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Study Role of Coenzyme Q_{10} in Improvement of Reproduction Efficiency in Female Wister Rats

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Abstract

CoQ_{10} have the ability to reduce the harmful effect of lead acetate. Therefore, the role of the CoQ_{10} on the reproductive system of female rats was studied. Gene expression for ovarian estrogen hormone Aromatase cytochrome P450 gene, progesterone (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase genes were investigated. cDNA were estimated from 4 group of mature rate that treated with different types of material were (corn oil, CoQ_{10} lead acetate and lead acetate for30 days then CoQ_{10}). Results showed clear difference in fold change of Aromatase cytochrome P450 and Hydroxy-delta-5-steroid dehydrogenase (Hsd3β5) expression levels among the groups at level ($p<0.05$). Gene expression results were revealed that CoQ_{10} improve the efficiency of reproductive system that exposed to Oxidative stress by lead acetate

Key words: ***Coenzyme Q_{10}, lead acetate, ovary, gene expiration***

Introduction

Coenzyme Q10 (CoQ10) or ubiquinone is essentially vitamin-like substance (1). CoQ10 is a crystalline powder that is insoluble in water. Absorption of CoQ10 follows the same process as that of lipids and the uptake mechanism appears to be similar to that of vitamin E. The biosynthesis of CoQ10 from the amino acid tyrosine is a 17-step process requiring at least eight vitamins and several trace elements (2,3), CoQ10 is found in small amounts in a wide variety of foods and is synthesized in all tissues. CoenzymeQ10 is compound that functions as an electron carrier in the mitochondrial respiratory chain (4).

Then ATP production acting as an essential antioxidant and supporting the regeneration of other antioxidants, influencing the stability and permeability of membranes; also, stimulating cell growth and inhibiting cell death (5). Deficits in energy at the cellular level could induce various disease processes such as cardiovascular disease and diabetes; the consequent increase in ROS production can further damage mitochondrial DNA, creating a vicious cycle (6).

Lead acetate is a chemical compound, that is, a white crystalline substance having slight acetic acid odor with a sweetish taste. Like other lead compounds, it is very toxic (7). Lead being one of the reproductive toxicant, can affect the gonadal structure, functions and can cause alterations in fertility (8). The effects on the physiology, histomorphology, development and biomarkers have been observed on different organs of animals and humans. In most of the previous studies, the harmful effects of lead were noted (9, 10). Lead can cause changes in implantation, embryonic development and reproductive organs in mammals (11). A study in male rats showed a variety of adverse reproductive effects including impaired sperm motility (11).CoQ10 appears to play multiple roles in cells. Therefore the present study aims to evaluate the effectiveness of the CoQ10 on the female reproductive system and the ability to reduce the harmful effect of lead acetate.

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Material and Methods

Experimental animals

The study was carried out on twenty four mature Wistar albino females rats at (190± 20) gm average weight, at the age 4-5months). Animals were purchased from animal house of Biology Department, Scientific College, Thi-Qa University.

Experimental design

Female rats were randomly assigned in to four equal groups (6 rats in each group). All the groups were administration orally by gavage needle as follows:

Group1: Animals in this group were daily administered 1ml/ kg corn oil for 60 days and served as control group.

Group2: Animals in this group were daily administered CoQ10 at dose 200 mg/kg for 60 days (12).

Group3: Animals in this group were daily administered lead acetate at dose 8 mg /kg for 60 days.

Group 4 : Animals in this group were daily administered lead acetate at dose 8 mg /kg for 30 days then orally administered by CoQ10 200 mg/kg for another 30 days.

Three primers were used in this study Aromatase cytochrome P450 gene and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5 (Hsd3β5) gene , primers used as target gene, as well as β-actin gene primers used Housekeeping gene for normalization of gene expression. These primers were designed by using NCBI- Gene Bank data base and Primer 3 design online, the primers used in quantification of gene expression using qRT-PCR techniques based SYBER Green DNA binding dye, and supported from (Bioneer, Korea) company.

Table (1): The primers, sequence and amplicon.

Primer	Sequence		Amplicon
Aromatase	F	AGCCAAACTACTGCTTTGCG	120bp
cytochrome P450	R	TGCATGGGGTTCAGCATTTC	
Hsd3β5	F	TGCAAGAGAAAGAGCTGCAG	93bp
	R	TGGCTTTTGTCTGCAGCTTG	
β-actin	F	CTAGGCACCAGGGTGTGATG	85bp
	R	GTCAGGATGCCTCTCTTGCTC	

Aromatase cytochrome P450 (NM33986.1), Hsd3β5 (NM_012584.1), and β-actin (NM_031144.3).

Animal sacrificing:

The females were sacrificed after anesthetizing by ether (4%) in the day (60) for all groups. The abdominal lumen was opened the ovaries were removed, one of the ovaries had been frozen by liquid nitrogen at (-960 C) for gen expiration (12).

Molecular analysis

1. Total RNA extraction

Total RNA were extracted from rat ovary tissue by using (TRIzol® reagent kit)(korea) and done according to company instructions .

2. The purity of RNA, also determined by reading the absorbance in Nanodrop spectrophotometer at 260 nm and 280 nm.

3. cDNA synthesis from all sample by using DNase I enzyme kit as described by (promega company, USA).

4. Quantitative Real-Time PCR (qPCR) master mix preparation qPCR master mix was prepared by using AccuPowerTM Green Star Real-Time PCR kit that dependant syber green dye detection of gene amplification in Real-Time PCR system and include the follow.

qPCR master mix	Volume
cDNA template (100ng)	3µL
Forward primer(10pmol)	1 µL
Reverse primer (10pmol)	1 µL
DEPC water	15 µL
Total	20 µL

After that, these qPCR master mix component that mentioned above Accopwer Green star qPCR premix standard plate tubes that contain the syber green dye and other PCR amplification components, then the plate mixed by Exispin vortex centrifuge for 3 minutes, then placed in Exicycler Real-Time PCR system.

After that, the qPCR plate was loaded and thermocycler began:

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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 (14). 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 as following equations.

Gene	Test (Treatment group)	Cal. (Control group)
Target gene	CT (target, test)	CT (target, cal)
Reference gene	CT (ref, test)	CT (ref, cal)

Statistical Analyses

Statistical analyses was made by using (SPSS) computer software according (ANOVA) and least significant differences (L.S.D) at P-Value less than (0.05) level of significant was considered statistically significant.(15).

Results

Molecular analysis

The Concentrations and Purity of extracted total RNA:
Total RNA concentrations (ng /µl) and purity was estimated using Nano drop spectrophotometer in absorbance readings (260-280nm). All ovary tissue samples that used in the this study gave high concentrations of total RNA and appeared quantitatively enough to proceed in quantitative reverse transcriptase real-time PCR as show in table(2).

Table(2);Total RNA concentration and purity in treatment and control groups of ovary tissue after 60 days in female rats:

Isolate	Total RNA concentration ng/µl	Purity 260-280nm
G1	754.4	1.88
G1	551.2	1.71
G1	764.4	1.85
G1	614.3	1.92
G1	564.9	1.86
G1	591.8	1.83
G2	981.1	1.69
G2	453.9	1.82
G2	875.3	1.79
G2	774.5	1.68
G2	590.8	1.53
G2	587.8	1.89
G3	512.2	1.88
G3	741.9	1.65
G3	584.1	1.85
G3	624.5	1.72
G3	594.3	1.86
G3	734.8	1.89
G4	671.1	1.69
G4	645.5	1.83
G4	713.3	1.89
G4	854.5	1.78
G4	589.8	1.93
G4	534.2	1.79

Quantitative Reverse Transecriptase Real -Time PCR
Quantitative Reverse Transcriptase Real Time (RT—qPCR) was performed for measurement of relative quantification (gene expression analysis) for ovarian estrogen hormone (Aromatase cytochrome P450 gene), progesterone (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5 (Hsd3β5) genes expression levels normalized by housekeeping gene expression(β-actin).Rt –qPCR quantification method in real-time PCR system was dependent on the values threshold cycle numbers(CT) of amplification plot of target genes and housekeeping gene as show in the following figures:

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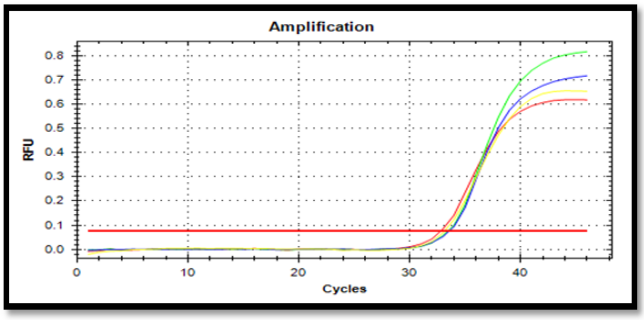

Figure(1): Real-Time PCR amplification plot for β-actin housekeeping gene that show no differences in threshold cycle numbers (CTvalue) between treatment and control groups .

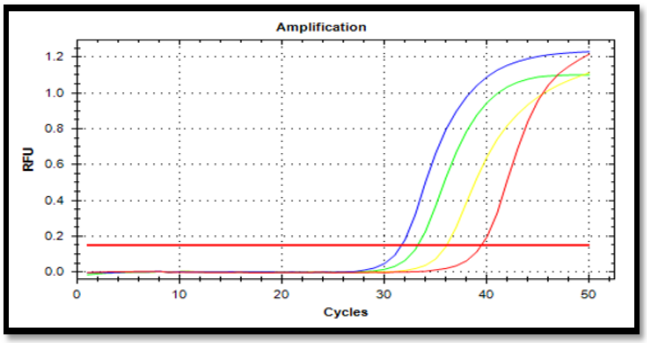

Figure (2): Real-Time PCR amplification plot for (aromatase gene) that show differences in threshold cycle numbers (CT value) between treatment and control groups.

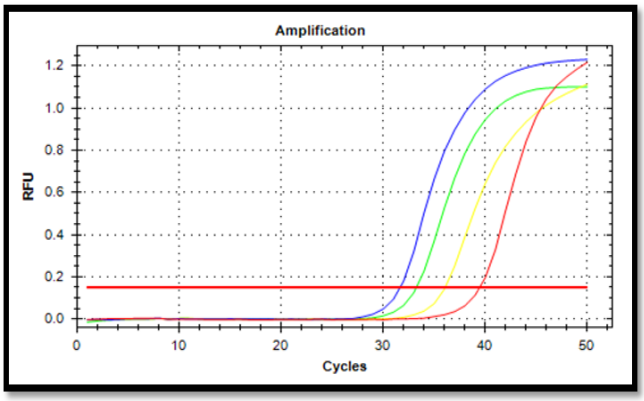

Figure (3): Real-Time PCR amplification plot for (3β-HSD gene) that show differences in threshold cycle numbers (CT value) between treatment and control groups.

Yellow plot: Group 1 animals received (corn oils) for 60 days Blue plot: Group 2 animals received (200 mg/kg) CoQ10 for 60 days. Red plot: Group 3 animals received (8 mg/kg) Lead acetatefor 60 days Green plot:Group4 animals received(8 mg/kg) Lead acetate then (200mg/kg) CoQ10for 30 days

Relative Gene Expression Results

The relative expression of target Genes(Aromatase cytochrome P450 gene and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta isomerase 5 (Hsd3β5) gene) in rats ovaries tissue were calculated by using Livak Methed (2ˆ-ΔΔ CT) that dependent on normalization of RT-qPCR (CTvalues) of target genes with housekeeping gene (β-actin) as reference gene in control and treated groups .The results of relative Gene expression in (Aromatase cytochrome P450 gene)gene which appeared clear difference in fold change of gene expression levels between positive and negative control groups (G1and G2) and treated groups, group2 was appeared up regulation at (12.2367 ± 79406), group 3was appeared down regulation at (0.0071 ± 0.0188) compared to group 4, group 4 was appeared up regulation at (4.6059 ± 53569) relative to group1 that is equal to 1 fold change of gene expression levels as in table (3) and figure (4) the statistical analysis of relative gene expression in aromatase cytochrome P450 gene was found significant differences in treated groups compared with control groups at level $p<0.05$.

The results of relative Gene expression in Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5 (Hsd3β5) gene which appeared clear difference in fold change of gene expression levels between group1,group2,group3 and group4, group2 was appeared up regulation at (5.1472 ± 67280).group 3 group was appeared down regulation at (0.0787 ± 0.0906) compared to group 4, group 4 group was appeared up regulation at(2.5316 ± 46018) relative to group1 that is equal to 1 fold change of gene expression levels as in table (3) and figure (5) the statistical analysis of relative gene expression in(Hsd3β5) gene was found significant differences in treated groups compared with control groups at level $p<0.05$.

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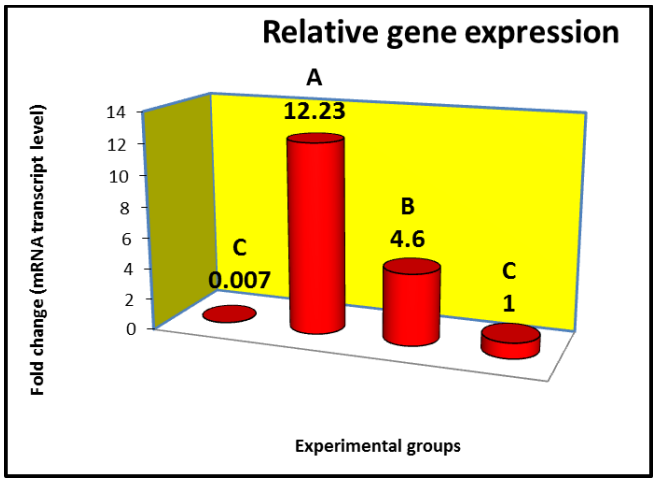

Figure (4) Relative Gene Expression of Gene aromatase cytochrome P450 Values are means ± S.E.
Different litters refer to significant differences (p<0.05).
Same litters refer to no significant differences (p>0.05).

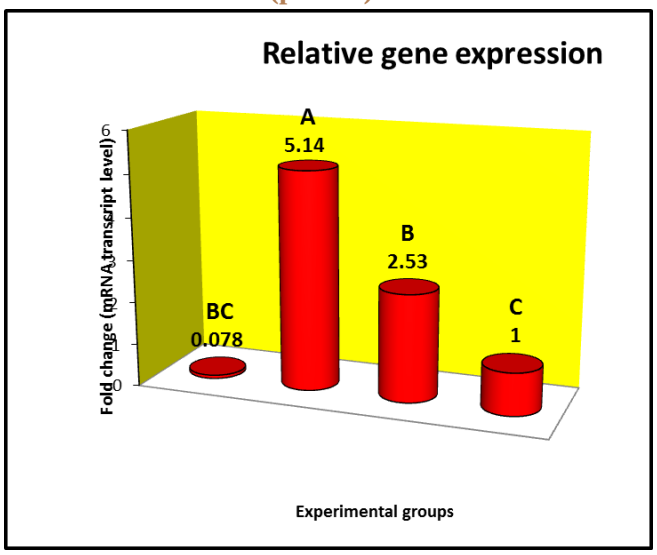

Figure (5) Relative Gene Expression of Gene Hsd3β5 Values are means ± S.E.
Different litters refer to significant differences (p<0.05).
Same litters refer to no significant differences (p>0.05)

Discussion

The mechanisms of Co Q10 in ameliorating the oxidation effects by it action as power full antioxidant was test. Based on that, we tried to reach the destination of our decision to investigate the role of Co Q10 using female rats as a mammalian model. In the present study we have studied mRNA expression levels of such genes in ovary tissues, related to ovary hormone secretion, as an alternative way to investigate the powerful of CoQ10 in effect of aromatase and Hsd3β5 genes which responsible for biosynthesis of ovary hormones. Coenzyme Q10 has a universal role in the cell, whereby its redox poise in the different sub cellular compartments acts as a redox sensing, signaling mechanism affecting gene expression and proteome composition for the maintenance of optimum sub-cellular regional metabolism. redox poisegene regulation is a universal phenomenon and that Coenzyme Q10 and its analogues are the orchestra leaders in most cells.(16).

P450aromatase It is a key enzyme for estrogen biosynthesis by ovarian granulosa cells, The P450 aromatase is essential for follicular maturation, oogenesis, ovulation, and normal luteal functions in females (17) 3β-hydroxysteroid dehydrogenase (3β-HSD) is a steroid-metabolising enzyme that converts pregnenolone to progesterone.3β-HSD has a wide tissue distribution with cell-specific expression in the placenta, adrenal gland, ovary and testis(18).

The result of present study found up regulation of aromates and 3β-HSD genes and statistical analysis significant increase ($p < 0.05$) in CoQ10 treated animals in positive control group compare with other groups. We suggest that the CoQ10 in positive control group effect to up regulation of aromates and 3β-HSD genes by acting as potent antioxidant to redaction the ROS and activated the factors which responsible for release aromatase and 3β-HSD. CoQ10 as antioxidants substance like vitamin E trigger reactive oxygen species-sensitive intracellular pathways that regulate the induction of specific gene. Free radicals are viewed as harmful by-products of cell metabolism, and it is well known that the accumulation

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of ROS in cells will induce the oxidation of DNA, lipids, and proteins, which results in cell damage and causes genomic instability. However, a number of studies have identified a critical physiological role of ROS in intracellular signaling (19).

Result showed down regulation of aromates and 3β-HSD genes and significant decrease ($p<0.05$) in lead acetate intubation in group 3 compare with other groups this result indicated of negative effect of lead acetate on these genes Because it is a source of oxidative stress factors, Lead is reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides (20, 21).Lead could accumulate in cell nuclei associated with nuclear proteins and chromatin and change their structure (22) .An excess of ROS has the ability to damage lipid ,proteins ,nucleic acids .DNA and RNA (23)OS exerts toxic effects by altering cellular molecules such as lipids, proteins and nucleic acids. This can lead to an increase in membrane permeability, loss of membrane integrity, enzyme inactivation, structural damage to DNA, mitochondrial alterations (24). Lead decreased basal aromatase activity, P450 aromtase protein, and transcript production. The mRNA was also reduced in chronically lead-intoxicated rhesus monkeys, primordial follicles are damaged and follicular development is inhibited (25).Lead is known as an environmental endocrine disrupter affecting ovarian steroidogenesis in rats (26). Its action on P450 arom expression provides further evidence that lead might be considered as a potential endocrine disrupter in Female rhesus monkeys that received lead acetate in drinking water showed a significant decrease in serum progesterone levels, indicating that lead blocks luteal function and decrease in serum levels of LH and FSH, indicating that lead inhibits ovarian function.

The result of present study found up regulation of aromates and 3β-HSD genes and statistical analysis significant increase ($p< 0.05$) in(lead acetate than Co Q10) animals in group4 compare with other groups agreement with (27) suggested that Co Q10 as an antioxidant, it protects cells and tissues from oxidative damages caused by reactive oxygen species. The present elevation of total RNA concentration in ovary tissues obtained from Co Q10 treated female rats was a result of increment of ovary cellular function in protein synthesis as the results revealed high level of ovary regeneration events which can be confirmed by high expression level of genes in end of experiment, and found that this expression was increased in parallel to the improvement that seen in histological sections. Ovary 2 gene, in CoQ10 treated female rats, showed high level of expression while decrease group 3.

Our data demonstrated a significant increase in serum estrogen and progesterone level in group 2 and 4 after 2 months oral treatment with Co Q10. This increase in the ovary hormone level resulted from activation of granulose cells and theca cells of the ovary from the CoQ10 treated female rats as demonstrated by mRNA expression level of aromatase and 3β-HSDgenes.

Conclusions

CoenzymeQ10 has beneficial therapeutic effects on lead acetate toxicity Coenzyme Q10 possess antioxidant activity and has a role in increasing the reproductive efficiency by increased levels of FSH, LH, estrogen and progesterone hormones.

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