Asian J. Adv. Basic Sci.: **2016, 4***(2),* 27-40
ISSN (Print): 2454 – 7492
ISSN (Online): 2347 – 4114
www.ajabs.org

Potential Mutagenicity of Some Artificial Sweeteners Using *Allium* Test

Rehab M. Rizk[1*], Magda I. Soliman[2] and Aya A. Rashed [3]

[1, 2 & 3] *Botany Department, Faculty of Science, Mansoura University, EGYPT*
[*] *Correspondance: E-mail: new_fm8@yahoo.com*

(Received 02 Apr, 2016; Accepted 14 Apr, 2016; Published 18 Apr, 2016)

ABSTRACT: Different concentrations of three artificial sweeteners (aspartame, sorbitol and sucralose) as well as two different exposure times (24 and 48 hrs) were applied to investigate the cytogenetical, biochemical and molecular alterations in onion (*Allium cepa* L.) meristematic cells. The results indicated that all the treatments induced different types of chromosomal abnormalities in non dividing cells (interphase) as well as different mitotic stages. The abnormality observed in interphase was micronucleus. At prophase stage small or large micronucleus appeared. Metaphase aberrations represent the most conspicuous type of abnormalities. A number of abnormalities at anaphase and telophase were noticed such as bridges, diagonal, disturbed and late separation. Biochemical study includes protein profile using polyacrylamide gel electrophoresis (SDS-PAGE) technique. The electrophoretic analysis of extracted proteins showed 18 bands with the molecular weight ranging from 41.70 to 96.36 KDa. There are 8 monomorphic bands and 10 polymorphic bands. Molecular study using inter simple sequence repeats DNA (ISSR-DNA) technique. Four primers were used and the total number of bands 34 from which 12 were common in control and all treatments of the artificial sweeteners and 22 were polymorphic bands.

Keywords: Aspartame; sorbitol; sucralose; artificial sweetener; abnormalities; ISSR and protein profile.

INTRODUCTION: Sugars are inseparable part of our life as these add sweetness in our food, but it cannot be consumed in large amount as it can be one of the potent reasons of many degenerative diseases. So, artificial sugar is the option which provides us sweetness with less or no calories. Due to potently sweetness, sweeteners have been continued to attract consumers as sugar replacer in food, beverages and other applications as flavour enhancer. It has been proven beneficial in weight loss and diabetes management. They are hypoglycaemic, non mutagenic and non-carcinogenic in nature (Jain and Grover, 2015). The US Food and Drug Administration regulate artificial sweeteners as food additives.

In recent years there has been a steady and important increase in consumer demand for low calorie products in the forms of food and drinks. As a result there is growing interest among healthcare professionals and the general public to learn more about low calorie sweeteners. Consuming artificial sweeteners increasing every year and more than 6,000 food products contain these sweeteners (Myers, 2007). The global market of artificial sweeteners is more than 500 million dollars (Bennett, 2013). Artificial sweeteners / low calorie sweeteners are synthetic sugar substitutes but may be derived from naturally occurring substances, including herbs or sugar itself.

Sweeteners are being categorized as nutritive and non-nutritive sweeteners relying on whether these are the calorie source. Nutritive sweeteners consists mainly the monosaccharide polyols for example sorbitol, mannitol and the disaccharide polyols such as maltitol and lactitol. These are approximately similar in sweetness to sucrose. Non-nutritive sweeteners are regarded as artificial sweeteners and provide about negligible calories examplified as saccharin, aspartame, sucralose, acesulfame-K, stevioside, cyclamate. The non-nutritive sweeteners are being commonly used in foods such as diet sodas, cereals and sugar-free desserts, and are being recommended for weight loss and for individuals suffering from glucose intolerance and type 2 diabetes mellitus (Nishal, 2015).

The most popular artificial sweeteners: acesulfame k (acesulfame potassium), aspartame, cyclamates, neotame, saccharin, steviol glycosides and sucralose.

Aspartame is non nutritive sweetener, which is produced by combining the two amino acids L- phenylalanine and L- aspartic acid by a methyl- ester link Fig. (1) (Lean and Hankey, 2004). It has a sweet taste and 180-200 times sweeter than sucrose. However it is 200 times sweeter than sugar but contains almost zero calories per serving. It is sold under brand name “NutraSweet” and “Equal”. It accounts for 32% of the global high intensity sweetener market. Major market for it is soft drinks and table top sweetener. It is also used in pharmaceutical tablets and dry syrups, yogurt, dairy products, dry mix products and bars.7

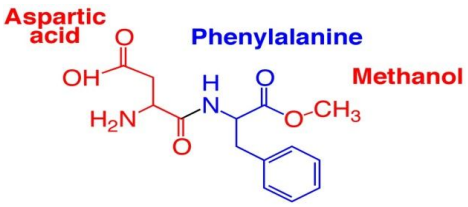

Figure 1: Chemical structure of aspartame.

Sorbitol, which has a systematic name d-glucitol is a 6 carbon sugar alcohol Fig. (2). This polyol can be naturally found in apples, pears, peaches, apricots and nectarines as well as in dried fruits, such as prunes, dates and in some vegetables (Ortiz *et al.*, 2013 and Barbieri *et al.*, 2014).

Sorbitol is resistant to digestion by oral bacteria which break down sugars and starches to release acids that may lead to cavities or erode tooth, according to the Us, FDA and European Commission, products containing this sugar alcohol can have a health claim on the labeling stating " does not promote tooth decay" (EFSA, 2011) .

Due to the non carcinogenic properties, it is used in products for special nutritional purposes designate for people with diabetes and it also finds its applications in pharmaceuticals and cosmetics. Sorbitol can be added to a wide variety of products, including sugar free candies chewing gums and sugar free foods such as frozen desserts and baked goods. Sorbitol similar to xylitol and erythritol, it has a negative heat of solution and thus it gives cooling sensation in the mouth. Sorbitol has a sweet, cool pleasant taste. Besides acting as a sweetener, it is also an excellent humectant, softener, texturizing and anti-crystallizing agent (Jonas and Silverira, 2004 and Ortiz *et al.*, 2013).

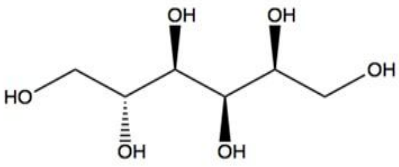

Figure 2: Chemical structure of sorbitol.

Sucralose is a white crystalline powder and is almost odorless. It has high melting temperature (Grotz and Munro 2009; Tollefsen *et al.*, 2012 and Lange *et al.*, 2012) and is considered thermally stable. It is high potency sweetener, made up from sucrose by a process of chemical modification that results in the enhancement of the sweetness intensity, retention of a pleasure sugar like taste and creation of a very stable molecule. Pure sucralose is white, free flowing powder, intensely sweet, practically odour less and freely soluble in water. It is most commonly sold under brand “Splenda”. It is appropriate for beverages, canned fruit, chewing gum, dairy, confectionary and baked products.

Sucralose is generally prepared by selectively substituting three hydroxyl groups of sucrose with three chlorine atoms Fig.(3). Sucralose is nearly 600 times sweeter than sucrose and this intense sweetness result in its use in food product and pharmaceuticals. The recent consumption of sucralose is ~2,000 tons per year in which the USA leads the consumption by using more than ~1,500 tons per year. The consumption in Europe is ~400 tons per year.

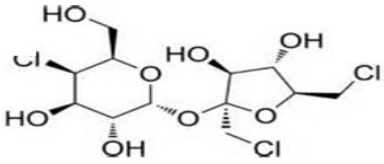

Figure 3: Chemical structure of sucralose.

Higher plant bioassays have been recommended for use in mutation screening and monitoring environment pollutants. They are now recognized as excellent indicators of cytogenetic and mutagenic effects of environmental chemical and are applicable for the detection of environmental mutagens (Sang and Li, 2004). Mutagenic environmental effects may be analyzed by microscopic parameters such as the types and frequencies of chromosomal aberrations and abnormal cell division (Yi and Meng, 2003).

Cytogenetic tests are considered to be indicator of cytotoxicity, genotoxicity, genetic variability and estimation of the mutagen potency in meiotic division (Maluszynska and Juchimiuk, 2005). On the other hand, proteins being primary gene products of plant’s DNA hence, any observed variation in protein systems induced by oxidative stresses or any mutagen is considered as a mirror for genetic variations (Yadav, 2008). Variation in electrophoretic pattern of proteins of the plant organs has been used very successfully to identify mutants (Stegemann, 1984). Also electrophoretic SDS- protein profiles were successfully used by some authors to establish biochemical genetic finger print of many plants (Ghareeb *et al.*, 1999; Soliman *et al.*, 2014a &b). Determination of protein molecular weights via polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) is a universally used method in biomedical research (Rath *et al.*, 2009). Proteins represent the direct products of DNA code (Jones and Luchsinger, 1987).

ISSR is a kind of simple and quick technique and a reliable marker system for many organisms especially plants (Zietkiewicz *et al.*, 1994). ISSR markers have a better reproducibility and a greater number of total polymorphic and lower relative costs compared with RAPD (Ge and Sun, 1999 & Mattioni *et al.*, 2002 and

Reddy *et al*., 2002). ISSR markers are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (Reddy *et al.*, 2002).

Food sweeteners are widely used in food, but little is known about their genotoxic effects. Thus the purpose of this study was to evaluate the potential genotoxic effects of food sweeteners such as aspartame, sorbitol and sucralose on *Allium cepa* which used as a test material. This goal is achieved by using a variety of tools as mitotic index, phase index, chromosome abnormalities, protein profile by SDS –PAGE and Inter simple sequence repeats (ISSR) technique.

MATERIALS AND METHODS:

Sample collection: The common onion bulbs (*Allium cepa* L.) (c.v. Giza 20) of appropriate size were obtained from the Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt. *Allium cepa* is a test plant and used to study the cytological effects of three artificial sweetners are aspartame, sucralose and sorbitol.

Chromosomal aberration assay: The bulbs of *Allium cepa* treated with three artificial sweetners. Four concentrations for each artificial sweetener applied for two different exposure times 24h. and 48h. The root tips were fixed in carny's solution (1glacial acetic acid/ 3 ethanol ratio) and stored in refrigerator at least for 48 h., then hydrolyzed in 1N HCL at 60^{o} c for 3-5 min. The root tips were stained using a double staining method combining the modified carbol fuchsin reaction (Koa, 1975 a&b) where the root tips were put in carbol fuchsin overnight then in 2 % aceto-orcein stain for 2-4 h. the mitotic zones were immersed in a drop of 45 % acetic acid on a clean slide and squashed. At least 2000 cells from 20 slides of each treatment were examined. The cells were recorded as normal or aberrant in the different stages of mitotic division: interphase, prophase, metaphase, anaphase and telophase. All cells with aberration were counted and photographed using Olympus camera (SC35 type 12 mode). Data of the differently treated groups of root tips were statistically analysed using T- test to determine the significance of the difference between treatments and control at 0.05 level of probability (Snedecor and Cochran, 1976).

Protein analysis: Seed proteins electrophoresis followed the method for discontinuous SDS-PAGE technique of Laemmli (1970).

DNA extraction: Genomic DNA of *Allium cepa* treated with aspartame, sucralose and sorbitol were extracted according to Dellaporta *et al.,* (1983).

Inter Simple Sequence Repeats DNA (ISSR-DNA): Four primers were tested to amplify the isolated DNA. These primers listed in Table (1) and their composition has been arbitrarily established.

Table 1: List of primers and their nucleotide sequence used in ISSR analysis.

Primers	Sequence
841	$(GA)_8$ CC
845	$(CT)_8$ AGG
848	$(AC)_8$ AGC
856	(AC)8 CTA

RESULTS AND DISCUSSION: Sugar free food products are sweetened by sugar substitutes that are commonly referred to as non- nutritive sweeteners, low calorie sweeteners or alternative sweeteners. All sugar substitutes taste similar to sugar but contain few to no calories and produce a low glycemic response. These sweeteners are widely used in processed foods including baked goods, carbonated beverages, powdered drink mixes, candy, puddings, canned foods, jams, jellies and dairy products (Findikli and Turkoglu, 2014).

Food additives are widely used in factory- made foods. Therefore, they must be completely safe for human consumption. Nevertheless, scientific studies on these additives have yielded unfavorable results especially in gene toxicity and carcinogenicity tests. Genotoxicity pertains to all types of DNA damage. Agents that interact with DNA and /or associated cellular components (e.g. the spindle apparatus) or enzymes (e.g. topoisomerases) are considered genotoxins (Robinson, 2010 and Jouyban and Parsa, 2012).

Allium cepa root tips were treated with three artificial sweeteners with four concentrations for each one. Also it was treated for two different exposure time (24h and 48h). The effect of different treatments of those artificial sweeteners on mitotic index (%MI), phase index (%PI), types and total abnormalities (%Tab) are given in Tables (2&3&4) and expressed graphically in Figs (4&5). For each treatment, about two thousands cells were examined in each sample and the mean of mitotic index was calculated in each treatment.

The results demonstrate that the majority of the treatments induced considerable decreasing or increasing effect on mitotic indices than that of control of exposure time 24h and 48h (6.13% & 12.01%) respectively, whereas aspartame 800 mg for 24h showed increasing of MI% (7.24%), while aspartame 1000 mg for 48h showed decreasing of MI% (4.06%) compared with control. Sorbitol 7.5gm for 24h showed increasing of MI% (8.28%), while sorbitol 10gm for 48h

showed decreasing of MI% (5.24%) compared with control. According to sucralose, 3gm for 24h showed increasing of MI% (9.47%) compared with control (6.13%), while sucralose 7gm for 48h showed decreasing of MI% (6.46%) compared with control (12.01%).

The cytotoxicity levels of an agent can be determined by the increase or decrease in the mitotic index (Fernandes *et al.*, 2007). Decreasing mitotic activity may be due to a slower progression of cells from S (DNA synthesis phase) to M (mitosis) phases (Wu *et al.*, 2010). On the other hand, MIs higher than the control indicating an increase in cell division that can be harmful to the cells leading to a disordered cell proliferation and to formation of tumour tissues. MI measures the proportion of cells in the M-phase of cell cycle and its suppression could be interpreted as cellular death or delay in the cell proliferation kinetics (Rojas *et al.*, 1993).

According to the percentage of different mitotic phases, it could be observed that increase or decrease in percentages of prophase, metaphase, anaphase and telophase.

According to aspartame, the highest percentage of prophase (31.46%) after treating *Allium cepa* root tips with aspartame 400mg for 24h but the lowest percentage (20.18%) recorded in aspartame 1000mg for 48h compared with control (27.94% & 36.47%) for 24h & 48h respectively. The lowest percentage of metaphase (13.33%) recorded in 400 mg for 24h, while the highest percentage (45.61%) at 1000mg aspartame for 48h as compared with control (29% & 17%) for 24h & 48h respectively. The percentage of anaphase reached the maximum value after treating *Allium cepa* root tips with aspartame 400mg for 24h as (43.99%) and the lowest value at aspartame 1000mg for 48h (19.74%) compared with control (30.83% & 24.36%) for 24h & 48h respectively. The minimum percentage of telophase (11.21%) recorded in 400mg for 24h and the maximum percentage (24.14%) at 400mg aspartame for 48h compared with control (12.23% & 22.17%) for 24h and 48h respectively.

According to sorbitol, the percentage of prophase reached a minimum value after treating *Allium cepa* root tips with sorbitol 10gm for 48h (8.85%) compared with control (36.47%) for 48h but the highest percentage of prophase (28.13%) recorded in 5 gm sorbitol for 48h. The maximum percentage of metaphase (59.38%) observed in 10gm sorbitol for 24h compared with control (29%) for 24h. For anaphase, the minimum value was (19.27%) at sorbitol 10gm for 24h and reached the maximum value at sorbitol 7.5gm for 48h as (30.21%) compared with control (30.83% & 24.36%) for 24h and 48h respectively. The percentage of telophase reached the minimum and the maximum value at 10gm sorbitol were (11.94% & 26.56%) for 24h and 48h respectively compared with control (12.23% & 22.17%) for 24h and 48h respectively.

According to sucralose, the percentage of prophase reached a minimum value after treating *Allium cepa* with sucralose 1gm for 24h as (13.80%) & at 3gm for 48h (16.93%) compared with control (27.94% & 36.47%) for 24h & 48h respectively. The lowest percentage of metaphase (24.72%) at sucralose 5gm for 24h and the maximum percentage (53.07%) recorded at sucralose 3gm for 48h compared with control (29% & 17%) for 24h & 48h respectively. The lowest percentage at anaphase (18.23%) in 1gm sucralose for 24h and the highest value (32.61%) recorded in sucralose 1gm for 48h as compared with control (30.83% & 24.36%) for 24h and 48h respectively. The maximum percentage of telophase (28.08%) found in 5gm sucralose for 24h and the minimum percentage (10.36%) found in 3gm sucralose for 48h compared with control (12.23% & 22.17%) for 24h and 48h respectively.

The results obtained in this investigation showed that the different treatments with three artificial sweeteners induced different mitotic changes on root tips cells of *Allium cepa*. Such changes vary from changes of mitotic index of meristematic cells, changes in phase index and the production of large number of chromosomal aberrations. These changes appeared in varying degrees depending on the duration and the concentration of the treatment. Mitotic inhibition has been attributed to number of factors among these factors is the blocking of mitotic cycle during interphase (Soliman and Ghoneam, 2004).

It could be observed that, there is a slight decrease or increase in the percentages of prophase, metaphase, anaphase and telophase. This may indicate that the treatments with artificial sweeteners affected the relative duration of each stage of mitosis as suggested by (Barakat and Hassan, 1997). The production of chromosome abnormalities by chemical compounds is regarded as a reliable evidence of the genotoxicity (Grant, 1982). Chromosomal aberrations occur due to lesions in both DNA and chromosomal spindle protein causing genetic damage (Amin, 2001). The results indicated that all the treatments induced different types of chromosomal abnormalities in non dividing cells (interphase) as well as different mitotic stages.

These abnormalities affected almost all the stages of mitosis. The abnormalities observed in interphase were the formation of small and or large micronucleus. At prophase stage small or large micronucleus

appeared. Metaphase aberrations represent the most conspicuous type of abnormalities. The most common abnormalities at this stage were non congression, stickiness, chromosome ring, oblique, disturbed and two groups. A number of abnormalities at anaphase and telophase were noticed. These abnormalities were bridges, laggard, diagonal, disturbed and late separation.

The abnormalities may be of three types; one as a result of an action on the spindle formation and thus resulted in cell division disturbance such as c- metaphase, lagging chromosomes and multipolar mitosis (Badr, 1983; Grant, 1999 and Badr *et al.*, 2013). Their presence may be attributed to the failure of the spindle apparatus to organize and function in a normal way. C- metaphase was regarded as indicative of a weak toxic effect which may be irreversible (Fiskesjo, 1985). The effect of the spindle apparatus may be as a result of the effect on the proteins constituting the spindle apparatus or because of interference with tubulin and /or the polymerization of microtubular subunit forming the spindle apparatus (Amer and Farah, 1974; Kabarity and Nahas, 1979 and Bartels *et al.*, 1981).

The second type is stickiness that may result from improper folding of chromosome fibers which makes the chromatids connected by means of sub-chromatid bridges (McGill et al., 1974 and Klasterska *et al.*, 1976) or may interpreted as a result of depolymerisation of DNA, partial dissolution of nucleoproteins or even breakage and exchanges of the basic folded fiber units of chromatids and the stripping of the protein associated with DNA in chromosomes (Mercykutty and Stephen, 1980). Stickiness may indicate irreversible highly toxic effect, which may probably lead to cell death (Fiskesjo, 1985).

The third type of chromosome abnormalities includes breakage and bridges; bridges were commonly observed during anaphase and telophase indicating clastogenic effect caused by breakage and fusion of chromatids or sub-chromatids (Badr, 1983 and Grant, 1999) indicating stable structural aberrations.

Protein electrophoresis: The electrophoretic analysis of extracted proteins using discontinuous SDS-PAGE gel for the root tips of *Allium cepa* treated with three artificial sweeteners, are shown in Table (5). The scanning of SDS-PAGE gel is shown in Fig (6). The total number of the bands was 18 bands with the molecular weight ranging from 41.70 to 96.36 KDa. There are 8 monomorphic bands and 10 polymorphic bands. The band of molecular weight 66.63 KDa was absent from the control and all treatments except aspartame 800 mg. The band of molecular weight 59.71KDa was absent from the control and all treatments except aspartame 400 mg. The band of molecular weight 41.70KDa was absent from the control and all treatments except sorbitol 2.5gm.

The appearance of unique bands may result from different DNA structural changes (breaks and deletions) which led to change in amino acids and consequently protein formed (Mondini *et al.*, 2009).

In Table (5), the highest percentage of polymorphism 22.22% was found in 400 mg, 600 mg aspartame, 2.5gm, 10gm sorbitol and 7gm sucralose, while the lowest value 11.11% was found in 1000mg aspartame and 7.5gm sorbitol. The changes of protein pattern following treatments with different chemicals were obtained by (Maslam, 2004 and El Nahas, 2005).

Inter simple sequence repeats (ISSRs) analysis: In this study, four tested primers were used to study the effect of artificial sweeteners on *Allium cepa* (Giza 20). As recorded in Table (6) and Plate (4) a total of 34 well-defined and scorable ISSRs markers were obtained as a result of fingerprinting of root tips of *Allium cepa* treated with three artificial sweeteners. The amplicon size varying from 340 to 5100 bp .Of the 34 amplified bands, 12 were common in control and all treatments with artificial sweeteners (aspartame, sorbitol and sucralose) and 22 were polymorphic bands. The number of polymorphic bands varied from five bands in 841 primer, one band in 845 primer, five bands in 848 primer and eleven bands in 856 primer.

The band of a molecular size 790 bp was recorded in control and the treatments with sorbitol and sucralose but absent from the treatment with aspartame. The band with the molecular size 1800 bp was recorded in treatments with aspartame and sorbitol but absent from sucralose, so that this band could be used as a negative molecular marker for sucralose. The band of a molecular weight 890 bp was found only in the treatment with sucralose and absent from aspartame and sorbitol, so that this band could be used as a positive molecular marker for sucralose.

The percentages of polymorphic bands of the studied artificial sweeteners are given in Table (7). Regarding to percentage of the polymorphism of all treatments of artificial sweeteners, the maximum value of polymorphism 100% recorded in primer 856 with sequence $(AC)_8$ CTA and the minimum value 25% recorded in primer 845 with sequence $(CT)_8$ AGG. According to Guo *et al.*, (2009) ISSR has several advantages including high annealing temperature, repetition and lower cost. DNA can be damaged by chemicals. The DNA damage caused by sweeteners may be associated with the generation of free radicals (reactive oxygen

species) which cause DNA strand breaks and irreversible damage to proteins involved in DNA replication, repair, recombination and transcription (Lin *et al.*, 2007).

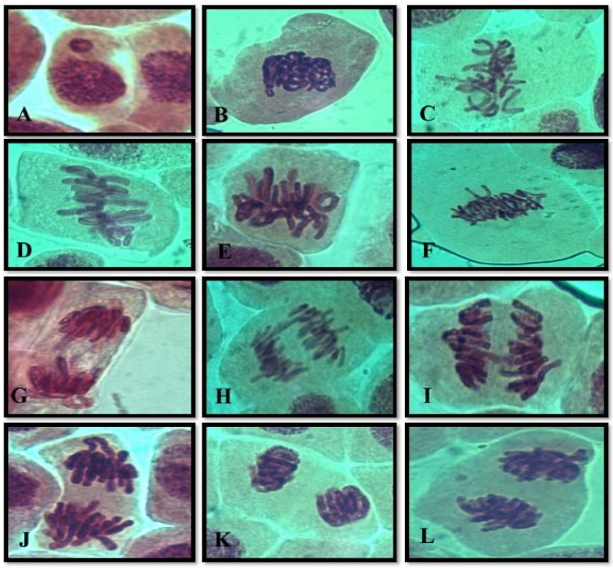

Plate 1: From (A-L): Types of mitotic abnormalities induced by treatment of *Allium cepa* root tips by artificial sweetener aspartame. (A) Micronucleus at interphase (aspartame 800 mg - 48h),(B) Stickiness at metaphase (aspartame 600 mg – 24h) , (C) Disturbed at metaphase (aspartame 600- 48h) , (D) Oblique at metaphase (aspartame 400 mg – 48h) , (E) Chromosome ring at metaphase (aspartame 800 mg – 24h) , (F) Disturbed metaphase (aspartame 600 mg – 48h) , (G) Late separation at anaphase (aspartame 400 mg – 48h) , (H) Bridge at anaphase (aspartame 600 mg – 48h) , (I) Bridge at anaphase (aspartame 800 mg- 24h) , (J) Disturbed at anaphase (aspartame 800 mg – 48h) , (K) Diagonal telophase (aspartame 400 mg – 24h) and (L) Disturbed telophase (aspartame 600 mg- 48h), (X=1000).

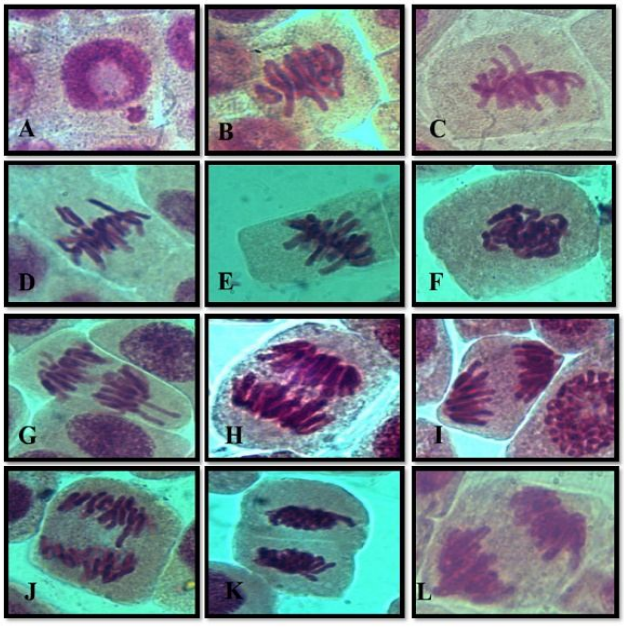

Plate 2: From (A-L): Types of mitotic abnormalities induced by treatment of *Allium cepa* root tips by artificial sweetener sorbitol. (A) Micronucleus at interphase (sorbitol 10 gm – 24h) ,(B) Ring chromosome at metaphase (sorbitol 10 gm – 24h) , (C) Disturbed metaphase (sorbitol 10 gm – 24h) , (D) Non congression at metaphase (sorbitol 10 gm – 48h) ,(E) Oblique metaphase (sorbitol 7.5gm- 48h) , (F) Stickiness metaphase (sorbitol 5gm- 24h), (G) Disturbed anaphase (sorbitol 7.5gm – 48h) , ,(H) Bridge anaphase (sorbitol 7.5gm – 24h) ,(I) Diagonal anaphase (sorbitol 5gm – 24h), (J) Late separation anaphase (sorbitol 7.5gm – 24h) , (K) Disturbed telophase (sorbitol 5gm – 24h) and (L) Diagonal telophase (sorbitol 10gm- 48h) (X=1000).

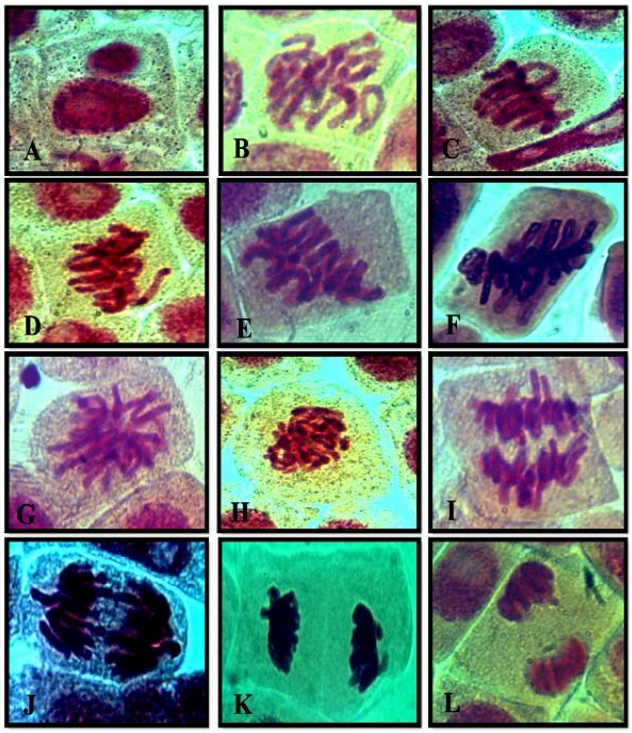

Plate 3: From (A-L): Types of mitotic abnormalities induced by treatment of *Allium cepa* root tips by artificial sweetener sucralose. (A) Large micronucleus at interphase (sucralose 7gm – 48h) , (B) Ring chromosome at metaphase (sucralose 1gm – 48h) , (C) Ring chromosome at metaphase (sucralose 7gm – 48h) , (D) Disturbed metaphase (sucralose 1gm – 48h) , (E) Oblique metaphase (sucralose 5gm – 24h) , (F) Oblique metaphase (sucralose 7gm – 48h) , (G) Star metaphase (sucralose 3gm – 24h) , (H) Stickiness metaphase (sucralose 1gm – 48h) , (I) Disturbed anaphase (sucralose 7gm – 24h) , (J) Bridge anaphase (sucralose 3gm – 24h) , (K) Disturbed telophase (sucralose 5gm – 48h) and (L) Diagonal telophase (sucralose 5gm – 24h), (X=1000).

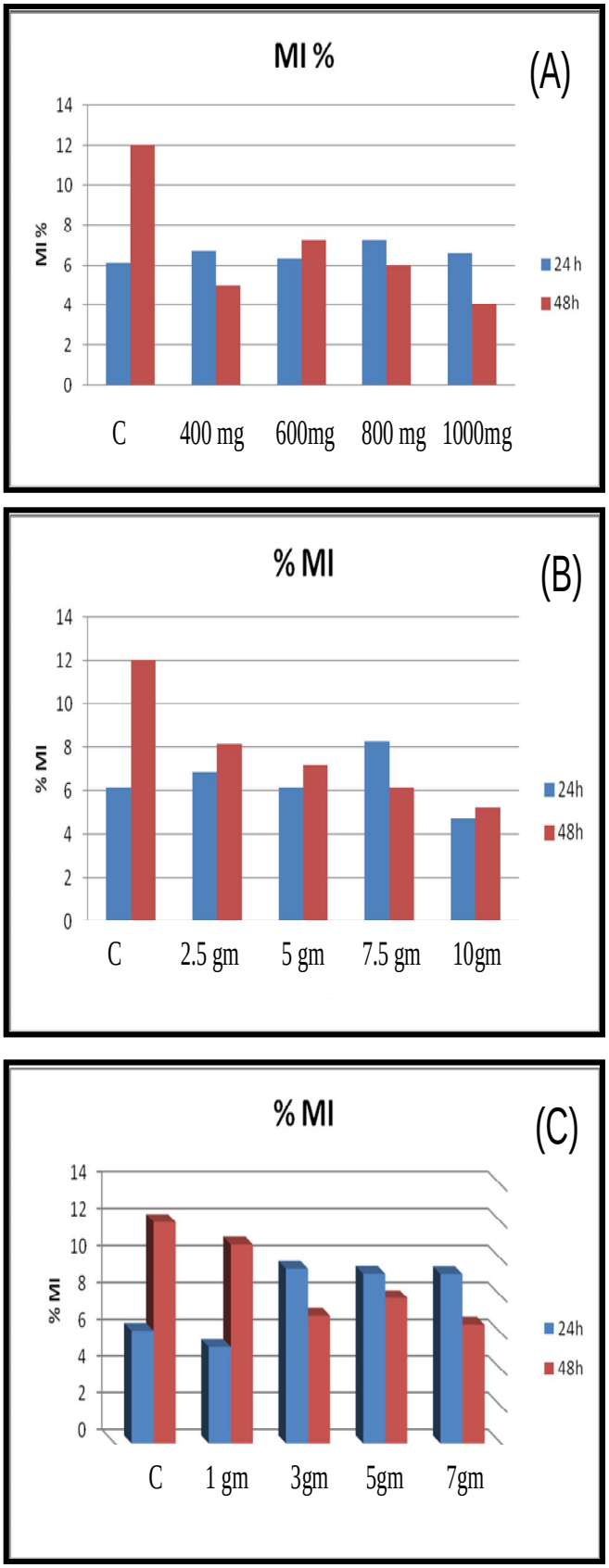

Figure 4: Show the percentage of mitotic index after treating *Allium cepa* root tips with (A) aspartame, (B) sorbitol and (C) sucralose.

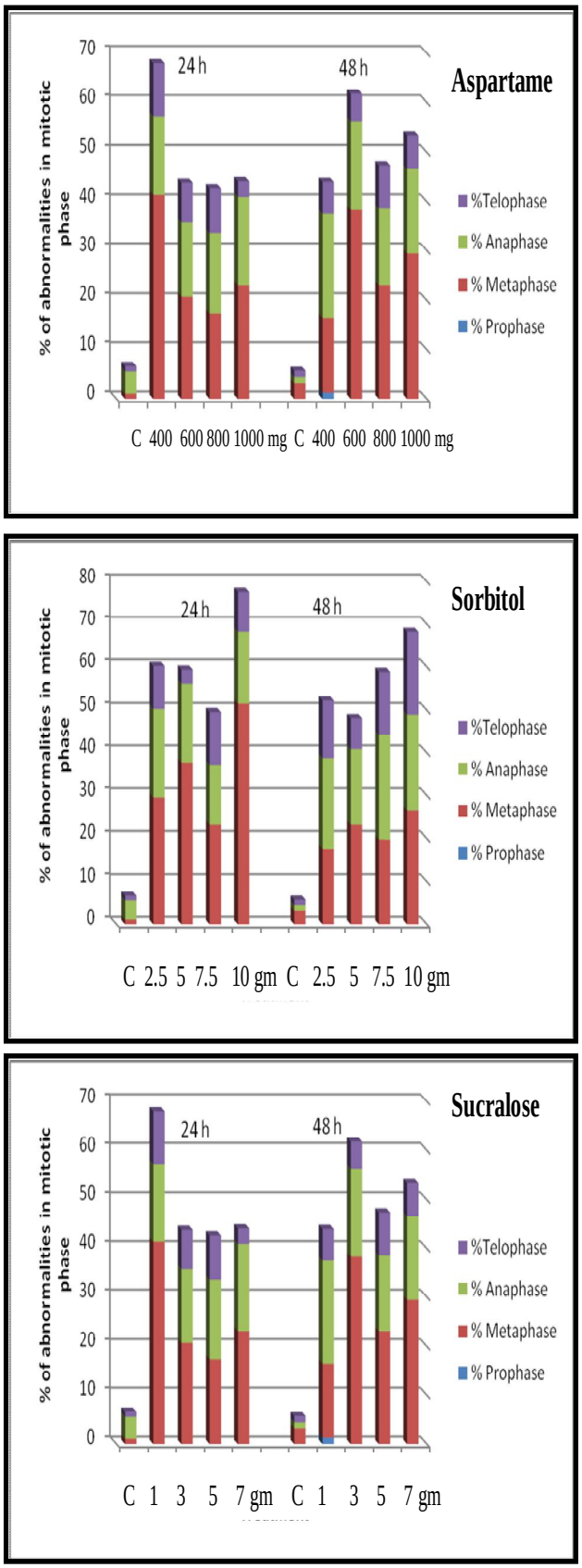

Figure 5: Show the percentage of abnormalities in mitotic phase after treating *Allium cepa* root tips with three artificial sweeteners.

Table 2: Mitotic index , normal and abnormal phase indices , total abnormalities in non-dividing and dividing cells after treating *Allium cepa* root tips with aspartame, Et= Exposure time (hours).

Treatment		%MI	Phase index								% Total abnormal	
			% prophase		% Metaphase		% Anaphase		% Telophase		Interphase	Mitosis
Concn.	ET		mitotic	Abn.	mitotic	Abn.	mitotic	Abn.	mitotic	Abn.		
Control	24	6.13±0.52	27.94	0.00	29.00	1.11	30.83	4.44	12.23	1.11	0.00±0.00	6.66±3.60
	48	12.01±1.18	36.47	0.00	17	3.15	24.36	1.27	22.17	0.00	0.00±0.00	4.42±1.37
400 mg	24	6.71±0.74ns	31.46	0.00	13.33	14.75	43.99	39.24	11.21	10.45	0.11±0.11ns	64.55±6.71*
	48	4.96±0.42*	23.76	0.00	28.23	3.23	23.87	17.85	24.14	10.70	0.06±0.06ns	31.84±6.77*
600 mg	24	6.37±0.44ns	26.30	1.56	28.91	10.00	28.65	30.21	16.14	9.64	0.56±0.24*	41.97±8.35*
	48	7.24±0.50*	25.51	1.39	25.52	11.77	25.32	20.82	23.65	11.55	0.00±0.00	45.53±3.81*
800 mg	24	7.24±0.50*	27.59	1.39	25.52	27.43	25.32	16.41	21.57	11.55	0.00±0.00	56.78±5.65*
	48	6.01±0.32*	24.55	0.00	33.03	22.07	23.59	18.54	18.83	12.58	0.07±0.07ns	53.26±4.08*
1000mg	24	6.62±0.47ns	22.66	0.00	33.59	21.88	22.40	17.19	21.35	16.15	0.00±0.00	55.22±4.86*
	48	4.06±0.29*	20.18	1.32	45.61	47.37	19.74	13.16	14.47	14.47	0.10±0.07ns	76.42±5.82*

Note: *Total number of examined cells = 2000, ns = not significant at 0.05 level from control, *= the two means are significantly different at the 0.05 level.*

Table 3: Mitotic index , normal and abnormal phase indices , total abnormalities in non-dividing and dividing cells after treating *Allium cepa* root tips with sorbitol, Et= Exposure time (hours).

Treatment		%MI	Phase index								% Total abnormal	
			% prophase		% Metaphase		% Anaphase		% Telophase		Interphase	Mitosis
Concn.	ET		mitotic	Abn.	mitotic	Abn.	mitotic	Abn.	mitotic	Abn.		
Control	24	6.13±0.52	27.94	0.00	29.00	1.11	30.83	4.44	12.23	1.11	0.00±0.00	6.67±3.60
	48	12.01±1.18	36.47	0.00	17	3.15	24.36	1.27	22.17	0.00	0.00±0.00	4.42±1.37
2.5 gm	24	6.86±0.57ns	18.07	0.00	38.65	29.53	26.04	20.83	17.24	9.90	0.00±0.00	60.26±4.74*
	48	8.15±0.59*	17.97	0.00	36.98	17.71	25.26	21.09	19.79	13.54	0.22±0.14ns	52.56±3.78*
5 gm	24	6.15±0.47ns	13.28	0.00	48.70	37.76	26.04	18.49	11.97	3.13	0.35±0.17ns	59.73±5.28*
	48	7.20±0.54*	28.13	0.00	35.67	23.18	23.70	17.71	12.50	7.29	0.56±0.17ns	48.74±3.66*
7.5 gm	24	8.28±0.76*	19.53	0.00	40.57	23.18	21.98	14.06	17.92	12.34	0.22±0.16ns	49.80±4.90*
	48	6.12±0.46*	21.35	0.00	29.17	19.79	30.21	24.48	19.27	14.58	0.00±0.00	58.85±4.10*
10 gm	24	4.74±0.72ns	9.41	0.00	59.38	51.56	19.27	16.67	11.94	9.38	0.43±0.31ns	78.04±4.33*
	48	5.24±0.47*	8.85	0.00	38.02	26.56	26.57	22.40	26.56	19.27	0.00±0.00	68.23±5.31*

Note: *Total number of examined cells = 2000, ns = not significant at 0.05 level from control, *= the two means are significantly different at the 0.05 level.*

Table 4: Mitotic index , normal and abnormal phase indices , total abnormalities in non-dividing and dividing cells after treating *Allium cepa* root tips with sucralose, Et= Exposure time (hours).

Treatment		%MI	Phase index								% Total abnormal	
			% prophase		% Metaphase		% Anaphase		% Telophase		Interphase	Mitosis
Concn.	ET		mitotic	Abn.	mitotic	Abn.	mitotic	Abn.	mitotic	Abn.		
Control	24	6.13±0.52	27.94	0.00	29.00	1.11	30.83	4.44	12.23	1.11	0.00±0.00	6.66±3.60
	48	12.01±1.18	36.47	0.00	17	3.15	24.36	1.27	22.17	0.00	0.00±0.00	4.42±1.37
1gm	24	5.23±0.51ns	13.80	0.00	52.86	41.41	18.23	15.89	15.10	10.68	0.00±0.00	67.98±5.27*
	48	10.80±0.80ns	23.44	1.34	25.21	15.16	32.61	20.99	18.70	6.51	0.00±0.00	44.00±4.25*
3gm	24	9.47±0.97*	23.20	0.00	32.72	20.76	21.24	15.00	22.85	8.07	0.00±0.00	43.83±3.96*
	48	6.94±0.65*	16.93	0.00	53.07	38.28	19.64	18.07	10.36	5.47	0.15±0.10ns	61.97±5.13*
5gm	24	9.20±0.54*	22.95	0.00	24.72	17.40	24.25	16.18	28.08	9.11	0.00±0.00	42.69±3.39*
	48	7.90±0.69*	29.90	0.00	33.23	23.18	20.89	15.36	15.98	8.85	0.00±0.00	47.39±4.68*
7gm	24	9.18±0.83*	19.22	0.00	35.15	23.18	28.18	17.81	17.45	3.13	0.25±0.18ns	44.37±5.10*
	48	6.46±0.47*	23.70	0.00	40.63	29.43	21.87	17.19	13.80	6.77	0.07±0.07ns	53.46±4.30*

Note: *Total number of examined cells = 2000, ns = not significant at 0.05 level from control, *= the two means are significantly different at the 0.05 level.*

Table 5: SDS-PAGE of protein banding pattern of *Allium cepa root* tips treated with aspartame, sorbitol and sucralose for 24 hr.

Band No.	KDa.	Control	Aspartame				Sorbitol				Sucralose			
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	96.36	1	1	1	1	1	1	1	1	1	1	1	1	1
2	88.53	1	1	1	0	1	0	0	0	1	1	1	1	1
3	85.75	1	0	1	1	0	1	0	1	0	0	0	0	0
4	83.39	0	1	0	0	0	0	1	0	0	0	1	0	0
5	79.00	1	1	1	1	1	1	1	1	1	1	1	1	1
6	70.39	1	1	1	1	1	1	1	1	1	1	1	1	1
7	66.63	0	0	0	1	0	0	0	0	0	0	0	0	0
8	61.83	1	0	1	1	0	1	0	0	1	1	1	0	1
9	59.71	0	1	0	0	0	0	0	0	0	0	0	0	0
10	57.38	1	1	1	1	1	1	1	1	1	1	1	1	1
11	55.68	0	0	0	0	0	0	0	0	1	0	0	1	0
12	53.24	1	1	1	1	1	1	1	1	1	1	1	1	1
13	51.67	1	1	1	1	1	1	1	1	1	1	1	1	1
14	50.66	0	1	0	0	1	0	1	0	1	1	0	1	1
15	48.19	0	0	1	0	0	1	1	1	0	0	0	0	1
16	47.00	1	1	1	1	1	1	1	1	1	1	1	1	1
17	43.40	1	1	1	1	1	1	1	1	1	1	1	1	1
18	41.70	0	0	0	0	0	1	0	0	0	0	0	0	0
Total		11	12	12	11	10	12	11	10	12	11	11	11	12
%of polymorphism		16.67	22.22	22.22	16.67	11.11	22.22	16.67	11.11	22.22	16.67	16.67	16.67	22.22

Table 6: Data matrix of ISSR-PCR for the treatments of *Allium cepa* root tips with three artificial sweeteners.

Artificial sweetener	control	Aspartame				Sorbitol				Sucralose			
code	1	2	3	4	5	6	7	8	9	10	11	12	13
bp	Primer 841												
4300	1	1	1	1	1	1	1	1	1	0	0	1	1
1700	1	1	1	1	1	1	1	1	1	1	0	1	1
1400	1	1	1	1	1	1	1	1	1	1	0	1	1
950	1	1	1	1	1	1	1	1	1	1	1	1	1
830	1	1	1	1	1	1	1	1	1	1	1	1	1
790	1	0	0	0	0	1	1	1	1	1	1	1	1
760	1	1	1	1	1	1	1	1	1	1	1	1	1
660	1	1	1	1	1	1	1	1	1	1	1	1	1
600	1	1	0	0	0	0	1	1	1	1	1	1	1
Amplified bands	9	8	7	7	7	8	9	9	9	8	6	9	9
bp	Pimer 845												
4400	1	1	1	1	1	1	1	1	1	1	1	1	1
1720	1	0	1	1	1	1	1	1	1	1	1	1	1
1640	1	1	1	1	1	1	1	1	1	1	1	1	1
1350	1	1	1	1	1	1	1	1	1	1	1	1	1
Amplified bands	4	3	4	4	4	4	4	4	4	4	4	4	4
bp	Primer 848												
5100	0	0	1	1	1	0	1	1	1	0	0	0	0
1800	1	1	1	1	1	1	1	1	1	0	0	0	0
1600	1	1	1	1	1	1	1	1	1	1	1	1	1
1100	0	0	1	1	1	0	0	0	0	0	0	0	0
930	1	1	1	1	1	1	1	1	1	1	1	1	1
860	1	1	1	1	1	1	1	1	1	1	1	1	1
810	1	1	1	1	1	1	1	1	1	1	1	1	1
780	1	1	1	1	1	1	1	1	1	1	1	1	1
710	1	1	1	1	1	1	1	1	1	1	1	0	0
640	1	1	1	1	0	1	1	1	1	0	1	0	0
Amplified bands	8	8	10	10	9	8	9	9	9	6	7	5	5
bp	Primer 856												
5040	0	1	1	0	0	1	1	0	0	0	1	1	1
1840	0	0	0	0	0	0	0	0	0	0	0	1	1
1750	0	0	0	0	0	0	0	1	0	1	1	1	1
1630	0	0	1	0	0	0	0	0	0	0	0	0	0
970	1	1	1	0	0	1	1	1	1	1	0	0	0
890	0	0	0	0	0	0	0	0	0	1	1	1	1
830	0	0	0	0	0	0	0	0	0	0	1	1	0
760	0	1	1	1	1	1	1	1	0	0	1	1	1
690	1	1	1	1	1	1	1	0	0	0	0	0	0
630	0	0	0	0	0	0	0	1	1	1	1	1	1
340	0	0	0	0	0	0	0	0	0	0	0	0	1
Amplified bands	2	4	5	2	2	4	4	4	2	4	6	7	7

Table 7: ISSR fragments per primer, fragment length, polymorphic fragments and percentage of polymorphism in *Allium cepa* root tips treated with three artificial sweeteners (aspartame, sorbitol and sucralose) based on ISSR-PCR analysis.

primers code	Amplified fragments			
	Total amplified fragments	Length range (bp)	Polymorphic fragments	Percentage of polymorphism (%)
ISSR-841	9	600-4300	5	55.55
ISSR-845	4	1350-4400	1	25
ISSR-848	10	640-5100	5	50
ISSR-856	11	340-5040	11	100
Total	**40**	**340-6520**	**25**	**62.5**

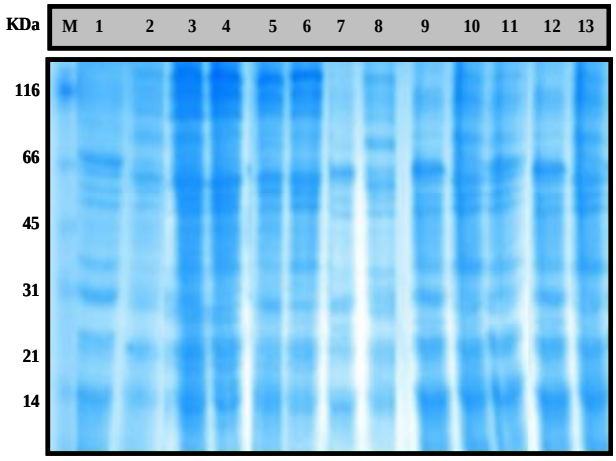

Figure 6: SDS- PAGE of protein banding pattern of *Allium cepa* root tips treated with three artificial sweeteners (aspartame, sorbitol and sucralose).
{M= Marker; 1= control; 2= 400mg aspartame; 3= 600mg aspartame; 4= 800mg aspartame; 5=1000mg aspartame; 6= 2.5gm sorbitol; 7= 5gm sorbitol; 8= 7.5gm sorbitol; 9= 10gm sorbitol; 10= 1gm sucralose; 11= 3gm sucralose; 12= 5gm sucralose; 13= 7gm sucralose}

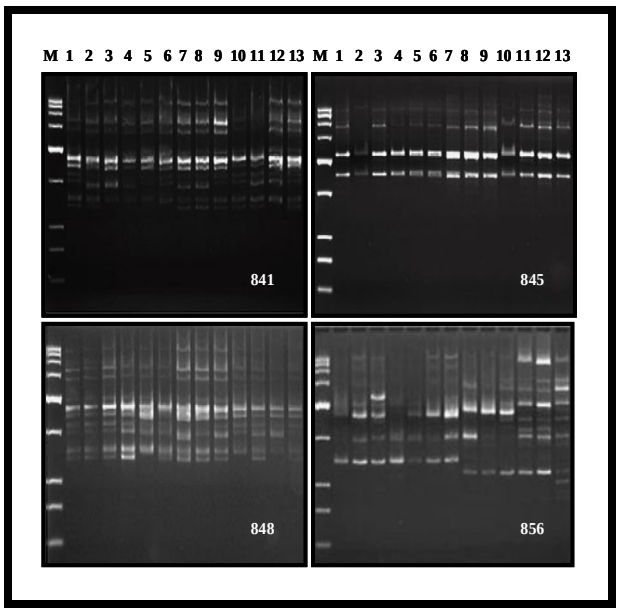

Plate 4: The amplification profiles of the treatments of *Allium cepa* with three artificial sweeteners (aspartame, sorbitol and sucralose) generated by four primers (M – indicates molecular size marker.
{1= control; 2= 400mg aspartame; 3= 600mg aspartame; 4= 800mg aspartame; 5=1000mg aspartame; 6= 2.5gm sorbitol; 7= 5gm sorbitol; 8= 7.5gm sorbitol; 9= 10gm sorbitol; 10= 1gm sucralose; 11= 3gm sucralose; 12= 5gm sucralose; 13= 7gm sucralose}

CONCLUSION: Sweeteners or sugar alternatives replace traditional sugars from our diet. It is an attractive alternative to sugar because they add virtually no/low calories to diet. This study demonstrated that the different treatments with three artificial sweeteners aspartame, sorbitol and sucralose induced different mitotic changes on root tips cells of *Allium cepa*. Such changes vary from changes of mitotic index of meristematic cells, changes in phase index and the production of large number of chromosomal aberrations. These changes appeared in varying degrees depending on the duration and the concentration of the treatment. Therefore, the effects of sweeteners on human health should be extensively investigated, especially when used at high concentrations in foods and beverages. It should be used within narrow limits and at very low concentrations to avoid their mutagenic effects on human health.

REFERENCES:

1. Amer S. M. and Farah O. R. (1974) Cytological effect of pesticides. VII- Mitotic effect of isopropyl-n- phenylcarbamate and duphar, *Cytologia*, 40, 21-29.

2. Amin A. W. (2001) Heavy metals content in maize affected by sewage sludge application. I-cytological effects, *Egypt. J. Genet. Cytol*., 29, 131–140.

3. Badr A. (1983) Mitodepressive and chromotoxic activities of two herbicides in *Allium cepa*, *Cytologia*, 48, 451-457.

4. Badr A., Zaki H., Germoush M. O., Tawfeek A. Q. and El-Tayeb M. A. (2013) Cytophysiological impacts of Metosulam herbicide on *Vicia faba* plants, *Acta Physiol Plant*, 35,1933–1941

5. Barakat H. M. and Hassan H. Z. (1997) Mutagenic effects of pendimethalin herbicide on *Vicia faba* and *Allium cepa* plants, *Egypt. J. Bot*., 37, 13 – 29.

6. Barbieri G., Barone C., Bhagat A., Caruso G., Conley Z. R. and Parisi S (2014) Sweet compounds in foods: Sugar alcohols. In: Springer (ed). The influence of chemistry on new foods and traditional products. Springer International Publishing, Berlin.

7. Bartels P. C., Hess F. O. and bayer D. F. (1981) Cytological and biochemical effects of trifluralin on mitosis, *Tech. bull. Haway Agric. Exptl*., State Univ. Haway, 64.

8. Bennett D. (2013) The intense sweetener world, Ehrenberg center for research in marketing. (http:/ www. Isbu.ac.uk/bus-ehrenberg/documents/high % 20 intensity % 20 sweeteners. Pdf. Accessed on 28 May 2013.

9. Dellaporta S. L., Wood J. and Hicks J. B. (1983) A plant DNA mini preparation.Version III. *Plant Mol Biol Rep*, 1, 19-21.

10. Deysson P. (1968) Antimitotic substances, *Inter. Rev. of Cytol*., 24, 1 -99.

11. EFSA (2011) Scientific opinion on the substantiation of health claims related to the sugar replacers xylitol, D-tagatose, xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt, erythritol, D-tagatose, isomaltulose, sucralose and polydextrose and maintenance of tooth mineralization by decreasing tooth demineralization. EFSA Journal 9 (4), 2076. http:// www.efsa.europa.eu/en/efsa- journal/doc/2076.pdf. Accessed 10 June 2014.

12. EL-Nahas N. M. (2005) Evaluation of sewage sludge use in agriculture: Cytogenetic Point of View. M. Sc. Fac. of Science, Alexandria Univer. Egypt.

13. Fernandes T. C. C., Mazzeo D. E. C. and Marin-Morales M. A. (2007) Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide, *Pestic Biochem Physiol*, 88, 252–259.

14. Findikli Z. and Turkoglu S. (2014) Determination of the effects of some artificial sweeteners on human peripheral lymphocytes using the comet assay, *Journal of Toxicology and Environmental Health Sciences*, 6(8), 147-153.

15. Fiskesjo G. (1985) The *Allium* test as a standard in environmental monitoring, *Hereditas*, 102 (1), 99-112.

16. Ge X. J. and Sun M. (1999) Reproductive biology and genetic diversity of *Cryptoviviparous Mangrove Aegiceras Corniculatum* (Myrsinaceae) using allozyme and inter-simple sequence repeat (ISSR) analysis, *Mol Ecol*, 8, 2061-9.

17. Ghareeb A., Khalifa S.F. and Nael F. (1999) Molecular systematics of some *Cassia* species, *Cytologia*, 64, 11-16.

18. Grant W. F. (1978) Chromosome aberrations in plants as a monitoring system, *Environ. Health perspectives*, 27, 37-43.

19. Grant W.F. (1982). Chromosome aberration assays in *Allium*. A report of the U. S. Environmental Protection Agency, *Gene-Tox. Program. J. Mutat. Res*., 93, 161 – 168.

20. Grant W. F. (1999) Higher plant assays for the detection of chromosomal aberrations and gene mutations- a brief historical background on their use for screening and monitoring environmental chemicals, *Mutat. Res.*, 426 (2), 107- 112.

21. Grotz V. L. and Munro I. C. (2009) An overview of the safety of sucralose. *Regul Toxicol Pharmacol*., 55, 1-5.

22. Guo H. B., Huang K. Y., Zhou T. S., Wu Q. H., Zhang Y. J. and Liang Z. S. (2009) DNA isolation, optimization of ISSR-PCR system and primers screening of *Scutellaria baicalensis*, *Journal of Medicinal Plants Research*, 3(11), 898-901.

23. Jain T. and Grover K. (2015) Sweeteners in human nutrition, *Int. J. Health Sci. & Res.*, 5, 439-451.

24. Jonas R. and Silveira M. M. (2004) Sorbitol can be produced not only chemically but also biotechnologically, *Appl Biochem Biotechnol*, 118, 321-336.

25. Jones S. B. and Luchsinger A. E. (1987) Plant Systematic, 2nd Edition, McGraw-Hill Book Company, New York, 512.

26. Jouyban A. and Parsa H. (2012) Genotoxic impurities in pharmaceuticals. In: Acree Jr. WE (Ed.), Toxicity and Drug Testing. In Tech, Rijeka, 387-414.

27. Kabarity A. and Nahas A. (1979) Induction of polyploidy and c-tumors after treating *Allium* with the herbicide Treflan, Ibd., 21, 253-258.

28. Klasterska I., Natarajan A. T. and Ramel C. (1976) An interpretation of the origin of subchromatid aberrations and chromosome stickiness as category of chromatid aberrations, *Hereditas*, 83(2), 153-169.

29. Koa K. N. (1975a) A nuclear staining method for plant protoplasts. in plant tissue culture methods

ch. 10, O. L. Gamborg and L. R. Wetter (eds.) National Research Council of Canada, Ottawa, Ontara.

30. Koa K. N. (1975b) A chromosomal staining method for cultured cells. In plant tissue culture methods ch. 10, O. L. Gamborg and L. R. Wetter (eds.) National Research Council of Canada, Ottawa, Ontara.

31. Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature.* 227, 680-685.

32. Lange F. T., Scheurer M. and Brauch H.J. (2012) Artificial sweeteners- a recently recognized class of emerging environmental contaminants: a review, *Anal Bioanal. Chem.*, 403, 2503-2518.

33. Lean M. E. J. and Hankey C. R. (2004) Aspartame and its effect on health, *BMJ*, 329 (7469), 755-756.

34. Lin A. J., Zhang X., Chen M. and Cao Q. (2007) Oxidative stress and DNA damages induced by Cadmium accumulation, *J. Environ. Sci.*, (China) 19, 596-602.

35. Maluszynska J. and Juchimiuk J. (2005) Plant genotoxicity: a molecular cytogenetic approach in plant bioassays, *Arh. Hig.Rada Toksikol*., 56 (2), 177–184.

36. Maslam S. S. M. (2004) Molecular ultrastructure and cytological characteristics of *Allium cepa* meristems treated with some medicinal plant extract. M. Sc. Fac. Of girls, Ain shams University, Egypt.

37. Mattioni C., Casasoli M. and Gonzalez M. (2002) Comparsion of ISSR and RaPD markers to characterize three *Chilean Nothofagus* species, *Theoretical and Applied Genetics*, 104, 1064-70.

38. McGill M., Pathak S. and Hsu T. C. (1974) Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness, *Chromosoma*, 47 (2), 157-167.

39. Mercykutty V.C. and Stephen J. (1980) Adriamycin induced genetic toxicity as demonstrated by the *Allium* test, *Cytologia*. 45, 769–777.

40. Mondini L., Noorani A. and Pagnotta M. A. (2009) Assessing plant genetic diversity by molecular tools, *Diversity*, 1 (1), 19-35.

41. Myers R. L. (2007) The 100 most important chemical compounds: a reference guide. Greenwood Publishing Group, Westport.

42. Nishal S. (2015) A review of artificial sweeteners, *World J. Pharma.Res*., 4, 776-792.

43. Ortiz M. E., Bleckwedel J., Raya R. R. and Mozzi F. (2013) Biotechnological and in situ food production of polyols by lactic acid bacteria, *Appl Microbiol Biotechnol*, 97, 4713-4726.

44. Rath A., Glibowicka M., Nadeau V. G., Chen G. and Deber C. M. (2009) Detergent binding explains anomalous SDS-PAGE migration of membrane proteins. Proceedings of the National Academy of Sciences of the United States of America, 106(6), 1760–1765.

45. Reddy M. P., Sarla N. and Siddiq E. A. (2002) Inter simple sequence repeat (ISSR) polymorphism an dits application in plant breeding, *Euphytica*, 128, 9-17.

46. Robinson D. I. (2010) Control of genotoxic impurities in active pharmaceutical ingredients: a review and perspective, *Org. Process Res. Dev.*, 14, 946-959.

47. Rojas M. R., Gilbertson R. L., Russell D. R. and Maxwell D. P. (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted Gemini viruses, *Plant Disease*, 77, 340-347.

48. Sang N. and Li G. (2004) Genotoxicity of municipal landfill leachate on root tips of *Vicia faba*, *J. Mutat. Res.*, 560, 159-165.

49. Snedecor G. W. and Cochran W. G. (1976) Statistical method 6th ed-Oxford and IBH publishing Co. New Delhi.

50. Soliman M. I. and Ghoneam, G. T. (2004) The mutagenic potentialities of some herbicides using *Vicia faba* as a biological system, *J. Biotechnol*., 3 (2), 140 – 154.

51. Soliman M. I., Rizk R. M., Reicha E. F. and Ramadan N. S. (2014b) Comparative cytological and biochemical studies on the effects of three types of chitosan on *Vicia faba*, *J. Environ. Sci.*, Mansoura Univ., 43(4), 501-525.

52. Soliman M. I., Soliman H. M., El- Metwally M. A. and E. l. Geoshi, B. F. (2014a) Assessment of cytological effects of some antioxidants using *Vicia faba* chromosomal aberration assay, *J. Environ. Sci.*, 43(3), 479-499.

53. Stegemann H. (1984) Retrospect on 25 years of cultivar identification by protein patterns and prospects for future. In Draper, S.R. and Cooke, R. J. (eds.) "Biochemical tests for cultivar identification "The International Seed Testing Association, Zurich, Switzerland, 20-31.

54. Tollefsen K. E., Nizzetto L. and Huggett D. B. (2012) Presence, fate and effects of the intense sweetener sucralose in the aquatic environment, *Sci Total Environ*, 438, 510-516.

55. Wu L., Yi H. and Yi M. (2010) Assessment of arsenic toxicity using *Allium cepa / Vicia* root tip micronucleus assays, *Journal of Hazardous Materials*, 176, 952-956.

56. Yadav R. K. (2008) Seed protein electrophoresis studies in Cucurbits-A review. *Agricultural Revolution*., 29(1), 21–30.

57. Yi H. L. and Meng Z. Q. (2003) Genotoxicity of hydrated sulphur dioxide on root tips of *Allium sativum* and *Vicia faba*, *J. Mutat. Res.*, 537, 109-114.

58. Ziekiewicz E, Rafalski A, Labuda D. (1994) Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase Chain reaction amplification, *Genome*, 20, 176-83.