss [2]. Polyploid cells indicate that the cells might have entered into endoreduplication followed by cell division arrest [39].

4. CONCLUSION: - It is quite possible that the Atenolol provoke disturbances in the cell division cycle affecting the MI, phase index and chromotoxicity. The present work is the first of its kind to monitor the possible genotoxic potential of antihypertensive drug Atenolol, making use of exclusive treatments during different phases of CDC and evaluating the consequences *in toto*. The results obtained in the study by using different end points are quite interesting. The preliminary results suggest that there is a significant damage caused by the drug. More in-depth studies are warranted.

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