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The protective role of curcumin on perfluorooctane sulfonate-induced genotoxicity: Single cell gel electrophoresis and micronucleus test

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ABSTRACT

Perfluorooctane sulfonate (PFOS) is a man-made fluorosurfactant and global pollutant. PFOS a persistent and bioaccumulative compound, 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 days at 48 h intervals. Here, we evaluated the DNA damage via single cell gel electrophoresis or comet assay and micronucleus test in bone marrow in vivo. PFOS induced micronucleus frequency and decreased the ratio of polychromatic erythrocyte to normochromatic erythrocyte in bone marrow. Using the alkaline comet assay, we showed that all doses of the PFOS strongly induced DNA damage in rat bone marrow and curcumin prevented the formation of DNA damage induced by PFOS.

1. Introduction

Perfluorinated organic compounds (PFOCs) have been widely used as lubricant paints cosmetic and fire-fighting foams (Kim et al., 2010). Among PFOCs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been detected in environment and variety of living organism worldwide (Kim et al., 2010; Giesy and Kannan, 2002). They are environmentally persistent because these compounds are resistant to hydrolysis, photolysis, microbial degradation and metabolism. Perfluorooctane sulfonate (PFOS), is a man-made fluorosurfactant and global pollutant. Therefore, it was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in May 2009 (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; EPA, 2002).

Curcumin, a natural polyphenolic compound abundant in the rhizome of the perennial herb turmeric, *Curcuma longa*, is known to possess comprehensive anti-inflammatory and anti-cancerous properties following topical or oral administration. It is commonly used as a dietary spice and coloring agent in cooking and anecdotally as an herb in traditional Indian and Chinese medicine (Miquel et al., 2002; Maheshwari et al., 2006). Curcumin has been shown to act as an antioxidant through modulation of glutathione (GSH) levels (Biswas et al., 2005). Curcumin is the most active component of turmeric, a botanical agent derived from the dried rhizome of the turmeric plant (*C. longa*), a perennial herb belonging to the ginger family that is cultivated extensively in south and southeast tropical Asia. Turmeric'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 and Wahl, 1991).

The micronucleus assay is a mutagenic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments, i.e. micronuclei in the cytoplasm of interphase cells. Micronucleus test has been used to evaluate the exposure to chemicals for decades in many investigations in in vitro and in vivo studies in different organism (Çelik et al., 2003, 2005; Çelik and Kanık, 2006; Çavaş, 2011).

The single-cell gel electrophoresis assay (comet assay) is capable of detecting DNA damage with great sensitivity and has been used widely both in vitro and in vivo protocols to identify potentially environmental genotoxins (Tsuda et al., 2000). 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 (Narendra, 2000; Çavaş, 2011).

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

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sample (<10,000) (c) sensitivity for detecting DNA damage and (d) use of any eukaryote single cell population both in vitro and in vivo, including cells obtained from exposed human populations and aquatic organisms for eco-genotoxicological studies and environmental monitoring (Collins et al., 1997).

In the present study, we aimed; (1) to evaluate the genotoxic and cytotoxic effects of PFOS at three different doses in rat bone marrow using micronucleus test system, single gel electrophoresis/comet assay and determining polychromatic erythrocyte (PCE)/normochromatic erythrocyte (NCE) ratio, respectively, (2) to evaluate the protective effects of curcumin against damages occurred by PFOS.

2. Materials and methods

2.1. Chemicals

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

2.2. 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, 2002). Therefore, in this study, the highest dose was set at 2.50 mg/kg body wt, i.e. 1% of the LD50 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 and Wahl, 1991).

Mitomycin C (MMC) (2 mg/kg), a single i.p. dose, was used as a positive control in this study. 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.

2.3. Animal and experimental design

Healthy adult female Swiss albino rats (Wistar rat) [6–8 weeks of age and average body weight (body wt) of 180–200 g] were used in this study. 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 ± 2 °C temperature and 50–70% humidity with free access to pelleted feed and fresh tap water. The animals were allowed to acclimate for 14 days before treatment. In this study, rats were divided to nine groups including six rats. Therefore, the study was performed by nine different diet regimen one dose per 48 h for 4 weeks (~30 days).

The rats were treated by gavage with

(1) 0.6 mg/kg body wt only PFOS dissolved in saline.
(2) 1.25 mg/kg body wt only PFOS dissolved in saline.
(3) 2.50 mg/kg body wt only PFOS dissolved in saline.
(4) 0.6 mg/kg dose of PFOS (dissolved in saline) with curcumin (80 mg/kg body wt).
(5) 1.25 mg/kg dose of PFOS (dissolved in saline) with curcumin (80 mg/kg) body wt).
(6) 2.50 mg/kg dose of PFOS (dissolved in saline) with curcumin (80 mg/kg body wt).
(7) The rats were given only curcumin (80 mg/kg body wt).
(8) The untreated control rats were treated identically with equal volumes of normal saline only via gavage throughout the study.

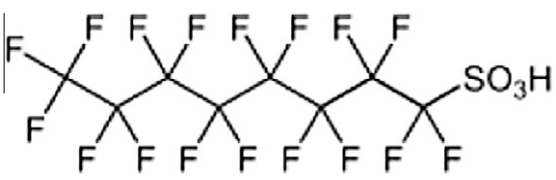
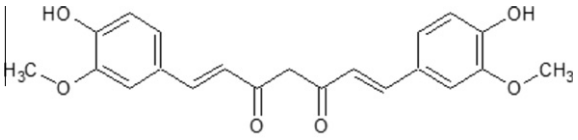


Fig. 1. Chemical structure of perfluorooctane sulfonate.



Curcumin

Fig. 2. Chemical structure of curcumin.

(9) Since positive controls may be administered by a different route and treatment schedule than the test agent (Hayashi et al., 1994) a single dose of MMC (2 mg/kg, i.p.) was administered at the 16th week dosing time.

2.4. Comet assay in bone marrow

The two femurs were removed and bone marrow cells were flushed from the femur into 1 ml of fetal bovine serum (FBS). Isolated bone marrow 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 covered by a thin layer of 0.5% normal melting agarose (NMA) at about 50 °C (dissolved in $Ca^{2+}$and Mg^{2+} free phosphate buffer saline (PBS). Eppendorf tubes were placed in water bath at 40 °C. One hundred microliters of bone marrow 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 spreaded on NMA-coated slides using micropipette and immediately was covered with coverslip. 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 1.5 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 (300 mM NaOH + 1 mM EDTA) to allow DNA unwinding before electrophoresis for 20 min Electrophoresis was conducted at 20 °C using 25 V and 185 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 (0.048 g/ml) (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, Japan). Each group should be six parallel samples.

2.5. Bone marrow micronucleus test

Rats were anaesthetized with Ketalar (Ketamine-HCl, Pfizer, Istanbul), 30 h after the last treatment and sacrificed. The frequency of micronucleated erythrocytes in femoral bone marrow was evaluated according to the procedure of Schmid (1973), with the slight modifications of Agarwal et al. (1994). The bone marrow was flushed out from both femora using 1 ml of fetal calf serum and centrifuged at 336 g for 10 min and the supernatant was discarded. Bone marrow smears were prepared on clean microscope slides, air dried, fixed in methanol and stained with acridine orange (125 μg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluorescence microscope (Olympus BX51) using a 40× objective.

2.6. Scoring of micronucleus frequency

The frequency of PCEs per total erythrocytes was determined using a sample size of 200 erythrocytes per animal. 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 by their green color and micronuclei by their yellowish color.

2.7. Scoring of comet images

Comet images were analyzed according to Collins et al. (1995). One hundred comet images were scored for each treatment by two scorers (S.Y.E. and S.Y.) visually under fluorescence microscopy (BX51 Olympus). An intensity score from class 0 (undamaged) to class 4 (ultra high damage) (Sun et al., 2004) was assigned to each cell. The method of the observation was barred in a blind way during which the observer had no knowledge of the identity of the slide.

2.8. Statistical analysis

The "arbitrary units (AU)" was used to express the extent of DNA damage and calculated following formula.

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

N_i = the number of scored cell in i level, i = the level of DNA damage (0–4).

Data were compared by one-way variance analysis. All statistical analysis was performed using the SPSS for Windows 11.5 package program. Multiple comparisons were performed by Tukey test. $P < 0.05$ was considered as level of significance.

3. Results

Tables 1 and 2 represent the frequencies of micronucleated polychromatic erythrocyte and the ratio of polychromatic erythrocytes to normochromatic erythrocytes (the number of polychromatic erythrocytes within total erythrocytes) in rat bone marrow, respectively. Table 3 indicates the comet levels of control group and groups treated with PFOS, curcumin and PFOS–curcumin combinations in rat bone marrow. Fig. 3, presents the micronucleated polychromatic erythrocytes (a) and comet assay views (b).

One-way variance analysis shows that there is a significant difference between groups and within groups for MN frequency, PCE and AU values in this study. It was observed that PFOS and PFOS plus curcumin treatments induced a dose-related increase in MN frequency (Table 1). There was a linear dose–response relationship between the frequency of micronucleated cells and the concentration of PFOS (Fig. 4). The results of the MNPCE analysis indicate that there was statistically a significant difference between negative control and all the concentration of PFOS ($p < 0.01$ or $p < 0.001$). MN frequency reached to 1.83 ± 0.16 in control group and to 1.66 ± 0.21 in group treated with curcumin. There is no statistically a significant difference between rat group treated with only curcumin and control group for MN frequency ($p > 0.05$, $p = 1{,}000$). There is no significant difference between the administration of 0.6 mg/kg of PFOS and negative control ($p = 0.05$) and between the administration of 0.6 mg/kg of PFOS plus curcumin and negative control ($p = 0.894$). The statistical difference between the administration of 1.25 mg/kg of PFOS plus curcumin and control reached at level of $p < 0.01$. It is not found the significant difference between the administration of 2.5 mg/kg of PFOS and the administration of 2.5 mg/kg of PFOS plus curcumin. MMC administered intraperitoneally as positive control induced a statistically significant increase in the mean number of cells with micronuclei ($p < 0.05$).

The cytotoxic effects of PFOS, curcumin and their combinations were evaluated by the determination of PCEs in total erythrocytes (PCEs and NCEs) in cytogenetic assays, such as the in vivo MN test on rat bone-marrow. Regarding the cytotoxicity index in all cytogenetic end-points considered, statistically significant differences were observed between controls and PFOS, and PFOS–curcumin combinations treated animals (Table 2) ($p < 0.001$). Only, there is no significant difference between curcumin and negative control. There was a linear dose–response relationship between the frequency of micronucleated cells and the concentration of PFOS (Fig. 4). The PCE/NCE ratio was statistically different from the negative control in all the PFOS and PFOS plus curcumin treatments ($p < 0.001$).

Table 3 shows AU values 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. According to comet analysis, Table 3 represents the results of DNA damages in rat bone marrow induced by PFOS and PFOS plus curcumin or curcumin and statistical results. All the doses of PFOS and PFOS plus curcumin combinations could induce DNA damage at different levels, and a statistically significant difference was observed comparing with control ($p < 0.001$). It is also demonstrates that DNA damage increased with the increase of dose of PFOS. Only curcumin treatment did not induce the DNA damage in bone marrow. Even, DNA damage induced by curcumin is at lower level than induced by control.

The number of MNPCEs in the positive control group receiving mitomycin C showed a significant increase compared to 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 and Hayashi (2000).

4. Discussion

This study is the first in vivo genotoxicity assessment for the PFOS and curcumin combination in bone marrow of Wistar rats. The fundamental similarities in cell structure and biochemistry between animals and humans provide a general valid basis for prediction of likely effects of chemicals on the human population (Meyer, 1993). An advantage of animal studies is that they provide a complete biological system which can evaluate the overall effect of subtle changes observed in cell systems. Carefully controlled animal studies are an essential step in the extrapolation of biological effects to human health safety.

In the present study, DNA damage levels were evaluated via two different assays. The first step was carried out using "COMET" the assay which measures low levels of DNA damage such as strand breaks, in the second, cytogenetic assay were performed to measure clastogenic/aneugenic effects using micronucleus assay/PCE ratio cytotoxicity test. Our results indicated that PFOS had genotoxic effects on rat bone marrow cells at chronic exposure. PFOS induced a dose-dependent increase in the frequency of micronucleated PCEs (Fig. 4), which indicated that chromosome breaks occurred in rat bone marrow cells after treatment and decreased the PCE/total erythrocytes ratio (Fig. 5). Also, in the SCGE assay a significant increase in AU values was observed in bone marrow cells (Fig. 6) exposed to PFOS, which indicated that PFOS induced remarkable DNA strand breaks in rat bone marrow cells.

Table 1
Micronucleus induction in bone marrow of rats exposed to PFOS, PFOS + curcumin and curcumin combination (MNPCE/2000 PCE) by gavage.

Rat number/ treatments	Negative control	PFOS 0.6 mg/kg	PFOS 1.25 mg/kg	PFOS 2.50 mg/kg	PFOS + C 0.6 + 80 mg/kg	PFOS + C 1.25 + 80 mg/kg	PFOS+C 2.50 + 80 mg/kg	C 80 mg/kg	PC MMC 2 mg/kg
1	1	3	3	6	2	3	4	1	22
2	2	3	4	5	2	4	4	2	20
3	2	3	4	4	2	3	5	2	20
4	2	3	4	5	3	3	4	1	21
5	2	3	3	5	2	3	6	2	19
6	2	3	3	4	3	3	5	2	20
Mean ± S.E.	1.83 ± 0.16	3.00 ± 0.00	3.50 ± 0.22[b]	4.83 ± 0.30[b]	2.33 ± 0.21	3.16 ± 0.16[a]	4.66 ± 0.33[b]	1.66 ± 0.21	20.3 ± 0.42[c]

PFOS: perfluorooctane sulfonate, C: curcumin, MNPCE: micronucleated polychromatic erythrocyte, PCE: polychromatic erythrocyte.
[a] $p < 0.05$ Compared with negative control.
[b] $p < 0.01$ Compared with negative control.
[c] $p < 0.001$ Compared with negative control.

Table 2
The number of PCEs in bone marrow of rats exposed to PFOS, PFOS + curcumin combination and curcumin (PCE/200 total erythrocytes) by gavage.

Rat number/ treatments	Control	PFOS 0.6 mg/ kg	PFOS 1.25 mg/ kg	PFOS 2.50 mg/ kg	PFOS + C 0.6 + 80 mg/ kg	PFOS + C 1.25 + 80 mg/ kg	PFOS+C 2.50 + 80 mg/ kg	C 80 mg/ kg	PC MMC 2 mg/ kg
1	100	92	80	72	95	90	78	100	47
2	101	93	80	75	91	85	85	101	45
3	98	88	75	69	90	85	85	98	46
4	99	86	75	72	92	83	82	99	40
5	98	85	76	74	88	81	81	98	44
6	102	90	77	73	91	80	80	102	45
Mean ± S.E.	101.1 ± 1.10	89.0 ± 1.31[a]	77.1 ± 0.94[a]	72.5 ± 0.84[a]	93.0 ± 0.93[a]	88.1 ± 1.19[a]	81.8 ± 1.13[a]	99.6 ± 0.66	44.5 ± 0.99[a]

PFOS: perfluorooctane sulfonate, C: curcumin, PCE: polychromatic erythrocyte.
[a] $p < 0.001$ Compared with negative control.

Table 3
Comet assay scores in bone marrow of rats exposed to PFOS, PFOS + curcumin combination and curcumin by gavage (mean ± S.E.).

	0	1	2	3	4	Mean arbitrary units ± S.E.
Control	45.3 ± 0.88	38.0 ± 0.57	9.50 ± 0.84	4.00 ± 0.63	3.16 ± 0.47	81.6 ± 3.35
PFOS (0.6 mg/kg body wt)	35.8 ± 1.27	36.3 ± 0.76	17.1 ± 0.60	8.83 ± 0.40	1.83 ± 0.60	104.5 ± 2.94**
PFOS 1.25 mg/kg body wt)	26.0 ± 0.57	33.5 ± 1.14	21.0 ± 0.63	12.3 ± 0.61	7.16 ± 0.74	139.5 ± 3.34***
PFOS 2.50 (mg/kg body wt)	19.3 ± 1.33	34.1 ± 2.73	22.0 ± 1.34	14.6 ± 0.61	9.83 ± 1.47	161.5 ± 6.92***
PFOS + C (0.6 + 80 mg/kg body wt	55.3 ± 1.35	31.6 ± 2.06	9.50 ± 1.11	3.16 ± 0.47	0.33 ± 0.21	61.5 ± 2.51
PFOS + C(1.25 + 80 mg/kg body wt)	32.6 ± 2.18	55.3 ± 1.45	8.00 ± 0.57	3.33 ± 0.88	0.66 ± 0.33	84.0 ± 4.16
PFOS + C(2.50 + 80 mg/kg body wt	34.5 ± 3.55	41.6 ± 1.28	18.0 ± 2.48	4.33 ± 0.42	1.50 ± 0.42	96.6 ± 7.33
C (80 mg/kg body wt)	46.1 ± 3.46	47.3 ± 2.02	6.66 ± 1.20	0.66 ± 0.33	0.00 ± 0.00	63.6 ± 3.95*
PK (MMC 2 mg/kg)	11.0 ± 0.73	19.1 ± 2.52	27.0 ± 1.03	23.1 ± 1.32	18.0 ± 1.65	226.1 ± 5.50***

Data were expressed as mean ± S.E. PFOS; perfluorooctane sulfonate, C; curcumin.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

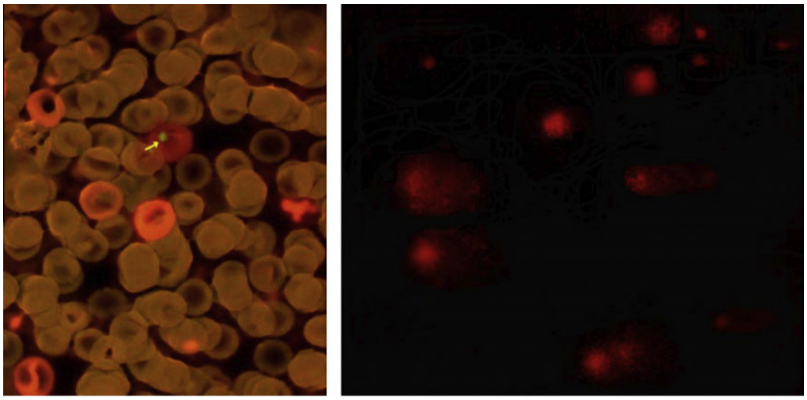


Fig. 3. Acridine orange stained-micronucleated polychromatic erythrocytes (a) and ethidium bromide stained-comet views (b).

Recently, there is been increasing scientific researches regarding the occurrence and dispersion of perfluorinated chemicals in environment. Although there are many epidemiological studies related with toxicity of these compounds to humans is far less conclusive. Bogdanska et al. (2011) researched the tissue distribution of PFOS in at several different tissues of mice. They found that levels of PFOS reached at the 287 nmol/kg in whole blood including bone marrow (femora and tibia) and monitorized autoradiographically the PFOS in bone marrow. In our study, we determined that PCE/NCE ratio decreased in all the PFOS doses. These data shows that PFOS may give rise to toxicologic effects on bone marrow at cytotoxic basic and supports results of study performed by Bogdanska et al. (2011).

Fernández Freire et al. (2008) investigated cytotoxic and mutagenic potential of perfluorooctanoic acid (PFOA) and they found that PFOA was not mutagenic in Ames test in presence and absence of rat S9 metabolic activation. It is found that the high concentration of PFOA caused oxidative stress in Vero cells. It is determined that the formation of oxidative stress was linked to cell cycle at G1 phase and induction of apoptosis pathway. Yao and Zhong (2005) investigated the genotoxic effects of perfluorooctanoic acid (PFOA) on HepG2 cells using alkaline comet assay. They found that PFOA caused a concentration-dependent increase in the level of strand breaks and PFOA exerts genotoxic effects on HepG2 cells, probably through oxidative DNA damage induced by intracellular ROS. Apart from mammalian organisms, there are some evidences that PFOCs, particularly PFOA and PFOS, have toxicological effects in studies performed in aquatic organisms. Kim et al. (2010) evaluated toxicological effects of PFOA and PFOS toward common carp, *Cyprinus carpio*, by assessing the responses of five biomarkers including

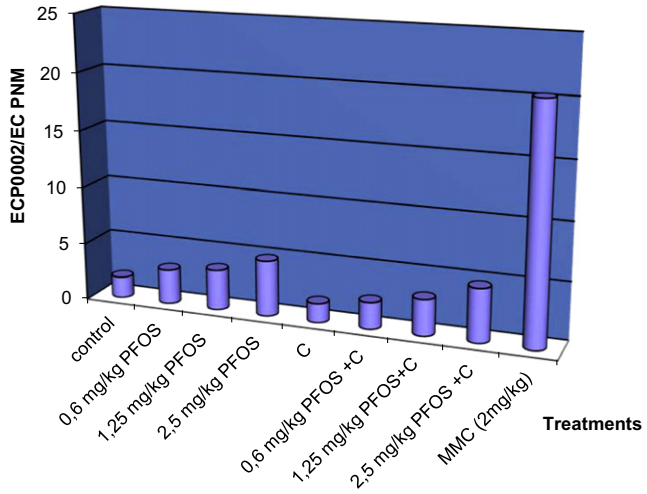


Fig. 4. The frequency of micronucleated polychromatic erythrocytes in rat bone marrow exposed PFOS, curcumin and PFOS + curcumin. MNPCE: micronucleated polychromatic erythrocyte; C: curcumin; MMC: mitomycin C; PFOS: perfluorooctane sulfonate.

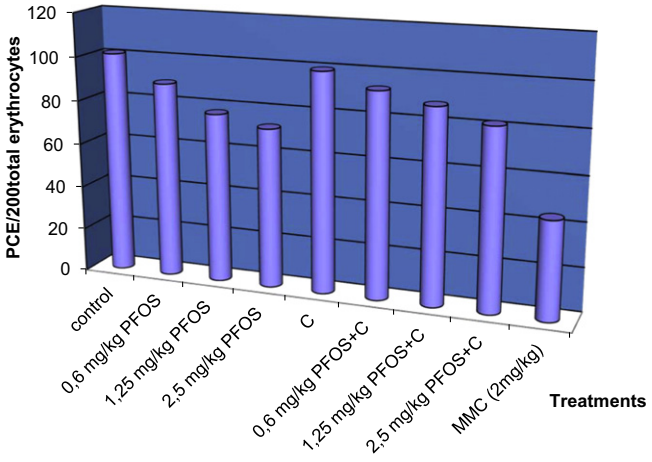


Fig. 5. The number of polychromatic erythrocytes in 200 total erythrocytes in rat bone marrow exposed PFOS, curcumin and PFOS + curcumin. PCE: polychromatic erythrocyte; C: curcumin; MMC: mitomycin C; PFOS: perfluorooctane sulfonate.

DNA single-strand breaks. On the PFOS exposure, a significant increase in DNA damages were observed while other biomarkers were not significantly altered compared the control group. PFOS-induced apoptosis has been reported in many cell types in in vivo and in vitro studies performed by many researchers. Lee et al. (2012) investigated the effect PFOS on cerebellar granule cells prepared from the cerebella of 7 days old Sprague–Dawley (SD) rat pups. They found that PFOS may induced apoptosis of cerebellar granule cells via on ROS-mediated PKC signal transduction pathway is pivotal in learning and memory and apoptosis of neuronal cells is a critical event in neurotoxicity and they reported that PFOS increased caspase-3 activity and nucleosomal DNA fragmentation in a dose dependent manner. Also Fang et al. (2010) reported that perfluorononanoic acid induced apoptosis in rat spleen increasing oxidative stress and determined the activation of caspase-independent death pathway. They indicated the decrease in superoxide dismutase (SOD) activity. In other study performed in primary cultured hepatocytes of freshwater tilapia (*Oreochromis niloticus*), Liu et al. (2007) reported that PFOS lead to significant induction of

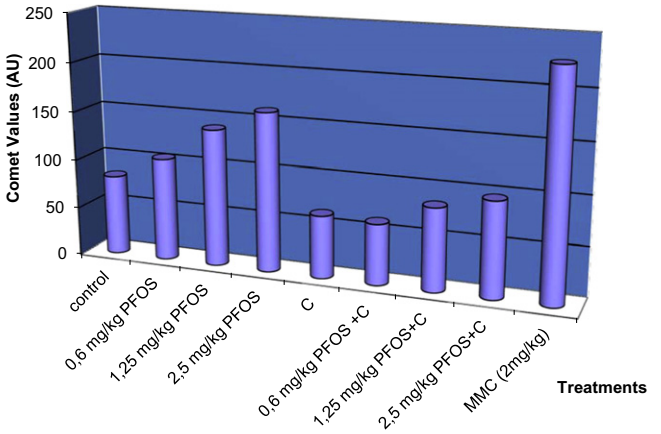


Fig. 6. DNA damage values (AU = arbitrary units) in rat bone marrow exposed PFOS, Curcumin and PFOS + Curcumin. C: curcumin; MMC: mitomycin C; PFOS: perfluorooctane sulfonate.

reactive oxygen species (ROS) accompanied by increases in activities of antioxidant enzymes such as SOD, catalase (CAT) while activities of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were decreased. Besides, they found a dose-dependent increase in lipid peroxidation level. On the basis of results obtained in the present study, comet assay thus clearly indicated that PFOS played a significant genotoxic role on the bone marrow of male rats. Therefore, these findings seem to support results obtained from studies performed by Lee et al. (2012), Fang et al. (2010) and Liu et al. (2007).

Curcumin is a well-known antioxidant (Chattopadhyay et al., 2004). The antioxidant mechanism of curcumin is attributed to its unique conjugated structure that includes two methoxylated phenols (Masuda et al., 2001). Curcumin inhibits the generation of reactive oxygen species (ROS) that are responsible for the DNA and membrane damage (Joe and Lokesh, 1994). Siddique et al. (2010) investigated the effect of curcumin against the anticancerous drug, i.e. mitomycin C and found that the concentrations of curcumin used in the present study were not genotoxic itself and reduced the genotoxic effect of mitomycin C. They found that MMC with curcumin treatments decreased at a certain level the DNA damage compared only MMC treatment. It has previously been shown that both curcumin is effective antioxidants and has contributory roles in protecting cells from oxidative stress and resulting DNA damage (Balu et al., 2006; Sharma et al., 2005). It is thought that the effectiveness of this compound as free radical scavenger can be directly attributed to its structural characteristics. The phenolic and methoxy groups on the curcumin benzene rings and the 1,3-diketone system are thought to be important structures for effective oxygen free radical scavenging (Sreejayan and Rao, 1996, 1997).

In this study, all the doses of PFOS induced the micronucleus formation in bone marrow of rats. However, the treatment with all the doses of PFOS with curcumin results in the increase of PCE, decrease of micronucleus frequency and DNA damage. Thus, curcumin has a protective role against the damage induced by PFOS in bone marrow. It is shown that the 80 mg/kg concentration of curcumin used in the present study was not genotoxic and prevented the genotoxic effect induced by PFOS. We chose to study with curcumin, because of its antioxidant properties indicated by many studies compared to the other phenolic compounds (Sreejayan and Rao, 1994). Ak and Gülçin (2008) investigated the antioxidant and radical scavenging capacity of curcumin by employing various in vitro antioxidant assay. They found that curcumin lead to inhibition on lipid peroxidation at

97.3% ratio. In this study, the administration of curcumin, or curcumin + 0.6 mg/kg of PFOS per se did not produce any significant change in the parameters investigated when compared to controls. The results obtained by the treatment with curcumin showed a significant reduction in the total micronucleus frequency and in the Comet values (AU) induced in bone marrow cells by PFOS. Curcumin reduces PFOS-induced DNA damage in Wistar rat bone marrow cells under the present experimental conditions. It indicates to activate preserving against physiological the DNA damage that even single curcumin treatment fairly decreased level of DNA damage. Data obtained from our study and other studies may be useful to evaluate the interaction between curcumin and certain compounds.

According to the results of this study, PFOS having a potential genotoxic character caused significant DNA damage in bone marrow tissue of male rats. The supplementation of curcumin significantly antagonized the PFOS-induced genotoxic effect and DNA damage as evident from the observations. The more research is needed to improve our understanding of what determines people's food choices in those northern regions where exposure levels are of concern to public health authorities, and to what extent contaminants are factors in these choices. Particular reference needs to be made to mothers, pregnant women, other women of childbearing age, and children. To complement this, research is needed to evaluate food substitution and other management programs that reduce contaminant exposure in high exposure regions but still encourage consumption of highly valued traditional/country foods and other nutritional food sources.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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