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2, 3, 7, 8-tetrachloro-dibenzo-p-dioxin induced testicular toxicity in rats and the protective effect of quercetin: Biochemical, histopathological and immunohistochemical studies

Mohamed SA. El-Gerbed[1], Ahmed M. Abu El-Saad[2], Abdullah Bedeer Haussein[3]

[1]Zoology Department, Faculty of Science, Damenhour University, Damenhour, Egypt. [2]Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt. Department of Biology, College of Medicine, University of Dammam, Kingdom Saudi Arabia, 3Department of Zoology, Faculty of Science, Mansura University, Mansura, Egypt. Department of Biology, College of Medicine, University of Dammam, Kingdom Saudi Arabia.

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ABSTRACT

Humans and animals are most sensitive to toxicant exposure during development. Dioxin, as an endocrine disruptor, is known to impair testicular functions and fertility. The present study was carried out to investigate the effects of quercetin on TCDD-induced toxicity in the testicular tissue of rats. Forty male albino rats were randomly divided into four groups (n = 10/group). Group I represent the control group; Group II administrated TCDD (27.5 µg/kg) via gavage for four week; Group III received quercetin (20 mg/kg bw.) Via gavage before TCDD administration; Group IV received quercetin alone (20 mg/kg bw). Biochemical markers included levels of testicular malondialdehyde formation and reduced glutathione as well as monitoring the activities of testicular superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase were studied. Also, serum hormonal profiles of luteinizing hormone and testosterone were reported. Our results show that administration of TCDD induces testicular damage concerning oxidative stress parameters, serum hormone level and sperm parameters. In addition, the microscopic structures of the testis, including histological and immunohistochemical studies were evaluated. Exposure to TCDD induces histopathological changes in rats testis including degeneration of seminiferous tubules, tubular necrosis, intratubular vacuolization, widened lumen and deshaped germ cells. Marked increase of apoptotic activity was observed. Also, our results clearly demonstrate the ameliorative potential of quercetin in dioxin induced testicular damage.

INTRODUCTION

There has been increased awareness of the possible effects of environmental contaminants on male reproduction. An environmental agent should disrupt reproductive function in the male at several potential target sites, the most important being the testes, the male gonads, which are the sites of spermatogenesis and androgen production (Rebourcet et al., 2010). The risk to human health posed by exposure to these persistent organic pollutants has been one of worldwide concerns (Humblet et al., 2008; Collins et al., 2009). Numerous environmental toxicants have been shown to adversely affect spermatogenesis in rodents and humans which can lead to low sperm count, abnormal sperm morphology and poor semen quality (Sharpe et al., 2010; Wong et al., 2010).

2, 3, 7, 8-tetrachloro-dibenzo-p-dioxin (TCDD) is the by-products of various industrial processes (i.e., bleaching of paper pulp and the manufacture of chemicals and pesticides) and combustion activities (i.e., burning household trash, forest fires, and waste incineration). Dioxins are found at low levels throughout the world in air, soil, water, and sediment and in foods such as dairy products, meats, fish, and shellfish (Pavan et al., 2006). Dioxins with very slow biodegradability have long persisted in the environment. These compounds can be concentrated in the adipose tissues of animals in the food chain, and are often found in human breast milk (Sharara et al., 1998).

Among various TCDD toxicities, reproductive toxicity may be seen in both males and females. The testis has been shown to be one of the sensitive targets of dioxin (Ohsako et al., 2001). The toxic effects of TCDD in male reproductive systems include a reduction in the size of the testes, prostate gland and seminal vesicle, decrease in sperm count, as well as an increase in the number of abnormal sperm (Latchoumycandane et al., 2003). It has also been reported that TCDD causes atrophy and testicular damage and the study suggested that Sertoli cells might be a target for TCDD in guinea pig testes, which in turn leads to changes in

* *Corresponding Author*
Email: m.elgerbed@yahoo.com

the germ cells (Kim et al., 1999).

Flavonoids are polyphenolic compounds with strong antioxidant properties widely distributed in dietary fruits; vegetables and herb medicine (Anjaneyulu et al., 2004). It is particularly abundant in onions (0.3 mg/g fresh weight) and tea (10–25 mg/L) (Scalbert et al., 2000). It has been reported that quercetin possess a number of biological effects such as its antioxidant properties against many diseases, including ischemic heart disease, atherosclerosis, liver fibrosis, renal injury and biliary obstruction (Ghosh et al., 2009; Liu et al., 2010). Also, quercetin possesses a lot of activities including anti-oxidative activity (Abdel-Raheem et al., 2009), anti-inflammation (Morales et al., 2006) and anti-apoptosis (Liu et al., 2010). Increasing evidence shows that quercetin can protect kidney from injury induced by nephrotoxins (Morales et al., 2006). And exhibits favorable hepatoprotective effect against ethanol hepatotoxicity by counteracting oxidative stress *in vivo* (Chen, 2010) and *in vitro* (Yao et al., 2008). Flavonoids can prevent oxidative damage as a result of their ability to scavenge reactive oxygen species such as hydroxyl radical and superoxide anion (Galati et al., 2002) and metal chelating (Pedrielli et al., 2002). The present investigation was carried out to explore the protective effect and the possible ameliorative role of quercetin on TCDD -induced reproductive toxicity.

MATERIALS AND METHODS

Chemicals

TCDD was obtained from Accu Standard Inc. (New Haven, CT, USA). Quercetin and dimethyl sulfoxide (DMSO) was supplied by Merck (Germany). Olive oil and all other chemicals were supplied by Sigma Chemicals (St. Louis, MO, USA).

Animals

Adult sexually mature male albino rats (weighing 170 ± 6g; 90 days old) were purchased from the animal breeding colony of the High Institute of Public Health, Alexandria University, Alexandria, Egypt, were used. Rats were housed in clean polypropylene cages, and allowed to acclimatize to their new environment for 14 days prior to initiation of treatment. Throughout the study, this experiment was performed in a controlled environment (maintained at 23 ±3 °C, a relative humidity of 60 ±10 °C, in 12-h light–dark cycle). They were fed standard rat chow and tap water *ad libitum*. Experiments were initiated only when the feeding and drinking of the rats had stabilized at the normal level.

Experimental design

Rats were randomized into four experimental groups of 10 males.

GroupI: The normal control group administration of olive oil once per day and administered *via* gavage for four weeks (28 days) of treatment.

Group II: The TCDD treatment group received 27.5µg / kg TCDD (2, 3, 7, 8-tetrachloro-dibenzo-p-dioxin once per day, which had been dissolved in acetone and then it was diluted with corn oil (1:6, v/v) in volumes of 5 ml/kg body weight. The acetone was evaporated under nitrogen before administration. TCDD was orally administered *via* gavage for four weeks. The test dose was determined on the basis of findings from previous studies (Pohjanvirta et al., 1993).

Group III: Co- treated TCDD with quercetin, rats were given quercetin dissolved in 0.5% DMSO (20 mg/kg bw per day) once per day *via* gavage (Kalender et al., 2012). Thirty minutes later, TCDD dissolved in olive oil (27.5 µg/ml) was administered, also *via* gavage for four weeks of treatment.

Group IV: administration for the quercetin treated group, the rats were given quercetin dissolved in 0.5% DMSO at a dose of 20 mg/kg bw once per day *via* gavage for four week of treatment.

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Local Committee on Animal Research of the Alexandria University, Alexandria, Egypt.

Tissue preparation

At the end of the fourth week of treatment, testes of each rat were quickly excised, rinsed with a cold 1.15% potassium chloride, and weighed. One testis was homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride and the homogenate was centrifuged at 10,000 g for 15 min at 4 ºC and the supernatant immediately collected and processed for biochemical studies and enzyme activity assays.

Lipid peroxidation assay

Lipid peroxidation process is determined by the thiobarbituric acid (TBA) method which estimates the malondialdehyde formation (MDA) according to Nair and Turner (1984). Briefly, a 0.33 ml of testes homogenate was mixed well with 3 ml of TBA reagent. The mixture was incubated for 20 min in a boiling water bath. After cooling, the mixture was centrifuged at 3000g for 20 min. The supernatant was measured at 532 nm. Lipid peroxidation is expressed as n moles MDA/g tissue.

Reduced glutathione (GSH) assay

Levels of GSH were determined in the testes homogenates (10%) according to the Ellman (1959). One milliliter of supernatant was treated with 0.5 ml of Ellman's reagent (19.8mg of 5, 5`dithiobisnitro benzoic acid in 100 ml of 0.1% sodium citrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm in spectrophotometer. To prevent the autoxidation of GSH, the samples were reduced with

potassium borohydride prior to analysis (Kleinman and Richie, 2001).

Enzyme Assays

Superoxide dismutase (SOD) activity was determined by the method of Kakkar et al. (1984). Superoxide radicals react with nitroblue tetrazolium in the presence of NADH and produce formazan blue. SOD removes the superoxide radicals and inhibits the formation of formazan blue. The intensity of colour is inversely proportional to the activity of the enzyme.

The activity of catalase (CAT) was determined by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. Glutathione peroxidase (GSH-Px) was estimated by the method of Rotruck et al. (1973). A known amount of enzyme preparation was allowed to react with hydrogen peroxide in the presence of GSH for a specified time period. Then the remaining GSH was measured by the method of (Ellman, 1959).

The glutathione *S*-transferase (GST) activity was determined spectrophotometrically by the method of Habig et al. (1974) in which 1-chloro-2, 4-dinitrobenzenewas used as a substrate. Lactate dehydrogenase (LDH) activity was determined by the method of Wahlefeld (1983) using sodium-lactate and NAD as the substrate. The reaction was initiated by the addition of the substrate and the increase in absorbance at 340 nm resulting from the formation of NADH was used for the calculation for LDH activity.

Ascorbate determination

Ascorbate was determined according to Omaye et al. (1997) and expressed as mmol ascorbic acid/g wet tissue.

Protein assay

Contents of protein were measured in the testicular homogenates (10%) according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

Evaluation of sperm characteristics

The spermatozoa were counted by the method described by Yokoi et al. (2003). Testicular sperm head numbers were assessed by the procedure of Meistrich and Van Beek (1993) to evaluate the numbers of mature elongate spermatids in the testis. The progressive spermatozoa motility was evaluated according to the method described by Zemjanis (1970). An aliquot of sperm suspension was stained using Wells and Awa's (0.2 g of eosin and 0.6 g of fast green dissolved in distilled water and ethanol in ratio 2:1) for sperm morphology and live-dead examination (Wells and Awa, 1970). Also, Two thousand sperms were evaluated on each slide and results were recorded as percentage of abnormal sperm for each slide. Abnormal heads and tails were evaluated using the criteria by Okamura et al. (2005) .

Hormonal assays

The blood samples were obtained from the animals by heart puncture. The serum level of testosterone was determined according to Abraham (1970); luteinizing hormone was detected according to Jaffe and Behrman (1974).

Histopathological examination

To assess histological changes, the fixed testicular tissues from 10 surviving rats in each group were paraffin embedded. Then, they were prepared as 4-mm-thick sections using a microtome. They were double stained with hematoxylin and eosin (Bancroft and Cook, 1994) and were observed by light microscope.

PCNA immunoreactivity (PCNA-ir)

Testicular distribution of PCNA receptor subunits were examined in deparaffinized sections (5 µm) of Bouin's fixed testes of rats using an Avidin-Biotin-Peroxidase (ABC) immunohistochemical method (Elite–ABC, Vector Laboratories, CA, USA) against PCNA (dilution 1:100, DAKO Japan Co, Ltd, Tokyo, Japan) were employed (Tousson et al., 2011) .

PCNA-Labeling Index (PCNA-LI)

Slides were examined under the light microscope with a magnification X 200 and thin sections were evaluated for PCNA immunostaining. Microscopic fields were chosen at random. Five fields per slide were evaluated. Only the basal germ cells of the seminiferous tubules were counted as the PCNA-LI for each seminiferous tubule and was estimated as a percentage of immunolabeled cells (cells with brown nuclear staining was positive PCNA-ir) to all basal cells according to (Tousson et al., 2011).

Statistical analysis

The data were expressed as the mean ± SE and were analyzed using SPSS 11.0 for windows. Statistical significance was calculated using one-way analysis of variance (ANOVA). Statistical evaluation of data was done following DMRT procedure for multiple comparisons. A difference was considered significant at $p < 0.05$.

RESULTS

Clinical findings

Death was not observed in any of the experimental groups during the treatment period. However, in dioxin treated group, few clinical signs such as alterations in feeding and drinking behaviors, wasting syndrome, reduced activity, increasing weakness, slight diarrhea and fur loss were observed.

Markers of oxidative damage

Testicular MDA concentration was significantly ($p < 0.05$) increased in the testis of rats treated with TCDD. In the quercetin + TCDD-treated animals, MDA level was significantly ($p < 0.05$) decreased compared with animals administered TCDD

alone (Table 1). Our results indicated that the GSH content was decreased in TCDD or quercetin exposure alone (Table 1).

Table 1: Effect of quercetin on the lipid peroxidation levels and antioxidant defence system in testicular tissue of rats treated with TCDD.

Parameters	Control	TCDD	QE + TCDD	QE
MDA (nmol/g tissue)	26.74 ±2.11 [a]	37.44 ± 4.15 [b]	32.35 ± 3.25 [c]	30.26 ± 4.23 [a]
GSH (mg/g tissue)	77.25 ± 5.21 [a]	53.72 ±3.14 [b]	31.26 ± 2.44 [c]	56.22 ±4.11 [b]
Ascorbate (µg/ml/g tissue)	10.38 ±2.13 [a]	11.34 ± 2.16 [a]	11.82 ± 2.45 [a]	15.37 ± 3.22 [b]

Values are mean ± SE for ten rats in each group. Values within rows with no common superscripts are significantly different (LSD multiple range test, p<0.05).

Table 2: Effect of TCDD and quercetin on spermatozoa parameters.

Parameters	Control	TCDD	QE + TCDD	QE
Sperm count (x10^6/g)	43.81 ± 3.34 [a]	28.17 ± 2.12 [b]	31.26 ± 2.44 [b]	38.74 ± 2.13 [a]
Sperm motility (%)	82.14 ± 6.41 [a]	49.32 ± 4.26 [b]	54.73 ± 3.26 [c]	77.67 ± 5.26 [a]
Dead sperms (%)	3.11 ± 0.58 [a]	33.12 ± 2.21 [b]	27.24 ± 2.31 [c]	5.33 ± 0.31 [a]
Abnormal sperm rate (%)	5.25 ± 0.62 [a]	17.23 ± 1.65 [b]	15.40 ± 0.85 [b]	6.81 ± 0.54 [a]

Values are mean ± SE for ten rats in each group. Values within rows with no common superscripts are significantly different (LSD multiple range test, p<0.05).

TCDD group and up to 27.2 % in the quercetin group relative to the corresponding control. Moreover, the TCDD-induced decrease of GSH in the testicular tissue was partially prevented by quercetin. The results shown in table 1 indicate that ascorbate content remained unaltered in the testicular tissue either when TCDD was administered alone or when co-administered with quercetin. The administration of quercetin alone had the tendency to increase ascorbate content in the testis.

Enzyme assay

The results obtained in this study show that TCDD exposure significantly ($p < 0.05$) decreased SOD activity but in the quercetin +TCDD animals, the SOD activity showed a significant reversal to the control value in the testicular tissue (Fig.1). In animals that were administered quercetin alone, the testicular SOD activity was increased when compared with the corresponding control. Testicular CAT activity was significantly decreased ($p < 0.05$) in TCDD intoxicated rats. However, co-administration with quercetin showed a restorative response of CAT activity in the testes as values returned to that of control (Fig.2). A significant increase ($p < 0.05$) in the activity of GSH-Px was observed in the testis in animals exposed to TCDD. In the quercetin +TCDD animals, this increase subsided, but was still higher than the corresponding control value in the testicular tissue. Quercetin alone increased the GSH-Px activity beyond the control value ($p < 0.05$) (Fig.3). GST activity in the testicular tissue was significantly decreased in animals exposed to TCDD. Co-administration with quercetin partially restored GST activity. However, quercetin administration alone had no effect on GST activity (Fig.4). Moreover, LDH activity increased significantly ($p < 0.05$) in the testicular tissue by TCDD exposure. Co-administration with quercetin normalized LDH to the control value. Interestingly, animals that received quercetin alone showed an increase in testicular LDH activity (Fig.5).

Spermatological analysis

Administration of TCDD reduced the number of sperms in the testis. The motility of the spermatozoa dropped below the control value by 40 %. A significant increase in the number of variables in the quercetin + TCDD animals were not normalized to the corresponding control value. Although, there was a tendency for the recovery in the spermatozoa viability (vs TCDD. group, $p < 0.05$), quercetin administered alone had no effects on these parameters of spermatozoa function (Table 2).

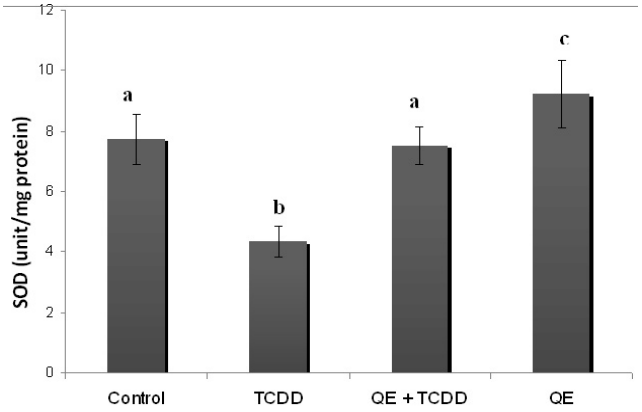

Fig. 1: Effects of quercetin on TCDD-induced changes in superoxide dismutase (SOD) activity in the testis of rats. Values are mean ± SE; n=7. Values not sharing common superscript letters (a-c) differ significantly at $p<0.05$ (DMRT).

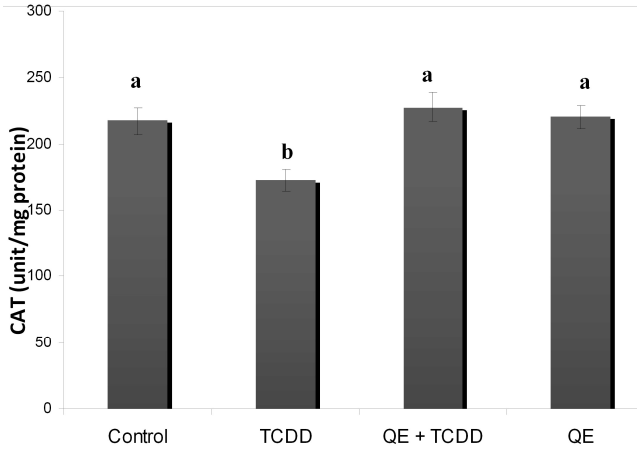

Fig. 2: Effects of quercetin on TCDD-induced changes in catalase (CAT) activity in the testis of rats. Values are mean ± SE; n=7. Values not sharing common superscript letters (a-c) differ significantly at $p<0.05$ (DMRT).

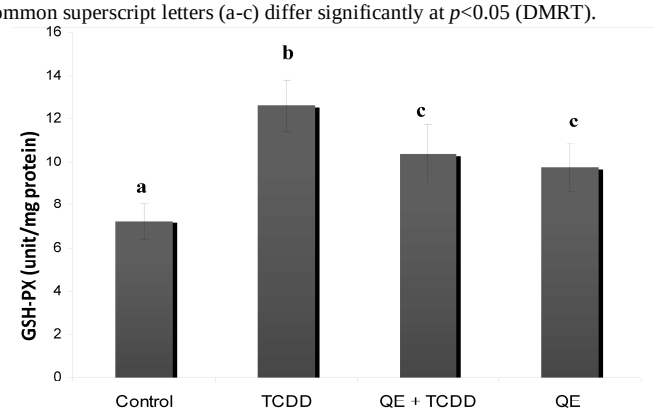

Fig. 3: Effects of quercetin on TCDD-induced changes in Glutathione peroxidase (GSH-Px) activity in the testis of rats. Values are mean ± SE; n=7. Values not sharing common superscript letters (a-c) differ significantly at $p<0.05$ (DMRT).

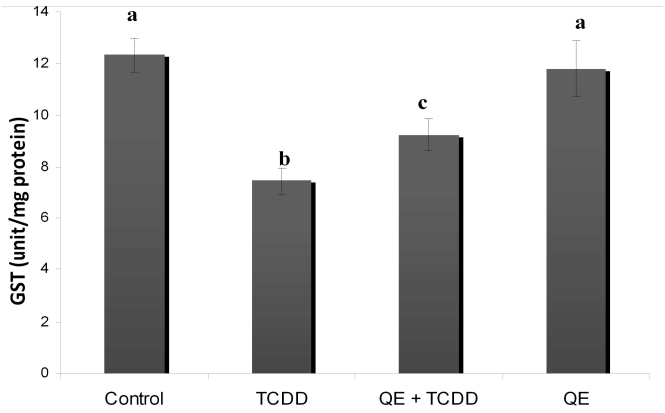

Fig. 4: Effects of quercetin on TCDD-induced changes in glutathione *S*-transferase (GST) activity in the testis of rats. Values are mean ± SE; n=7. Values not sharing common superscript letters (a-c) differ significantly at $p<0.05$ (DMRT).

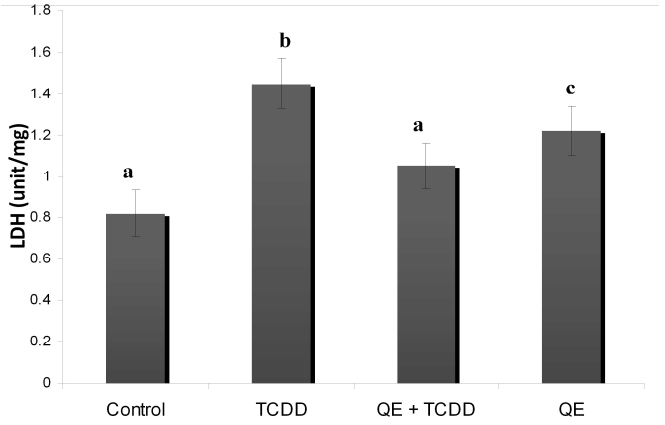

Fig. 5: Effects of quercetin on TCDD-induced changes in Lactate dehydrogenase (LDH) activity in the testis of rats. Values are mean ± SE; n=7. Values not sharing common superscript letters (a-c) differ significantly at $p<0.05$ (DMRT).

Evaluation of Testosterone hormone and luteinizing hormone

Treating animals with TCDD for 4 weeks showed significant reduction ($P < 0.05$) in serum testosterone hormone and luteinizing hormone in comparison with control group (Fig.6 a&b). Treatment with TCDD + Quercetin caused significant increase ($P < 0.001$) in serum testosterone and LH in comparison with rats treated with TCDD. There was insignificant increase in levels of these hormones in sera of animals treated with quercetin alone in comparison with control group.

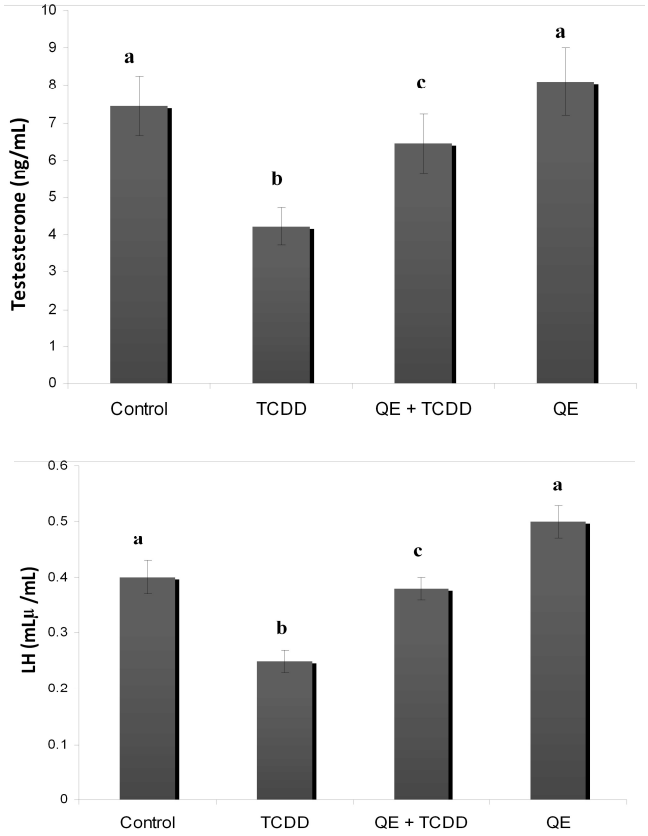

Fig. 6: Effects of quercetin on TCDD-induced changes in serum level of (a) testosterone hormone and (b) luteinizing hormone in rats. Values are mean ± SE; n=7. Values not sharing common superscript letters (a-c) differ significantly at $p<0.05$ (DMRT).

Histopathological findings

Figures (7A–D) shows photomicrographs of testes from the different experimental groups. In control and quercetin groups, there was no evidence of testicular abnormality. Light microscopic examination revealed normal cytoarchitecture and maturation of germinal epithelium. The seminiferous tubules appeared uniform in size and shape. Leydig cells and blood vessels were found in the interstitial connective tissue between the tubules. A regular arrangement of spermatogenic epithelium existed in seminiferous tubules (Fig. 7A&D).

Testicular histopathology showed a detectable effect of TCDD on spermatogenesis (Fig. 7B) at the end of 4th week. Administration of dioxin caused severe histopathologic lesion. Seminiferous tubules showed loss of normal histoarchitecture widely separated from each other with loose arrangements due to reduction in diameter and atrophy of the tubules. The majority of

the seminiferous tubules possessed irregular or ruptured basement membranes. Testicular atrophy leading to degenerative changes in the germinal epithelium, the testes with partial loss of the spermatogenic cells, Moreover, the germ cell arrangement within the seminiferous tubules was disrupted by exposure to dioxin. In the group III (quercetin plus TCDD) treated group, the majority of the seminiferous tubules possessed regular basement membranes with normal histoarchitecture, These tubules with no evidence of testicular abnormalities (Fig. 7 C)**.**

Immunohistochemical results

Only the spermatogonia in control and quercetin groups (GI and GIV respectively) showed a positive strong reaction for PCNA-ir while the other spermatogenic cell types showed negative reaction (Fig.8 A-D). The lumen of the seminiferous tubules in the control and quercetin groups was fully packed with sperms that showed a positive reaction for PCNA-ir (Fig.8 A&D). Testes section in rats that treated with TCDD (G II) showed decreased in the numbers of spermatogonia that have PCNA-ir positive reaction as compared to control group (Fig.8 B). Testes section in co-treated TCDD with quercetin showed increased in the numbers of spermatogonia that have PCNA-ir positive reaction as compared to TCDD group (Fig.8 C). PCNA labeling index (PCNA-ir positive germ cells per tubule cross-section) were significantly decreased following TCDD treatment group (53±6%) in comparison to the control group (79±4%). On the other hand, PCNA labeling index were increased in testes section in co-treated TCDD with quercetin (63±5%) as compared to TCDD treatment group (53±6%) and decreased when compared with quercetin group (71±5 %).

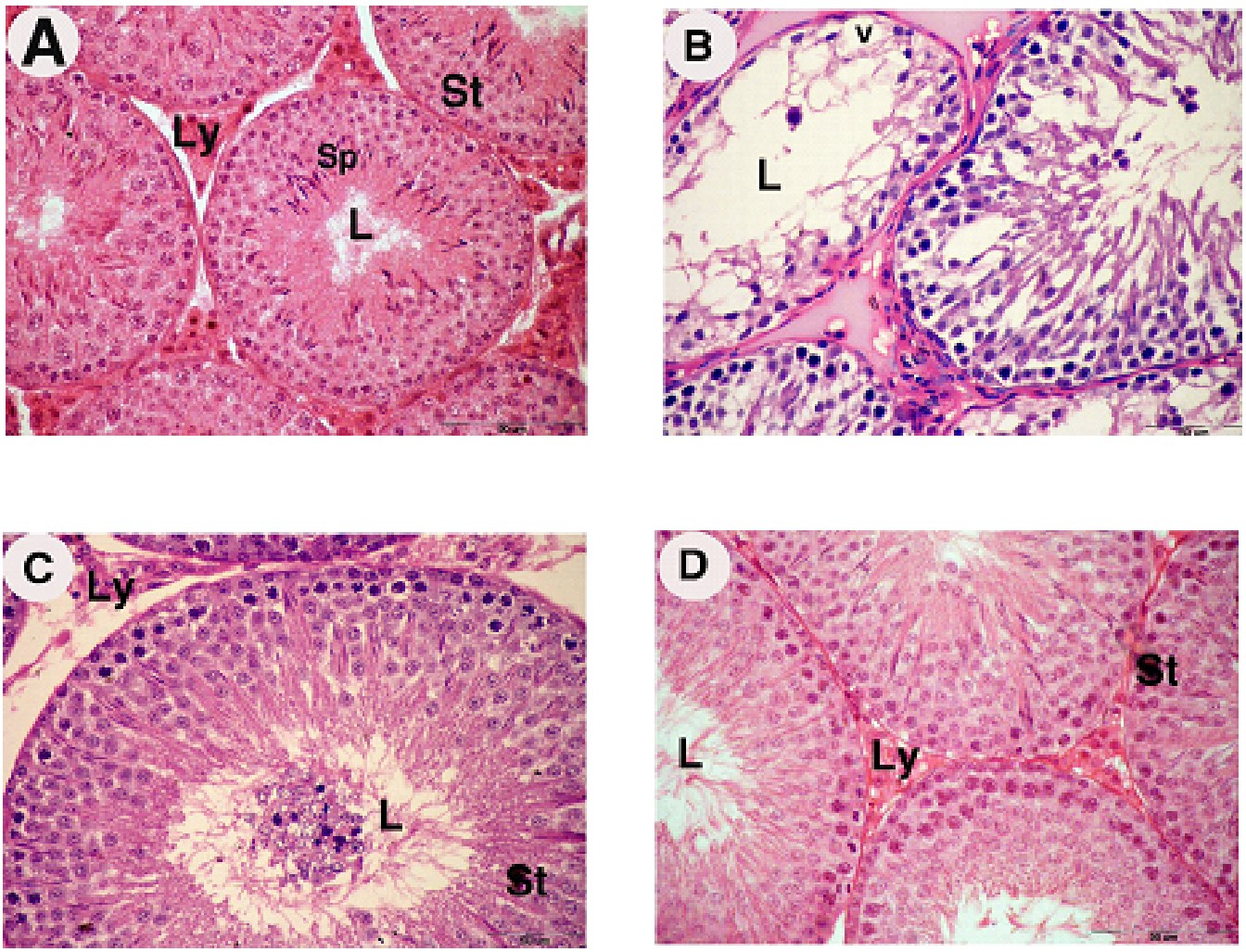

Fig. 7: Photomicrographs of testicular sections from control and other experimental groups. In control (Fig. A) and, quercetin (Fig. D) - treated groups showing The seminiferous tubules were well developed with active spermatogenesis without any structural changes. Section of testes from rat treated with TCDD (Fig.B). Seminiferous tubules possess irregular basal lamina and are separated from each other. Also, wide gaps between neighboring cells and enlargement of the intercellular spaces. Interstitial tissues showed edema, hemorrhage and vacuolation. Many seminiferous tubules were edematous with intact germinal layer and undergoing degeneration along with loss of spermatogenesis. Cellular alterations at different spermatogenic stages. In quercetin plus TCDD (Fig.C) treated groups, the normal tubules were seen, Abbrev: seminiferous tubules (St), elongated spermatids (Sp), Leydig cells (Ly), lumen (L), vacuole (V)

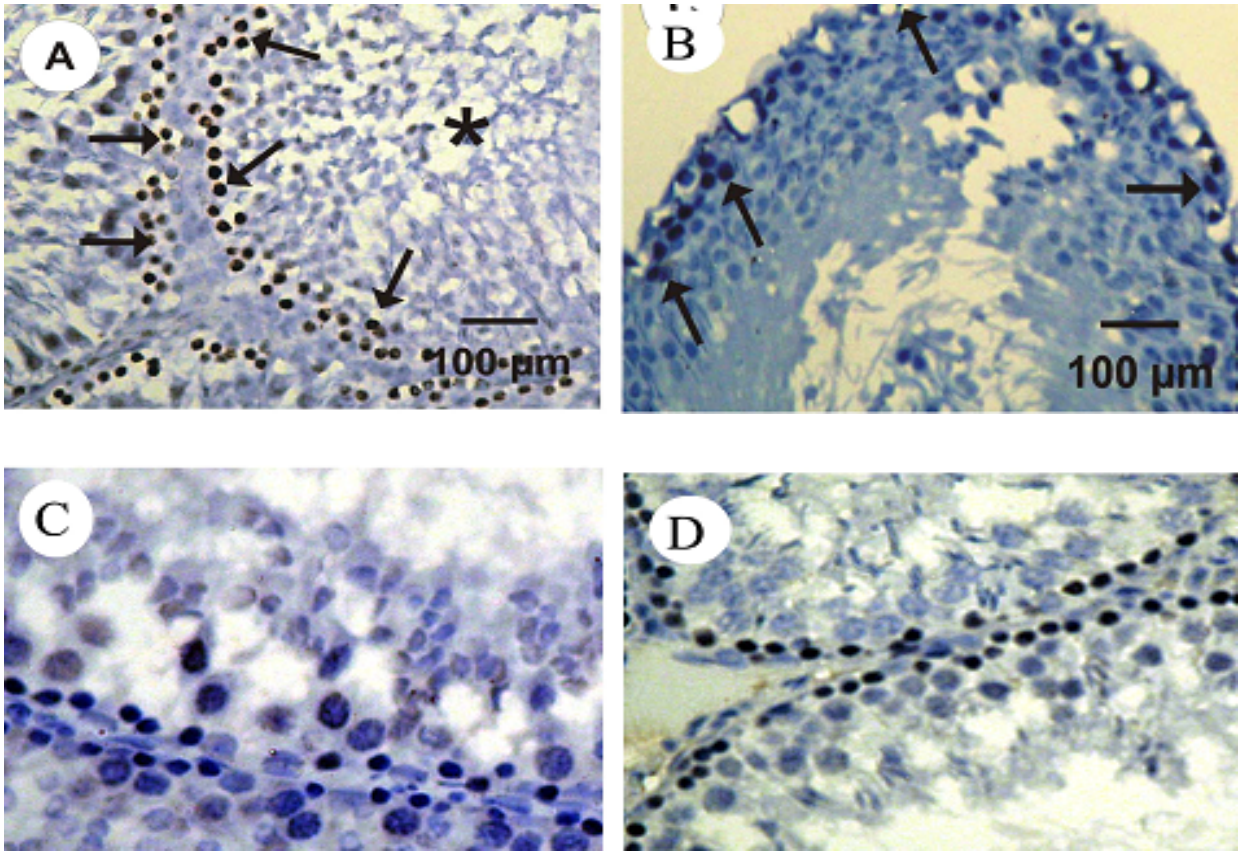

Fig. 8: Photomicrographs of PCNA-ir (immunoperoxidase labelling) in the testes cross sections from control and other experimental groups. Control (Fig. A) and quercetin (Fig. D) - treated groups showing different stages of spermatogenic cells in the seminiferous tubules with normal distribution of spermatogonia in seminiferous tubules of rats were seen. (Fig. B) Section of testes from rat treated with TCDD, showing only a few numbers of spermatogonia have negative reaction for PCNA-ir. Note that the lumen of the tubules was clear and the spermatozoa were not seen. (Fig. C) showings, few numbers of the spermatogonia in the seminiferous tubules have positive reaction for PCNA. Note, normal distributions of spermatogonia in seminiferous tubules of quercetin plus TCDD rats were seen.

DISCUSSION

Reproductive toxicology has been receiving increasing interest and concern in recent years (Amin, 2008). Environmental contaminants are known to induce reproductive toxicity by perturbing the prooxidant and antioxidant balance, which could induce oxidative stress (Elissa et al., 2011). TCDD is one of such environmental contaminants and has been shown to induce reproductive abnormalities in both wild life and humans by reduction in fertility (Sharpe et al., 2011).

Lipid peroxidation is a process of oxidative degradation of polyunsaturated fatty acids that result in impaired membrane structure and function (Goel et al., 2005). ROS, otherwise damage cellular lipids by binding to membrane anionic phospholipids (Sayed-Ahmed and Nagi, 2007), protein and DNA, finally the entire cell (Matés, 2000). Therefore, the increase in the testicular MDA in the present study could be due to the concomitant increase in the generation of ROS in TCDD treated group. Recent studies have shown that TCDD induced oxidative stress in mouse testis by decreasing enzymatic antioxidants, which resulted in enhanced level of ROS. This could impair testicular function in the

mice (Shi et al., 2008). These data are consistent with our findings. TCDD treatment increased ROS generation and LPO and decreased the specific activities of cellular antioxidant enzymes such as SOD, CAT, GST and the non enzymatic antioxidant GSH, indicating an imbalance in prooxidant and antioxidant system, leading to oxidative stress. ROS generated can cause DNA damage by directly attacking DNA or by activation of endonucleases that degrade DNA, which ultimately contribute to cell death (Floyd and Carney, 1992). Cells are equipped with antioxidant defense system to counteract the effect of ROS (Halliwell, 1994). Antioxidant enzymes are considered to be a primary defense that prevents biological macromolecules from oxidative damage. SOD is considered the first line of defense against deleterious effects of oxyradicals in the cell by catalyzing the conversion of superoxide anion to less dangerous H_2O_2, which is further degraded by CAT and GPx to water. CAT activity was significantly decreased. Decreased CAT activity may compromise the overall antioxidant enzyme defense system. CAT, along with GPx, exerts a major role in metabolizing hydrogen peroxide in cells and protecting cells against oxidative damage (Sun, 1990). Decrease in CAT levels

would result in accumulation of hydrogen peroxide. Also, CAT and GPx protect SOD against effects of hydrogen peroxide (Marklund and Marklund, 1974). The decrease in activities of enzymatic antioxidants in TCDD-treated group may be related to increased LPO and ROS production. GSH is important in the regulation of cellular redox and a decline in its level is considered to be a good indicator of oxidative stress (Bray et al., 1993). This observation supports our finding where of a decline in GSH levels as evidenced by increased LPO. The decrease in testicular GSH level might be in part attributed to the inhibition of GR activity, which is responsible for regeneration of GSH from its oxidized form. GR is known to be inactivated by superoxide anion and hence, testicular cells showing reduced SOD activity might have enhanced flux of superoxide radicals that could potentially damage GR and decrease GSH content in these germ cells. All these antioxidant enzymes and GSH are extensively used as an index of unbalanced reactive oxygen species production and oxidative stress in physiological systems (Matés, 2000). Also TCDD decreased the antioxidant activity and increased the levels of hydrogen dioxide and MDA in the testis tissue in mice (Latchoumycandane et al., 2002).

In the present study, cell damage was evaluated by increase the activity of testicular lactate dehydrogenase in TCDD treated group. The hypersecretion of lactate may indicate a disturbance in the regulatory mechanism of intermediary metabolic pathway of Sertoli cells, finally resulting in detrimental effect on developing germ cells (Mita et al., 1982). However, an increase of lactate secretion by TCDD indicates a deleterious effect on the cell that may lead to lactate deficiency and infertility (Trejo et al., 1995). An expected effect of TCDD seems to be disturbing the physiological supply of lactate to the developing germ cells (Courtens and Plöen, 1999). The increase in LDH activity in the testicular tissue is usually viewed as an evidence of increased glycolysis and/or cell cytotoxicty (Raychoudhury and Kubinski, 2003).

Luteinizing hormone secreted by the anterior pituitary can affects Sertoli cells and Leydig cells and, in turn, increase the secretion of testosterone from Leydig cells. The secretion of LH is regulated by hormones secreted from the testes. This feedback loop maintains a balance in the level of androgenic hormone secretion (Yao, 2005). In the present study, it was observed that serum testosterone and LH had been significantly decreased by TCDD administered for four weeks in contrast to that of the vehicle control group. This finding is consistent with the results obtained from previous studies in male rats (Bookstaff et al., 1990). However, testosterone and LH of the blood plasma were not affected in rats exposed to TCDD (Li et al., 1997). This difference in the toxic effect was presumed to be due to the dissimilarity of the dose, duration of exposure and the species of animal. In the present study, suppression of serum testosterone is accompanied by a significant decline in the activities of antioxidant enzymes and increase in the levels of lipid peroxidation in the testis indicating excess ROS generation and induction of oxidative stress. The observed decline in the serum levels of testosterone following TCDD exposure may be due to adverse effect on the Leydig cells. In addition, the inhibitory effects of TCDD on pituitary luteinizing hormone (LH) secretion (Young et al., 2004) could also have contributed to the observed decline in serum testosterone levels. Several environmental toxicants have been reported to impair the testicular steroidogenesis via disruption of steroidogenic acute regulatory (StAR) protein expression and or by altering the activities of steroidogenic enzymes like cytochrome P450scc, 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase (Saradha et al., 2008). Oxidative changes in primary cultured Leydig cells have been observed following exposure to 2-bromopropane, an intermediate used in the production of pesticides, and cadmium which resulted in decreased testosterone secretion (Yang et al., 2003). Exposure of adult male rats to a single intraperitoneal injection of TCDD resulted in a dose-dependent reduction in Leydig cell volume per testis, which indicates Leydig cell as a potential target for TCDD toxicity (Johnson et al., 1992). TCDD has been shown to inhibit human chorionic gonadotropin (hCG)-induced testosterone secretion in rat Leydig cell primary cultures by decreasing the expression of steroiodogenic enzymes (StAR, P450scc and 3β-hydroxysteroid dehydrogenase) and cellular levels of cAMP (Lai et al., 2005). TCDD has antiandrogenic effects on both humans and animals, and it could therefore affect the form and function of testes. TCDD inhibited the growth and differentiation of seminal vesicle epithelium during postnatal development (Hamm et al., 2005) and caused atrophy and decreases in the diameter of the seminiferous tubules and in the spermatogonial population (El-Sabeawy et al., 1998). It also caused the feminization of male mice and as well as their sexual dysfunction (Gray et al., 1997). An epidemiology study found that the testosterone level had a negative correlation with serum TCDD, and the number of spermatozoa decreased to half that of the normal levels in men exposed chronically to TCDD (Egeland et al., 1994). In the present study, we found that TCDD resulted in a decrease in the spermatozoa number and sperm motility and increase in dead and abnormal sperm rate indicative damage of the testis structure in rats. Most toxic effects induced by TCDD were mediated by binding to the AhR, which binds together with a second protein, aryl hydrocarbon receptor nuclear translocator, to the response elements of a number of target genes and thus modulates gene expression (Nau, 2006). The activated AhR induces the expression of various genes with xenobiotic response elements in their enhancer regions, such as the gene for cytochrome P450. Esser et al. (2005) found that TCDD interfered with the physiological signaling of the AhR, led to cell-specific changes in gene transcription and cell differentiation, and thereby producing toxic effects. TCDD combined with AhR, and thereby induced a change in the expression of monoamine oxidase related to CYP450 enzymes activated by AhR (Poland and Kimbrough, 1984). TCDD could have caused the decrease in testosterone by damaging the smooth endoplasmic reticulum or mitochondria of the Leydig cells, thereby interfering with enzymes involved in androgen synthesis (Roman et al., 1995).

Kim et al. (1999) reported that TCDD may cause testicular morphological lesions and a decrease in steroidogenesis. Similarly, in this study, treatment of TCDD caused morphological and immunohistochemical changes. Seminiferous tubules, where only the Sertoli cells exist with few germ cells found, were shown to be necrosed and completely damaged. Thus, it suggests that the toxicity of TCDD in the testicles caused abnormal spermatogenesis. There is much concern that exposure to environmental contaminants can produce major pathological effects in the reproductive systems of both humans and animals. 2, 3, 7, 8-TCDD is the most toxic environmental contaminant known and has been shown to induce reproductive abnormalities (Chia, 2000).

The current study showed histopathologic changes in the seminiferous tubules of testis ranging from vascular congestion to focally diffuse interstitial edema, focal areas of tubular degeneration and coagulative necrosis of spermatozoa were observed following dioxin administration to the experimental rats. Furthermore, interstitial tissue in the treated rats showed a relative increase in edema of the interstitium and congested blood vessels suggesting inflammation. In addition, there were apoptotic signs in Leydig cells. Later, seminiferous tubules were relatively widely separated from each other with fragments of degenerated peritubular tissue detected inbetween. In agreement with the present results, TCDD was found to produce atrophy, morphological changes, impaired spermatogenesis, and epididymal lesions in the testes of experimental animals (Al-Bayati et al., 1988). Further, when 2,3,7,8-TCDD was administered to rats, histopathologic examination revealed a decrease in the diameter of seminiferous tubules and the number of testicular sperm (El-Sabeawy et al., 1998).

Antioxidants are exogenous or endogenous compounds acting in several ways, including scavenging reactive oxygen species or their precursors, inhibiting ROS formation, and binding metal ions needed for the catalysis of ROS generation (Bhatt and Flora, 2009). Flavonoids can prevent oxidative damage as a result of their ability to scavenge reactive oxygen species such as hydroxyl radical and superoxide anion (Galati et al., 2002). The antioxidant properties of flavonoids depend on both metal-chelating properties and free radical scavenging of reactive oxygen species (Pedrielli and Skibsted, 2002). Quercetin is one of the most frequently studied dietary flavonoids, distributed in vegetables, fruits and many other dietary sources (Bhatt and Flora, 2009). Quercetin, a potent anti-oxidant, scavengers' free radicals directly, inhibits lipid peroxidation and alters anti-oxidant defence pathway *in vivo* and *in vitro* (Anjaneyulu and Chopra, 2004). Previous studies have shown that flavonoids have protective effects against oxidative damage induced by organophosphorous pesticides (Panemangalore and Bebe, 2009; Sadowska, 2010). In addition, it has been reported that quercetin has protective effects on antioxidant enzyme metabolism and histopathological changes induced by chlorpyrifos in rat lung tissues (Uzun et al., 2010). Our results clearly showed that quercetin treatment successfully prevent oxidative and histological damage caused by TCDD. When TCDD and quercetin given together, quercetin reduced lipid peroxidation induced by TCDD treatment via decreasing the levels of MDA in testicular tissue of TCDD-treated rats. Although there are a few studies about the protective effect of quercetin against testicular toxicity by TCDD, there are many studies about the antioxidant properties of quercetin against lipid peroxidation (Pushpavalli et al., 2010). Recently Ciftci et al. (2012) demonstrated that quercetin induced the testicular antioxidant enzyme capacity in TCDD intoxicated rats. However, our results in the present work included a mutibiochemical markers concerning testicular damage such as assays of ascorbate content, GST, LDH activities, luteinizing hormone level, in addition to immunohistochemical studies which were not included in the previous work of Ciftci et al. (2012). Our results confirmed the findings of this earlier study concerning the toxic effects of TCDD in testicular tissue and protective role of quercetin. Renugadevi and Prabu (2009) determined that quercetin treatment significantly protected kidney tissue of rat against toxicity of cadmium by decreasing TBARS formation. These results are in agreement with our findings and confirmed our study. Quercetin treatment in the current study prevented a decrease in GSH level and the activities of SOD, CAT and GST in testis by TCDD. Similarly, Abdel-Raheem et al. (2009) showed as many other studies that quercetin treatment induced detoxifying enzymes and thereby prevented toxicity in different organs of rats induced by gentamicin. On the other hand, it was indicated that quercetin treatment together with TCDD prevented adverse effects of TCDD in terms of histological alternations. Similarly, Yousef et al. (2010) showed that quercetin treatment exerts potent protective effects in histological damage caused by different agents such as cadmium, paracetamol and ethanol. These beneficial effects (in terms of oxidative and immunohistological results) of quercetin on testicular toxicity of TCDD are related with their high antioxidant capacity.

In conclusion, the current study demonstrated that exposure to TCDD caused deterioration in semen quality, decrease of fertility indexes as well as alternation in lipid profile and decreased the enzyme activities of serum. Moreover, the testosterone decreased and there were many histological changes in the tests. Our results reveal that quercetin has a marked ameliorative effect on testis toxicity, as rats receiving this flavonoid in addition to TCDD. Therefore, the present study elucidated the therapeutic effects of quercetin administered in combination with TCDD to minimize its reproductive toxicity.

REFERENCES

Abdel-Raheem, I.T., Abdel-Ghany, A.A., Mohamed, G.A. Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats. Biol. Pharm. Bull. 2009; 32: 61–67.

Abraham, G. E. Handbook of radioimmunoassay. 1977; New York: Marcel Dekker, Inc.

Al-Bayati, Z.A., Wahba, Z.Z., Stohs, S.J. 2, 3, 7, 8-Tetrachlorodibenzo- p-dioxin (TCDD)-induced alterations in lipid peroxidation, enzymes, and divalent cations in rat testis. Xenobiotica. 1988; 18: 1281–1289.

Amin, A. Ketoconazole-induced testicular damage in rats reduced by Gentiana extract. Exp Toxicol Pathol. 2008; 59: 377–384

Anjaneyulu, M., Chopra, K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clinical and Experimental Pharmacology and Physiology. 2004; 31: 244–248.

Bancroft, J.D., Cook, H.C. Manual of histological techniques and their diagnostic application. Churchill Livingstone, Edinburgh, London, New York, Tokyo1994; 23-26.

Bhatt, K., Flora, S.J.S. Oral cp-administration of _-lipoic acid, quercetin and captopril prevents gallium arsenide toxicity in rats. Environmental Toxicology and Pharmacology. 2009; 28:140–146.

Bookstaff, R.C., Moore, R.W., Peterson, R.E. 2,3,7,8-tetrachlorodibenzo- p-dioxin increases the potency of androgens and estrogens as feedback inhibitors of luteinizing hormone secretion in male rats. Toxicol.Appl.Pharmacol. 1990; 104: 212–224.

Bray, T.M., Taylor, C.G. Tissue glutathione, nutrition, and oxidative stress. Can J Physiol Pharmacol. 1993; 71: 746–751.

Chen, X. Protective effects of quercetin on liver injury induced by ethanol. Pharmacogn. Mag. 2010; 6: 135–141.

Chia, S.E. Endocrine disruptors and male reproductive functions – a short review. Int J Androl. 2000; 23 (Suppl 2): 45–46.

Ciftci, O., Aydin, M., Ozdemir, I., Vardi, N. Quercetin prevents 2,3,7, 8 tetrachlorodibenzop- dioxin-induced testicular damage in rats. Andrologia. 2012; 44:164-173.

Collins, J.J., Bodner, K., Aylward, L.L., Wilken, M., Bodnar, C.M. Mortality rates among trichlorophenol workers with exposure to 2,3,7, 8 tetrachlorodibenzop- dioxin. Am. J. Epidemiol. 2009;170:501–6.

Courtens, J.L., Plöen, L. Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. Biol Reprod. 1999; 61: 154–161.

Ellman, G.L. Tissue sulfhydryl groups. Arch.Biochem. Biophys. 1959; 82: 70–77.

Elissa, W.P. Wong and C. Yan Cheng. Trends in Pharmacological Sciences. 2011; 32, (5)

El-Sabeawy, F., Wang, S., Overstreet, J., Miller, M., Lasley, B., Enan, E. Treatment of rats during pubertal development with 2,3,7,8-tetrachlorodibenzo-p-dioxin alters both signaling kinase activities and epidermal growth factor receptor binding in the testis and themotility and acrosomal reaction of sperm. Toxicol. Appl. Pharmacol. 1998; 150: 427–442.

Egeland, G.M., Sweeney, M.H., Fingerhu, M.A., et al. Total serum testosterone and gonadotropinsin workers exposed to dioxin. American Journal of Epidemiology. 1994; 139: 272–281.

Esser, C., Steinwachs, S., Herder, C., et al. Effects of a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin, given at post-puberty, in senescent mice. Toxicology Letters. 2005; 157(2): 89–98.

Floyd, R.A., Carney, J.M., Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Ann Neurol. 1992; 32 (Suppl): S22–S27.

Galati, G., Sabzevari, O., Wilson, J.X., O'Brien, P.J. Prooxidant activity and cellular effects of phenoxyl radicals of dietary flavonoids and other polyphenolics. Toxicology. 2002; 177: 91–104.

Ghosh, A., Mandal, A.K., Sarkar, S., Panda, S., Das, N. Nanoencapsulation of quercetin enhances its dietary effcacy in combating arsenic-induced oxidative damage in liver and brain of rats. Life Sci. 2009; 84: 75–80.

Goel, A., Dani, V., Dhawan, D.K. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chem Biol Interact. 2005; 156:131–140.

Gray, L.E., Ostby, J.S., Kelce, W.R. A dose-response analysis of the reproductive effects of a single gestational dose of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in male Long Evans Hooded rat offspring. Toxicol Appl Pharmacol. 1997; 146 (1):11–20.

Habig, W.H., Pabst, M.L., Jakpoly, W.B. Glutathionetransferase: a first enzymatic step inmercapturic acid and formation. J. Biol.Chem. 1974; 249: 7130–7139.

Hamm, J.T., Sparrow, B.R., Wolf, D., Birnbaum, L.S. In utero and lactational exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin alters postnatal development of seminal vesicle epithelium. Toxicological Sciences. 2000; 54 (2): 424–430.

Halliwell, B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence. Lancet. 1994; 344:721–724.

Humblet , O., Birnbaum, L., Rimm ,E., Mittleman, M .A., Hauser, R. Dioxins and cardiovascular disease mortality. Environmental Health Perspectives. 2008; 116(11): 1443–1448.

Jaffe, B. M,, Behrman, N. R. Methods of hormone radio-immunoassay. 1974; New York: Academic Press.

Jin, M.H., Hong, C.H., Lee, H.Y., Kang, H.J., Han, S.W. Enhanced TGF-beta1 is involved in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced oxidative stress in C57BL/6 mouse testis. Toxicol Lett. 2008; 178:202–209.

Johnson, L., Dickerson,R., Safe, S.H., Nyberg, C.L., Lewis, R.P., Welsh, J.r.T.H. Reduced Leydig cell volume and function in adult rats exposedto2, 3, 7,8tetrachlorodibenzo-p-dioxin without a significant effect on spermatogenesis. Toxicology. 1992; 76:103–18.

Kalender, Y., Kaya, S., Durak, D., Uzun, F. G., Demir, F. Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis -histoarchitecture induced by chlorpyrifos in male rats. Environmental toxicology and pharmacology. 2012; 33:141-148.

Kakkar, P., Das, B., Viswanathan, P.N. A modified spectroscopic assay of superoxidedismustase, Indian J. Biochem. Biophys. 1984; 21:130–132.

Kim, W., Hwang, S., Lee, H., Song, H., Kim, S. Panax ginseng protects the testis against 2, 3, 7; 8-tetrachlorodibenzo-p-dioxin induced testicular damage in guinea pigs. BJU Int. 1999; 83: 842–849.

Kleinman, W.A., Richie, J.P. Status of glutathioneand other thiols and disulfides in humanplasma, Biochem. Pharmacol. 2001;60:93-99.

Latchoumycandane, C., Chitra, K.C., Mathur, P.P. 2,3,7,8 tetrachlorodibenzo-pdioxin (TCDD) induces oxidative stress in the epididymis and epididymal sperm of adult rats. Arch Topical. 2003; 77: 280–284.

Latchoumycandane, C., Chitra, K.C., Mathur, P.P. The effect of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin on the antioxidant system in mitochondrial and microsomal fractions of rat testis. Toxicology. 2002; 171: 127–135.

Lai, K.P., Wong, M.H., Wong, C.K. Inhibition of CYP450scc expression in dioxinexposed rat Leydig cells. J. Endocrinol. 2005; 185 (3): 519–527.

Li, X., Johnson, D.C., Rozman, K.K. 2,3,7,8-Tetrachlorodibenzop- dioxin (TCDD) increases release of luteinizing hormone and folliclestimulating hormone from the pituitary of immature female rats in vivo and in vitro. Toxicol. Appl. Pharmacol. 1997; 142: 264–269.

Liu, C.M., Zheng, Y.L., Lu, J., Zhang, Z.F., Fan, S.H., Wu, D.M., Ma, J.Q., Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environ. Toxicol. Pharmacol. 2010; 29: 158–166.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. Protein measurement with the Folinphenol reagent. J. Biol. Chem. 1951; 193: 265–275.

Marklund, S., Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47:469–474.

Matés, J. M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000;153:83–104.

Meistrich, M.L., Van Beek, EAB. 1993. Spermatogonial stem cells: assessing their survival and ability to produce differentiated cells. In: Chapin RE, Heindel JJ, editors. Methods in Toxicology, vol. 3, Part A: Male Reproductive Toxicology. San Diego, CA: Academic Press. 106–123.

Mita, M., Price, J.M., Hall, P.F. Stimulation by follicle-stimulating hormone of synthesis of lactate by Sertoli cells from rat testis. Endocrinology. 1982; 110: 1535–1541.

Morales, A.I., Vicente-S.nchez, C., Santiago Sandoval, J.M., Egido, J., Mayoral, P., Arffalo, M.A., Fern.ndez-Tagarro, M., L.pez-Novoa, J.M., P ez-Barriocanal, F. Protective effect of quercetin on

experimental chronic cadmium nephrotoxicity in rats is based on its antioxidant properties. Food Chem. Toxicol. 2006; 44: 2092–2100.

Nair, V., Turner, G.E. The thiobarbituric acid testfor lipid peroxidation structure of the adductwith malondialdehyde. Lipids. 1984; 19: 84–95.

Nau, H. Impacts and impact mechanisms of ‘‘dioxins’’ in humans and animals. Dtsch Tierarztl Wochenschr. 2006; 113(8): 292-297.

Omaye, S.T., Turbull, J.D., Sauberlich, H.E. Selected methods for the determination ofascorbic acid in animal cells, tissues andfluids. Method.Enzymo. 1979; 62: 3–11.

Ohsako, S., Miyabara, Y., Nishimura, N., Kurosawa, S., Sakaue, M., Ishimura, R., Sato, M., Taketa, K., Aoki, Y., Sone, H., Tohyama, C., Yonemoto, J. Maternal exposure to a low dose 2,3,7,8 tetra chlorodibenzo-p-dioxin (TCDD)suppressed the development of reproductive organs of male rats: dose- dependent increase of mRNA levels of 5a-reductase type 2 in contrast to decrease of androgen receptorin the pubertal ventralprostate. Toxicol, Sci. 2001; 60:132–43.

Okamura, A., Kamijima, M., Shibata, E., Ohtani, K., Takagi, K., Ueyama, J., Watanabe, Y., Omura, M., Wang, H., Ichihara, G., Kondo, T. and Nakajima, T. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicol. 2005; 213: 129–137.

Pavan, M., Todeschini, R., Orlandi, M. Data mining by total ranking methods, a case study on optimisation of the “pulp and bleaching” process in the paper industry. Ann. Chim. 2006; 96:13–27.

Panemangalore, M., Bebe, F.N. Short- and long-term exposure to low levels of pesticide and flavonoid mixtures modify endogenous antioxidants in tissues of rats. Journal of Environmental Science and Health. 2009; B 44: 357–364.

Pedrielli, P., Skibsted, L.H. Antioxidant synergy and regeneration effect of quercetin, (−)-epicatechin, and (+)-catechin on άtocopherol in homogeneous solutions of peroxidating methyl linoleate. Journal of Agricultural and Food Chemistry. 2002; 50: 7138–7144.

Poland, A., Kimbrough, R.D. Biological mechanisms of dioxin action. Bambury Report 18. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1984; p. 500.

Pedrielli, P., Skibsted, L.H. Antioxidant synergy and regeneration effect of quercetin, (−)-epicatechin, and (+)-catechin on _-tocopherol in homogeneous solutions of peroxidating methyl linoleate. Journal of Agricultural and Food Chemistry. 2002; 50:7138–7144.

Pohjanvirta, R., Unkila ,M., Tuomisto, J. Comparative acute lethality of 2, 3, 7, 8-tetrachlorodibenzo- p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin and 1,2,3,4,7,8- hexachlorodibenzo-p-dioxin in the most TCDD-susceptible and the most TCDDresistant rat strain. Pharmacol Toxicol. 1993; 73:52–56.

Pushpavalli, G., Kalaiarasi, P., Veeramani, C., Pugalendi, K.V . Effect of chrysin on hepatoprotective and antioxidant status in D-galactosamine-induced hepatitis in rats. European Journal of Pharmacology. 2010; 631(1-3): 36–41.

Sinha, A.K. Colorimetric assay of catalase, Anal. Biochem. 1972; 47: 389–394.

Raychoudhury, S.S., Kubinski, D. Polycyclic aromatic hydrocarbon-induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. Environ Health Perspect. 2003;111: 33–38.

Rebourcet, D., Odet, F., Vérot, A., Combe, E., Meugnier, E., Pesenti, S., Leduque, P., Déchaud, H., Magre, S., Le Magueresse-Battistoni, B. The effects of an in utero exposure to 2,3,7,8-tetrachloro-dibenzo-p-dioxin on male reproductive function: identification of Ccl5 as a potential marker. Int J Androl. 2010; 33 (2): 413–424.

Renugadevi, J., Milton Prabu, S., in press. Quercetin protects against oxidative stressrelated renal dysfunction by cadmium in rats. Exp. Toxicol. Pathol. 2009.

Rotruck, J.T., Pope, A.L., Ganther, H.E. Selenium:biochemical role as a component ofglutathione peroxidase purification assay. Science. 1973; 179: 588–590.

Roman, B.L., Sommer, R.J., Shinomiya, K., Peterson, R.E. In utero and lactational exposure of the male rat to 2, 3, 7, 8-tetrachlorodibenzo- p-dioxin: impaired prostate growth and development without inhibited androgen production. Toxicol. Appl. Pharmacol. 1995; 134: 241–250.

Sadowska-Woda, I., Popowicz, D., Karowicz-Bilin´ ska, A. Bifenthrin-induced oxidative stress in human erythrocytes *in vitro* and protective effect of selected flavonols. Toxicology in Vitro. 2010; 24: 879–884.

Saradha, B., Vaithinathan, S., Mathur, P.P. Single exposure to low dose of lindane causes transient decrease in testicular steroidogenesis in adult male Wistar rats. Toxicology. 2008; 244:190–197.

Sayed-Ahmed, M.M., Nagi, M.N. Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats. Clin Exp Pharmacol Physiol. 2007; 34: 399–405.

Scalbert, A., Williamson, G. Dietary intake and bioavailability of polyphenols. Journal of Nutrition. 2000; 130: 2073S–2085S.

Sharpe, R.M. Environmental/lifestyle effects on spermato-genesis. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 2010; 365: 1697–1712.

Sharara, F.I., Seifer, D.B., Flaws, J.A. Environmental toxicants and female reproduction. Fertil. Steril. 1998; 70: 613– 622.

Spencer, J.P., Jenner, A., Aruoma, O.I., Evans, P.J., Kaur, H., Dexter, D.T., Jenner, P., Lees, A.J., Marsden, D.C., Halliwell, B. Intense oxidative DNA damage promoted by L-dopa and its metabolites. Implications for neurodegenerative disease. FEBS Lett. 1994; 353:246–250.

Sun, Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med. 1990; 8: 583–599.

Tousson, E., Alm-Eldeen, A., El-Moghazy, M. p53 and Bcl-2expression in response to boldenone induced liver cells injury. Toxicology and Industrial Health. 2011; 27: 711–718

Trejo, R., Valadéz-Salazar, A., Delhumeau, G. Effects of quercetin on rat testis aerobic glycolysis. Can J Physiol Pharmacol. 1995; 73: 1605–1615.

Uzun, F.G., Demir, F., Kalender, S., Bas, H., Kalender, Y. Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food and Chemical Toxicology. 2010;48:1714–20.

Wahlefeld, A.W. Lactate dehydrogenase. UV-method with L-Lactate and NAD. Methods of Enzymatic Analysis. 1983; 3:126–32.

Wells, M.E., Awa, O.A. New technique for assessing acrosomal characteristics of spermatozoa. J Diary Sci. 1970; 53:227.

Wong, E.W. et al. Cell junctions in the testis as targets for toxicants. In Comprehesive toxicology (Richburg, J.H. and Hoyer, P., eds), 2010; 167–188, Academic Press.

Yao, P., Nussler, A., Liu, L., Hao, L., Song, F., Schirmeier, A., Nussler, N. Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. J. Hepatol. 2007; 47: 253–261.

Yao, T. 2005. Physiology. Beijing: People hygiene press. pp. 602–603.

Yang JM, Arush M, Chen QY, Wu XD, Pang B, Jiang XZ. Cadmium induced damage to primary cultures of rat Leydig cells. Reprod Toxicol. 2003; 17:55–560.

Yokoi, K., Uthus, E.O., Nielsen, F.H. Nickel deficiency diminches sperm quantity and movement in rats. Biol. Trace Element Res. 2003; 93: 141–153.

Young-Il Kwon, Je-Deuk Yeon, Seung-Min Oh, and Kyu-Hyuck Chung. Protective effects of ursodeoxycholic acid against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced testicular damage in mice. Toxicology and Applied Pharmacology. 2004; 194: 239– 247.

Yousef, M.I, Omar, S.A, El-Guendi, M.I, Abdelmegid, L.A. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food and Chemical Toxicology. 2010; 48(11): 3246–3261.

Zemjanis, R. 1970. Collection and evaluation of semen. In: Diagnostic and therapeutic technique in animal reproduction. 2nd ed. Baltimore: The William and Wilkins Company. 139–53.

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