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Evaluation of genotoxic effects of five flavour enhancers (glutamates) on the root meristem cells of *Allium cepa*

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Abstract

The effects of different treatments with flavour enhancers monosodium glutamate, monopotassium glutamate, calcium diglutamate, monoammonium glutamate, and magnesium diglutamate on the cytology, DNA content, and interphase nuclear volume (INV) of *A. cepa* were investigated. Three concentrations of these additives – 20, 40, and 60 ppm – were applied for 6, 12, and 24 h. All the concentrations of these chemicals showed an inhibitory effect on cell division in root tips of *A. cepa* and caused a decrease in mitotic index values. Additionally, all the treatments changed the frequency of mitotic phases when compared with the control groups. These compounds increased chromosome abnormalities, among them are micronuclei, c-mitosis, anaphase bridges, stickiness, binucleus, laggards, and breaks. The nuclear DNA content and INV decreased when compared with control groups.

Keywords

Allium cepa, DNA content, genotoxic effect, interphase nuclear volume, magnesium diglutamate, monoammonium glutamate, monopotassium glutamate, monosodium glutamate

Introduction

Flavour enhancers are used in savoury foods to enhance the existing flavour. Food flavour enhancers are commercially produced in the form of instant soups, frozen dinners, and snacks. Flavour enhancers are labelled on food ingredient packets with E numbers from 600 to 699 and help to improve the taste of both sweet and savoury foods. For example, the sweet foods that they are commonly found in include cakes, biscuits, sweet snacks, breads, and bakery products. As far as savoury products go, they are widely found in soups, packet soups, pork pies, sausages, pre-cooked dried rice, sauces, crisps, snacks, and gravy granules.

Flavour enhancers can come from natural, synthetic, or manmade sources. Glutamic acids are used as a flavour enhancer in a variety of foods. Monosodium glutamate (MSG-E621), monopotassium glutamate (MPG-E622), calcium diglutamate (CDG-E623), monoammonium glutamate (MAG-E624), and magnesium diglutamate (MDG-E625) are salts of glutamic acid. There are used as food additives and are commonly marketed as flavour enhancers (FAO/WHO, 2012). Glutamic acid is one of a chain of 20 amino acids that make up a protein molecule. It is a non-essential amino acid, which means that the body produces what is needed and we do not need to make it up in our diet. The brain uses glutamic acid as a neurotransmitter.

Although glutamates are used worldwide and work effectively as flavour enhancers, they also carry some side effects. The side effects have gained the title of 'Chinese Restaurant syndrome', as MSG has been used a lot in Chinese food in the past. Some of the symptoms include palpitations, dizziness, headaches, muscle pain, nausea, migraine-like headaches, and pains in the neck and arms (Leber, 2008; Yang et al., 1997).

It has been demonstrated that high concentrations of MSG in the central nervous system induce neuronal necrosis and damage the retina and circumventricular

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organs (Ortiz et al., 2006). It is reported that MSG may be linked to brain tumours and neurodegenerative diseases, such as Amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's diseases (Andreas and Pullanipally, 2000). According to Blaylock (1996), author of a book titled, *Excitotoxins: The Taste That Kills*, MSG is an excitotoxin, which basically means it is a chemical substance that excites your neurons (brain cells) and may cause them to die. Blaylock proposes that excitotoxins, like MSG, may aggravate many neurological disorders, such as Alzheimer's disease and Parkinson's disease. MSG (and excitotoxins) side effects may include seizures, brain cell damage, allergies, rashes, asthma attacks, headaches, obesity, and brain tumours. The stress-induced abnormalities in blood–brain barrier permeability suggest differing MSG effects dependent on existing states of relaxation or stresses. The suggestive evidence for MSG-induced neuroendocrine effects is substantial, coupled with the observation of increased obesity in children. Glutamates are considered the major excitatory neurotransmitter into the mammalian central nervous system, where it is an important mediator of sensory information, motor coordination, emotions, and cognition, including the formation and retrieval of memory (Schwartz, 2000).

In fact, when flavour enhancers are given to organisms in excessive amounts, they may cause toxic reactions. There have been many studies on the nervous system and especially MSG in mice and humans. However, there are many flavour enhancers whose possible side effects are unknown.

The Ministry of Agriculture of Turkey (General Directorate of Protection and Control) (2003) suggested that food additives may be used at 10 g/kg in food. No studies have been carried out, to our knowledge, on the cytological effects of MSG, MPG, CDG, MAG, and MDG in plant systems despite the fact that they are commonly used. For this reason, the aim of this study is to investigate the effects of MSG, MPG, CDG, MAG, and MDG on chromosomes, mitotic index (MI), mitotic phases, nuclear DNA content, and interphase nuclear volume (INV) induction in *Allium cepa* root tip cells.

There are a number of higher plants such as *Vicia faba*, *Tradescantia*, *Hordeum vulgare*, and *A. cepa* that can be used in the study of the cytogenetic and mutagenetic effect of chemicals (Mišik et al., 2006; Sang and Li, 2004; Türkoğlu, 2009a, 2009b). Among them, the *Allium* test is one of the best-established test systems in order to determine toxicity in laboratories (Fiskesjö, 1997; Liman et al., 2010; Türkoğlu, 2008). *A. cepa* has been used to evaluate DNA damage, such as chromosome aberrations and disturbances in the mitotic cycle. *A cepa* is characterized as a low-cost test. It is easily handled and has advantages over other short-term tests that require previous presentations of tested samples as well as the addition of the exogenous metabolic system.

The present study has reported the cytogenetic effects of five food additives (flavour enhancer) on the root meristem cells of *A. cepa*, with an aim to provide (1) information that authenticates the genotoxicity potential that these additives are composed of and (2) a possible explanation to validate the use of *Allium* test as alternative model to mammalian test systems for similar studies, by comparing the results of the present study with the outcome of earlier reports published on the mammalian tests.

Material and methods

A. cepa (n = 16) onion bulbs, 25–30 mm diameter, without any treatment, were purchased from a local supermarket.

The following enhancers were used in this study: MSG-E621 (Cas No: 142-47-2), MPG-E622 (Cas No: 540778-10-7), CDG-E623 (Cas No: 5996-22-5, MAG-E624 (Cas No: 15673-81-1), MDG-E625 (Cas No: 18543-68-5).

EC_{50} determination

To determine the appropriate concentrations for the genotoxicity assay, an *Allium* root inhibition test was carried out. The procedure of the root inhibition test as described by Fiskesjö (1997) was followed with few modifications (Rencüzoğulları et al., 2001). The onions were grown in freshly made distilled water for the first 24 h and afterwards were exposed for 4 day to the chemical solutions (20, 40, 60, 80, and 100 ppm respectively). In order to assess the effective concentration (EC_{50}) value, roots from each bundle were cut off on the fourth day and length of each root was measured. EC_{50} value was considered as the concentration that retards the growth of root 50%, when compared with the control. EC_{50} value was found as 80 ppm.

Experimental design, mitotic activity, aberrations, nuclear DNA content, and INV

The preparation of slide, analyses of MI and chromosomal abnormalities, nuclear DNA content, and INV

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Table 1. Cytogenetic analysis of *Allium cepa* root tips exposed to different concentrations of monosodium glutamate for different periods.

Time of treatment	Doses (ppm)	Examined total cells	% Prophase	% Metaphase	% Anaphase–telophase	MI (mean ± SE)[a]	c-M	AB	MN	L	S	% Total abnormalities[a]
6 h	Control	1512	47.01	38.88	14.11	30.29 ± 0.24[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1521	25.13	30.00	44.87	13.93 ± 0.48[c]	2.14	1.05	1.23	1.14	0.00	5.56[c]
	40	1500	14.18	25.77	60.05	12.86 ± 1.25[c,d]	1.35	1.54	2.41	0.60	1.12	7.02[d]
	60	1508	38.21	25.05	36.74	11.33 ± 1.79[d,e]	3.26	4.32	4.51	1.94	0.00	14.03[e]
12 h	Control	1510	46.05	37.23	16.72	29.80 ± 3.11[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1513	30.01	29.18	40.81	13.08 ± 2.18[c]	1.42	1.35	3.10	0.00	2.11	7.98[d]
	40	1507	47.23	27.47	25.30	10.68 ± 2.00[e]	0.61	0.39	2.00	0.00	1.27	4.27[f]
	60	1504	41.11	28.41	30.48	11.50 ± 1.14[d,e]	3.13	3.51	4.19	0.00	2.30	13.13[e,g]
24 h	Control	1511	48.28	30.13	21.59	30.44 ± 0.98[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1500	30.30	21.17	48.53	8.13 ± 1.31[f]	3.41	2.65	3.27	1.24	1.98	12.55[g]
	40	1504	27.51	20.00	52.49	6.91 ± 1.10[f,g]	7.16	3.65	12.14	3.77	4.51	31.23[h]
	60	1502	20.23	20.19	59.64	6.12 ± 0.85[g]	10.45	5.54	16.32	2.18	3.87	38.36[i]

c-M: C-mitosis; AB: anaphase bridges; MN: micronucleus; L: laggards; S: stickiness; MI: mitotic index; SE: standard error.
[a]Means with the same letters do not significantly differ at 0.05 level.

Table 2. Cytogenetic analysis of *Allium cepa* root tips exposed to different concentrations of monopotassium glutamate for different periods.

Time of treatment	Doses (ppm)	Examined total cells	% Prophase	% Metaphase	% Anaphase–telophase	MI (mean ± SE)[a]	c-M	AB	MN	L	S	% Total abnormalities[a]
6 h	Control	1504	73.20	10.31	16.49	27.46 ± 0.45[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1502	81.00	3.50	15.50	12.18 ± 1.92[c,d]	4.15	0.00	1.78	0.00	0.00	5.93[c]
	40	1513	58.90	5.40	35.70	13.35 ± 0.65[c]	5.50	0.00	7.48	0.00	3.66	16.64[d]
	60	1510	82.96	1.77	12.27	9.07 ± 3.42[d]	6.00	0.00	13.48	2.44	0.00	21.92[f]
12 h	Control	1502	72.34	4.37	23.29	25.03 ± 1.19[f]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1500	79.78	3.21	17.01	13.20 ± 2.54[c]	5.17	4.51	4.23	0.00	0.00	13.91[d]
	40	1510	74.34	1.23	24.43	12.11 ± 3.79[c,d]	6.01	4.32	5.31	0.00	3.30	18.94[g]
	60	1521	97.79	1.20	1.01	11.11 ± 1.10[d]	6.33	4.82	8.51	0.00	4.00	23.66[h]
24 h	Control	1509	73.12	6.28	20.60	24.90 ± 2.41[f]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1515	80.01	4.50	15.49	11.35 ± 3.65[d]	5.00	0.00	3.54	2.08	4.65	15.27[i]
	40	1504	86.00	4.93	9.07	9.24 ± 1.14[d]	6.22	5.13	0.85	0.00	5.98	20.19[g]
	60	1500	93.03	3.00	0.97	8.00 ± 0.18[d]	6.50	4.08	16.27	0.00	5.04	31.89[j]

c-M: C-mitosis; AB: anaphase bridges; MN: micronucleus; L: laggards; S: stickiness; MI: mitotic index; SE: standard error.
[a]Means with the same letters do not significantly differ at 0.05 level.

were made by the methods described in the author's previous works (Türkoğlu, 2009).

Statistics

Analysis of variance (ANOVA) of the data was carried out with SPSS computer program. For statistical analysis, the one-way ANOVA and Duncan's mean range test were used.

Results

The effects on the MI, frequency of mitotic phases, and chromosomal abnormalities are given in Tables 1 to 5 for the treatments with MSG, MPG, CDG, MAG, and MDG, respectively. As can be seen, these flavour enhancers significantly decreased MI in the treatment groups compared with the control in all the concentrations and treatment periods. All the concentrations of MSG, MPG, CDG, MAG, and MDG used in the study caused changes in the percentage of phase distribution in comparison to the control. Assays carried out with the MSG, MPG, CDG, MAG, and MDG revealed several chromosomal alterations. Some of the alterations seemed to be derived from the effects that occurred in different phases of the cell cycle and/or due to the persistence of the flavour enhancer's action in more than a consecutive cell cycle. The chromosomal abnormalities observed in the present study were visualized in all stages of the cell cycle: interphase, prophase, metaphase, anaphase, and telophase. The cells in interphase and prophase showed micronuclei (MNs) at the treatments with MSG, MPG, CDG, MAG, and MDG

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Table 3. Cytogenetic analysis of *Allium cepa* root tips exposed to different concentrations of calcium diglutamate for different periods.

Time of treatment	Doses (ppm)	Examined total cells	% Prophase	% Metaphase	% Anaphase–telophase	MI (mean ± SE)[a]	c-M	AB	MN	Break	S	% Total abnormalities[a]
6 h	Control	1510	41.25	37.52	21.23	26.29 ± 0.04[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1504	45.00	19.35	35.65	18.00 ± 0.25[c]	1.39	6.34	0.98	0.60	0.00	9.31[c]
	40	1512	51.26	23.82	24.92	14.08 ± 0.08[d]	3.51	1.96	1.84	3.81	0.00	11.12[d]
	60	1500	60.32	28.71	10.97	14.80 ± 1.37[d]	6.27	0.77	1.00	1.30	0.70	10.04[c,d]
12 h	Control	1513	45.18	30.97	23.85	26.43 ± 1.59[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1521	54.20	23.03	22.77	14.59 ± 1.09[d]	1.67	3.52	4.25	3.51	0.30	13.25[e]
	40	1504	52.31	24.75	22.94	16.48 ± 0.25[e]	3.48	4.25	8.13	4.31	0.00	20.17[f]
	60	1505	57.71	22.35	19.94	14.21 ± 0.16[d]	8.51	0.00	6.74	5.87	0.00	21.12[f]
24 h	Control	1500	43.13	31.31	25.56	26.33 ± 0.36[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1502	53.26	21.01	25.73	7.78 ± 0.12[f]	3.73	4.38	16.13	3.15	0.00	27.39[g]
	40	1513	56.32	28.85	14.83	11.96 ± 2.84[g]	7.14	9.81	17.51	2.18	0.00	36.64[h]
	60	1508	58.00	26.40	15.60	12.73 ± 1.97[g]	10.00	9.32	21.16	0.00	0.00	40.48[i]

c-M: C-mitosis; AB: anaphase bridges; MN: micronucleus; S: stickiness; MI: mitotic index; SE: standard error.
[a]Means with the same letters do not significantly differ at 0.05 level.

Table 4. Cytogenetic analysis of *Allium cepa* root tips exposed to different concentrations of monoammonium glutamate for different periods.

Time of treatment	Doses (ppm)	Examined total cells	% Prophase	% Metaphase	% Anaphase–telophase	MI (mean ± SE)[a]	c-M	AB	MN	Bi	S	% Total abnormalities[a]
6 h	Control	1500	28.27	37.90	33.83	28.46 ± 2.75[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1506	28.14	27.57	44.29	21.31 ± 1.26[c]	3.21	0.00	2.53	0.00	0.00	5.74[c]
	40	1521	21.62	29.47	48.91	19.72 ± 0.38[d,e,f,g]	1.19	0.00	5.22	0.00	0.00	6.41[c]
	60	1533	42.70	15.32	41.98	20.28 ± 4.00[c,d]	2.41	0.00	3.68	0.00	0.00	6.09[c]
12 h	Control	1501	34.13	22.00	43.87	28.64 ± 2.81[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1511	33.56	30.12	46.32	19.85 ± 2.71[d,e,f]	3.14	1.25	1.25	0.00	0.00	8.00[d]
	40	1510	39.41	34.70	25.89	19.07 ± 1.44[c,h]	5.00	4.53	4.19	0.00	3.51	17.23[h]
	60	1509	28.60	25.48	45.92	16.56 ± 1.18[i]	7.32	5.10	8.12	0.00	3.65	24.19[e]
24 h	Control	1504	30.82	42.82	26.36	26.59 ± 2.80[j]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1513	21.13	23.13	55.74	20.55 ± 2.41[c,f]	0.00	0.00	4.01	4.17	0.00	8.18[d]
	40	1500	25.00	26.00	49.00	18.60 ± 2.06[h,e]	3.17	2.92	12.11	8.14	0.00	26.34[i]
	60	1522	18.20	37.18	44.62	18.46 ± 1.11[h,g]	9.47	5.19	18.98	5.85	6.51	46.00[j]

c-M: C-mitosis; AB: anaphase bridges; MN: micronucleus; Bi: binucleus; S: stickiness; MI: mitotic index; SE: standard error.
[a]Means with the same letters do not significantly differ at 0.05 level.

(Tables 1 to 5, Figure 1(a)) and binucleus (Tables 4 and 5; Figure 1(e)). In metaphase, we observed c-mitosis (Tables 1 to 5; Figure 1(b)), stickiness (Tables 1 to 5; Figure 1(d)), and chromosomal breaks (Table 3; Figure 1(g)). Cells in anaphase and telophase showed chromosomal bridges (Tables 1 to 5; Figure 1(c)) and laggards (Tables 1 and 2; Figure 1(f)).

Table 6 represents the DNA contents in the root tip cells of *A. cepa* treated with various concentrations of MSG, MPG, CDG, MAG, and MDG for different time intervals. DNA contents in the root cells of *A. cepa* decreased drastically after all the treatment groups. One-way ANOVA of the nuclear DNA contents of cells of the control and treated groups was significant at $p < 0.05$ in all concentrations and time intervals studied.

The effects of the different treatments of MSG, MPG, CDG, MAG, and MDG on the INV are shown in Table 7. Increased concentration and/or prolonged periods of treatment resulted in increased reductions in INV.

Discussion

The *Allium* test has been widely used throughout the world to detect mutagenic activity of food additives (Dönbak et al., 2002; Gömürgen, 2005; Rencüzoğulları et al., 2001; Türkoğlu, 2007, 2008, 2009a, 2009b). MI is summarized in Tables 1 to 5. The MI reflects the frequency of cell division. The inhibition of cellular cycle by the decline of the MI indicates the occurrence of a cytotoxic effect. MI frequencies

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Table 5. Cytogenetic analysis of *Allium cepa* root tips exposed to different concentrations of magnesium diglutamate for different periods.

Time of treatment	Doses (ppm)	Examined total cells	% Prophase	% Metaphase	% Anaphase–telophase	MI (mean ± SE)[a]	c-M	AB	MN	Bi	S	% Total abnormalities[a]
6 h	Control	1505	38.00	19.23	42.77	33.08 ± 2.15[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1512	52.04	26.00	21.96	21.36 ± 1.36[c]	1.00	0.00	1.78	0.70	0.00	3.48[c]
	40	1521	49.72	22.13	28.15	13.87 ± 1.12[d]	2.05	0.00	3.14	0.00	1.11	6.30[d]
	60	1548	35.00	42.82	22.18	19.18 ± 3.75[e,f]	2.13	1.12	1.00	0.99	0.00	5.24[d]
12 h	Control	1506	40.07	27.60	32.33	32.53 ± 2.84[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1510	42.44	37.41	20.15	20.99 ± 1.24[c,g]	1.63	2.11	3.14	0.00	2.25	9.13[e]
	40	1507	41.87	32.56	25.57	18.91 ± 1.51[e,f]	5.21	2.59	3.54	0.00	0.00	11.34[g]
	60	1518	32.64	48.87	18.49	19.76 ± 1.18[g,f]	6.12	2.68	3.24	5.10	0.00	17.14[f]
24 h	Control	1500	42.00	40.77	17.23	32.13 ± 1.18[b]	0.00	0.00	0.00	0.00	0.00	0.00[b]
	20	1500	48.00	21.62	30.38	19.20 ± 3.15[e,f]	4.60	0.00	4.54	0.00	3.21	12.35[g]
	40	1531	44.11	29.41	26.48	9.47 ± 1.05[h]	5.15	4.26	9.87	4.85	0.00	24.13[h]
	60	1527	35.91	29.00	35.09	8.31 ± 1.09[h]	5.32	4.43	10.00	4.25	0.00	24.00[h]

c-M: C-mitosis; AB: anaphase bridges; MN: micronucleus; Bi: binucleus; S: stickiness; MI: mitotic index; SE: standard error.
[a]Means with the same letters do not significantly differ at 0.05 level.

decreased with increasing concentrations of MSG, MPG, CDG, MAG, and MDG. Present findings are in agreement with earlier studies on other food additives demonstrating mitotic inhibition in root meristem cells of *A. cepa* (Dönbak et al., 2002; Gömürgen, 2005; Rencüzoğulları et al., 2001; Türkoğlu, 2007, 2008, 2009a, 2009b) and cultured human peripheral lymphocyte (Zengin et al., 2011). Due to the reduced number of dividing cells, it is suggested that these chemicals might have a mitodepressive effect on the cell division of *A. cepa*. Mitodepression blocks the synthesis of DNA and nuclear proteins (Schulze and Kirschner, 1986). More cells were arrested at the interphase stage, which consequently led to a reduction in the rates of cell division. Considering the three sub-phases of interphase, there are a number of reports showing that only the synthetic phase (S) or gap$_2$ (G$_2$) was frequently disturbed by genotoxic substances (Kwankua et al., 2010; Salehzadeh et al., 2003). According to Macleod (1969) and Brunori (1971), disturbance of interphase stage at S or G$_2$ could occur through sub-phase inhibition or an increase in phase duration.

From the results in Tables 1 to 5, it was noted that the rate of each of the mitotic stages in *A. cepa* was affected by the treatments. Generally, the increase in prophase index and the simultaneous decrease in the metaphase and anaphase–telophase index were counted by exposing them to these chemicals. This might be an indication of the blockage of the CHFR (checkpoint with forkhead associated and ring finger) point (control point between prophase/metaphase) (Liman et al., 2011). Scolnick and Halazonetis (2000) reported that CHFR defines a checkpoint that delays entry into metaphase in response to mitotic stress. Similar results have been reported after the treatment of *A. cepa* root tip cells with various other food additives (Dönbak et al., 2002; Gömürgen, 2005; Rencüzoğulları et al., 2001; Türkoğlu, 2007, 2008, 2009a, 2009b). MSG, MPG, CDG, MAG, and MDG induced very few abnormal cells in the *A. cepa* root tips, and the main effect appears to be clastogenicity (Tables 1 to 5; Figure 1). The most frequent chromosome abnormalities were MNs (Figure 1(a)), c-mitosis (Figure 1(b)), anaphase bridges (Figure 1(c)), stickiness (Figure 1(d)), binucleus (Figure 1(e)), laggards (Figure 1(f)), and chromosome breaks (Figure 1(g)).

For all flavour enhancers, the most frequent type of abnormalities was MNs. The percentage of micronucleated cells in *A. cepa* indicates an aneugenic and/or clastogenic activities, according to some authors, such as Mamur et al., (2010). The large MN would indicate an aneugenic effect resulting from a chromosome loss, whereas small MN may indicate a clastogenic action resulting from a chromosomal break. The occurrence of MN may result in the loss of genetic materials. MNs were recorded by many investigators following treatment with different food additives (Rasgele and Kaymak, 2010; Severin et al., 2010; Zengin et al., 2011). This means MSG, MPG, CDG, MAG, and MDG are clastogens that induce chromosome breaks and/or aneugens that induce lagging chromosomes.

As shown in Tables 1 to 5, significant increases in c-mitosis frequency were detected in *A. cepa* roots after exposuring to the five flavour enhancers. These results are in agreement with *A. cepa* root tips with different food additives (Dönbak et al., 2002;

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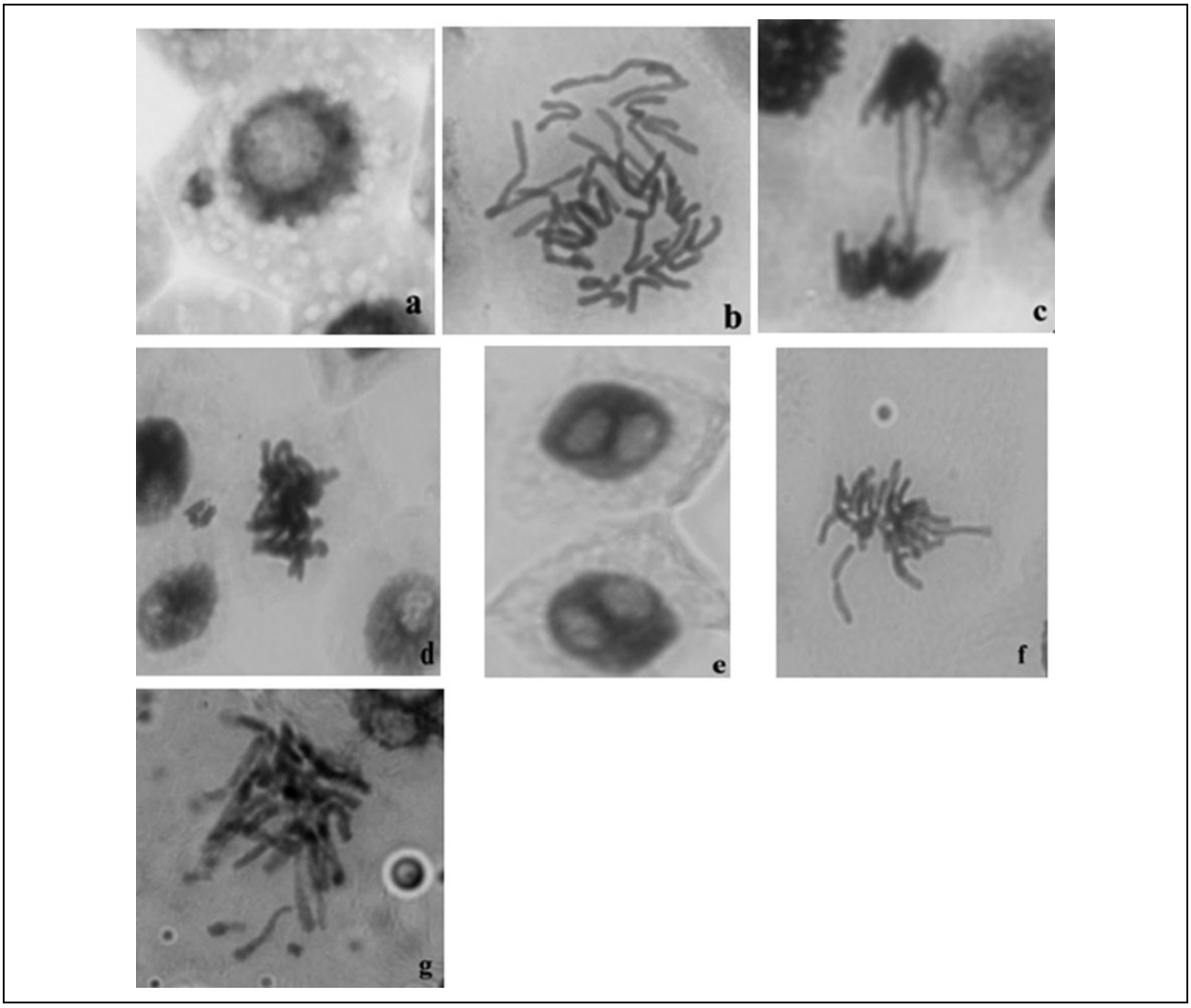

Figure 1. Different types of aberrations induced by the food additives in *Allium cepa* root tips. (a). Micronucleus at interphase (treated with MSG, MPG, CDG, MAG, MDG), (b) C-mitosis (treated with MSG, MPG, CDG, MAG, MDG), (c) anaphase bridge (treated with MSG, MPG, CDG, MAG, MDG), (d) sticky metaphase (treated with MSG, MPG, CDG, MAG, MDG), (e) binucleated cells (treated with MAG and MDG), (f) laggard (treated with MSG and MPG), and (g) break (treated with CDG). MSG: monosodium glutamate; MPG: monopotassium glutamate; CDG: calcium diglutamate; MAG: monoammonium glutamate; MDG: magnesium diglutamate.

Gömürgen, 2005; Rencüzoğulları et al., 2001; Türkoğlu, 2007, 2008, 2009a, 2009b). The formation of c-mitosis may be due to the disturbance in the spindle formation, which affected the flavour enhancers. C-Mitosis indicates a chemical-inhibited spindle formation similar to the effect of colchicines (Badr, 1983) and induction of c-mitosis commonly associated with spindle poisons, indicating a turbogenic effect (Shahin and El-Amoodi, 1991). The significant presence of c-mitosis provided remarkable evidence of turbogenic action of the chemicals, which was observed in this study.

The anaphase bridges were also observed in root tips treated with MSG, MPG, CDG, MAG, and MDG (Tables 1 to 5). Anaphase bridges could form during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence or due to the breakage and fusion of chromosomes and chromatids. These bridges cause structural chromosome mutations (El-Ghamery et al., 2000, Luo et al., 2004). The induction of bridges may lead to loss of genetic material (Salam et al., 1993). Such chromosome bridges were also reported to be induced by several other food additives (Gömürgen, 2005; Njagi

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Table 6. Effect of different concentrations of monosodium glutamate, monopotassium glutamate, calcium diglutamate, monoammonium glutamate, and magnesium diglutamate on DNA contents in *Allium cepa* root tips after treatments for different durations.

Time of Treatment	Doses (ppm)	Monosodium glutamate (pg ± SE[a])	Monopotassium glutamate (pg ± SE[a])	Calcium diglutamate (pg ± SE[a])	Monoamonnium glutamate (pg ± SE[a])	Magnesium glutamate (pg ± SE[a])
6 h	Control	69.82 ± 0.12^{b}	61.05 ± 3.35^{b}	69.82 ± 3.51^{b}	65.88 ± 0.12^{b}	64.74 ± 2.18^{b}
	20	53.41 ± 1.24^{c}	64.21 ± 2.22^{c}	55.78 ± 2.17^{c}	57.95 ± 1.28^{c}	61.57 ± 1.25^{c}
	40	50.15 ± 2.18^{c,d}	53.05 ± 1.18^{d,e}	47.14 ± 1.28^{d}	51.24 ± 2.57^{d}	54.27 ± 3.11^{d,f}
	60	49.18 ± 2.24^{c,d}	52.01 ± 2.87^{d}	39.23 ± 3.45^{f}	46.41 ± 3.04^{f}	48.00 ± 1.07^{d,e}
12 h	Control	69.00 ± 0.98^{b}	66.21 ± 1.13^{f}	67.87 ± 2.19^{e}	66.13 ± 2.11^{b}	66.80 ± 2.11^{b,c}
	20	50.00 ± 3.11^{c,d}	54.18 ± 2.52^{e}	45.14 ± 3.24^{g}	50.23 ± 1.29^{d}	56.95 ± 3.04^{f,g}
	40	48.12 ± 2.15^{d}	42.50 ± 2.21^{g}	42.21 ± 1.00^{h}	48.81 ± 3.10^{e}	51.41 ± 1.18^{d,f,i}
	60	45.71 ± 1.98^{d,f}	47.25 ± 1.00^{h}	39.00 ± 2.11^{f}	41.12 ± 0.96^{g}	47.23 ± 2.23^{e,i}
24 h	Control	68.19 ± 0.08^{b}	64.24 ± 2.11^{c}	67.00 ± 3.18^{e}	65.07 ± 3.01^{b}	68.13 ± 1.12^{b,c}
	20	50.18 ± 2.09^{c,d}	53.50 ± 1.19^{d,e}	44.35 ± 2.15^{g}	43.35 ± 2.18^{h}	50.24 ± 2.14^{d,g,i}
	40	40.98 ± 1.26^{f}	41.00 ± 1.23^{g}	38.28 ± 3.00^{f}	39.16 ± 2.12^{i}	40.00 ± 2.07^{h}
	60	38.22 ± 1.15^{e}	42.50 ± 2.02^{g}	31.13 ± 3.29^{i}	31.21 ± 1.23^{j}	45.18 ± 1.03^{e,g,i}

SE: standard error.
[a]Significant at $p < 0.05$ level.

Table 7. Effect of different concentrations of monosodium glutamate, monopotassium glutamate, calcium diglutamate, monoammonium glutamate, and magnesium diglutamate on interphase nuclear volumes in *Allium cepa* root tips after treatments for different durations.

Time of treatment	Doses (ppm)	Monosodium glutamate (m^3 ± SE[a])	Monopotassium glutamate (m^3 ± SE[a])	Calcium diglutamate (m^3 ± SE[a])	Monoamonnium glutamate (m^3 ± SE[a])	Magnesium glutamate (m^3 ± SE[a])
6 h	Control	198.38 ± 1.29^{b}	194.33 ± 0.19^{b}	195.22 ± 0.06^{b}	197.44 ± 0.49^{b}	194.51 ± 2.51^{b}
	20	173.81 ± 3.34^{c}	191.38 ± 2.54^{c}	183.27 ± 1.21^{c}	131.89 ± 0.55^{c}	173.45 ± 1.48^{c}
	40	135.33 ± 2.45^{d}	172.96 ± 2.10^{d}	165.18 ± 1.87^{d}	130.00 ± 1.25^{c}	157.15 ± 1.53^{d}
	60	130.28 ± 1.79^{e}	110.16 ± 1.19^{e}	150.04 ± 2.46^{e}	113.88 ± 1.79^{e}	160.00 ± 2.00^{e}
12 h	Control	194.36 ± 1.00^{f}	197.66 ± 3.46^{f}	192.27 ± 2.18^{f}	189.78 ± 3.54^{f}	193.33 ± 1.76^{b}
	20	158.87 ± 2.15^{g}	145.81 ± 2.83^{g}	171.77 ± 3.05^{g}	112.97 ± 2.85^{e}	171.15 ± 1.64^{f}
	40	103.45 ± 2.46^{h}	126.13 ± 2.47^{h}	150.26 ± 2.49^{e}	104.81 ± 1.29^{g}	162.27 ± 2.53^{g}
	60	96.04 ± 1.33^{i}	179.28 ± 1.95^{i}	135.18 ± 2.04^{h}	91.13 ± 3.16^{h}	144.00 ± 1.19^{h}
24 h	Control	199.00 ± 3.18^{b}	196.88 ± 0.51^{f}	195.23 ± 1.19^{b}	191.32 ± 1.52^{f}	198.00 ± 0.94^{i}
	20	120.13 ± 2.45^{j}	147.21 ± 2.13^{j}	150.09 ± 2.14^{e}	94.61 ± 2.45^{h}	145.72 ± 1.45^{j}
	40	95.25 ± 3.96^{i}	145.82 ± 1.47^{g}	121.18 ± 3.51^{i}	81.06 ± 1.32^{i}	140.93 ± 3.75^{k}
	60	83.18 ± 4.00^{k}	137.40 ± 1.14^{k}	130.05 ± 3.54^{j}	72.13 ± 1.44^{j}	120.14 ± 2.64^{l}

SE: standard error.
[a]Significant at $p < 0.05$ level.

and Gopalan 1982; Türkoğlu 2007, 2008, 2009a, 2009b).

In addition to the above-mentioned type of abnormalities, stickiness was observed in the present investigation (Tables 1 to 5, Figure 1). Sticky chromosomes might have resulted from increased chromosome contraction and condensation or possibly from depolymerization of DNA and partial dissolution of nucleoproteins. This effect is related to a disturbed balance in the quantity of histones or other proteins responsible for controlling the proper structure of nuclear chromatin (Kuras et al., 2006). Stickiness is considered a common sign of toxic effects on chromosomes probably leading to cell death (Fiskesjö, 1997). Sticky chromosomes have been reported in *Allium* roots after treatment with various food additives, such as sodium sulphite, potassium sulphite, calcium sulphite, sodium propionate, calcium propionate, potassium propionate,

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and mono-, di-, and trisodium phosphate (Türkoğlu, 2007, 2008, 2009b). Chromosome stickiness causes extremely shortened and thickened chromosomes found in prophase and metaphase. This means that flavour enhancers regarding organization of chromatin may be related to the impact on the physical and chemical properties of DNA, protein, or both and may lead to improper folding of the chromatin (El-Ghamery et al., 2003).

Binucleus (Tables 4 and 5, Figure 1(e)), laggards (Tables 1 and 2, Figure 1(f)), and breaks (Table 3, Figure 1(g)) were also observed in root tips treated with these flavour enhancers. Binucleated cells could be an indicative of possible interference with normal cytokinesis or cell plate formation. Microtubules have been implicated in cell plate formation and food additives might affect these microtubules resulting in the inhibition of cytokinesis. Such inhibition of binucleated cells was also reported by Borah and Talukdar (2002), Gömürgen (2005), Türkoğlu (2008). Laggards are the chromosome segregation defects that may be linked to the mutagenic activity of the chemicals. The occurrence of lagging chromosomes at anaphase was due to the failure of the chromosomes or acentric chromosome fragments to move to either of the poles. Such mitotic abnormalities may lead to genomic instability and cause mutagenic/cancerous effects. Earlier studies also demonstrated similar abnormalities in plant cells exposed to other food additives (Gömürgen, 2005; Türkoğlu, 2007). In addition to the above-mentioned aberrations, chromosome breaks were observed in the present study. Chemicals that induce chromosome breakage are known as clastogens and their action on chromosomes is generally regarded to involve an action on DNA (Chauhan and Sundararaman, 1990). Thus, induction of chromosome breaks indicates the clastogenic potential of CDG. Breaks were reported in diverse materials as a result of treatment with various food additives (Gömürgen, 2005; Türkoğlu, 2007; Zengin et al., 2011).

The effects of different treatments of MSG, MPG, CDG, MAG, and MDG on the amounts of DNA and INV are shown is Tables 6 and 7. Increased concentration and/or prolonged periods of treatment resulted in increased reductions in the amount of DNA and INV. A remarkable decline in the 4C DNA content and INV in the plants exposed to these flavour enhancers might be attributed to the intercalation of sodium, potassium, calcium, ammonium, and magnesium into the DNA double helix and the inhibition of DNA synthesis. Njagi and Gopalan (1982) reported that sodium benzoate and sodium sulphite inhibited DNA synthesis in *V. faba* root meristems. Additionally, Mohanty et al. (2004) and Bennet et al. (1985) suggested that a low dose of aluminium decreased INV. In this study, these food additives decreased the INV. However, the mechanisms of the damaging effects of these substances need to be clarified by more detailed studies.

From the present test, it appears that MSG, MPG, CDG, MAG, and MDG, which are used frequently in the food industry, have clear chromotoxic effects. The *Allium* assay is a good and sensitive test system for monitoring clastogenic effects. Hence, the *Allium* test should be considered as a warning and also an indication that the tested chemical may be a risk to human health. Glutamate is naturally produced in human bodies and also exists in many of the foods we eat, such as cheese, tomatoes, mushrooms, walnuts, eggs, and chicken. Glutamate is also involved in the synthesis of proteins. Studies have shown that the body uses glutamate as a nerve impulse transmitter in the brain and that there are glutamate-responsive tissues in other parts of the body as well. The abnormal function of glutamate receptors has been linked with certain neurological diseases, such as Alzheimer's disease, ALS, Parkinson's disease, and Huntington's chorea. The implications for children are that MSG in particular could seriously affect their cognitive skills and cause learning difficulties (Leber, 2008). For this reason, it is necessary to be careful when using these chemicals as food additives. Additionally, there is a need to investigate the long-term effects of continuous exposure to glutamates on humans from a global perspective. Children are more vulnerable to the effects of additives than adults. Eliminating as much MSG, MPG, CDG, MAG, and MDG as possible from our diet will result in great improvement in our and our families' health, especially children, both today and in the future.

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