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Protective action of vitamin C against mutagenic effects of synthetic food color tartrazine

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The present study has been carried out to investigate the possible mutagenic effects of the synthetic food color tartrazine on mitosis, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contents of *Allium cepa* roots and protein banding pattern of *A. cepa* seeds. The obtained results indicated that the synthetic food color tartrazine (E102) had the ability to cause different mitotic changes varying from reduction in mitotic index to the production of a large number of mitotic abnormalities. These changes appeared in varying degrees depending on the applied concentration and duration of treatment. The types of abnormalities produced were laggards, bridges, stickiness, C-metaphase and disturbed phases as well as micronuclei. The amounts of both DNA and RNA were generally decreased with increasing of most concentrations and time of treatment. At electrophoretic level, E102 induced alternations in the protein banding pattern of *A. cepa* seeds as compared with the control. These alternations were expressed as disappearance of some characteristic bands, appearance of new bands, and changes in band intensities. The administration of vitamin C was found to be very helpful in minimizing the toxic effects induced by E102.

Key words: *Allium cepa*, tartrazine, chromosome aberration, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content, protein banding patterns, vitamin C.

INTRODUCTION

Human beings are using a variety of color additives for a long time where most of colors are of synthetic origin. Many food industries started using synthetic food dyes without knowledge of their safety. Tartrazine (E102) is a monoazo dye, popularly used as colorant in food, drugs and industrial manufacturing products intended for human consumption. The ADI for tartrazine is 7.5 mg/kg/day (Walton et al., 1999). Tartrazine is used mainly to color several foods such as breakfast cereals, chocolate chips, biscuits, ice creams, juices, sweets, jams, cereals, snack foods, canned fish and soft drinks. Generally, detailed toxicity studies on various food colors and additives products are missing (Food Reactions, 2010). The metabolite of Tartrazine can generate reactive oxygen species (ROS), which in turn, accelerate the oxidative stress (Bansal, 2005).

A variety of immunologic responses have been attributed to tartrazine including: neurobehavioral toxicity, anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heat waves, feeling of suffocation, purple skin patches and sleep disturbance (FSA, 2007; Park et al., 2009). Amin et al. (2010) concluded that Tartrazine and carmosine affect adversely and alter biochemical markers in vital organs (liver and kidney) not only at higher doses but also at low doses. Allergy leading to asthma attacks, cellular damage, and interaction of food additive tartrazine with common drug products such as aspirin and ventolin were reported by various researchers leading to ban on some of the additives in Norway and Austria Food Reactions (2010). Tartrazine (E102) is still popularly used as colorant in food, drugs and many different industrial products, intended

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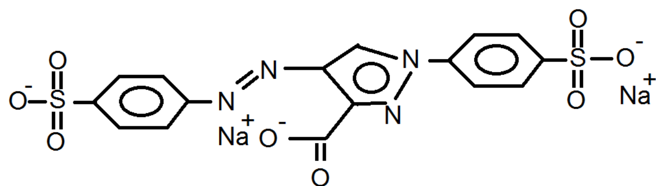

Figure 1. Chemical structure of Tartrazine

for human consumption (Mpountoukas et al., 2010). Mervat and Heba (2011) concluded that a causal link truly exists between tartrazine and inflection of hyperactivity, anxiety and depression-like behaviours in rats and points to the hazardous impacts of tartrazine on puplic health. Yonglin et al. (2011) reported that decline in the activities of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) as well as a rise in the level of monoaldehyde (MDA) was observed in the brain of tartrazine treated rats, and these changes were associated with the brain oxidative damage. The dose levels of tartrazine in the study produced a few adverse effects in learning and memory functions in treated rats. The mechanisms might be attributed to promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense system and the brain tissue damage. Epidemiological evidences suggest that dyes might possess carcinogenic potential under certain circumstances (Axon et al., 2012).

Tartrazine is capable of producing free radicals, which in turn cause damage to the cellular compartment system of rat testis (Amin et al., 2010; Visweswaran and Krishnamoorthy, 2012). There has been increasing concern in recent years about the assay methods and mutagenic potential of a variety of food additives and food colors, and it is believed that such substances may present a possible hazard to man by causing gene mutations and/or chromosomal aberrations.

Vitamin C (VC) can protect indispensable molecules in the body, such as protein, lipids, carbohydrates and nucleic acids (Sanchez-Moreno et al., 2003). VC can promote the removal of oxidative DNA damage from the DNA and/or nucleotide pool, through the up regulation of repair enzymes (Arraiga-Alba et al., 2008). This inhibitory effect of VC towards a number of mutagens/carcinogens was shown by many authors in humans, animals and plants (Fahmy et al., 2008; Jennifer et al., 2009). VC is one of the leading compounds used as antioxidant. VC as a highly effective antioxidant acts as a reducing agent that can terminate free radical driven oxidation by being converted to a resonance-stabilized free radical (Ambali et al., 2011; Assia et al., 2012).

Higher plants, particularly *Vicia faba* and *Allium cepa* possess many advantages that make them ideal for use by scientists for screening and monitoring of genotoxic agents according to the standard protocol for the plant assay established by the International Program on Chemical Safety (IPCS) and the World Health Organization (WHO). Plant cells undergo mitosis and meiosis and can mutate in a manner similar to human and animal cells (Grant, 1994).

Allium test is one of the best-established test systems used in order to determine the toxicity in the laboratories. Moreover, this system is well correlated with the data obtained from eukaryotic and prokaryotic systems (Matsumoto et al., 2006). Many authors investigated the potentialities of higher plant genetic systems for monitoring and screening chemical mutagens (Konuk et al., 2007; Liman et al., 2010). Some investigations were carried out to indicate the relation between changes in mitotic and meiotic activities with changes in nucleic acid contents as a result of treatment with synthetic food colors (Giri, 1991; Tsuda et al., 2001). Electrophoretic techniques of protein have been used as a successful tool to estimate the possible mutagenic potentialities produced due to continuous and accumulative pollution by chemicals and correlate the produced variation with chromosomal aberrations caused by these pollutants (Badr, 1995; George and Ghareeb, 2001).

The present investigation was carried out to diminish the genotoxicity of the synthetic food color E102 by using the natural antioxidant compound Vitamin C. The search for new safer types of food additives, less harmful and non toxic products, have recently attracted the attention of many scientists all over the world. Thus, this work aimed to evaluate the cytotoxic effects of tartrazine and the protective effect of VC against the DNA damage induced by this food coloring agent (E102) using *A. cepa* as a biological system. For this purpose, cytological studies included mitotic index, chromosomal aberrations, as well as nucleic acids contents (DNA and RNA) and protein profiles were done.

MATERIALS AND METHODS

Plant

Bulbs and seeds of *A. cepa* (Giza 20) were supplied by the Agricultural Research Center, Giza, Egypt. They were used as experimental plants for both mitotic and biochemical analysis.

Test chemicals

Tartrazine, also known as E102, FD&C yellow No.5 was used. Tartrazine is trisodium (E)-5-oxo-1-(4-sulfonatophenyl)-4-((4-sulfonatophenyl) diazenyl)-4, 5-dihydro-1H-pyrazole-3-carboxylate. The molecular formula for tartrazine is $C_{16}H_9N_4Na_3O_9S_2$ (Figure 1). Vitamin C (L. ascorbic acid) was purchased from Memphis Co. The dose of vitamin C used in the present study was 100 mg/L.

Mitotic analysis

A. cepa bulbs were germinated in tap water, till the roots reached 2 to 3 cm in length, and then were divided into three groups. The first group was left as a control in distilled water and the second group was treated with freshly prepared test solutions of different

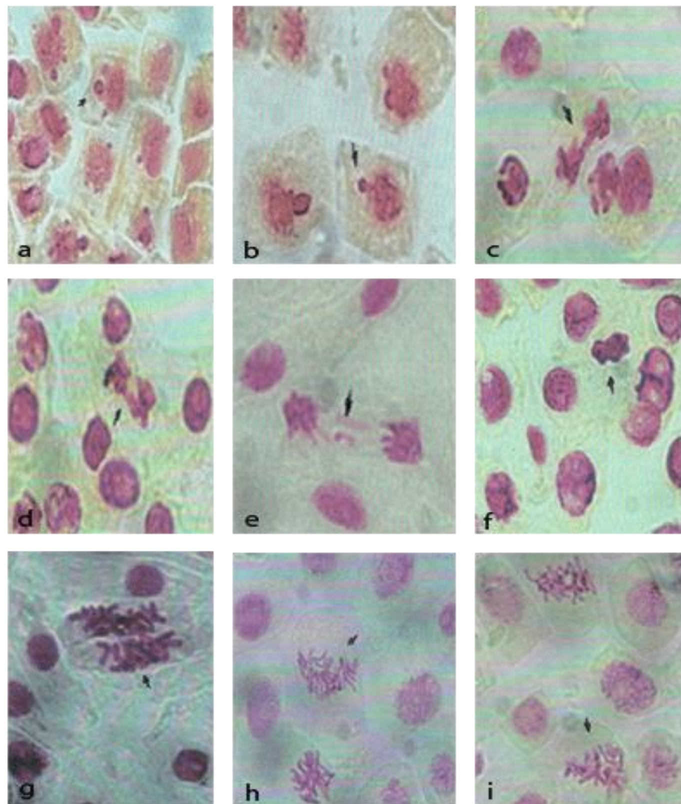

Figure 2. Some types of chromosomal abnormalities after treating *A. cepa* root tips with different concentrations of E102.
(a and b) Micronuclei; (c and d) telophase bridge; (e) lagging chromosome in telophase; (f) sticky metaphase; (g) C-metaphase; (h and i) disturbed metaphase.

concentrations of E102 for 3, 6, 12 and 24 h. E102 concentration differed according to each period. The third group was treated with the same concentrations used for 24 h by E102 alone for the same period and then treated with Vitamin C (100 mg/L) for 3 h. Following treatments, roots were detached, washed and fixed in ethanol: glacial acetic acid (3:1) for 24 h and then stored in 70% alcohol in a refrigerator until use. Cytological preparations were carried out using Faulgen squash technique according to Darlington and La-Cour (1976). Mitotic index and total abnormalities in each concentration were statistically analyzed using paired Student's t –test.

Biochemical analysis

Estimation of nucleic acids content

Roots of *A. cepa* (2 to 3 cm in length) were subjected to the same treatment mentioned before, and a method based on that of Shibko et al. (1967) was performed to estimate nucleic acid contents. RNA content was determined according to Ashwell (1957), while the method of Burton (1968) was used to determine DNA content.

SDS-PAGE of M2 seed storage protein

Characterization of protein profiles was carried out using one dimensional sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide slab gel (12.5%) was prepared according to (Laemmil, 1970). Seed samples of A. cepa previously treated with tartrazine under study were prepared for electrophoresis. For protein extraction, 0.2 ml of sample buffer was added to 0.02 g of seed meal (homogenate) and stored overnight at 4°C. Centrifugation was performed at 9000 rpm for 6 min and the supernatant was collected for analysis. Protein samples were prepared by mixing the clear supernatant with treatment buffer in 1:1 ratio, with a drop of bromophenol blue and denatured by heating at 90°C for 3 min. Equal amount of samples (20 to 30 µl) was loaded carefully through electrode buffer into the sample wells in the stacking gel layer. Wide range standard protein marker was also loaded. The apparatus was turned on and a current of 15 mA was applied till protein sample passes stacking gel, then the current was increased to 25 mA for 4 to 6 h. The bromophenol blue dye could be used to monitor the rate of migration. At the end of electrophoresis, protein bands were visualized by staining the gel with silver nitrate according to Sammons et al. (1981). The gel was removed carefully, left in the staining solution with gentle agitation until the dye penetrated the gel and then de-stained several times in de-staining solution. The gels were first photographed using a camera and documented for further analysis.

RESULTS

Concerning the effect of E102 on mitotic division of *A. cepa* root tips, it was obvious that the synthetic food color E102 had a marked reducing effect on mitotic index values. The mitotic index values were progressively decreased as the concentrations and duration of treatments were increased. The lowest value of mitotic index (MI), 1.80%, was recorded after treatment for 24 h with 0.789 mg/L as compared with the control value (4.86%). Such decrease in the MI was found to be statistically significant in most treatments (Table 1). In addition to the reduction of mitotic index, the synthetic food color caused a change in the frequencies of the different mitotic stages in all used concentrations. The results of the present study clearly demonstrated that as the concentration of E102 increased, the frequencies of both prophase and ana-telophase decreased, with a corresponding increase in the percentage of metaphase. The metaphase increased gradually until it reached a maximum value of 87.92% after 3 h treatment compared with control value of 35.92%. The frequency of prophase decreased gradually until it reached a minimum value of 4.83% after 3 h treatment compared with the control value of 34.48%. This indicates that E102 affects the relative duration at each stage as compared with the control.

The synthetic food color tartrazine showed a wide range of mitotic abnormalities and their frequencies increased as the concentration of E102 and the duration of the treatment increased as compared to the control (Table 2 and Figure 2). The most common types of abnormalities observed were stickiness, C-metaphase, laggards, disturbed chromosomes, bridges and micro-nuclei. The percentages of mitotic abnormalities were highly significant in most of treatment with the high concentrations. Low concentrations induced either a statistically significant percentage or had non- significant effect as compared to the control. The maximum value of

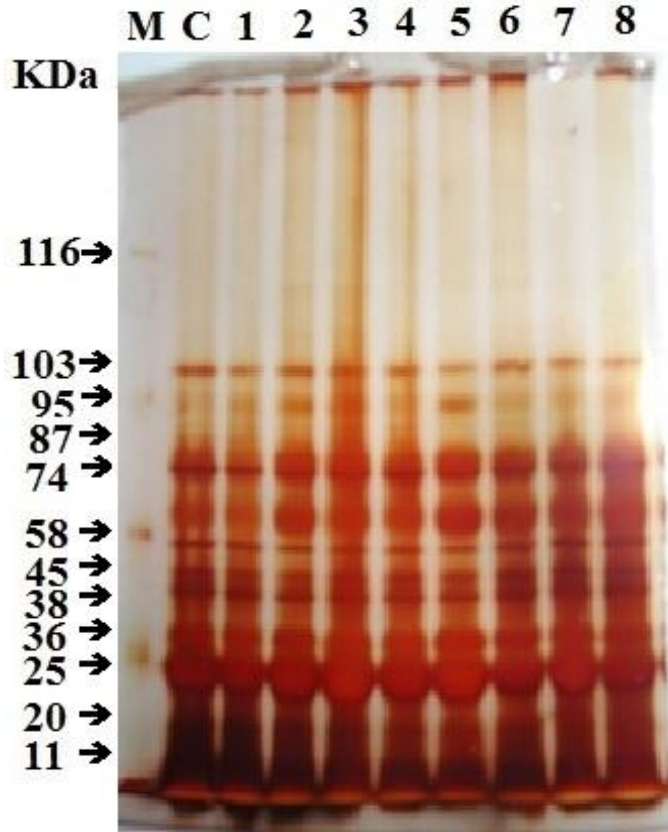

Figure 3. Electrophotograph produced by SDS-PAGE analysis of protein pattern of *A. cepa* seeds after treatment with different concentrations of the synthetic food additive color E102 for 24 h without or with further treatment by vitamin C (100 mg/L) for 3 h. M: Marker; C: Control; Lane 1: Conc. 0.097 mg/L of E102 for 24 h; Lane 2: Conc. 0.195 mg/L of E102 for 24 h; Lane 3: Conc. 0.339 mg/L of E102 for 24 h; Lane 4: Conc. 0.789 mg/L of E102 for 24 h; Lane 5: Conc. 0.097 mg/L of E102 for 24 h. + treatment with 100 mg/L of vitamin C for 3 h; Lane 6: Conc. 0.195 mg/L of E102 for 24 h. + treatment with 100 mg/L of vitamin C for 3 h; Lane 7: Conc. 0.339 mg/L of E102 for 24 h. + treatment with 100 mg/L of vitamin C for 3 h; Lane 8: Conc. 0.789 mg/L of E102 for 24 h. + treatment with 100 mg/L of vitamin C for 3 h.

the percentage of mitotic chromosomal abnormalities reached 96.44%, with the highest concentration of E102 (0.789 mg/L) for 24 h as compared with control value (1.20%). This might indicate toxic effects of E102 on the chromosomes of *A. cepa* root cells.

When different concentration of E102 were applied for 24 h and then terminated by treatment with vitamin C (100 mg/L) for 3 h (E102 + vitamin C), the results showed a marked protective effect against the mito-inhibition effect of the synthetic food color E102. The reduction of mitotic index was still observed in all treatments, but the intensity of reduction was much less in comparison to the treatment with E102 only (Table 1). The mitotic index reached 2.98% at the concentration 0.789 mg/L of E102 with vitamin C while it reached 1.80% at the same concentration without vitamin C. Vitamin C successfully reduced the effect of E102 on the percentage of chromosomal abnormalities in the roots treated with different concentrations of (E102 + VC), as compared to corresponding treatment with E102 alone. The maximum value of mitotic abnormalities was 96.44% after treatment with the highest concentration of E102 (0.789 mg/L) for 24h without vitamin C and could be reduced to 84.85% in the presence of VC (E102 for 24 h and vitamin C for 3 h).

Generally, E102 treatments resulted in a significant decrease in nucleic acid contents as compared to the control. Nucleic acid contents de-creased as the concentration of E102 and time of treatment increased as compared with the untreated roots. After vitamin C administration, only RNA contents increased in comparison with 24 h treatment with E102 alone (Table 3). The effect of synthetic food color on protein banding patterns of *A. cepa* seeds have been depicted in Table 4 and Figure 3. The total number of protein bands was 15 bands; 10 of which were common to the control and treated roots. The most visible changes in SDS-PAGE patterns were the disappearance of few bands such as: the band with molecular weight 95, 87, 74 and 11 KD found in E102 treated roots. Band with molecular weight 25 KD appeared in E102 treated roots. Over expression of bands with molecular weights 45, 20 and 25 KD was observed in E102 treatment group.

DISCUSSION

The inhibition of mitotic activity has been regarded as a common effect by numerous food colours as reported by many investigators (Palani Kumar and Panneerselvam, 2007; Shipra et al., 2008). Inhibition of mitotic division in plants has been attributed to inhibition of certain types of nuclear proteins essential in mitotic cycle and/or the inhibition of DNA synthesis (Kim and Bendixen, 1987; Lamsal et al., 2010). In addition to the reduction of mitotic index, the synthetic food color caused a change in the frequencies of the different mitotic stages with all the used concentrations. As the concentration of E102 increased, the frequencies of both prophase and ana-telophase decreased, with a corresponding increase in the percentage of metaphase (Table 1). This indicates that E102 affects the relative duration of each stage, as compared with the control.

The synthetic food color E102 produced several types of chromosomal abnormalities such as stickiness, c-metaphase, laggards, disturbed chromosomes, bridges and micronuclei after all treatments used in this investigation (Table 2). These results indicate the potentiality of the investigated synthetic food color to induce mitotic irregularities that is in accordance with other studies carried out for assessing genotoxic effects of food colors or other agents (Palani Kumar and Panneerselvam, 2007;

Table 1. Total cells examined, total mitoses, percentage of mitotic phases, percentage of total abnormal mitotic phases, and mean mitotic index after treating of *A. cepa* root tip cells with different concentrations of synthetic food color E102 for 3, 6, 12 and 24 h. Two groups of root tip cells subjected to E102 concentrations used at the last period for 24 h, and one of them terminated by vitamin C (100 mg/L) for 3 h.

Time of treatment (h)	Conc. mg/L	Total cells examined	Total mitoses	Prophase		Metaphase		Ana-telophase		Mean MI%±SE
				Prophase (%)	Abnormal prophase (%)	Metaphase (%)	Abnormal Metaphase (%)	Ana-telophase (%)	Abnormal Ana-telophase (%)	
3	Control	11953	696	34.48	0.00	35.92	0.80	29.60	0.00	5.82±0.03
	0.789	15826	591	23.18	1.46	37.56	21.17	39.26	0.43	4.43±1.08
	1.560	14144	441	53.51	4.24	30.84	97.06	15.65	49.28	3.08±0.07**
	3.125	15087	467	50.75	7.59	33.19	83.87	16.06	50.67	3.10±0.05**
	6.250	11479	207	4.83	100.00	87.92	100.00	7.25	93.33	1.82±0.11**
6	Control	12640	632	36.55	3.46	25.95	9.76	37.50	2.53	5.00±0.07
	0.339	11555	520	34.62	4.44	29.81	25.16	35.58	8.65	4.50±0.39
	0.789	12843	513	25.34	1.54	37.82	46.91	36.84	10.58	4.00±0.46
	1.56	17065	505	32.67	17.58	54.46	69.45	12.87	55.38	2.96±0.03**
	3.125	14853	553	32.19	26.40	56.60	70.93	11.21	58.06	3.73±0.02**
12	Control	17713	886	27.99	0.00	39.95	3.67	32.05	0.00	5.01±0.25
	0.195	16869	639	35.21	0.89	25.35	11.11	39.44	0.00	3.80±0.14
	0.399	15402	552	33.15	3.28	42.39	25.21	24.46	2.22	3.57±0.30*
	0.789	17700	600	28.00	2.38	28.00	27.38	44.00	3.79	3.39±0.07*
	1.56	16284	326	19.94	40.00	42.94	96.43	37.12	49.59	2.01±0.05
24	Control	18864	918	27.67	0.00	31.37	2.43	40.96	1.06	4.86±0.05
	0.097	14019	308	22.73	2.86	33.44	22.33	43.83	3.70	2.19±0.40**
	0.195	15991	344	26.16	4.44	30.52	27.62	43.31	4.70	2.14±0.10**
	0.399	15051	295	25.42	4.00	30.51	57.78	44.07	23.85	1.95±0.02**
	0.789	17039	307	6.84	100.00	63.84	99.49	29.32	90.00	1.80±0.06**
24 + 3 h vitamin C	Control	12886	780	27.69	---	37.31	0.69	35.00	---	6.05±0.23
	0.097	13260	575	25.74	---	34.09	17.86	40.17	0.87	4.33±0.16*
	0.195	13100	547	27.61	---	42.41	37.07	29.98	4.88	4.24±0.30
	0.399	16768	670	22.84	---	35.07	39.57	42.09	2.13	4.00±0.09*
	0.789	21255	636	19.18	28.69	54.87	95.42	25.94	24.85	2.98±0.10**

*Significant from control at 0.05 level (t. test). **Significant from control at 0.01 level (t. test).

Shipra et al., 2008). The most common type of abnormalities observed with nearly all treatments was stickiness (Table 2). The number of sticky cells increased in all stages of mitotic division as the concentration of E102 increased during the same period.

Table 2. Frequency of different types of metaphase and ana-telophase abnormalities and mean % of abnormal mitoses after treating of *A. cepa* root tip cells with different concentrations of synthetic food color E102 for 3, 6, 12 and 24 h. Two groups of root tip cells subjected to E102 concentrations used at the last period for 24 h, and one of them terminated by vitamin C (100 mg/L) for 3 h.

Time of treatment	Conc. in mg/L	% of metaphase abnormalities							% of ana-telophase abnormalities					Mean% of abnormal Mitoses±SE
		c-m 2n	c-m 4n	Star	Dist.	Lagg.	Stick.	Total abnormality (%)	Bridge	Dist.	Lagg.	Stick.	Total abnormality (%)	
3	Control	---	---	---	0.80	---	---	0.80	---	---	---	---	---	0.29±0.12
	0.789	14.42	---	0.90	5.41	0.45	---	21.17	---	---	---	0.43	0.43	8.45±0.64
	1.56	25.74	1.47	2.21	38.24	1.47	27.94	97.06	---	5.80	1.45	39.13	49.28	39.51±0.94
	3.125	26.45	---	---	16.13	---	41.29	83.87	---	2.67	1.33	46.67	50.67	39.84±0.18**
	6.250	---	---	1.65	---	---	98.35	100.0	13.33	---	---	80.0	93.33	99.52±0.39**
6	Control	1.83	0.61	1.83	4.88	0.61	---	9.76	---	2.53	---	---	2.53	5.19±0.97
	0.339	0.65	---	0.65	17.42	1.94	4.52	25.18	1.08	4.86	1.62	1.08	8.65	12.25±0.96
	0.789	3.61	1.03	---	20.62	1.03	20.62	46.91	4.76	3.70	2.12	---	10.58	22.64±1.67*
	1.56	18.18	4.00	1.08	9.82	3.27	33.10	69.45	4.62	6.14	4.62	40.0	55.38	51.81±0.23*
	3.125	7.67	3.51	8.95	8.95	0.32	48.56	70.93	6.54	4.84	6.45	40.32	58.06	53.15±0.56*
12	Control	---	---	---	3.67	---	---	3.67	---		---	---	---	1.46±0.11
	0.195	---	---	---	11.11	---	---	11.11	---		---	---	---	3.07±0.94
	0.399	---	---	---	18.80	---	6.41	25.21	---		---	2.22	22.22	12.42±0.57
	0.789	---	---	---	27.38	---	---	27.38	1.14		---	2.65	3.79	10.01±0.23
	1.56	29.29	---	---	7.14	---	60.0	96.43	---		8.27	---	45.59	67.83±1.75**
24	Control	---	---	---	2.43	---	---	2.43	---	1.06	---	---	1.06	1.20±0.08
	0.097	1.94	---	---	20.39	---	---	22.33	---	3.70	---	---	3.70	9.95±1.20
	0.195	---	---	---	24.76	---	2.86	27.62	0.67	4.03	---	---	4.70	12.38±1.28
	0.399	55.56	---	---	---	---	2.22	57.78	11.54	0.77	---	11.54	23.85	29.12±0.72**
	0.789	8.16	---	---	2.04	---	89.29	99.49	1.11	1.11	---	87.78	90.00	96.44±0.67**
24 + 3 h vitamin C	Control	---	---	---	0.69	---	---	0.69	---	---	---	---	---	---
	0.097	---	---	---	17.35	0.51	---	17.86	---	0.87	---	---	---	0.87
	0.195	2.59	---	---	32.76	---	1.72	37.07	---	4.88	---	---	---	4.88
	0.399	7.23	---	---	32.34	---	---	39.57	---	2.13	---	---	---	2.13
	0.789	25.21	---	0.58	24.36	---	45.27	95.42	---	15.15	---	---	---	84.85

*Significant from control at 0.05 level (t. test). **Significant from control at 0.01 level (t. test). Stick: stickiness, lag: laggards, dist: disturbed chromosomes.

Liu et al. (1992) suggested that sticky chromosomes reflect highly toxic effects, usually of an irreversible type, and probably lead to cell death. Stickiness has been suggested as a type of physical adhesion involving mainly the proteinceous matrix of chromatin material (Lamsal et al., 2010). Induction of chromosomal and chromatin bridges at anaphase and telophase stages was also observed after treatment with E102.

Table 3. Percentage of DNA and RNA content after treating of *A. cepa* root tip cells with different concentrations of the synthetic food color E102 for 3, 6, 12 and 24 h. Two groups of root tip cells subjected to E102 concentrations used at the last period for 24 h, and one of them terminated by vitamin C (100 mg/L) for 3 h.

Concentration (mg/L)	DNA (%)		RNA (%)	
	mg/g	Content (%)	mg/g	Content (%)
3 h				
Control	11.47	100	3.24	100
0.789	10.67	93.03	2.86	88.27
1.560	8.80	76.72	2.48	76.54
3.125	7.46	65.04	2.10	64.81
6.250	6.13	53.44	1.71	52.78
6 h				
Control	11.20	100	3.62	100
0.339	10.13	90.45	3.05	84.25
0.789	8.80	78.57	1.90	52.49
1.56	6.67	59.55	1.71	47.24
3.125	5.60	50.00	1.33	36.74
12 h				
Control	9.86	100	3.24	100
0.195	9.85	99.90	3.43	105.86
0.399	8.27	83.87	2.10	64.81
0.789	7.20	73.02	1.33	41.05
1.56	5.87	59.53	0.35	10.80
24 h				
Control	8.80	100	2.67	100
0.097	8.27	93.98	1.90	71.16
0.195	6.94	78.86	1.33	49.81
0.399	6.67	75.80	0.76	28.46
0.789	5.60	63.64	0.76	28.46
Treatment with E102 for 24 h followed by 3 h treatment with vitamin C				
Control	9.14	100	3.47	100
0.097	8.00	87.53	3.11	89.63
0.195	6.29	68.82	2.67	76.95
0.399	5.71	62.47	2.31	66.57
0.789	5.14	56.24	2.04	58.79

Formation of bridges could be attributed to the breakage and reunion or due to the general stickiness of chromosomes (El-Khodary et al., 1990; Lamsal et al., 2010). Sifa (2005) reported that chromosome bridges might be due to chromosomal stickiness and subsequent failure of free anaphase separation. The presence of both chromosome stickiness and bridges (Table 2) supports this conclusion.

Large number of C-metaphase where chromosomes appear scattered in the cytoplasm was found after treatment with E102 (Table 2). Such type of anomaly is an indication of the action of E102 on the inhibition of spindle fiber formation by their action on microtubules which play a major role in the formation of spindle fiber (Jain and Sarbhoy, 1987). Considerable percentages of disturbed mitotic phases were induced by treatment with E102. Disturbed phases may be due to disturbance in the function of the mechanism of chromosomes movement and the orientation of these chromosomes at the equatorial plate (Shehata et al., 2000). Among aberrations that appeared frequently after treatment with E102 was lagging chromosomes at metaphase, anaphase and

Table 4. Effect of different concentrations of the synthetic food color E102 on the protein banding pattern of *A. cepa* seeds using SDS – PAGE technique, which subjected for 24 h, without or with further treatment by and vitamin C (100 mg/L) for 3 h.

Band No.	M.wt (KDa)	Band %									
		Marker	Control	E 102				E102 + Vitamin C			
				Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
1	116	+									
2	103		+	+	+	+	+	+	+	+	+
3	95	+	+	+	+	+	+	+	+	+	
4	87		+	+	+	+	+				
5	74		+	+	+	+	+				
6	66		+	+	+	+	+	+	+	+	+
7	64		+	+	+	+	+	+	+	+	+
8	58	+	+	+	+	+	+	+	+	+	+
9	52		+	+	+	+	+	+	+	+	+
10	48		+	+	+	+	+	+	+	+	+
11	45		++	++	++	++	++	++	++	++	++
12	38		+	+	+	+	+	+	+	+	+
13	36		+	+	+	+	+	+	+	+	+
14	25	++		++	++	++	++	++	++	++	++
15	20		+	++	++	++	++	++	++	++	++
16	11		+	+	+	+	+	+	+		

Specifications of lanes

Lane 1	Conc. 0.097 mg/L of E102 for 24 h
Lane 2	Conc. 0.195 mg/L of E102 for 24 h
Lane 3	Conc. 0.339 mg/L of E102 for 24 h
Lane 4	Conc. 0.789 mg/L of E102 for 24 h
Lane 5	Conc. 0.097 mg/L of E102 for 24 h + treatment with 100 mg/L of vitamin C for 3 h
Lane 6	Conc. 0.195 mg/L of E102 for 24 h + treatment with 100 mg/L of vitamin C for 3 h
Lane 7	Conc. 0.339 mg/L of E102 for 24 h + treatment with 100 mg/L of vitamin C for 3 h
Lane 8	Conc. 0.789 mg/L of E102 for 24 h + treatment with 100 mg/L of vitamin C for 3 h

telophase stages. These laggards might be distributed randomly to either pole. They might result in the formation of micronuclei (Abd-El-Salam et al., 1993a). Considerable frequencies of micronuclei were observed in the cells of mitotic division at anaphase. Micronuclei can originate either from chromosome fragments or from lagging chromosomes. In some instances, the lagging chromosomes or fragments might have either dissolved in the cytoplasm or gradually clumped and might be surrounded by nuclear membrane to form micronuclei (El-Ghamery et al., 2003). The formation of micronuclei is regarded as an induction of mutagenicity of their inducers (Abd-El-Salam et al., 1996). Multinucleated cells were recorded at few percentages.

VC is a highly effective antioxidant. It acts as a reducing agent that can terminate free radical driven oxidation by being converted to a resonance-stabilized free radical. It is well established that VC can protect indispensable molecules in the body, such as protein, lipids, carbohydrates and nucleic acids (DNA and RNA) (Schneider et al., 2001). Our results showed that administration of VC ameliorated the DNA damage and chromosome aberrations induced by E102 at all tested doses. This ameliorative effect by VC might have resulted from enhancement of detoxification pathways that converted reactive compounds to less toxic and more easily excreted products (Vijayalaxim and Venu, 1999) and/or through its VC action as a free radical scavenging agent (Chaudiere and Ferrari-Iliou, 1999). It might also be due to the formation of complex compounds with mutagens or modulation of their metabolism (Mark et al., 2008). In addition, numerous *in vitro* and *in vivo* studies have evaluated the protective effects of VC against several radical generating chemicals (Robichova et al., 2004; Arranz et al., 2007; Rudrama and Kusum, 2011).

The protective effect of VC against E102 induced genotoxicity might be due to one of the following properties of VC: antioxidant action, trapping of free radicals, formation of complex with mutagens or modulation of mutagen metabolism (Mark et al., 2008; Nancy et al., 2011). However,

the definite molecular mechanisms of antimutagenic effects or antigenotoxicity of vitamin C in *A. cepa* root meristem cells needs further investigations.

Tartrazine induces oxidative stress and DNA damages (Soheila and Zeidali, 2011). The inhibition of the nucleic acid contents could be attributed to the inhibition of DNA synthesis. Most of the treatments with E102 resulted in a progressive significant decrease in the nucleic acid contents with the increase of E102 concentration and the time of treatment, and also as compared with the untreated roots (Table 3), but still lower than the control values. The inhibition of DNA and RNA synthesis might be due to inhibition of DNA replication as suggested by Scott (1968). The inhibition of the nucleic acid contents could be attributed to inhibition of DNA synthesis. These results are in accordance with those obtained by Badr (1987). However, the reduction in DNA content could be presumably attributed to the reduction of oxidative phosphorylation that would lead to lowering adenosinetriphosphate (ATP) level in the cell (Gruenhagen and Moreland, 1971).

The present results indicated that the decrease in the rate of the cell division is also accompanied by a decrease in the DNA content of the cells. Ibrahim (1991), Tsuda et al. (2001) and Mpountoukas et al. (2010) indicated that there should be a positive correlation between the rate of cell division and DNA synthesis.

It was well concluded earlier that the synthetic food color under study caused disappearance of some bands in *A. cepa*. The vanishing of some electrophoretic bands could be attributed to the loss of the genetic materials due to fragmentation, laggards and micronuclei as found in the present study. The results of the present study are in full agreement with the earlier reports of Hassan (2000) and Soliman and Ghoneam (2004). In addition, another earlier report also clearly showed that the absence of some bands represent the deletion of their corresponding genes (Hassan, 2000). It was interesting to notice in the present study that E102 caused the appearance of new bands which were absent in untreated *A. cepa*. The appearance of new characteristic bands could be explained on the basis of mutational events at the regulatory system of an unexpected gene(s) that activated it (El-Nahas, 2000). It is well known that gene mutation and changes in gene expression are responsible for changing the banding pattern. E102 treatment induced cytological abnormalities which gave rise to changes in band intensity due to induction of gene mutation at the regulatory system. Gene expression is changed due to gene mutation (Soliman and Ghoneam, 2004). The increase in band colour intensity provide basis to represent gene duplication resulting due to cytological abnormalities (Hassan, 2000; Soliman and Ghoneam, 2004). The alternations in the electrophoretic profiles of seed proteins are indicative of the ability of E102 to alter the gene expression of the exposed cells.

There is considerable evidence that the effects of mutagenic and carcinogenic agents can be altered by many dietary constituents. VC is an essential dietary nutrient required as a co-factor for many enzymes and a very efficient antioxidant, scavenging reactive oxygen and nitrogen species and protecting cells against free radical-mediated damage. Besides exerting antioxidant influence directly, VC can promote the removal of oxidative DNA damage from the DNA and/or nucleotide pool through the up regulation of repair enzymes. Also, VC has inhibitory effect towards a number of mutagens/ carcinogens (Fahmy et al., 2008; Jennifer et al., 2009; Sanchez et al., 2003).

The supplementation of vitamin C showed to be very much helpful in minimizing the toxic effects induced by E102. Although the mutagenic potential of E102 was not significantly high, but it clearly indicates that continuous or prolonged exposure and consumption of E102 can pose a potential risk to human health. It was found that the synthetic food color E102 caused harmful effects at both cytogenetic and biochemical levels. Therefore, it is suggested to test the mutagenic potential of synthetic food colors on more intensive and extensive basis, especially on non target systems before it is recommended for wider use in foods.

REFERENCES

Abd-El-Salam AZE, Hassan HZ, Badawy FMI, Abdel-Naby WM (1993a). The mutagenic potentialities of three pesticides on three biological systems. Egypt J. Gent. Cytol. 22:109-128.

Abd-El-Salam AZE, Hassan HZ, Soliman KH (1996). The mutagenicity of two aromatic systemic pesticides using three biological systems. Egypt. J. Genet. Cytol. 25:171-184.

Ambali SF, Shittu M, Ayo JO, Esievo KAN, Ojo SA (2011). Vitamin C alleviates chronic chlorpyrifos induced alterations in serum lipids and oxidative parameters in male wistar rats. Am. J. Pharmacol. Toxicol. 6(4):109-118.

Amin KA, Abdel Hameid H, AbdElsttar AH (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem.Toxicol. 48(10):2994-2999.

Arranz N, Haza AI, Garcia A, Rafter J, Morales P (2007). Protective effect of vitamin C towards N -nitrosamine-induced DNA damage in the single cell gel electrophoresis (SCGE)/HepG2 assay. Toxicol. In vitro 21:1311-1317.

Arriaga-Alba M, Rivera-Sanchez R, Ruiz-Pérez JN (2008). Comparative study of the antimutagenic properties of vitamins C and E against mutation induced by norfloxacin. BMC Pharmacology. http://link.springer.com/article/10.1186%2F1471-2210-8-2.

Ashwell G (1957). Methods in Enzymol *3*. Academic Press New York. P 87.

Assia D, Mahfoud M, Amel B, Lilia K, Abdelfattah EF (2012). Protective effects of vitamin C and selenium supplementation on methomyl-induced tissue oxidative stress in adult rats. Toxicol. Ind. Health PMID. 23222694.

Axon A, May FE, Gaughan LE, Williams FM, Blain PG, Wright MC (2012). Tartrazine and sunset yellow are xenoestrogens in a new screening assay to identify modulators of human oestrogen receptor transcriptional activity. Toxicology 298(1-3):40-51.

Badr A (1987). Effects of the S-triazine herbicide terbutryn on mitosis chromosomes and nuclecic acids in root tips of *Vicia faba.* Cytologya 51:571-578.

Badr A (1995). Electrophoretic studies of seed proteins in relation to chromosomal criteria and relationships of some taxa of *Trifolium.* Taxon 44:183-191.

Bansal AK (2005). Modulation of N-nitrosodiethylamine induced

oxidative stress by vitamin E in rat erythrocytes. Hum. Exp. Toxicol. 2:297-302.

Burton K (1968). Determination of DNA concentration with diphenylamine. Methods Enzymol. 12B:163-166.

Chaudiere J, Ferrari Iliou R (1999). Intracellular antioxidants: from chemical to biochemical mechanisms. Food Chem. Toxicol. 37:949-962.

Darlington CD, La-Cour LF (1976). The handling of chromosomes, Sixth ed. George Allene and Unwin., Ltd, London.

El Khodary S, Habib A, Haleim A (1990). Effect of the herbicide tribunal on root-mitoses of *Allium cepa*. Cytologia 55: 209-215.

El -Nahas AI (2000). Mutagenic potential of imazethapyr herbicide (Pursuit) on *Vicia faba* in the presence of urea fertilizer. Pak. J. Biol. Sci. 3(5):900-905.

El-Ghmery AA, El kholy MA, ELyousser *A* (2003). Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticumae stivum* L. Mutat. Res. 537:2941.

Fahmy MA, Hassan NHA, Farghaly AA, Hassan EES (2008). Studies on the genotoxic effect of beryllium chloride and the possible protective role of selenium/vitamins A, C and E. Mutat. Res. 652:102-111.

Food Reactions (2010). http://home.iprimus.com.au/foo7/additives.html

FSA (2007). Food Standards Agency U.K. http://www.food.gov.uk/news/newsarchive/2007/sep/foodcolours#content

George NM, Ghareeb A (2001). Genotoxicity of insecticide cyolan on mitosis, meiosis and seed storage proteins of *Vicia faba.* Cytologia 66:77-84.

Giri AK (1991). Food dyes in India: mutagenic and clastogenic potentials- A review. Proc. Indian Nat. Sci. Acad. B57:183-198.

Grant WF (1994). The present status of higher plants bioassays for the detection of environmental mutagens. Mutat. Res. 310:175-185.

Gruenhagen RD, Moreland DE (1971). Effects of herbicides on ATP levels in excised *Soya bean* hypocotyls. Weed Sci. 19:319-330.

Hassan HZ (2000). Effects of stimufol fertilizer on bost cytological abnormalities and protein profile alternations induced by Nuvacron insecticide. Proceedings of the 1st International Conference on Biological Science (ICBS). Faculty of Science, Tanta University 1:448- 466.

Ibrahim AG (1991). Cytological effects of the herbicide Cycluron on root tip mitosis and nucleic acids in *Vicia faba.* Egypt .J. Appl .Sci. 6:225-231.

Jain AK, Sarbhoy RK (1987). Cytogenetical studies on the effect of some chlorinated pesticides 1. Effect on somatic chromosomes of *Len a*nd *Pisum* spp. Cytologia. 52:47-53.

Jennifer A, Nancy RC, Christine A, Elaine Z, Michael JG, Martin VD, Julie EB, Joann EM (2009). Vitamins C and E and Beta Carotene Supplementation and Cancer Risk. A Randomized Controlled Trial. J. Natl Cancer Inst. 101(1):14-23.

Kim JC, Bendixen EL (1987). Effects of haloxyfop and CGA- 82725 on cell cycle and cell division of oat (*Avena sativa*) root tips. Weed Sci. 35:769-774.

Konuk M, Liman R, Cigerci IH (2007). Determination of genotoxic effect of Boron on *Allium cepa* root meristematic cells. Pak. J. Bot. 39:73-79.

Lamsal K, Ghimire BK, Sharma P, Ghimirar AK, Kim SW, Lee YS, Kim JS, Shakya SR (2010). Genotoxicity evalution of the insecticide ethion in root of *Allium cepa* L. Afr. J. Biotechnol. 9(27):4204-4210.

Liman R, Akyil D, Eren Y, Konuk M (2010). Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/*Salmonella* and *Allium* test. Chemosphere 80:1056-1061.

Liu D, Jiang W, Li M (1992). Effects of trivalent and bexavalent chromium on root growth and cell division of *Allium cepa*. Hereditas 117:2329.

Mark LH, Jeffry RG, Nicos K, David WO, David AS, Emily AS, Owen AO (2008). Vitamin C antagonizes the cytotoxic effects of antineoplastic drug. Cancer Res. 68:8031-8038.

Matsumoto ST, Mantovani MS, Malamute MI, Dias AL, Fonseca IC, Marin-Morales MA (2006). Assessment of the genotoxic and mutagenic effect of chromium residues present in tannery effluents using the micronucleus and comet assay in *Oreochromis niloticus* and chrromosome aberrations of *Allium cepa* . Genet. Mol. Biol. 29:148-158.

Mervat MK, Heba SE (2011). The Potential Health Hazard of Tartrazine and Levels of hyperactivity, anxiety-like symptoms, depression and anti-social behaviour in rats. J. Am. Sci. 7(6):1211-1218.

Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, Mourelatos C, Lambropoulou V, Lialiaris T (2010). Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem. Toxicol. 48(10):2934-2944.

Nancy NM, Edward LG, Kevin TM, John BM (2011). Dietary, but not supplemental, intakes of carotenoids and vitamin C are associated with decreased odds of lower urinary tract symptoms in men. J. Nutr. 141(2):267-273.

Palani Kumar L, Panneerselvam N (2007). Cytogenetic studies of food presservative in *Allium cepa* root meristem cells. Med. Biol. 14(2):60-63.

Park M, Park HR, Kim SJ, Kim MS, Kong KH, Kim HS, Gong EJ, Kim ME, Kim H.S, Lee BM, Lee J (2009). Risk assessment for the combinational effects of food color additives: neural progenitor cells and hippocampal neurogenesis. J. Toxicol. Environ. Health A. 72(21-22):1412-1423.

Robichova S, Slamenova D, Chalupa I, Sebova L (2004). DNA lesions and cytogenetic changes induced by N-nitrosomporpholine in HepG2, V79 and VH10 cells: the protective effects of vitamins A, C and E. Mutat. Res. 560:91-99.

Rudrama DK, Kusum LC (2011). Protective role of vitamin c against cytotoxicity induced by adriamycin in mice. Int. J. Pharm. Life Sci. 2(11):1195-1202.

Sammons DW, Adamas LD, Nishizawe EF (1981). Ultrasensitive silver based color staining of polypeptides in polyacrylamide gels. Electrophoresis 2:135-40.

Sanchez-Moreno C, Paniague M, Madrid A, Martin A (2003). Protective effect of vitamin C against the ethanol mediated toxic effects on human brain glial cells. J. Nutr. Biochem. 14:606-613.

Schneider M, Diemer K, Engelhart K, Zankl H, Trommer WE, Biesalski K (2001). Protective effects of vitamins C and E on the number of micronuclei in lymphocytes in smokers and their role in ascorbate free radical formation in plasma. Free Radic. Res. 34(3):209-219.

Scott D (1968).The additive effect of X-rays and maleic hydrazide in inducing chromosome aberrations at different stages of mitotic cycle in *Vicia faba*. Mutat. Res. 5:65-92.

Shehata MM, Habib A, Khalifa NS, Salama MS (2000). Cytological and biochemical effects of 5-florouracil and colchicine on *Vicia faba* plants. Egypt J. Biotechnol. 7:218-233.

Shibko S, Koivistoven P, Tratnyek CA, Newhall AR, Friedman L (1967). Method for sequential quantitative separation and determination of protein, RNA, DNA, lipid and glycogen from a single liver homogenate or from subcellular fraction. Annal. Biochem. 19:415-528.

Shipra S, Rajendra PG, Geetanjali C, Anjali S (2008). Toxicity of tomato red, a popular food dye blend on male albino mice. Exp. Toxicol. Pathol. 60(1):51-57.

Sifa T (2005). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutat. Res. 626:4-14.

Soheila K, Zeidali SH (2011). DNA binding studies of tartrazine food additive. DNA Cell Biol. 30(7):499-505.

Soliman MI, Ghoneam GT (2004). The mutagenic potentialities of some herbicides using *Vicia faba* as a biological system. Biotechnology 3(2):140-154.

Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchio K, Sasali YF (2001). DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicol. Sci. 61:92-99.

Vijayalaxmi KK, Venu R (1999). *In vivo* anticlastogentic effects of L-ascorbic acid in mice. Mutat. Res. 438:47-51.

Visweswaran B, Krishnamoorthy G (2012). Oxidative Stress by Tartrazine in the Testis of Wistar Rats. IOSR J. Pharm. Biol. Sci. 2(3):44-49.

Walton K, Walker R, Van DS, Jjm S, Castell JV (1999). The application of in vitro in the derivation of the acceptable daily intake of food additives. Food Chem. Toxicol. 37:1175-1197.

Yonglin G, Chunmei L, Jingyu S, Huaxian Y, Xiulin A, Haizhu J (2011). Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. J. Food Sci. 76(67):T125-T129.