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HISTOPATHOLOGICAL EVALUATION ON THE EFFECT OF COENZYME Q10 AGAINST EXPERIMENTALLY INDUCED CRYPTORCHIDISM IN RATS

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ABSTRACT

Cryptorchidism is the absence of one or both testes from the scrotum. It is a common birth defect regarding male genitalia. CoQ is synthesized endogenously through a complex and only partially elucidated metabolic pathway. The aim of present study was to histopathological evaluation on the effect of coenzyme Q10 against experimentally induced cryptorchidism in rats.In this study, 40 male Wistar rats (120–90 g and 28 days old) were selected for the study then were divided into 4 equal groups: group1; healthy control rats received standard diet; group 2 induced cryptorchidism; Group 3 were undertake orchidopexy using Q10 and group 4 were undertake orchidopexy plus olive oil. For induction of experimental cryptorchidism, a longitudinal incision was made on abdomen, so that testes were fixed in abdominal cavity. Orchidopexy was done 35 days after induction of cryptorchidism was done. A longitudinal incision was made on abdomenTestes were dissected from the peritoneum and were planned in their normal position and fixed in scrotum. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. A small piece of testes was removed for histological analysis. In histological studies it has been shown that 35 days later in groups those were undertook cryptorchidism surgery, depletion of the seminiferous tubules and reduce the number of germinal epithelium were obvious. Small amounts of spermatogenesis cells which are mostly spermatogonia are seen in the wall of the seminiferous tubules. No significance changes were seen in sertoli cells and extended hyperemia in all over the testicular tissue was obvious. Diameter of seminiferous tubules was decreased because of

extension of interstitial tissue. In rats those received olive oil after orchidopexy, negligible changes was seen in thickness of epithelium of seminiferous tubules and repairing process was slow in these tubes. Connective tissue was still extended and small amount of spermatogenesis cells were seen in the tubules. But, in rats those received Co-Q10, repairing process were rapid so that diameter and thickness of epithelium of seminiferous tubules were normal which shows healing process. Also, the number of germinal cells was increased.

Keywords: Coenzyme Q10, Testis, Cryptorchidism, Orchidopexy, Olive Oil, Rat

INTRODUCTION

Cryptorchidism is the absence of one or both testes from the scrotum. It is a common birth defect regarding male genitalia. In unique cases, cryptorchidism can develop later in life, often as late as young adulthood. About 3% of full-term and 30% of premature infant boys are born with at least one undescended testis. However, about 80% of cryptorchid testes descend by the first year of life (the majority within three months), making the true incidence of cryptorchidism around 1% overall.

Coenzyme Q10, also known as ubiquinone is an oil-soluble, vitamin-like substance is present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, generating energy in the form of ATP. Ninety-five percent of the human body's energy is generated this way **(Ernster and Dallner, 1995; Dutton *et al.*, 2000)**. Therefore, those organs with the highest energy requirements—such as the heart, liver and kidney—have the highest CoQ10 concentrations **(Okamoto *et al.*, 1989; Aberg *et al.*, 1992; Shindo *et al.*, 1994)**. There are three redox states of CoQ10: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). The capacity of this molecule to exist in a completely oxidized form and a completely reduced form enables it to perform its functions in the electron transport chain, and as an antioxidant, respectively.

CoQ is synthesized endogenously through a complex and only partially elucidated metabolic pathway. Most available information derives from yeast studies, where initially 9 genes (coq1-9) have been characterized as essential for CoQ biosynthesis **(Tran and Clarke, 2007)**. Moreover, recently mitochondrial ferredoxin Yah1 and ferredoxin reductase Arh1 have been found to be also required for CoQ biosynthesis (Pierrel et al., 2010). CoQ is composed of a benzoquinone ring, derived from tyrosine, and an isoprenoid side chain (which contains 6-10 isoprene

units in different species) generated from acetyl-CoA via the mevalonate pathway **(Bentinger *et al*., 2010)**. Briefly, the polyisoprenoid tail is assembled by polyprenyl diphosphate synthase and then covalently bound to the benzoquinone head group producing the 4-hydroxy-3-polyprenyl benzoic acid (4-HB). This is followed by several modifications of the aromatic ring, such as C-hydroxylations, decarboxylation, O-methylations, and C-methylation leading to CoQ (Tran and Clarke, 2007). The aim of present study was to histopathological evaluation on the effect of coenzyme Q10 against experimentally induced cryptorchidism in rats.

MATERIALS AND METHODS

Study design

In this study, 40 male Wistar rats (120–90 g and 28days old) were selected for the study and were purchased from Animal House, Islamic Azad University and randomly divided into 4 equal groups: group1; healthy control rats received standard diet; group 2 induced cryptorchidism; Group 3 were undertake orchidopexy using Q10 and group 4 were undertake orchidopexy plus olive oil. In group 3, 30 days after orchidopexy surgery, Q10 was administrated at a dose of 300mg dissolved in olive oil as gastric gavage (Nehadet al.,2003). In group 4, 30 days after orchidopexy surgery, olive oil was administrated as gastric gavage.

Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*.

Induction of cryptorchidism

For induction of experimental cryptorchidism first of all we used Xylazine at a dose of 1-2mg/kg as i.p. route and pre-anesthesia drug. After 10 minutes, ketamine was administrated at a dose of 90-100mg/kg as i.p. route and anesthetic drug. Then the hairs of abdomen area were shaved and sterilized. A longitudinal incision was made on abdomen, skin, muscular layer and peritoneum were incised so abdominal cavity was exposed. Testes were brought out of scrotum and sutured in peritoneum using silk suture no. 3-0 so that testes were fixed in abdominal cavity. For prevention of testes returning into the scrotum, Gubernaculum was cut. At the end, muscular and skin layers were sutured using catgut 3-0 and continue mattress and silk 3-0 and horizontal mattress respectively. For prevention of secondary infections,

chloramphenicol was administrated as spray on the surgery area.

Orchidopexy procedure

35 days after induction of cryptorchidism was done. Xylazine was administrated at a dose of 1-2mg/kg as i.p. route and pre-anesthesia drug. After 10 minutes, ketamine was administrated at a dose of 90-100mg/kg as i.p. route and anesthetic drug. Then the hairs of abdomen area were shaved and sterilized. A longitudinal incision was made on abdomen, skin, muscular layer and peritoneum were incised so abdominal cavity was exposed. Testes were dissected from the peritoneum and were planned in their normal position and fixed in scrotum. At the end, muscular and skin layers were sutured using catgut 3-0 and continue mattress and silk 3-0 and horizontal mattress respectively. For prevention of secondary infections, chloramphenicol was administrated as spray on the surgery area.

Histopathological studies

The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. A small piece of testes was removed for histological analysis. The sample was fixed by immersing it in 10% neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin-eosin for blinded histological assessment. The histological changes were evaluated in nonconsecutive, randomly chosen ×200 histological fields using light microscope, NIKON ECLIPSE E200.

RESULTS

The normal view of testes of rats of control group is depicted in figure 1 which shows the normal structure of testis. In histological studies it has been shown that 35 days later in groups those were undertook cryptorchidism surgery, depletion of the seminiferous tubules and reduce the number of germinal epithelium were obvious **(Figure 2)**. Small amounts of spermatogenesis cells which are mostly spermatogonia are seen in the wall of the seminiferous tubules. No significance changes were seen in sertolicells and extended hyperemia in all over the testicular tissue was obvious. Diameter of seminiferous tubules was decreased because of extension of interstitial tissue. In rats those received olive oil after orchidopexy, negligible changes was seen in thickness of epithelium of seminiferous tubules and repairing process was slow in these tubes. Connective tissue was still extended and small amount of spermatogenesis cells were seen in the tubules **(Table 3)**.

But, in rats those received Co-Q10, repairing process were rapid so that diameter and thickness of epithelium of seminiferous tubules were normal which

shows healing process. Also, the number of germinal cells was increased **(Figure 4)**.

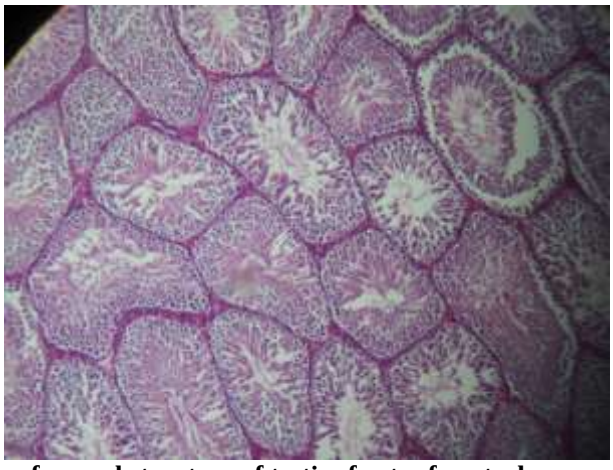

Figure 1: microscopic view ofnormal structure of testis of rats of control group, H&E, 200X.

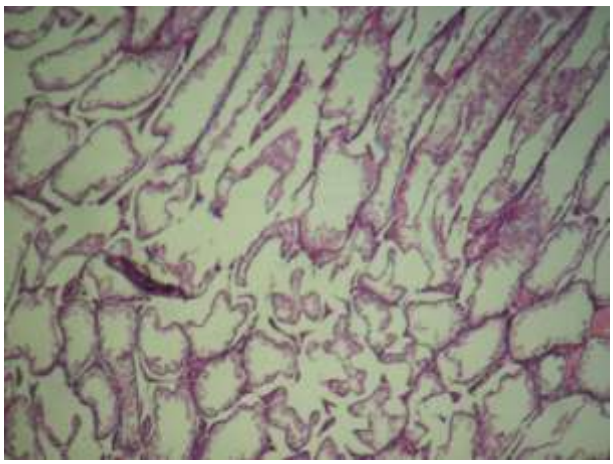

Figure 2: microscopic view of testis of rats of group 2 in which they underwent cryptorchidism, H&E, 200X

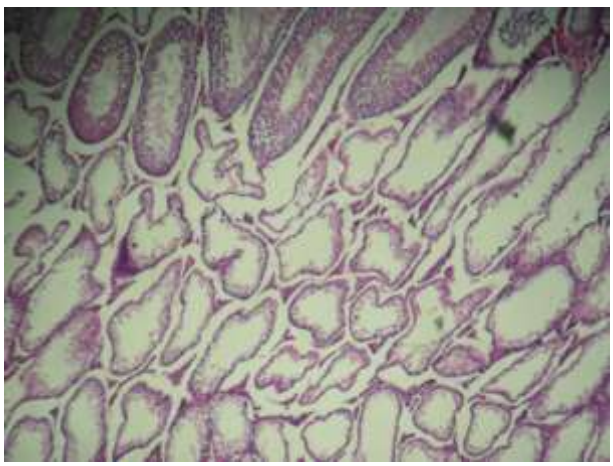

Figure 3: microscopic view of testis of rats of group 4 in which they underwent cryptorchidism followed by 30 days olive oil treatment, H&E, 200X.

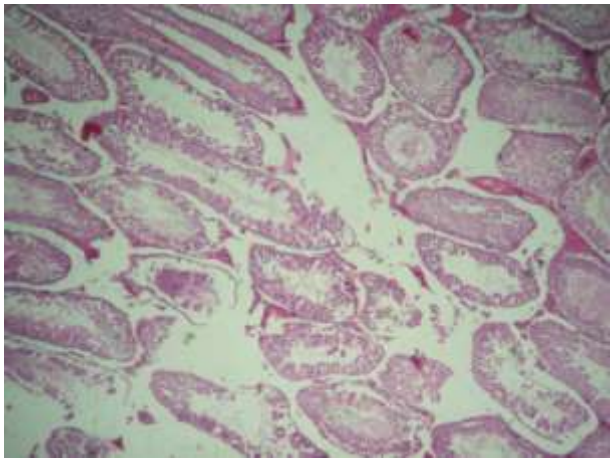

Figure 4: microscopic view of testis of rats of group 3 in which they underwent cryptorchidism followed by 30 days Q10 treatment, H&E, 200X.

DISCUSSION AND CONCLUSION

It has been suggested that cryptorchidism is associated with a decrease in antioxidant enzyme activity **(Zini & Schlegel 1997)** or an increase in the production of ROS, such as superoxide anion, hydroxyl radical, nitric oxide and hydrogen peroxide **(Zini & Schlegel 1997; Kumagai *et al*. 2002; Ishii *et al*. 2005)**, which stimulates lipoperoxidation **(Janero 1990)**. Antioxidant treatments could increase a cell's endogenous antioxidant defense system, inhibiting ROS production or working to trap free radicals and impede lipoperoxidation **(Tilly & Tilly 1995)**. Attempts have been made to decrease the damage caused by free radicals in cryptorchidism **(Kumagai *et al*. 2002; DeFoor *et al*. 2004)**, but there are no reports on the use of αT as a powerful antioxidant in this pathology.

Cryptorchidism is associated with high levels of apoptosis in germ cells **(Shikone *et al*. 1994)**. In this work, we showed that Q10 protects germ cells. The mechanism through which this is believed to occur is through Q10 inhibition of the production of lipoxygenases, enzymes that initiate membrane lipoperoxidation and activate apoptosis **(Maccarrone *et al*. 2001)**, promoting cell survival and reducing histological alterations to the seminiferous epithelium. There are many studies in the field of cryptorchidism in which short-term observation of animals with cryptorchidism without the administration of Q10 had a number of alterations similar to those described previously in testicles subjected to elevated temperature **(Huff *et al*. 1989; Brehm & Steger 2005)**, such as the reduction in the EA, maturation index and testicular weight. These alterations are possibly owing to the fact that spermatocytes and spermatids are the germ cells most vulnerable to damage caused by ROS because, even though they are capable of converting superoxide anion to hydrogen peroxide, they have difficulty metabolizing

peroxide, which saturates its protective system against peroxide that becomes a highly toxic hydroxyl radical **(Bauché et al. 1994**)

The results of this study confirm that the generation of lipoperoxidation plays an important role in the cryptorchid testicular damage mechanism. The administration of Q10 significantly reduces lipoperoxidation because of the Q10 action mechanism. This mechanism has been suggested to decrease lipoperoxidation by ceding hydrogen from the hydroxyl group in position 6 of its ring, in addition to its capacity to stabilize radicals produced during this process **(Traber & Kayden 1987)**, reducing the ROS and the histological damage caused by them, leading to the conservation of spermatogenesis and increasing fertility. It has also been reported that αT reduces damage caused by ROS generated by exposure to metals and toxic substances inside the testicle **(Lucesoli & Fraga 1999; Jedlinska-Krakowska *et al*. 2006** .

In conclusion, treatment with Q10 prior to orchidopexia, particularly at 300 mg, partially protects the undescended testicle from damage caused by ROS (demonstrated by the significant improvement in seminiferous epithelium damage), restored spermatogenesis and increased fertility.

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