Clastogenic effects of food additive citric acid in human peripheral lymphocytes

Serkan Yılmaz · Fatma Ünal · Deniz Yüzbaşıoğlu · Hüseyin Aksoy

Received: 9 August 2007 / Accepted: 8 February 2008 / Published online: 21 February 2008
© Springer Science+Business Media B.V. 2008

Abstract Clastogenic properties of the food additive citric acid, commonly used as an antioxidant, were analysed in human peripheral blood lymphocytes. Citric acid induced a significant increase of chromosomal aberrations (CAs) at all the concentrations and treatment periods tested. Citric acid significantly decreased mitotic index (MI) at 100 and 200 $\mu\text{g ml}^{-1}$ concentrations at 24 h, and in all concentrations at 48 h. However, it did not decrease the replication index (RI) significantly. Citric acid also significantly increased sister chromatid exchanges (SCEs) at 100 and 200 $\mu\text{g ml}^{-1}$ concentrations at 24 h, and in all concentrations at 48 h. This chemical significantly increased the micronuclei frequency (MN) compared to the negative control. It also decreased the cytokinesis-block proliferation index (CBPI), but this result was not statistically significant.

Keywords Clastogenic effect · Citric acid · Human lymphocytes · Chromosome aberrations (CAs) ·

Sister chromatid exchanges (SCEs) · Micronucleus assay (MN)

Abbreviations

CA	Chromosome aberration
SCE	Sister chromatid exchanges
MN	Micronucleus assay
BN	Binucleate
MMC	Mitomycin-C
Cyt-B	Cytochalasin B
MI	Mitotic index
RI	Replication index
CBPI	Cytokinesis-block proliferation index

Introduction

Food additives are used to increase nutrient value or to protect decaying nutrients during the preparation of factory-made foods. So, humans are unavoidably exposed to these complex mixtures in their foods. Some food additives have been prohibited from use due to their toxicity. For example, AF-2 (2-[2-furyl]-3-[5-nitro-2-furyl] acrylamide) is proved to induce DNA damage in bacteria and human cells and to cause mutations in bacteria, fungi, insects and mammalian cells in vivo and in vitro. It also causes chromosomal anomalies in mammalian cells, including human cells. AF-2 has been used in Japan since 1965 but it is banned presently (IARC 1983). Another food additive,

S. Yılmaz (✉) · F. Ünal · D. Yüzbaşıoğlu
Arts and Sciences Faculty, Department of Biology, Gazi University, 06500 Ankara, Turkey
e-mail: syilmaz@gazi.edu.tr

H. Aksoy
Arts and Sciences Faculty, Department of Biology,
Sakarya University, Sakarya, Turkey

butter yellow (p-dimethylamino-azobenzene-an azo compound) was also banned first in the USA and following in Europe, because of its carcinogenicity in experimental animals (IARC 1975).

Citric acid is used widely as an acidulant, pH regulator, flavour enhancer, preservative and antioxidant synergist in many foods, like soft drinks, jelly sweet, baked nutrients, jam, marmalade, candy, tinned vegetable and fruit food (Gürsoy 2002). Global production is estimated by industry to be approaching 1,000,000 t/a (OECD 2001). Although citric acid has widespread usage, some authors have reported that it causes damage to dental cells (Lan et al. 1999) and necrotic changes such as vacuolated and glassy cytoplasm in hepatocytes, chromatin decrease and increase of collagen fibers amongst hepatocytes in mouse liver (Aktaş et al. 2003). Koçak (2005) reported that citric acid significantly increased micronucleus frequency in the erythrocytes of *Tinca tinca* at all doses used. Türkoğlu (2007) showed that citric acid significantly increased the chromosomal aberrations and decreased the mitotic index in *Allium cepa* chromosomes. Contrasting with these results, Ishidate et al. (1984) reported that citric acid was negative in *Salmonella* microsome assays. They also observed that chromosomal aberration assay with Chinese hamster fibroblast cell line yielded negative results.

Studies carried out showed that there are some chemicals that give negative results in bacteria but show as mutagenic when tested in the other organisms and the other test systems (Ishidate et al. 1984; Rencüzoğulları et al. 2004). In addition positive and negative effects were published in the same test system in different studies both in bacterial and mammalian cells (Hsia et al. 1979; Goggelmann and Schimmer 1983; Ramos-Ocampo and Hsia 1988). Even in the same test system, for example in the Ames/*Salmonella*, different strains give different responses to the same chemical (Pagano and Zeiger 1987). On the other hand, bacteria, plant and animal assays are differentially responsive to most chemicals and these differences may be due to their metabolism.

Contradictory results as mentioned above and widespread usage of citric acid prompted us to investigate genetic effects of citric acid in human lymphocytes in vitro using three genetic endpoints; sister chromatid exchanges (SCEs), chromosomal aberrations (CAs) and micronucleus (MN) formation.

Materials and methods

Citric acid (pure) as the test substance was obtained from Merck (Cas. No. Merck 5949-29-1). The chemical properties of citric acid are as follows: chemical formula is $C_6H_8O_7 \cdot H_2O$, molecule weight is 210,14 g/mol. Mitomycin-C (MMC, Cas. No. 50-07-7) and Cytochalasin B (Cyt-B, Cas. No. 14930-96-2) were obtained from Sigma.

In this study human peripheral blood cells were used as the test material. Peripheral blood (0.2 ml) was obtained from two healthy (1 male and 1 female) non smoking donors (aged 27). Whole blood was added to 2.5 ml Chromosome Medium B (Biochrom 5025) supplemented with 10 µg/ml bromodeoxyuridine. The cultures were incubated at 37°C for 72 h. The cells were treated with 50, 100, 200 and 3,000 µg ml⁻¹ (the concentration used in foods) of citric acid for 24 h and 48 h. In addition, a negative and a positive (response to the standard mutagen mitomycin-C, 0.10 µg ml⁻¹) controls were included for each experiment to ensure validity of the assay. The test substance citric acid and positive control mitomycin-C (MMC) were dissolved in distilled water. 0.06 µg ml⁻¹ colchicine was added 2 h prior to the harvesting of the culture. At 72nd h the cells were harvested by centrifugation (216×g, 10 min), and the pellet was resuspended in a hypotonic solution of 0.075 M KCl for 30 min at 37°C. Cells were again centrifuged and fixed in cold methanol acetic acid (3:1) for 20 min. The treatment with fixative was repeated 3 times. At last, slides were made by dropping and air drying.

For chromosome aberrations, slides were stained with 5% Giemsa (pH = 6.8) prepared in Sörensen buffer solution, for 20–25 min and then washed in distilled water, dried at room temperature and mounted with Depex. Chromosomal abnormalities were scored from 100 well spread metaphases per donor (total 200 metaphases per concentration). The mitotic index (MI) was determined by scoring 1,000 cells from each donor.

For the SCE study, slides were stained with Giemsa according to Speit and Houptner (1985)'s method with some modifications. The number of SCE's was scored from a total of 50 cells (25 cells from each donor) in second metaphases for each treatment. In addition, a total of 200 cells (100 cells from each donor) were scored for the determination

of the replication index (RI). The RI was calculated according to the following formula; $[1 \times M_1] + [2 \times M_2] + [3 \times M_3]/N$ (N = number of observed cells) where M_1 , M_2 , and M_3 represent the number of cells undergoing first, second and third mitosis, respectively (Schneider et al. 1981).

For the MN assay, the human lymphocyte cultures were incubated at 37°C for 72 h, 44 h from the initiation; cytochalasin B (Cyt-B) at a final concentration of $5.2 \mu\text{g ml}^{-1}$ was added to arrest cytokinesis. Citric acid was added after 24 h of the initiation of culture. Micronuclei were scored from 1,000 binucleated cells (BN) per donor (total 2,000 binucleated cells per concentration). Cell proliferation was evaluated using the cytokinesis-block proliferation index (CBPI) which indicates the average number of cell cycles undergone by a given cell (Scarf et al. 1997). 500 lymphocytes (total 1,000 lymphocytes) were scored to evaluate the percentage of cells with 1, 2, 3 and 4 nuclei. CBPI was calculated according to Surralles et al. (1995) as follows; $[1 \times N_1] + [2 \times N_2] + [3 \times [N_3 + N_4]]/N$ where N_1 – N_4 represent the number of cells with 1–4 nuclei, respectively, and N is the total number of cells scored.

The significance of the difference of percentage of abnormal cells, CA/cell, RI, % MN, CBPI and MI in treated cultures and their controls was determined using the z-test. The significance of the difference between mean SCE in treated cultures and their controls were determined using the t-test. Dose-response relationships were determined from the correlation and regression coefficients for the percentage of abnormal cells, CA/cell, SCE/cell, % MN and MI.

Results

Chromosomal aberrations

Citric acid induced a significant increase in the frequency of CAs and CA/cell in all concentrations and treatment periods as compared with negative control (Table 1). The increase in the frequency in CAs and CA/cell were dose-dependent in both 24 h and 48 h treatments ($r = 0.87$, $r = 0.67$ respectively). Seven types of aberrations, structural and numerical, were observed. Sister chromatid union was the most pronounced aberrations in all

Table 1 The chromosome aberrations in cultured human lymphocytes treated with citric acid

Test substance	Treatment		Aberrations							Abnormal cell ± SE (%)	CA/Cell ± SE
	Period (hour)	Dose (µg ml ⁻¹)	Structural					Numerical			
			scu	ctb	dic	csb	cte	p	er		
Negative control	24	0.00	2	–	–	–	–	–	–	2.50 ± 0.70	0.025 ± 0.01
Positive control	24	0.10	18	25	7	3	12	3	–	30.00 ± 3.24	0.325 ± 0.03
Citric acid	24	50	15	4	5	1	–	1	1	12.50 ± 2.34*	0.135 ± 0.02*
		100	25	5	7	1	–	5	–	19.00 ± 2.77*	0.190 ± 0.03*
		200	35	5	10	–	–	5	–	20.00 ± 2.83*	0.250 ± 0.03*
		3,000	–	–	–	–	–	–	–	Toxic	Toxic
Negative control	48	0.00	3	1	–	–	–	–	–	2.00 ± 0.98	0.020 ± 0.01
Positive control	48	0.10	42	7	6	–	31	3	–	34.00 ± 3.35	0.430 ± 0.035
Citric acid	48	50	34	2	2	–	–	1	–	19.00 ± 2.77*	0.070 ± 0.03*
		100	27	9	–	–	1	1	–	18.50 ± 2.75*	0.100 ± 0.03*
		200	30	2	3	1	1	4	–	18.50 ± 2.75*	0.105 ± 0.03*
		3,000	–	–	–	–	–	–	–	Toxic	Toxic

Scu, Sister chromatid union; ctb, chromatid break; dic, dicentric; csb, chromosome break; cte, chromatid exchange; p, polyploidy; er, endoreduplication; CA/cell, (Chromosome aberrations/cell)

200 Metaphases were scored for each treatment

*Significant from the control $P < 0.001$ (z test)

experimental groups except 3,000 $\mu\text{g ml}^{-1}$. Chromatid breaks and dicentrics were also observed in most treatments. Chromosome breaks and chromatid exchanges were other structural aberrations. Polyploidy and endoreduplication were two numerical aberrations found in citric acid-treated lymphocytes.

The effectiveness of citric acid on the induction of CAs was lower than that found in positive control. Neither dividing nor aberrant cells in 3,000 $\mu\text{g ml}^{-1}$ concentration was determined.

Sister chromatid exchanges, cell cycle and mitotic index

The results of SCE analysis are shown in Table 2. Citric acid increased the frequency of SCEs/cell. This increase was significant in all concentrations at 48 h treatment and 100 and 200 $\mu\text{g ml}^{-1}$ concentrations at 24 h treatment. These effects were dose dependent at both 24 h ($r = 0.95$) and 48 h ($r = 0.94$) treatments. The increase in SCEs/cell reached two-fold the negative control value at 200 $\mu\text{g ml}^{-1}$ at 48 h period.

The performance of citric acid in the induction of SCEs/cell was lower than that found in the positive control. In this assay, there was no dividing cell and hence, no SCEs/cell could be scored in 3,000 $\mu\text{g ml}^{-1}$ concentration at both 24 h and 48 h. Citric acid had any significant effect in RI, compared to negative control (Table 2).

Citric acid decreased MI in a dose-dependent manner at 24 h treatment ($r = -0.88$). However, 50 $\mu\text{g ml}^{-1}$ concentration was not significantly different from the negative control in this treatment. At 48 h, 50, 100 and 200 $\mu\text{g ml}^{-1}$ concentrations significantly decreased the MI dose dependently ($r = -0.67$). There were no scorable lymphocytes in 3,000 $\mu\text{g ml}^{-1}$ concentration and no cells in metaphase for either treatment periods (Table 2).

Lymphocytes with micronucleus

Table 3 shows that citric acid increased the frequency of binucleate cells with micronucleus in all treatment groups as compared to the negative control. This

Table 2 The number of SCEs/cell, RI and MI in cultured human lymphocytes treated with citric acid

Test substance	Treatment		Minimum–maximum SCE	SCEs/cell $\pm$ SE	M ₁	M ₂	M ₃	RI $\pm$ SE	MI $\pm$ SE
	Period (hour)	Dose ($\mu\text{g ml}^{-1}$)							
Negative control	24	0.00	1–19	4.70 $\pm$ 0.45	44	78	78	2.17 $\pm$ 0.074	7.60 $\pm$ 0.59
Positive control	24	0.10	9–53	32.20 $\pm$ 1.65	48	99	53	1.83 $\pm$ 0.049	4.80 $\pm$ 0.47
Citric acid	24	50	1–17	5.52 $\pm$ 0.43	22	61	117	2.43 $\pm$ 0.069	6.70 $\pm$ 0.56
		100	2–23	5.82 $\pm$ 0.46*	46	64	91	2.24 $\pm$ 0.056	5.10 $\pm$ 0.49**
		200	1–23	6.32 $\pm$ 0.56*	52	69	79	2.14 $\pm$ 0.045	5.00 $\pm$ 0.48***
		3,000	–	Toxic	–	–	–	Toxic	Toxic
Negative control	48	0	1–13	3.36 $\pm$ 0.27	34	58	108	2.37 $\pm$ 0.072	10.40 $\pm$ 0.68
Positive control	48	0.10	22–69	46.38 $\pm$ 1.72	110	72	18	1.54 $\pm$ 0.046	6.30 $\pm$ 0.54
Citric acid	48	50	1–14	5.48 $\pm$ 0.36*	49	79	72	2.12 $\pm$ 0.054	7.20 $\pm$ 0.57***
		100	3–14	5.76 $\pm$ 0.32*	39	66	95	2.28 $\pm$ 0.083	6.60 $\pm$ 0.53***
		200	3–15	7.20 $\pm$ 0.43*	50	68	82	2.16 $\pm$ 0.056	7.10 $\pm$ 0.57***
		3,000	–	Toxic	–	–	–	Toxic	Toxic

SCEs/cell, Sister chromatid exchanges/cell, M₁: mitosis 1, M₂: mitosis 2, M₃: mitosis 3, RI: replication index, MI: Mitotic index
50 metaphases were scored for each concentration in SCE

200 metaphases were scored for each concentration in RI

2,000 metaphases were scored for each concentration in MI

*Significant from the control $P < 0.05$ (t test)

**Significant from the control $P < 0.05$ (z test)

***Significant from the control $P < 0.001$ (z -test)

increase was dose-dependent ($r = 0.88$). The increase in MN (%) at $200 \mu\text{g ml}^{-1}$ nearly reached nine-fold the negative control. Most of the cells showed just one micronucleus, but one which had two micronuclei and one showing four micronuclei. The frequencies of MN were in all cases lower than those of the positive control. This chemical also decreased the CBPI but these results were not statistically significant. Here, $3,000 \mu\text{g ml}^{-1}$ was toxic again and no dividing cell was observed.

Discussion

Chromosomal aberrations, sister chromatid exchanges and micronucleus analysis of human lymphocytes as well as single cell gel electrophoresis (SCGE) or “Comet” assay are used as the most useful assays to detect the potential genotoxicity of chemicals (Rencüzoğulları et al. 2004; Meng and Zhang 1994; Blasczyk et al. 2003). They have been considered to be markers of early biological effects of carcinogen exposure (Liou et al. 2002). Since such assays have not been previously carried out, this study was planned to find genotoxic potential of citric acid, a food preservative, in human peripheral lymphocytes. Citric acid significantly increased the frequency of CAs, SCEs (except $50 \mu\text{g ml}^{-1}$ for 24 h) and MN in all treatment groups as compared to their negative controls without changing the pH of the medium. Citric acid induced five types of structural aberration in lymphocytes. These are sister chromatid union, chromatid and chromosome breaks,

dicentric chromosomes and chromatid exchange. Numerical aberrations such as polyploidy and endoreduplication were also recorded. In this study, sister chromatid union as a result of terminal deletion (Kayraldız and Topaktaş 2001) has been observed as the most common aberration. Chromatid breaks resulting from DNA double-strand breaks (Bryant 1998) was the second common abnormality. The third common aberration was dicentric chromosomes which are well known to have serious biological consequence (Little Inc 1990). Although polyploidy was not a frequent abnormality, such abnormalities showed that this chemical most probably inhibited the function of DNA topoisomerase II on DNA. Since this enzyme especially plays an important role in the mitotic chromosome segregation after DNA replication, if such an error has existed in this event, aberrant mitosis such as endoreduplication, polyploidy and eventually cell death occur (Cortes and Pastor 2003). Türkoğlu (2007) reported that citric acid induced anaphase bridges, c-mitosis, laggards and stickiness in *Allium cepa* chromosomes. Similar results were obtained with other food additives. Rencüzoğulları et al. (2004) reported that aspartame that is used as sweetener induced CAs at all the concentrations and treatment periods. Sodium bisulfite, an antimicrobial agent, induced an increase of chromatid type aberrations in human lymphocytes in a dose-dependent manner (Meng and Zhang 1992). Kaya and Topaktaş (2007) reported that potassium bromate which is used as a bleaching agent in flour induced significant amounts of CAs. Other food additives such as maltitol and sodium

Table 3 Induction of micronuclei in cultured human lymphocytes treated with citric acid

Test substance	Treatment		BN cells scored	Distribution of BN cells according to the no. of MN				MN (%)	Cytokinesis-block proliferation index (CBPI)
	Period (hour)	Dose ($\mu\text{g ml}^{-1}$)		(1)	(2)	(3)	(4)		
Negative control	48	0	2,000	6	0	0	0	0.30 ± 0.12	1.84 ± 0.30
Positive control	48	0.1	2,000	220	20	0	0	13.0 ± 0.75	1.30 ± 0.25
Citric acid	48	50	2,000	33	0	0	0	$1.65 \pm 0.28^*$	1.43 ± 0.27
		100	2,000	45	1	0	0	$2.35 \pm 0.34^*$	1.41 ± 0.26
		200	2,000	48	0	0	1	$2.60 \pm 0.36^*$	1.34 ± 0.26
		3,000	–	–	–	–	–	Toxic	Toxic

BN, Binucleate; MN, Micronucleus

*Significant from the control $P < 0.001$ (z-test)

metabisulfite induced CAs in human lymphocytes as well (Rencüzoğulları et al. 2001; Canımoğlu and Rencüzoğulları 2006).

The SCE analysis was used as genotoxicity indicator (Meng and Zhang 1992; Kaya and Topaktaş 2007; Xing and Zhang 1990). Our results show that citric acid increased SCE frequency in human lymphocytes (except $50 \mu\text{g ml}^{-1}$ for 24 h). Although the exact mechanism that leads to an increased exchange of segments between sister chromatids is not known in detail (Bolognesi 2003), present studies showed that the nucleotide pool imbalance is critical in SCE formation. The modulation of SCE by DNA precursors raises the possibility that DNA changes are responsible for the induction of SCE and mutations in mammalian cells (Bolognesi 2003; Ashman and Davidson 1981; Popescu 1999). Studies conducted with other additives such as sodium bisulfite, potassium bromate, sodium metabisulfite and sodium benzoate have shown that they increased the SCE yield in human lymphocytes (Meng and Zhang 1992; Kaya and Topaktaş 2007; Rencüzoğulları et al. 2001; Xing and Zhang 1990).

Citric acid also increased the MN frequency in a dose dependent manner. MN assay detects both clastogenicity (chromosome breakage) and aneugenicity (chromosome lagging due to dysfunction of the mitotic apparatus) (Albertini et al. 2000). Similar results were observed in several studies using different food additives. Aspartame, increased the MN frequency in human lymphocytes at the highest concentration (Rencüzoğulları et al. 2004) and so did sodium bisulfite (Meng and Zhang 1992). Maltitol increased the MN frequency at all doses and treatment periods in a non dose-dependent manner (Canımoğlu and Rencüzoğulları 2006).

In the results of our experiments, citric acid induced mitotic delay and decreased the mitotic index. This reduction was significant in all treatments (except $50 \mu\text{g ml}^{-1}$ for 24 h). Similar results have been also reported from other studies (Rencüzoğulları et al. 2001, 2004; Meng and Zhang 1994, 1992). The RI and CBPI values, however, were not affected by citric acid. Comparing of RI and MI values shows that citric acid affected the cells especially in G_2 or early prophase.

Not only human lymphocytes, but also other test systems have been also used to detect potential genotoxicity of additives. In a cytogenetic study of

sodium nitrite, it was shown that this chemical increases the frequency of aberrant metaphases in all treatment groups in vivo, and also produced a significant increase in the percentage of chromosomal aberrations (Luca et al. 1987). In a report of Pagano and Zeiger (1987), sodium bisulfite was shown as mutagenic in strains of *S. typhimurium*. Sasaki et al (2002) studied the genotoxicity of 39 chemicals currently in use as food additives. Among them, seven food dyes, namely amaranth, allura red, new coccine, tartrazine, erythrosine, phloxine, rose bengal, two antioxidants [butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)], three fungicides (biphenyl, sodium o-phenylphenol, and thiabendazole), and four sweeteners (sodium cyclamate, saccharin, sodium saccharin, and sucralose) induced DNA damage as assessed by the comet assay on the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow in mice. Roychoudhury and Giri (1989) reported that fast green FCF, indigo carmine, orange G and tartrazine induced chromosome aberrations and micronuclei at high doses in *Allium cepa*. Dönbak et al. (2002) reported that boric acid slightly increased the percentage of abnormal cells in *Allium cepa*.

Citric acid occurs widely in a great amount of plants and fruits. It is widely synthesized by *Aspergillus niger* and also synthesized in the metabolism of mammalian cells. Citric acid is an important chemical that plays an important role in the physiological oxidation of glucose and other carbohydrates, as well as fats and proteins. The citric acid concentration controls several metabolite pathways and in higher organisms it regulates the utilization of calcium contained in foods (Gautier-luneau et al. 2007). Ferric citrate (iron and citric acid) plays a paramount role in iron metabolism in living systems (Gautier-luneau et al. 2007). Citrate is present in blood plasma in submillimolar concentration and promotes the bioavailability of dietary iron (Gautier-luneau et al. 2007, Parkes et al. 1991). Owing to fact that the mobilization of iron may lead to the formation of highly toxic hydrogen radicals (Pierre and Fontecave 1999, Gautier-luneau et al. 2007), one can question concerning the innocence of adding citric acid in large amounts in foods or drinks (Gautier-luneau et al. 2007). Gautier-luneau et al. (2007) observed that iron:citrate and H_2O_2 solution caused high amount of OH^- radicals. The authors also suggested

that the presence of citric acid in the drinks favors the formation of hydroxyl radical. Also, it has been showed that mitochondrial lipid peroxidation could be induced by iron-citrate (Castilho et al. 1999; Santos et al. 2001, Gautier-luneau et al. 2007). In addition, hydroxyl radical formation from the auto reduction of a ferric-citrate complex has been evidenced (Gutteridge 1991; Gautier-luneau et al. 2007). Because of these reasons above mentioned we can conclude that although citric acid plays an important role in the intermediary metabolism in the cell, the present study shows that this acid possesses clastogenic and cytotoxic activity, especially in high doses, in cultured human lymphocytes. The clastogenic activity of citric acid is most probably due to formation of OH^- radicals. These radicals are highly reactive oxygen-centered radical which attacks all molecules including DNA in the human body (Gautier-luneau et al. 2007). Such damaging effects of OH^- radicals might cause different type of aberrations in human lymphocytes.

Similar results about damaging effects of citric acid were also obtained in *A. cepa* by Türkoğlu (2007) and in *Tinca tinca* erythrocytes by Koçak (2005). This acid, although it is a weak organic acid, damaged dental cells in human and hepatocytes in mouse liver too (Lan et al. 1999, Aktaş et al. 2003). OECD (2001) reported that citric acid did not cause chromosomal aberrations in rat bone marrow. But the differences between ours and OECD might be due to following reasons: In in vivo studies, numbers of factors may effects the genotoxicity of chemicals such as compound solubility, rate and distribution of biotransport, availability at the target site as influenced by time and cell permeability (McFee and Tice 1990). However, the mechanism of such damaging effects to lymphocytes need more detailed studies. The results obtained in this and the other studies carried out warrant a more extensive safety assessment of food additives.

Acknowledgements The authors thanks to the Gazi University for founding this research [grant 05/2006–41].

References

- Aktaş T, Kaboğlu A, Ertan F, Ekinci F, Huseyinova G (2003) The effects of citric acid [antioxidant] and benzoic acid [antimicrobial agent] on the mouse liver: biochemical and histopathological study. *Biologia Bratisl* 58:343–347
- Albertini RJ, Aderson D, Dauglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Waters MD, Atio A (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in human. *Mutat Res* 463:111–172
- Ashman CR, Davidson RL (1981) Bromodeoxyuridine mutagenesis in mammalian cells is related to deoxyribonucleotide pool imbalance. *Mol Cell Biol* 1:254–260
- Blaszczyk A, Osiecka R, Skolimowski J (2003) Induction of chromosome aberrations in cultured human lymphocytes treated with ethoxyquin. *Mutat Res* 542:117–128
- Bolognesi C (2003) Genotoxicity of pesticides: a review of human biomonitoring studies. *Mutat Res* 543:251–272
- Bryant PE (1998) Mechanisms of radiation-induced chromatid breaks. *Mutat Res* 404:107–111
- Canımoglu S, Rencüzoğulları E (2006) The Cytogenetic effects of Food Sweetener Maltitol in Human Peripheral Lymphocytes. *Drug Chem Toxicol* 29:269–278
- Castilho RF, Meinicke AR, Vercesi AE, Hermes-Lima M (1999) Role of Fe(III) in Fe(II) citrate-mediated peroxidation of mitochondrial membrane lipids. *Mol Cell Biochem* 196:163–168
- Cortes F, Pastor N (2003) Induction of endoreduplication by topoisomerase II catalytic inhibitors. *Mutagenesis* 18: 105–112
- Dönbak L, Rencüzoğulları E, Topaktaş M (2002) The Cytogenetic Effects of the Food Additive Boric Acid in *Allium cepa* L. *Cytologia* 67:153–157
- Gautier-luneau I, Bertet P, Jeunet A, Serratrice G, Pierre JL (2007) Iron-citrate complexes and free radicals generation: Is citric acid an innocent additive in foods and drinks? *Bio Metals* 20:793–796
- Goggelmann W, Schimmer O (1983) Mutagenicity testing of β -asarone and commercial calamus drugs with *Salmonella typhimurium*. *Mutat Res* 121:191–194
- Gutteridge JMC (1991) Hydroxyl radical formation from the auto-reduction of a ferric citrate complex. *Free Radic Biol Med* 11:401–406
- Gürsoy S (2002) Besinlerde katkı maddelerinin kullanımı ve sitrik asit toksisitesi. Trakya Üniversitesi Fen Bilimleri Enstitüsü. MSc Thesis
- Hsia MTS, Adamovics JAA, Kreamer LW (1979) Microbial mutagenicity studies of insect growth regulators and other potential insecticidal compounds in *Salmonella typhimurium*. *Chemosphere* 8:521–529
- International Agency for Research on Cancer [IARC] (1975) p-Dimethylaminoazobenzene. In: IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans: some aromatic azo compounds. World Health Organization, vol. 8. pp 125–139
- International Agency for Research on Cancer [IARC] (1983) 2-[2-Furyl]-3-[5-nitro-2-furyl] acrylamide [AF-2]. In: IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: some food additives, feed additives and naturally Occurring Substances. World Health Organization vol. 31. p 47
- Ishidate M, Sofuni Jr T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A (1984) Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol* 22:623–636

- Kaya FF, Topaktaş M (2007) Genotoxic effects of potassium bromate on human peripheral lymphocytes in vitro. *Mutat Res* 626:48–52
- Kayraldız A, Topaktaş M (2001) Indirect genotoxic effect of gamma rays in human peripheral lymphocytes. *Cytologia* 66:25–31
- Koçak Y (2005) Sitrik Asitin Tinca tinca [L., 1758] [Pisces: Cyprinidae] üzerindeki genotoksik etkisinin mikronükleus testi ile belirlenmesi. Gazi Üniversitesi Fen Bilimleri Enstitüsü. MSc Thesis
- Lan WC, Lan WH, Chan CP, Hsieh CC, Chang MC, Jeng JH (1999) The effects of extracellular citric acid acidosis on the viability, cellular adhesion capacity and protein synthesis of cultured human gingival fibroblasts. *Aust Dent J* 44(2):123–130
- Liou SH, Chen YH, Loh CH, Yang T, Wu TN, Chen CJ, Hsieh LL (2002) The association between frequencies of mitomycin C-induced sister chromatid exchange and cancer risk in arseniasis. *Toxicol Lett* 129:237–243
- Little Inc AD (1990) Chemical evaluation committee draft report, ethoxyquin, CAS number 91–53–2, Submitted to National Toxicology Program, Executive Summary of Safety and Toxicity Information
- Luca D, Luca V, Cotor F, Răileanu L (1987) In vivo and in vitro cytogenetic damage induced by sodium nitrite. *Mutat Res* 189:333–339
- McFee AF, Tice RR (1990) Influence of treatment to sacrifice time and the presence of BrdUrd on chemically-induced aberration rates in mouse marrow cells. *Mutat Res* 241: 95–108
- Meng Z, Zhang L (1992) Cytogenetic damage induced in human lymphocytes by sodium bisulfite. *Mutat Res* 298: 63–69
- Meng Z, Zhang L (1994) Chromosomal aberrations, sister chromatid exchanges and micronuclei induced human lymphocytes by sodium bisulfite [sulfur dioxide]. *Acta Genet Sin* 21:1–6
- OECD SIDS (2001) Initial Assessment Report. Citric acid
- Pagano DA, Zeiger E (1987) Conditions affecting the mutagenicity of sodium bisulfite. *Mutat Res* 179:159–166
- Parkes HG, Allen RE, Furst A, Blake DR, Grootveld MC (1991) Speciation of non-transferrin bound iron ions in synovial fluid from patients with rheumatoid arthritis by proton nuclear magnetic resonance spectroscopy. *J Pharm Biomed Anal* 9:29–32
- Pierre JL, Fontecave M (1999) Iron and activated oxygen species in biology: the basic chemistry. *Biometals* 13:91–96
- Popescu NC (1999) Sister chromatid exchange formation in mammalian cells is modulated by deoxyribonucleotide pool imbalance. *Somat Cell Mol Genet* 25:101–108
- Ramos-Ocampo VE, Hsia MTS (1988) Mutagenic and DNA-damaging activity of calamus oil, asarone isomers and dimethoxypropenylbenzenes analogues. *Phillip Ent* 7: 275–291
- Rencüzoğulları E, İla HB, Kayraldız A, Topaktaş M (2001) Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfite, a food preservative. *Mutat Res* 490:107–112
- Rencüzoğulları E, Tüylü BA, Topaktaş M, İla HB, Kayraldız A, Arslan M, Diler S (2004) Genotoxicity of Aspartame. *Drug Chem Toxicol* 27(3):257–268
- Roychoudhury A, Giri K (1989) Effects of certain food dyes on chromosomes of *Allium cepa*. *Mutat Res* 223:313–319
- Santos NCJ, Castilho RF, Meinicke AR, Hermes-Lima M (2001) The iron chelator pyridoxal isonicotinoyl hydrazone inhibits mitochondrial lipid peroxidation induced by Fe(II)-citrate. *Eur J Pharmacol* 428:37–44
- Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S (2002) The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res* 519(1–2):103–119
- Scarfı MR, Lioi MB, Noce MD, Zeni O, Franceschi C, Monti D, Castellani G, Bersani F (1997) Exposure to 100 Hz pulsed magnetic fields increases micronucleus frequency and cell proliferation in human lymphocytes. *Bioelectrochemistry* 43:77–81
- Schneider EL, Nakanishi Y, Lewis J, Sternberg H (1981) Simultaneous examination of sister chromatid exchanges and cell replication kinetics in tumor and normal cells in vivo. *Cancer Res* 41:4973–4975
- Speit G, Houptter S (1985) On the mechanisms of differential giemsa staining of bromodeoxyuridine-substituted chromosomes. II. Differences between the demonstrations of sister chromatid differentiation and replication patterns. *Human Genet* 70:126–129
- Surrales J, Xamena N, Creus A, Catalan J, Norppa H, Marcos R (1995) Induction of micronuclei by five pyrethroid insecticides in whole blood and isolated human lymphocytes cultures. *Mutat Res* 341:169–184
- Türkoğlu Ş (2007) Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutat Res* 626(1–2): 4–14
- Xing W, Zhang Z (1990) A comparison of SCE test in human lymphocytes and *Vicia faba*: a hopeful technique using plants to detect mutagens and carcinogens. *Mutat Res* 241:109–113