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Induction of Oxidative Stress in the Reproductive System of Rats after Subchronic Exposure to 2,3,7,8-Tetrachlorodibenzo-*P*-Dioxin

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Polychlorinated dibenzo-*p*-dioxins (PCDDs) are widespread, persistent environmental contaminants. The most potent and extensively studied congener of all PCDDs isomers is 2,3,7,8-tetrachloro-dibenz-*p*-dioxin (TCDD) (Safe, 1990). TCDD is formed as unwanted by-product in the manufacture of chlorinated hydrocarbons. This compound is also formed in the incineration process of waste, paper and pulp bleaching, emission from steel foundries and motor vehicles (Skene et al., 1989). TCDD elicited a number of toxic effects in different animal species. It increased the mortality and induced many clinical changes in female mink (Hochstein et al., 2001) and fish (Walter et al., 2000). Also, TCDD impaired fertility and suppressed systemic lymphocyte proliferation in immature rats (El-Sabeawy et al., 1998; Wade et al., 2002) and hamsters (Yellon et al., 2000). In utero and lactational exposure to TCDD at different doses induced various functional effects on offspring (Mann, 1997; Stanton, 2001). TCDD impaired the development of reproductive system in male and female mice (Theobald and Peterson, 1997);affect male reproductive capability in rats at puberty and at adulthood (Faqi et al., 1998; Ohsako et al., 2001). TCDD exposure resulted in an oxidative stress in multiple tissues and different animal species. TCDD has been reported to induce superoxide formation, lipid peroxidation and DNA damage in the hepatic and brain tissues of rat (Hassoun et al., 2001). In addition, complex changes in the oxidative stress enzymes activity of both adipocytes and liver were found (Kern et al., 2002). Several mechanisms have been proposed to explain the toxicity of TCDD and its congeners (Safe, 1990). Recently, the toxic mechanism of TCDD is mediated through combining with aryl-hydrocarbon (Ah) receptor (Sugihara et al., 2001). Moreover, induction of oxidative stress upon exposure to TCDD is also considered an important mechanism (Slezak et al., 2000). Most of the studies have focused on the in utero, lactational and pre-pubertal exposure of TCDD on male reproduction (Gray et al., 1997; El-Sabeawy et al., 1998). Further details about TCDD testicular toxicity on mature animals are still needed. Therefore, the present study was conducted to evaluate the effect of TCDD on the testicular function of mature male rats. In addition, the effect of TCDD on the antioxidant system of testicular mitochondrial and microsomal fractions was also evaluated.

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MATERIALS AND METHODS

Healthy mature male Sprague-Dawley rats weighing (120 ± 20 g) were used in this study. They were kept under good ventilation and standard hygienic conditions with 12-hour darkness schedule in cages containing four to five animals. Food and water are supplied *ad libitum*. The animals were randomly divided into four equal groups. The first one served as control, while the other three groups orally received different doses of TCDD dissolved in olive oil (50, 100 or 200 ng/kg body weight per day) for 60 days. TCDD was generously provided from the Department of Pharmacology, National Research Center, Cairo, Egypt. The selected doses of TCDD are appropriate for the subchronic study (Michalek et al., 1995). In the meantime, the selected doses are resembled to the repeated environmental exposure found in forages near to waste incinerations and manufacturing plants (Feil et al., 2000).

At the end of the time course, the animals were fasted overnight, weighed and killed using anesthetic ether (The using of ether is controlled according to the occupational health and safety regulations methods). Postmortem inspection was performed in all animals. Testis, epididymis, seminal vesicles and prostate were removed and their relative weights were calculated (g/100 g body weight). Sperm samples were obtained from cauda epididymis and their count and motility as well as the percentage of live sperms and abnormal forms were assessed according to the method of Bearden and Fuquay (1980). Left testis was used for the histopathological study (Bancroft et al., 1994). The right one was processed for subcellular fractionation and biochemical studies.

Mitochondrial and microsomal fractions of the testis were obtained by the differential centrifugation method as described by Chainy et al. (1997). Briefly, testicular tissue was homogenated in ice-cold 0.25 M sucrose solution. The homogenate was centrifuged at 1000 x g for 10 min to obtain the nuclear pellet. Mitochondrial pellet was obtained by centrifugating the post-nuclear supernatant at 10000 x g for 10 min. while, the microsomal pellet was obtained by resuspended the post mitochondrial supernatant with ice-cold CaCl2 (1M) then centrifugated at 100000 x g for 10 min. All the fractions were washed with ice-cold 1.15% potassium chloride solution and resuspended in 0.25 M sucrose solution. The mitochondrial and microsomal fractions were used for determination of antioxidant enzymes activity [such as superoxide dismutase (SOD), catalase (CAT) and glutathione perioxidase (GSH-PX)] and the level of lipid peroxidation. The activity of SOD and GSH-Px and CAT was assessed according to the methods of Marklund and Marklund (1974), Paglia and Valentine (1967) and (Claiborne, 1985) respectively. The level of lipid peroxidation in mitochondrial and microsomal fractions was determined by measuring the level of thiobarbituric acid reactive substance (TBARS); using malondaldehyde for polating the standard curve (Buege and Aust, 1976). The value expressed in umol of malondaldehyde/min/mg protein. Protein concentration was determined by the method of Lowry et al. (1951) using bovine albumin as standard. The statistical assessment of the results was performed using one-way ANOVA procedure

followed by Tukey-Kramer multiple comparison tests using Software GRAPHPAD INSTAT (Version 2). The 0.05 level of probability was used as the criterion for significance.

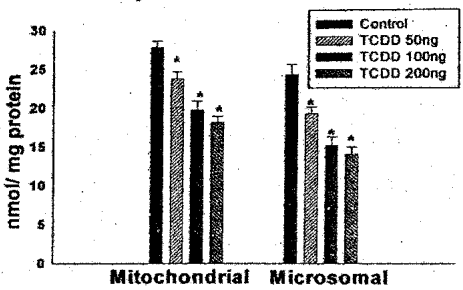
RESULTS AND DISCUSSION

Animals orally received different doses of TCDD did not show any clinical toxic signs. There was no marked change in food intake or body weight gains when compared with the control group indicating that the general metabolic conditions of the animals were within normal range. Administration of TCDD caused significant decrease in the activities of the antioxidant enzymes (Figs.1-3) and significant increase in the level of lipid peroxidation of the testis (Fig. 4). This effect was in a dose related manner. The difference was not statistically significant between the doses 100 and 200 ng/kg. Thus the dose 100 ng/kg may be considered as the maximum observable adverse effect level for the oxidative stress induced by TCDD. The level of oxidative stress was more prominent in microsomal fraction than that in mitochondrial fraction in all given doses. This was manifested by calculation of the percentage of changes from their respective control in the antioxidative parameters. The percentages of the change were higher in microsomal fraction than that of the mitochondrial.

The reduction in the activities of antioxidant enzymes and increased lipid peroxidation could reflect the oxidative stress induced by TCDD in testis subcellular fractions. In normal physiological condition free radicals and reactive oxygen species (ROS) such as H_2O_2 generated in tissues are efficiently scavenged by the antioxidant defense system, which mainly constituted from antioxidant enzymes. An imbalance in the generation of ROS and antioxidant system produces lipid peroxidation and oxidative stress (Murray et al., 2000). Generated ROS lead to cell damage and diseases. Lipid peroxidation disrupts the structural integrity of cell membrane and also leads to the formation of aldehydes, which in turn further damage the lipid, protein and DNA (Kern et al., 2002).

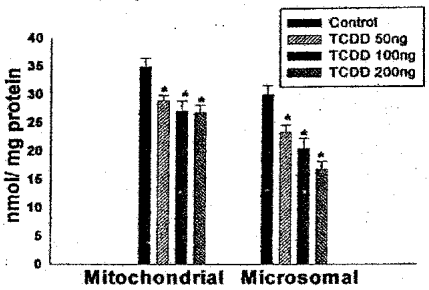
Animals exposed to TCDD showed significant decrease in the relative weight of sex organs and its accessory glands in dose-dependent manner (Table 1). Reduced relative weight of the testis may be attributed to the atrophic changes recorded by histopathological examination (Figs. 5, 6). The severity of the histopathological lesions was directly proportional to TCDD doses. Testicular atrophy may be due to the direct effect of TCDD. Faqi et al., (1998) recorded high TCDD concentration in the testis of rat in utero exposed to TCDD during adulthood. TCDD has been shown to act through Ah receptor, a ligand activated nuclear transcription factor (Safe, 1990). Therefore, the presence of Ah receptor at high level in testis (Johnson et al., 1992) reveals that the testis is a possible target for TCDD induced responses. It has been reported that TCDD induced androgen deficiency and decreased androgen receptor (Johnson et al., 1992) that may explain the recorded decrease in the relative weights of androgen sensitive organs (seminal vesicles, prostate and epididymis). The present study also revealed that the epididymal sperm cell count and the percentage of the progressive motility

Figure 1. Effect of TCDD on the SOD activity in rat testis fractions



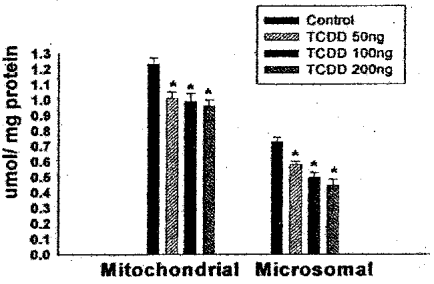
Data expressed as mean + S.E (n= 10).
* Significantly different from respective control, one-Way ANOVA (p< 0.05).

Figure 2. Effect of TCDD on the GSH-PX activity in rat testis fractions



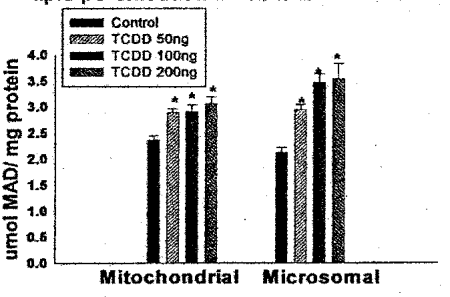
Data expressed as mean + S.E (n= 10).
* Significantly different from respective control,one-way ANOVA (p< 0.05).

Figure 3. Effect of TCDD on the CAT activity in rat testis fractions



Data expressed as mean + S.E (n = 10).
* Significantly different from respective control, one-way ANOVA (p< 0.05).

Figure 4. Effect of TCDD on the levels of lipid peroxidation in rat testis fractions



Data expressed as mean + S.E (n = 10).
* Significantly different from respective control, one-way ANOVA (p< 0.05).

were significantly decreased. In addition, the percentages of sperm mortality and abnormalities were increased (Table 2; Fig. 7). Since TCDD altered the histopathological architecture of the testis, its function will subsequently affect. The reduction in the epididymal sperm counts is consistent with the results of Gray et al. (1997). Oxidative stress recorded in this study appears to be a causative agent in the cytotoxic effects on the testis. Decreased antioxidant enzyme activities and increased lipid peroxidation reflected the generation of ROS by TCDD that cannot be eliminated by the exhausted antioxidant defense mechanisms. Besides, polyunsaturated fatty acids of the sperm plasma membrane are very susceptible to ROS attack (Ichikawa et al., 1999). Lipid peroxidation changes the permeability of testicular cells leading to disruption of testicular function. In this respect, Sommer et al. (1996) mentioned that sperm loss was due to an increase in sperm phagocytosis in the excurrent duct system of rats exposed to TCDD. Recently, Ohbayashi et al. (2001) identified a novel TCDD target gene, that is implicated in spermatogenesis and mediating the reproductive toxicity induced by TCDD. From the present results it documented that TCDD reduced the reproductive capabilities and caused exhaustion of the antioxidant defense system in rat testis inducing an oxidative stress.

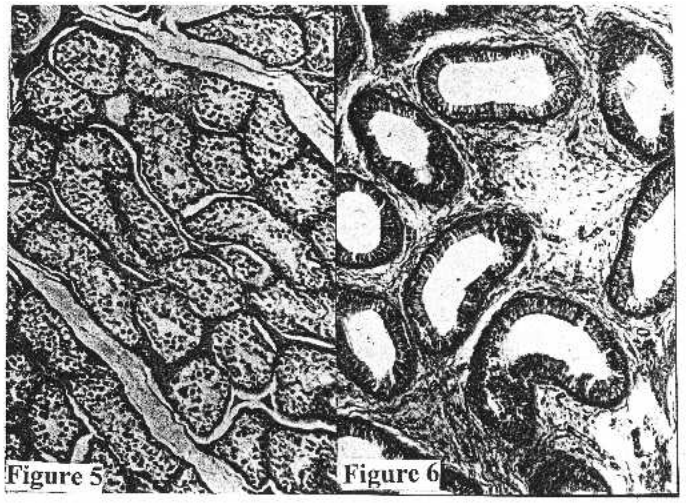


Figure 5 Figure 6

Figure 5. Testes of rat exposed to 200ng TCDD showing degeneration and atrophy of all of the seminiferous tubules (H & E X 40).
Figure 6. Epididymis of rat exposed to 200ng TCDD showing the epididymal tubules lumen completely empty and free from sperms (H & E X 40).

Table 1. Effect of TCDD on the body weight (g) and the relative weights of sexual organs (g /100g body weight) of mature rats.

Groups / Parameters	Control	TCDD (ng/kg b.wt.)		
		50	100	200
Body weight	153 ± 2.51	152 ± 3.10	150 ± 3.50	149 ± 4.20
Testis	1.54 ± 0.03	1.36 ± 0.02*	1.24 ± 0.02*	1.11 ± 0.03*
Epididymis	0.50 ± 0.03	0.37 ± 0.02*	0.29 ± 0.02*	0.22 ± 0.02*
Seminal vesicles	0.82 ± 0.02	0.74 ± 0.02*	0.69 ± 0.01*	0.62 ± 0.02*
Prostate	0.32 ± 0.02	0.25 ± 0.01*	0.23 ± 0.01*	0.20 ± 0.01*

Data are presented as mean ± S.E (n=10).
(*) Significantly different from control at $p < 0.05$ (One-Way ANOVA).

Table 2. Effect of TCDD on the sperm characteristics of mature rats.

Groups / Parameters	Control	TCDD (ng/kg b.wt.)		
		50	100	200
Sperm count (x 10^8/ml)	6.10 ± 0.23	4.70 ± 0.42*	4.15 ± 0.24*	3.80 ± 0.35*
Motility (%)	84.2 ± 1.86	73.1 ± 1.51*	68.7 ± 2.69*	63.1 ± 2.06*
Mortality (%)	9.60 ± 0.40	13.2 ± 0.41*	15.4 ± 0.37*	16.6 ± 0.33*
Total Abnormalities (%)	3.00 ± 0.21	7.20 ± 0.38*	10.0 ± 0.51*	13.2 ± 0.48*
Head Abnormalities (%)	1.20 ± 0.20	2.90 ± 0.27*	4.10 ± 0.43*	4.70 ± 0.55*
Tail Abnormalities (%)	1.80 ± 0.13	4.30 ± 0.30*	5.90 ± 0.37*	8.50 ± 0.54*

Data are presented as mean ± S.E (n=10).
(*) Significantly different from control at $p < 0.05$ (One-Way ANOVA).

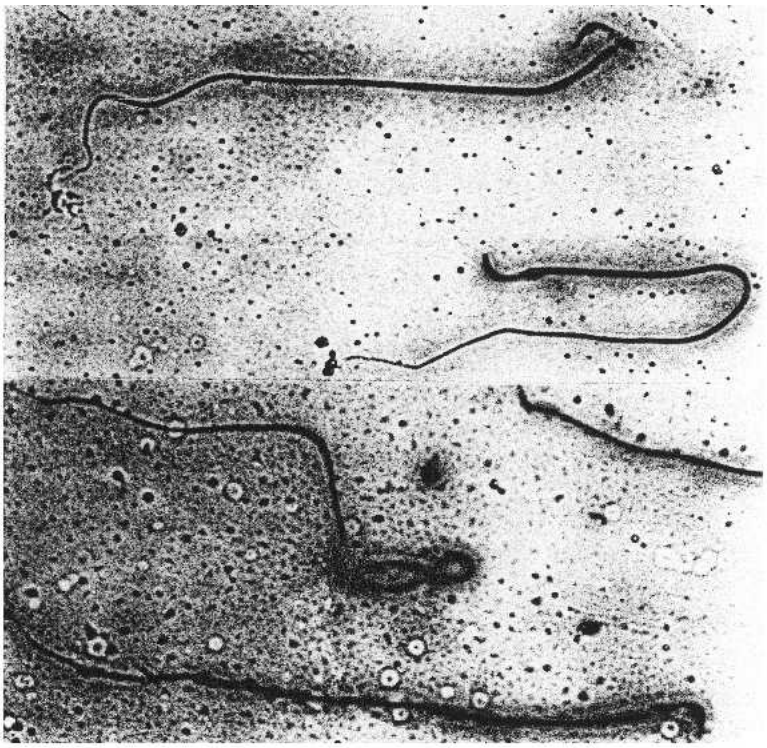


Figure 7. Sperm of rat exposed to 200ng TCDD showing head deformity and tail deformities (curved and coiled).

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