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Role of Vitamin C and CO-Q10 in controlling Doxorubicin-Induced Female fertility in a Rat model

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Abstract:

The prevalence of female infertility cases has been increasing at a frightening rate, affecting approximately 48 million women across the world. However, oxidative stress induced by chemotherapy has been recognized as one of the main factors of female infertility by causing various reproductive pathologies in females such as endometriosis, polycystic ovary syndrome (PCOS), preeclampsia, spontaneous abortion, and unexplained infertility. Doxorubicin is regarded as a many chemo responsive tumors a widely used for various cancers, particularly for the female such as ovarian cancer, liver cancer, lung cancer, and lymphomas, breast cancer patients. Reactive oxygen species ROS and Antioxidant has been embroiled in female infertility, If the antioxidant system has exhausted can be Female genital tract function disrupted due to over production of ROS. Antioxidants are compounds at act on oppose formation of the actions ROS, Nowadays, concerned women prefer dietary supplements with antioxidant properties over synthetic drugs as a natural way to lessen the oxidative stress and enhance their fertility. The aim of the present study is to evaluate the effects of Coenzyme Q10 (CO-Q10) and vitamin C on doxorubicin (DOX) induced oxidative stress in the female reproductive system in a rat model, (42) female rats (with age ranged between 12-16 weeks and weight of 200 gm) were divided into 4 groups depending on the body weight including (control , DOX, Vitamin C , CO-Q10).Biochemical parameters measured in the serum after 21 day: treatment are Catalase (CAT) , Super Oxide Dismutase (SOD) , Malondialdehyde (MDA) . The results have demonstrated the reactive effects of CO-Q10 and vitamin .C on doxorubicin which reveal positive impact on female fertility and improving reproductive capacity , as well results have shown high significant difference (p=0.002) in the level of SOD enzyme activity in Vit C , CO-Q10 groups , also Results of analysis are showed significant decrease activity level of SOD in disease group .But There was no significant difference in the level of each CAT , MDA compared with control group.

Key words: Doxorubicin, Oxidative Stress, Antioxidants, Vitamin C.

دور فيتامين C و CO-Q10 في السيطرة على خصوبة الإناث التي يسببها الدوكسوروبيسين في نموذج الفئران

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الخلاصة:

يتزايد انتشار حالات العقم عند النساء بمعدل مخيف وهو يؤثر على ما يقرب 48 مليون امرأة في جميع أنحاء العالم. ومع ذلك ، فقد تم التعرف على الإجهاد التأكسدي الناجم عن العلاج الكيميائي كواحد من اهم العوامل الرئيسية لعقم النساء من خلال التسبب في أمراض تناسلية مختلفة في الإناث مثل التهاب بطانة الرحم ومتلازمة المبيض المتعدد الكيسات (PCOS) وتسمم الحمل والإجهاض التلقائي والعقم غير المبرر. يعتبر Doxorubicin من الأورام المستجيبة للعلاج الكيميائي والتي تستخدم على نطاق واسع لمختلف أنواع السرطان ، وخاصة للإناث مثل سرطان المبيض وسرطان الكبد وسرطان الرئة والأورام اللمفاوية ومرضى سرطان الثدي. ان أنواع الأكسجين التفاعلية ROS ومضادات الأكسدة دور في عقم الإناث، إذا استنفد نظام مضادات الأكسدة يمكن أن تتعطل وظيفة الجهاز التناسلي للإناث بسبب زيادة إنتاج (ROS.) مضادات الأكسدة هي مركبات تعمل على معارضة تشكيل إجراءات ROS. في الوقت الحاضر ، تفضل النساء المعنيات المكملات الغذائية ذات الخصائص المضادة للأكسدة على العقاقير الاصطناعية كطريقة طبيعية لتقليل الإجهاد التأكسدي وتعزيز الخصوبة. الهدف من هذه الدراسة هو تقييم آثار الإنزيم المساعد Q10 (CO-Q10) وفيتامين C على الإجهاد التأكسدي الناجم عن دوكسوروبيسين (DOX) في الجهاز التناسلي الأنثوي في نموذج الفئران ، (42) إناث الجرذان (مع أعمار تتراوح بين 16-12 أسبوعًا ووزن 200 جرام) تم تقسيمها إلى 4 مجموعات حسب وزن الجسم بما في ذلك (المجموعة الضابطة ، DOX ، فيتامين (C ، CO-Q10) المقاييس البيوكيميائية المقاسة في المصل بعد 21 يومًا: العلاج هو كاتالاز (CAT) ، سوبر أوكسيد ديسموتاز (SOD)) ، مالونديالديهيد (MDA)). لقد أظهرت النتائج التأثيرات التفاعلية لـ CO-Q10 وفيتامين .C على دوكسوروبيسين مما يكشف عن تأثير إيجابي على خصوبة الإناث وتحسين القدرة الإنجابية ، كما أظهرت النتائج فرقًا كبيرًا معنويًا ($p = 0.002$) في مستوى نشاط إنزيم SOD في وفيتامين C ومجموعات CO-Q10 كما أظهرت نتائج التحليل انخفاضًا معنويًا في مستوى نشاط SOD في مجموعة المرض ولكن لم يكن هناك فرق معنوي في مستوى كل من CAT و MDA مقارنة مع مجموعة التحكم.

الكلمات المفتاحية: دوكسوروبيسين ، الإجهاد التأكسدي ، مضادات الأكسدة ، فيتامين سي.

Introduction

As a reproductive health disease, infertility is defined as an incapacity to generate and maintain a full-term pregnancy after a year of regular sexual intercourse without the use of any contraceptive method, according to the World Health Organization (WHO), around 9% of the couples worldwide present fertility issues, Females or males may have infertility problems. For many years, infertility issues were mainly attributed to women, but nowadays, such problems are attributed to both sexes in almost equal parts [(1)]. The (WHO) performed a large multinational study to determine gender distribution and infertility etiologies , the most common identifiable factors of female infertility are as follows:

- Ovulatory disorders - 25%
- Endometriosis - 15%
- Pelvic adhesions - 12%
- Tubal blockage - 11%
- Other tubal/uterine abnormalities - 11% [(2)].

Infertility cases are associated with biochemical changes in the body more over oxidative stress caused by chemotherapy has a well-established role in causing justified and unexplained infertility, as the presence of reactive oxygen species in the female reproductive system clearly affects the physiology of sex cells, Ovaries and fallopian tubes[(3)] .the current cancer treatment protocols, including chemotherapy, are gonadotoxic and can destroy the women's reproductive potential by accelerating the loss of ovarian reserve and atrophy, leading to premature ovarian failure (POF) and early menopause , Along with this, the uterine complications caused by cancer-directed treatments can also lead to reproductive difficulties , Chemotherapy-induced infertility can have several secondary adverse effects, due to the ovarian hormones' influence on the maintenance of physiological metabolism[(4)]. To prevent the damaging effect of excessive ROS, enzymatic and non-enzymatic antioxidant pathways plays a key role in scavenge in excess ROS and allow a balance to be achieved between beneficial oxidant generation and damaging harmful ROS , antioxidants are the most important defense substances against free radical induced infertility [(5)]. Anti-oxidants exert their fundamental role, through four levels of defense, starting from hindering free radicals' formation, and a scavenging mechanism to conquer the propagation reactions. In this regard vitamin C is very potent in factor. DNA repair systems constitutes a strong defense against oxidative damage. Adaptation is the fourth level of defense where the signal for the production and reactions of free radicals prompts creation and transport of specific antioxidant to the right site [(6)].

Doxorubicin is an anthracycline chemotherapeutic drug that cures several types of cancers, including breast cancer. A young female who has breast cancer might often face a problem of infertility and premature oocyte after

the treatment of obtained forty-two chemotherapeutic drugs. Besides, they also induce female reproductive toxicity characterized as a (POF), DOX directly affects the follicles and oocytes. It causes some genotoxic effects, which results as side effect, The anticancer drug doxorubicin has been associated with several adverse side-effects including reproductive toxicity in both genders [7].

Materials and methods

Female white rats, aged 12-16 weeks, with an average weight of 200 gm), from the animal house of the College of Veterinary Medicine/ Tikrit University, The animals were placed in the experiment stages under laboratory conditions in terms of ventilation and lighting 12 hours, and a temperature of 28-22 °C, and they were given water and ration ad libitum Length of experiment, After they reach social maturity after 6-5 months, They were acclimatized to the laboratory environment for 14 days prior to the start of experiment; then posteriorly rats entered the dosing phase.

The rats were randomly divided into Four groups as follows:

Group I: Control included subject given normal saline

Group II: Doxorubicin-treated (DOX) at a concentration (15 mg/kg) of body weight dissolved in normal saline.

Group III: Vitamin .C at a concentration (100 mg/kg) dissolved in normal saline in addition to DOX.

Group IV: Co-Q10) at a concentration of (150 mg/kg) dissolved in normal saline in addition to DOX.

All doses were selected based on previous studies that had determined the best performance of these compounds.

Normal saline for control group, Vitamin C, and Co-Q10 were administered orally, While DOX was given by subcutaneous injection. All groups were dosed (100 µl) for 21 days.

Estimation of biochemical parameters Deeply frozen serum samples were thawed and allowed to reach room temperature for biochemical analysis. MDA was measured using a colorimetric method depending on the reaction of thiobarbituric acid (TBA) with MDA[8] **.** CAT activity was determined method depends on the dissociability of hydrogen peroxide and the formation of water and oxygen [9] . and SOD activity was performed according to [10] .

Statistical analysis

All clinical examination results were analyzed using the MINITYPE statistical program according to the analysis of variance (ANOVA) test, and a probability level of (0.05) and (0.001).

Results and discussion

Results of **Table 1** and **Fig. 1.** have indicated a significant decrease SOD enzyme activity in the serum of group II (70.38±6.41 U /ml) compared with group I (79.53±5.59 U /ml), One the other hand no significant difference was shown in MDA, CAT levels in DOX

activity compared with control group.

Table 1. antioxidant analysis variables of DOX and control groups.

Parameters/ Groups	Control Group	disease group (DOX)	P value
SOD U/ml	79.53±5.59	70.38±6.41	0.002**
MDA mol/l	21.65±3.91	23.25±7.63	n.s
CAT KU/l	62.2±14.34	20.65±64.1	n.s

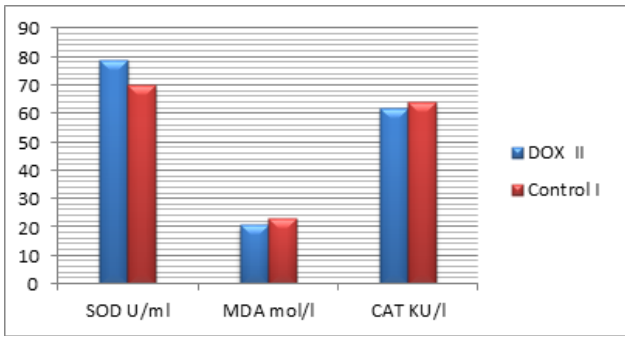

Fig. 1. Effect of treatment DOX on the efficacy of antioxidants

Many chemotherapy and radiotherapy applied for a woman's pelvis contributed to the rapid acceleration frequent auto-killing of cells predominantly leading to ovarian failure in female cancer survivors. DOX has been shown to cause gonadotropin-induced toxicity by stimulating OS [11]. The reason for the decrease, as indicated by Al-Youzbaki [12] .may be attributed to the deterioration of the enzymatic and non-enzymatic Antioxidants system by the effect during the process of removing free radicals and their metabolites caused by the toxicity of DOX due to its residues, activating OS that cause tissue damage, as well as the weakness of the antioxidant system in the treated patients and through an increase in the level offer radical and the increase in oxidative reactions in the body , which may caused by an increase in ROS, and this occure through the inhibition of some enzymatic system is including SOD [13]. has indicated that the positive relationship between excess OS and decreased of Antioxidant enzyme activity leads to infertility because it is scientifically and physiologically negatively effects a number of reproductive processes, including in females, including: follicle formation, oocyte maturation, endometriosis and causes disruption in the development of the fetus farther more oxidation causes significant damage to the cell membrane, and thus infertility[14] .

In Table 2 and **Fig. 2** SOD activity was significantly increased in sera of group III(85.73±8.72 U /ml) compared with control group I (79.53±5.59 U/ml) while no significant difference was reported in MDA and CAT activity in group III compared with control group I .

Table 2. antioxidant analysis variables of DOX/ vitamin C and control groups

Parameters/ Groups	Control Group	DOX/Vit C	P value
SOD u/ml	79.53±5.59	85.73±8.72	0.002**
MDA mol/l	21.65±3.91	25.18±3.83	n.s
CAT ku/l	62.2±14.34	69.0 ± 23.1	n.s

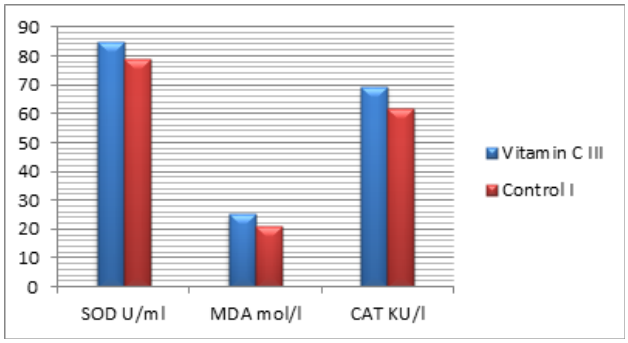

Fig. 2. Effect of treatment DOX / Vit C on the efficacy of antioxidants

Loss of reproductive function, gonadal dysfunction, and impaired fertility are among the most adverse effects of chemotherapy. Treatment can lead to gonadotropin toxicity, ovarian failure, leading to premature menopause due to oxidative stress generation, which is the most important source of chemotherapy for its potentially harmful effects on cells. It can attack cellular targets including ovarian cells ,Ovarian toxicity induced by chemotherapy results in oocyte digenesis based on evidence of disturbances in redox balance and through drug performance Drugs that cause oxidative stress-induced cell death may be beneficial for killing cancer cells. Moreover, vitamin C supplementation appears to be very beneficial in preventing the harmful effects of chemotherapy and that antioxidant therapy mitigates the ill effects of cells, indicating that its role as an reactive antioxidant has helped mitigate oxidative damage, as it is indirectly able to reduce oxidative damage to the reproductive system. In the case of malignancy, nowadays cancer patients widely use antioxidants as supplements after diagnosis or during treatment [15,16].

As result SOD activity was significantly increased in the group treated with vitamin C and this appears to be natural as far as its antioxidant effects which have been proven. Strong oxidation reduces the amount of free radicals after tissue damage and has been shown to have a positive effect in wound healing and improvement of tissue function after tissue ischemia. Remarkably vitamin also has anti-inflammatory properties due to the inhibition of phospholipase A2; Thus, it can contribute to reduce the harmful effects of ovarian tissue (ROS) and thus improving reproductive function[17] .

Results in **Table 3** and **Fig. 3** have indicated a significant increase of SOD activity in the sera of group IV (85.518.32± U /ml) compared with group I(79.53±5.59 U/ml) While no significant difference was shown in MDA and CAT in CO-Q10 group.

Table 3. antioxidant analysis variables of DOX /CO-Q10 and control groups

Parameters/ Groups	Control Group	DOX/CO-Q10	P value
SOD u/ml	79.53±5.59	85.51±8.32	0.002**
MDA mol /l	21.65±3.91	26.09±9.09	n.s
CAT ku/l	62.2±14.34	72.9 ± 27.0	n.s

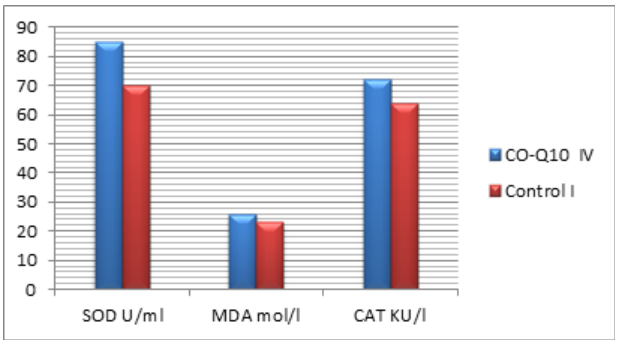

Fig. 3. Effect of treatment DOX / CO-Q10 on the efficacy of antioxidants

The reason for the high efficacy of the enzyme SOD may be attributed to the animals being dosed with CoQ10, because it is a vitamin-like substance that is important to complete of vital functions and chemical reactions inside the cells of the body, It is found mainly within the mitochondrial membranes, where it is synthesized in the mitochondria and is an essential component in the field of energy production. It works to produce ATP that the body cells need to perform their functions normally and properly. In females, CoQ10 helps in the process of producing the egg, as this process needs a lot of energy to take place. Definitely. it is one of the most important and powerful Antioxidants essential in the body and works to support the regeneration of other antioxidants, affecting their stability and permeability of membrane also stimulating cell growth and inhibiting cell death and thus protecting cells from the so-called free radical resulting from the treatment of animals with a chemotherapy drug for cancer, In this regard CoQ10 plays multiple roles in cells as it fights the harmful oxidative effects of free parts on the cell SOD deficiency activates antioxidants through its ability to reduce the harmful effect and activate antioxidant enzymes, especially (SOD)[18, 19] .

Additionally , it acts as a redox agent for protons and electrons via the electron transport chain and prevents the oxidation of lipids, protein and DNA, and is continually restored by cellular reductase [20] .Thus, the increased activity of SOD in the CoQ10-treated groups in this study indicates the critical role of CoQ10 in protecting ovarian follicles from oxidative stress, the drug-induced imbalance between oxidants and antioxidants in cells can be controlled via the efficacy of the SOD enzyme through the conversion of ROS into neutralizing products and non-enzymatic factors via ROS collection and deactivation of radical chain reactions. These biochemical processes are complex because SOD is located in the cytosol and mitochondria, as it works to convert the superoxide radical into hydrogen peroxide and molecular oxygen, it then reduces the effect of oxidative stress [21].

Conclusions

Supplementation of antioxidants (Vit C , CO-Q10) may have positive impact on female fertility and improving reproductive capacity in doxorubicin induced female rats and this effect is more remarkable in the term of SOD activity

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