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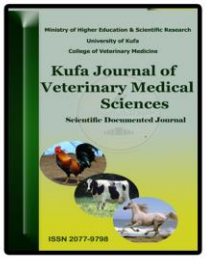

Study the role CoQ10 in improvement of reproductionefficiency in male wister rats

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Abstract

The current study was carried in physiology and pharmacology department at college of veterinary medicine, Al-Qadiysia University. The aim of this study was clarified the effect of Coenzyme Q10 in improvement of reproductive efficiency in male Wister rats after exposed to oxidative stress by lead acetate.40 male rats at 60 days old with 135±11gm in weight were divided into four equal groups, The first group was given distilled water with orally gavage for 60 days as control group (C).The second group (T1)wasgiven CoQ10 (200mg/kg/b.w)for 60 days , while the third group (T2) given lead acetate (10mg/kg/b.w) for 60 days. The forth(T4) group was gives lead acetate (10mg/kg/b.w) for 30 days then given CoQ10 for 30 days. At 60 days of experiment all animals were sacrified, sample of testis taken for measure the gene expression of(CYP17 and CPY11), gene expression results were revealed that there is up regulation ($p \leq 0.05$) in T1 compared with T2.

Key words: CoQ10, CYP17 and CPY11 gene, qRT-PC

دراسة دور CoQ10في تحسين الكفاءة التناسلية لذكور جرذان الوستر

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الخلاصة

أجريت الدراسة الحالية في فرع الفسلجة والأدوية في كلية الطب البيطري بجامعة القادسية بهدف الكشف عن دور المرافق الإنزيمي CoQ10 في رفع الكفاءة التناسلية لذكور جرذان ويستر المعرضة للإجهاد ألتأكسدي لمادة خلات الرصاص . تم توزيع 40 جرذاً بعمر 60 يوماً ومعدل وزن 135 ∓ 11 غرام عشوائياً الى أربعة مجاميع متساوية أعطيت الأولى الماء المقطر بوصفها مجموعه السيطرة (C) وأعطيت المجموعة الثانية (T1) مادة المرافق الإنزيمي CoQ10 200 ملغم / كغم من وزن الجسم يومياً لمدة 60 يوم أما المجموعة الثالثة(T2) أعطيت مادة خلات الرصاص 10 ملغم / كغم من وزن الجسم لمدة 60 يوم ، أما المجموعة الرابعة(T3) أعطيت مادة خلات الرصاص 10 ملغم / كغم من وزن الجسم لمدة 30 يوم ثم أعطيت مادة المرافق الإنزيمي 200 ملغم / كغم من وزن الجسم لمدة 30 يوم . تمت التضحية بجميع الحيوانات في اليوم 60 من التجربة ، وأخذ جزء من الخصية لغرض قياس التعبير الجيني لجينات (CYP11وCYP17). أظهرت نتائج الدراسة في عينة التعبير الجيني زيادة ($p \leq 0.05$) في التعبير الجيني لكل من (CYP11 و CYP17) في مجموعةT1 مقارنة مع مجموعةT2

Introduction

It is well known that Coenzyme Q (CoQ) or ubiquinone is an essential vitamin-like compound which found in most organisms (1). Interestingly, CoQ10 has many biochemical roles. It can work as an intermediate role of the electron transport system in mitochondria, plays a crucial role in production of adenosine triphosphate (ATP) and cellular respiration (2). Another study hypothesized that CoQ10 prevent lipid peroxidation in most subcellular membranes (3,4) . The effect of ubiquinol could be extending to proteins and DNA . Furthermore, CoQ10 has direct role as an antioxidant function. There is many evidence proved that CoQ10 can take role in antioxidant therapy, due to its role in mitochondria and antioxidant action (5). Further study in human revealed that CoQ10 has been shown to be a valued element in treating cardiovascular (6,7), male infertility (8), neurodegenerative (9) and cancer (10). Interestingly, studies revealed that CoQ10 has the ability to inhibit oxidative damage and enhance DNA repair enzyme activity in human cultured lymphocytes (11). Co-enzyme Q10 could make ATP and performing as a vital antioxidant. Furthermore, it could associate with the revival of many antioxidants and restrain cell death by induce cell growth 12).

Lead is a toxic metal, which can work in organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in environment. It can release from industrial discharge, automobile fuels and paints (13). The effect of this metal is less danger in the public than the industries. It was reported that, human can exposure to lead through inhalation (15–30%) as well as 70–85% of lead from food and drinks through the gastrointestinal tract (14). Consequently,

lead acetate can affect the development and maturation of the ovarian follicles in female(15).While in men, mainly in professional workers, lead exposure results in many discord such as infertility, cellular degeneration , sterility testicular atrophy, reductions in somniferous tubule diameter (STD) and sperm count. All of these discords depending on the dose and time of exposure to lead acetate .In animal models, lead exposure consistently decreases male reproductive function. (McGivern, 1991) report that the effects of lead on adult rat testis caused testicular necrosis and atrophy in rodents. It is well known that exposure to some materials or elements through the critical period of development might affect the adult life. The aim of this studies was to investigate the possibility of using Co enzyme Q10 to improve the efficiency of reproductive system that exposed to Oxidative stress (lead acetate) by using estimating the CYP17,CYP11 Gen expression using R-T_PCR for testis.

Materials and Methods

Cells and culture conditions

In this experiment we used (40) adult male Wister rat (average weight was 135+_11) were obtained from animal house in veterinary medicine collgein AL-Qadysia university. These animal reared under controlled conditions (12L:12D at 22C*) , fed standard laboratory food (19% protein ratio and 3000kilocalories energy) and drinking distilled water(Shittuet al.,

2007). 40 adult male wister rats divided randomly into 4 groups each group consist of (10) males and we recorded the primary weight of animals after 5 days of acclimatization, that all groups treated as fallowing .

1- Control Group : given the standard food and distilled water.

2- The first treated Group (T1) given co enzyme Q10 in a dose of 200mg \kg b.w\day. For 60 consecutive days.(Safarinejad,2009)

3- The second Treated Group (T2) given lead acetate in a dose 10 mg /kg /b.w. for 60 days (Yousif,2010)

4- The Third Treated Group (T3) given lead acetate for 30 days then given co-enzyme Q10 (200mg/kg/b.w)for 30 days

Primers:-

The primers were used in this study, GAPDH gene primers used as Housekeeping gene, CYP17 gene and CYP11A1 gene primers used as target genes for testosterone gene expression. The sequence of genes was obtained from the online database, NCBI- Gene Bank data base. The CYP17 primer sequences were (ACAACAACAGCTGTGAAGGC) forward and (AGGATTGTGCACCAGGAAAG) reverse. The CYP11A1 primer sequences were (GACGCATCAAGCAGCAAAAC) forward and (ATGGACTCAAAGGCAAAGCG) reverse. The GAPDH primer sequences were (ATGCCCCCATGTTTGTGATG) forward and (TCCACGATGCCAAAGTTGTC) reverse. All primer design used in quantification of gene expression using qRT-PCR techniques based SYBER Green DNA binding dye, and supported from (Bioneer, Korea) company.

Quantitative Reverse Transcription Real-Time PCR:-

Quantitative Reveres Transcription Real-Time PCR technique was performed for estimation of relative quantification (gene expression analysis).

1- Total RNA extraction:-

Total RNA were extracted from rat testes tissue by using (TRIzol® reagent kit) and done according to company instructions. The extracted total RNA was assessed and measurement by Nanodrop spectrophotometer (THERMO. USA).

2- DNase I Treatment:-

The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by promega company, USA instructions.After that, The mixture was incubated at 37C° for 30 minutes. Then, 1µl 25mM EDTA was added and incubated at 65C° for 10 minutes for inactivation of DNase enzyme action.

3- CDNA synthesis:-

DNase-I treatment total RNA samples were used in cDNA synthesis step by using AccuPower® RocktScript RT PreMix kit that provided from Bioneer company, Korea and done according to company instructions .

4-Quantitative Real-Time PCR (qPCR) master mix preparation:-

QPCR master mix was prepared by using AccuPowerTM Green Star Real-Time PCR kit that dependantsyber green dye detection of gene amplification in Real-Time PCR system

5- Data analysis of qRT-PCR:-

The data results of q RT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) Livak method that described by (Livak and Schmittgen, 2001). The relative quantification method, quantities obtained from q). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. In this method, one of the experimental samples is the calibrator such as (Control samples) each of the normalized target values (CT values) is divided by the calibrator normalized target value to generate the relative expression levels. After that, the ΔCT Method with a Reference Gene was used.

Results

Molecular analysis

We isolate total RNA and the purity were estimated using Nanodrop spectrophotometer in absorbance reading (260/280 nm). Successfully, all studied testicular tissue samples provides us high concentration of total RNA. Interestingly, group that treated with Co-enzyme Q10 recorded significant higher concentration throughout experimental.

In a real time PCR assay a positive reaction could be done byaccumulation of a fluorescent signal. The Ct (cycle threshold) is the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Inorder to make our data biologically meaningful,Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level means the greater amount of target nucleic acid in the sample). In this study, the cycle threshold (Ct) values were obtained from the samples (Figure 1).

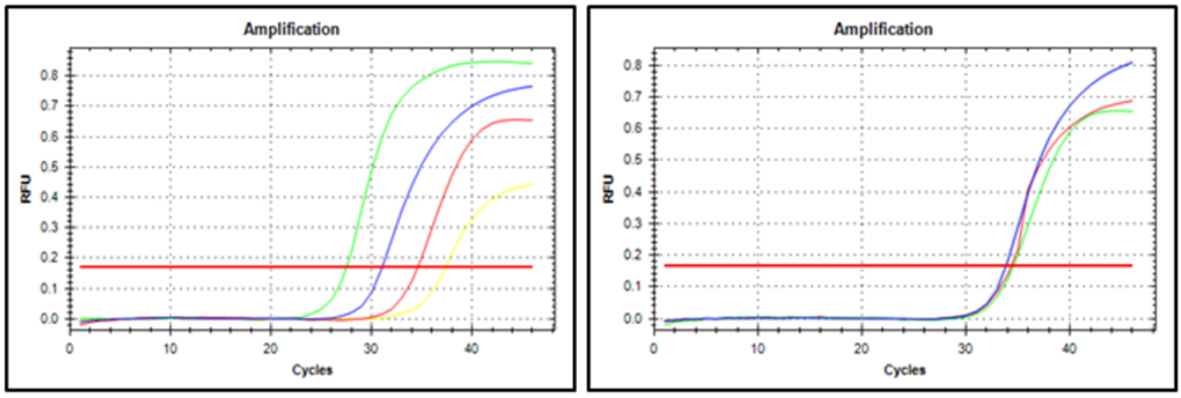

Figure (1): Real time PCR amplification plot for CYP17 and CYP 11 housekeeping gene that show no difference in threshold cycle numbers (Ct value) between treatment and control groups.

Red plot : control group

Green plot: T1group animal received (200mg/kg/b.w) CoQ10 for 60 days.
Yellow plot:T2 group animal received (10mg/kg/b.w) lead acetate for 60 days.
Blue plot:T3 group animal received (10mg/kg/b.w)lead acetate for 30 days then (200mg/kg/b.w) for 30 days .

Relative quantification of CYP17 gene expration :

Figure (2), showed that the mRNA expression level for the CYP17 gene change in (T1, T2 and T3) groups comparing to control group. There was highly significant increase of gene expression (up to 13.3) in T1 group compared with T2 and control. T2 group showed significant decrease (0.06) comparing with control, T1 and T3 groups. while (T3) group showed significant increase(10.16) compared with C and T2 group table 1**.**

Table (1)Effect of co-enzyme Q10 on testicular section of CYP17 gene of Wister rats treated with lead acetate .

Exp. Group	CT (CYP17)	CT (GAPDH)	ΔCT (Test)	ΔCT (control)	ΔΔCT	Fold change (2^-ΔΔCT)	**Mean**
T1	30.27	32.27	-2.005	0.897	-2.902	7.473	**13.309**
T1	30.44	32.12	-1.684	0.897	-2.581	5.983	
T1	29.53	32.34	-2.813	0.897	-3.710	13.084	
T1	29.18	32.13	-2.948	0.897	-3.844	14.364	
T1	28.74	32.32	-3.584	0.897	-4.480	22.322	
T1	29.12	32.28	-3.159	0.897	-4.055	16.626	
T2	38.54	32.14	6.400	0.897	5.503	0.022	**0.067**
T2	36.15	32.36	3.793	0.897	2.896	0.134	
T2	37.12	32.42	4.702	0.897	3.805	0.072	
T2	37.42	32.28	5.140	0.897	4.244	0.053	
T2	37.12	32.45	4.667	0.897	3.770	0.073	
T2	37.66	32.33	5.334	0.897	4.437	0.046	
T3	29.73	32.45	-2.716	0.897	-3.612	12.231	**10.160**
T3	29.21	32.22	-3.006	0.897	-3.903	14.957	

T3	30.16	32.64	-2.480	0.897	-3.377	10.389	
T3	30.64	32.56	-1.921	0.897	-2.818	7.050	
T3	30.16	32.37	-2.211	0.897	-3.108	8.619	
T3	30.26	32.31	-2.051	0.897	-2.948	7.715	
Mean C	33.36	32.46	0.897	0.897	0.000	1.000	**1**

C=control group .
T1=orally gavage co-enzyme Q10 (200mg/kg/b.w) for 60 days
T2=orally gavage lead acetate (10mg/kg/b.w) for 60 days
T3=orally gavage lead acetate (10mg/kg/b.w) for 30 days then given co-enzyme Q10(200mg/kg/b.w) for 30 days.

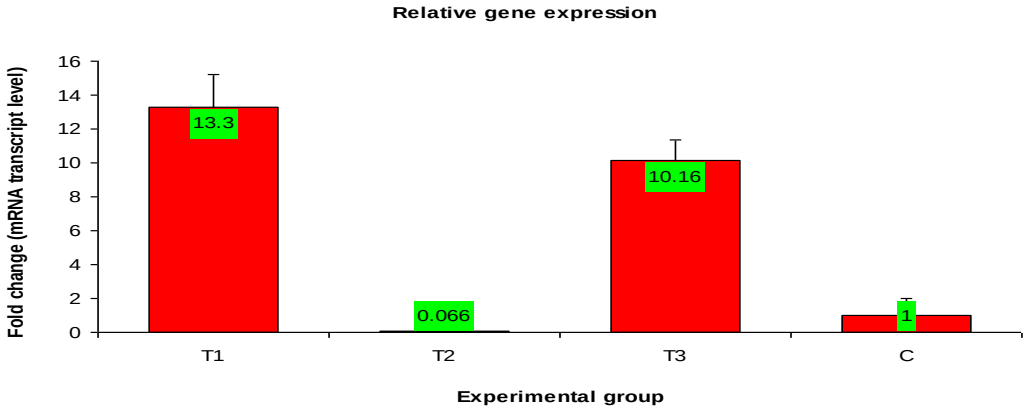

Figure (2) show relative gene exprssion of CYP17 gene

Relative quantification of CYP11 gene expression:

Figure (2), showed the mRNA expression level from CYP11 gene change in (T1,T2 and T3) groups comparing to control group . T1 group showed significant increase (15.17) compared with C and T2 group . While T2 showed significant decrease (0.05) compared with other studied groups. T3) group showed significant increase (7.41) compared with C and T2 group.

Table (2) Effect of Co-enzyme Q10 on CYP11gene of testicular section of Wester rats treated with lead acetate .

Exp. Group	CT (CYP11)	CT (GAPDH)	ΔCT (Test)	ΔCT (control)	ΔΔCT	Fold change (2^-ΔΔCT)	**Mean**
T1	28.57	32.27	-3.705	0.208	-3.913	15.066	**15.171**
T1	28.44	32.12	-3.684	0.208	-3.893	14.852	

T1	28.13	32.34	-4.213	0.208	-4.421	21.427	
T1	29.08	32.13	-3.048	0.208	-3.256	9.554	
T1	28.24	32.32	-4.084	0.208	-4.292	19.590	
T1	29.09	32.28	-3.189	0.208	-3.397	10.535	
T2	38.12	32.14	5.980	0.208	5.772	0.018	**0.026**
T2	37.45	32.36	5.093	0.208	4.885	0.034	
T2	38.82	32.42	6.402	0.208	6.194	0.014	
T2	37.82	32.28	5.540	0.208	5.332	0.025	
T2	38.02	32.45	5.567	0.208	5.358	0.024	
T2	37.16	32.33	4.834	0.208	4.626	0.041	
T3	29.73	32.45	-2.716	0.208	-2.924	7.590	**7.410**
T3	29.31	32.22	-2.906	0.208	-3.114	8.660	
T3	30.06	32.64	-2.580	0.208	-2.789	6.910	
T3	30.14	32.56	-2.421	0.208	-2.629	6.187	
T3	29.26	32.37	-3.111	0.208	-3.319	9.982	
T3	30.16	32.31	-2.151	0.208	-2.359	5.131	
Mean C	32.67	32.46	0.208	0.208	0.000	1.000	**1**

C=control group .
T1=orally gavage co-enzyme Q10 (200mg/kg/b.w) for 60 days
T2=orally gavage lead acetate (10mg/kg/b.w) for 60 days
T3=orally gavage lead acetate (10mg/kg/b.w) for 30 days then given co-enzyme Q10(200mg/kg/b.w) for 30 days

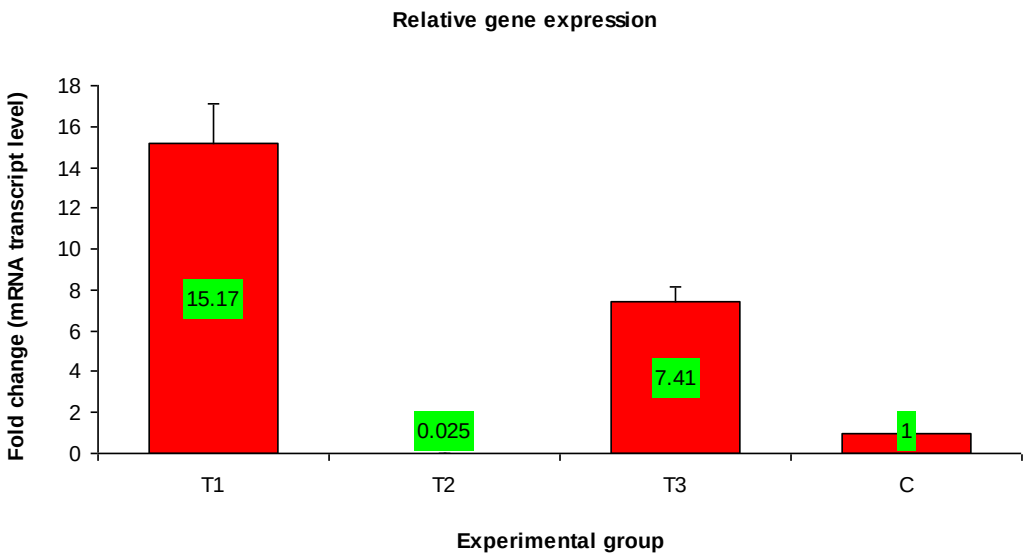

Figure (3) show relative gene expression of CYP 11 gene

Discussion

CoQ10 has essential role as an antioxidant function and infertility. In the present study, We attempt to explore the role of Co-Q10 by using a mammalian model (male rats) . We have studied mRNA expression levels of such gene CYP17 and CYP11 in testis tissue (16). Both of CYP11 and CYP17 aresteroidogenic enzyme that participate in testosterone production (17). They have some effects on the hypothalamic-pituitary-gonadal axis (18). Basically, Testosterone biosynthesis involves steroidogenic proteins: steroidogenic acute regulatory protein, cholesterol side-chain cleavage enzyme (CYP11A), 3b-hydroxysteroid dehydrogenase (3b-HSD type II), 17a-hydroxylase (CYP17) (19). These steroidogenic enzymes are critical for the creation of androgens and up-regulation the mRNA expression of genes coding for these enzymes. They can rise the efficiency of testosterone and estradiol synthesis. On other hand, CoQ10 is an important molecule due to their role in modulates the transcription of hundreds of human genes Schmelzer et al 2007 (20). CoQ10 increased the expression of genes connected to cell signaling, metabolism, transport and control of transcription, and oxidative phosphorylation. The CO-Q10 improve of lydig's cells make antioxidant action lead to Cholesterol transport within the Leydig cells steroidogenesis requires the transportof free cholesterol from the outer to the inner mitochondrial membrane enzyme reaction occurs, catalyzed by the CYP11A gene and CYP17 gene lead to increase production of testosterone(21). Result showed up regulation of CYP11A gene and CYP17 gene animal roup that gavage with CO-Q10(T1) while there was significant decrease ($p<0.05$) in animal group that gavage with lead acetate (T3) compare with other groups. These result showed the negative effect of lead acetate on gene expression for these genes for its role

as a source of oxidative stress factors. This stress happened by Lead is reported to by creating reactive oxygen species (ROS) for instance superoxide radicals, hydrogen peroxide and lipid peroxides (22). An excess of ROS has the ability to damage lipid , proteins ,nucleic acids. Our result showed up regated to gene expression in animal group that gavage with CO-Q10 after treat with lead acetate. We observe that Co Q10 acting as antioxidant role to reduce the effect of ROS. These results agree with with(23). They proposed that Co Q10 protect cells and tissues from oxidative damages caused by reactive oxygen species. We hypothesize that Co Q10 has a role in regulationof tests hormone by icreasing the rates of CYP11A gene and CYP17 gene epression , the testserone hormone production and effect on male infertilit .

References

1- Littarru GP. (2009). Coenzyme Q10: from biochemistry to medicine. http://www.st-hs.it/tma_MAForum_0201.htm (14.01.)

2- Bentinger M, Brismar K, Dallner G. The antioxidant role of coenzyme Q. Mitochondrion 2007; 7(Suppl 1): S41-S50.

3- Kitano M, Watanabe D and Oda S.(2008). Subchronic oral toxicity of ubiquinol in rats and dogs. Int J Toxicol; 27: 189-215.

4- Crane FL.(2001). Biochemical functions of coenzyme Q10 . J Am CollNutr; 20: 591-8.

5- Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK and Kumar MNVD. (2006).Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. J Control Release; 113: 189-207.

6- Weant KA and smith KM. (2005).The role of coenzyme Q10 in heart failure. Ann Pharmacother; 39: 1522-6.

7- Mandelker L. (2008). Cellular effects of common antioxidants. Vet Clin North Am Small AnimPract; 38: 199- 211.

8- Shults CW.(2003).Coenzyme Q10 in neurodegenerative diseases. Curr Med Chem; 10: 1917-21.

9- ChaturvediRK ,and Beal MF.(2008).Mitochondrial approaches for neuroprotection. Ann N Y AcadSci; 1147: 395-412.

10- Tomasetti M, Alleva R and Borghi B, (2001). Collins AR. In vivo supplementation with coenzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage. FASEB J; 15: 1425-7.

11- Hoppe U, Bergemann J and Diembeck W,. (1999).Coenzyme Q10, a cutaneous antioxidant and energizer.Biofactors; 9: 371-8.

12- Jones K, Hughes K, Mischley L and McKenna DJ: (2002).Coenzyme Q-10: efficacy, safety, and use. AlternTher Health Med, 8(3):42-55. Quiz56, 138

13- Shalan MG, Mostafa MS, Hassouna MM, Nabi Se and Rafie A. (2005). Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology.;206:1–15.

14- Levin SM and Goldberg M. (2000). Clinical evaluation and management of lead exposed construction workers. Am J Ind Med;37:23-43.

15- Wong WY, Thomas CMG, Merkus JMWM and Zielhuis GA, (2000). SteegersTheunissen RPM. Male factor subfertility: possible causes and the impact of nutritional factor. FertilSteril; 73:435-442

16- Echtay, K. S., Winkler, E and Klingenberg, M. (2000). Coenzyme Q is an obligatory cofactor for uncoupling protein function.Nature, 408, 609–613.

17- Schmelzer, C., G. Lorenz, I. Lindner, G. Rimbach, P. Niklowitz, T. Menke and F. Döring, (2007). Effects of Coenzyme Q10 on TNF-alpha secretion inhuman and murine monocytic cell lines. Biofactors, 31: 35-41.

18- 20- Blystone, C. R., Lambright, C. S., Howdeshell, K. L., Furr, J.,Sternberg, R. M., Butterworth, B. C., Durhan, E. J., Makynen, E. A., Ankley, G. T and Wilson, V. S. (2007). Sensitivity of fetal rat testicular steroidogenesis to maternal prochloraz exposure and the underlying mechanism of inhibition. Toxicol. Sci. 97, 512–519.

19- Gray, L. E., Jr, Wilson, V. S., Stoker, T., Lambright, C., Furr, J., Noriega, N.,
Howdeshell, K., Ankley, G. T., and Guillette, L. (2006).Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. Int. J. Androl. 29, 96–104.

20- Sundaram, K. & Kumar, N. (1996) in The Leydig Cell, eds. Payne, A., Hardy,
M. & Russell, L. (Cache River Press, Vienna, IL), pp. 287.

21- Schmelzer, C., G. Lorenz, I. Lindner, G. Rimbach, P. Niklowitz, T. Menke and F. Döring, (2007). Effects of Coenzyme Q10 on TNF-alpha secretion inhuman and murine monocytic cell lines. Biofactors, 31: 35-41.

22- Thompson CJ, Ross SM, Gaido KW 2004 Di(n-butyl) phthalate impairs cholesterol transport and steroidogenesis in the fetal rat testis through a rapid and reversible mechanism.Endocrinology 145:1227–1237

Littarru GP and Tiano L. (2007). Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. MolBiotechnol; 37: 31-7