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Protective Effect of Coenzyme Q10 on Cadmium-Induced Testicular Damage in Male Rabbits

[1]El-Sayed K. Abdel-Hady and [2]Gamal H. Abdel-Rahman

[1,2]Department of Biology, Faculty of Science, Taif University, Taif, KSA
[1]Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt
[2]Department of Zoology, Faculty of Science, Assiut University, Assiut, Egypt

Abstract: The present study was designed to investigate the potential protective effect of Coenzyme Q10 against the testicular toxicity of cadmium in male rabbits. Four groups of animals were used in this study (control, Coenzyme Q10, cadmium chloride-treated (5 mg/kg), cadmium chloride plus Coenzyme Q10 (10 mg/kg) for 30 days). Results showed normal testicular tissue in control and coenzyme Q10 groups. Histopathological observations of cadmium chloride group showed severe damage in testicular tissue such as atrophy in many seminiferous tubules, vacuolations and cellular debris. The germinal epithelium was sloughed off and the spermatogenic cell layers were disturbed. Morphometrical data displayed significant decrease in both seminiferous tubules diameter and the counted number of the different spermatogenic cells. Additionally, testosterone and luteinizing hormones level were decreased. Coenzyme Q10 co-treatment to the cadmium-administered rabbits reduced testis histopathological changes and increased the levels of testosterone and luteinizing hormones. The present study suggests that coenzyme Q10 may be ameliorate testicular damage due to cadmium toxicity.

Key words: Cadmium · Coenzyme Q10 · Testis · Morphometry · Histopathology · Rabbit

INTRODUCTION

Cadmium (Cd) is a well-known heavy metal widely used in industry and It has been ranked among the 10 most toxic compounds for human health [1]. Tobacco smoke and agriculture phosphate fertilizers are another important sources of cadmium. Cd is characterized by its toxicity to various organs, including kidney, liver, lung, testis, brain, bone and blood [2]. Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins, DNA and initiating various pathological conditions in humans and animals [3, 4]. In recent years, there has been increasing concern about this metal as an environmental pollutant mainly derived from its extremely long biological half life, so long-term exposures could still result in direct toxic effects from the residual metal [5]. After acute exposure, cadmium-induced testicular damage in interstitial and tubular levels and cause oedemas, hemorrhage and necrosis, seem to be clearly implicated in the histopathological mechanism [6]. Although it is well known that long-term cadmium exposure has carcinogenic effects on the male reproductive organs [7, 8] and causes a diminution of reproductive capacities [9, 10]. Cadmium causes alterations in the serum levels of androgens and other reproductive hormones as well as, histopathological damages to reproductive organs [11-13]. Cadmium exposure can adversely affect male fertility and results in severe impairment of testicular functions including germ cell death and inhibition of testicular steroidogenesis [14-16].

Luteinizing hormone (LH) is required for quantitatively normal spermatogenesis in pubertal rats [17] and it is a prime regulator of testicular androgenic enzyme activities [18]. In male rats, circulating LH is responsible for maintaining normal plasma testosterone concentrations [19]. Adult mammalian spermatogenesis is a testosterone dependent process [20]. Massive testicular germ cell apoptosis is known to result directly either from exposure to cadmium [21] or alterations of hormonal support from Leydig cells [22].

Coenzyme Q10 (CoQ10) is an endogenous lipid-soluble benzo- quinone compound that functions as a diffusible electron carrier in the mitochondrial respiratory chain [23], then ATP production acting as an essential antioxidant and supporting the regeneration of other

Corresponding Author: Gamal Hasan Abdel-Rahman, Department of Biology, Faculty of Science, Taif University, Taif, KSA.

antioxidants, influencing the stability and permeability of membranes; also, stimulating cell growth and inhibiting cell death [24-26]. CoQ10 acts as a powerful antioxidant which scavenges free radicals, prevents the initiation and propagation of lipid peroxidation in cellular biomembranes and helps regeneration of tocopherol [24, 27]. In addition, CoQ10 has anti-inflammatory properties decreasing the production of pro-inflammatory cytokines as tumor necrosis factor [28, 29]. In recent years, CoQ10 has gained considerable attention as a dietary supplement capable of influencing cellular bioenergetics and counteracting some of the damage caused by free radicals [30-32].

The aim of the present study was to show the protective role of Coenzyme Q10 against cadmium-induced testicular damage in male rabbits.

MATERIALS AND METHODS

Chemicals: Coenzyme Q10 (MEPACO, Egypt) was presented as 30 mg tablets. Daily oral dose for each animal was 10 mg/kg-body weight according to Singh *et al*. [33]. Cadmium chloride ($CdCl_2$, 99% pure) was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA).

Animals: Adult male New Zealand White rabbits (2.40 ± 0.08 kg) were obtained from Animals Farm in Taif city, KSA. Animals were kept at standard housing facilities (24±1°C, 45±5% humidity and 12 h light/dark cycle). They were supplied with standard laboratory food and water *ad-libitum* and left to acclimatize for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. Animals were randomly divided into four equal groups (n=6, each):

Group 1: Control; received normal saline solution orally.

Group 2: Eeceived a daily dose of Coenzyme Q10 (10 mg/kg b.w) orally.

Group3: Was injected i. p. with cadmium chloride (5 mg/kg b.w) daily.

Group 4: was injected i. p. with cadmium chloride (5 mg/kg b.w) plus Coenzyme Q10 (10 mg/kg BW) orally. Time of the experiment was 30 days.

Histopathology: Animals were sacrificed under ether anaesthesia. Testes were carefully separated from animals and immediately fixed in neutral formalin. Paraffin sections of (5ìm thickness) were prepared for histopathological examinations and seminiferous tubules diameter. Sections were stained with hematoxylin and eosin (H&E) using the standard techniques [34] and then examined under light microscope.

Morphometry: For seminiferous tubules diameter measurements, five slides from testis of each group (6 sections per slide) were measured. Sections were examined by using a research microscope equipped with digital camera and connected to a PC based image analysis system. Sigma Scan Pro (version 4.0, Jendel Scientific, SPSS Inc., Chicago, USA) was used for image analysis and morphometrical data acquisition. For spermatogenic count, testes was fixed in a specific fixative (10 ml glycerol, 10 ml glacial acetic acid and 80 ml distilled water) according to Meistrich [35] and were examined by using a phase contrast microscope.

Serum Testosterone and Luteinizing Hormone: Blood samples were collected from the marginal ear vein of the rabbits and were placed into plain Vacutainer® silicone-coated tubes then, allowed to clot at room temperature. The blood samples were centrifuged at 3000 rpm for 15 minutes. Serum samples were frozen immediately at 20°C and stored until required for analysis. Quantitative determination of serum testosterone and luteinizing hormones level were carried out on thawed serum using testosterone enzyme immunoassay (EIA) test kit (Teco® Diagnostics, USA). An enzyme-linked immunosorbent assay (ELISA) reader was used to quantify hormones concentration.

Statistical Analysis: For statistical analysis, Quantitative results were expressed as means ± S.D. Differences between means were tested by univariate Analysis of Variance followed by Mann-Whitney Rank Sum Test. The values were considered significantly when $P<0.05$. All statistical analysis were performed using SPSS (version 9.0, 1998).

RESULTS

Histopathological Observation: Histological observation of the testes of control animals (Group I) showed normal features and arrangement of seminiferous tubules with

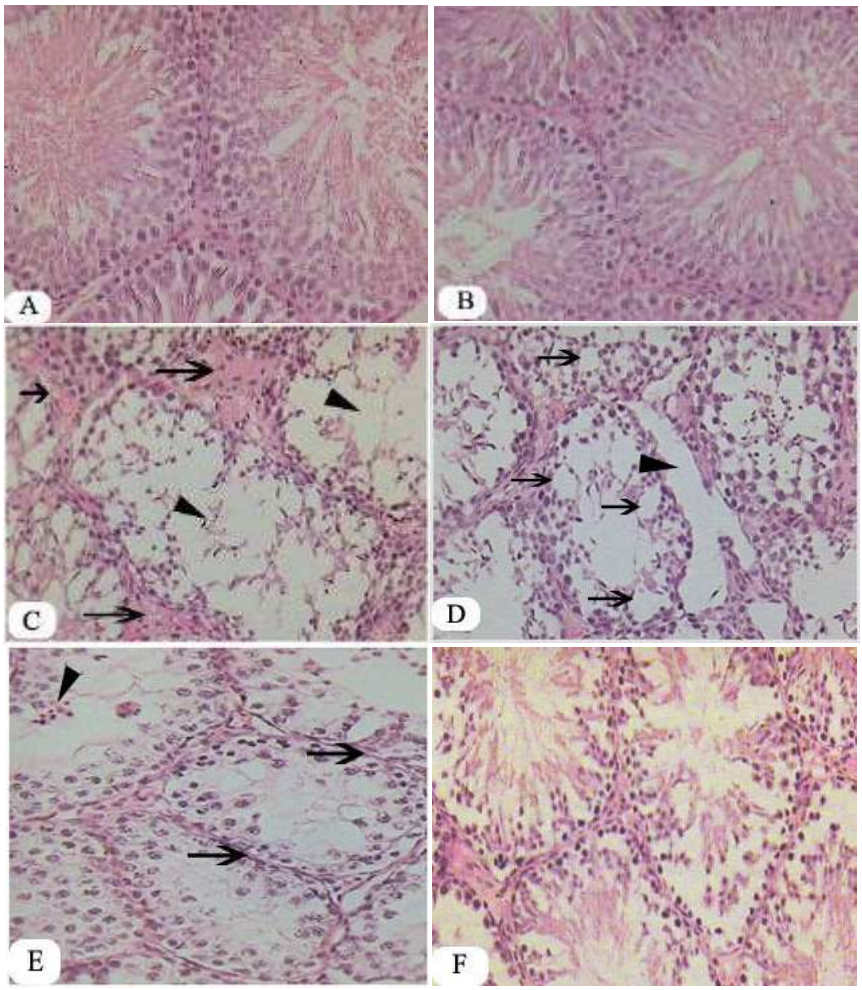

Fig. (1A): Photomicrograph of the testis of control rabbits showing normal structure of seminiferous tubules containing different types of spermatogenic cells. (H& E, X 400)

(B): Photomicrograph of the testis of rabbits received Coenzyme Q10 showing seminiferous tubules which more or less similar to that of control. (H& E, X 400)

(C): Photomicrograph of the testis of rabbits treated with Cadmium chloride for four weeks illustrating Hemorrhage in interstitial cells (arrows) and atrophy of the tissue and disturbance in spermatogenic cell layers (arrow head). (H& E, X 400)

(D): Photomicrograph of the testis of rabbits treated with Cadmium chloride for four weeks showing sloughing of the spermatogenic layer (arrow head) and vacuolation (arrows). (H& E., X 400)

(E): Photomicrograph of the testis of rabbits treated with Cadmium chloride for four weeks displaying cellular debris (arrow head) in the lumen of the seminiferous tubules and thinner epithelia (arrows). (H& E., X 400)

(F): Photomicrograph of the testis of rabbits treated with Cd+Coenzyme Q10 for four weeks illustrating some improvement in the seminiferous tubules compared to that of Cd treated group. (H& E., X 400)

Table 1: Means ± standard deviations of seminiferous tubules diameter (µm) of different groups of male rabbits, n = 6 animals

	Groups			
Parameter	Control	Coenzyme	Cd	Cd+ Coenzyme
Seminiferous tubules Diameter	262±2.19	258.33±2.16	214.16±1.83*	232.55±2.73*

*(P < 0.05)

Table 2: Means±standard deviations of different spermatogenic cells count of different groups of male rabbits (X 10^7), n = 6 animals.

	Groups			
Parameters	Control	Coenzyme	Cd	Cd+ Coenzyme
Spermatoginia	2.08±0.07	1.9±0.080	0.90±0.08*	1.31±0.09*
Spermatocyte	14.96±0.29	13.48±0.39	9.10±0.75*	11.03±0.49*
Spermatids	18.43±0.49	16.03±0.59	10.90±0.28*	13.37±0.32*
Spermatozoa	27.83±0.70	24.73±0.58	19.67±0.72*	22.37±0.69*
Sertoli cell	0.96±0.01	0.78±0.07	0.45±0.05*	0.65±0.05*

*(P < 0.05)

Table 3: Means±standard deviations of serum testosterone and luteinizing hormone of different groups of male rabbits, n = 6 animals.

	Groups			
Parameter	Control	Coenzyme	Cd	Cd+ Coenzyme
Testosterone (nmol/l)	15.30±0.36	14.92±0.56	8.19±0.59*	11.76±0.61*
Luteinizing hormone (mIU/ml)	0.24±0.007	0.22±0.005	0.13±0.004*	0.17±0.006*

*(P < 0.05)

spermatogenic cells (Fig. 1A). No histopathological alterations were detected in the testes of animals received Coenzyme Q10 (Group II) and the testicular morphology as in case of the control group (Fig. 1B). After four weeks treated rabbits with Cd (Group III), complete atrophy of some seminiferous tubules, with loss of the spermatogenic cells and heamorhage in the interstitial tissue (Fig. 1C). In some semineferous tubules, vacuolization and few sloughed spermatogenic cell layers were observed (Fig. 1D). Epithelia became thinner compared to those of the control and cellular debris appeared in the lumen of seminiferous tubules (Fig. 1E).

In Cd+Coenzyme Q10 treated group, toxic effects were ameliorated and the histological picture of the testis showed partial improvement, with restoration of the normal arrangement of cells in many tubules. Most of the tubules contained spermatogonia, spermatocytes and few spermatids (Fig. 1F).

Morphometry

Mean Diameter of Seminiferous Tubules: There are obvious variations in the mean diameter of the seminiferous tubules among different groups (Table 1). Data showed that there were significant decreases of the mean diameter of the seminiferous tubules of Cd group in comparison with control one (*P<0.05*). However, there was significant increase of diameter of seminiferous tubules of Cd+ Coenzyme Q10 group in comparison with Cd group (*P<0.05*).

Spermatogenic Cells Count: There are pronounced variations in the number of spermatogonia, spermatocytes, spermatids, Sertoli cells and sperms among different groups (Table 2). Data showed that there was significant decrease of all spermatogenic cells of Cd treated group in comparison with control one (*P<0.05*). However, there was significant increase of all spermatogenic cells in Cd+ Coenzyme group in comparison with Cd group (*P<0.05*).

Hormonal Levels: Table 3 shows that the average levels of serum testosterone and Luteinizing hormone were not significantly different (*P<0.05*) between control and Coenzyme groups. In case of Cd treated group, average levels of serum testosterone and Luteinizing hormone were significantly decreased (*P<0.05*) in comparison with the control group. In Cd plus coenzyme group, the two hormones were significantly increased in comparison with the cd group (P < 0.05).

DISCUSSION

It is well known that testis is very sensitive to acute Cd toxicity. Previous studies reported that cadmium is one of the most abundant elements due to its large usage in various industrial applications [36]. Cd promotes oxidative stress that contributes pathogenesis because of its long retention in some tissues [37]. Cadmium toxicity is reported to be associated with oxidative damage in

testicular Leydig cells through the production of reactive oxygen species (ROS) [38, 39]. Cadmium causes loss in the ability of the plasma membrane to act as a barrier, leading to the loss of catalytic enzymes and substrates from intracellular stores [40]. Shen and Sangiah [41] found that $CdCl_2$ induced testicular toxicity and this could possibly be mediated by a significant increase in hydroxyl free radical formation. It has been shown that production of ROS may mediate a signal for apoptotic cell death [42]. Stohs *et al*. [39] reported that cadmium exposure increases oxidative stress through the generation of free radicals such as superoxide anion radicals, hydroxyl radicals, nitric oxide and hydrogen peroxide. These radicals are transitory due to their high chemical reactivity and thus can stimulate lipid peroxidation and deleterious modification of complex lipoprotein assemblies in biomembranes and cellular dysfunction.

The present study showed that administration of cadmium for four weeks produced several histopathological changes in seminiferous tubules such as atrophy in many tubules, vacuolations and cellular debris. The germinal epithelium was sloughed off at many points and the spermatogenic cell layers were disturbed.

Histopathological changes observed in the present study is in agreement with the findings of El-Ashmawy and Youssef, [43] who demonstrated that a single dose of Cd induced severe necrosis and degeneration of seminiferous tubules with complete loss of the spermatogenic cell layers and absence of the centrally located spermatozoa. The present results are also in accordance with El-Missiry and Shalaby [44] who illustrated that Cd can induce lipid peroxidation and testicular tissue necrosis and apoptosis in rats.

The present study showed also that the number of spermatogonial cells were decreased in cadmium treated animals. This may be due to the effect of cadmium on RNA and consequently protein synthesis which affect the number of sperms. These morphometric data are in line with the previously studies about cadmium-induced impairment of spermatogenesis [45-47]. Testosterone is required for the attachment of different generations of germ cells in seminiferous tubules and therefore a low level of intratesticular testosterone may lead to detachment of germ cells from seminiferous epithelium and may initiate germ cell apoptosis [47]. The present study showed a decrease in the hormonal levels of testosterone and lutenizing hormones. This is in agreement with the findings of Gunnarsson *et al*. [16] who found that Cd caused a decrease in testosterone production through the reduction of testicular luteinizing hormone (LH) receptor. Laskey and Phelps [49] demonstrated that Cd administration can affect steroidogenesis even at concentrations that do not cause any testicular necrosis, indicating specific disruptive mechanisms in male rats.

Previous studies reported that CoQ10 is a naturally occurring hydrophobic compound that is not only a critical component of the mitochondrial respiratory chain, but also a powerful antioxidant. CoQ10 suppresses the generation of reactive oxygen species by blunting the expression of NADPH oxidase [50] and scavenges lipid peroxidation products during free radical reactions [51]. CoQ10 also suppresses excess NO production and prevents nutritive tissue stress [52]. The reduced form of CoQ10-ubiquinol, acts as an antioxidant, preventing lipid peroxidation in biological membranes and in serum low-density lipoprotein [53].

Results obtained from the present work showed that CoQ10 ameliorated some pathological changes of the testis produced by cadmium. Administration of CoQ10 also improve the levels testosterone and luteinizing hormone in the animals treated with cadmium. Th present findings therefore could suggested the ability of CoQ10 to ameliorate cadmium-induced testicular damage. The effective role of CoQ10 in the present study is in accordance with Sayed-Ahmed, *et al*. [54] who reported the protective role of CoQ10 against high magnetic field-induced testicular toxicity.

The present study concluded that coenzyme Q10 may be useful to scavenge free radical-induced testicular damage due to cadmium toxicity. Therefore, drugs or food containing this antioxidant can protect against cadmium-induced testicular damage.

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