Genotoxicity of food preservative sodium sorbate in human lymphocytes in vitro

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Abstract The genotoxic effects of antimicrobial food additive sodium sorbate (SS) was assessed by using chromosome aberrations (CAs), sister-chromatid exchanges (SCEs), and micronucleus (MN) in cultured human lymphocytes and comet assay in isolated human lymphocytes. Lymphocytes were treated with four concentrations (100, 200, 400 and 800 µg/ml) of SS as well as a negative (sterile distilled water) and a positive control (Mitomycin-C: MMC for cultured lymphocytes and H₂O₂ for isolated lymphocytes). The result of this study indicated that SS increased the frequency of CAs at both 24 and 48 h period compared to control. When gaps were included, this increase was significant at 200, 400 and 800 µg/ml concentrations at 24 h and, at all concentrations at 48 h treatment time. When gaps were excluded, this increase was significant at only 800 µg/ml concentration at both 24 and 48 h treatments. In addition, SS increased SCEs/cell and MN frequency at 400 and 800 µg/ml concentrations at both 24 and 48 h compared to negative control. Furthermore, this additive caused DNA damage at all concentrations in isolated human lymphocytes after 1 h in vitro exposure. The present results show that SS is genotoxic to the

human peripheral blood lymphocytes in vitro at the highest concentrations.

Keywords Genotoxic effects · Food preservative · Sodium sorbate · Human lymphocytes

Introduction

Man has a long history of adding chemical substances to foods for various reasons, including improving shelf life, flavor, appearance or texture (Altuğ 2003). No highly developed society could exist today without food additives. Food additives immediately become necessary when areas of food production are separated from areas of population concentration and the food must be stored or transported under conditions that can affect spoilage. These food additives are preservative in character (Potter 1984). Preservatives are compounds that delay or prevent microbiological spoilage of foods. They do not only act against visible spoilage by yeasts, molds and bacteria, but also prevent the formation of toxins, especially those produced by bacteria and molds (Altuğ 2003). The most widely used preservatives are sorbates (sorbic acid, sodium sorbate, potassium sorbate), benzoates (benzoic acid, sodium benzoate, potassium benzoate), propionates, sulfur dioxide and sulfites, sodium nitrate and sodium nitrite (Altuğ 2003). Sodium sorbate (E 201), which is the sodium salt of sorbic acid, is widely used as food

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preservatives on cheese, meat, ketchup, mayonnaise and marmalade (Potter 1984; Warth 1985). The levels at which it is used cover the range from 100 to 2,000 mg per litre or per kilogram (Ferrand et al. 2000). The acceptable daily intake (ADI) levels recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for sorbates is 25 mg/kg body-weight (i.e. 1,500 mg for a 60 kg adult) (WHO 1974; Walker 1990). In general the sodium and potassium salts are preferred over the acid form because they are more soluble in water (Liewen and Marth 1985).

Though food preservatives play an important role in the safety of food supply, many studies revealed the potential genotoxic and mutagenic effects of the additives (Hasegawa et al. 1984; Schlatter et al. 1992; Schiffmann and Schlatter 1992; Yilmaz et al. 2008, 2009; Mamur et al. 2010; Zengin et al. 2011). Hasegawa et al. (1984) reported that SS showed genotoxic effects in Chinese Hamster V79 cells at 200–800 µg/ml concentrations in CAs, SCEs and gene mutation tests. This genotoxic effect is consistent with other results obtained in Syrian Hamster embryo (SHE) fibroblast cells at 120–1,200 µg/ml concentrations, with MN and cell transformation tests in stored solution (Schiffmann and Schlatter 1992) and in Chinese Hamster V79 cells at 2,500 µg/ml concentration, with evaluation of mitotic index (MI) (Hasegawa et al. 1984). Münzner et al. (1990) demonstrated that stored SS (100 mg/kg body weight) weakly increased number of CAs and MN frequency. Mukherjee et al. (1988) revealed that sorbic acid increased SCEs and frequency of MN at the highest dose (150 mg/kg body weight) in bone marrow cells of mice. Similar results about damaging effects of sorbic acid salts potassium sorbate were also obtained by Mpountoukas et al. (2008) and by Mamur et al. (2010) in human lymphocytes in vitro. Some authors compared the genotoxic effect of sorbic acid and its sodium and potassium salts. They found that SS was more genotoxic than sorbic acid and potassium sorbate (Hasegawa et al. 1984; Münzner et al. 1990).

On the other hand, SS was tested as negative in Chinese Hamster ovary cells at 200–800 µg/ml concentrations with AMES (*Salmonella*/mammalian microsomes test), Hypoxanthine-guanine-phosphoribosyl transferase (HPGRT) and SCEs tests; in Chinese Hamster and mouse bone marrow cells at 200 mg/kg bw (body weight) concentration with MN test and in

Chinese Hamster at 500–1,000 µg/ml concentrations with CAs and SCEs tests (Münzner et al. 1990). In addition, Schiffmann and Schlatter (1992) reported that fresh SS had no genotoxic effects in Syrian Hamster embryo (SHE) fibroblast cells at concentrations of 120–1200 µg/ml using MN and cell transformation tests. Banerjee and Giri (1986) reported that sorbic acid (15 mg/kg bw) did not induce CAs in bone marrow cells of mice. Münzner et al. (1990) indicated that no mutagenicity was found in mice or hamsters after an oral administration of freshly prepared solution of sodium sorbate and potassium sorbate (200 mg/kg bw) using CAs and MN tests. In addition, using comet assay, sorbic acid and potassium sorbate (2,000 mg/kg) did not induce DNA damage in a lot of organs (Sasaki et al. 2002). This result of potassium sorbate is compatible with Kawachi et al.'s (1980) results.

According to the literature survey, no study has been conducted on genotoxicity of SS in human peripheral lymphocytes using CAs, SCEs, MN and comet tests. Moreover previous investigations revealed that SS showed conflicting results. For these reasons, the aim of this study was to investigate the genotoxic and cytotoxic effects of SS by using CAs, SCEs, MN and comet assays in human lymphocytes in vitro. The analyses of CAs, SCEs and MN in human peripheral blood lymphocytes as well as comet assay are popular biomarkers for genotoxic, carcinogenic, and mutagenic effects (Garaj-Vrhovac and Zeljezic 2000; Yüzbaşıoğlu et al. 2006; Blaszczyk 2006; Yilmaz et al. 2009; Mamur et al. 2010; Zengin et al. 2011).

Materials and methods

Chemicals

The test substance SS was obtained from sorbic acid (Cas. No: 110-44-1, Applichem). SS was prepared according the Schiffmann and Schlatter (1992) and Schlatter et al. (1992) with some modifications. To obtain SS, 1 g sorbic acid was suspended in 40 ml bidistilled water and dissolved by adding 10 N NaOH (at room temperature). In order to obtain a clear stock solution it was necessary to heat the mixture (30 °C) and to sonicate it (after cooling) in Vibra-Cell (Vibra-Cell, Sonics & Materials Inc. Danbury, CT USA) sonifier, at 50 MHz for 15 min. Finally, the clear

solution was adjusted to pH: 7.4. Mitomycin-C (Cas. No: 50-07-7), cytochalasin-B (Cas. No: 14930-96-2), bromodeoxyuridine (Cas. No: 59-14-3) and NaCl (Cas. No: 7647-14-5) were obtained from Sigma. DMSO (Cas. No: 67-68-5), NaOH (Cas. No: 1310-73-2), EDTA (Cas. No: 6381-92-6), Tris (Cas. No: 77-86-1), Triton X-100 (Cas. No: 9002-93-1), Normal Melting Agarose (Cas. No: 9012-36-6), Low Melting Agarose (Cas. No: 9012-36-6), EtBr (Cas. No: 1239-45-8) were obtained from Applichem.

Lymphocyte cultures and isolations

In this study, human peripheral blood lymphocytes were used as the test material. Peripheral venous blood was obtained from two healthy donors (a male and a female, nonsmokers, aged 24–25 years) not exposed to any drug therapy or known mutagenic agent over the past 2 years and not exposed to X-rays for the previous 6 months, with no history of chromosome fragility or recent viral infection. The experiments were conducted using the same blood samples, divided into two fractions: CAs, SCEs and MN were evaluated in whole blood, whereas the comet assay was used to measure the SS-induced DNA strand breakage in isolated lymphocytes.

Chromosomal aberrations and sister chromatid exchange assay

Heparinized whole blood sample (0.2 ml) was added to Chromosome Medium B (Biochrom) supplemented with 10 µg/ml bromodeoxyuridine for CAs and SCEs and incubated in the dark at 37 °C for 72 h and the cells were treated with SS at 100, 200, 400 and 800 µg/ml concentrations for 24 and 48 h. In addition, a negative control and a positive control (Mitomycin-C; MMC, 0.20 µg/ml) were included for each experiment to ensure validity of the assay. For CA and SCE analysis 0.06 µg/ml colchicine was present in the cultures during the last 2 h. SS did not change the pH of the culture medium. The cultured peripheral blood lymphocytes were harvested by treating with KCl (75 mM), which spreads chromosomes and hemolyzes the red blood cells, and then fixed with freshly prepared cold methanol:glacial acetic acid (3:1 v/v). Fixation process was repeated for three times. At last, metaphase spreads were prepared by dropping the concentrated cell suspension onto slides. Slides for CAs were conventionally stained

with 5% Giemsa (pH = 6.8). Slides for SCEs were stained according to Speit and Houppter's (1985) FPG (fluorescence plus Giemsa) technique. The slides were dried at room temperature, and mounted in Depex. A hundred well spread metaphases per donor (totally 200 metaphases per concentration) were analyzed for the CA assays, 50 second mitosis for the SCE assays for each experimental concentration. The MI was determined by scoring 1,000 cells from each donor. In addition, a total of 200 cells (100 cells from each donor) were scored to determine the replication index (RI). Each metaphase was classified as being in the first (M_1), second (M_2) and third (M_3) division. The RI was calculated as follows: $RI = ([1 \times M_1] + [2 \times M_2] + [3 \times M_3])/N$. Here, N is the total number of metaphases scored (Schneider and Lewis 1981).

Micronucleus assay

For the MN assay, the preparation was performed according to Palus et al.'s (2003) method, with some modifications (Mamur et al. 2010). Whole blood was added to 2.5 ml Chromosome Medium B (Biochrome). Human lymphocytes were incubated at 37 °C for 72 h and treated with SS at 100, 200, 400 and 800 µg/ml during the last 48 h. The SS did not alter the pH of the culture medium. Mitomycin-C (MMC, 0.20 µM) was used as positive control. Cytochalasin-B (5.2 µg/ml) was added at 44 h of incubation to block cytokinesis. Then the cells were harvested by centrifugation (216×g, 10 min), and the pellet was re-suspended in a hypotonic solution of 0.075 M KCl for 5 min at 4 °C. Cells were re-centrifuged and fixed three times in cold methanol:glacial acetic acid (3:1 v/v). To the last fixative, 1% formaldehyde was added to preserve the cytoplasm. The slides were air-dried and stained with 5% Giemsa (pH = 6.8) and washed in distilled water, dried at room temperature and mounted in depex. MN was scored from 1,000 binucleated cells (BN) per donor (totally 2,000 BN per concentration). Cell proliferation was evaluated using the cytokinesis-block proliferation index (CBPI), which indicates the average number of cell cycles. 500 lymphocytes (totally 1,000 lymphocytes per concentration) were scored to evaluate the percentage of cells with 1, 2, 3 or 4 nuclei. The CBPI was calculated according to Surrales et al. (1995) as follows: $([1 \times N1] + [2 \times N2] + [3 \times (N3 + N4)])/N$, where $N1$ – $N4$ represent

the number of cells with 1–4 nuclei, respectively, and N is the total number of cells scored.

Comet assay (SCGE)

Primary DNA damaging effect caused by the SS was determined using comet assay according to the method by Singh et al. (1988) with some modifications. Peripheral blood was obtained with a heparinized syringe, immediately before conducting the test. Lymphocytes were isolated using Biocoll separating solution. To detect viability of cells, trypan blue exclusion test was used. Cell viability was >97%. Isolated human lymphocytes were incubated with four concentrations of SS (100, 200, 400, 800 µg/ml) for one hour at 37 °C. Negative and positive controls (H₂O₂, 40 µM) were also included at the same temperature and exposure time in parallel with SS. Other procedure was applied according to Mamur et al. (2010).

The slides were examined using a fluorescent microscope (Olympus) equipped with an excitation filter of 546 nm and a barrier filter of 590 nm at 400× magnification. Two slides were prepared for each concentration of SS. The tail intensity (%) of 100 comets on each slide (a total of 200 comets per concentration) were determined, using the specialized Image Analysis System (“Comet Assay IV”, Perceptive Instruments Ltd., UK).

Statistical analysis

For the statistical analysis of the results, z test for percentage of abnormal cells, CAs/cell, RI, CBPI, MI, MN were used. Students’s t test was applied for SCEs and comet assay results induced by SS. Concentration–response relationships were determined from the correlation and regression coefficients for the percentage of abnormal cells, CAs/cell, SCEs, mean MN, mean comet tail intensity.

Results

Chromosomal aberrations assay

SS significantly induced the frequency of CAs and CA/cell including gaps at all concentrations (except at

100 µg/ml for 24 h treatment for CA) and treatments. Also SS significantly increased the frequency of CAs and CA/cell excluding gaps at 800 µg/ml concentration for both treatments compared to negative control. These effects were concentration dependent at both 24 and 48 h treatments with gaps (in abnormal cell percentage $r = 0.79$, $r = 0.92$ and in CAs/cell $r = 0.88$, $r = 0.97$, at 24 and 48 h, respectively) and without gaps (in abnormal cell percentage $r = 0.93$, $r = 0.97$ and in CAs/cell $r = 0.96$, $r = 0.98$, at 24 and 48 h, respectively) (Table 1). Six types of structural chromosomal abnormality were recorded such as chromatid break, chromosome break, fragment, sister union, chromatid exchange and dicentric chromosomes. In addition, SS caused gaps at all concentrations and treatment periods (66.3%). Chromatid breaks (23.7%) and dicentric chromosomes (5%) were more frequent than the other types of aberrations in total at 24 and 48 h treatments together. Polyploidy was recorded as numerical chromosomal abnormality. According to these result, SS was capable of inducing structural CAs rather than numerical CAs (Table 1).

Sister chromatid exchanges, cell cycle and mitotic index

Sodium sorbate significantly increased SCEs at 400 and 800 µg/ml concentrations at both 24 and 48 h treatment periods compared to negative control (Table 2). This effect was concentration dependent ($r = 0.90$ at 24 h, $r = 0.95$ at 48 h). The role of SS in the induction of SCEs was lower than that found in positive control, mitomycin-C. On the other hand, SS significantly decreased MI at 200 µg/ml concentration at 48 h compared to negative control (Table 2). However, it did not decrease replicative index (RI) significantly (Table 2).

Micronucleus assay

According to the MN result, SS increased the micronucleus frequency at 400 and 800 µg/ml concentrations compared to negative control (Table 3). The effect was concentration dependent ($r = 0.97$). The role of SS in the induction of MN frequency was lower than that of the positive control, mitomycin-C. The CBPI was not affected by the treatments of SS (Table 3).

Table 1 Chromosomal aberrations in cultured human lymphocytes treated with sodium sorbate

Test substance	Treatment		Aberrations						Abnormal cell $\pm$ SE (%)		Abnormal cell $\pm$ SE (%)		CAs/cell $\pm$ SE	
	Time (h)	Concent. (μ g/ml)	ctb	csb	f	scu	cte	dic	p	g	(+G)	(-G)	(+G)	(-G)
Negative control	24	0.00	6	1	-	-	-	2	-	12	8.50 $\pm$ 1.97	4.50 $\pm$ 1.46	0.105 $\pm$ 0.021	0.045 $\pm$ 0.014
MMC	24	0.20	26	4	1	1	-	5	2	42	28.00 $\pm$ 3.20	17.50 $\pm$ 2.68	0.405 $\pm$ 0.034	0.195 $\pm$ 0.028
Sodium sorbate	24	100	9	-	-	-	-	3	1	25	14.50 $\pm$ 2.48	6.00 $\pm$ 1.67	0.190 $\pm$ 0.027 ^a	0.065 $\pm$ 0.017
	200	200	8	2	-	1	-	4	-	42	19.50 $\pm$ 2.80 ^b	7.00 $\pm$ 1.80	0.285 $\pm$ 0.031 ^c	0.075 $\pm$ 0.018
	400	400	13	-	-	-	-	4	-	46	22.00 $\pm$ 2.92 ^c	8.50 $\pm$ 1.97	0.315 $\pm$ 0.032 ^c	0.085 $\pm$ 0.019
	800	800	16	2	1	1	1	1	-	51	22.00 $\pm$ 2.92 ^c	9.50 $\pm$ 2.07 ^a	0.365 $\pm$ 0.034 ^c	0.110 $\pm$ 0.022 ^a
Negative control	48	0.00	6	1	-	-	-	2	-	12	8.50 $\pm$ 1.97	4.50 $\pm$ 1.46	0.105 $\pm$ 0.021	0.045 $\pm$ 0.014
MMC	48	0.20	24	12	5	-	6	2	-	42	31.00 $\pm$ 3.27	19.00 $\pm$ 2.77	0.455 $\pm$ 0.035	0.245 $\pm$ 0.030
Sodium sorbate	48	100	10	-	-	-	-	2	-	27	16.00 $\pm$ 2.59 ^a	5.50 $\pm$ 1.61	0.195 $\pm$ 0.028 ^a	0.060 $\pm$ 0.016
	200	200	6	2	1	-	-	3	-	20	15.00 $\pm$ 2.52 ^a	5.00 $\pm$ 1.54	0.175 $\pm$ 0.026 ^a	0.060 $\pm$ 0.016
	400	400	16	1	1	-	-	-	-	38	22.00 $\pm$ 2.92 ^c	8.50 $\pm$ 1.97	0.280 $\pm$ 0.031 ^c	0.090 $\pm$ 0.022
	800	800	26	4	1	1	-	5	2	42	29.00 $\pm$ 3.20 ^c	11.50 $\pm$ 2.25 ^b	0.410 $\pm$ 0.034 ^c	0.120 $\pm$ 0.022 ^b
Frequency of total abnormalities (%) (24 h + 48 h)			23.7	2.5	0.9	0.68	0.23	5	0.68	66.3				

Totally 200 cells were scored for each treatments

ctb chromatid break, *csb* chromosome break, *f* fragment, *scu* sister chromatid union, *cte* chromatid exchange, *dic* dicentric chromosome, *p* polyploidy, *g* gap, *MMC* mitomycin-C, *Concent* Concentration

(+G): Gaps were included, (-G): Gaps were excluded

^a Significantly different from the control $P < 0.05$ (z test)

^b Significantly different from the control $P < 0.01$ (z test)

^c Significantly different from the control $P < 0.001$ (z test)

Table 2 Sister chromatid exchanges, replication and mitotic indices in cultured human lymphocytes treated with sodium sorbate

Test substance	Treatment		Min.–max. SCE	SCEs/ cell $\pm$ SE	M ₁	M ₂	M ₃	RI $\pm$ SE	MI $\pm$ SE
	Time (h)	Concentration (μ g/ml)							
Negative control	24	0.00	2–16	6.82 $\pm$ 0.42	30	31	139	2.545 $\pm$ 0.055	6.50 $\pm$ 0.55
MMC	24	0.20	19–55	35.88 $\pm$ 1.26	42	72	86	2.220 $\pm$ 0.054	5.10 $\pm$ 0.49
Sodium sorbate	24	100	3–12	7.82 $\pm$ 0.34	35	53	112	2.385 $\pm$ 0.054	5.75 $\pm$ 0.52
		200	2–20	8.32 $\pm$ 0.54	37	59	104	2.335 $\pm$ 0.054	5.80 $\pm$ 0.52
		400	4–16	8.40 $\pm$ 0.43 ^a	46	61	93	2.235 $\pm$ 0.056	5.50 $\pm$ 0.50
		800	3–19	9.22 $\pm$ 0.50 ^a	32	48	120	2.440 $\pm$ 0.053	5.70 $\pm$ 0.51
Negative control	48	0.00	2–16	6.82 $\pm$ 0.42	30	31	139	2.545 $\pm$ 0.055	6.50 $\pm$ 0.55
MMC	48	0.20	33–83	58.22 $\pm$ 1.76	75	104	21	1.730 $\pm$ 0.045	4.80 $\pm$ 0.47
Sodium sorbate	48	100	2–16	6.76 $\pm$ 0.44	35	43	122	2.435 $\pm$ 0.054	6.45 $\pm$ 0.54
		200	3–18	7.96 $\pm$ 0.45	27	51	122	2.475 $\pm$ 0.051	4.90 $\pm$ 0.48 ^b
		400	3–21	9.44 $\pm$ 0.52 ^a	26	54	120	2.470 $\pm$ 0.050	5.25 $\pm$ 0.49
		800	5–20	10.36 $\pm$ 0.56 ^a	44	59	97	2.265 $\pm$ 0.056	5.40 $\pm$ 0.50

Totally 50 second mitosis were scored for SCEs/cell

Totally 200 cells were scored for RI

Totally 2,000 cells were scored MI

MMC mitomycin-C

^a Significantly different from the control $P < 0.05$ (t test)

^b Significantly different from the control $P < 0.05$ (z test)

Table 3 Micronucleus frequency and cytokinesis-block proliferation index in cultured human lymphocytes treated with sodium sorbate

Test substance	Treatment		Distribution of BN cells according to the no. of MN				(%) MN $\pm$ SE	CBPI $\pm$ SE
	Time (h)	Concentration (μ g/ml)	(1)	(2)	(3)	(4)		
Negative control	48	0.00	6	–	–	–	0.30 $\pm$ 0.12	1.85 $\pm$ 0.42
MMC	48	0.20	62	8	4	1	4.70 $\pm$ 0.47	1.59 $\pm$ 0.39
Sodium sorbate	48	100	11	1	–	–	0.65 $\pm$ 0.17	1.64 $\pm$ 0.40
		200	6	2	–	–	0.50 $\pm$ 0.15	1.66 $\pm$ 0.40
		400	10	4	–	–	0.90 $\pm$ 0.21 ^a	1.85 $\pm$ 0.42
		800	13	3	–	–	1.95 $\pm$ 0.30 ^b	1.78 $\pm$ 0.41

Totally 2,000 binucleated cells were scored for each treatment

MMC mitomycin-C

^a Significantly different from the control $P < 0.05$ (z test)

^b Significantly different from the control $P < 0.001$ (z test)

Comet assay

The results of comet assay analysis are shown in Table 4. SS caused DNA damage at all concentrations in isolated human lymphocytes after 1 h in vitro

exposure. Although, SS significantly increased the mean tail intensity at all concentrations, this increase was not concentration dependent. The performance of SS on the induction of main tail intensity was lower than that found in H₂O₂ treatment.

Table 4 Effect of sodium sorbate on DNA mean comet tail intensity

Compound	Concentration (µg/ml)	Average tail intensity $\pm$ SE (%)
Negative control	0.00	2.73 $\pm$ 0.21
H ₂ O ₂	40 µM	20.04 $\pm$ 1.65
Sodium sorbate	100	10.30 $\pm$ 1.11 ^a
	200	7.89 $\pm$ 0.91 ^a
	400	10.91 $\pm$ 1.30 ^a
	800	5.97 $\pm$ 0.60 ^a

Totally 200 comets were scored

H₂O₂ hydrogen peroxide

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

Discussion

Chromosomal aberrations, sister chromatid exchanges and micronucleus analysis of human lymphocytes as well as single cell gel electrophoresis (SCGE) are used as the most useful assays to detect the potential genotoxicity of chemicals (Yüzbaşıoğlu et al. 2006; Mpountoukas et al. 2008; Çelik et al. 2009; Yılmaz et al. 2009; Mamur et al. 2010; Zengin et al. 2011). The genome damage to the lymphocytes of peripheral blood has been widely used as a biomarker of carcinogenesis from genotoxic environmental factors, and long-term studies have demonstrated its validity and high clinical predictivity (Hagmar et al. 2004). SS significantly increased the chromosomal aberration, sister chromatid exchanges, micronucleus frequency and DNA damage in cultured and isolated human lymphocytes especially at the two highest concentrations without changing of the pH of the medium. In this study, the highest concentration of sodium sorbate (800 µg/ml) was lower than its level in food. But we found that this concentration was genotoxic for human lymphocytes in vitro.

An increase in the frequency of chromosomal aberrations is associated with an increased risk of cancer (Hagmar et al. 1998). In this study SS significantly increased the frequency of CAs together with gaps at 100, 200, 400 and 800 µg/ml concentrations (except 100 µg/ml for 24 h) and also increased CAs without gaps at 800 µg/ml concentration for 24 and 48 h. SS induced six types of structural aberration in lymphocytes. These are chromatid breaks, chromosome breaks, fragment, sister chromatid union, chromatid exchange, dicentric chromosome. Polyploidy

was recorded as numerical chromosomal abnormality. Chromatid breaks resulting from DNA double-strand breaks was the first common abnormality. The second common aberration was dicentric chromosome. Chromosome breaks and fragments were also recorded in low frequencies. SS also caused gaps at all concentrations and treatment periods. Hasegawa et al. (1984) reported that SS showed genotoxic effect at 200–800 µg/ml concentrations in Chinese Hamster V79 cells, using CAs. Chromatid breaks and chromatid exchanges have been observed as the most common aberrations (Hasegawa et al. 1984). On the other hand Münzner et al. (1990) showed that SS tested as negative in Chinese Hamster and mouse bone marrow cells at 200 mg/kg bw (body weight) concentration, using CAs test. Chromatid and chromosome breaks were observed as the most common aberrations (Münzner et al. 1990). SS has also led to the formation of gap in the above-mentioned investigations (Hasegawa et al. 1984; Münzner et al. 1990). SS had a cytotoxic effect via decreasing the MI. Epel (1963) and Jain and Andsorbhoy (1988) found that the decrease of the mitotic index or the inhibition of the DNA synthesis might be caused by the decreasing ATP level and the pressure from the functioning of the energy production center. However SS showed genotoxic effect in Chinese Hamster V79 cells at a concentration of 2500 µg/ml, with evaluation of MI (Hasegawa et al. 1984).

The sister chromatid exchange technic is used as a sensitive means of monitoring DNA damage. It is useful for assessing the cytogenic impact of clastogenic agents on chromosomes. Many agents found to induce SCEs are well-known mutagens and/or carcinogens (Latt and Schreck 1980). SS significantly increased SCEs at 400 and 800 µg/ml concentrations for 24 and 48 h treatment periods compared to negative control. This genotoxic effect is consistent with another result obtained by SCEs test in Chinese Hamster V79 cells at concentrations of 200–800 µg/ml (Hasegawa et al. 1984). In contrast, SS was tested as negative in Chinese Hamster ovary cells at concentrations of 200–800 µg/ml, in Chinese Hamster and mouse bone marrow cells at a concentration of 200 mg/kg bw (body weight), with SCEs tests (Münzner et al. 1990).

The micronucleus technique has also been proposed as a useful tool for measuring genotoxicity in vitro cultures (Fenech and Morley 1985). MN can be

formed from acentric chromosomal fragments or whole chromosomes left behind during mitotic cellular division. Both clastogenic and aneugenic effects can be determined with the MN test (Kirsch-Volders et al. 1997; Norppa and Falck 2003). Furthermore, an increased MN frequency in peripheral blood lymphocytes implies cancer risk in humans (Bonassi et al. 2005, 2007). Our study showed that SS significantly increased MN formation at 400 and 800 µg/ml concentrations, but it did not change significantly CBPI at all treatment concentrations. Likewise, Schiffmann and Schlatter (1992) showed that SS was genotoxic at 120–1,200 µg/ml concentrations in SHE fibroblast cells with MN test in stored solution. There are many studies that showed no genotoxicity of SS in different cell lines such as, in Chinese Hamster and mouse bone marrow cells at a concentration of 200 mg/kg bw (body weight) (Münzner et al. 1990), in Syrian Hamster embryo fibroblast cells at concentrations 120–1200 µg/ml using MN test with freshly prepared SS (Schiffmann and Schlatter 1992).

The alkaline Comet assay is a widely used SCGE technique for the quantification of DNA strand breaks, crosslinks and alkali-labile sites induced by a series of physical and chemical agents. DNA migration in an electric field, supposed proportional to strand breakage, is a proposed estimation of genotoxicity. Breaks are quantified from geometric and fluorescence measurements by image analysis of comet-shaped DNA (Duez et al. 2003). The Comet assay is a rapid, simple and sensitive technique for measuring DNA breakage with a small number of cells and detects intercellular differences in DNA damage (Ostling and Johanson 1984, 1987; Singh et al. 1988). SS induced DNA damage at all concentrations in isolated human lymphocytes after 1 h in vitro exposure. To our knowledge, no investigations of genotoxicity of SS have been done so far in human lymphocytes with Comet assay.

The genotoxicity studies related to sorbic acid and sorbates are limited. Sorbic acid increased frequency of SCEs and MN at the highest dose (150 mg/kg bw) in bone marrow cells of mice (Mukherjee et al. 1988). In addition, stored SS (100 mg/kg bw) revealed weak clastogenic activity as shown by the increased number of CAs and MN frequency (Münzner et al. (1990). Mpountoukas et al. (2008) reported that sorbic acid salts potassium sorbate showed a weak genotoxic effect at two concentrations (4 and 8 mM) in human lymphocytes using the SCE test. Besides, potassium

sorbate increased CAs, SCEs and DNA damage at 500 and 1,000 µg/ml in human lymphocytes in vitro (Mamur et al. 2010). Hasegawa et al. (1984) reported that, SS is a genotoxic agent, although its potency seems to be weak, and that sorbic acid and potassium sorbate are less genotoxic than the sodium salt. Furthermore, Jung et al. (1992) found that sorbic acid and its potassium salt were not genotoxic in vivo and in vitro. Sasaki et al. (2002) determined that sorbic acid and its potassium salts (2,000 mg/kg) did not induce DNA damage studied in a lot of organs, using the Comet assay. In addition, no chromosomal damage was seen in bone marrow cells of mice given 15 mg sorbic acid/kg bw by stomach tube for 30 days (Banerjee and Giri 1986). Another in vivo study demonstrated that potassium sorbate did not cause DNA damage in rats (Kawachi et al. 1980). Finally, sorbic acid, potassium sorbate and a fresh solution of sodium sorbate tested in SHE fibroblast cells using micronucleus and SHE cell transformation tests gave negative genotoxicity (Schiffmann and Schlatter 1992).

Some authors reported positive results using different food additives; benzoic acid at concentrations of 200 and 500 µg/mL (Yilmaz et al. 2009) and sodium benzoate at concentrations of 6.25, 12.5, 25, 50, 100 µg/mL (Zengin et al. 2011) significantly increased the CAs, SCEs and MN frequency in human lymphocytes. Thakur et al. (1994) reported that sorbates were more efficient and less toxic than benzoate. Boric acid used as an antimicrobial agent induced an increase of chromatid type aberrations at concentrations of 600, 800, 1000 µg/ml in human lymphocytes (Arslan 2004). Additionally, citric acid increased the CAs, SCEs and MN frequency at concentrations of 62.5, 125, 250, 500, 1000 µg/mL in cultured human lymphocytes (Yilmaz et al. 2008). Zengin et al. (2011) reported that potassium benzoate increased CAs, SCEs and MN frequency at 62.5, 125, 250, 500, 1,000 µg/ml concentrations in cultured human lymphocytes. Potassium bromate, which is used as a bleaching agent in flour, induced CAs, SCEs and MN formation in human peripheral blood lymphocytes in vitro (Kaya and Topaktaş 2007). Biswas et al. (2000) reported that, sodium selenite (2.9×10^{-6} , 1.16×10^{-6} and 2.32×10^{-7} M) and sodium selenate (5.3×10^{-6} , 2.65×10^{-6} and 1.06×10^{-6} M) which are used inorganic salts in food, induced CAs and reduced cell division in proportions directly related to the dose.

Sodium selenite (2.9×10^{-5} M) and sodium selenate (2.65×10^{-5} M) were also found to be lethal in human peripheral blood lymphocytes in vitro. On the contrary, potassium nitrate did not affect the SCE frequency at 0.02, 0.2, 2, 4 and 8 mM concentrations in human lymphocytes (Mpountoukas et al. 2008).

Results of the study revealed that SS, which is commonly used in the food industry, has genotoxic and clastogenic effects in human peripheral lymphocytes. Madle et al. (1993) reported that using human lymphocytes could provide the best results for human mutagenicity studies. According to these data, it can be concluded that SS may also cause cancer because of its mutagenic and genotoxic effects. But the exact mechanism of genotoxicity of SS is currently unknown. The mechanism of toxicity of sorbic acid and its salts are probably related to the alkylating activity. Sorbic acid + sorbate system showed alkylating activity on the nucleophile 4-(*p*-nitrobenzyl) pyridine (NBP), which is used as a trap for alkylating agents having nucleophilic characteristics similar to DNA bases (Pérez-Prior et al. 2005). Alkylating agents cause gross mitotic abnormalities and can effect gene mutations (Warwick 1963). A correlation was found between their alkylating capacity and carcinogenicity (Manso et al. 2005). Although many aspects related to the worldwide use of sorbic acid and its salts as food preservatives have long been known, there is little quantitative knowledge about their alkylating potential (Pérez-Prior et al. 2008). Finally, we should say that sodium sorbate is genotoxic to human lymphocytes. But this genotoxic effect should be supported with in vivo studies.

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Conflict of interest The authors declare that there are no conflicts of interest.

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