J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (5): 91 – 103, 2015

EVALUATION OF HIGH FRUCTOSE CORN SYRUP (HFC) GENOTOXIC EFFECT ON *Allium cepa* GENOME.

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ABSTRACT

High fructose corn syrup is the favorable sweetener for food and beverage production because of its advantages over table sugar in some applications. Previous studies showed that it causes metabolic disorders and obesity. In this study, the genotoxic effect of HFCS was investigated using the *A. cepa* root tips as a genetic model. Five concentrations, 2 mL/L, 1 mL/L, 0.5 mL/L, 0.25 mL/L, 0.125 mL/L, were employed. Because the highest two concentrations greatly inhibited mitosis they were excluded. Results of the other three concentrations showed inhibition of mitosis. This was reflected by decreasing of the mitotic index with increasing HFCS concentration. Also, increasing of HFCS concentration increased the number of prophase cells compared to the other three mitotic stages indicating that HFCS inhibited the progression from prophase to metaphase stage. Different types of chromosomal aberrations were detected including stickiness, vagrant and lagging chromosomes, bridge, disturbed metaphase, pole to pole metaphase. These types of chromosomal aberrations increased with increasing of the concentration of HFCS.

Keywords: chromosome aberrations, mitotic index, corn syrup, vagrant chromosome, pole to pole metaphase.

INTRODUCTION

High-fructose corn syrup (HFCS) is a liquid sweetener alternative to table sugar (sucrose) used in many foods, desserts, and beverages.It was developed in 1950s and used commercially during the 1960s of the last century. It has become the most successful food ingredients in modern history (Buck *et al*, 2001) because it overcomes the technical difficulties of using sucrose. It contains 42 – 55% of fructose and the rest is glucose (White, 2008). Its similarity to sucrose suggested that it would be metabolized in the same manner. The safety of HFCS had not seriously considered for long time simply because the scientific community used to draw the same conclusion that sucrose, fructose, glucose, and, lately, HFCS did not pose a significant health risk(Glinsmann and Bowman, 1993 Glinsmann *et al*, 1986;) although there was considerable speculation in the 1980s that fructose was responsible for several metabolic anomalies (Reiser *et al*, 1985; Hallfrisch *et al*, 1986). It was a surprise when HFCS was transformed from a favorable food ingredientinto a cause of obesity around the world in a short time. Recent research data showed that fructose coming from HFCS promotes over consumption of energy, weight gain, and the development of insulin resistance. Also, it was reported that it increases uric acid levels and is considered as one main factor of obesity (Angelopoulos *et al*, 2009).

Alium cepa has been used to evaluate chromosome aberrations during the mitotic cycle. It has been used to assess a great number of genotoxic and antigenotoxic agents, which contributes to its increasing application in

environmental monitoring. The *A. cepa* is commonly used as a test organism because it is cheap, easily available and handled and has advantages over other short-term tests. The mitotic index and chromosomal abnormalities are used to evaluate genotoxicity and micronucleus analysis used to verify mutagenicity of different chemicals. The *A. cepa* root chromosomal aberration assay was widely used to determine genotoxic and antigenotoxic effects of different plant extracts (Khanna and Sharma, 2013).

A. cepa is considered the most efficient plant genome to indicate the presence of mutagnic chemicals (Matsumoto, 2004; Matsumoto, 2006; Levan, 1938; Fiskesjo, 1985). *A. cepa* root chromosomal aberration assay has been used to evaluate the genotoxic potential of chemicals in theenvironment because of its sensitivity andgood correlation with mammalian test systems (Grant, 1982; Chauhan *et al*, 1999). Thus *A. cepa*is considered an efficient test organism for environmental monitoring, especially in contaminated aquatic environments (Parry and Tweats, 1976; Rank and Nielson, 1994; Jiang and Liu, 1999).The classical*A. Cepa* test was introduced by Levan (1938) to examine the effect of colchicines onmitotic spindles, howeverweak contaminations innaturally occurring watergave very little effectsin the original form of the test (Fiskesjo, 1979).After that, technical modifications in the *A.cepa*test have been designed toallow more comprehensive assessmentof weak and unknown contaminations, such as the complex mixtures, whichrepresent most of the environmentaland the pure samples (Fiskesjo, 1985; Grant, 1982; Rank, 2003).

A. cepa root chromosomal test was modified for environmental monitoring for evaluation of soluble and in soluble compounds in water and complex mixtures (Fiskesjo, 1985).Other modifications included germinating onion bulbs in the chemical to betested and take the final observations within few days without initialtreatment with pure water. This method of treatment is more similar to conditions in nature. This new modification of the *A.cepa* root chromosomal aberration assay has also been convenient for studying the action of different concentrations of known toxic chemicals. More modifications were made to the *A.cepa* test, making it even more efficient to analyze various known complex mixtures. The system was simplified in a way that aberration in the anaphase and telophase stages is considered (Rank and Nielson, 1994; Rank, 2003).*A. cepa* assay was used to test the genotoxic effect of food preservatives including sodium metabisulfite (Rencuzogullari *et al*, 2001), sodium benzoate, boric acid, citric acid, potassium citrate and sodium citrate (Turkoglu *et al*, 2007), potassium metabisulp-hite (Kumar *et al*, 2007), monosodium glutamate (Adeyemo and Farinmade, 2013); pesticides (Bolle *et al*, 2004; Badr, 1983; Sakamoto *et al*, 1981); metals (Achary *et al*, 2007; Marcano *et al*, 2006; Ivanova *et al*, 2005; Wierzbicka *et al*, 1999); waste water cleaning (Firbas*, et al*, 2013; Sik *et al*, 2009); and herbicides (Jabee *et al*, 2008; Srivastava *et al*, 2009).

In this study, the controversial genotoxic effect of HFCS was evaluated using *A. cepa* modified root chromosomal assay by estimating mitotic index and chromosomal aberrations in the anaphase and telophase stages.

MATERIALS AND METHODS

A number of 60 onion bulbs of about the same size were selected and cleaned by peeling the outer brown layer of each bulb and removing the dried basal root plate. High Fructose Corn Syrup (HFCS) (Goody, USA) was purchased from the local market. The average daily consumption of HFCS is 32 g per capta per day (USDA, ERS, Sugar and Sweeteners Outlook, http://www.ers.usda.gov) in 2013. This gives an estimate of about 0.45 g/kg/day for the average of 70 kg person. Assuming 1 kg is equivalent to 1 L of water. Based on this data, four concentrations (2 mL/L, 1 mL/L, 0.5 mL/L, 0.25 mL/L, 0.125 mL/L) were prepared from HFCS and distributed into culture bottles. The 60 bulbs were distributed on the 5 treatments, 10 bulbs per each, and 10 bulbs were grown in water as control. Using anaphase–telophase assay (Rank, 2003) chromosomal aberrations were recorded.

Fixation and Staining

Adventitious roots were cut and fixed in 3:1 ethanol: glacial acetic acid and kept for 24 hrs. Roots were transferred to 70% ethanol and kept at 4ºC until used. Acetocarmin, 1%, was prepared in 45% acetic acid and boiled for 30 min, filtered, and used for staining. Roots were boiled in 45% acetic acid for 5 min for softening. About 2 mm of the root tip was cut and squashed in one drop of acetocarmin.

Microscopic Investigation

One thousand cells were investigated from three roots of each treatment. Various aberrations were detected. Vagarant chromosome is detected as fast moving chromosome beyond its chromosome group toward the poles in the anaphase or telophase stages. Pole to pole metaphase was recognized as the alignment of chromosomes on the pole to pole line not the equator of the cell. C-mitosis also was recorded as the random distribution of metaphase chromosomes in the cell lacking their alignment at the cell equator.

Statistical Analysis

Data were scored in triplicates. SPSS16.0 software was used for statistical analysis.

RESULTS AND DISCUSSION

Different concentrations of HFCS were administered on *A. cepa* adventitious roots to investigate its mutagenic effect. Five concentrations were used; 2 mL/L, 1 mL/L, 0.5 mL/L, 0.25 mL/L, and 0.125 mL/L. The higher two concentrations of 2 mL/L and 1 mL/L showed high inhibition of mitotic division, therefore they were excluded and used the data of the other three concentrations. All concentration of HFCS showed a degree of inhibition for mitotic activity. The lower concentration 0.125 showed less inhibition whereas the two higher concentrations (0.5 mL/L, 0.25 mL/L) showed significant inhibition of mitotic index compared to the negative control (Table 1). The two higher concentrations also revealed higher inhibition of all mitotic stages. This was indicated in the mitotic index estimations. Mitotic index ranged from 8.9% in the control group to 4.5% in the highest concentration of HFCS (0.5 mL/L),

whereas it was 7.9% and 7.1% in the 0.125 mL/L and 0.25 mL/L consecutively (Table 1). Although the total number of cells in each mitotic stage is very close, there are some differences in the number of divided cells in each concentration compared to the control group (Table 1).In the control group prophase cells were very close to the number of cells in the other three mitotic stages. With increasing HFCS concentration there is a general decrease in the total dividing cells and more interestingly, there is an increase in the number of cells in the prophase stage compared to the other three stages. This may indicate that the HFCS inhibit the progression from one mitotic stage to the following stage, prophase in this case. This led to the accumulation of prophase cells and may cause to inhibition of whole mitotic process afterwards. The cytotoxicity of a test chemical or compound can be determined based on the increase or decrease in the mitotic index (MI) (Smaka-Kincl *et al*, 1996). Reduction in mitotic index may be due to the inhibition of DNA synthesis or the blocking in the G2 phase of the cell cycle (Sudhakar *et al*, 2001). Several other chemicals have been reported to inhibit mitosis (Turkoglu, 2007). The cytotoxic level can be determined by the decreased rate of mitotic index. A mitotic index decrease below 22% of negativecontrol causes lethal effects on test organism while a decrease below 50% has sub lethal effects (Sharma and Vig, 2012) and is called cytotoxic limit value. Several investigators have used mitotic index as an end point for the evaluation of genotoxicity or antigenotoxicity of different chemical treatments (Sharma *et al*, 2012; Panda and Sahu, 1985).

Table (1): Number of dividing cells in the four mitotic stages in a 1000 counted cells / concentrations and miotic index (MI) in *A. cepa* roots after treatment with HFCS.

	Prophase	Metaphase	Anaphase	Telophase	Total	MI
Control	20	23	24	22	89	8.9%[a]
0.12	22	19	18	20	79	7.9%[ab]
0.25	21	17	17	16	71	7.1%[b]
0.5	16	10	9	12	47	4.5%[c]
Total	79	69	68	70	286	
%	27.6%	24.1%	23.8%	24.5%		

Numbers with the same letter in MI column are not significant. Means were compared based on the LSD value.

Mitotic chromosomal aberrations

Chromosomal aberrations (CAs) caused by the three concentrations of HFCS were investigated on the anphase-telophase stages. Various types of chromosomal aberrations were recorded in the HFCS treated roots. This include vagrant chromosome (VC), lagging chromosome (LC), stickiness (S), c-mitosis (C-M), pole to pole metaphase (PPM), bridge (B), and disturbed metaphase (DM).Total chromosomal aberrations ranged from 0.5% in the negative control to 2.9% in the highest concentration of HFCS. The aberration percentages were 1.1% and 2% in the 0.125 mL/L and 0.5 mL/L concentrations consequently. Only the higher two concentrations (0.25 mL/L and 0.5 mL/L) showed significant rates of chromosomal aberrations (Table 2). To evaluate the different chromosomal abnormalities, several types of CAs

are considered in different stages of mitosis (Prophase, metaphase, anaphase and telophase). CAs are grouped into two types, clastogenic and physiological aberrations. Clastogenic aberrations include bridges, chromosomal breaks and ring chromosomes whereas physiological aberrations included stickiness, vagrant chromosome, lagging chromosomes, c-mitosis, pole to pole metaphase, and disturbed metaphase.

Different levels of stickiness were recorded in this study. One stickiness was recorded in the control compared to 2, 3, 5 for 0.125, 0.25, 0.5 mL/L concentration of HFCS (Table 2, Figure 1). Stickiness of chromosomes is a result of increased chromosomal contraction and condensation or might from the depolymerization of DNA and partial dissolution of nucleoproteins. Chromosome stickiness indicates the toxic effects of a chemical or a compound. It is usually an irreversible type and may lead to cell death. Induction of stickiness by HFCS is in agreement with results obtained from previous studies that examined the effects of different chemicals on different genomes (Turkoglu, 2007; Rencuzogullari *et al*, 2001).

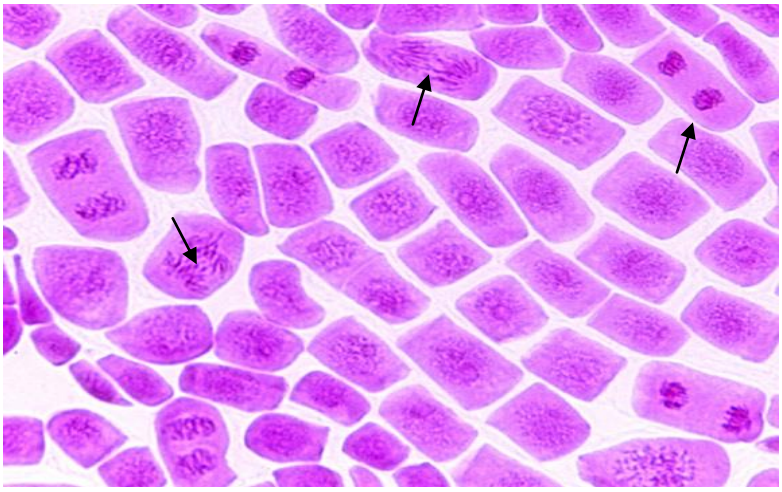

Figure (1): Anaphase bridge and telophase stickiness in *A. cepa* root tips after treatment with HFCS.

Vagrant chromosome (VC) was detected in all concentration of HFCS as well as in the control group. One VC was recorded in the control group, whereas 2, 3, 5 VCs were recorded for 0.125, 0.25, 0.5 mL/L concentrations of HFCS (Table 2, Figure 6a, b,c) Vagrant chromosome is chromosome which moves faster than its chromosome group to either pole of the cell. This could lead to unequal separation of number of chromosomes in the daughter cell. Vagrant chromosomes have been observed by many workers indifferent studies (Sondhi *et al*, 2008), (Seehy, 1989).

Lagging chromosome is the result of the failure of a chromosome to get attached to the spindle fiber and to move to one of the two cellular poles. Lagging chromosomes were recorded in this study. The control group showed 1 lagging chromosome, whereas the 0.125, 0.25, 0.5 mL/L concentrations of HFCS gave

1, 2, 4 lagging chromosomes consequently (Table 2, Figure 6c). (Turkoglu, 2007) reported the induction of lagging chromosomal aberrations in response to food additives treatments.

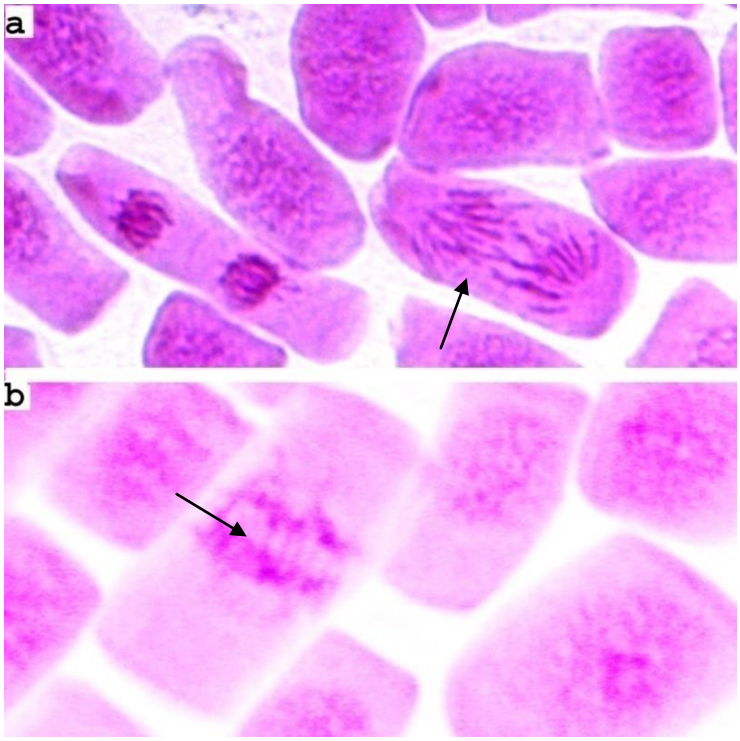

Figure (2): Anaphase bridge *A. cepa* root tips after treatment with HFCS.

Table (2): Chromosomal aberrations in *A. cepa* roots after treatment with HFCS,1000 cells were counted/treatment.

Treatment	Type of aberration							%CA
	S	VC	LC	c-M	PPM	B	DM	
control1	1	1	1	2	0	0	0	0.5%[a]
0.12	2	2	1	2	2	1	1	1.1%[a]
0.25	3	3	2	4	4	2	2	2%[b]
0.5	5	5	4	6	4	2	3	2.9%[c]

Numbers with same letter in the %CA column are not significant. Means were compared based on the LSD value.S: Stickiness, VC: Vagrant chromosome, LC: lagging chromosome, PPM: pole to pole metaphase, B: bridge, DM: disturbed metaphase, CA: chromosomal aberrations.

The term c-mitosis was introduced by (Levan, 1938) and described that colchicines prevents the assembly of the spindle fibers and results in scattering of the chromosomes over the cells. In this study, 1 C-mitosis was recorded in the negative control group and 6 were recorded in the highest concentration (0.5 mL/L), whereas 2 and 4 were recorded in the 0.125 mL/L and 0.25 mL/L concentrations (Table2, Figure 4a). C-mitosis was induced by

a number of pesticides. Mercury,carbamates, and dieldrin.propham, chlorpropham, carbaryl, and benomylareare extremely active C- mitotic inducer chemicals(Chauhan *et al*, 1999). Several investigators reported that C-mitosis was induced by different types of food additives in plant cells (Turkoglu, 2007; Rencuzogullari *et al*, 2001).

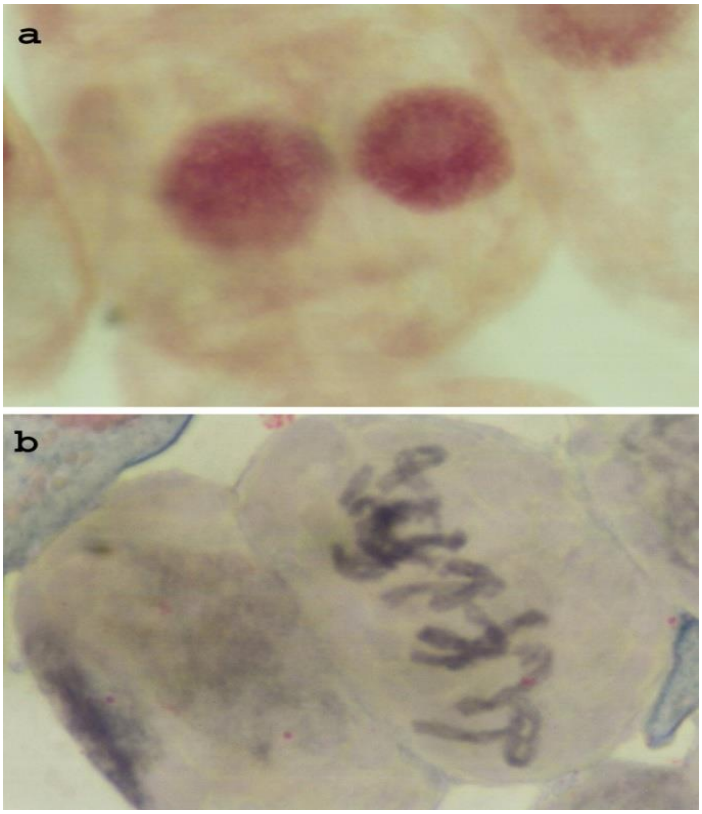

Figure (3): a. Binucleate, b. ghost cell and pole to pole metaphase in *A. cepa* root tips after treatment with HFCS.

Pole to pole metaphase (PPM) is another aberration that was detected in the HFCS treated roots. There were 2, 4, and 4 PPM in the 0.125 mL/L, 0.25 mL/L, and 0.5 mL/L concentrations consecutively (Table 2, Fig 3b, 4b), whereas it was not detected in the control group. PPM is an abnormal alignment of chromosomes during metaphase where chromosomes are not aligned on the cell equator but aligned from pole to pole.

Chromosome bridge was not recorded in the control group, whereas 0.125, 0.25, 0.5 mL/L concentrations of HFCS gave 1, 2, 2, bridges consequently (Table 2, Figure 1, 2a, b). Similar results were obtained of the effect of sodium benzoate, boric acid, citric acid, potassium citrate and sodiumcitrate (Turkoglu, 2007). Disturbed metaphase was one of the recorded chromosome abnormalities in the current study. It was not detected in the control group. The

0.125, 0.25, 0.5 mL/L concentration of HFCS showed 1, 2, 3, of disturbed metaphases consequently (Table 2, Figure 5a, b, c).

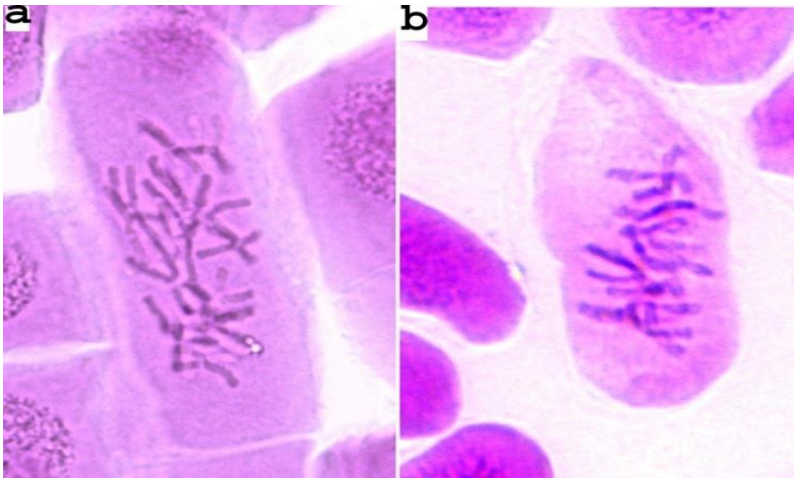

Figure (4): a. C-mitosis, b. pole to pole metaphase in *A. cepa* root tips after treatment with HFCS.

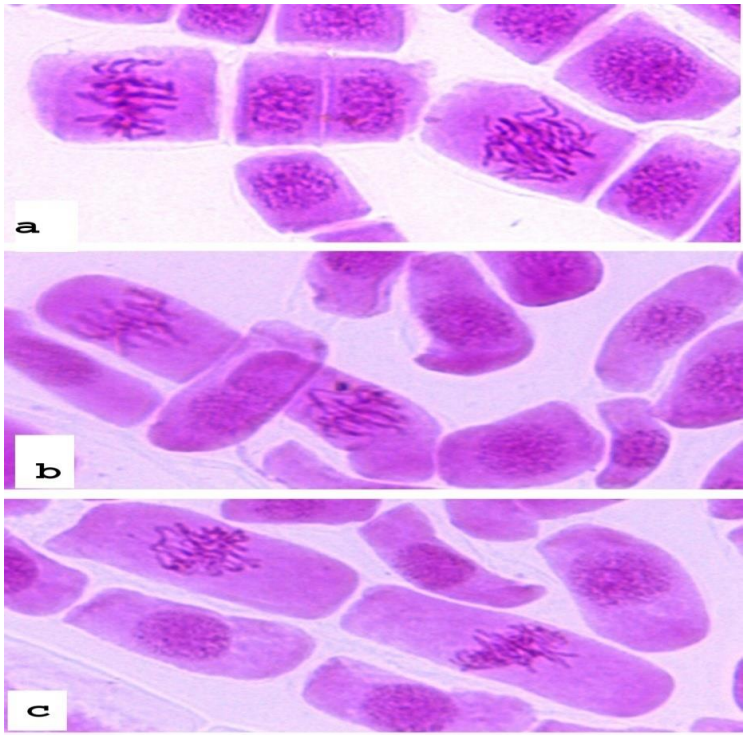

Figure (5): Disturbed metaphase in *A. cepa* root tips after treatment with HFCS.

Binucleated cells were also detected in this study (Figure 3a). Binucleated cells were reported by several investigators in various organisms after treatments with different chemicals (Gomurgen, 2005). Binucleated cells usually appear when the treatment with a chemical or a compound causes inhibition of cytokinesis after mitosis.Ghost cells were observed in the highest concentrations (0.5 mL/L) (Figure 3b). Ghost cell is defined as a dead cell of which the general shape is visible but nucleus and structures are not stainable. Cell death known as apoptosis is a biological process that occurs in living organisms. It was induced by high concentrations of toxic chemicals. Ghost cells were observed by the Celik and Aslanturk (2006) during their evaluation of the genotoxicity of *Inula viscosa* leaf extract using *A. cepa* test.

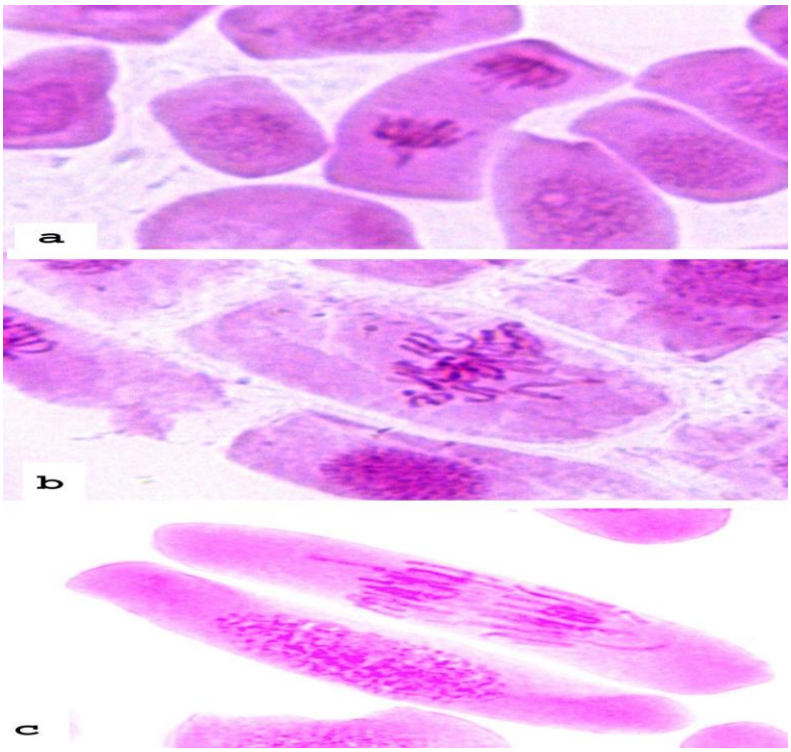

Figure (6): Vagrant chromosome in teleopahse (a), vagrant chromosome in metaphase (b), and vagrant and lagging chromsosome in anaphase (c) in *A. cepa* root tips after treatment with HFCS.

Recommendation: The results of this study recommend that we have to decrease if not exclude the use of HFCS from human usage..

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تقييم الاثرالضار وراثيا لشراب الذرة عالي الفراكتوز علي جينوم نبات البصل *Allium cepa*

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يعد شراب الذرة عالي الفراكتوز المحلي المفضل في صناعة الاغذية والمشروبات بسبب مميزاته في بعض التطبيقات الصناعية مقارنة بسكر المائده. أوضحت الدراسات السابقة أن شراب الذره عالي الفراكتوز يسبب إختلالات ميتابولزمية وله دور أساسي في السمنه. في هذه الدراسة تم تقييم التأثير الضار وراثيا لشراب الذره عالي الفركتوز بإستخدام جذور نبات البصل وهو اختيار معتمد من وكاله حمايه البيئه الامريكيه. تم إستخدام خمسة تركيزات من شراب الذرة وهي 2 مل/لتر، 1 مل/لتر، 0.5 مل/لتر، 0.25 مل/لتر، 0.125 مل/لتر. التركيزان الأول والثاني (2 مل/لتر، 1 مل/لتر) تسببا في خفض معدل الإنقسام بدرجة كبيرة لذلك تم إستبعادهما.أظهرت نتائج الثلاثة تركيزات الأخري تثبيط معدل الإنقسام الميتوزي في صورة خفض معدل الإنقسام بزيادة تركيز شراب الذرة. كذلك أدت زيادة تركيز شراب الذرة إلي زيادة نسبة الخلايا في مرحلة الدور التمهيدي مقارنة بباقي أدوار الإنقسام، مما يدل علي أن شراب الذره يثبط إنتقال الخلايا من الدور التمهيدي إلي الدور الإستوائي. تم رصد أنواع مختلفة من الشذوذات الكروموسومية منها اللزوجة الكروموسومية، الكروموسوم المتسارع، الكروموسوم المتلكىء، الجسر الكروموسومي، الدور الإستوائي المختل، الدور الإستوائي المنتظم قطبيا. أوضحت النتائج أن هناك زيادة معنوية في هذه الشذوذات بزيادة تركيز شراب الذرة عالي الفراكتوز. ويوصى البحث الحالى بعدم استخدام هذا الشراب فى صناعه الاغذيه والمشروبات.