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Protective Effects of Coenzyme Q10 Along with Fe2O3 Nanoparticles On Sperm Parameters in Rats with Scrotal Hyperthermia

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Abstract

Background: One of the most important factors in reducing the birth rate is male infertility, and one of the main reasons for male infertility is scrotal hyperthermia (SH). Therefore, this study aimed to investigate the protective effect of coenzyme Q10 (CoQ10) along with Fe2O3 nanoparticles on semen parameters in rats with SH. **Materials and Methods:** Forty-eight adult male Wistar rats were divided into eight groups: healthy control, control group receiving Fe2O3 nanoparticles, control group receiving CoQ10, control group receiving Fe2O3 nanoparticles plus CoQ10, SH group, SH group receiving CoQ10, SH group receiving Fe2O3 nanoparticle, and SH group receiving Fe2O3 nanoparticles plus CoQ10. After killing rats, semen was collected from epididymal tissue, and parameters such as sperm viability, motility, concentration, and morphology were studied. **Results:** SH significantly reduced sperm concentration, motility, and viability, as well as altering sperm morphology in rats. Nevertheless, CoQ10 strongly improved sperm parameters in SH rats. Fe2O3 nanoparticles led to a sharp decrease in sperm parameters; however, during the simultaneous administration of Fe2O3 nanoparticles with CoQ10, improvement in sperm parameters was seen in the SH rats. **Conclusion:** Our findings suggest that CoQ10, along with Fe2O3 nanoparticles, has a protective effect against spermatogenic cell death induced by SH. Thus, green synthesis of nanoparticles with the administration of antioxidants, including CoQ10 is recommended for the treatment of SH. **[GMJ.2022;11:e2046] DOI:10.31661/gmj.v11i0.2046**

Keywords: Scrotal Hyperthermia; Sperm; CoQ10; Fe2O3; Nanoparticle

Introduction

In general, infertility is an active sexual dysfunction. Couples who are unable to conceive within one year without the use of contraceptives are called infertile couples [1]. The prevalence of infertility is about 15%, of which 10-15% of infertility is idiopathic [2]. Malefactors are responsible for 50% of infertility cases [3].

The most important causes of male infertility are congenital or acquired anomalies,

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malignancies, increased scrotal temperature, endocrine disorders, genetic, immunological, and idiopathic causes [4]. Despite extensive research on male infertility, the etiology of many infertile men is unknown [5], which may be due to various causes such as environmental pollution, reactive oxygen species (ROS), genetic, and epigenetic abnormalities.

As mentioned, testicular hyperthermia is one of the causes of infertility in men. The main reason for testicular hyperthermia is the lack of scrotal temperature regulation, which eventually leads to thermal stress [6]. This can severely impair spermatogenesis and eventually lead to infertility [7]. Causes of thermal imbalance include lifestyle and behavioral factors, occupational and environmental factors, and clinical factors caused by other comorbidities [8]. These factors can alter gene expression, and one of the main mechanisms of cell damage is the overproduction of free radicals [9]. While it has been shown that low ROS in the testicles can improve spermatogenic functions such as capacitation, acrosome reaction, hyperactivation, and sperm-oocyte fusion [10], high concentrations of ROS can cause severe damage to testicular tissue. To maintain ROS at the proper level, antioxidants are found in the testicles [11]. However, when ROS levels within the testicles increase and the balance of free radicals and antioxidants is disturbed, oxidative stress occurs, leading to apoptosis of testicular cells [12].

Among the important factors in the treatment of male infertility is proper nutrition and consumption of antioxidants, which play a critical role in the growth and development of the reproductive system [13]. Coenzyme Q10 (Co Q10) is an antioxidant that prevents cells from being damaged by free radicals, thus reducing necrosis and apoptosis [14]. Studies have shown that taking antioxidants reduces male infertility, improves semen parameters, and increases fertility rates by four times [15]. Reducing compounds is a pioneering field to restore and regenerate the function of damaged cells, organs, and tissues. Nanoparticles have unique physical and chemical properties compared to their bulk counterparts. The small size and high surface area of the nanoparticles increase their chemical activity, allowing them to act as high-performance catalysts [16, 17]. The unique chemical and physical activities of nanoparticles have led to their widespread use in processes such as drug delivery, vaccination, diagnosis or treatment of a variety of diseases, tissue regeneration, immunometric, excretion of biological fluids toxication, cancer cell heat therapy, and dental alloys, bladder catheters, etc. [18]. Due to the ability of these nanoparticles to pass through cell membranes, these substances have antioxidant properties in low doses and toxic properties in high doses [19]. Some nanoparticles can prevent hormonal dysfunction through various mechanisms such as antioxidant properties [20]. In addition, studies have shown that the concomitant use of nanoparticles with antioxidants increases the antioxidant effect and improves their performance [21].

Therefore, this study aimed to identify the sperm's response to stress caused by rising temperatures. Also, we investigate the effect of CoQ10 along with Fe2O3 nanoparticles, providing more accurate knowledge of cell damage that can be used as new methods for treating infertility in men.

Materials and Methods

Materials and Agents

Fe2O3 nanoparticles (Sigma Aldrich, German) were purchased. The current study used injectable CoQ10 (Antiaging Institute, California, USA). The dose of Fe2O3 nanoparticles was determined based on LD50, namely, the concentration that caused the death of half of the rats. Accordingly, concentrations (0.005, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/kg body weight [BW]) were given to the rats and LD50 was determined as 0.02 mg/kg BW. Therefore, this concentration was used in subsequent experiments.

Animals

Forty-eight adult male rat was purchased from the Pasteur Institute Tehran-Iran. The animals were kept under standard conditions of 12 hours of light and 12 hours of darkness,

25±2°C, and relative humidity of 50±10%. All animals were fed the same proportions of corn, wheat, barley, and pellets under the same nutritional conditions, and free access to water was available to all.

Induction of Scrotal Hyperthermia (SH)
A hot water bath (Memmert, Germany) was used for induction of SH at the temperature of 43°C for 30min once a day for six consecutive days, and for control rats, a hot water bath at a temperature of 22°C was used. Then, the rats were dried and examined for any damage on the scrota and then placed in cages. Studies have shown that no animals were harmed.

Groups
After the rats were randomly divided into eight groups as follow (n=6 per group):
1. Control group
2. Control group receiving magnetic Fe2O3 nanoparticle (0.02 mg/kg BW)
3. Control group receiving CoQ10 (0.02 mg/kg BW)
4. Control group receiving magnetic Fe2O3 nanoparticles (0.03 mg/kg BW) and CoQ10 (0.02 mg/kg BW) simultaneously
5. SH group
6. SH group receiving Fe2O3 nanoparticle (0.03 mg/kg BW)
7. SH group receiving CoQ10 (0.02 mg/kg BW)
8. SH group receiving Fe2O3 nanoparticles (0.03 mg/kg BW) and CoQ10 (0.02 mg/kg BW) simultaneously

Ethical Consideration
This project has been done with the approval code IR.IAU.PS.REC.1399.209 in accordance with ethical principles and national norms and standards for conducting in Islamic Azad University.

Sperm Analysis
Sperm morphology, viability, concentration, and motility were studied. After completing the treatments, all the animals were killed by an overdose of anesthesia, and then the testicular tissue was removed. The semen was collected from the epididymis tissue. Then 10µL of sperm was transferred to a hemocytometer, and sperm counts were performed under an optical microscope with a magnification of 40× after diluting the sperm. Sperm motility was evaluated by a microscope in ten fields based on the World Health Organization recommendation. For this purpose, 200 sperm cells were counted, and the percentage of sperm motility was determined [3].
The Sperm MTT (Sigma-Aldrich, USA) viability assay introduced by Nasr-Esfahani *et al.* (2002) was used to evaluate sperm viability [22]. The morphology of sperms was studied using the standard Papanicolaou stain protocol (Sigma-Aldrich, USA) [23], and normal and abnormal forms of sperm were studied under a microscope at 100× magnification. Abnormal sperm morphology, including sperm with two heads, large head, small head, round head, no acrosome, no head, long or short tail, no tail or twisted tail, and cytoplasmic diameter was observed and reported in 200 sperm.

Statistical Analysis
One-way analysis of variance (ANOVA) was used to identify significant differences in the studied characteristics among the rats' groups. SPSS software version 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used for data analysis.

Results

Sperm Viability
Sperm viability in rats was greatly affected by SH, and a sharp decrease in this parameter was observed. Also, the results showed the toxic effect of Fe2O3 nanoparticles on sperm viability in control and SH rats. However, CoQ10 increased sperm viability in healthy rats and rats with SH receiving Fe2O3 nanoparticles. Therefore, the results of the current study indicate the positive effect of CoQ10 on increasing sperm viability (Figure-1).

Sperm Concentration
The present study results showed significant differences in sperm concentration in different groups of rats ($P<0.001$). Under SH conditions, a relative decrease in sperm concentration was observed. However, CoQ10 was found to have beneficial effects on sperm concentration in rats with SH. Also, Fe2O3 nanoparticles

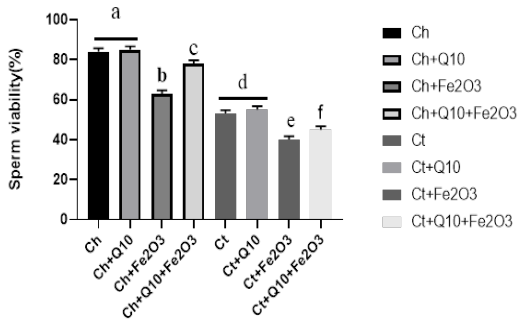

Figure 1. The effect of CoQ10 and Fe2O3 nanoparticles on sperm viability percentage. The different letters indicate significant differences between the groups according to the One-way analysis of variance (ANOVA) test (P<0.001). **Ch:** Healthy control; ***Ch+Q10:*** Healthy control receiving CoQ10; **Ch+Fe2O3:** Healthy control receiving Fe2O3 nanoparticles; **Ch+ Q10+Fe2O3:** Healthy control receiving CoQ10 and Fe2O3 nanoparticles; **Ct:** Rats with scrotal hyperthermia; **Ct+ Q10:** Rats with scrotal hyperthermia receiving CoQ10; **Ct+ Fe2O3:** Rats with scrotal hyperthermia receiving Fe2O3 nanoparticles; **Ct+ Q10+ Fe2O3:** Rats with scrotal hyperthermia receiving CoQ10 and Fe2O3 nanoparticles

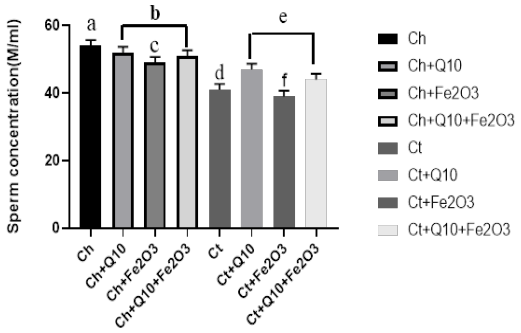

Figure 2. The effect of CoQ10 and Fe2O3 nanoparticles on sperm concentration in different rat groups. The asterisks indicate significant differences between the groups according to the One-way analysis of variance (ANOVA) test (P<0.001). **Ch:** Healthy control; ***Ch+Q10:*** Healthy control receiving CoQ10; **Ch+Fe2O3:** Healthy control receiving Fe2O3 nanoparticles; **Ch+ Q10+Fe2O3:** Healthy control receiving CoQ10 and Fe2O3 nanoparticles; **Ct:** Rats with scrotal hyperthermia; **Ct+ Q10:** Rats with scrotal hyperthermia receiving CoQ10; **Ct+ Fe2O3:** Rats with scrotal hyperthermia receiving Fe2O3 nanoparticles; **Ct+ Q10+ Fe2O3:** Rats with scrotal hyperthermia receiving CoQ10 and Fe2O3 nanoparticles

reduced sperm concentration in control and SH rats. However, co-administration of Fe2O3 nanoparticles with CoQ10 resulted in increased sperm concentration (Figure-2).

Sperm Motility

A sharp decrease in sperm motility occurred due to SH. Fe2O3 nanoparticles also had a relatively toxic effect on sperm motility. However, CoQ10 could compensate for this reduction and increased sperm motility. The effect of CoQ10 on increasing sperm motility was observed in SH rats as well as rats receiving Fe2O3 nanoparticles (Figure-3).

Normal Sperm Morphology

A relative decrease in the percentage of sperm with normal morphology was observed due to SH in rats. However, the treatments had significant effects on the percentage of sperm with normal morphology (P<0.001). The positive effect of CoQ10 on the percentage of sperm with normal morphology was observed in control and SH rats. However, Fe2O3 nanoparticles decreased normal sperm in control and SH rats. While with simultaneous administration of Fe2O3 nanoparticles and CoQ10, an increase in the percentage of sperm with normal morphology was observed (Figure-4).

Discussion

In recent years, the birth rate has fallen sharply [24]. Nearly one-fifth of current couples have been reported to be infertile, almost half of them due to male infertility [25]. One of the causes of male infertility is high temperature, which disrupts the process of spermatogenesis, and studies have shown that SH leads to severe disruption in this physiological process that eventually leads to infertility [26]. Therefore, it is very important to study preventive strategies. Current research has shown the protective effects of CoQ10 on improving semen parameters in rats with SH. CoQ10 is an important part of the electron transport chain and has been shown to be involved in the aerobic respiration of cells and energy production in these conditions [27]. It has been reported that CoQ10 concentration is closely related to semen parameters such as concentration, semen motility, and the beneficial effects of CoQ10 on semen parameters have been attributed to the antioxidant activity of this compound and the improvement of total antioxidant capacity [28].

Therefore, the effects of CoQ10 on the semen parameters in this study can be attributed to the improvement of antioxidant capacity [27].

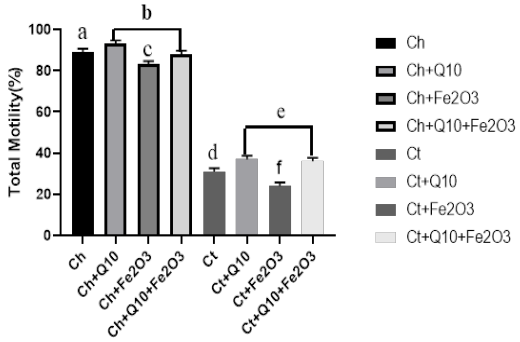

Figure 3. The effect of CoQ10 and Fe2O3 nanoparticles on sperm motility percentage. The asterisks indicate significant differences between the groups according to the One-way analysis of variance (ANOVA) test (P<0.001). **Ch:** Healthy control; ***Ch+Q10:*** Healthy control receiving CoQ10; **Ch+Fe2O3:** Healthy control receiving Fe2O3 nanoparticles; **Ch+ Q10+Fe2O3:** Healthy control receiving CoQ10 and Fe2O3 nanoparticles; **Ct:** Rats with scrotal hyperthermia; **Ct+ Q10:** Rats with scrotal hyperthermia receiving CoQ10; **Ct+ Fe2O3:** Rats with scrotal hyperthermia receiving Fe2O3 nanoparticles; **Ct+ Q10+ Fe2O3:** Rats with scrotal hyperthermia receiving CoQ10 and Fe2O3 nanoparticles

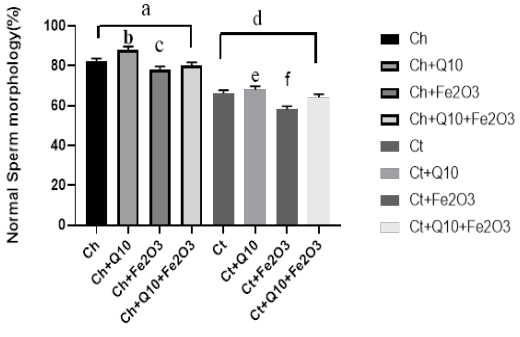

Figure 4. The effect of CoQ10 and Fe2O3 nanoparticles on the percentage of sperms with normal morphology. The asterisks indicate significant differences between the groups according to the One-way analysis of variance (ANOVA) test (P<0.001). **Ch:** Healthy control; ***Ch+Q10:*** Healthy control receiving CoQ10; **Ch+Fe2O3:** Healthy control receiving Fe2O3 nanoparticles; **Ch+ Q10+Fe2O3:** Healthy control receiving CoQ10 and Fe2O3 nanoparticles; **Ct:** Rats with scrotal hyperthermia; **Ct+ Q10:** Rats with scrotal hyperthermia receiving CoQ10; **Ct+ Fe2O3:** Rats with scrotal hyperthermia receiving Fe2O3 nanoparticles; **Ct+ Q10+ Fe2O3:** Rats with scrotal hyperthermia receiving CoQ10 and Fe2O3 nanoparticles

SH has been shown to cause germ cell death and disrupt spermatogenesis [9]. Germ cell death due to SH is caused by cell apoptosis. This cell death occurs in various organs such as the testicles, prostate, and penis [14, 29]. Apoptosis has been shown to play an important role in removing unwanted cells while also contributing to the development of many disorders [30]. Short-term exposure of the testicles to temperatures above 43°C for 15 to 20 min also killed cells due to apoptosis [29]. The toxic effect of Fe2O3 nanoparticles on sperm parameters in healthy and SH rats in the current study was due to the induction of oxidative stress and ROS production. The toxic effects of nanoparticles on male reproductive cells were reported in previous studies [31, 32]. The sensitivity of spermatogonial stem cells to nanoparticles seems to play an important role [33].

The induction of inflammation or edema in the interstitial tissue and oxidative stress are the mechanisms of damage to reproductive cells [34]. Other studies have also reported damage to cellular DNA and cell dysfunction due to nanoparticles [35]. Autophagy-induced cell death seems to play an important role [36]. Therefore, the toxic effects of Fe2O3 magnetic nanoparticles observed in the present study can be attributed to these mechanisms. However, co-administration of CoQ10 with Fe2O3 nanoparticles greatly improved sperm parameters, which can be attributed to the antioxidant properties of CoQ10. Therefore, it can be stated that CoQ10 can improve sperm parameters by reducing the autophagy of sperm cells. However, more research is needed in this regard. In recent years, the green synthesis of nanoparticles by plants has attracted much attention and is being considered as an alternative to the chemical methods of nanoparticle synthesis [37]. Also, green synthesis of nanoparticles is very affordable. Therefore, green synthesis of Fe2O3 nanoparticles and concomitant use of CoQ10 in patients with SH is recommended.

Conclusion

The results of the current study suggested that CoQ10 along with Fe2O3 nanoparticles could strongly improve semen parameters, and these effects were attributed to their antioxidant properties; therefore, it is suggested as a treatment option to reduce infertility caused by SH damage.

Conflict of Interest

The authors declare no conflict of interest.

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