QUERCETIN REDUCES OXIDATIVE STRESS DAMAGE TO REPRODUCTIVE PROFILE INDUCED BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN MALE ALBINO RATS (*RATTUS NORVEGICUS* L.)

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Abstract. The article aimed to investigate the effect of the antioxidant quercetin (QCT) on male Wistar albino rats under oxidative stress caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), focusing on their reproductive system, in order to find a way to combat declining male productivity. Thirty adult male Wistar rats of body weight ranged from 230 to 250 g were randomly divided into five equal groups (6 per group), designated as normal control (corn oil) and treatments: 10 µg/kg TCDD; 20 mg/kg QCT; 20 mg/kg QCT + 10 µg/kg TCDD (pretreatment of QCT); 10 µg/kg TCDD + 20 mg/kg QCT (post-treatment of QCT), and the treatments were administered intragastrically by gavage for twelve weeks and the study was conducted in Salahaddin University-Erbil, Iraq. In each group, three males were mated with untreated fertile females, and the reproductive and biochemical parameters were evaluated for each group. TCDD significantly reduced the values of sperm quality (motility, viability, and count), serum testosterone, sex ratio, body weight, gonads weight, and testicular antioxidant enzymes (superoxide dismutase, catalase, and glutathione reductase). However, increased sperm abnormality and oxidant biomarker (malondialdehyde) were observed. Concomitant treatment with QCT ameliorated these affected values, especially when QCT treatment was administered 30 min before TCDD dosing.
Keywords: *TCDD, QCT, sperm quality, male fertility, antioxidants*

Introduction

It is believed that biological factors such as contamination of the environment have a considerable impact on male fertility. Various environmental contaminants responsible for the global decline in male reproductive health have been suggested (Erthal et al., 2018). One of these environmental polluters is dioxins, and dioxin-like compounds that are known as endocrine-disrupting and reprotoxic pollutants (Dhanabalan et al., 2011). These substances exerted their cellular and metabolic effects through interactions with the aryl hydrocarbon receptor (AhR) associated with the xenobiotic- (XRE) and antioxidant responsive (ARE) DNA elements (Nguyen et al., 2009). Upstream genes such as the promoter of the cytochrome P450 1A1 (CYP1A1) and another Ah-responsive gene that organizes the expression of the genes participating in the metabolism and detoxification of polycyclic aromatic hydrocarbons such as dioxins and dioxin like-compounds in experimental animals and humans (Mandal, 2005).

Dioxins suchlike 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are leaked as unwanted by-products to the environment from several industrial processes such as the production of certain herbicides, pesticides and plastics, and combustion of plastics and medical waste (Anderson and Fisher, 2002; Hewitt et al., 2006; Lin et al., 2006). Home heating systems, cars exhaust, and cigarette smoke have been found to contain trace

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quantities of dioxin (Fiedler et al., 1990). TCDD is a toxic chemical compound and a widespread potent environmental contaminant, and because of its lipophilicity and long biological half-lives, it bioaccumulates in organisms and biomagnifies in the food web. They enter to human body primarily by eating food rich in animal fat and accumulating in human fatty tissues (Jackson et al., 2017). TCDD can affect male fertility by reducing the number of sperm (Pilsner et al., 2017), changes in motility and in sperm transit time (Sanabria et al., 2016), serum testosterone (Karman et al., 2012), lowering reproductive organs weight (Jin et al., 2010), delayed sexual maturation (Takeda et al., 2012), and can also change the “male/female” sexual ratio of offspring (You et al., 2018).

Quercetin (3,5,7,3’,4’-pentahydroxyflavone, QCT), a compound of yellow powder extracted from natural sources and have a potent antioxidant, due to the existence of two pharmacophores, the catechol group in the B-ring and the OH group at situation 3 within the molecule scavenging the free radicals (Nabavi et al., 2015). It is found in several daily foods such as onion, grape, nuts, tea, berries, cabbage, and cauliflower (Azzi et al., 2018). Inal and Kahraman (2000) reported the following biological effects of QCT: anti-cancerous, antiviral, anti-ischemic, anti-inflammatory, and anti-allergenic, in addition to the protective power against atherosclerosis and coronary heart disease.

In animal studies, TCDD exposure has been shown to induce testicular damage and QCT may reduce the male reproductive toxicity of TCDD due to its antagonistic activity against AhR which is activated by TCDD (Ciftci et al., 2012). Melekoglu et al. (2016) reported that TCDD and related substances promote the production of reactive oxygen species (ROS) in the reproductive system. Additionally, several studies have shown that TCDD exposure results in oxidative damage to many tissues, such as the kidney, liver, and testis (Palaniswamy et al., 2014; Slezak et al., 2000).

Though to our knowledge, there are some literary works on the mitigating role of QCT in testicular damage caused by TCDD. Therefore, the purpose of this study was to assess the role of QCT in alleviating the testicular damage caused by TCDD in rats by estimating serum testosterone, sperm qualities, and determining the levels of the oxidant-antioxidant biomarkers activity in the testis.

Materials and methods

Chemicals

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; purity > 99%) and quercetin (QCT; purity > 99%) were purchased from Beijing Solarbio Science & Technology Company, Ltd. (Beijing, China), and corn oil (Afia, Saudi Arabia) was obtained from local markets. TCDD was dissolved in corn oil at a dose of 10 μg/kg body weight, and QCT at a dose of 20 mg/kg body weight was also dissolved in corn oil.

Experimental animals

Thirty adult male Wistar rats, *Rattus norvegicus*, used for this study were procured from the Laboratory Animal Center (College of Science, University of Zakho, Duhok Province, Iraq). The rats were acclimated to the animal housing for two weeks before the start of dosing. They were preserved in plastic cages with wire mesh coverings and kept in an air-conditioned and well-ventilated room in a controlled light environment (12-h light/dark cycle) and temperature (22 ± 2 °C). Rats were fed on a balanced rat chow, and water (tap water) provided ad libitum. The local Committees approved the

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experimental protocols for using animals of the Salahaddin University-Erbil, Erbil city, Kurdistan Region of Iraq. The care and handling of the experimental animals were performed according to the precepts of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Experimental design

The body weight (BW) of the animals ranged between 230 to 250 g. After weighing, the animals randomly divided into five equal groups (n = 6), and the treatment was given intragastrically by gavage for twelve weeks: The 1st group was kept as a normal group and was given corn oil (vehicle). The 2nd group (TCDD group) was orally administered TCDD at a dose of 10 µg/kg BW/day. A dose of 20 mg/kg BW/day of QCT was treated to rats in group 3 (QCT group). In group 4 (QCT-TCDD), rats were treated with TCDD (10 µg/kg BW/day) and QCT (20 mg/kg BW/day) 30 min before TCDD treatment (pre-treatment group), while in group 5 (TCDD-QCT), after 30 min of TCDD administration (10 µg/kg BW/day), animals were treated with 20 mg/kg BW/day of QCT (post-treatment group).

Body weight and gonadosomatic index (GSI)

The rats weighed once per week throughout the experiment and the weight gain determined at the end of the study. The testes and cauda epididymis were removed and weighed to measure the gonado-somatic index (GSI) and epididymal-somatic index (ESI) by the following equation:

$$GSI = \frac{Gonad\ weight \times 100}{Body\ weight}$$

$$ESI = \frac{Weight\ of\ epididymis \times 100}{Body\ weight}$$

Serum testosterone concentration

Serum testosterone concentration was measured by the enzyme-linked immunosorbent assay (ELISA) method using DRG ELISA testosterone kit (ELISA EIA-1559, 96 wells; DRG Instruments, GmbH, Marburg, Germany), as directed by the manufacturer’s instructions.

Preparation of tissue homogenates

The testis tissue samples were homogenized in extraction solution specialized for each test, using an Ultra-Turrax (Janke and Kunkel IKA, Labortechnik, Germany) homogenizer at 20000 rpm/min. The resulting homogenate was centrifuged at 8000 rpm for 10 min at 4 °C using a cooling centrifuge (Biobase, China). The resulting cell-free supernatant was used for the biochemical analysis. The product was assessed spectrophotometrically according to the manufacturer’s protocol.

Lipid peroxidation and anti-oxidant assay

The level of lipid peroxidation of testis tissue was measured spectrophotometrically (EMC^2 GmbH, Germany) by determining the malondialdehyde (MDA) production and

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the antioxidant profiles such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH-R) levels were assayed using the kit method supplied by Beijing Solarbio Science & Technology Company, Ltd, (Beijing, China). The results are expressed per unit weight of tissue.

Sperm quality analysis

Spermatozoa were obtained from the right cauda epididymis, which was carefully removed, cleaned, and immediately placed into a pre-warmed container with 1 ml of phosphate-buffered saline (PBS, pH 7.2) at 37 °C and minced with scissors (Narayana et al., 2005). Subsequently, sliced tissue was incubated at 37 °C for 10 min. All epididymal samples were examined for the following parameters:

Sperm motility analysis

Sperm motility was evaluated by counting 200 sperms per rat counted throughout at least 10 fields of view of the sample on a pre-warmed slide (37 °C) under prewarmed coverslip (37 °C) using binocular microscope (Nikon, Japan) with a warmed stage (400× magnification).

Total sperm count

The sperm counting was carried out by diluting the sperm suspension with PBS (1:20) in the pipette with white bead, then mixed gently, after that a drop of the suspension was delivered into the Neubauer hemocytometer in each side of the counting chamber. The hemocytometer was allowed to stand for 5 min for sedimentation, and then sperms were counted under binocular microscope (Nikon, Japan) in large eight squares of 1 mm^2 each area with the exception of the central area for erythrocyte counting of Neubauer's chamber was performed and multiplied by a 5×10^4 factor to calculate the total count of spermatozoa in a million/epididymis (Narayana et al., 2005). Counting was only done for those sperms with sperm head located in squared areas.

Sperm viability

In order to determine live-dead spermatozoa, 10 μL of pre-heated eosin-nigrosin stain was placed on a pre-warmed slide (37 °C) then 10 μL of sample suspension was added and mixed gently. A smear of the sample-stain mixture was prepared and dried at room temperature. Samples were analyzed at 1000-fold magnification under a binocular microscope (Nikon, Japan). Two hundred sperms per rat were used for the determination of sperm viability. Unstained (white) sperms are viable, while stained (pink) sperms are dead sperms (Kledmanee et al., 2013).

Sperm abnormality

Sperm morphology was evaluated following smear staining with eosin-nigrosin stain. Slides were viewed with an oil immersion (100× magnification) under a microscope (Nikon, Japan). On each slide, a total of two hundred sperms were examined for each animal, and the total sperm abnormality rates were expressed as percentages (Ciftci et al., 2012).

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Fertility test

At the end of the experiment, fertility and sex ratio was estimated in adult male rats of treated groups and the control of male counterparts. In each group, three males were randomly selected, and each one was mated with three untreated fertile females in separated plastic cages. Detection of sperm in the vaginal smear supposes an early indicator of successful mating. After five days of mixing, every three days, female rats were checked for pregnancy in three days intervals (by checking abdominal size) with calculating the pregnancy percentage for each group. Also, the number and sex ratio (male/female ratio) of newborns in each group was calculated.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for this experiment. Analyzing of results performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences between the experimental groups using GraphPad Prism 6 version 6.01 for Windows (GraphPad Software 2012). Accepting a conventional 5% level of significance ($P \leq 0.05$), hence, all tests are statistically significant. Significant differences between treatment groups (or comparison of mean values) and appropriate vehicle control were denoted by superscript asterisks symbols *, **, ***; significant differences between TCDD group and the rest treatment groups were denoted by superscript hash symbols #, ##, ###; significant differences between QCT group and the treatment groups were denoted by superscript symbols $, $$, $$$ which represent significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Results

Metabolic results

Body weight and GSI

Results of mean (±SD) body weight (g), the GSI (%), and the ESI (%) are given in *Table 1*. There was a statistically significant decrease in body weight after twelve weeks of TCDD administration in TCDD group rats in comparison to control and QCT group rats (TCDD: 244.16 ± 13.92 g; Control: 294.83 ± 7.60 g; 286.83 ± 8.18 g, $p < 0.001$). However, co-treatment of QCT with TCDD in both QCT-TCDD and TCDD-QCT group rats reversed this effect of TCDD, but administration of QCT 30 min after TCDD administration was more effective than administration of QCT 30 min before TCDD administration (QCT-TCDD: 303.66 ± 8.71 g; TCDD-QCT: 320.66 ± 10.48 g; TCDD: 244.16 ± 13.92 g, $p < 0.001$).

The GSI and ESI of the TCDD treated rat were significantly lower than that of the control rat (TCDD: 0.387 ± 0.012%, 0.056 ± 0.01%; Control: 0.419 ± 0.021%, 0.075 ± 0.01, $p < 0.05$). The GSI and ESI of the QCT group rats were insignificantly higher than that of the control rats ($p > 0.05$) and significantly greater than that of the TCDD group ($p < 0.001$, $p < 0.01$). Rats received QCT in combination with TCDD (QCT-TCDD) significantly ($p < 0.001$) reduced the adverse effects of TCDD in rats received TCDD alone, which demonstrate increasing of GSIs in comparison to TCDD group. While QCT administration in combination with TCDD (TCDD-QCT) showed a reduction in the adverse effects of TCDD compared to rats received TCDD alone, but the values did not meet any statistical significance ($p > 0.05$).

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Table 1. *Body weight, GSI, and ESI of control and treated groups (n = 6)*

Groups	Body weight (g)	GSI (%)	ESI (%)
Control	294.83 ± 7.60	0.419 ± 0.021	0.075 ± 0.010
TCDD	$244.16 \pm 13.92^{***}$	$0.387 \pm 0.012^{*}$	$0.056 \pm 0.010^{*}$
QCT	$286.83 \pm 8.18^{\#\#\#}$	$0.444 \pm 0.010^{\#\#\#}$	$0.076 \pm 0.011^{\#}$
QCT-TCDD	$303.66 \pm 8.71^{\#\#\#}$	$0.427 \pm 0.029^{\#\#\#}$	0.065 ± 0.005
TCDD-QCT	$320.66 \pm 10.48^{**\#\#\#\$\$\$}$	$0.411 \pm 0.033^{\$}$	0.062 ± 0.009

Testosterone concentration

Relative to the rats of the control group, the plasma testosterone level is significantly reduced in rats administrated with TCDD, whereas the testosterone levels in rats in QCT group were not markedly changed in comparison to control rats (Control: 1.83 ± 0.26; TCDD: 0.31 ± 0.19; QCT: 2.25 ± 0.38, $p < 0.001$, $p > 0.05$).

The results obtained in this study exhibit a significant ($p < 0.001$) decrease of plasma testosterone levels in rats treated with TCDD, but in the QCT-TCDD and TCDD-QCT rats, the value of hormone testosterone is significantly reversed in the blood plasma compared to the TCDD treated group (TCDD: 0.31 ± 0.19; QCT-TCDD: 1.18 ± 0.24; TCDD-QCT: 1.01 ± 0.18, $p < 0.001$, $p > 0.01$). This increase was grown, but it was still lower than the corresponding value in the blood plasma of control group. Plasma testosterone started to increase again after subsiding to approximately 1/6 of the corresponding control value. In rats with QCT administered alone, the testosterone concentration was insignificantly ($p > 0.05$) increased when compared with the corresponding control (*Table 2*).

Table 2. *Testosterone hormone of control and treated groups (n = 6)*

Groups	Testosterone concentration (ng/dl)
Control	1.83 ± 0.26
TCDD	$0.31 \pm 0.19^{***}$
QCT	$2.25 \pm 0.38^{\#\#\#}$
QCT-TCDD	$1.18 \pm 0.24^{**\#\#\#\$\$\$}$
TCDD-QCT	$1.01 \pm 0.18^{***\#\#\$\$\$}$

Lipid peroxidation and anti-oxidant assay

Values of the antioxidant enzymes (SOD, CAT, and GSH) and the oxidative biomarker (MDA) in the testis are shown in *Table 3*. A significant elevation in MDA levels was observed in the rats exposed to TCDD, whereas GSH levels, SOD, and CAT activities were meaningfully declined in testis tissues in opposition to the control group rats. Also, there were statistically insignificant changes between the QCT and control groups regarding MDA, SOD, CAT, and GSH levels. Otherwise, the decline in MDA levels and rises in SOD and CAT activities and GSH levels in the group pretreated with QCT (QCT-TCDD) were observed in comparison to the rats exposed to only TCDD. Administration of QCT 30 min after TCDD administration (post-treatment group) improved SOD, CAT, GSH, and MDA levels but these improvements (except MDA level) did not reach the statistical significance when compared with the rats of TCDD group.

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Table 3. *Oxidant and antioxidant assay of control and treated groups (n = 6)*

Groups	MDA (nmol/g)	GSH-R (µg/g)	SOD (U/g)	CAT (U/g)
Control	22.63 ± 7.57	82.09 ± 8.85	83.32 ± 12.39	47.43 ± 8.54
TCDD	$54.73 \pm 8.66^{***}$	$39.58 \pm 9.45^{***}$	$45.02 \pm 11.94^{***}$	$22.95 \pm 9.18^{**}$
QCT	$19.88 \pm 4.89^{\#\#\#}$	$84.19 \pm 9.97^{\#\#\#}$	$89.84 \pm 7.02^{\#\#\#}$	$55.85 \pm 10.63^{\#\#\#}$
QCT-TCDD	$34.33 \pm 10.09^{\#\#\$}$	$56.07 \pm 9.13^{***\#\$\$\$}$	$69.64 \pm 16.66^{\#}$	$38.25 \pm 10.33^{\#\$}$
TCDD-QCT	$39.36 \pm 8.83^{*\#\$\$}$	$49.33 \pm 8.03^{***\$\$\$}$	$64.38 \pm 12.34^{\$}$	$33.66 \pm 7.49^{\$\$}$

Sperm analysis

TCDD-treated rats exhibited a significant reduction in sperm count, motility, and viability percentage, while increased morphologically abnormal sperms in the TCDD group compared with the control and other experimental groups. The sperm numbers, motility, viability, and abnormality were $58.25 \times 10^6 \pm 5.53$, $61.33 \pm 8.93\%$, $51.33 \pm 6.47\%$, and $12.83 \pm 1.94\%$, respectively, in the TCDD group. The corresponding values in the control group were $71.33 \times 10^6 \pm 6.96$, $86.33 \pm 7.28\%$, $84.50 \pm 8.33\%$, and $6.50 \pm 1.87\%$. However, QCT consumption by rats treated with TCDD resulted in significant (QCT-TCDD: $p < 0.001$, TCDD-QCT: $p < 0.01$) ameliorations in all sperm parameters when compared with the TCDD group rats. The corresponding values in the QCT-TCDD group were $67.66 \times 10^6 \pm 5.90$, $78.33 \pm 8.16\%$, $69.83 \pm 5.23\%$, and $7.16 \pm 1.47\%$, and similar amounts in the TCDD-QCT group were $64.25 \times 10^6 \pm 5.98$, $71.67 \pm 7.11\%$, $66.67 \pm 4.50\%$, and $8.66 \pm 1.03\%$, but the results showed that administration of QCT 30 min before TCDD administration was more effective than QCT administration 30 min after TCDD administration. On the other hand, the sperm number, sperm motility, sperm viability, and sperm abnormality were not significantly changed in rats orally received QCT when compared with the rats of the control group, while a considerable change was observed in comparison to TCDD group rats (*Table 4*).

Table 4. *Sperm analysis of control and treated groups (n = 6)*

Groups	Sperm count (million/epididymis)	Sperm motility %	Sperm viability %	Sperm abnormality %
Control	71.33 ± 6.96	86.33 ± 7.28	84.50 ± 8.33	6.50 ± 1.87
TCDD	$58.25 \pm 5.53^{*}$	$61.33 \pm 8.93^{***}$	$51.33 \pm 6.47^{***}$	$12.83 \pm 1.94^{***}$
QCT	$71.91 \pm 7.76^{\#\#}$	$88.50 \pm 7.03^{\#\#\#}$	$86.17 \pm 7.19^{\#\#\#}$	$5.16 \pm 1.60^{\#\#\#}$
QCT-TCDD	67.66 ± 5.90	$78.33 \pm 8.16^{\#\#}$	$69.83 \pm 5.23^{**\#\#\#\$\$}$	$7.16 \pm 1.47^{\#\#\#}$
TCDD-QCT	64.25 ± 5.98	$71.67 \pm 7.11^{*\$}$	$66.67 \pm 4.5^{***\#\#\$\$\$}$	$8.66 \pm 1.03^{\#\#\$\$}$

Fertility test

The percentage of pregnant female rats in the group received TCDD was significantly lower than that of the control and QCT group (TCDD: 66.66%; Control: 100%; QCT: 100%, $p < 0.05$). Whereas in the QCT-TCDD and TCDD-QCT group, the pregnancy rate was reversed compared to the TCDD group, but the difference did not attend any statistical significance, and this increase grew but was remain lower than the corresponding value in the control group (QCT-TCDD: 88.88%; TCDD-QCT: 88.88%, $p > 0.05$).

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The sex ratio of the control group's 9 litters (89 embryos), 6 litters of the TCDD group (51 embryos), 9 litters of the QCT group (92 embryos), 8 litters of the QCT-TCDD group (77 embryos) and 8 litters of the TCDD-QCT group (74 embryos) was linked to the visual examination of anogenital distance away and the presence of male genitals (*Table 5*). Our results showed a statistically significant decrease in the sex ratio of embryos in the TCDD group against the control and QCT group rats (TCDD: 27.62 ± 21.14%; Control: 52.01 ± 8.58%; QCT: 53.98 ± 10.26%, $P < 0.05$). In general, QCT treatment significantly increased pregnancy percentage, mean litter size, and sex ratio (male/female ratio) in comparison to TCDD treatment. Additionally, the results exhibited that QCT administration along with TCDD in both QCT-TCDD and TCDD-QCT groups in contrast to TCDD group insignificantly increased pregnancy percentage, mean litter size, and sex ratio percentage. Also, there were no significant changes between QCT-TCDD and TCDD-QCT groups in means of pregnancy percentage, mean litter size, and sex ratio percentage. The QCT substance when given together with TCDD, especially in a group when pre-treated with QCT, brought pregnancy percentage, mean litter size, and sex ratio percentage closer to the control level.

Table 5. *Fertility test of control and treated groups (n = 6)*

Groups	Pregnant female rats (%)	Total No. of male newborns	Total No. of female newborns	Mean litter size	Total sex ratio (%)
Control	100 ± 0.00	46 ± 0.78	43 ± 1.09	9.88 ± 1.05	52.01 ± 8.58
TCDD	66.66 ± 0.50*	21 ± 1.80*	30 ± 2.64	5.66 ± 4.38*	27.62 ± 21.14*
QCT	100 ± 0.00#	50 ± 1.50##	42 ± 1.00	10.22 ± 1.64#	53.98 ± 10.26#
QCT-TCDD	88.88 ± 0.33	42 ± 2.12#	35 ± 1.69	8.55 ± 3.46	48.27 ± 19.83
TCDD-QCT	88.88 ± 0.33	38 ± 1.92	36 ± 1.65	8.22 ± 3.30	40.47 ± 24.15

Discussion

Male reproductive disorders, such as a diminution in sperm quality and quantity, have shown an increasing trend in recent years. Several environmental/lifestyle factors (like exposure to dioxins, traffic exhaust fumes, smoking, and obesity) appear to negatively affect both the perinatal and adult testes (Sharpe, 2010; Wang et al., 2017). TCDD was found to increase oxidative stress and inhibit the activity of the antioxidant enzymes in testis and influences the serum level of testosterone hormone. It has also been reported that TCDD decelerates the progressive motility of epididymal sperm (Dhanabalan et al., 2015).

The current data showed the beneficial properties of QCT on TCDD induced reproductive impairment in male rats. We found a reduction in the sperm characteristics of TCDD treated rats compared to control rats. TCDD induced significant oxidative disorders in the testis tissue. Administration of QCT to rats received TCDD markedly decreased the reproductive damage and its oxidative stress induced by TCDD. Similarly, other researchers (Ciftci et al., 2012) found that TCDD produced oxidative stress in rat testes tissue. In addition to significantly decreasing serum testosterone levels, sperm count with its motility as well as increasing abnormal sperm percentage and testicular damages with TCDD dosing. It was thought that spermatological effects of TCDD might be due to its oxidative and histopathological effects.

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The results of the study showed that body weight in TCDD group less gained in comparison to control rats and also GSI and ESI in the rats treated with TCDD decreased. The results of the study are in agreement with previous findings obtained by Seefeld et al. (1984) and Ciftci et al. (2010). They showed that oral TCDD administration decreased body weight compared to control rats. However, Awal et al. (2001) reported that in male rats, exposure to TCDD during adulthood resulted in a reduction in the weights of testes and accessory sex organs, but it still remains unclear which cells of the testis are particularly affected by TCDD. This adverse effects of TCDD, including those related to the endocrine-disrupting activities (Le Magueresse-Battistoni et al., 2017; Decherf and Demeneix, 2011). While, pre and post-treatment with QCT could improve the gonad and body weight in the rats treated with TCDD.

Furthermore, antioxidant defence systems consist of SOD, glutathione peroxidase (GPx), CAT (enzymatic), and GSH (non-enzymatic) protects the cell against oxidative damage in normal physiological conditions. TCDD treatment caused a significant increase in testicular MDA levels in the present study with a decrease in testicular SOD, GSH, and CAT activity compared to the control group. An increased MDA concentration is maybe a consequence of the reduced production of antioxidants in the TCDD treated rats' tissues thereby shifting the accurate balance in favour of ROS, thus leading to an excess of pathologic damage to sperm cells and coincident loss of function (Oyeyipo et al., 2014). The antioxidant superoxide dismutase enzyme dramatically speeds up the dismutation of the superoxide anion to hydrogen peroxide (H_2O_2). Catalase breaks down H_2O_2 to H_2O and O_2, and the glutathione peroxidase/reductase system catalyzes H_2O_2 and lipid hydroperoxide degradation by reduced glutathione. Reducing the activity of SOD, CAT, GSH-R, and GPx could reflect the negative effect of TCDD on antioxidant enzymes in epididymal sperm (Latchoumycandane et al., 2003). Due to the direct damaging effect of TCDD, reduced activity of one or more antioxidant systems leads to increased lipid peroxidation and oxidative stress, resulting in testicular and sperm toxicity.

As pointed out in the introduction that TCDD binds to an AhR in the cytoplasm, the receptor complex (AhR-TCDD) translocates into the nucleus, where AhR heterodimerizes with the Ah receptor nuclear translocator (ARNT), connects to dioxin-responsive enhancer elements and regulates the transcription of target genes (Baba et al., 2005). The mechanisms of TCDD-induced disorders in the reproductive system of mammals have been shown to be multiple, including impairment of spermatogenesis (Foster et al., 2010), steroidogenesis (Adamsson et al., 2009), dropping in the number of testicular cells (Johnson et al., 1994), and induction of cytochrome P450 (CYP) 1 family enzymes, leading to inactivation of steroid hormones (Badawi et al., 2000). The treatment of QCT ameliorated these detrimental effects. These results corroborate the previous findings of Ciftci et al. (2012) and El-Gerbed et al. (2015). Ciftci et al. (2012) reported that QCT could reduce testicular damage caused by TCDD in rats. El-Gerbed et al. (2015) said that exposure to TCDD triggered semen quality to deteriorate, a reduction in fertility indexes, decreased the activity of serum enzymes, and decreased testosterone, in addition to histological changes in the tests. The team also highlighted the effect of QCT in reducing the adverse impact generated by TCDD.

Our results showed decreased male/female ratio in TCDD group rats compared with control group rats. Several studies have shown that exposure to TCDD in humans and animal reduces the male sex ratio of offspring (You et al., 2018; Mocarelli et al., 2000; Rowlands et al., 2006; Ishihara et al., 2007). James (2006) supposes that before

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conception, elevated gonadotropin and low levels of testosterone would have injured and distorted Y- bearing gametes. Song et al. (2018) found that Y chromosome-bearing human spermatozoa were more susceptible to EDCs than spermatozoa with the X chromosome. Ishihara et al. (2010) investigated the effects on sex ratio, sperm concentration, and motility of TCDD exposure. They found that TCDD leads to decreasing Y-bearing/X-bearing sperm ratio. Although it is still unclear why Y spermatozoa are more susceptible to high TCDD levels than X spermatozoa, You et al. (2018) proposed that after exposure to stressful conditions such as endocrine-disrupting chemicals (EDCs), the fertilization capacity according to their chromosome constitution may differ. Interestingly, Y spermatozoa survived at high TCDD concentrations for a shorter time than X spermatozoa. Furthermore, the reduced male sex ratio of births was related to the limited lifespan of Y spermatozoa and they attributed that to the antiandrogenic properties in utero or during lactation in adult rats that might be exposed to dioxin-like compounds and polychlorinated biphenyls (PCBs), leading to permanently changed sperm-transit time through the epididymis and the extra-testicular excurrent duct system (Jongbloet et al., 2002).

Conclusion

In summary, that we really found that rats subjected to TCDD showed reduced sexual activity, low motility with sperm count inhibition, viability, decreased antioxidant enzymes activity, additionally increased sperm abnormality and oxidative biomarker (MDA) in testis tissue. QCT treatment could minimize the effects of TCDD in rats, in part by improving sexual performance and eliminating oxidative damage. Thus, these results suggested that QCT could be used as protective agents against reproductive toxicity induced by TCDD in male rats. Further studies are required to clarify the mechanism of QCT as a therapeutic agent against the toxic effects of TCDD.

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