

Original Research Article

Clastogenic effect of soft drink on root tip of *Allium cepa*

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A B S T R A C T

Keywords

Clastogenic Effects, Mitotic Index, Stickiness, Adherent Chromosome, Laggard of Chromosomes.

The aim of present investigation is to check the clastogenic effects of soft drink at different concentrations on mitosis of *Allium cepa* L. In the present study the two different concentrations of soft drink was taken in beaker (50% and 100%) and dipped the *Allium cepa* root tip for different time exposure (6 hrs. and 8 hrs.) was carried out and it was found that both the concentration of soft drink and time exposure with *Allium cepa* affect the mitotic index of *Allium cepa*. The result further showed that Soft drink induced mitotic abnormalities like C-mitosis, Stickiness, Adherent chromosome and Laggard of chromosomes in 50% concentration whereas in 100% concentration the chromosomes destroyed. All the readings were compared with the normal mitotic index.

Introduction

The plant's name comes from the Latin *unio*, or *annianus*, and is associated with the Welsh *einion*, meaning "anvil." ("Onion" *Online Etymology Dictionary* November 2001. 28, February, 2008). It has long been known that chemical agents as well as ionizing radiation can induce mutations and chromosome aberrations in both plant and animal cells. This early work was reviewed by (Auerbach and by Levan in 1951). The mechanism leading to the formation of daughter cells and the retention of identical chromosome numbers and other hereditary factors in the newly formed cells, treatments with various reagents have been studied by

several workers such as (Shanthamurthy & Rangaswamy, 1979, Okoli & Russom, 1987, Okagbue, 1990 and Umar,2004).

Allium cepa bulbs were selected for research material because it is easy to obtain root meristem and have a small number of chromosome ($2n = 16$) for this experiment Soft drink (soft drink).

It has been shown that plant meristem have a well regulated structure of mitotic cycle. The first induction of this was discovering of the quiescent in root apices. The onion is a subspecies and primary member of the genus *Allium*. Because

many *Allium* species share the common name onion, the “garden onion”- also known as the “bulb onion” and “shallot” is referred to as *Allium cepa*. (Jones, Henry A. and Louis K. Mann., 1963).

Scientific Classification

Plant	- <i>Allium cepa</i>
Phylum	- magnoliophyta
Class	- Liliopsida
Oder	- Asparagales
Family	- Alliaceae
Genus	- <i>Allium</i>
Species	- <i>cepa</i>

Definition of soft drink

Soft drinks are non-alcoholic water-based flavored drinks that are optionally sweetened, acidulated, carbonated and which may contain fruit, fruit juice and/or salts; their flavor may derive from vegetable extracts or other aromatic substances. They constitute a defined and homogenous range, designated by a generic denomination and utilizing a single common list of additives, such as fruit juices and nectars, dairy drinks, mineral waters, etc. (Mathur, Johnson and Avinash Kumar. 2003).

The market

Globally, carbonated soft drinks are third most consumed beverages. Per capita annual consumption of carbonated soft drinks is nearly four times the per capita consumption of fruit beverages. (Source: Data from the Beverage marketing Corporation, as reported by the Canadian soft drink Association) soft drink consumption is growing by around 5% a year, according to Global soft drinks 2002. (Zenith International, 2002). Total volume reached 412,000 million litres in 2001,

giving a global per capita consumption of around 67.5 litres per year.

Karl Sax *et al* .(1966) had reported that when coca cola dissolved in a cup (6 oz) of water, they are as potent as moderately strong coffee. Coca Cola had to be diluted 50 per cent to permit growth of the onion root tips. The radiomimetic effect indicates that pure Coca Cola is about a fourth as potent as weak coffee. Cocoa contains theobromine, which is closely related to caffeine, and is as potent as coffee when prepared at the same concentration. The fact that Sanka coffee, and the soft drink Tab, which contain very little or no caffeine, do not induce chromosome aberrations, and that cafergot and No Doz tablets, which contain 0.1 gm of caffeine, are radiomimetic, is evidence that caffeine is the radiomimetic agent in coffee, tea, and Coca Cola.

Palani Kumar *et al* . (2007) had studied that the cytogenetic effects of food preservative i.e. Potassium metabisulphite on root tip cells of *Allium cepa* L. The root tips have been treated with a series of concentrations, ranging from 25 to 150µg/ml for 1, 3, 5, 7 and 9 h. The results indicate that the food preservative has reduced mitotic division in *A. cepa* compared with the respective control. The percentage of Mitotic index has decreased with increasing dose and time. Chromosomal abnormalities increase with increasing concentrations of the test chemical and the longer period of treatment. We have found that chromatid break and multiple breaks increase with dosage.

Ahmet Kayraldiz *et al*. (2001) had investigated that the effects of sodium meta bisulfite (SMB) on mitosis in *Allium cepa* L. The roots of *A. cepa* were treated with SMB concentrations of 7.5 mg/lt, 15

mg/lit and 30 mg/lit for 10- and 20-hour treatment periods. SMB significantly decreased the mitotic index (MI) at all concentrations and treatment periods. While the decreasing of the MI was dose-dependent at 10 hours treatment time, SMB increased the mitotic abnormalities dose dependently.

Materials and Methods

Materials

Fixative: Chloroform - 30%, Glacial acetic acid - 10 %, Ethanol - 60%

Aceto-orcein stain

2% Orcein dissolved in 45% glacial acetic acid acetic acid.

Onion bulbs

Stain preparation

5 g of orcein is added 150 ml of hot acetic acid. Keep the stain solution in a dark bottle for 2-3 days, shaking several times to saturate the solution. After that treatment, add 150 ml of distilled water, filter and store in a dark bottle. (Babich, Segall and Fox, 1997)

Allium cepa Assay:

Onion bulbs (*Allium cepa* L., 2n=16) of average size (15-22 mm diameter) were placed in distilled water to multiply primodial cells (Rank and Nielsen, 1993). For root growth inhibition evaluation, 50% and 100% concentrations of soft drink were used.

Submerged the base of an onion in water at a constant temperature to grown roots for 48 hours *Allium cepa* become rooted, than the onion bulbs were kept in the 50% and 100% concentrations of Soft drink (soft drink) for 6 and 8hrs, cut onion root tip in morning (5:30am) added a very small drop of 1M HCL acid to the root tip and let it for 4 min. Covered the root tip with a drop staining solution for 2 minutes, observe mitotic stages in individual cells.

Results and Discussion

The data presented in Table.1 and 2 show chromosomal aberrations induced by Soft drink (soft drink) in *Allium cepa* root cells. The mitotic index (MI) and the frequency of chromosomal aberrations (CA) was calculated (Fiskesjo 1997; Bakare *et al.* 2000).

The result reveals that most of the aberrations observed in chromosomes at metaphase and anaphase, very few in prophase and telophase. When *Allium cepa* root treated with two different concentrations of Soft drink (soft drink) 50% and 100%. In the 50% concentration of Soft drink (soft drink) treatment mitotic index was found to be 1.8%, (6hrs.) and 1.7%, (8hrs.) and in the control group, 3.3, (6hrs.) and 3.4%, (8hrs.) In the case of 100% concentration, onion roots were destroying. From this it was cleared that the rate of cell division decreases on increasing the concentration and time exposure of tested soft drinks.

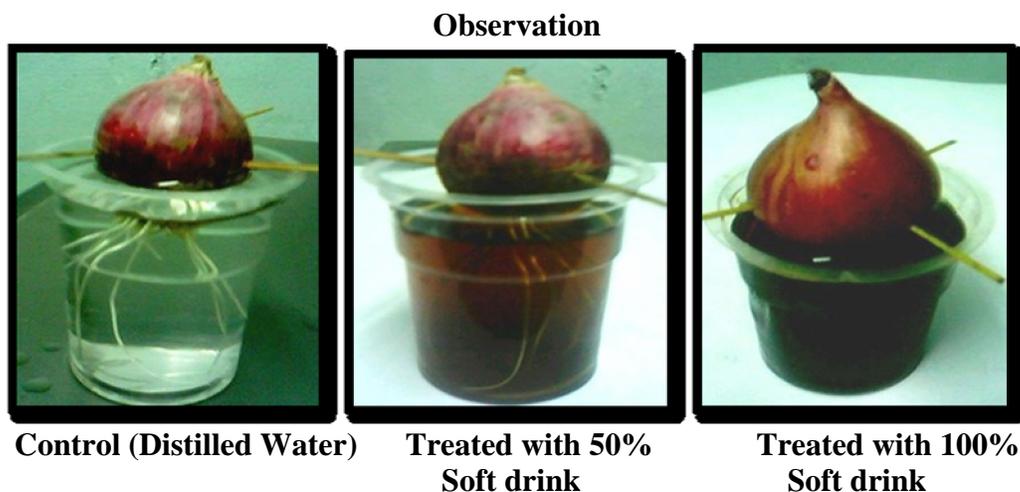


Figure 1- *Allium cepa* become rooted, than the onion bulbs were kept in the 50% and 100% concentrations of Soft drink (soft drink) for 6 and 8hrs.

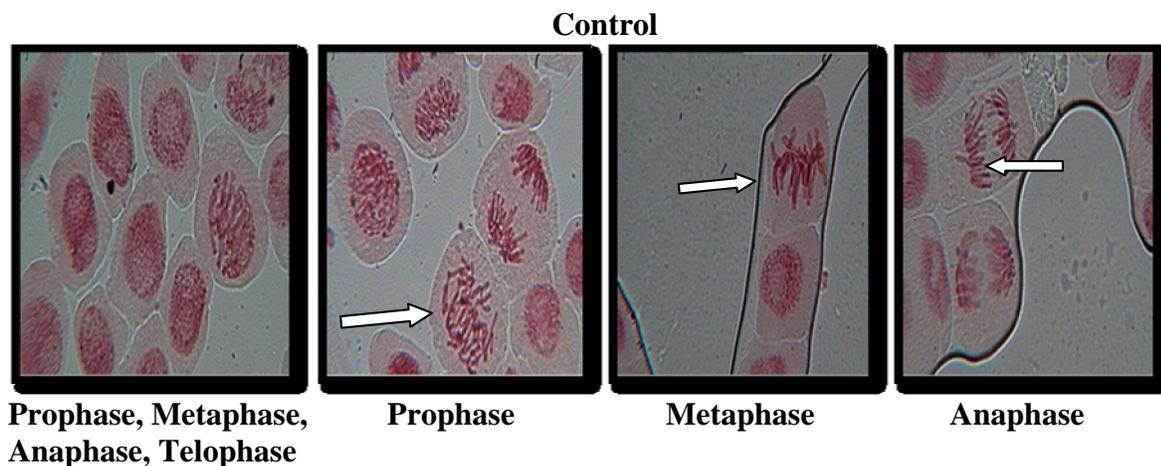


Figure.2 Different stages of mitotic cell division (Control)

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells(n)}}{\text{Total number of cells counted(N)}} \times 100$$

$$\text{Frequency of chromosomal aberration (CA)} = \frac{\text{Number of aberrant cell}}{\text{Total number of cells counted}} \times 100$$

Abbreviations;* CA= chromosomal aberration, MI = Mitotic index, N = The total number of Cells, n = The total number of dividing cells, I = Interphase, P= Prophase, M = Metaphase, A = Anaphase, T = Telophase.

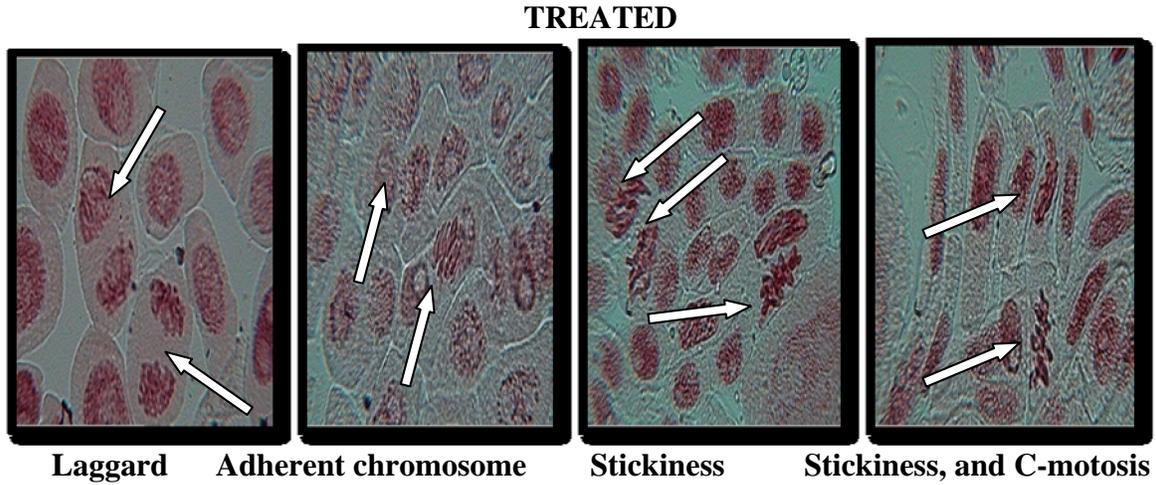


Figure.3 Effect of mitosis cells after treated by 50% Soft drink (soft drink)

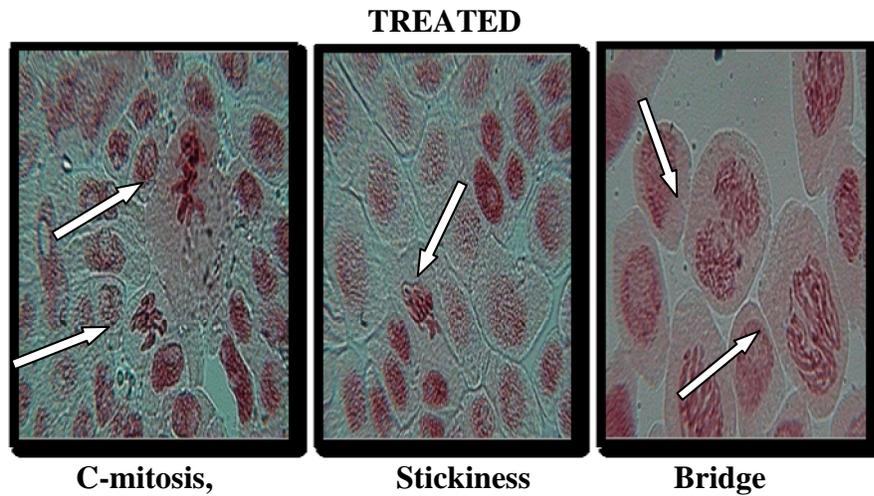


Figure.4 Effect of mitosis cells after treated by 100% Soft drink (soft drink)

Table.1 Showing mitotic index in root tip cells of *Allium Cepa L.* induced by Soft drink .
Mitotic index

Exp.	After 6 hrs.		After 8 hrs.	
	Control	50%	Control	50%
1.	3.41	1.90	3.20	1.93
2.	3.54	1.42	3.49	1.75
3.	3.84	1.90	3.20	1.93
4.	3.41	2.91	3.65	1.94
5.	4.03	1.93	3.68	1.85
Mean	3.63 ± 0.083	1.994 ± 0.155	3.204 ± 0.1820	1.848 ± 0.02

Table.2 Showing chromosomal abnormalities induced by soft drink (Soft drink) in root tip cell of *Allium Cepa L.*

Time (Hrs.)	Thums up Conc.	Total No. of Abnormal cells	MI	P	M	A	T
After 6 Hrs	Control	0	3.63±0.08 3	0	0	0	0
	50%	26.20± 2.88	1.99± 0.15	7.0±1.99	64.15±1.8 7	20.87±2.8 9	7.89± 2.80
After 8 Hrs	Control	0	3.20±0.18	0	0	0	0
	50%	23.60±2,4 5	1.84±0.02	11.71±1. 17	58.49±2.8 9	22.59±1.7 6	7.18± 1.02

The aberration also increased with duration of treatment. At 6 hrs. 50% concentration Soft drink treatment produced 60% metaphase, 22% anaphase, 10%prophase and 8% telophase chromosomal aberrations. whereas at 8hrs 50% concentration Soft drink treatment produced 53% metaphase, 25% anaphase, 8% prophase and 14% telophase chromosomal aberrations. Fig.7-13 showed that Soft drink induced different type of cell mitotic abnormalities like C-motosis, Stickiness, Adherent chromosome and Laggard of chromosomes at 50% concentrations.

According to Karl Sax. 1966. Cocoa contains theobromine, which is closely related to caffeine, and is as potent as coffee when prepared at the same concentration. The fact that Sanka coffee, and the soft drink Tab, which contain very little or no caffeine, do not induce chromosome aberrations, and that cafergot and No Doz tablets, which contain 0.1 gm of caffeine, are radiomimetic, is evidence that caffeine is the radiomimetic agent in coffee, tea, and Coca Cola.

In contrast to Karl Sax, in the present investigation it was found that the Soft drink (soft drink) which is a product of Coca cola at 50% concentration induced chromosomal abnormalities such as C-mitosis, Stickiness, Adherent chromosome and Laggard of chromosomes during 6hrs and 8hrs treatment.

From the present study it is clear that the soft drinks are harmful to the health and their regular intake must be avoided.

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