



Protective effects of quercetin supplementation against short-term toxicity of cadmium-induced hematological impairment, hypothyroidism, and testicular disturbances in albino rats

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Abstract

The aim of this study was to evaluate the probable protective effect of quercetin (QUE) against cadmium (Cd)-induced sub-chronic toxicity in rats. Adult male rats were given either Cd (as cadmium chloride; 5 mg/kg) alone or in combination with QUE (50 mg/kg) daily for 4 weeks by oral gavage. At the end of the experimental period, Cd accumulation, and selected hematological, thyroid, and reproductive markers were assessed. Results revealed that Cd treatment significantly increased Cd concentrations in blood, thyroid gland, and testicular tissue of rats. Cd also caused a decline in hemoglobin content, hematocrit value, and total erythrocyte and leucocyte counts. Further, significant suppressions in the blood levels of hormones related to thyroid gland function, and male reproductive hormones (i.e., testosterone, luteinizing hormone and follicle-stimulating hormone), were observed in Cd-treated rats compared to the control. In parallel, low sperm count and sperm motility, increased sperm abnormalities, and marked pathology occurred in testis. Combination with QUE recorded amelioration of the deleterious effects of Cd, involving regulation of hematological toxicity and thyroid hormonal levels and subsequently modulation of testicular function. In conclusion, it appears that dietary QUE can rescue from Cd-induced hematological dysfunctions and testicular damage by reversing the hypothyroid state.

Keywords Cadmium chloride · Quercetin · Hematological markers · Thyroid hormones · Reproductive dysfunction

Introduction

Cadmium (Cd) is a hazardous heavy metal largely available in the environment, especially in industrial and urbanized regions (Järup and Åkesson 2009). Cd has a very long biological half-life ranging from 15 to 30 years (Satarug et al. 2010).

Available epidemiological and experimental investigations revealed that Cd can disrupt thyroid function even at low environmental doses (Nie et al. 2017; Buha et al. 2018). It has been shown that cadmium toxicity has deleterious testicular effects including apoptotic events, decreased steroidogenesis, and male infertility (Marettová et al. 2015). In addition, in vivo

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experiments showed that high levels of Cd had cytotoxic and genotoxic effects on peripheral blood and bone marrow cells of rats (Çelik et al. 2009; Curcic et al. 2017; Aly et al. 2018). Anemia has been suggested as a risk factor for increased hepatic and renal Cd accumulation after oral Cd intake (Min et al. 2008). Many studies reported the association between Cd intoxication and a biologically relevant inhibition of white blood cell function or formation (Colacino et al. 2014; El-Boshy et al. 2015).

It is well known that Cd exposure results in generation of large amount of reactive oxygen species (ROS) which may contribute to oxidant/antioxidant imbalance, inflammation, membrane protein damage, altered gene expression, and various pathological conditions in humans and animals (Stohs et al. 2001). Therefore, the effects of natural antioxidants such as caffeic acid phenethyl ester (Kobroob et al. 2012) and proanthocyanidins (He et al. 2018) were tested in animal models and demonstrated cytoprotection against Cd-mediated oxidative insult. Quercetin (QUE) is classified as one flavonoid antioxidant of the five subclasses and major dietary flavonoids distributed in both cultivated and wild plants (D'Andrea 2015). The family members of flavonoids include flavones, flavonol, isoflavones, flavanones, flavanonol, flavanol, and anthocyanidin. Flavonols are represented abundantly by QUE (Shahidi and Ambigaipalan 2015). QUE is found in dietary plants like vegetables, onion, broccoli kale and fruits, red grapes, cherries, and blueberries (D'Andrea 2015; Ozgen et al. 2016). QUE is considered as a phytochemical that can modulate different mitochondrial pathways (de Oliveira et al. 2016) and has pivotal role in treatment of diabetes, cancers, and some cardiovascular diseases (Ozgen et al. 2016). Although the antioxidant capacity of QUE has been tested for providing protection to germ cells against the adverse effects of Cd (Bu et al. 2011; Farombi et al. 2012; Kanter et al. 2016; Nna et al. 2017; Ujah et al. 2018), there is a scarcity of information in literature regarding the effect of QUE on Cd-induced thyrotoxicity. Also, the role of QUE in protecting the hematological parameters of Cd-treated animals is still not well elucidated. In view of these considerations, the current study was designed to evaluate the possible ameliorative role of QUE on Cd-induced hematological disorders of adult male rats and its potential as a protector of the thyroid function abnormalities. Furthermore, we also investigated the contribution of QUE in Cd-induced sperm defects and testicular pathology.

Materials and methods

Chemicals

CdCl₂ and QUE were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of the highest analytical quality.

Animals and experimental design

A total of 24 male albino rats, weighing 180–200 g (3 months old) were used in the present study. The experimental protocol conforms to the ethical standards of the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Rats were housed under standard laboratory conditions. They were given rat chow and water ad libitum. After 2 weeks of acclimatization, animals were divided into four equal groups ($n = 6$ in each group) as follow:

- Group 1 served as control (CTRL) and received dimethyl sulfoxide (DMSO)/saline (0.5 ml per rat), with the final concentration of 1% DMSO (vehicle).
- Group 2 was treated with CdCl₂ (5-mg/kg b.w.).
- Group 3 was treated with QUE (50-mg/kg b.w.).
- Group 4 was treated at same time with CdCl₂ (5-mg/kg b.w.) in combination with QUE (50-mg/kg b.w.).

Rats were orally (i.e., orogastric gavage) administered by their respective doses every day for 4 weeks on the basis of a previous study by Renugadevi and Prabu (2010).

Blood collection

At the end of the experimental period, animals were sacrificed and trunk blood samples were collected for analysis. The collected blood from each animal was divided into two parts. Ten percent EDTA was added to one part for the evaluation of Cd level and hematological parameters while the second portion was centrifuged at 5000 rpm for 10 min; the separated sera were subjected to different biochemical analysis.

Assay for Cd concentration in serum, thyroid gland, and testis

The concentration of Cd was assessed in serum, thyroid gland, and testis by spectrophotometric method as previously described (Jin et al. 2000).

Hematological analysis

In the whole blood, hemoglobin content (Hb) was determined using a commercial kit according to supplier's protocol (Biodiagnostics, Cairo, Egypt). Packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) counts were determined according to reported methods (Faulkner and King 1970).

Estimation of serum hormonal concentrations

Thyroid hormones were determined by measuring the serum levels of thyroxine (T₄), triiodothyronine (T₃), free thyroxine

(fT₄), free triiodothyronine (fT₃), and thyroid-stimulating hormone (TSH). T₄ was determined following the protocol described in T₄ ELISA kit (Cat. No. 60863), Kamiya Biomedical Company, WA, USA. The assay was relied on the competitive enzyme immunoassay technique utilizing a monoclonal anti-T₄ antibody and a T₄-HRP conjugate. The developed color was measured at 450 nm in a microplate reader (Elisa Microplate Reader (x18), BioTeck, Bad Friedrichshall, Germany). The data of T₄ were measured as ng/ml. The sensitivity of the assay was 1.0 ng/ml, and coefficient of variance (CV) was less than 10%. T₃ was estimated using a solid-phase competitive ELISA kit (Cat. No. T3043T-100) obtained from CalbioTech Inc., Spring Valley, Canada. The T₃ values were expressed as pg/dl. Levels of fT₄ and fT₃ were determined by using ELISA kits, Cat. No. MB S700784, 1640, respectively (Alpha Diagnostic Intl. TX, USA). The assays employed the competitive inhibition enzyme immunoassay technique. Values of fT₄ and fT₃ were expressed as pg/ml. The sensitivity of the assay was less than 1.08 pmol/l for fT₄ and 0.3 pg/ml for fT₃. The intra- and inter-assay CV was <15% for both fT₄ and fT₃. TSH was estimated using ELISA kit (Cat. No. CSB-E05115r) obtained from CUSABIO Biotech Co., LTD, Wuhan, China. The records of TSH were given in μ IU/ml. The detection range was 0.78–50 μ IU/ml, and no significant cross-reactivity or interference was observed with this assay.

Serum levels of testosterone, LH, and FSH were detected according to Sakuma (2009), Shioya and Wakabayashi (1998), and Teerds et al. (1989), respectively. The values of testosterone were expressed as pg/ml whereas LH and FSH values were expressed as ng/ml.

Assessment of sperm concentration, motility, and abnormality

The left testis of each rat was harvested, then minced in pre-warmed saline (37 °C), and the resulted suspension was used in semen analysis. To analyze sperm motility, 1 drop of sperm suspension was placed on a glass slide to analyze 200 motile sperm in 4 different fields. The motility of the sperm was evaluated microscopically within 2–4 min of their isolation from the testis, and data were expressed as percentage motility (Morrissey et al. 1988). The sperms were counted using a hemocytometer following the method of Freud and Carol (1964). The technique Evans and Maxwell (1987) was adopted for sperm abnormality study. Briefly, smears were made by placing a drop from the sperm suspension and one or two drops of the previously warmed (37 °C) eosin–nigrosin stain. The smears were allowed to dry in the air and then examined under the microscope using a high power (100 \times) oil immersion objective. The normal sperm cells were counted and the percentage calculated.

Light microscopy of the rat testis

Right testis of each experimental animal was fixed in 10% neutral buffered formalin for 24 h, and standard paraffin preparations were stained with conventional hematoxylin and eosin (HE) dye. The testicular tissue was analyzed in random order with standard light microscopy by a pathologist who was unaware as to which group the rat had belonged, and the microscopic scoring was graded on a scale of absence of lesion (–), mild (+), moderate (++), and severe (+++). Also, Johnsen's tubular biopsy score (JTBS) (Johnsen 1970) was used to evaluate the degree of testicular and spermatogenesis damage in 20 seminiferous tubules (magnification \times 400) for each sample. In this respect, a numerical score of 0–10 was assigned to each seminiferous tubule indicating level of germinal epithelial maturation as follow: score (1), no seminiferous epithelium; score (2), Sertoli cells without germ cells; score (3), only spermatogonia; score (4), few spermatocytes; score (5), many spermatocytes; score (6), few early spermatids; score (7), many early spermatids without differentiation; score (8), few late spermatids; score (9), slightly impaired spermatogenesis, many late spermatids; and score (10), full spermatogenesis with regular tubules.

Statistical analysis

All values were expressed as mean \pm standard error (SE). Results were analyzed by one-way analysis of variance (ANOVA) followed by LSD post hoc test. In all the cases, statistical significance was accepted when $p \leq 0.05$. We used SPSS for Windows (version 17; SPSS Inc., Chicago, IL, USA) for statistical calculations.

Results

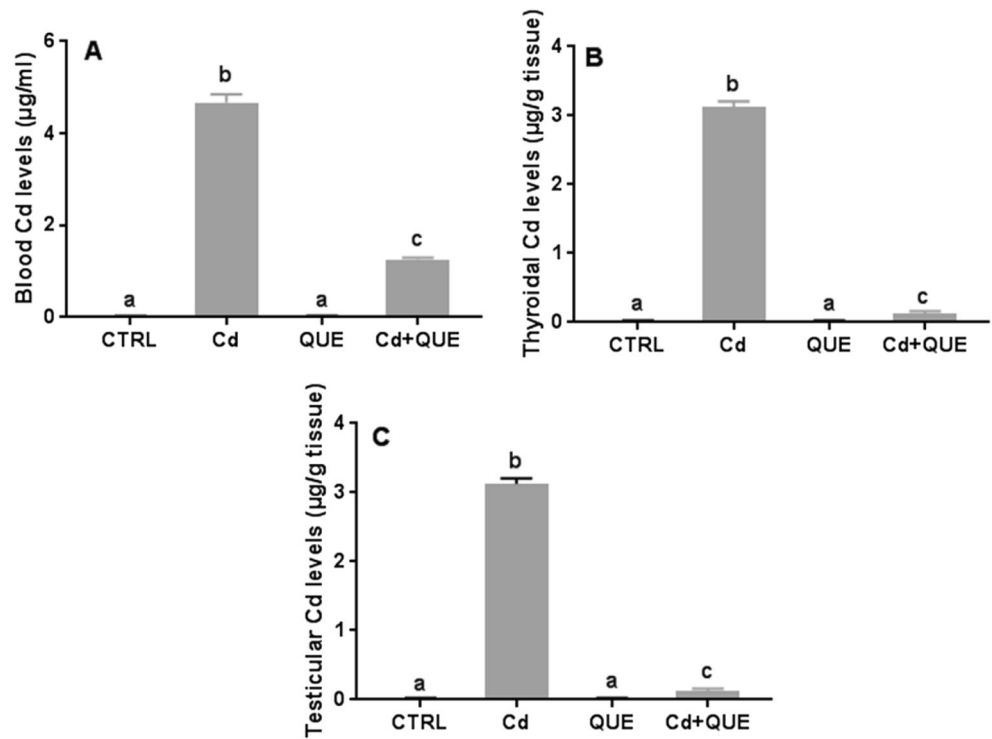
Cd accumulation

The oral administration of CdCl₂ caused a significant increase in Cd level in serum, thyroid gland, and testis compared with the CTRL group. The Cd concentration was also high in Cd+QUE group, but to lesser extent than in Cd-treated rats (Fig. 1).

Hematology

Data in Fig. 2 showed significant decrease in Hb concentration, PCV and RBCs, and WBCs count in the Cd group as compared to the CTRL. But for Cd+QUE group, values of the abovementioned parameters were significantly increased when compared with the Cd group. Specifically, the treatment of Cd-exposed rats with QUE was able to reverse and restore the decrease in PCV and WBCs count to normal levels.

Fig. 1 Cd accumulation levels in (a) serum, (b) thyroid gland, and (c) testis of male rats after oral administration of Cd and/or QUE for 4 weeks. Data are expressed as the mean \pm SE of $n = 6$ rats/group. Bars with different letters show significant differences between the groups ($p \leq 0.05$)



Hormonal measurements

Treating rats with Cd led to a significant decrease in serum T_4 , T_3 , fT_4 , fT_3 , and TSH when compared to the corresponding

CTRL animals (Fig. 3). In fact, thyroid hormone levels of the Cd+QUE group were significantly higher in comparison to the Cd-treated rats but significantly lower when compared to the CTRL (unchallenged) rats.

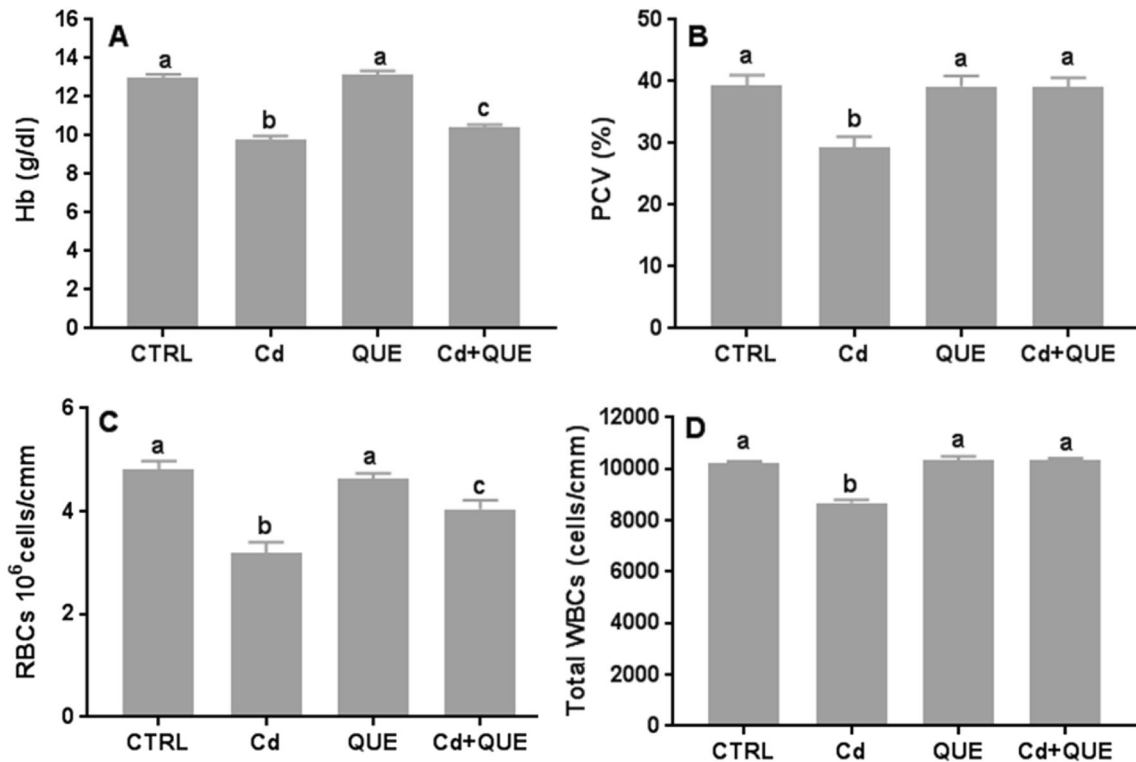
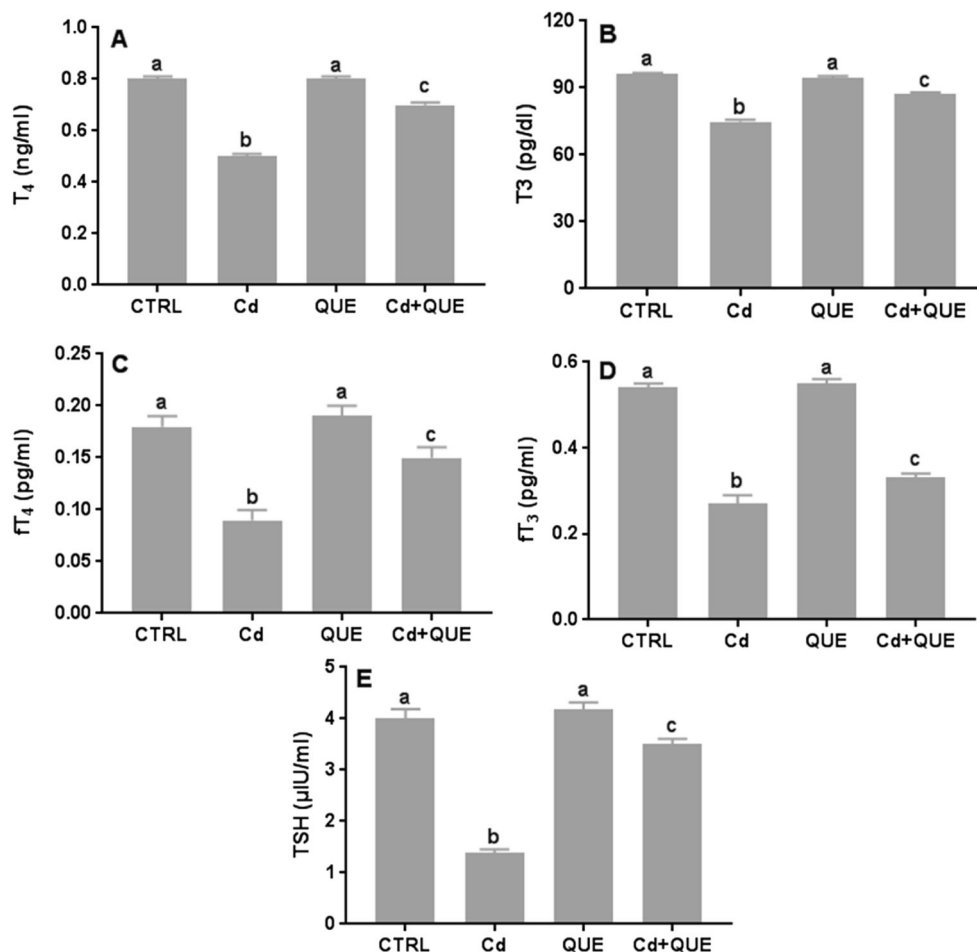


Fig. 2 Hematological indices in male rats after oral administration of Cd and/or QUE for 4 weeks. a Hb, (b) PCV, (c) RBCs count, and (d) total WBCs count. Data are expressed as the mean \pm SE of $n = 6$ rats/group. Bars with different letters show significant differences between the groups ($p \leq 0.05$)

Fig. 3 Serum thyroid hormones in male rats after oral administration of Cd and/or QUE for 4 weeks. **a** T_4 , **(b)** T_3 , **(c)** fT_4 , **(d)** fT_3 , and **(e)** TSH. Data are expressed as the mean \pm SE of $n = 6$ rats/group. Bars with different letters show significant differences between the groups ($p \leq 0.05$)



As can be seen in Fig. 4, Cd administration significantly decreased serum testosterone, LH and FSH when compared to the normal CTRL. Co-treatment by QUE resulted in significant increment in steroid hormone levels versus the Cd-treated rats. Interestingly, simultaneous supplementation of QUE with Cd has shown to restore LH concentration to near the CTRL value.

Sperm quality

Relative to the CTRL, Cd treatment induced a significant decrease in sperm count and sperm motility. While sperm abnormalities increased significantly in response to Cd. However, a significant restoration of sperm parameters was found in animals that received Cd plus QUE when compared to rats treated with Cd alone, although still not reaching the CTRL group levels (Fig. 5).

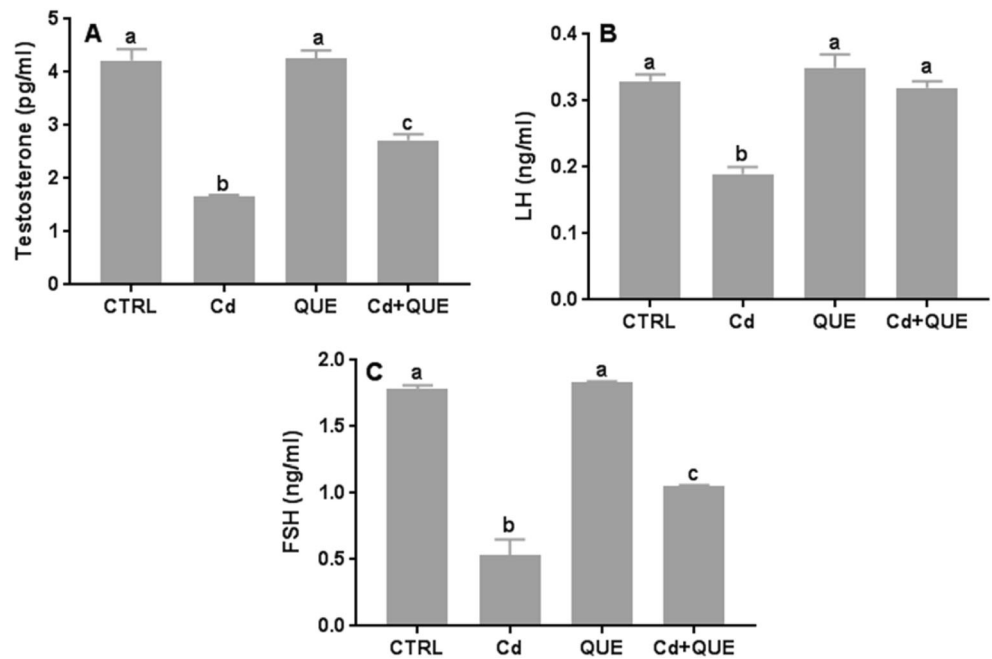
Testicular histopathology and spermatogenesis

The mean JTBS values and semiquantitative scoring of lesions magnitude for testes in each group are shown in

Table 1. The cytoarchitecture of the testicular tissue of CTRL animals exhibited well-preserved seminiferous tubules with active spermatogenesis, as well as prominent interstitial cellularity (Fig. 6a, b). For rats treated with Cd, the affected seminiferous tubules displayed structural atrophy, irregular outlines, spermatogenic dissociation of basal laminae (i.e., desquamation), vacuolization, epithelial gaps, and germ cell destruction (Fig. 6c, d). Moreover, hypocellularity, edema, and congestion were detected in the testicular stroma. In contrast, testes showed better tissue appearance with improved histological findings in the Cd+QUE co-exposed group (Fig. 6e, f). The histological data revealed normal germinal epithelium with considerable numbers of sperms in the seminiferous tubules in rats treated with QUE alone (Suppl. Fig. 1).

When testicular spermatogenesis scores (i.e., JTBS) were compared among the groups, the mean JTBS of the Cd group testes was significantly lower than in the CTRL, indicating a substantial maturation arrest or hypospermatogenesis. However, concurrent administration of QUE significantly increased JTBS values over the Cd group.

Fig. 4 Serum reproductive hormones in male rats after oral administration of Cd and/or QUE for 4 weeks. **a** Testosterone, **(b)** LH, and **(c)** FSH. Data are expressed as the mean \pm SE of $n = 6$ rats/group. Bars with different letters show significant differences between the groups ($p \leq 0.05$)



Discussion

In this study, Cd concentrations significantly increased in blood, thyroid gland, as well as in testis of Cd-treated rats. Cd bioaccumulation had negative impacts on the biological system due to its toxicity at a very low concentration (ATSDR 2012). Previous reports have shown that exposure to Cd causes renal injury (Gonick 2008) thus affecting the rate of elimination of the metal from the body. QUE is considered the most efficient antioxidants of the flavonoids (Ozgen et al. 2016) and recommended for dietary supplements against Cd

toxicity (Zhai et al. 2015). According to our results, the combined treatment of Cd+QUE decreased Cd level in serum, thyroid gland, and testis. A probable mechanism for this is through metal chelating ability of QUE (Ravichandran et al. 2014). In addition, recent studies indicated that QUE prevents renal injury in Cd-exposed rats by quenching ROS and/or enhancing cellular antioxidant defenses in the kidneys, thus increasing creatinine, urea, and Cd clearance (Morales et al. 2006; Renugadevi and Prabu 2010). This may partly contribute to decreased Cd accumulation in Cd+QUE-treated rats (Nna et al. 2017).

Fig. 5 Sperm parameters in male rats after oral administration of Cd and/or QUE for 4 weeks. **a** Sperm counts, **(b)** sperm motility, and **(c)** sperm abnormality. Data are expressed as the mean \pm SE of $n = 6$ rats/group. Bars with different letters show significant differences between the groups ($p \leq 0.05$)

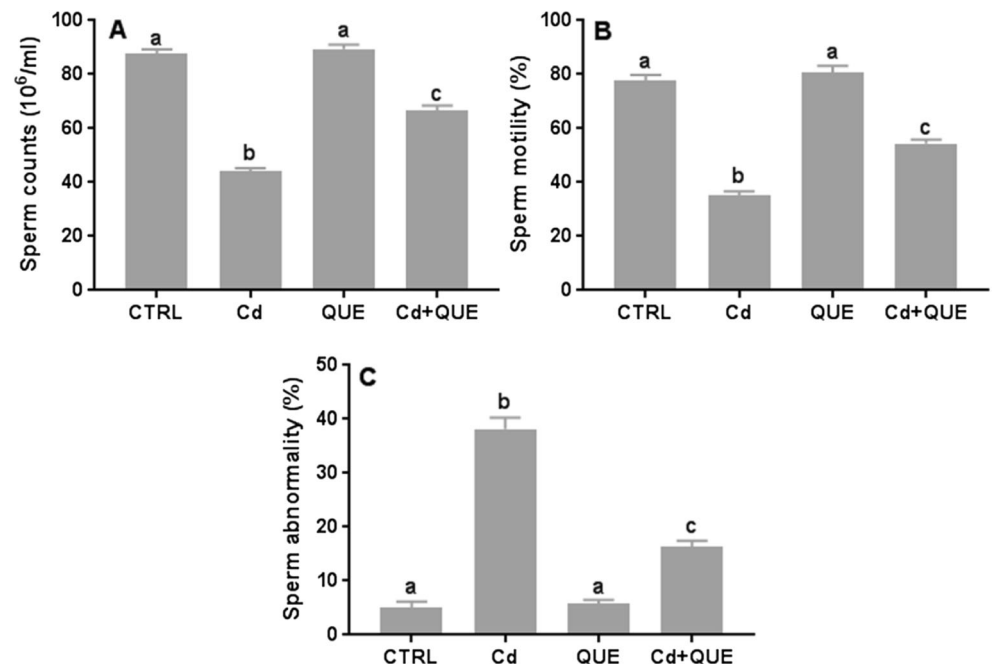


Table 1 Johnsen's tubular biopsy score (JTBS) and histopathological lesions in the testes of rats after oral administration of Cd and/or QUE for 4 weeks

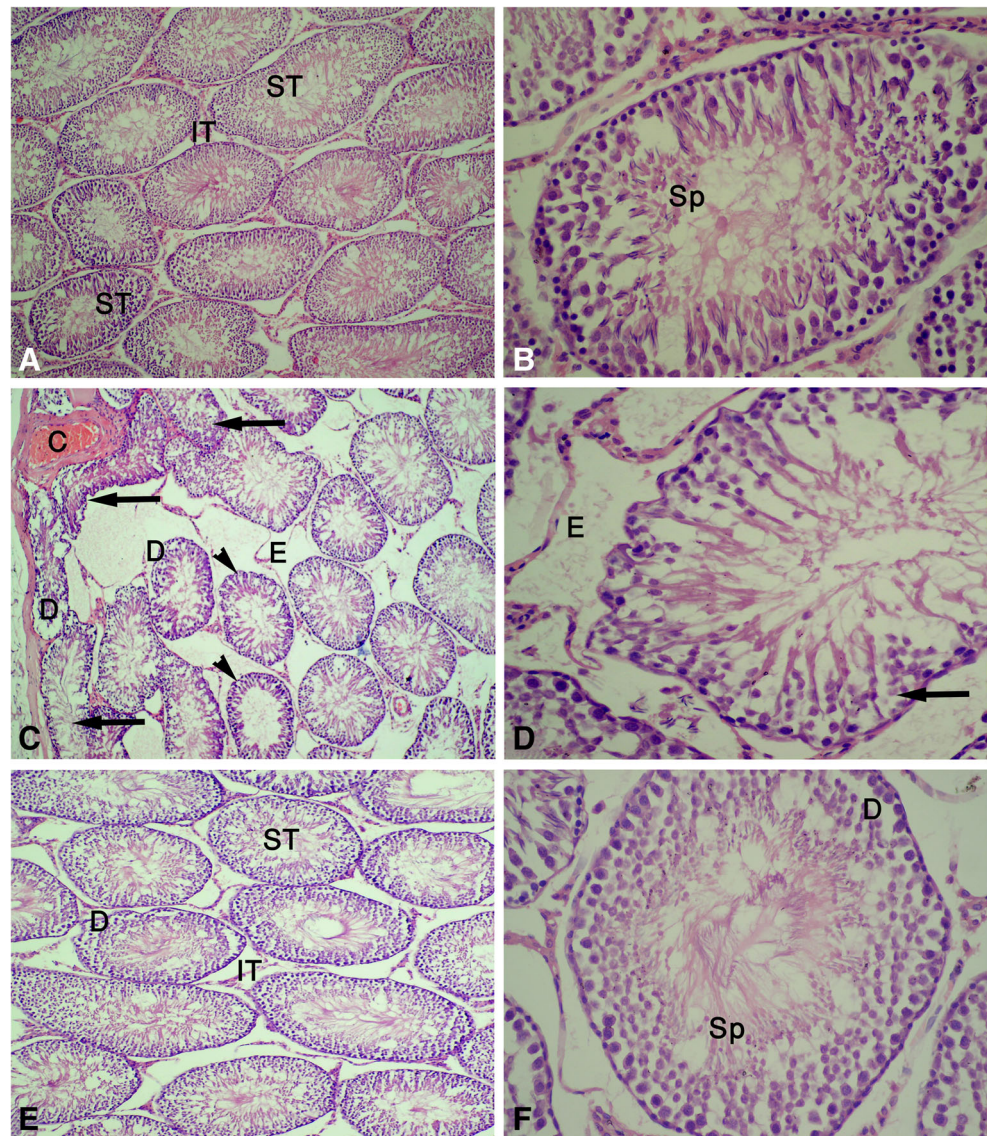
Parameters	CNRL	Cd	QUE	Cd+QUE
JTBS	9.58 ± 0.17a	3.24 ± 0.09b	8.91 ± 0.19a	6.98 ± 0.54c
Testicular lesions				
Atrophied tubules	–	+++	–	+
Desquamation	+	+++	+	++
Germ cell degeneration	–	+++	–	+
Interstitial edema and congestion	–	+++	–	+

Data are expressed as the mean ± SE of $n = 6$ rats/group. Lowercase letters within the same row show significant differences between the groups ($p \leq 0.05$). Absence of lesion (–), mild (+), moderate (++) and severe (+++)

During the 4-week Cd treatment, we found a significant decrease in the RBCs count, Hb concentration, and PCV. This reflects a sign of anemia related to Cd toxicity. Cd-induced anemia is multifactorial and could be attributed to the following: (1) impairment of erythropoietin formation

due to renal failure, (2) inhibition of delta-aminolevulinatase dehydratase (δ -ALAD) which is involved in heme biosynthesis, (3) accelerated erythrocyte destruction because of the altered erythrocyte membrane permeability, (4) increased mechanical fragility, and/or (5) failure of the intestinal uptake of

Fig. 6 Hematoxylin and eosin staining of testicular tissue of different experimental groups. **a**, **b** CTRL viewing normal structure of seminiferous tubules with complete spermatogenic series and interstitial tissue. **c**, **d** Cd group demonstrating testicular disruption. **e**, **f** Cd+QUE group indicating the improvement in the seminiferous tubule structure. Abbreviations/labels inside the image: ST, seminiferous tubules; Sp, spermatozoa; IT, interstitial tissue; arrowheads, atrophied tubules with spermatogenic disturbance; D, desquamation of germ cells; E, interstitial edema; C, congestion; arrows, marked damage of seminiferous tubules. Original magnification $\times 100$ (**a**, **c**, **e**), $\times 400$ (**b**, **d**, **f**)



Fe because of mucosal lesions (Horiguchi et al. 2011; Dwivedi et al. 2012; Yuan et al. 2014). Furthermore, we observed significant action of Cd, which decreased WBC count. This also was revealed in another study (Simsek et al. 2009). Administration of QUE along with Cd increased the reduced levels of Hb concentration, PCV, and RBCs and WBCs counts, providing protection against Cd-induced hematotoxicity. These findings are supported by the results of Selvakumar et al. (2013) who demonstrated that QUE mitigated the alterations in the RBC, Hb, PCV, and WBC in rats exposed to polychlorinated biphenyls. QUE was shown to inhibit RBC hemolysis and oxidative damage to membrane lipoprotein (Kitagawa et al. 2004; Asgary et al. 2005).

The results obtained in this investigation showed that treating rats with Cd induced a decrease in T_4 , T_3 , fT_4 , fT_3 , and TSH. Similarly, Buha et al. (2013) reported that exposure to low levels of Cd interferes with thyroid function. Cd has been reported to affect synthesis and release of T_4 via oxidative phosphorylation disorders in mitochondria of follicular cells (Yoshizuka et al. 1991). Prakash et al. (1997) proposed that thyroid dysfunction under Cd exposure is due to impaired thyroid hormonogenesis under an oxidative stress. Cd also inhibits hepatic peripheral deiodination of T_4 to T_3 by binding to sulfhydryl groups of 50-monodeiodinase, a seleno-enzyme containing a selenocysteine active site (Matović et al. 2015). It was established that abnormal thyroid hormone levels induce diverse effects on blood cells such as anemia, thrombocytopenia, and leukopenia and even in rare cases cause pancytopenia (in hypothyroidism) (Bremner et al. 2012; Dorgalaleh et al. 2013). Likewise, other blood indices such as MCV, MCH, MCHC, and Hb could change during thyroid dysfunction (Kawa et al. 2010). Furthermore, it is proposed that the prolonged insufficiency of thyroid hormones may be directly related to the impedance of the cellular immune system (Pillay 1998). In this context, studies have suggested that hypothyroidism is associated with a decrease in male sex hormones, abnormal spermatogenesis, and retardation of Sertoli cell differentiation (Krajewska-Kulak and Sengupta 2013; Mohamed and Gawad 2017). T_3 hormone increases LH receptors and steroidogenesis of Leydig cells, thus stimulating basal testosterone biosynthesis (Maran et al. 2000). T_3 also plays an important role in the regulation of mitochondrial function in several tissues; therefore, hypothyroid condition disturbs intra-mitochondrial glutathione redox status and causes oxidative stress in testis that cannot even be reversed with T_3 treatment (Chattopadhyay et al. 2010). Noteworthy, in the present study, QUE showed better improvement of the thyroidal function at the concurrent treatment with Cd. Similarly, earlier study of Nabavi et al. (2011) has verified that QUE may reduce the severity of NaF-induced thyroid dysfunction in male rats.

As expected, the results of the present study clearly demonstrated that oral Cd administration significantly decreased

sperm count and motility and increased the percentage of sperm abnormalities compared to CTRL. In addition, it was observed that, in the Cd group, there was a sharp decrease in total serum testosterone, LH, and FSH levels. Cd causes male infertility through alteration in histopathology, sperm characteristics, and oxidant/antioxidant balance (El-Demerdash et al. 2004; El-Neweshy et al. 2013; Djuric et al. 2015). In fact, Cd is likely to substitute Zn^{2+} in the testicular tissue, which may lead to negative implication on sperm development process (Amara et al. 2008). Cd negatively influences testis structure, by damaging vascular endothelium and blood-testis barrier integrity, and by inducing inflammation and apoptosis within the testis (de Angelis et al. 2017). As a known endocrine disruptor, Cd can modify reproductive hormone levels by affecting the hypothalamic–pituitary–testicular axis (Lafuente 2013). In analyzing the results, it was seen that QUE counteracted the negative effects of Cd on sperm quality and sex hormones production. Other authors have also reported that QUE can attenuate testicular damage in rats following exposure to free-radical promoting agents/drugs such as dioxins (Ciftci et al. 2012), bisphenol A (Jahan et al. 2016), and docetaxel (Altintas et al. 2015). According to Bharti et al. (2014), antioxidant therapy with QUE has the potential to stimulate androgen production, restore redox balance, and prevent germ cell apoptosis subsequently leading to restoration of testicular function in estrogenized rats. Abarikwu et al. (2012) demonstrated that QUE by decreasing atrazine-induced lipid peroxidation and ROS production could promote cell survival in Sertoli/germ cells co-culture. Based on the *in vitro* study of Hu et al. (2015), QUE is proved to augment Leydig cell viability by downregulating mitochondrial/apoptotic protein-induced by triptolide. Additionally, QUE has high antigenotoxic capacity via diminishing DNA damage of sperm upon *in vitro* exposure to food mutagens (Anderson et al. 1998).

In conclusion, this study showed that QUE is a powerful/safe phytotherapy to overcome Cd toxicity in rat tissues. On the basis of our results, the regulatory effects of QUE on thyroidal hormones might be the mechanism (at least in part) for its hematological and testicular protective effect against Cd toxicity.

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Compliance with ethical standards

Disclosure The authors declare that they have no conflicts of interest.

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