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MORPHOMETRIC STUDY OF *CO Q10* ON TESTIS IN DIABETIC RATS

JAHANGIRFARD R[1], HEJAZI S[2*] AND REYHANI-RAD S[3]

1: Veterinary Student, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

2: Department of Anatomy, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

3: Department of Laboratory Sciences, Marand Branch, Islamic Azad University, Marand, Iran

***Corresponding Author's E Mail: sajjad.hejazi@iaut.ac.ir**

ABSTRACT

Diabetes is a chronic disorder in fats, carbohydrates and protein metabolism which is characterized by increased blood glucose level. In the present study, the effect of CoQ10 supplementation was examined on the structural changes in the testis of Alloxan-induced diabetic rats. In present study, 40 male Wistar rats (about 200-300 g body weight) were purchased from Animal House, Islamic Azad University. The rats were randomly divided into 4 groups (10 rats each) as the following: Group 1, healthy control rats received isotonic saline solution (ISS, 10 ml/kg) intraperitoneally; Group 2, rats were treated with 120 mg/kg b.w. /day intraperitoneal (i.p.) injection of Aloxan; in Group 3, rats administered by CoQ10 (75 ml/kg) was given through oral Gavage; Group 4, diabetic rats were treated with Alloxan and CoQ10. For this mean, rats were induced diabetes by i.p. injection of alloxan at the dose of 120mg/kg then were received CoQ10 at the dose of 75mg/kg orally for a month. Diabetes was induced by intraperitoneal injection of Alloxan (Sigma, St. Louis, Mo, USA) at a dose of 120 mg/kg body weight dissolved in distilled water. 72 hours later, animals with fasting blood glucose levels measured by glucometer and levels greater than 130 mg/dl were considered diabetic and then included in this study. Based on data obtained from present study, Q10 acts as an antioxidant which significantly protects spermatogenic cells, testicular capsule, weight and diameter of

seminiferous tubules in diabetic rats received Q10 in compared with non-treated diabetic rats. Therefore, Q10 supplementation is useful in protection of testicular tissue in diabetic rats, so, Q10 is considered as an anti-diabetic drug supplemented in food. However it needs more pharmacological and biochemical studies in use of it.

Keywords: Coenzyme Q10, Alloxan, Diabetes, Testis, Rats

INTRODUCTION

Diabetes is a chronic disorder in fats, carbohydrates and protein metabolism which is characterized by increased blood glucose level. Disorder is caused by inability of cells in absorption of blood sugar because of low secretion of insulin or cell's resistance against insulin **[1, 2]**.

Despite the insulin and synthetic oral medications such as biguanides, sulfonilureas, thiazolidinones and α-glucosidase inhibitors are the basic treatment in diabetes, have significant side effects that cannot decrease diabetes complications perfectly **[3]**.

CoQ10 was first discovered by Professor Fredrick. CoQ10 is a vitamin like substance and is found in many food resources but in a little amount **[4]**.

Studies showed that the levels of CoQ10 is decreased by aging, heart diseases, muscular dystrophy, Parkinson's disease, cancer, diabetes and HIV. Also, it protects membrane proteins from oxidative stresses. Decreasing in CoQ10 levels has been observed in diabetic patients, cancer and heart diseases **[4]**. Recent years, several drugs have been found useful for the treatment of diabetes, but the therapeutic or preventive effect of few of them have been studied microscopically. In the present study, the effect of CoQ10 supplementation was examined on the structural changes in the testis of Alloxan-induced diabetic rats.

MATERIALS AND METHODS

In present study, 40 male Wistar rats (about 200-300 g body weight) were purchased from Animal House, Islamic Azad University. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment execution. 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.

The rats were randomly divided into 4 groups (10 rats each) as the following: Group 1, healthy control rats received isotonic saline solution (ISS, 10 ml/kg) intraperitoneally; Group 2, rats were treated with 120 mg/kg b.w. /day intraperitoneal (i.p.) injection of Aloxan; in Group 3, rats administered by CoQ10 (75 ml/kg) was given through oral Gavage; Group 4, diabetic rats were treated with Alloxan and CoQ10. For this mean, rats were induced diabetes by i.p. injection of alloxan at the dose of 120mg/kg then were received CoQ10 at the dose of 75mg/kg orally for a month.

Diabetes was induced by intraperitoneal injection of Alloxan (Sigma, St. Louis, Mo, USA) at a dose of 120 mg/kg body weight dissolved in distilled water **[5, 6]**.

72 hours later, animals with fasting blood glucose levels measured by glucometer **[7]** and levels greater than 130 mg/dl were considered diabetic and then included in this study **[8]**. Fasting blood glucose was estimated by using one touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany.

At the end of the study, rats' testis was sampled and weighing accurately by a digital scale. Samples were evaluated by light microscope after fixing in formalin 10% and staining by H&E method.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

Histomorphometric Evaluation

Effects on Testis Weight

The mean value of testis' weight in control group was 1.53±0.035, in Q10 group was 1.75±0.03, alloxan group was 1.1±0.037 and alloxan+Q10 was 1.53±0.089. Obtained data showed that alloxan cause significant decrease in testis' weight in compared with other groups ($P<0.001$). Also, Q10 had significant compensatory effects in compared with diabetic group ($P<0.05$) **(Table and Diagram 1)**.

Effects on Testis Length

The mean value of testis' lenght in control group was 20.66±0.33, in Q10 group was 21.9±0.31, alloxan group was 17±0.84 and alloxan+Q10 was 20.3±0.47. Obtained data showed that use of Q10 increases the length of testis significantly in compared with other

groups (P<0.001). Also, Alloxan had significant decreasingly effects in compared with alloxan+Q10 group (P<0.001) **(Table 1 and Diagram 2)**.

Effects on Seminiferous Tubule's Diameter:

The mean value of seminiferous tubule's diameter in control group was 36.45±0.73, in Q10 group was 36.65±0.55, alloxan group was 30.9±0.69 and alloxan+Q10 was 33.95±0.42. Obtained data showed that alloxan decreases the seminiferous tubule's diameter significantly in compared with control and Q10 groups (P<0.001). Also, Q10 does not affect in compensation of decreased values **(Table 1 and Diagram 3)**.

Effects on Thickness of Seminiferous Tubule's Epithelium

The mean value of thickness of seminiferous tubule's epithelium in control group was 29.7±0.97, in Q10 group was 31.9±0.8, alloxan group was 16.5±0.73 and alloxan+Q10 was 21±0.9. Obtained data showed that alloxan decreases the thickness of seminiferous tubule's epithelium significantly in compared with groups (P<0.001) while, by administration of Q10 (alloxan+Q10 group) significantly increases observed than alloxan group (P<0.001) **(Table 1 and Diagram 4)**.

Effects on Thickness of Testicular Capsule

The mean value of thickness of testicular capsule in control group was 8.4±0.36, in Q10 group was 8.8±0.35, alloxan group was 16.4±0.81 and alloxan+Q10 was 14.6±0.65. Obtained data showed that alloxan increases the thickness of testicular capsule significantly in compared with groups (P<0.001). While, by administration of Q10 (alloxan+Q10 group) significantly decreases observed than alloxan group but could not arise to the normal ranges (P<0.05) **(Table 1 and Diagram 5)**.

Table 1: Comparison of Data as Mean±SD in Parameters Measured in Groups

Group / Parameters	No	Q10	Aloxane	Alox+ Q10	Control
Testicular Weight	12	**1.53±0.35 [b]**	**1.53±0.089 [b]**	**1.60±0.037 [ab]**	**1.75±0.030 [ab]**
Testicular Length	12	**20.66±0.33 [b]**	**20.3±0.47 [b]**	**17±0.74 [c]**	**21.9±0.31 [a]**
Epithelium thickness	20	**29.7±0.97 [a]**	**21±0.9 [b]**	**16.5±0.73 [c]**	**31.1±0.8 [a]**
Testicular capsule thickness	20	**8.4±0.37 [c]**	**14.6±0.65 [b]**	**16.4±0.81 [a]**	**8.8±0.35 [c]**
Diameter of seminiferous tubules	20	**36.45±0.73 [a]**	**33.95±0.42 [c]**	**30.9±0.69 [b]**	**36.65±0.55 [a]**

Dissimilar Letters Show Significant Difference Among Groups (P<0.001)

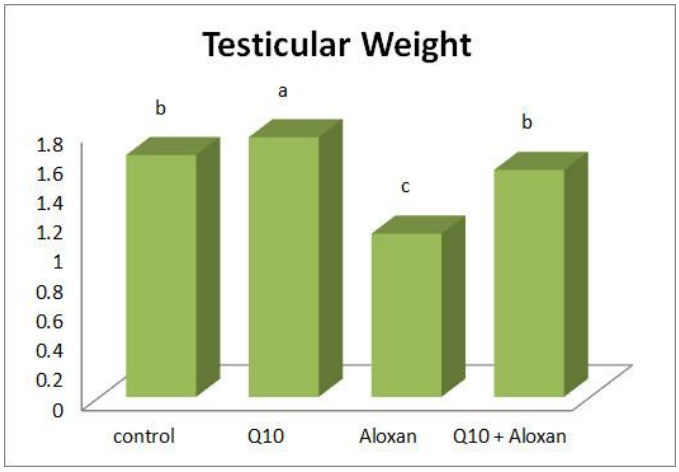

Diagram 1: Comparison of Data as Mean±SD in Term of Testicular Weight in Groups One Month Later. Dissimilar Letters Show Significant Difference Among Groups (P<0.05)

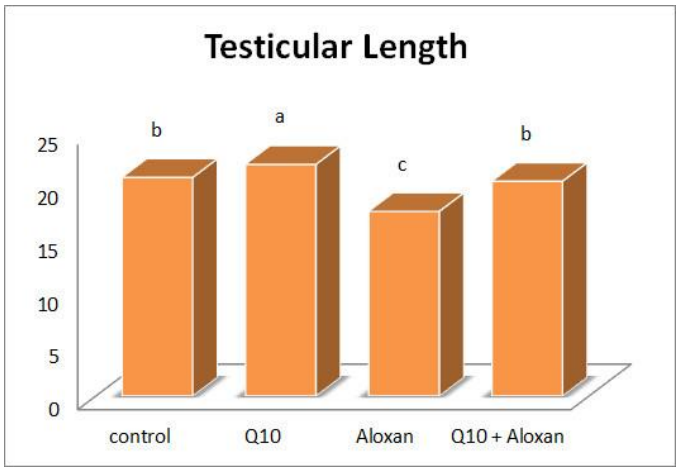

Diagram 2: Comparison of Data as Mean±SD in Term of Testicular Length in Groups One Month Later, Dissimilar Letters Show Significant Difference Among Groups (P<0.001)

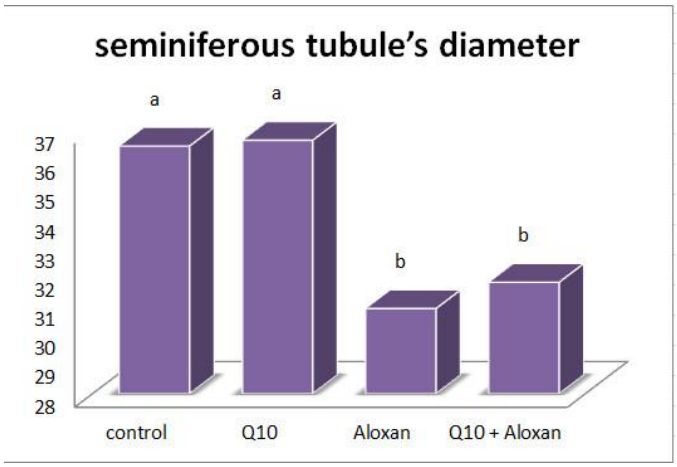

Diagram 3: Comparison of Data as Mean±SD in Term of Seminiferous Tubule's Diameter in Groups One Month Later. Dissimilar Letters Show Significant Difference Among Groups (P<0.05)

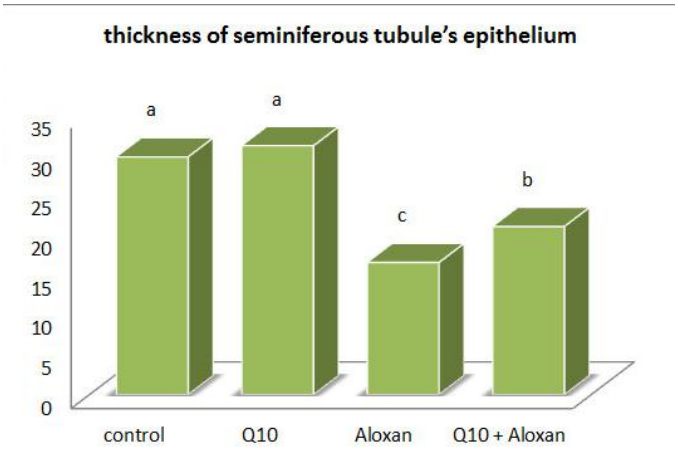

Diagram 4: Comparison of Data as Mean±SD in Term of Thickness of Seminiferous Tubule's Epithelium in Groups One Month Later. Dissimilar Letters Show Significant Difference Among Groups (P<0.05)

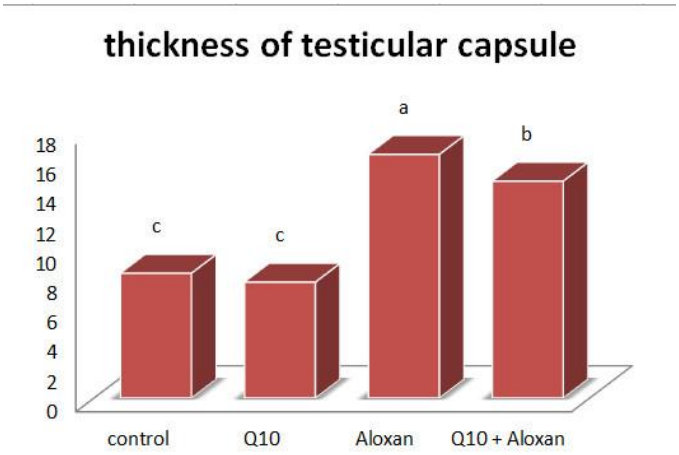

Diagram 5: Comparison of Data as Mean±SD in term of Thickness of Testicular Capsule in Groups One Month later. Dissimilar Letters Show Significant Difference Among Groups (P<0.05)

DISCUSSION

Hyperglycemia is a top sign of diabetes as a disturbance of metabolism **[9]**. Prolonged high levels of blood glucose is accompanied with other diseases such as neuropathy and emerging of injuries in cardiovascular system, kidneys and eyes. Disorder in genital system is another complication of diabetes **[10]**. Diabetes may cause disturbances in spermatogenesis process by inducing changes in seminiferous tubules **[11]**.

Of data obtained from present study in term of thickness of seminiferous tubule's epithelium can be concluded that there was significant decrease in epithelium thickness in alloxan diabetic group which is increased and raised next to the normal ranges by injection of Q10, therefore, Q10 is useful in testicular injuries due to diabetes.

In a study, **[12]** have reported the thickness of epithelium in control and Aloe vera groups greater than diabetic rats.

In present study, alloxan increases the thickness of testicular capsule which is compensated by administration of Q10 in alloxan+Q10 group. Thus, Q10 can be modulator in testicular injuries due to diabetes. **[12]** have reported the thickness of testicular capsule in Alloxan group greater than other groups.

In present study, alloxan decreased the diameter of seminiferous tubules in diabetic rats. Administration of Q10 cause relative increase in diameter of seminiferous tubules in diabetic rats but it was not significant. **[12]** have reported the diameter of seminiferous tubules in control and Aloe vera groups greater than diabetic rats.

[13] showed the decrease in the diameter of seminiferous tubules in alloxan-induced diabetic rats.

In our study, alloxan caused significant decrease in testicular weight in diabetic groups. Also, Q10 could compensate this change in alloxan+Q10 group. Therefore, Q10 can be beneficial in diabetic rats' testis tissue. **[14]**revealed that testicular weight in diabetic rats is lesser than control group. As well as, observed significant decrease in diameter of seminiferous tubules of diabetic rats. **[12]** also reported the significant decrease of testicular weight in diabetic rats.

CONCLUSION

Based on data obtained from present study, Q10 acts as an antioxidant which significantly protects spermatogenic cells, testicular capsule, weight and diameter of seminiferous tubules in diabetic rats received Q10 in compared with non-treated diabetic rats. Therefore, Q10 supplementation is useful in protection of testicular tissue in diabetic rats, so, Q10 is considered as an anti-diabetic drug supplemented in food. However it needs more pharmacological and biochemical studies in use of it.

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