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Genotoxicity of Caffeinated Soft Drinks on Meristematic Root Cells of *Allium Cepa*

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ABSTRACT: The potential genotoxicity of caffeinated soft drinks was investigated by *Allium cepa* assay. Three types of soft drinks used with varying caffeine content. Four different concentrations of the soft drinks (100%, 75%, 50% and 25%) were treated with the roots at three different times of exposure (1h, 3h and 5h). The concentration and duration of exposure affected the mitotic division in root tip cells and produced various chromosomal aberrations. The abnormalities scored were spindle abnormalities, bridges, fragments, condensed chromosome and bi-nucleated cells. Exposure to soft drinks inhibited mitotic index in a concentration dependent manner.

KEYWORDS: Soft drink, Mitotic index, *Allium cepa* test.

I. INTRODUCTION

Soft drinks are nonalcoholic water based flavoured drinks that are optionally sweetened, acidulated, carbonated and may contain fruit juice and salts, their flavor may derive from vegetable extracts or other aromatic substances as per Chandraker et al. [1]. In certain soft drinks caffeine is also present. This include certain soft drinks (primarily Cola drinks) and energy drinks (Moster, Red Bull and Bison) designed as stimulants and to perpetuates activity at times when the user might be asleep. The consumption of caffeinated drinks is often intended entirely or partly for the physical and mental effects of caffeine. Caffeine overdoses has been linked to serious health problems including arrhythmia, arterial fibrillation, myocardial infection which can lead to death. According to Ito et al. [2] caffeine in soft drinks causes changes in the DNA repair system, which leads to disease in various organs, especially cardiovascular abnormalities and pancreatic cancer.

Cytological aberrations in plants are an excellent monitoring system for the detection of chemicals that may pose a genetic hazard. A number of studies which have been carried out in chemicals indicate that there is an excellent correlation between chromosome abnormalities found in root tip systems and those of mammalian cell systems. It is true that the type of chromosomal aberration induced by a specific chemical may not be the same as in plant cells, but if a particular chemical will induce chromosome aberration in one group, generally it will do same in the other relative group as well. The *Allium cepa* test is an easy fast genetic assay to detect the potential cytotoxicity and genotoxicity of polluted water and other chemicals on mitosis and chromosome structure.

II. METHODOLOGY

Healthy *Allium cepa* bulbs and soft drinks such as Coca Cola, Red Bull and Pepsi were purchased from the local market. The loose outer scales of bulbs and old roots were removed with the help of sharp and pointed forceps so as to expose root primordial cells. Onion bulbs (*Allium cepa* 2n=16) of average size (15-22 mm in diameter) were placed in distilled water to multiply primordial root cells. Root growth was observed till reached 1.5 – 2cm in length, then the bulbs placed in a series of concentrations of soft drinks. The various concentrations used are 25, 50, 75 and 100% for 1h, 3h and 5h durations. Root tips were fixed in ethanol and hydrolysed in 1N HCl, stained with acetocarmine and squashed on microscopic slides. The mitotic stages and chromosomal aberrations in the root cells were determined using light microscope.

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III. RESULTS AND DISCUSSION

a. Effect of soft drink on mitotic index: Exposure to soft drinks inhibited mitotic index in a concentration dependent manner when compared to the mitotic index of the control. The lowest mitotic index value was recorded for 5 hrs of exposure to Red bull (26%). Mitotic index decreased as the concentrations of soft drinks increased from 25% to 100% (Table 1, 2, 3). In Coca Cola the mitotic index is 65% at 25% of concentration and it is reduced to 50% when treated with 100% concentration. According to Karaismailoglu [3] decline in mitotic index in *Allium cepa* root cells with increasing treatment periods with soft drinks may reflect cytotoxicity of soft drinks through the inhibition of cell cycle from the S (synthetic) phase to the M (mitosis) phase, or might be due to the prevention of DNA synthesis and holding the cell from entering mitosis.

Table 1: Mitotic Index of *Allium cepa* root cells exposed to Red bull.

Concentration of Red Bull	Duration of exposure	P	M	A	T	Mitotic Index (%)
100 %	1h	8	9	10	7	34
	3h	8	7	8	6	29
	5h	7	7	8	4	26
75%	1h	9	11	12	8	40
	3h	7	7	10	9	33
	5h	6	6	10	8	30
50%	1h	10	13	13	10	46
	3h	9	12	12	7	40
	5h	7	11	11	7	36
25%	1h	14	13	13	12	50
	3h	14	12	14	11	51
	5h	10	11	12	10	44

Table 2: Mitotic Index of *Allium cepa* root cells exposed to Pepsi

Concentration of Pepsi	Duration of exposure	P	M	A	T	Mitotic Index (%)
100 %	1h	11	13	12	10	46
	3h	9	11	11	9	40
	5h	8	10	11	8	37
75%	1h	14	14	15	9	52
	3h	11	14	13	8	46
	5h	11	12	12	7	42
50%	1h	17	13	14	10	54
	3h	15	13	12	9	49
	5h	14	12	12	8	46
25%	1h	20	15	15	8	58
	3h	18	14	14	7	53
	5h	17	13	13	7	50

Where, P = Prophase; M =Metaphase; A = Anaphase; T = Telophase.

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Table 3: Mitotic Index of *Allium cepa* root cells exposed to Coca Cola.

b. Chromosomal aberration: It was observed that exposure to Red Bull at 100% of concentration leads to

Concentration of Coca Cola	Duration of exposure	P	M	A	T	Mitotic Index (%)
100 %	1h	14	12	13	11	50
	3h	12	11	12	10	45
	5h	10	11	11	9	41
75%	1h	15	14	13	10	52
	3h	13	13	12	10	48
	5h	12	12	12	9	45
50%	1h	17	14	15	12	58
	3h	16	12	14	11	53
	5h	14	12	13	10	49
25%	1h	20	16	15	14	65
	3h	19	14	13	13	59
	5h	18	13	13	13	57

chromosome bridges, breaks, condensation, spindle abnormalities, lagging and binucleated cells at 1, 3 and 5h of exposure (Table 4). The aberrations observed during the treatment increases over increased duration and concentration of exposure to soft drinks (Table 5, 6). The concentration here implies the amount of caffeine exposure to the *Allium cepa* cells. In this study three types of soft drinks were used. The caffeine content in the soft drinks varied. Red Bull contains 80 mg of caffeine while Pepsi contains 38g and Coca Cola contains 34g of caffeine in 250 ml. The present study agreed with the study of Kenneth [4] that Red Bull treatment produced higher number of aberrated cells which contain bi-nucleated cells, bridged chromosomes, spindle abnormalities and fragments. In the case of 100% concentration and 5 hour of exposure in Red bull, onion root tips were found to be destroying. It is true in the case of cells treated with Pepsi also.

The prophase stages are usually not much affected as compared with metaphase and anaphase. In the *Allium cepa* assay cells which are treated with all the three soft drinks reported the presence of chromosomal abnormalities in the metaphase and anaphase stages. The most common aberration is spindle abnormalities in metaphase and it is because the chromosomes are fail to form the metaphase plate correctly or some of the chromosomes are not aligned in the equatorial line when other chromosomes are arranged at the equator. Instead they are scattered over the cell and forms chromosome fragments and bridges. The genotoxicity of caffeine is proved in human cells also. Timson [5] reported that caffeine treatment on cultured human lymphocytes have reduced the rate of mitosis. From the present study it is clear that the soft drinks are harmful to health and their regular intake must be avoided.

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Table 4: Cyto-genetic analysis for chromosomal aberrations on the cells of *Allium cepa* in four different concentrations of Red bull and three periods of treatment

Concentration of Red bull	No. of cells observed						
	Time of exposure	Bridges	Breaks	Condensation	Spindle abnormalities	Lagging	Bi Nucleated cell
100%	1h	4	2	2	5	2	4
	3h	6	2	1	7	3	5
	5h	6	1	1	9	4	6
75%	1h	3	2	2	6	-	4
	3h	4	1	2	7	1	6
	5h	5	2	2	8	1	6
50%	1h	2	1	1	6	-	3
	3h	3	2	2	7	-	4
	5h	4	3	2	8	1	5
25%	1h	1	1	-	3	-	3
	3h	2	1	-	5	-	5
	5h	4	1	-	7	-	5

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Table 5: Cyto-genetic analysis for chromosomal aberrations on the cells of *Allium cepa* in four different concentrations of Pepsi at three periods of treatment.

Concentration of Pepsi	No. of cells observed						
	Time of exposure	Bridges	Breaks	Condensation	Spindle abnormalities	Lagging	Bi Nucleated cell
100%	1h	4	3	3	7	4	7
	3h	4	3	2	9	5	8
	5h	5	4	4	8	3	9
75%	1h	2	2	1	6	3	5
	3h	3	2	2	7	2	7
	5h	3	3	4	9	2	8
50%	1h	1	-	1	5	-	4
	3h	2	2	1	6	-	6
	5h	3	2	2	7	1	7
25%	1h	1	-	-	3	-	2
	3h	1	1	-	4	-	3
	5h	2	1	-	6	-	4

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Table 6: Cyto-genetic analysis for chromosomal aberrations on the cells of *Allium cepa* in four different concentrations of Coca Cola at three periods of treatment.

Concentration of Coca Cola	No. of cells observed						
	Time of exposure	Bridges	Breaks	Condensation	Spindle abnormalities	Lagging	Bi Nucleated cell
100%	1h	4	2	1	6	2	1
	3h	5	2	2	7	3	3
	5h	7	3	3	8	1	4
75%	1h	3	1	-	5	-	-
	3h	3	-	1	6	-	2
	5h	2	-	2	7	-	3
50%	1h	1	-	-	3	-	-
	3h	2	-	-	4	-	1
	5h	3	-	1	5	-	2
25%	1h	1	-	-	1	-	-
	3h	1	-	-	3	-	-
	5h	2	-	-	5	-	1

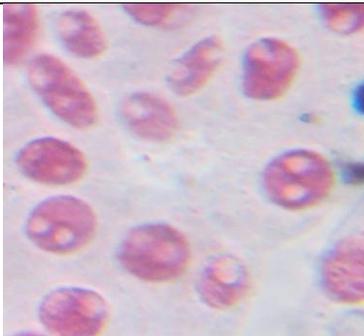
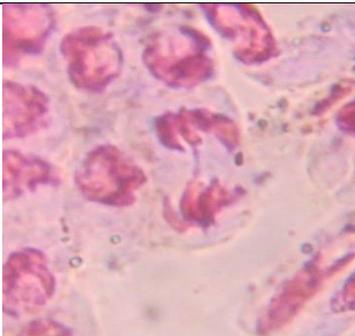
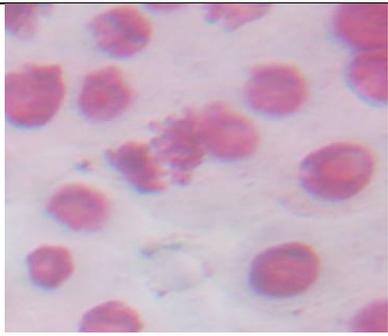
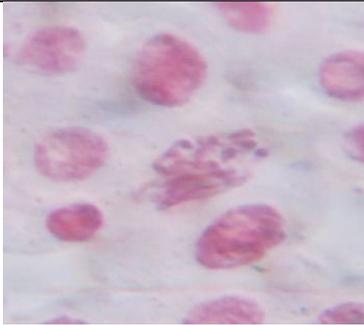
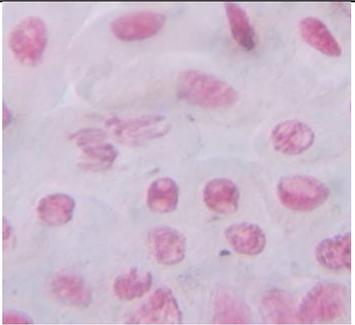
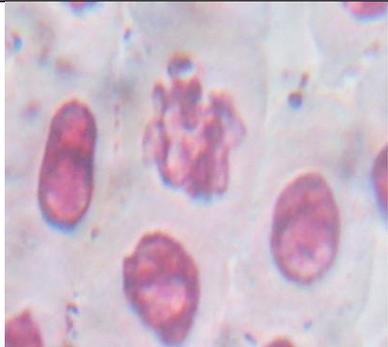
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Effect of mitotic cells after treatment with soft drinks

		
Plate 1: Binucleated cell	Plate 2: single bridge	Plate 3: multiple bridge
		
Plate 4: chromosome fragments	Plate 5: chromosome lagging	Plate 6: spindle abnormality

IV. CONCLUSION

Soft drinks such as Red bull, Pepsi and Coca cola produced chromosomal aberrations in *Allium cepa* and thus it gives a warning to the consumption of these drinks.

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