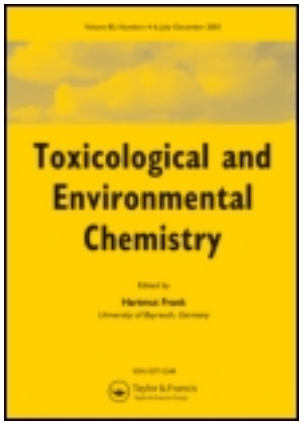

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Protective effects of curcumin on antioxidant status, body weight gain, and reproductive parameters in male rats exposed to subchronic 2,3,7,8-tetrachlorodibenzo-p-dioxin

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Protective effects of curcumin on antioxidant status, body weight gain, and reproductive parameters in male rats exposed to subchronic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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The aim of this study was to investigate the effects of curcumin (CUR) on antioxidant status, body weight (BW) gains, and some reproductive parameters in male rats exposed to subchronic doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Thirty-two rats were divided into four groups. The first group was kept as control. The second group (TCDD group) was given TCDD at a dose of 50 $ng \cdot kg^{-1}$ BW per day; the third group (CUR group) was treated with CUR at a dose of 80 $mg \cdot kg^{-1}$ BW per day. The fourth group (TCDD + CUR group) was given TCDD and CUR at the same doses simultaneously. Malondialdehyde (MDA) levels were significantly increased in the TCDD group. In addition, TCDD exposure decreased liver superoxide dismutase (SOD) activity, catalase (CAT) activities of kidney and brain, glutathione peroxidase (GSH-Px) activities of liver, kidney, and brain, and glutathione levels of liver, kidney, and heart. However, CUR treatment with TCDD exposure decreased MDA levels in all tissues and increased SOD activities of liver, kidney, and brain, CAT activity of heart, and GSH-Px activities of heart and brain. TCDD caused a decrease in BW gain, and CUR partially eliminated this effect of TCDD. In addition, while reproductive organ weights, sperm concentration, and sperm motility tended to decrease with TCDD exposure, these effects tended to be close to normal levels by CUR treatment. In conclusion, CUR was seen to be effective in the treatment and prevention of toxicity induced by subchronic TCDD exposure.

Keywords: curcumin; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; antioxidant status; body weight gain; reproductive parameters

Introduction

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic congener among the polychlorinated dibenzo-*p*-dioxins, which are widespread, persistent and highly toxic environmental pollutants, and TCDD has been widely investigated as a model compound of this class of chemicals (Ishida et al. 2004; Niittynen, Tuomisto, and Pohjanvirta 2008). Prolonged exposure to TCDD may result in a wide variety of adverse health effects in laboratory animals and in humans, including wasting syndrome, dermal toxicity, reproductive toxicity, immunotoxicity, neurotoxicity, hepatotoxicity, teratogenesis, and carcinogenesis (National Toxicology Program 2006).

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There are many studies on the toxicity of TCDD, but the mechanism is not fully understood. It has been reported that nearly all major toxic effects of dioxins are mediated by the specific binding of TCDD to a cytosolic protein, the aryl hydrocarbon receptor (AhR) (Umannova et al. 2008). The interaction of TCDD with the AhR and the subsequent activation of the cytochrome P450 (CYP) 1A and 1B subfamilies contribute significantly to the formation of reactive oxygen species (ROS) and promotion of liver tumors (Moennikes et al. 2004). It has been demonstrated that oxidative stress occurs in various tissues of TCDD-treated animals, and it is considered an important mechanism in the TCDD toxicity (Hassoun et al. 2000; Slezak et al. 2000). Oxidative stress following TCDD exposure in laboratory animals has been demonstrated to increase the production of ROS, lipid peroxidation, DNA and membrane damage, and possibly enzyme inhibition (Shertzer et al. 1998; Slezak et al. 2000). In addition, TCDD causes reduced fertility, delayed puberty, and reduced testicular weight in the reproductive system of male rats (Gray, Ostby, and Kelce 1997; Latchoumycandane, Chitra, and Mathur 2002). TCDD-induced oxidative stress also decreases sperm count (Latchoumycandane, Chitra, and Mathur 2002) and increases the number of abnormal sperm (Faqi et al. 1998). In addition, studies on humans who were accidentally exposed to TCDD have reported a decrease in sperm quality (Mocarelli et al. 2008).

Curcumin (CUR) is a major yellow-orange pigment derived from the rhizomes of turmeric (*Curcuma longa*), which is widely used as a spice and food-coloring agent and in cosmetics and drugs (Joe, Vijaykumar, and Lokesh 2004). CUR exhibits a wide spectrum of biological activities, including anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive, hypocholesterolemic, and cardioprotective activities (Chattopadhyay et al. 2004; Kurup and Barrios 2008). However, it is unclear whether the antioxidant activity of CUR is the basis for most of its reported biological activities (Weber et al. 2005). Studies have shown CUR to be a powerful scavenger of nitrogen dioxide, the superoxide anion, and the hydroxyl radical and to inhibit lipid peroxidation, thereby protecting cellular macromolecules (including DNA) from oxidative damage (Sreejayan and Rao 1994; Eybl, Kotyzova, and Bludovska 2004).

The aim of this study was to investigate the possible protective effects of CUR on antioxidant status, body weight (BW) gain, and several reproductive parameters such as reproductive organ weight and sperm concentration and motility in male rats exposed to subchronic TCDD.

Material and methods

Chemicals

TCDD was purchased from AccuStandard (New Haven, CT, USA). CUR, glutathione (GSH), GSH-reductase, thiobarbituric acid (TBA), sodium bicarbonate, formalin, eosin, phosphotungstic acid, hydrogen peroxide, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide phosphate (NADPH), and other reagents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Animals and experimental design

The animal use protocol was approved by the Institutional Review Board of the National Institute of Health and Local Committee on Animal Research. The animals were obtained

from Fırat University, Experimental Research Centre (Elazığ, Turkey) and were housed under standard laboratory conditions (temperature 24 ± 3°C, humidity 40–60%, a 12-h light:12-h dark cycle). A commercial pellet diet (Elazığ Food Company, Elazığ, Turkey) and fresh drinking water were given *ad libitum*. The study was approved by the Experimental Animals Ethics Committee of Fırat University (report no: 2006/13).

Thirty-two healthy adult male Sprague-Dawley rats (6–8 weeks old) were used in this study. The rats were divided into four groups containing eight rats each. All treatments were applied by gavage as an emulsion with 0.5 mL corn oil. The experiment ran for 13 weeks.

Experimental groups were arranged as follows:

Group 1 (control) : 0.5 mL corn oil.
Group 2 (CUR) : Daily dose of 80 $mg \cdot kg^{-1}$ BW CUR.
Group 3 (TCDD) : Daily dose of 50 $ng \cdot kg^{-1}$ BW TCDD.
Group 4 (TCDD + CUR) : Daily doses of 50 $ng \cdot kg^{-1}$ BW TCDD and 80 $mg \cdot kg^{-1}$ BW CUR.

Biochemical assays (MDA, GSH levels, and CAT, SOD, and GSH-Px activities)

The rats were decapitated under slight ether anesthesia at the end of the experiment. The heart, liver, kidney, and brain were removed immediately and stored at −20 °C until the biochemical analyses.

The homogenization of tissues was carried out in a Teflon-glass homogenizer (B. Braun 853022, Germany) with a buffer containing 1.15% KCl to obtain a 1:10 (w/v) whole homogenate. Malondialdehyde (MDA) levels were directly measured in the homogenates. The homogenates were centrifuged (Hettich Universal 320R, Germany) at 2500 × *g* for 45 min before determining GSH concentrations and glutathione peroxidase (GSH-Px) and catalase (CAT) activities. The obtained supernatants were then centrifuged again at 2500 × *g* for 45 min before determining the superoxide dismutase (SOD) activities.

The MDA concentration was determined according to a modified method from Ohkawa, Ohishi, and Yagi (1979) based on reaction with TBA and expressed as $nmol \cdot g^{-1}$ protein. The tissue GSH concentration was measured using a kinetic assay with the dithionitrobenzoic acid recycling method described by Elman (1959) and was expressed as $nmol \cdot mg^{-1}$ protein. The GSH-Px activity was determined by the procedure described by Beutler (1975). The analysis performed was based on the oxidation of reduced GSH by GSH-Px coupled to the disappearance of NADPH by GSH-reductase measured at 37 °C and 340 nm, and the activity was expressed as units per gram protein (U g^{-1} protein). The CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (1984), and was expressed as k (U g^{-1} protein), where k is the first-order rate constant. SOD activity was determined according to the method of Sun, Oberley, and Li (1988), which is based on the inhibition of NBT reduction by the xanthine–xanthine oxidase system as a superoxide generator. SOD activity was also expressed as U g^{-1} protein. Protein concentrations were measured according to Lowry et al. (1951).

Evaluation of sperm parameters

The testes, epididymides, seminal vesicles, and prostate glands were removed, cleared of adhering connective tissue, and weighed.

The epididymal sperm concentration was determined with a hemocytometer using a modified method described by Türk et al. (2007). The right epididymis was finely minced using anatomical scissors in 1 mL of isotonic saline in a Petri dish, completely squashed with tweezers for 2 min, and then allowed to incubate at room temperature for 4 h for the migration of all spermatozoa from the epididymal tissue to the fluid. After incubation, the epididymal tissue–fluid mixture was filtered via a strainer to separate the supernatant from the tissue particles. The supernatant fluid containing all epididymal spermatozoa was drawn into a capillary tube up to the 0.5 line of a pipette designed for counting red blood cells. A solution containing 0.595 mol·L^{-1} sodium bicarbonate, 1% formalin, and 0.025% eosin was pulled into the bulb up to the 101 line of the pipette. The contents of the pipette were mixed by holding the ends of the pipette between the thumb and the index finger and shaking it vigorously in 100 back-and-forth 30-cm movements. The bulb of the pipette contains a small glass bead that makes thorough mixing possible. Sufficient solution was then blown from the pipette to ensure that the diluents containing no sperm were flushed from the capillary, resulting in a dilution rate of 1:200. Approximately 10 μL of the diluted sperm suspension was then transferred to both counting chambers of the improved Neubauer (Deep 1/10 mm, LABART, Darmstadt, Germany) and allowed to stand for 5 min. The sperm cells in both chambers were counted using a light microscope at 200× magnification.

The percentage of forward progressive sperm motility was evaluated using a light microscope with a heated stage as described by Sönmez, Türk, and Yüce (2005). For this process, a slide was placed on a light microscope with a heated stage that had been warmed to 37°C, several droplets of Tris buffer solution [0.3 mol L^{-1} Tris (hydroxymethyl) aminomethane, 0.027 mol L^{-1} glucose, 0.1 mol L^{-1} citric acid] were dropped on the slide, and a very small droplet of fluid obtained from left cauda epididymis was pipetted into the Tris buffer solution and mixed with the coverslip. The percentage of forward progressive sperm motility was evaluated visually at 400× magnification. Motility estimates were performed from three different fields in each sample. The mean of three successive estimations was used as the final motility score.

Statistical analysis

All values are presented as the mean ± SEM. Differences were considered to be significant at $P < 0.05$. One-way analysis of variance and *post hoc* Tukey-HSD (honestly significant difference) tests were used to determine the differences between the groups. All of the analyses were carried out using the SPSS/PC (Version 10.0; SPSS, Chicago, IL) software package program.

Results

BW gains

At the end of the study, a BW gain of 34% was observed in the control group compared to the weight at the beginning of the study, while a lower weight gain of 24% was observed in the TCDD group. In the CUR-administered group, BW gain was higher compared to the control group (39%). In the TCDD + CUR group, BW gain was higher compared to the TCDD group (29%).

MDA, GSH levels, and CAT, SOD, and GSH-Px activities of liver, kidney, heart, and brain tissues

Statistical evaluations of the MDA and GSH levels and the CAT, SOD, and GSH-Px activities in the liver, kidney, heart, and brain tissues are provided in Table 1.

The MDA levels in the TCDD group were higher compared to those of the control group in all tissues ($P < 0.001$). However, the MDA levels in the TCDD + CUR group were significantly lower than those in the TCDD group ($P < 0.001$), and the MDA levels in the liver, heart, and brain tissues were even lower than those of the control group ($P < 0.001$).

The SOD activity in the liver tissue was significantly lower in the TCDD group than that in the control group ($P < 0.01$). In addition, the SOD activities in the liver, kidney,

Table 1. Mean ± SEM values of MDA and GSH levels, and SOD, CAT, and GSH-Px activities in tissues belonging to each group.

Parameters	Groups	Tissues			
		Liver	Kidney	Heart	Brain
MDA (nmol·g^{-1} protein)	Control	34.2 ± 2.0^{b}	89.6 ± 3.9^{bc}	34.4 ± 4.6^{b}	63.9 ± 4.0^{b}
	TCDD	38.5 ± 3.8^{a}	135.8 ± 20.5^{a}	53.9 ± 8.1^{a}	78.7 ± 5.4^{a}
	CUR	33.0 ± 2.2^{b}	78.7 ± 9.7^{c}	28.8 ± 7.4^{b}	62.7 ± 5.7^{b}
	TCDD + CUR	27.2 ± 3.7^{c}	100.6 ± 19.5^{b}	19.5 ± 5.2^{c}	52.0 ± 5.8^{c}
	P value	0.000	0.000	0.000	0.000
SOD (U·g^{-1} protein)	Control	46.5 ± 6.7^{a}	33.5 ± 9.1^{ab}	83.0 ± 13.0	64.1 ± 16.0^{ab}
	TCDD	39.6 ± 4.7^{b}	32.3 ± 5.6^{b}	74.2 ± 8.4	58.3 ± 11.2^{b}
	CUR	45.6 ± 3.4^{ab}	36.3 ± 9.9^{ab}	78.1 ± 15.9	70.4 ± 6.8^{ab}
	TCDD + CUR	47.8 ± 3.8^{a}	43.0 ± 4.1^{a}	93.2 ± 19.7	76.1 ± 6.4^{a}
	P value	0.010	0.040	0.085	0.017
CAT (U·g^{-1} protein)	Control	67.2 ± 11.8^{ab}	22.1 ± 4.3^{a}	4.5 ± 1.0^{ab}	1.3 ± 0.5^{a}
	TCDD	55.8 ± 10.7^{b}	14.4 ± 3.8^{b}	3.3 ± 0.9^{b}	0.8 ± 0.1^{b}
	CUR	70.8 ± 6.1^{a}	27.6 ± 6.3^{a}	4.8 ± 1.3^{a}	1.3 ± 0.4^{a}
	TCDD + CUR	62.9 ± 12.8^{ab}	15.7 ± 3.1^{b}	4.9 ± 1.0^{a}	1.0 ± 0.2^{ab}
	P value	0.05	0.000	0.016	0.029
GSH-Px (U·g^{-1} protein)	Control	88.4 ± 14.7^{a}	45.1 ± 10.2^{a}	35.8 ± 3.8^{b}	32.6 ± 4.3^{a}
	TCDD	57.1 ± 7.7^{b}	35.5 ± 3.5^{b}	37.0 ± 6.1^{b}	27.1 ± 2.9^{b}
	CUR	91.6 ± 13.5^{a}	41.8 ± 5.1^{ab}	34.7 ± 3.9^{b}	34.3 ± 3.8^{a}
	TCDD + CUR	59.3 ± 8.0^{b}	40.4 ± 2.4^{ab}	55.1 ± 2.9^{a}	32.4 ± 3.0^{a}
	P value	0.000	0.030	0.000	0.003
GSH (nmol·mg^{-1} protein)	Control	3.7 ± 1.0^{a}	7.7 ± 1.6^{a}	1.9 ± 0.2^{a}	9.2 ± 1.1^{ab}
	TCDD	2.4 ± 0.6^{bc}	4.9 ± 1.2^{c}	1.5 ± 0.3^{b}	8.9 ± 1.5^{b}
	CUR	3.3 ± 1.0^{ab}	7.1 ± 1.7^{ab}	2.0 ± 0.3^{a}	11.6 ± 2.6^{a}
	TCDD + CUR	2.2 ± 0.2^{c}	5.3 ± 1.4^{bc}	1.4 ± 0.3^{b}	10.5 ± 1.7^{ab}
	P value	0.001	0.001	0.000	0.021

[a,b,c]Different letters in the same column are statistically significant ($P < 0.05$).

and brain tissues were significantly higher in the TCDD + CUR group than those in the TCDD group ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively).

The CAT activities in the kidney and brain tissues were significantly lower in the TCDD group than those of the control group ($P < 0.001$ and $P < 0.05$, respectively). In addition, the CAT activity in the heart tissue was higher in the TCDD + CUR group than that in the TCDD group ($P < 0.05$).

The GSH-Px activities in the liver, kidney, and brain tissues were lower in the TCDD group than those in the control group ($P < 0.001$, $P < 0.05$, and $P < 0.01$, respectively). In addition, the GSH-Px activities in the heart and brain tissues were higher in the TCDD + CUR group than those in the TCDD group ($P < 0.001$ and $P < 0.01$, respectively).

The GSH levels in the liver, kidney, and heart tissues were lower in the TCDD group than those in the control group ($P < 0.001$).

Reproductive organ weights and sperm parameters

Statistical evaluations regarding the testis, epididymides, seminal vesicles, and prostate weights, along with sperm concentrations and sperm motility, are provided in Table 2. No statistically significant differences were identified based on these values. However, it was determined that the testis, epididymis, seminal vesicles, and prostate weights were lower in the TCDD-administered groups than those in the control group and that these decreases were eliminated in the TCDD + CUR-administered group. In addition, it was determined that sperm concentrations and sperm motilities were lower in the TCDD group compared to those in the control group and that these decreases were partially eliminated in the TCDD + CUR-administered group.

Discussion

The effects of environmental pollutants on living organisms have resulted in significant health problems in parallel with developments in industry and technology. The toxicity of environmental pollutants is known to play a significant role in the development of numerous diseases, particularly cancer. The toxicity of most xenobiotics is associated with their ability to produce free radicals. It has been reported in numerous studies that oxidative stress plays an important role in the long-term toxicity of TCDD (Hassoun et al. 2000; Slezak et al. 2000; Latchoumycandane and Mathur 2002). Oxidative stress is caused by changes in the balance between pro-oxidants and antioxidants and ROS serve as an indicator of the oxidative damage resulting in oxidative stress (Alsharif and Hassoun 2004).

Table 2. Mean ± SEM values of reproductive organ weights, sperm concentration, and sperm motility belonging to each group.

Parameters	Control	TCDD	CUR	TCDD + CUR
Testes (g)	1.41 ± 0.09	1.35 ± 0.03	1.34 ± 0.04	1.43 ± 0.11
Epididymides (g)	0.47 ± 0.02	0.46 ± 0.03	0.47 ± 0.01	0.46 ± 0.05
Seminal vesicles (g)	0.91 ± 0.04	0.87 ± 0.12	1.09 ± 0.05	0.95 ± 0.16
Prostate (g)	0.40 ± 0.03	0.39 ± 0.07	0.49 ± 0.02	0.42 ± 0.07
Sperm concentration (million g^{-1} tissue)	377 ± 40	312 ± 15	374 ± 17	324 ± 36
Sperm motility (%)	74.4 ± 4.2	68.3 ± 6.1	85.0 ± 2.2	73.3 ± 6.4

Note: No statistically significant differences were found among any of the groups.

Lipid peroxidation, which occurs as a consequence of the presence of ROS, leads to cell death by disrupting the integrity of the cell membrane (Gurer et al. 1998). It has been demonstrated in numerous studies that the application of TCDD in laboratory animals causes oxidative stress by increasing lipid peroxidation in various tissues (Slezak et al. 2000; Shon et al. 2002; Ciftci et al. 2011). Supporting previous research, the current study demonstrated that the subchronic administration of TCDD led to lipid peroxidation and induced oxidative stress by significantly increasing MDA levels in the liver, kidney, heart, and brain tissues of rats ($P < 0.001$) (Table 1). TCDD has been reported to cause oxidative stress by binding to the AhR in cells, reducing membrane fluidity and increasing membrane damage (Alsharif et al. 1990).

CUR is a turmeric compound, which in addition to being an effective antioxidant, acts as a strong scavenger of free oxygen radicals (Chattopadhyay et al. 2004). The effect of CUR treatment in preventing oxidative damage by reducing lipid peroxidation has been reported in numerous studies (Eybl, Kotyzova, and Koutensky 2006; Kaur et al. 2006; Chandra et al. 2007; Kuhad et al. 2007). Supporting previous research, the current study demonstrated that the tissue MDA levels that were raised by TCDD administration decreased significantly ($P < 0.001$) through the concomitant administration of CUR with TCDD and that concomitant administration of CUR prevented lipid peroxidation (Table 1). Ciolino et al. (1998) reported that CUR has an antagonist effect against the AhR. Therefore, CUR is believed to prevent the oxidative damage caused by the binding of TCDD to the AhR within the cells by antagonizing these receptors.

The production of free radicals/ROS in tissues can be effectively countered by antioxidant defense mechanisms involving enzymes and compounds such as SOD, CAT, GSH-reductase, and GSH-Px. An imbalance between the free radicals/ROS and the antioxidant defense systems leads to oxidative stress. Numerous studies have demonstrated the effects of TCDD on antioxidant enzymes in different tissues. It has been previously reported that TCDD administration leads to a decrease in the activities of GSH-Px, SOD, CAT, and GSH-reductase in the testis tissue and rat epididymal sperm (Latchoumycandane, Chitra, and Mathur 2002). Furthermore, a study conducted by Kern et al. (2002) reported that TCDD administration caused a decrease in the CAT activity in adipose tissue and a significant decrease in the GSH-Px activity in the liver but had no effect on the SOD and GSH-Px activity levels. The current study showed that TCDD administration caused a significant decrease in SOD activity in liver tissue, CAT activity in kidney and brain tissues, and GSH-Px activity in liver, kidney, and brain tissues (Table 1). These decreases in the antioxidant defense enzymes and substances are believed to occur as they protect against the oxidative damage caused by TCDD administration.

In addition to being an oxygen free radical scavenger, CUR displays antioxidant effects by increasing the activity of endogenous antioxidant enzymes such as SOD, CAT, GSH-Px, and GSH (Sivalingam et al. 2007). In previous studies, CUR was reported to increase SOD, CAT, and GSH-Px activities in various tissues (Eybl, Kotyzova, and Koutensky 2006; Farombi and Ekor 2006; Chandra et al. 2007). The current study showed that concomitant administration of CUR with TCDD caused a significant increase in the SOD activity in liver, kidney, and brain tissues, CAT activity in heart tissue, and GSH-Px activity in heart and brain tissues (Table 1). In agreement with previous research, this study demonstrated that CUR supports the antioxidant defense systems within the body by increasing GSH-Px, CAT, and SOD activities.

GSH is the first line of defense against free radical damage. As a nonenzymatic antioxidant, GSH contains reactive sulfhydryl groups that protect cells against oxidative stress (Ishikawa and Sies 1989). Although several studies have reported that TCDD

reduces the GSH levels in various tissues (Slezak et al. 2000; Latchoumycandane and Mathur 2002), other studies have reported an increase in GSH levels with TCDD administration (Shertzer et al. 1998; Shon et al. 2002). However, decrease in GSH levels due to TCDD administration has been observed only in low-dose applications, and this decrease was not observed in high-dose applications (Slezak et al. 2000; Shon et al. 2002). This study demonstrated that GSH levels in the liver, kidney, and heart tissues were significantly reduced by TCDD administration (Table 1). These decreases in the GSH levels are believed to be a result of their depletion as they protect against the oxidative damage caused by TCDD administration.

Numerous studies have reported that CUR leads to an increase in the GSH levels of various tissues (Farombi and Ekor 2006; Kaur et al. 2006; Kuhad et al. 2007). This study also demonstrated that the concomitant administration of CUR with TCDD led to an increase in the GSH levels in the kidney and brain tissues; however, this increase was not statistically significant (Table 1). This lack of statistical significance is believed to be caused by the considerable decrease in the GSH levels in these tissues as a result of TCDD administration.

Wasting syndrome is one of the most distinctive symptoms of toxicity associated with TCDD exposure. Wasting syndrome and lethality in rodents are used as indices for TCDD toxicity. Typical characteristics of TCDD-induced wasting syndrome include the inability to feed, decreased gains in BW, and the depletion of energy reserves (Tuomisto et al. 1995). The current study identified a suppression of BW gain associated with TCDD exposure. However, this suppression of BW gain tended to be partly eliminated by the concomitant administration of CUR with TCDD. Similar results were reported in previous studies conducted in mice (Ishida et al. 2004) and rats (Ciftci, Tanyildizi, and Godekmerdan 2010). Although the mechanism has not yet been fully elucidated, it has also been reported that CUR may partially eliminate the reduction in BW gain without affecting AhR activation by TCDD.

In the reproductive system, TCDD has been reported to decrease antioxidant enzyme activity in the testis and epididymal sperm and to increase the formation of ROS (Latchoumycandane, Chitra, and Mathur 2002). TCDD exposure can also lead to biochemical and pathological changes within the reproductive systems of male rats (Sonmez et al. 2011; Beytur et al. 2012). TCDD exposure is known to lead to infertility in humans and animals by increasing tissue damage and oxidative stress in the testis tissue and by adversely affecting sperm characteristics. In agreement with previous research, TCDD exposure in the current study led to a decrease in the epididymis, seminal vesicle, and prostate weights in male rats and in the sperm concentration and motility. The concomitant administration of CUR with TCDD eliminated these decreases by raising these parameters back to normal levels (Table 2). Because antioxidant damage is the prominent cause and mechanism of impairment in the reproductive parameters induced by TCDD, CUR, a strong antioxidant, was able to eliminate this oxidative damage, thus alleviating the impairment in the reproductive parameters as well.

In conclusion, this study demonstrated that TCDD administered to rats for 13 weeks at a dose of 50 $ng \cdot kg^{-1}$ BW per day led to oxidative stress by increasing the MDA levels and by decreasing the activity of endogenous antioxidant enzymes. TCDD also led to a reduction in BW gain, male rat reproductive organ weights, sperm concentration, and sperm motility. However, treatment with CUR reduced the MDA levels raised by TCDD administration, increased the antioxidant enzyme activity, partially eliminated the decreases in BW gain, and eliminated the impairment of reproductive parameters. Therefore, CUR may be beneficial for the treatment and prevention of toxicity induced by subchronic TCDD exposure.

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