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ORIGINAL RESEARCH

Cytotoxicity and genotoxicity of butyl cyclohexyl phthalate

Çinel Köksal · Ayse Nalbantsoy · N. Ülkü Karabay Yavaşoğlu

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Abstract Butyl cyclohexyl phthalate (BCP) is frequently used in personal care products, medical and household applications. The aim of this study is therefore to evaluate possible cytotoxicity and genotoxicity of BCP using in vitro and in vivo assays. The in vitro cytotoxic effect of BCP was investigated on mouse fibroblastic cell line (L929 cells) by MTT assay. The result showed that BCP inhibits cell proliferation in a concentration-dependent manner (IC_{50} value = 0.29 μg/mL). For genotoxicity assessment, tested concentrations of BCP demonstrated mutagenic activity in the presence of S9 mix with the *Salmonella* strain TA100 in the Ames test. Results showed that BCP is a secondary mutagenic substance even in low concentrations. The data obtained from 28-days repeated toxicity tests on mice revealed that BCP caused abnormalities of chromosome number, in a dose-dependent manner. Additionally, DNA damage, particularly DNA strand breaks, was assessed by Comet assay. The test result shows that BCP seemed to have genotoxic potential at a high level of exposure.

Keywords Butyl cyclohexyl phthalate · Cytotoxicity · Genotoxicity · Mutagenicity · Chromosome aberration

Ç. Köksal
Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, 35100 Bornova, Izmir, Turkey

A. Nalbantsoy
Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova, Izmir, Turkey

N. Ü. Karabay Yavaşoğlu (✉)
Department of Biology, Faculty of Science, Ege University, 35100 Bornova, Izmir, Turkey
e-mail: ulku.karabay@ege.edu.tr

Introduction

Many new industrial compounds have been synthesized for commercial and industrial purposes due to their high production and widespread use. Despite of the dramatic increase in the use of these chemicals, little information is available on their potential toxic effects on human health and living organisms (Park and Choi 2007). As one of these chemicals phthalates are widely used as plasticizing agents in manufacture of defoaming agents, various organic syntheses, and vehicles for perfumes and cosmetics (Heudorfa et al. 2007; Lee and Lee 2007). Phthalates are considered more importantly, because they are used by industry in variable amounts (Harris et al. 1997). As the record of 2004, US manufacturers produced about 363 thousand tones (800 million pounds or 400,000 short tons) of phthalates each year. According to The European Council for Plasticizers and Intermediates (2008), in Western Europe about one million tones of phthalates are produced each year, of which approximately 900,000 tones are used. Phthalates contribute to

10–60 % of plastic products by weight (Committee on the Health Risks of Phthalates 2008). The large scale use of phthalate esters as industrial chemicals has a potential environmental importance. Phthalates are not covalently bound to the plastic product thus they can leach out over time from plastic products. Hence they can easily spread to the environment and humans are exposed through ingestion, inhalation, and dermal exposure during their whole lifetime, including intrauterine development (Lee and Lee 2007). After absorption, phthalates are rapidly hydrolyzed by esterases in the gut and other tissues into a monoester. Their metabolites are constantly detected in plasma, urine, amniotic fluid or breast milk, therefore reflecting substantial and constant exposure (Koch et al. 2003; Silva et al. 2004; Koo and Lee 2005).

Butyl cyclohexyl phthalate (BCP; CAS No. 84-64-0) is frequently used in personal care products, medical and household applications. It is a clear, colorless and practically odorless liquid (MSDS 2010). Because of being an oily liquid with slight water solubility and being soluble in or partially miscible with many of the organic molecules with fragrance properties, BCP provides a technical advantages as a vehicle for cosmetic products. One of common phthalates, BCP is also a widely used industrial plasticizer (Harris et al. 1997). Hazard identification of BCP is not available (MSDS 2010). Also, biodegradation data for BCP were not available. However, BCP may be subject to biodegradation in the environment. BCP is expected to adsorb to suspended solids, when released into water. Therefore, bioconcentration of BCP in aquatic organisms is very high. Occupational exposure to BCP may occur through inhalation and dermal contact with this compound at workplaces where BCP is produced or used. Data indicate that the general population may be exposed to BCP via dermal contact with products containing BCP (HSDB 2011).

A number of studies were published on phthalate toxicities. They have shown that some of them can produce severe developmental toxic effects in rodents, and more particularly affect the male reproductive organs and sexual development (Latini et al. 2006; Saillenfait et al. 2009). Exposure to some phthalates results in serious and irreversible changes in the development of reproductive tract in males. In particular, prenatal exposure to phthalates, by interfering with androgen signaling pathway seems to cause permanent adverse effects on reproductive development in male rats (Foster et al. 2001; Sharpe 2001; Ahbab et al. 2014). Few published human studies have examined the effect of environmental chemicals, such as phthalates, on DNA integrity. These studies have shown that some phthalate esters could produce DNA damage (Anderson et al. 1999; Hauser et al. 2007). However, in the literature, there are no in vitro cytotoxic or in vivo genotoxicity studies conducted on BCP. The aim of this study was to evaluate cytotoxicity and genotoxicity of BCP using in vitro and in vivo methods.

Materials and methods

Material

Butyl cyclohexyl phthalate (BCP; CAS No: 84-64-0) was obtained from Chemical Service Inc. (West Chester, PA, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Cell line and cell culture

Mouse fibroblastic cell line (L929 cells) were purchased from the HUKUK (Animal Cell Culture Collections) in the Foot-and-Mouth Disease Institute (Ankara) of the Ministry of Agriculture & Rural Affairs of Turkey. The cell line was maintained in RPMI 1640 (HyClone, Logan, UT, USA) medium supplemented with 10 % heat–inactivated fetal bovine serum, 1 % L-glutamine (Biochrome, Berlin, Germany) and 1 % gentamycine (Biochrome) in a humidified atmosphere with 5 % CO_2, at 37 °C. The cells were subcultured twice a week.

Butyl cyclohexyl phthalate was dissolved in dimethyl sulfoxide (DMSO) and subsequent dilutions were made in culture medium. The same amount of DMSO/culture medium was added to the controls. The final DMSO content was never above 0.1 %. MTT [3-(4, 5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide)] (Sigma Chemical Co. St. Louis, MO, USA) assay for cytotoxicity of BCP was carried out (Mossman 1983). The survival of viable cells after treatment of BCP in monolayer culture was determined. L929 cells were cultivated for 24 h in 96 well microplates with 2.3×10^5 cells/mL as initial concentration was cultivated for 24 h. After that the

culture was treated with different dilutions (0, 0.2, 0.4, 0.6, 0.8, 1 and 2 μL/100 μL) of BCP and incubated for 72 h. The growth inhibition was estimated as the 50 % effective concentration (IC_{50}). Optical density of the dissolved material was measured at 570 nm (reference filter, 690 nm) with an UV visible spectrophotometer (Molecular Devices, Winnersh, Berkshire, UK). Cytotoxicity was expressed as mean percentage increase relative to the unexposed control ± standard deviation (SD). Control values were set at 0 % cytotoxicity. Cytotoxicity data (where appropriate) were fitted to a sigmoidal curve and a four parameters logistic model was used to calculate the IC_{50}, which was the concentration of BCP causing a 50 % inhibition in comparison to untreated controls. This analysis was performed with GraphPad Prism (San Diego, CA, USA).

Bacterial reverse mutation assay (Ames test)

Bacterial reverse mutation assay (Ames test) was performed in two histidine-requiring strains of *Salmonella typhimurium*, tester strains TA98 and TA100, according to the OECD Guideline 471: Bacterial Reverse Mutation Test (1997). Two separate experiments were performed, using triplicate plates, in the presence and absence of metabolic activation by an Aroclor 1254-induced (500 mg/kg body weight) Swiss albino mice liver post-mitochondrial fraction (S9). The post mitochondrial fraction was used at a concentration of 10 % v/v in the S9 mixture (metabolic activation). The S9 mixture was freshly prepared for each experiment. Negative controls and positive controls were tested in all strains in both experiments. Benzo[a]pyrene (5 μg/plate), 2-nitrofluorene (5 μg/plate) and sodium azide (10 μg/plate) were used as positive controls. Fresh cultures of tester strains were grown to approximately 10^9 cell/mL in 5 mL nutrient broth (Oxoid Ltd, Basingstoke, Hampshire, UK). The cultures were incubated for 10–12 h at 37 °C in a gyratory incubator in order to insure adequate aeration. The strains were periodically raised from a single colony to check the genetic markers.

Four concentrations of BCP (0.5, 1, 2 and 5 μg per plate) were tested on TA98 and TA100. The recommended maximum test concentration, 5 μg/plate was used as the highest concentration according to the OECD Guideline 471: Bacterial Reverse Mutation Test (1997). BCP concentrations were plated in triplicate with 0.1 mL of overnight bacterial cultures per plate. The solutions were prepared in the absence (0.5 mL/plate phosphate buffer 1 M, pH = 7.4) and presence of S9 mixture (0.5 mL/plate). The mixture containing chemicals and bacteria with or without S9 was vortexed and pre-incubated at 37 °C for 30 min. It was then plated in 2 mL of top agar on glucose-supplemented minimal agar. After 48 h of incubation at 37 °C, revertant colonies (his^+) were counted. The results of the test were presented as the mean ± standard error means (SEM). Comparisons were made between control and treatment groups using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of $p \leq 0.05$ were regarded as statistically significant. Statistical analyses were performed using SPSS for Windows V.11.0.

Bone marrow chromosome aberration assay

For bone marrow chromosome aberration assay, the protocol was approved by the Animal Experiment Local Ethical Committee of the Ege University (date 23.11.2009, number 2009-165). All experiments were performed in accordance with ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann 1983). The study was conducted on Swiss albino mice (4–5 week old, 15–25 g in weight) obtained from Breeding Center of Experimental Animals in the Ege University. Animals were housed in cages (19 × 19 × 12 cm) with solid plastic sides and stainless-steel grid tops and floors. They were maintained in controlled laboratory conditions of 12 h dark/light cycle, 21 ± 1 °C temperature and 45–75 % humidity. Animals were orally fed daily with a normal diet in standard laboratory chow. Tap water was also available ad libitum. Repeated dose toxicity study was conducted on 20 male and 20 female Swiss albino mice. After 10 days of acclimation, the mice were assigned randomly to either the exposure groups (100, 200 and 400 mg/kg BCP) or the control group, each containing 5 male and 5 female mice.

The treatment dose of the BCP was calculated based on lethal dose (LD_{50} = 1,931 mg/kg body weight for mice) and body weight data. The up and down procedures was used to determine the lethal dose (LD_{50}) according to the OECD Guideline 425: Acute Oral Toxicity: Up-and-Down Procedure (2001). BCP is administered in increasing doses to animals orally. Mortalities were recorded within a given period, and

the LD_{50} was determined with the aid of statistical calculations (Probit analyses in SPSS for Windows 10.0). Therefore, 100 mg/kg (around 1/20 of LD_{50}) as low dose or non-toxic dose, 200 mg/kg (two fold of low dose) as medium dose, and 400 mg/kg (two fold of medium dose) as high dose or toxic dose were selected in the study. Animals of the control group were administered daily with physiological saline, while the animals of the treated groups were administered with BCP for five consecutive days per week by oral route during 28 days as described in the OECD guideline 407: Repeated Dose 28-day Oral Toxicity Study in Rodents (1995). All animals were weighed weekly throughout the study.

At the end of the experiment, all mice of each group were sacrificed by cervical dislocation and the genotoxic effect was evaluated by the bone marrow chromosome aberration assay (Adler 1984). About 2 h before sacrifice, colchicine (4 mg/kg) (Sigma Chemical Co. St. Louis, MO, USA) was injected intraperitoneally to produce mitotic arrest. Both femora were dissected out and cleaned of any adhering muscle. The femoral bone marrow was aspirated into 8 mL 0.075 M KCl (37 °C) and the cells were flushed out with a Pasteur pipette. The cell suspension was incubated at 37 °C for 20 min. The cells were centrifuged at 2,000*g* for 10 min, and the supernatant was removed. After centrifugation, the cells were resuspended in 7 mL fixative (3:1 methanol:glacial acetic acid). Tubes with the cell suspension were kept at +4 °C overnight. Centrifugation and fixation (in the cold) were repeated four times at intervals of 20 min. Slides were prepared by the air-drying method. The slides were stained on the following day for 10 min in 8.5 mL 5 % buffered Giemsa solution, pH 6.8. Twenty slides were prepared for each group. One hundred cells were examined to detect chromosomal aberrations in each slide. Numerical and structural chromosome aberrations were evaluated. The data were analyzed by the Fisher exact test to determine if there was significant increase in aberrant cells in the BCP-treated groups compared with the control.

Micronucleus test

The frequency of micronucleus was evaluated based on a technique developed by Schmid (1976). The femoral bone marrow was flushed out using 1 mL of fetal calf serum and centrifuged at 2,000*g* for 10 min. The supernatant was discarded. Smears were prepared for each animal, fixed in methanol and stained with 5 % Giemsa in Sørensen buffer. Smears were screened at a magnification of 1,000×, using a light microscope. The cells with one or more micronuclei were counted in at least 2,000 cells per animal. Micronucleus frequency (MN ‰) was calculated as follows: MN ‰ = (number of cells containing micronucleus/total number of cells counted) × 1,000. Statistical differences between control and concentration groups were determined by the Dunnet test. Statistical analyses were performed using SPSS for Windows V.11.0.

Comet assay

The comet assay was performed in accordance with Tice et al. (2000). Before sacrification, BCP treated mice of each group were anaesthetized with ether and blood samples were drawn from the heart of each animal. Lymphocytes were isolated from blood samples with Histopaque (Sigma Chemical Co. 1077-1) and Comet assay was performed on lymphocytes. Lymphocyte cells were embedded in 0.6 % low melting agarose layers on a microscope slide precoated with 0.5 % normal melting agarose. The slides were lysed with fresh lysing solution containing high salt and detergent concentrations (2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10), 10 % DMSO and 1 % Triton X-100) for at least 1 h at 4 °C. After lysis, slides were placed in an electrophoresis tank having fresh electrophoresis buffer (300 mM NaOH, 1 mM EDTA and distilled water) for 20 min to allow DNA unwinding. Electrophoresis was conducted at 25 V cm^{-1}, 300 mA (at 4 °C). Slides were then neutralized for 10 min with 0.4 M Tris–HCl (pH 7.5), fixed in methanol at −20 °C and dried. All these steps were carried out in dim light to minimize artifactual DNA damage. DNA of individual cells was stained with DAPI (1 mg/mL, Applichem, St. Louis, MO, USA) and analyzed using a fluorescence microscope. The positive control was not included in the evaluation. Slides were examined at 100× magnification using a fluorescence microscope (Leica, Wetzlar, Germany) equipped with a filter for DAPI (excitation λ 360 nm, emission λ 460 nm). For each experimental condition 100 randomly chosen cells from two duplicate slides were examined (50 from each slide). DNA damage was expressed as arbitrary

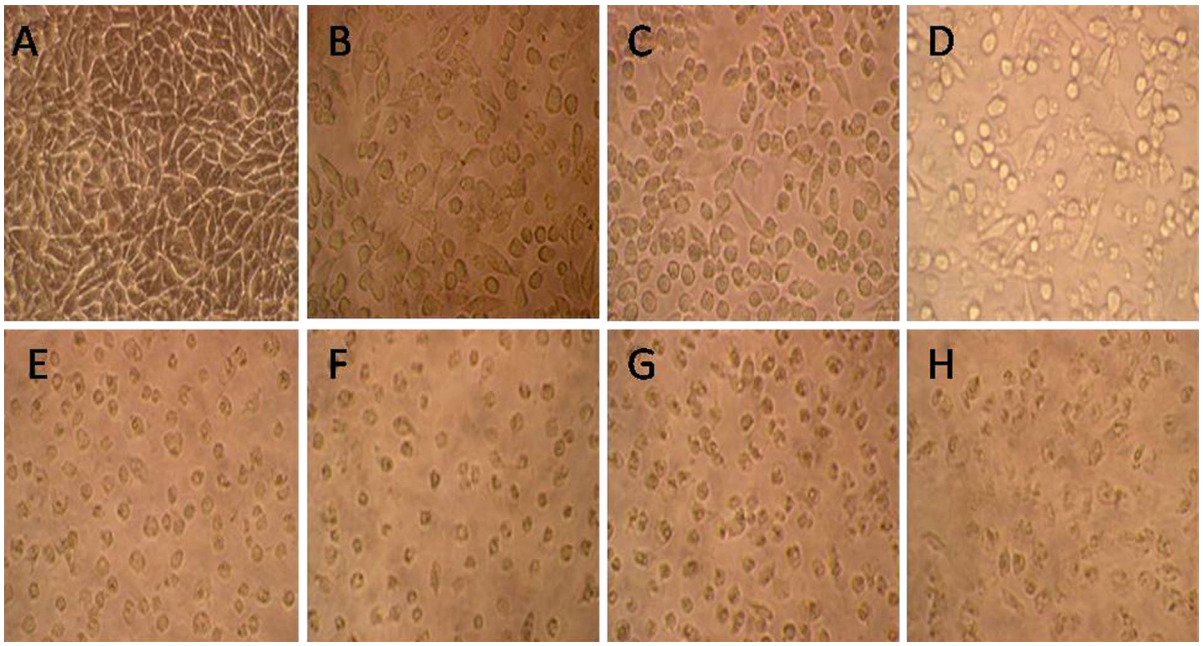

Fig. 1 Morphological changes towards L929 cells as observed by inverted microscope after the post-treatment with BCP. Cells were treated with different concentrations of BCP for 72 h at 37 °C. **a** Untreated cells, **b** treated cells with 0.15 µg/mL, **c** treated cells with 0.30 µg/mL, **d** treated cells with 0.45 µg/mL, **e** treated cells with 0.60 µg/mL, **f** treated cells with 0.75 µg/mL, **g** treated cells with 1.5 µg/mL, **h** treated cells with 2.25 µg/mL (magnification 10×)

units (AU) based on the classification of comets into five categories (0–4) proposed by Collins et al. (1997) and Collins and Dusinska (2009). In total 100 comets were scored visually according to the relative intensity of the fluorescence in the tail and given a value of 0, 1, 2, 3 or 4 (0: undamaged, 1: little damaged, 2: medium damaged, 3: damaged, 4: maximally damaged). Thus, the overall score for 100 Comets could range from 0 (all undamaged) to 400 (all damaged) arbitrary unit. Slides were analyzed by one observer to minimize the scoring variability. Higher scores indicate a significant positive genotoxic effect of the sample. $AU = (0 \times N_0) + (1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4)$, where N*i* is the number of nuclei scored in each category (Collins 2004; Collins and Dusinska 2009).

Results and discussion

In this study, cytotoxicity and genotoxicity tests were performed on BCP using several test systems. This finding is, to the best of our knowledge, the first scientific report to describe the cytotoxic and genotoxic induction potential of BCP. Screening of chemicals for cytotoxicity, based on metabolic cell viability, was carried out by using a modified MTT assay which affects the mitochondrial activity of viable cells (Mossman 1983). The cytototoxic compounds destroy cancer cells by interfering the cell division and growth. The affected cells become damaged and eventually die. In the present study, by using different concentrations the cytotoxic effects of BCP were tested on L929 cells. The result showed that BCP inhibits cell proliferation in a concentration-dependent manner. Morphological changes were obtained for L929 cells (Fig. 1), which were growing in logarithmic phase throughout the treatment with BCP. After the post-treatment of BCP, an increased number of rounded cells and growth inhibition were observed when compared with the untreated control cells (Fig. 2). The loss of normal morphology started appearing even within 24 h at a concentration of 0.29 µg/mL. The cells retracted into spherical shape with a consequent increase in exposure time. The IC_{50} value of BCP on L929 cells was calculated as 0.29 µg/mL. According to the obtained outcomes from the morphological observation and MTT test BCP showed

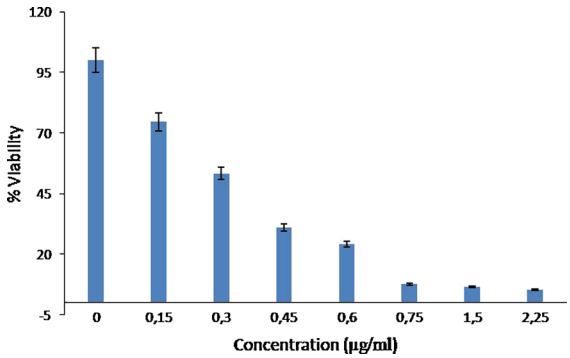

Fig. 2 Cytotoxic effect of BCP on L929 cells after 72-h exposure to different BCP concentrations. Cell viability was determined by MTT assay. Control was exposed to vehicle only which was taken as 100 % viability. Data are expressed as mean ± SD

significant cytotoxic activity against L929 cells dependent on concentrations.

The genotoxicities of various concentrations of BCP (0.5, 1, 2 and 5 µg) were evaluated on the *S. typhimurium* TA 98 and 100 strains, both in the presence and the absence of metabolic activation. Significant concentration-dependent increases in the number of his^+ revertants were observed with the TA100 strain at 1, 2 and 5 µg/plate concentrations in the presence of S9 mix as metabolic activation. BCP did not produce mutagenic activity in any of the bacterial strains tested under the condition without S9 mix. The test results are shown in Table 1. Chromosomal aberrations were determined as numerical chromosomal aberrations, which consisted of a decrease in chromosome number and structural chromosomal aberrations, which consisted of sticky chromosomes and fragments. The frequencies of numerical and structural chromosomal aberrations are shown in Table 2. Especially chromosomal aberrations including aneuploidy ($<2n = 40$) were induced by BCP at all tested concentrations versus the untreated controls (Fig. 3). Significant differences were detected between all BCP treated groups versus the control group ($p < 0.05$). The in vivo and in vitro genotoxicity results of the tests performed based on the guidelines indicated that BCP has no direct effect on mutagenesis and it is a secondary mutagenic substance even at low concentrations. In the Ames test, BCP showed increase in base-pair substitution mutation frequency in the TA 100 test strains with presence of S9 mix. The positive result in the Comet assay in the present study showed a clear response at lower dose levels. Also, BCP have a clastogenic potential. The presences of aneuploidy in the bone marrow cells of BCP treated mice show that BCP cause abnormal mitotic cell division.

DNA damage was measured by Comet assay to evaluate whether BCP induced any genotoxicity. As shown in Table 3 and Fig. 4, BCP seemed to induce DNA strand breaks in mice at the studied exposure levels. Oxidative stress is one of the possible mechanisms of toxicity and it results from an imbalance between the excessive formations of reactive oxygen species (ROS) and limited antioxidant defenses. Oxidative damage to DNA has been postulated to have biological importance. The role of ROS in production of DNA single strand breaks is well known (Collins 2009). There have been several investigations in vivo and in vitro on the correlation between toxicant-induced oxidative stress and DNA damage (Muniz et al. 2008; Azqueta et al. 2009). Furthermore,

Table 1 his^+ revertants in the Bacterial reverse mutation assay

Groups	Concentration (µg/plate)	Mean *his*$^+$ revertants/plate (X ± SEM)			
		TA 98		TA 100	
		+S9 mix	−S9 mix	+S9 mix	−S9 mix
Control (DMSO)	10 µL	40 ± 3.2	35 ± 4.6	182 ± 3.6	146 ± 7.0
BCP	0.5	31 ± 2.9	40 ± 1.5	186 ± 4.2	142 ± 2.5
	1	30 ± 2.3	31 ± 2.3	211 ± 2.9*	144 ± 3.1
	2	37 ± 1.7	39 ± 3.0	230 ± 7.2*	146 ± 6.5
	5	39 ± 2.6	37 ± 4.5	242 ± 6.4*	155 ± 5.9
Benzo[a]pyrene	5	672 ± 15*	–	764 ± 42*	–
2-Nitrofluorene	5	–	935 ± 25*	–	–
Sodium azide	10	–	–	–	1,157 ± 28*

* Significantly higher than the control group (DMSO) ($p < 0.05$)

Table 2 Frequency of numerical and structural chromosome aberrations and micronucleus formation in bone marrow cells of control and BCP treated groups

Groups	Doses (mg/kg)	Numerical aberrations (%)	Structural aberrations (%)		MN frequency (‰)
			Stickiness	Fragment	
Saline	100	2.97	1.02	0.34	1.85
BCP	100	11.52*	1.22	0.30	2.12
	200	12.08*	1.18	0.45	2.20
	400	12.27*	1.34	0.56	2.18

* Statistically significant according to control group (Saline) ($p < 0.05$)

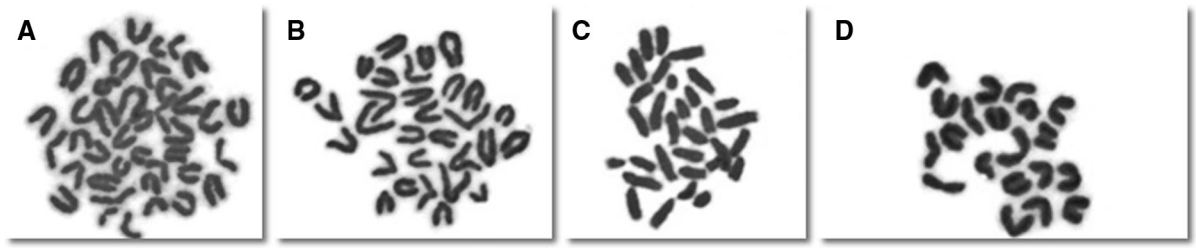

Fig. 3 Chromosomes images. **a** Control group (2n = 40); **b** 100 mg/kg BCP treated groups (2n = 30, aneuploidy); **c** 200 mg/kg BCP treated groups (2n = 21, aneuploidy); **d** 400 mg/kg BCP treated groups (2n = 20, aneuploidy)

Table 3 Comet assay results of control and BCP treated groups

Groups	Doses (mg/kg)	Comet score (AU)	Comet class (%)				
			0	1	2	3	4
Saline	100	26	76	22	2	0	0
BCP	100	132*	4	74	10	10	2
	200	202*	2	18	60	16	4
	400	356*	0	2	4	30	64

* Statistically significant according to control group (Saline) ($p < 0.05$)

some studies also showed that the elevated levels of ROS result in oxidation of cellular components with unsaturated fatty acids and further induce cell apoptosis and degeneration (Fujii et al. 2003). Comet assay results of this study clearly showed that the number of damaged nuclei increased resulting in many fold increase in the damage index on exposure with BCP. Our other study results supported that 28 days BCP exposure induces oxidative stress in mice and constitutes an imbalance between scavenging and forming of ROS (Karabay Yavasoglu et al. 2014). In the study, we showed that BCP is capable of inducing marked hazardous alteration by the imbalance of the pro/antioxidant system and the histopathology of liver, in addition to the lipid peroxidation which may be one of the molecular mechanisms involved in BCP-induced oxidative stress in mice. Our data suggest that oxidative stress might contribute to BCP cytotoxicity. Similarly, cytotoxic and genotoxic potential of other phthalates such as di-2-ethylhexyl phthalate (DEHP) has been demonstrated previously with various genotoxicity assays (Anderson et al. 1999; Erkekoglu et al. 2010).

Genotoxicity tests such as Ames test, chromosome aberration test and Comet assay are appropriate tests for screening of the potential hazardous effects of a large number of chemicals. DNA strand breaks are potential pre-mutagenic lesions and sensitive markers of genotoxic damages. Among the available genotoxicity indicator tests, the Comet assay has recently attracted much attention. The Comet assay primarily measures DNA strand breakage in single cells. The protocol has been increasingly used in different fields of study, and the DNA strand fragmentation as analyzed by the Comet assay is a common biomarker widely used to assess the genotoxic potential of chemical pollutants (Cotelle and Ferard 1999; Brendler-Schwaab et al. 2005; Møller 2006). The Comet

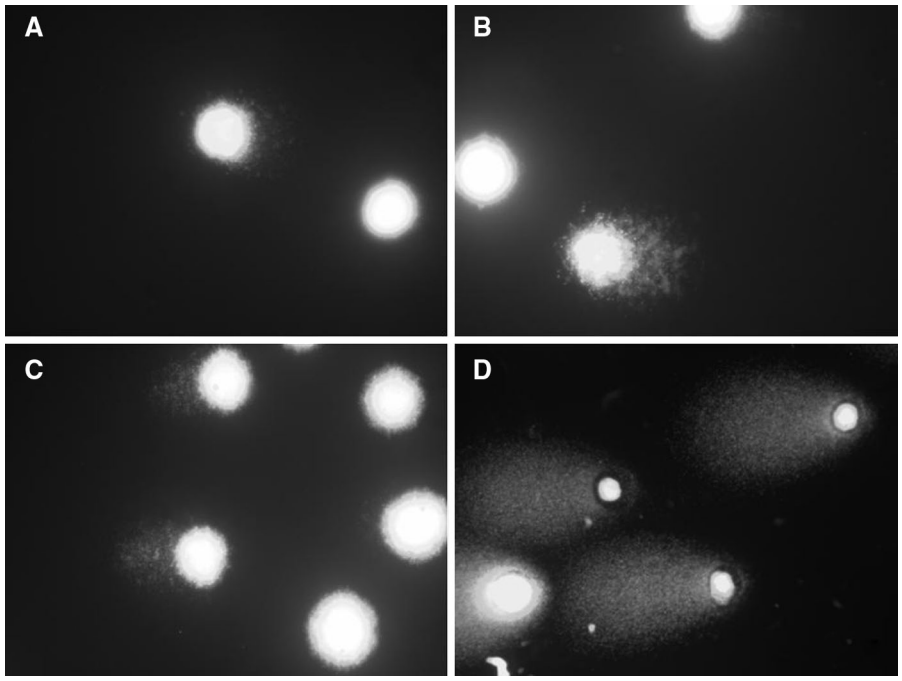

Fig. 4 Comet images. **a** Control group; **b** 100 mg/kg BCP treated group; **c** 200 mg/kg BCP treated group; **d** 400 mg/kg BCP treated group

assay indicated that repair by an excision repair mechanism was initiated. The Comet assay is able to detect DNA breaks, which could be repaired efficiently by an excision repair mechanism. However, extensive induction of DNA breaks could exceed the repair capacity of the cell, also resulting in chromosomal breaks (Collins 2004; Mladenov and Iliakis 2011). In our study, we could not observe induction of micronuclei or chromosomal aberrations except that aneuploidy, suggesting an efficient repair of BCP-induced DNA breaks.

The frequency of chromosomal aberrations have been used for decades as a biomarker of the early effects of genotoxic carcinogens in occupational and environmental settings (Carrano and Natarajan 1988; Norppa et al. 2006). Chromosomal aberrations are thought to represent a surrogate endpoint for more specific chromosome alterations in target tissues of carcinogenesis. In this study, we showed that a high frequency of numerical chromosome aberrations such as aneuploidy ($<2n = 40$), is associated with increased risk of cancer.

Some studies indicate that different agents may act at different stages in the carcinogenic process and that several different mechanisms may be involved. The agents cause molecular changes such as formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations and aneuploidy (IARC 2011). In conclusion, BCP causes the mutagenic effect by inducing DNA damage. These findings of the present study suggest that BCP has a genotoxic potential at a high level of exposure according to the different mutagenic tests utilized. The data obtained from the study revealed that BCP might be a potential carcinogenic substance to humans. Further studies are necessary to shed light on possible mechanisms of action of toxic effects caused by BCP.

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Conflict of interest The authors have declared that they have is no conflict of interest.

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