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RESEARCH ARTICLE

Curcumin prevents perfluorooctane sulfonate-induced genotoxicity and oxidative DNA damage in rat peripheral blood

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Abstract

Perfluorooctane sulfonate (PFOS) is a man-made fluorosurfactant and global pollutant. PFOS a persistent and bioaccumulative compound, and it is widely distributed in humans and wildlife. Therefore, it was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in May 2009. Curcumin is a natural polyphenolic compound abundant in the rhizome of the perennial herb turmeric. It is commonly used as a dietary spice and coloring agent in cooking and anecdotally as an herb in traditional Asian medicine. In this study, male rats were treated with three different PFOS doses (0.6, 1.25, and 2.5 mg/kg) and one dose of curcumin, from *Curcuma longa* (80 mg/kg), and combined three doses of PFOS with 80 mg/kg dose of curcumin by gavage for 30 d at 48 h intervals. Here, we investigated the DNA damage via single-cell gel electrophoresis/comet assay and micronucleus test in rat peripheral blood *in vivo*. It is found that all doses of PFOS increased micronucleus frequency ($p < 0.05$) and strongly induced DNA damage in peripheral blood in two different parameters; the damaged cell percent and genetically damage index, and curcumin prevented the formation of DNA damage induced by PFOS. Results showed that curcumin inhibited DNA damage including GDI at certain levels at statistical manner, 30.07%, 54.41%, and 36.99% for 0.6 mg/kg, 1.25 mg/kg, and 2.5 mg/kg.

Keywords

Curcumin, micronucleus, perfluorooctane sulfonate, rat peripheral blood, single cell gel electrophoresis

History

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Introduction

Perfluorooctane sulfonate (PFOS) is a member of perfluorinated organic compounds (PFOCs) family. Due to their unique physicochemical properties, including potent surfactant activity and resistance to chemical hydrolysis and heat, PFOS and its congeners have been used for decades in numerous industrial and consumer applications as cosmetics (Liu et al., 2007). With its exceptional resistance to both environmental and biological degradations, PFOSs, along with other organic compounds with alkyls have emerged as global environment pollutant. Approximately 5 years ago, it was added to Annex B (http://chm.pops.int/Convention/Pressrelease/COP4Geneva8May2009/tabid/542/language/enUS/Default.aspx).

PFOS can form from the degradation of precursors in addition to industrial production (Ye et al., 2008). The elimination period of PFOS varies among living organisms. It is indicated that the long serum half-life of PFOS in human, rats, and monkeys, 4–5 years, 50 d, and 200 d, respectively (Olsen et al., 2007). In human, PFOS has been detected in serum samples from general population (Olsen et al., 2003) and in human milk and blood samples in China (So et al., 2006). Furthermore, liver and blood are the major sites of accumulation for this chemical (Seacat et al., 2002).

Although the relative importance of different routes of human exposure to PFOS remain to be established, food intake and food packaging materials (Poothong et al., 2012), water (Domingo et al., 2012), house dust, and in-door air (Ericson Jogste et al., 2012) are all considered to be potential sources. Qazi et al. (2009) have demonstrated that short-term exposure to long-term exposure to this compound impairs the immune system. Çelik et al. (2013) showed that PFOS increased the DNA damage and has genotoxic/toxic effects on the bone marrow polychromatic erythrocytes of rats *in vivo*.

Curcumin is a natural compound that is present in turmeric that can be extracted from rhizome of *Curcuma longa* and used extensively in Eastern cuisine. Recently, it has attracted much attention due to its significant medicinal potential. Pharmacologically, curcumin exhibits a wide range of effects including anti-inflammatory, hypo-cholesterolemic, and anti-infection activities (Menon et al., 2007), as well as anti-carcinogenic (Balcerek & Matlawska, 2005) and anti-genotoxic (Çelik et al., 2013). It is found that curcumin has therapeutic and protective effects in many diseases such as colon cancer (Kalpana et al., 2007).

The micronucleus assay is a genotoxic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments, i.e. micronuclei in the

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cytoplasm of interphase cells. Micronucleus test has been used to evaluate the exposure to chemicals for decades in many investigations in different organisms (Çavaş, 2011; Çelik & Kanık, 2006; Çelik et al., 2005).

Comet assay has been widely used in different models from bacteria to man, employing diverse cell types to assess the DNA-damaging potential of chemicals and/or environmental conditions by many research groups and researchers (Çavaş, 2011; Narendra, 2000; Solanky & Haydel 2012). The main advantages of the comet assay include (a) the collection of data at the level of the individual cell, allowing more robust statistical analyses, (b) the need for a small number of cells per sample (<10 000), (c) sensitivity for detecting DNA damage, and (d) the use of any eukaryotic single-cell population both *in vitro* and *in vivo*, including cells obtained from exposed human populations for eco-genotoxicological studies and environmental monitoring (Collins et al., 1997).

The basic aim of this study was to investigate genotoxic effects of PFOS and the genoprotective effects of curcumin against to PFOS-induced DNA damage using single-cell gel electrophoresis and micronucleus test in rat peripheral blood erythrocytes in chronic period *in vivo*.

Material and methods

Chemicals

Perfluorooctane sulfonate (PFOS; CAS no. 1763-23-1) was supplied as grade form and purchased form Sigma (St. Louis, MO). Curcumin was purchased from Sigma (St. Louis, MO; chemical purity >99%). Figures 1 and 2 represent the molecular structure of PFOS and curcumin, respectively.

Dose selection

Doses were selected according to LD_{50} dose of PFOS for rats. LD_{50} dose of PFOS is 251 mg/kg for rats (OECD, 2005). Therefore, in this study, the highest dose was set at 2.50 mg/kg body wt, i.e. 1% of the LD_{50} dose. The lowest dose was determined via pre-study as 0.6 mg/kg body wt because this dose did not affect the animals neurologically and behaviorally. Curcumin dose was selected according to previous studies performed by other researchers (Balakrishnan et al., 2008; Sankar et al., 2010) and curcumin's pharmacological safety is accepted, considering that it has been consumed as a dietary spice, at doses up to 100 mg/d, for centuries (Ammon & Wahl, 1991).

In this study, mitomycin C (MMC) (2 mg/kg), a single i.p. dose, was used as a positive control. It is acceptable that a positive control is administered by a route different from or the same as the test substance and that it is given only a single time (Hayashi et al., 1994). The positive control and untreated control rats were treated identically with equal volumes of normal saline.

Animals and diet program

In this study, healthy adult male Swiss albino rats (Wistar rat) [6–8 weeks of age and average body weight (body wt) of 180–200 g] were used. Rats were obtained from the Experimental Animal Center, University of Mersin, Turkey. The study was approved by the research and ethical committee at the University of Mersin. The rats were randomly selected and housed in polycarbonate boxes (six rats per box) with steel wire tops and rice husk bedding. They were maintained with 12 h dark/light cycle in a controlled atmosphere of $22 \pm 2\,^{\circ}C$ temperatures and 50–70% humidity with free access to pelleted feed and fresh tap water. The animals were allowed to acclimate for 14 d before treatment. In this study, rats were divided to nine groups including six rats. Therefore, this study was performed by nine different diet programs one dose per 48 h for 4 weeks (~30 d).

The rats were treated by gavage with

(i) 0.6 mg/kg body wt only PFOS dissolved in saline.
(ii) 1.25 mg/kg body wt only PFOS dissolved in saline.
(iii) 2.50 mg/kg body wt only PFOS dissolved in saline.
(iv) 0.6 mg/kg dose of PFOS (dissolved in saline) plus curcumin (80 mg/kg body wt).
(v) 1.25 mg/kg dose of PFOS (dissolved in saline) plus curcumin (80 mg/kg body wt).
(vi) 2.50 mg/kg dose of PFOS (dissolved in saline) plus curcumin (80 mg/kg body wt).
(vii) The rats were treated only curcumin (80 mg/kg body wt).
(viii) The untreated control rats were treated identically with equal volumes of normal saline only via gavage throughout the study.
(ix) Since positive controls may be administered by a different route and treatment schedule than the test agent (Hayashi et al., 1994). In this study, a single dose of MMC (2 mg/kg, i.p.) was administered at the 10th week.

Experimental design

Comet assay in peripheral blood

Blood samples were obtained from rat heart by venipuncture at the end of the 4th week. Isolated blood cell suspension was washed two times with RPMI supplemented with 10% FBS. Part of the isolated cells was used directly for the comet assay. Comet assay was performed under alkaline conditions according to the method of Singh et al. (1988) with slight modifications. Completely frosted microscopic slides were

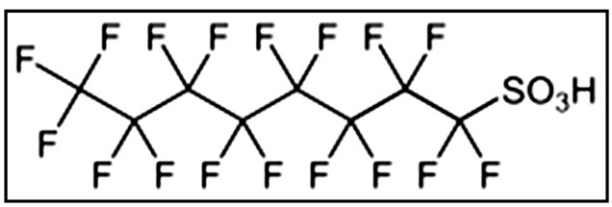

Figure 1. The molecular structure of PFOS.

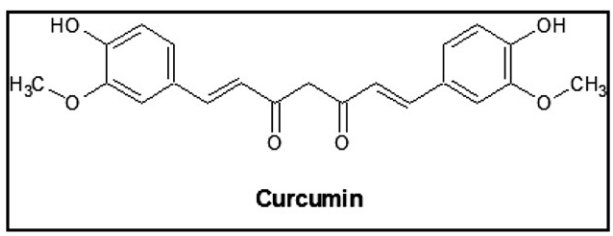

Figure 2. The molecular structure of curcumin.

covered by a thin layer of 0.5% normal melting agarose (NMA) dissolved in Ca^{2+} and Mg^{2+} free phosphate buffer saline (PBS) at about 50 °C. Eppendorf tubes were placed in water bath at 40 °C. One hundred microliters of blood suspension were diluted with 1 ml of PBS in eppendorf tube. Then 30 µl mixtures were mixed with 250 µl of LMA (0.5%). One hundred microliters of this mixture was spread on the NMA-coated slides using micropipette and immediately was covered with coverslips. Slides were preserved in refrigerators at +4 °C for 15 min. The coverslips were slowly removed from top of slides. Then slides were placed in chalets including lysis solution and preserved for 2 h in refrigerator in dark. Slides were washed with chilled distilled water and placed on a horizontal gel electrophoresis unit filled with fresh electrophoretic buffer (0.3 M NaOH + 1 mM EDTA) to allow DNA unwinding before electrophoresis for 20 min. Electrophoresis was conducted at 20 °C using 25 V and 300 mA for 20 min. The above steps should be carried out in dark to avoid DNA damage. After electrophoresis, slides were washed with chilled distilled water and placed in neutralizing buffer (for 5 min). Then again slides were washed with chilled distilled water and placed in chilled ethanol for 10 min. DNA was stained with ethidium bromide (0.1 mg/ml, 1:4) and the slides were examined with a fluorescent microscope (BX51, Olympus, Tokyo, Japan). Each group should be six parallel samples.

Two different parameters were evaluated for genetic damage in comet assay:

- Genetic damage index was calculated as AU values.

The ''Arbitrary units (AU)'' were used to express the extent of DNA damage and calculated following formula:

$$AU = \sum_{i=0}^{4} i \times N_i$$

N_i is the number of scored cells in *i level, i* is the level of DNA damage (0, 1, 2, 3, and 4).

Genetic damage index: [(0 × Type 0) + (1 × Type 1) + (2 × Type 2) + (3 × Type 3) + (4 × Type 4)]

AU values indicating the comet assay scores show levels of UD (undamaged, 0), Type 1 (low damaged, 1), Type 2 (moderate damaged, 2), Type 3 (high damaged, 3), and Type 4 (ultra high damaged, 4).

- Damaged cell percent (DCP): Type 2 + Type 3 + Type 4

The suppression percentage of DNA damage was calculated as for each dose treatment group in above following formula:

$$100 - \left[\frac{\left\{ \begin{array}{c} \text{incidance of DNA damage in} \\ \text{curcumin plus PFOS treated group} \end{array} \right\}}{\left\{ \begin{array}{c} \text{incidance of DNA damage in} \\ \text{PFOS alone treated group} \end{array} \right\}} \times 100 \right]$$

MN assay in peripheral blood

Whole blood smears were collected on the day following the PFOS, PFOS plus curcumin administration, or 1 d after MMC treatment. Whole blood smears were prepared on clean microscope slides, air dried, fixed in methanol, and stained with acridine orange (125 mg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluorescence microscope using a 40 × objective (Holden et al., 1997). The number of MNPCEs was determined using 2000 PCE per animal. Briefly, immature erythrocytes, i.e. PCEs were identified by their orange-red color, mature erythrocytes were identified by their green color, and micronuclei were identified by their yellowish color.

Statistical analysis

All statistical analysis was performed using the SPSS for Windows 17.0 package program (SPSS Inc., Chicago, IL). Micronucleus and comet-related data were controlled for normal distribution by the Shapiro-Wilk test. The Shapiro-Wilk test exhibited that data are not suitable in normal distribution ($p = 0.007$), therefore, the Kruskal–Wallis test was used for statistical calculation. $p < 0.05$ was considered as level of significance.

Results

Table 1 represents micronucleated polychromatic erythrocyte/micronucleus frequency and comet results including genetic damage index, damaged cell percent in peripheral blood rat treated with PFOS, curcumin, and PFOS–curcumin combinations, respectively. Figure 3 represents MN in rat peripheral blood treated by gavage with PFOS *in vivo*.

Statistical analysis shows that there is a significant difference between groups and within groups for MN

Table 1. Micronucleus frequency, genetic damage index (GDI), and damaged cell percent (DCP) in peripheral blood of rats exposed to PFOS–PFOS + curcumin and curcumin combinations by gavage for 4 week at 48 h intervals (mean ± SD).

Treatment groups	Micronucleus frequency, mean ± SD	Genetic damage index values (AU), mean ± SD	Damaged cell percent, mean ± SD
Negative control	1.81 ± 0.23	29.83 ± 8,01	21.83 ± 4.95
PFOS, 0.6 mg/kg	2.15 ± 0.12*	64.83 ± 2.31**	39.33 ± 1.21*
PFOS, 1.25 mg/kg	2.47 ± 0.23**	141.83 ± 17.05***	59.50 ± 4.72**
PFOS, 2.50 mg/kg	2.76 ± 0.14***	197.33 ± 9.37***	74.50 ± 3.93***
PFOS + C, 0.6 mg/kg + 80 mg/kg	2.15 ± 0.18*	45.33 ± 5.57*	30.66 ± 2.25
PFOS + C, 1.25 mg/kg + 80 mg/kg	2.63 ± 0.24***	64.66 ± 5.71**	42.16 ± 2.78*
PFOS + C, 2.50 mg/kg + 80 mg/kg	2.76 ± 0.22***	124.33 ± 4.92***	65.16 ± 2.63**
C, 80 mg/kg	1.86 ± 0.14	35.33 ± 7.31	25.33 ± 3.93
PC MMC, 2 mg/kg	3.58 ± 0.45***	237.00 ± 15.64***	84.83 ± 3.18***

C, curcumin; MMC, mitomycin C; PC, positive control; PFOS, perfluorooctane sulfonate.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

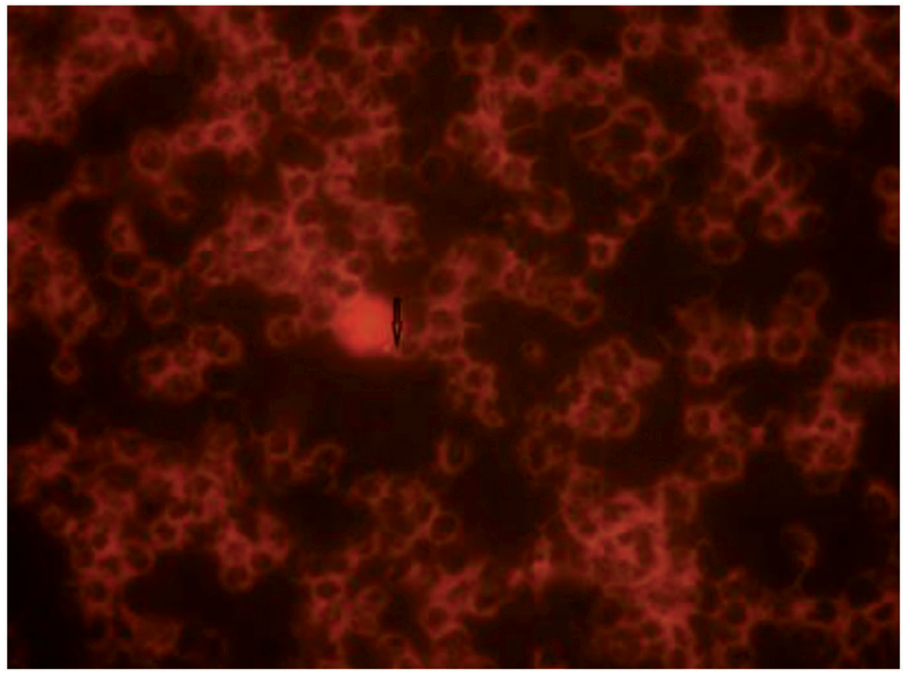

Figure 3. Arrow indicates the MN in rat peripheral blood treated by gavage with PFOS *in vivo*.

frequency and comet values in this study. It was observed that PFOS and PFOS plus Curcumin treatments induced a dose-related increase in MN frequency compared with negative control. There was a linear dose–response relationship between the frequency of micronucleated cells and the concentration of PFOS or PFOS plus curcumin. There is a significant difference between PFOS dose groups/PFOS plus curcumin and negative control for MN frequency. MN frequency reached to 1.81 ± 0.23 in the negative control group and to the 1.86 ± 0.14 in group treated with curcumin. There is no statistically a significant difference between the rat group treated with only curcumin and the control group for MN frequency ($p > 0.05$, $p = 1000$). The MN frequency in the positive control group receiving mitomycin C showed a significant increase compared with the negative control. This finding demonstrates the validity of the experiment and the sensitivity of the animal strain to the clastogenic agents as observed by Krishna & Hayashi (2000).

Table 1 shows AU values (Genetic damage index) indicating the comet assay scores showing levels of UD (undamaged, 0), LD (low damaged, 1), MD (moderate damaged, 2), HD (high damaged, 3), UHD (ultra high damaged, 4), comet figures, and damaged cell percent (DCP) (Type 2 + Type 3 + Type 4). According to the comet analysis, Table 1 represents the results of DNA damages in rat peripheral blood induced by PFOS and PFOS plus curcumin or curcumin and statistical results. All the doses of PFOS and PFOS plus curcumin combinations could induce the DNA damage at different levels, and statistically significant differences were observed compared with negative control ($p < 0.001$) for AU and DCP values. It is also demonstrated that DNA damage increased with the increase of dose of PFOS. Only curcumin treatment did not induce the DNA damage in rat peripheral blood. There is no significant difference between the negative control and the curcumin group for both AU and DCP values. Table 1 shows that curcumin prevented the effects induced by PFOS at all the dose at statistical levels. The dose 0.6 mg/kg of PFOS (64.83 ± 2.3) induced the AU values at statistical level compared with negative control (29.83 ± 8.01). There is a statistical difference between all the PFOS doses and PFOS plus curcumin combinations, 0.6 mg/kg PFOS/0.6 mg/kg PFOS + curcumin, 1.25 mg/kg PFOS/1.25 mg/kg PFOS + curcumin, 2.5 mg/kg PFOS/2.5 mg/kg PFOS + curcumin, respectively, $p < 0.05$, $p < 0.01$, and $p < 0.001$. DCP is 21.83 ± 4.95 in the control group. DCP reached to 39.33 ± 1.21, 59.50 ± 4.72, and 74.50 ± 3.93 in 0.6 mg/kg, 1.25 mg/kg, and 2.5 mg/kg dose groups, respectively. Curcumin decreased the DCP induced by PFOS at all the dose at statistical levels ($p < 0.05$). Comet levels (comets 0–4) are pictured in Figure 4.

The suppression percentage of DNA damage including GDI is 30.07%, 54.41%, and 36.99% for 0.6 mg/kg, 1.25 mg/kg, and 2.5 mg/kg, respectively.

Discussion

To our knowledge, this is first study that demonstrates that PFOS exposure induced genotoxicity and curcumin prevented the negative effects of PFOS in rat peripheral blood study. A carefully controlled animal studies is an advantage in the extrapolation of biological effects to human health safety of many chemicals such as pesticide, organic compounds, and perfluorinated chemicals. In the present study, the protective effects of curcumin on genotoxicity induced by PFOS were evaluated by the MN test and comey assay including in two parameters, such as GDI and DCP. PFOS induced the MN frequency, GDI, and DHI in a dose-dependent manner in rat peripheral blood.

Recently, there has been increasing scientific concern regarding the occurrence and dispersion of perfluorinated chemicals in the environment. Over the past decade, the

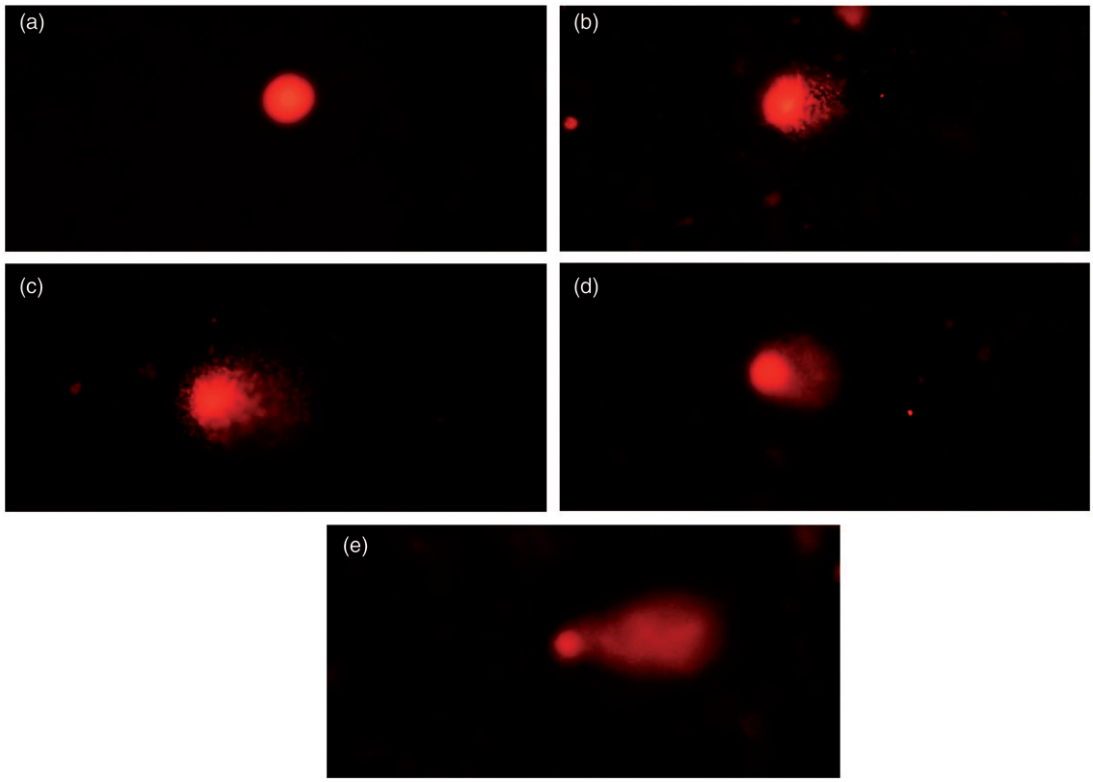

Figure 4. (a) Type 0 non-damaged. (b) Type 1 low damaged comet view. (c) Type 2 moderate damaged comet view. (d) Type 3 highly damaged comet view (e). Type 4 ultra highly damaged comet view.

possible genotoxic effects of PFOS exposure have been investigated in a variety of tissues of organism. Most of the data available in the literature regarding PFOS genotoxicity suggest a lack of effects. However, there is some limited evidence that exposure to PFOS directly causes genetic changes in biological systems via *in vitro* and *in vivo*.

There are *in vitro* studies that have addressed a possible association between exposure to different doses of perfluorinated chemicals such as PFOS and induction of genetic damage in the different cells. Eriksen et al. (2010) investigated the potential of five different perfluorinated chemicals to generate reactive oxygen species (ROS) and to induce oxidative DNA damage in HepG2 cells at a broad concentration range (0.4 μM–2 mM) under *in vitro* conditions. They found that PFOS increased the intracellular ROS production by 1.25-fold, but this increase was not dependent on the concentration. ROS formation may induce the genotoxicity via DNA damage. ROS originating from mitochondrion may be derived through respiratory chain and exist in all aerobic cell under normal condition to balance the metabolism. Alternatively, deficit mitochondrion may produce the increasing levels of ROS, creating a significant threat for major biomolecules primarily lipits, proteins, and DNA (Zorov et al., 2005). Lee et al. (2012) attempted to analyze apoptotic effects of PFOS on developing neuron. Cerebellar granule cells derived from 7-d old Sprague–Dawley rats and grown in culture for additional 7 d were used to mimic postnatal day-14 condition. They indicated that PFOS exposure increased the ROS production and induced caspase-3 activity and nucleosomal DNA fragmentation in a dose-dependent manner. Brieger et al. (2011) explored the impacts of PFOS and perfluorooctanoic acid (PFOA) on selected functions of human leucocytes *in vitro.* They indicated that PFOC exposure leads to natural killer-cell cytotoxicity by inducing a significant decrease of natural killer-cell activity.

Apart from mammalian organisms, there are some evidence that PFOCs, particularly PFOA and PFOS, have toxicological effects in studies performed in aquatic organisms. Kim et al. (2010) evaluated toxicological effects of PFOS toward to common carp, *Cyprinus carpio,* by assessing the responses of five biomarkers including DNA single-strand breaks. On the PFOS exposure, a significant increase in DNA damages were observed while other biomarkers were not significantly altered compared with the control group. Liu et al. (2007) investigated the cellular toxicology of PFOS and PFOA on oxidative stress and induction of apoptosis in primary cultured hepatocytes of freshwater tilapia (*Oreochromis niloticus*). Cultured hepatocytes were exposed to PFOS or PFOA (0, 1, 5, 15, and 30 mg L^{-1}) for 24 h, and a dose-dependent decrease in cell viability was determined using the trypan blue exclusion method. They observed that a significant activation of caspase-3, -8, and -9 activities was evident in both PFOS and PFOA exposure groups. Typical DNA fragmentation (DNA laddering) was further characterized by agarose gel electrophoresis and PFOS and PFOA are able to produce oxidative stress and induce apoptosis with the involvement of caspases in primary cultured tilapia hepatocytes.

Çelik et al. (2013) investigated the effects of PFOS by comet assay for the oxidative DNA damage and by bone marrow micronucleus test for genotoxicity at different doses. They found that PFOS induced DNA damage and

micronucleus frequency. Also they determined that PFOS has toxic effects on the bone marrow decreasing PCE/NCE ratio. In the present study, the comet assay and micronucleus test results show that PFOS exposure induced DNA damage in two parameter, GDI and DCP and MN frequencies at the significant statistical level. The results of the bone marrow genotoxicity study performed by Çelik et al (2013) are on the parallel line with the peripheral blood data obtained from this study.

Bogdanska et al. (2011) investigated the tissue distribution of PFOS in at different tissues of mice. They found that levels of PFOS reached at the 287 nmol/kg in whole blood and also monitored autoradiographically the PFOS in bone marrow. In the present study, presented data show that PFOS may give rise to genotoxicological effects on peripheral blood at DNA damage level and, therefore, supports results of the study performed by Bogdanska et al. (2011).

It is reported that chemicals that give rise to excess ROS production and lipid peroxidation will cause different types of toxicity, including genotoxicity and cell death (Cao et al., 2008). These adverse effects of chemicals can be suppressed by antioxidants, which can eliminate ROS production leading to oxidative stress (Weisburger, 2001). Cheng et al. (2003) have reported that curcumin and other some dietary compounds are beneficial to prevent the harmful adduct formation and to block the potential carcinogenesis induced by nicotin. In our study, all the PFOS doses increased both MN frequency and GDI or DCP (Table 1), but treatment with PFOS + curcumin prevented only this increase in GDI and DCP at a significant level (Table 1). In the present study, curcumin was used at a dose of 80 mg/kg body wt and has been found to inhibit the incidence of PFOS induced DNA damage in rats in a dose-dependent manner, suggesting its potential as an antigenotoxic agent. Balakrishnan et al. (2008) reported that oral treatment with curcumin reverted antioxidants enzymes and status of thiobarbituric acid in hamsters treated with 7–12-dimethylbenz (a) anthrancene. Menon & Sudheer (2007) indicated that the presence of an electron donating group like phenolic hydroxyl group in curcumin was responsible for the free radical scavenging activity.

In our study, curcumin treatment inhibited the DNA damage observed in single-cell gel electrophoresis assay at certain levels in all the doses. This inhibition reached to 30.07%, 54.41%, and 36.99% for 0.6 mg/kg, 1.25 mg/kg, and 2.5 mg/kg, respectively. The highest inhibition effect of curcumin was observed at 1.25 mg/kg dose of PFOS. Shukla et al. (2002) reported that curcumin decreased the percent incidence of DNA damage to 10.90 ± 1.10 and 8.0 ± 1.02, at dose of 100 mg/kg and 200 mg/kg of curcumin, respectively. Earlier studies reported that curcumin inhibits copper, cyclophosphamide, and cisplatin-induced genotoxicity in rats or mice (Corona et al., 2007; Mendonça et al., 2010; Shukla et al., 2002). Iqbal et al (2003) explained that curcumin and other phenolic compounds increased the activity of detoxification enzymes which appear to be crucial protection against the oxidative stress. Results obtained from present study and other studies will set light to studies performed with curcumin and certain compounds and the correlations between curcumin and various chemical may be determined at molecular level.

Conclusion

In this study, it is shown that chronic exposure to PFOS promoted the micronucleus frequency and oxidative DNA damage in rat peripheral blood. Our results support that oxidative stress and genotoxicity are relevant for potential adverse effects of PFOS. Besides, curcumin treatment inhibited DNA damage observed in single-cell gel electrophoresis assay at certain levels in all the doses.

Declaration of interest

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