Hormonal and Histological Study of the Effect of Co Enzyme Q10 on Male Reproductive System of Wister Rats Exposed to Cadmium Chloride

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Abstract

The aim of this study was to investigate at the effect of co enzyme CO 10, which acts as an antioxidant, on the male reproductive system of rats treated by cadmium chloride. (40) male rats were divided as follows: The control group received just drinking water, the T1 group received Q10 (10 mg/kg) for 60 days, the T2 group received cadmium chloride (25 mg/kg) for 60 days and the T3 group received cadmium chloride (25 mg/kg)+ Q10 (10 mg/kg) for 60 days. The results showed there were a significant increase ($p \leq 0.05$) in testosterone hormone level in T1 group compared with other groups (C,T2,T3). While there were a significant decrease in T2 group compared with control group**.** ICSH increased significantly in the T1 group as compared to the T2 group, but there were no significant differences between the other groups. GnRH levels were significantly increased in the T1 group compared to the other groups, but there were no significant differences between the C,T2, and T3 groups. Testicular sections from rats in the T2 group that were administered cadmium chloride revealed impairment in the function of the testes, epididymis, and vas deference, as well as a reduction in the number of sertoli cells .Testicular sections of rats in the T3 group that were administered cadmium chloride and treated with Q10 revealed full spermatogenesis with prominent sertoli cells. The epididymial duct has normal and high epithelial cells and stereo cilia, with a large amount of sperms in its lumen; the vas deference has high columnar epithelial cells, long and extended stereo cilia, and normal smooth muscle fiber. The present study shows that the antioxidant properties of CoQ10 the basis of the ameliorative role of male reproductive system. Evidence reveals that CoQ10 mainly improves male hormones and testicular function and protects it from oxidative damage by cadmium chloride.

Keywords: CoQ10 , Male Reproductive System ,Wister Rats , Cadmium Chloride**.**

دراسة هرمونية ونسيجة لتأثير مساعد الانزيم *Q10* على الجهاز التناسلي الذكري لجرذان الوستر المعرضة لكلوريد الكادميوم

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الخلاصة

الهدف من هذه الدراسة هو التحقق من تأثير مساعد الانزيم *CO10* ، الذي يعمل كمضاد للأكسدة ، على الجهاز التناسلي الذكري للجرذان المعالجة بكلوريد الكادميوم. استخدم 40 ذكر جرذ وقسمت على النحو التالي: تلقت مجموعة السيطرة ماء الشرب فقط ، تلقت المجموعة الاولى *T1* *Q10* 10 مغم / كغم لمدة 60 يومًا ، تلقت المجموعة الثانية *T2* كلوريد الكادميوم (25 مغم / كغم) لمدة 60 يومًا و تلقت المجموعة الثالثة *T3* كلوريد الكادميوم (25 مغم / كغم) + *Q10* (*10*) مجم / كجم) لمدة 60 يومًا. أظهرت النتائج وجود زيادة معنوية ($p \leq 0.05$) في مستوى هرمون التستوستيرون في مجموعة *T1* مقارنة بالمجموعات الأخرى (*C* ، *T2* ، *T3*). بينما كان هناك انخفاض معنوي في مجموعة *T2* مقارنة

مع مجموعة السيطرة. زاد *ICSH* بشكل ملحوظ في المجموعة *T1* مقارنة بالمجموعة *T2* ، ولكن لم تكن هناك فروق ذات دلالة معنوية بين المجموعات الأخرى. زادت مستويات *GnRH* بشكل ملحوظ في المجموعة *T1* مقارنة بالمجموعات الأخرى ، ولكن لم تكن هناك فروق ذات دلالة معنوية بين مجموعات *C* و *T2* و *T3*. أظهرت المقاطع النسيجية للخصية في المجموعة *T2* التي تم إعطاؤها كلوريد الكادميوم ضعفًا في وظيفة الخصيتين والبربخ الوعاء الناقل ، بالإضافة إلى انخفاض في عدد خلايا سيرتولي. أظهر تناول كلوريد الكادميوم ومعالجته بـ *Q10* تكوين حيوانات منوية كاملة مع خلايا سيرتولي بارزة واحتوت القناة البربخية على خلايا طلائية طبيعية وكثيرة وأهداب مجسمة ، مع وجود كمية كبيرة من الحيوانات المنوية في تجويفها ؛ احتوت قناة الوعاء الناقل على خلايا طلائية عمودية كثيرة وأهداب مجسمة طويلة وممتدة وألياف عضلية ملساء طبيعية. وضحت الدراسة الحالية أن الخصائص المضادة للأكسدة لـ *CoQ10* هي أساس الدور التحسيني للجهاز التناسلي الذكري المعرض لكلوريد الكادميوم. تظهر الأدلة أن *CoQ10* يحسن بشكل أساسي هرمونات الذكورة ووظيفة الخصية ويحميها من الأكسدة بواسطة كلوريد الكادميوم.

الكلمات المفتاحية: الجهاز التناسلي الذكري ، جرذان الوستر ، كلوريد الكادميوم ،كلوريد الكادميوم

Introduction

Coenzyme Q10 (CoQ10), also known as ubiquinone, is a vitamin-like molecule found in living organisms that consists of a redox active benzoquinone ring and a hydrophobic side chain [1]. CoQ10 exists in two redox forms: ubiquinone-10 (CoQ10, oxidized form) and ubiquinol-10 (CoQ10H2, reduced form) [2]. CoQ10 has several biological and chemical activates. As an electron transport system intermediate in mitochondria, it plays a vital roles in cellular respiration and ATP production [3]**.** CoQ10 deficiency is an autosomal recessive disorder characterized by at least five main symptoms:

1. Encephalomyopathy characterized by three signs ragged red fibers ,recurrent myoglobinuria and brain involvement.
2. A severe multi-systemic disease in infants.
3. Cerebellar ataxia
4. Leigh syndrome is characterized by deafness, growth retardation and ataxia.
5. Isolated myopathy.[4]

CoQ10 acts as a primary free radicals scavenger and therefore prevents lipid peroxidation in most sub cellular membranes[5]. CoC10 has a protective impact on proteins and DNA in addition to lipids [6]. CoQ10 is emerging as a preventive and therapeutic agent in addition to its direct antioxidant activity. Because of its important function in mitochondria and antioxidant activity, it is one of the most promising substances in antioxidant treatment [7]**.** Studies in the human showed CoQ10 has been to be a important component in treating cardiovascular diseases [8], neuropath [9] and renal diseases [10]. It can also suppress oxidative damage, increase the activity of DNA repair enzymes in human cultured lymphocytes, and prevent many of the negative consequences of photoaging on the skin [11]**.** CoQ10 help to production ATP and acting as a vital antioxidant and supporting the generation of further antioxidants, influencing the membranes permeability and stability, also stimulating cell growth and inhibiting cell death [12]. And helps regeneration of tocopherol [13]. Furthermore, CoQ10 has anti-inflammatory effects, since it reduces the production of pro-inflammatory cytokines such as tumor necrosis factor [14]. CoQ10 has received a lot of interest as a dietary supplement due to its ability to influence cellular bioenergetics and prevent some of the damage caused by free radicals [15]. Heavy metals, such as cadmium, induce an increase in the formation of free radicals, which increases

lipid peroxidation and causes an increase in the formation of unsaturated fatty acids [16]. Cadmium is a persistent heavy metal with several industrial implications [17]. It enters the body by inhalation of polluted air, gases and dusts, cigarette smoke, contaminated food and drink, and infrequently through ingestion at work [18]. The basic mechanism of cadmium toxicity includes an excess of reactive oxygen species (ROS), which leads to lipid peroxidation and subsequently, oxidative stress.[19]

The aim of this *in vivo* study was to investigate the efficacy of CoQ10 in combating the reproductive toxicity of Cd in male rats.

Materials and Methods

Experimental animals:

40 adult male Wister rats (average weight about 170±10gm) obtained from animal house of veterinary Medicine college /AL- Qadisiyah university **.**The animals were housed in well ventilated wire-plastic cages and reared under controlled conditions. The animals were allowed to acclimatize for seven days before experimentation.

The experiment continuous from 1/8-1/9/2021

Experiment design:

The animals were divided into four equal groups. And was treated for 60 days as follows: The first group was given just drinking water as a control, whereas the second group (T1) was given co enzyme Q10 (orally) once a day in a dose of 10 mg/kg [20]. The third group (T2) was given cadmium chloride (CdCl2) at a dose of 25 mg/kg, which is one-fifth of the LD50 [21]. The fourth group (T3) was given Cd + CoQ10 (25 mg/kg + 10 mg/kg) for 60 day.

Material: Q10 provide from Merk company / Germany

Animals Sacrificing:-

All animals were sacrificed 24 hours following the last dose, and blood samples were taken from the abdominal vein for assessment of male reproductive hormones (Testosterone, ICSH and GnRH,). The testis, epididymis, and vas deferens were excised and preserved in 10% formalin for histological examination.

Hormonal Assays In Blood Serum:

Testosterone, interstitial cell stimulating hormone (ICSH) and gonadotropin releasing hormone (GnRH) hormones were evaluated by using ELISA technique, the kits provide from Elabscince /USA and the assay done according to the company instruction**.**

Histological Study:

Five tissue samples (testis, epididymis, and vas deference) were collected from each group immediately after killing the animals and fixed in 10% formalin left for 72 hours. After fixation, the tissue was trimmed and the specimens were washed by tap water for 3-4 hours to remove the formalin solution and transferred to the following steps**:**

1. Dehydration : by upgrading the alcohol from 50% to absolute alcohol 100% for two hours in each concentration
2. Clearing: The clearing process by use xylene for ½ hours for each solution
3. Embedding : the samples put it in the oven (58C°). Then, it poured in a block of paraffin
4. Blocking : The specimen was poured in blocks of pure wax to be prepared for cutting. It was left for 24 hours in refrigerator to give the paraffin time to solidity.
5. Cutting :Cutting by using the rotary microtome. The thickness is about (5-8µm).
6. Staining :The sections are stained with the Alum Haematoxylin and Eosin stains.[22]

Statistical analysis :

A computerized program (SPSS) was used to calculated the statistics analysis.[23]

One way analysis of variance (ANOVA) with least significant difference LSD was detected to compare between groups.[24]

All data were presented as mean SR.

Results

Testosterone Hormone:

Result in table (1) showed: The serum testosterone hormone concentration of experimental groups revealed a significant increase ($p \leq 0.05$) in T1 group (90.03 ± 4.7) which administers CoQ10 (10mg/kg) for (60) days compared to other groups (C,T2,T3) which had (50.1 ± 2.5, 30.8 ± 2.1, and 40.25 ± 2.8 nmol/L) respectively. While there was a significant decrease in the T2 group as compared to the control group.

Interstitial Cell Stimulating Hormone (ICSH):

Result in table (1) showed: The serum ICSH concentration revealed that there was significant increase ($P \leq 0.05$) in group T1 (6.1 ±1.2) compared to T2 group (3.51 ± 1.1) and there were no significant differences ($p \leq 0.05$) between other groups.

Gonadotropin Releasing Hormone (GnRH):

Result in table (1) showed: there was a significant increase ($p \leq 0.05$) in GnRH concentration in group T1 (20 ± 2.4) compared to other groups , but there were no significant differences between groups C,T2 and T3.

Table (1) Effect Of Co-Enzyme Q10 On Male Reproductive Hormones Levels Of Wister Rats Exposed To The Cadmium Chloride

	C	T1	T2	T3
Testosterone (nmol/L)	c 50.1 ± 2.5	a 90.03 ± 4.7	b 30.8 ± 2.1	bc 40.25 ± 2.8
ICSH(ng/L)	ab 5.1 ± 0.2	a 6.1 ± 1.2	b 3.51 ± 1.1	ab 5.5 ± 0.5
GnRH (ng/L)	b 10.1± 0.1	a 20±2.4	b 6.3 ± 0.7	b 9.5 ± 0.4

Numbers =mean ± S.E

Different litters= significant Differences ($p \leq 0.05$).

C group =control group.

T1 group =orally gavage Q10 (10mg/Kg once daily, for 60 days).

T2 group =orally gavage cadmium chloride (25 mg/Kg once daily, for 60 days.

T3 group = orally gavage cadmium chloride (25 mg/kg) + Q10 (10 mg/kg) once daily, for 60 days.

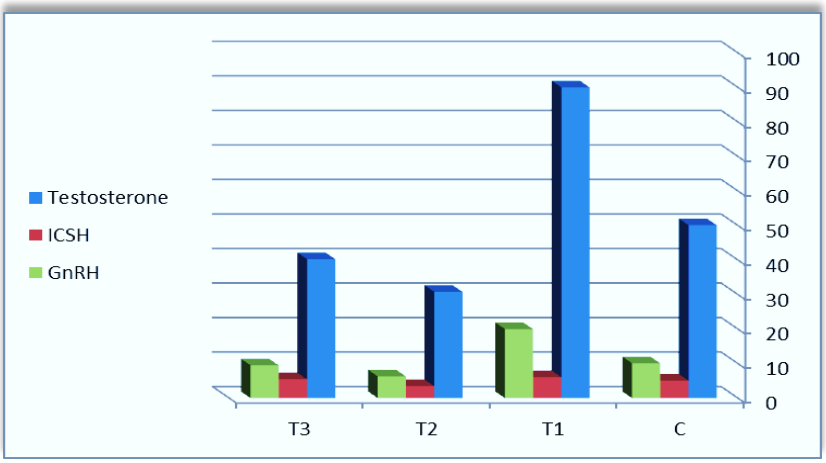

Figure (A) Effect of Co-Enzyme Q10 On Male Reproductive Hormones Levels Of Wister Rats Exposed To The Cadmium Chloride

Histopathological study:

Testis histopathology:

Testicular sections of rats in the control group revealed normal seminiferous tubules with a large number of spermatogonia, primary, secondary spermatocytes, sertoli cells, and spermatozoa, as well as the presence of leydig's in the interstitial tissue of the testes fig.(1) .

Testicular sections from rats in the T1 group administered CoQ10 reveal full spermatogenesis characteristics such as the existence of spermatogonia and a large number of primary and secondary spermatocytes, as well as the presence of spermatids and spermatozoa in the lumen of the seminiferous tubules fig.(2).

Testicular sections from rats in the T2 group that were given cadmium chloride showed vaculation of spermatogonia and a small number of primary and secondary spermatocytes, a small number of sperms produced, a wide lumen of seminiferous tubules, a few number of sertoli cells, and the presence of spermatid multinucleated giant cells within the seminiferous tubules fig.(3)

Testicular sections from rats in the T3 group that were given cadmium chloride and treated with Q10 revealed complete spermatogenesis characteristics such as the presence of spermatogonia, primary and secondary spermatocytes with spermatid, spermatozoa in the lumen of seminiferous tubules, and prominent sertoli cells that supported spermatogonia fig.(4)

Epididymis histopathology:

Sections of the epididymis of rats in the control group indicate a normal epididymial duct packed with sperms, a normal epithelium lining the epididymis duct, and the presence of long stereocilia fig.(5).

Epididymis sections from rats in the T1 group administered CoQ10 reveal the epididymis duct extended, dilated, and completely filled with sperms, as well as typical epididymis duct epithelium and the presence of long stereo cilia fig.(6).

Epididymis sections from rats in the T2 group treated with cadmium chloride revealed that the epididymis duct was empty, with degeneration and loss of stereo cilia and low epithelial cells lining the epididymial duct Fig.(7)

Epididymis sections of rats in T3 group which given cadmium chloride and treated with CoQ10 show the epididymial duct has normal and high epithelial cells as well as stereocilia. The epididymial duct wide with large amount of sperms in the lumen of it fig (8).

Vas deference histopathology:

Vas deference sections from rats in the control group revealed typical vas deference wall characteristics such as normal columnar epithelium with stereo cilia, normal smooth muscle fiber, , with sperms filling the lumen of the vas deference fig (9) .

Vas deference sections from rats in the T1 group that received Q10 had typical vas deference tissue characteristics such as normal epithelium lining it, normal smooth muscle fibers, and sperms filling the lumen of the vas deference fig (10).

Vas deference sections from rats in the T2 group that were given cadmium chlorid showed that the conductive tube of vas deference was empty of sperms and that there was mild destruction in the stereo cilia and sloughing of the epithelial layer of vas deference, as well as degeneration of vas deference smooth muscles (fig (11).

Vas deference sections from the tats in T3 group that received cadmium chloride and was treated with CoQ10 revealed a high columnar epithelial with long and extended stereo cilia and a thick vas deference wall due to typical smooth muscle fiber. fig (12).

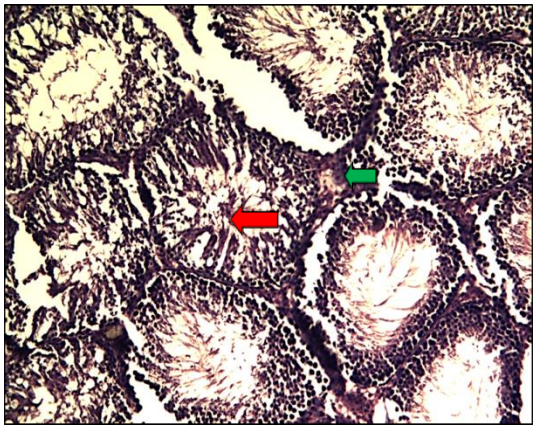

Fig. (1) Cross section of rat seminiferous tubules in control group. There are normal seminiferous tubules characterized by high numbers of spermatogonia, primary and secondary spermatocytes with spermatozoa (red arrow), there is presence of leydig's in the interstitial (green arrow). 100X H&E.

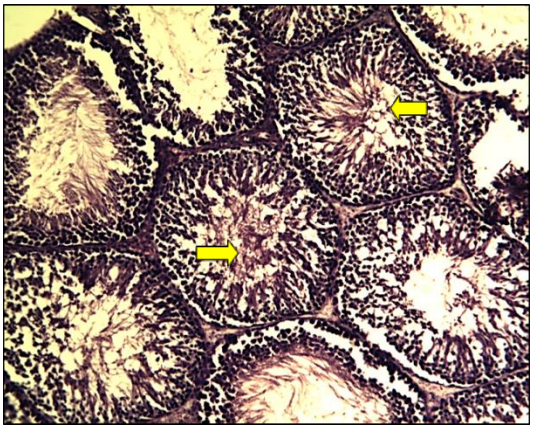

Fig. (2) Cross section of rat seminiferous tubules in T1 group Show complete spermatogenesis characterized by presence of spermatid and spermatozoa in the lumen of seminiferous tubules (yellow arrows). 100X H&E

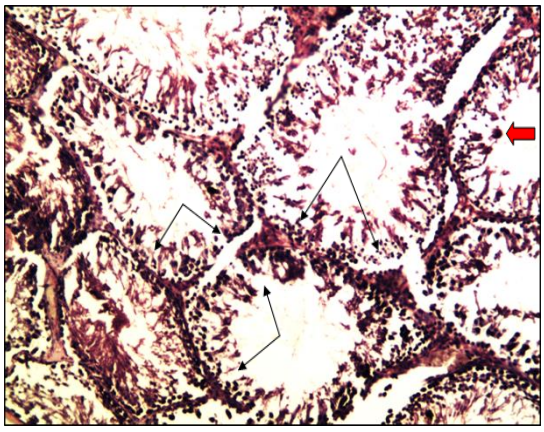

Fig. (3) Cross section of rat seminiferous tubules in T2 group . Show there is vacuolation of spermatogonia (thin arrows) and few numbers of spermatocytes, there are very little sperms production and the lumen of seminiferous tubules show wide. Presence of spermatid multinucleated

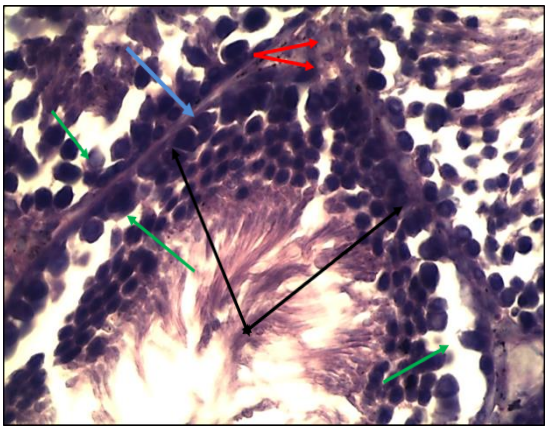

Fig.(4) Cross section of rat seminiferous tubules in T3 group .show there are complete spermatogenesis characterized by presence of spermatogonia, primary and secondary spermatocytes with spermatid (two head arrows) .Also there are spermatozoa in the lumen of seminiferous tubules also presence of leydig's cells in the interstitial tissue of testis (red arrows). There are prominent sertoli cells (thin green arrows). Basement membrane (blue arrow) 400X H&E

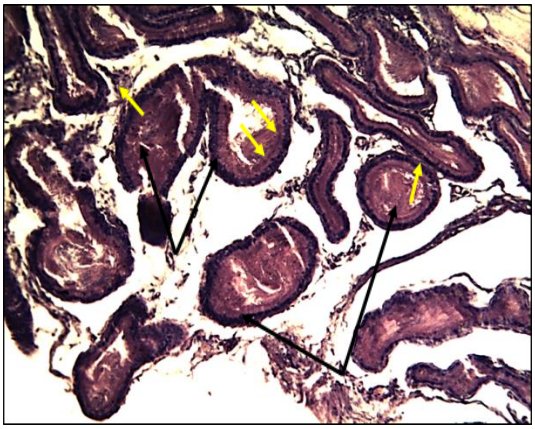

Fig.(5) cross section of rat epididymis in control group Show normal epididymial duct filled with the sperms (thin black arrows), normal epithelium which lining epididymis tubules (thin yellow arrows). 100X H&E

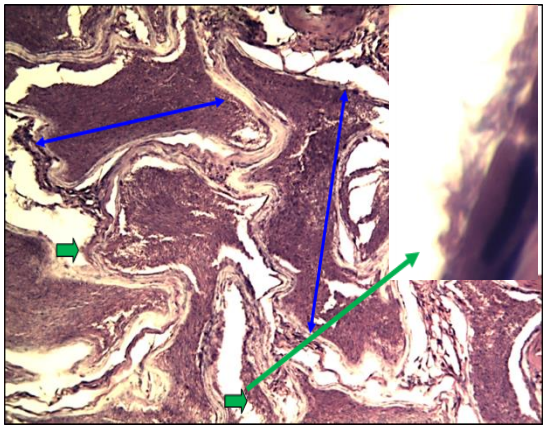

Fig.(6) cross section of rat epididymis in T1 group .Show there are extended, dilated and complete filled epididymal duct (two head blue arrow) with presence of long stereocilia (green arrows). 400X H&E

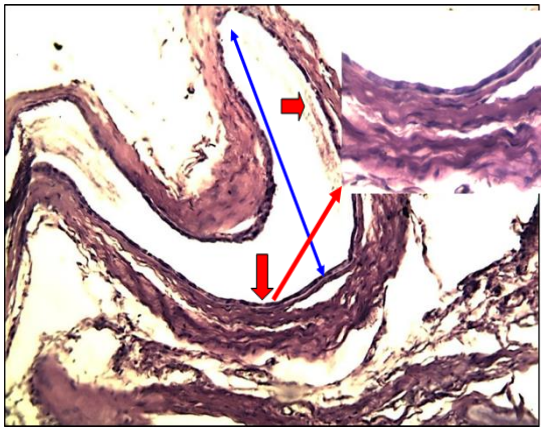

Fig.(7) cross section of rat epididymis in T2 group. Show the epididymal duct are empty (two head blue arrow) with complete absence of stereocilia and degeneration of epithelial cells which lining the epididymial duct (red arrows). 400X H&E.

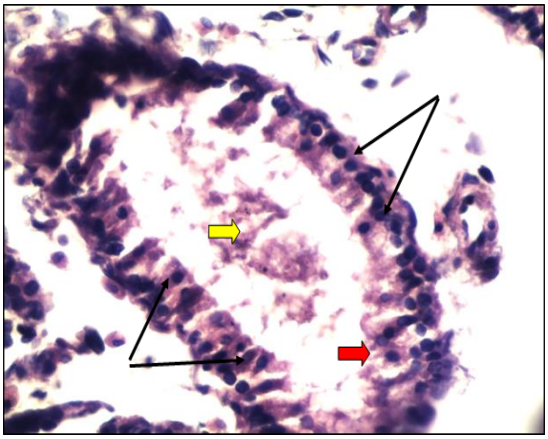

Fig.(8) cross section of rat epididymis in T3 group. Show the epididymial duct filled with sperms (yellow arrow) with normal and high epithelial cells (thin arrows) and presence of stereocilia (red arrow). 400X H&E

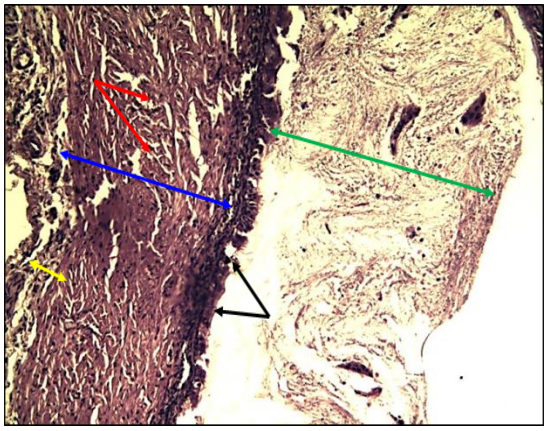

Fig.(9) Vas deference section of rat in control group. Show normal vas deference wall (blue arrow) which characterized by normal columnar epithelium (black arrows) with stereocilia and normal smooth muscles fibers (red arrows) and normal serosa (yellow arrow). The sperms filled the lumen of vas deference (tow head green arrow). 100X H&E.

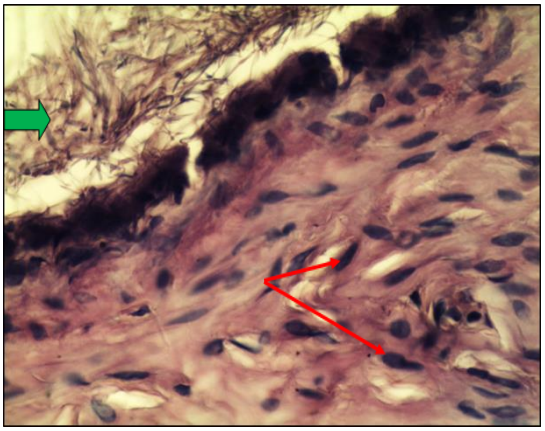

Fig.(10) Vas deference section of rat in T1 group. Note normal epithelium which lining it (black arrow). Normal smooth muscles fibers (red arrows) and sperms filled the lumen of vas deference (green arrow). 400X H&E.

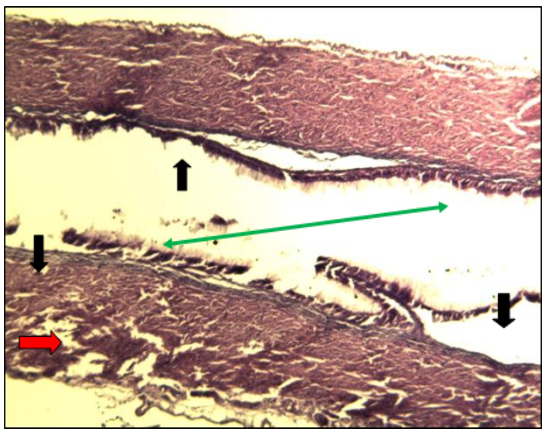

Fig.(11) Vas deference section of rat in T2 group. Note the conductive tube of vas deference empty from sperms (green arrow) and there is mild destruction in the stereocilia and sloughing the epithelial layer of vas deference (black arrows). There is degeneration of smooth muscles of vas deference (red arrow). 400X H&E.

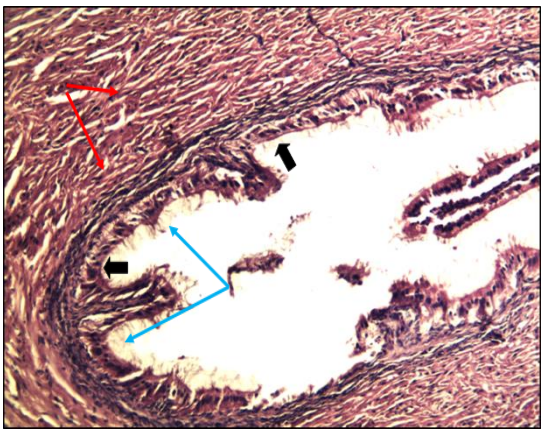

Fig.(12) Vas deference section of rat in T3 group. Show there is high columnar epithelium (black arrows) with long and extended stereocilia (blue arrows) and thick vas deference wall due to normal and proliferating smooth muscles fiber (red arrows). 400X H&E.

Discussion

In experimental models, Cd exposure can affect on the testis function and induce pathogenesis leading to reduced male hormone and impaired male fertility. Q10 was employed as an antioxidant to protect the male reproductive system from cadmium toxicity, which was accomplished by studying male reproductive hormone and histological sections of male reproductive organs, which is the same approach utilized by [24][25]. The findings supported previous research that found CoQ10 to be a strong antioxidant in protecting rat testes from oxidative damage caused by Cd [26]. Local study showed The co-enzyme Q10 be used to improve in testicular dysfunctions and return the level of reproductive hormones near to the normal after exposure to lead acetate.[27]

The current study revealed that the male rats in group T1 which received co- enzyme Q10 had significant increase ($P \leq 0.05$) in testosterone hormone concentration, This indicates stimulation of testosterone synthesis in the testes by co enzyme Q10, which increases the activity of the enzymes responsible for testosterone production, the epididymis is also abundant in androgen receptors, the site of action of testosterone and dihydrotestosterone [28]. CoQ10 treatment had an effect on the testosterone hormone, most likely via altering the equilibrium between oxygen radicals and antioxidant defense.

Such a relationship can be attributed to coenzyme Q10's active biological activity as a critical component of the energy generation pathway in humans and animals, which is considered to have a beneficial impact on testosterone production and therefore on infertility, particularly male infertility.

Studies of the consequences of Cd contamination have demonstrated that the testis is more sensitive to Cd than other important organs, in addition, Cd can interfere with testis function and significant increase in lipid peroxidation in the testes of rats [29]. This probably is the consequence of the intracellular accumulation of ROS with subsequent development of tissue injury. We presume that Cd also enters the mitochondria and inhibits the activities of many enzymes by binding to their –SH groups or by inhibiting the protein synthesis [30]. Pretreatment with CoQ10 was very effective in the prevention of oxidative damage induced by Cd, which resulted in significant increase in testosterone hormone ,these result can be explained by the important role of CoQ10 in the prevention of lipid peroxidation and in the protection of integrity and functioning of tissues and cells [31]. It is known that CoQ10 induces an elevation of Vit E concentration in tissues of rats and it is known, that the rate of O2 elimination is directly related to the Vit E concentration, indicating the role of Vit E in the elimination of radicals [32]. The presence of the antioxidants minimized the toxic effects of Cd on the affected enzymes CoQ10 by quenching ROS can be indirectly involved in the regulation of gene expression and in modulating the activities of most enzymes. At the same time, CoQ10 has an important role in the prevention of lipid peroxidation and oxidative damage of tissues, and thus induces changes in the activity of many enzymes [33]. CoQ10 in its reduced form as the hydroquinone (ubiquinol) is a potent lipophilic antioxidant and is capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate [32]. Co-Q10 acts on lydig's cells in the testis, which are responsible for testosterone levels, and CO-Q10 promotes the release of (GnRH) from the hypothalamus to the anterior pituitary gland via the portal system due to its antioxidant impact on free radicals[34].

The antiapoptotic effect of CoQ10 has been described by several studies [35][36]. And it was confirmed CoQ10 treatment upregulated the expression of the antiapoptotic genes *Bcl2* and downregulate proapoptotic genes *Casp3* and *Bax* [37]. CoQ10's antiapoptotic impact was linked to its prevention of DNA fragmentation and mitochondrial depolarization, as well as its increase in ATP levels. Furthermore, CoQ10 reduces apoptosis-inducing factor nuclear translocation and prevents cell death via inhibiting mitochondrial complex I activity [38]. Q10 have anti-inflammatory effect can now be extended to the testes, and reduced the concentrations of proinflammatory cytokines, TNF-α and IL-1β, consequently attenuating inflammation. Meanwhile, it activated the *Nfe212/Hmox1* pathway by upregulating both *Nfe212* and *Hmox1* gene expressions.[39]

Conclusion

The present study demonstrates that the antioxidant activities of CoQ10 are the basis of the male reproductive system's ameliorative activity. Evidence suggests that CoQ10 primarily enhances male hormones and testicular function while also protecting them from oxidative damage. Further research is required to identify the specific mode of action of CoQ10 and the suitable standardized dose and duration of CoQ10 supplementation in the treatment of various male infertility patients..

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