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Appraisement Role Of Ubiquinone On Testis Function, Hormonal Parameters And Morphometric Changes Under Oxidative Stress In Rat.

Alireza Shahriary*, Masood Khakzadihe, and Yunes Panahi.

Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

ABSTRACT

It is estimated that male factor of couple infertility is between25% to 50%. Oxidative stress is believed to be an important and plausible cause of idiopathic male infertility. First group as control rats with i.p. injection of saline daily. Second group fed on normal diet and supplemented by 10% fructose in drinking water ad libitum with i.p. injection of saline daily. Third group as Fructose fed rats treated with ubiquinone, 10 mg/kg i.p. daily. After 30 days blood serum samples and testis function were analyzed. Testicular Length, weight, capsule thickness, epithelium thickness, diameter of seminiferous tubules, interstitial thickness, connective diameter, TDI, RI, SPI and blood serum Testosterone level analyzed for investigating testicular function. Using ubiquinone in oxidative stress condition can protect testis tissue and improves testicular function.

Keywords: Rat, Testis, Oxidative stress, Fructose, Ubiquinone

Corresponding author

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INTRODUCTION

The World Health Organization (WHO) defines infertility as the inability to achieve pregnancy within 12 months of regular sexual intercourse for couples in conception [1]. It is estimated that male factor of couple infertility is between 25% to 50% [2, 3]. This problem is further compounded when no identifiable reason can be found. Currently, oxidative stress is believed to be an important and plausible cause of idiopathic male infertility [4].

Oxidative stress can increase reactive oxygen species (ROS) in high free fatty acid condition that mechanism of this oxidative stress depends on increase mitochondrial uncoupling of oxidative phosphorylation and β-oxidation leading to increasing production of reactive oxygen species [5]. Also through auto-oxidation, oxidative phosphorylation, glycosylation and glycosamine pathways in high glucose range can product mitochondrial reactive oxygen species that causes oxidative stress [6]. Ubiquinone or Coenzyme Q10 (2-methyl-5,6-dimethoxy-1,4-benzoquinone), soluble natural fat quinine, is a ubiquitous and endogenous lipid-soluble antioxidant found in plant as well as in human and animal organisms [7].

Ubiquinone is important for optimal biological function. It is a component of the oxidative phosphorylation process in mitochondria that converts the energy in fatty acids and carbohydrates into Adenosine Three Phosphate (ATP) to drive cellular synthesis [8].

Break the continuity of the extra production of oxidants by electron transport chain of mitochondria would normalize the pathways involved in development of oxidative stress [9]. In diabetes condition oxidative phosphorylation is significantly reduced, afterwards production of adenosine three phosphate is reduced along with decreased level of ubiquinone [9]. One of the other important roles of ubiquinone is inhibit certain enzymes that plays in formation of free radicals and so attenuates oxidative stress [10, 11, 12, 13].

Feeding fructose can provide model of hyperlipidemia, hyperglycemia and insulin resistance [12, 13, 14, 15]. This research conducted to investigate how ubiquinone can improve the antioxidant defense system and decrease of abnormalities in testicular function in rat.

MATERIAL AND METHODS

Animal and breeding

For this study 45 male adult rats were divided in to three groups (each group n=15). They were housed three per wire-bottomed, stainless steel cages to minimize coprophagy in a well ventilated. There were no significant differences on weight (The average body weight 180 to 220 gram), age, appearance and environment of rats. The environment was exposed to 12-hour lighting and 12-hour dark during the experiment for each group. The room temperature was 22±2 Degrees Celsius during the experimental period with 58-62% relative humidity. Using fed was according to NRC. The consumed water was the Tabriz city tap water which was re-refined using carbon and sand filters.

Experimental groups

First group as control rats fed on normal diet and water ad libitum with i.p. injection of saline daily. Second group as Fructose fed control rat group fed on normal diet and supplemented by 10% fructose in drinking water ad libitum with i.p. injection of saline daily. Third group as Fructose fed rats treated with ubiquinone, 10 mg/kg i.p. daily [7, 16, 17].The used ubiquinone in this study was from metagenic company. The injection amount of saline in first and second groups was as same as ubiquinone in third group.

Blood serum analyzing

After 29 days, blood sampling was taken on the 30th day from groups. The blood was added into the serum tube manufactured by Euro Tube ® Company. After clotting, the blood was centrifuged with the speed of 3000 rpm for 10 minutes and the serum was removed. The blood testosterone hormone analyzed by radioimmunoassay method.

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Tissue analyzing

At the end of study, testis was sampled and weighted by a digital scale. Tissue fixed in formalin 10% for 2 week. After tissue fixation slides prepared by H&E staining method and evaluated by light microscope.

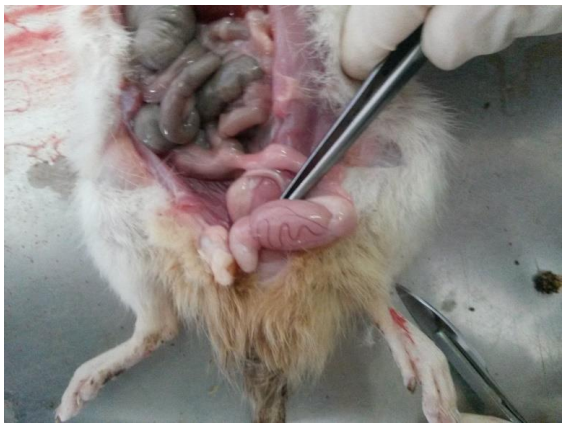

Figure1: testis sampling method for this study

RESULTS AND DISCUSSION

Table 1: results of studied morphometric parameters in groups

Parameter	Analyzed form	Experimental groups		
		Control	Fructose	Ubiquinone+Fructose
Testicular Length	Mean±SD	22.1±0.63[a]	20.4±0.36[b]	17.2±0.65[c]
	Percent of changes	-	-7.69%	-22.17%
Testicular Weight	Mean±SD	1.80±0.041[a]	1.60±0.078[b]	1.75±0.05[a]
	Percent of changes	-	-11.11%	-2.77%
Testicular Capsule Thickness	Mean±SD	9.1±0.25[a]	13.25±0.58[b]	15.96±0.52[c]
	Percent of changes	-	45.60%	75.38%
Epithelium thickness	Mean±SD	31.1±0.9[a]	17.1±0.51[c]	22.36±0.25[b]
	Percent of changes	-	-45.01%	-28.10%
Diameter of seminiferous tubules	Mean±SD	37.01±0.55[a]	31.02±0.25[b]	34.00±0.71[c]
	Percent of changes	-	-16.23%	-8.07%
Interstitial thickness	Mean±SD	14.7±1.08[a]	31.79±1.11[b]	21.17±1.31[c]
	Percent of changes	-	116.25%	44.01%
testis/body weight index	Mean±SD	0.0074[a]	0.0042[b]	0.0055[c]
	Percent of changes	-	-43.24%	-25.67%
Connective tissue diameter	Mean±SD	20.91±1.15[a]	17.18±0.94[b]	20.40±0.98[a]
	Percent of changes	-	-17.83%	-2.43%

Table2: RI, SPI and TDI parameters between groups

Parameter	Analyzed form	Experimental groups		
		Control	Fructose	Ubiquinone+Fructose
R.I	Mean±SD	99.09±0.04	70.01±0.05	91.4±0.06
	Percent of changes	-	-29.34	-7.76
S.P.I	Mean±SD	94.8±0.06	61.1±0.01	80.1±0.02
	Percent of changes	-	-35.54	-15.50
T.D.I	Mean±SD	94±0.94	66.41±1.09	80.3±1.04
	Percent of changes	-	-29.35	-14.57

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Table3: Testosterone concentrations between groups

Parameter	Analyzed form	Experimental groups		
		Control	Fructose	Ubiquinone+Fructose
Testosterone	Mean±SD	341±77[a]	150±71[b]	249±40[c]
	Percent of changes	-	-56.01%	-26.97%

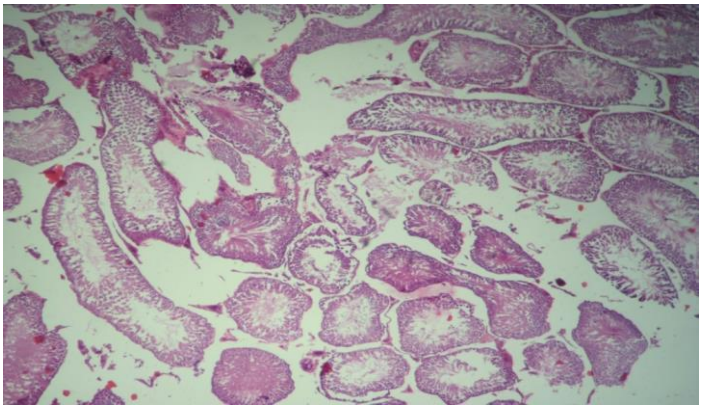

Figure 2: Testis tissue of under oxidative stress group (x100, H&E); decrease of seminiferous tubules diameter, deformity of seminiferous tubules and decrease of sperms

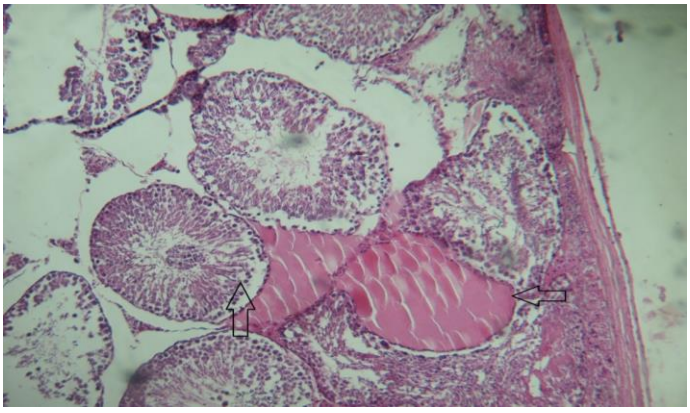

Figure 3: Testis tissue of under oxidative stress group (x100, H&E); secretion of exudate fibrin, Dislodge cells from the basement membrane of seminiferous tubules.

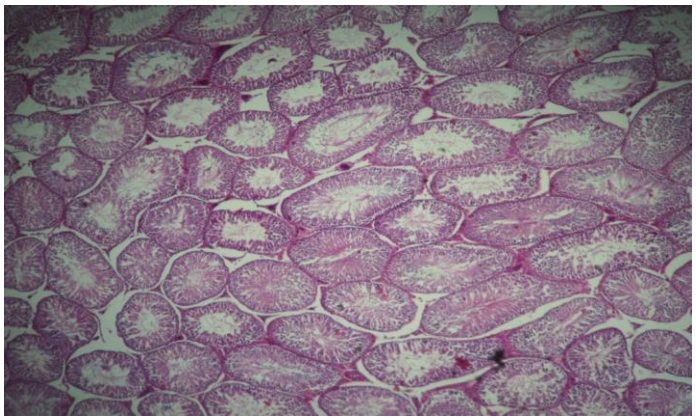

Figure 4: Testis tissue of under oxidative stress group treated with ubiquinone (x100, H&E); increase of seminiferous tubules diameter compared with under stress group, decrease of interstitial tissue.

Oxidative stress (OS) is outcome of disbalance between reactive oxygen species (ROS) and antioxidants in the both human and animal bodies. Oxidative stress has been implicated in the pathogenesis of many other diseases such as diabetes, inflammatory bowel disease, cancer, atherosclerosis, motor neuron disease, liver damage, rheumatoid arthritis, Parkinson disease, AIDS and cataracts. Oxidative stress can also lead to sperm damage, deformity and finally male infertility. This involves per oxidative damage to sperm membrane and DNA fragmentation at both nuclear and mitochondrial levels [18, 19].

Ubiquinone is a hydrophobic compound that is not only a mitochondrial respiratory chain critical component, but also a powerful antioxidant. Ubiquinone inhibits the expression of NADPH oxidase [20] and scavenges lipid peroxidation products during free radical reactions [21]. Ubiquinone also suppresses excess nitric oxide production and prevents nitrative tissue stress [22]. In addition, ubiquinone exhibits anti-inflammatory properties reducing proinflammatory cytokines release during inflammatory injury [23]. Treatment with ubiquinone attenuates the depletion of the antioxidant defense mechanisms by reducing glutathione level and superoxide dismutase activity, also suppresses lipid peroxidation, and decreases the elevations of tumor necrosis factor-α and nitric oxide levels in testicular tissue [24].

Seminiferous tubule germinal epithelium indicates high rates of mitochondrial oxygen consumption. However, the testis poor vascularization means that oxygen tension in tissue is low. Although the testis contains antioxidant enzymes and free radical scavengers, both spermatogenesis and steroidogenesis are susceptible to oxidative stress. Hence, wide range of conditions from testicular torsion and diabetes to xenobiotic exposure can result impaired testicular function. Excessive production of free radicals or reactive oxygen species can have harmful effect on spermatogenesis, where reactive oxygen species have been extensively studied as one of the mechanisms of impotency [25, 26].

Decrement in diameter of Seminiferous tubule was accompanied with depletion in the height of germinal epithelium which causes the atrophy of seminiferous tubules. These histological observations in seminiferous tubule illustrate the depressed cellular activity of spermatogenic cells in diabetic like conditions. Oxidative stress in testicular tissue has a direct relationship with abnormal spermatogenesis due to decrement of glutathione in male germ cells which leads to incomplete functional maturation and capacitation of spermatozoa [27, 28].

spermiogenesis (SPI) and Diminished tubular differentiation (TDI) indices in fructose fed rats indicates that, spermatogonia conversion to primary spermatocytes is reduced. Reduction of repopulation index in diabetic rats shows the number of inactive spermatogonia increased in fructose fed group. This process results a decline of the number of primary spermatocytes derived from spermatogonia cells. These alterations in cellular conversion and/or activity lead to reduction in production of spermatozoids. Oxidative stress can influence the developing spermatozoids normal structure because of induction of excessive lipid phosphorylation [28]. An excess of reactive oxygen species weakens sperm cell function and plays a negative role in male fertility. Ubiquinone may play a positive role in the treatment of asthenozoospermia because of its antioxidant properties. Has been proven that, ubiquinone levels increased in seminal plasma and in sperm cells after treatment [29]. According to Kianifard and Rezaee research administration of CoQ10 leads to improvement of cellular activity indices of testicular tissue and sperm analysis [30].

In a research, pretreatment with coenzyme Q10 and L-Carnitine attenuated the destructive effects of high LDL and oxidized LDL levels on spermatogenesis parameters in male rats. They reported, the percentage of motile sperm, motility grade, sperm viability and sperm count were decreased in both high cholesterol and oxidized cholesterol-fed groups [31]. In other study, Coenzyme Q10 treatment markedly ameliorated the arsenic-induced damage of testicular tissue and restored active spermatogenesis in most of seminiferous tubules [24].

Blood testosterone measurement results in this research showed that, the administration of ubiquinone leads to slight elevation of blood testosterone levels in treated fructose fed rats. In a research, rats received sodium arsenite without ubiquinone treatment showed a marked reduction in serum testosterone level as compared to the control group. Ubiquinone treatment significantly attenuated the arsenic-induced reduction in serum testosterone level [24]. It is stated that, function of Leydig cells may have a direct relationship with blood glucose levels [30].

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CONCLUSION

Using ubiquinone in oxidative stress condition can protect testis tissue and improves testicular function.

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