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Research Article

THE PROTECTIVE EFFECT OF *COENZYME Q10* ON NEPHROPATHY IN ALLOXAN-INDUCED DIABETIC RATS

Miad Marashi[1] and *Sajjad Hejazi[2]
[1]Department of Veterinary, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Anatomy, Tabriz Branch, Islamic Azad University, Tabriz, Iran
**Author for Correspondence*

ABSTRACT
Considering the various effects of Q10 especially hypoglycemic and antioxidant characteristics, it is assumed that Q10 can decrease diabetes nephropathy complications. Due to the antioxidant effects of Q10, it is assumed that it can decrease the nephropathy side effects of diabetes. In any case, considering that no study has been conducted so far about the Q10 effects on renal tissue damages resulted of diabetes, the present study was conducted in order to evaluate the protective effects of Q10 on serum levels of renal function indicators in diabetic rats induced by alloxan. The experimental model of diabetes type A in rats was induced by intraperitoneally injection of 120 mg Alloxan monohydrate per kg/bw and physiology serum was used as Alloxan solvent. Q10 Treatment group received 75 mg/kg Q10 via gavage for one month and the combined experimental group at first the rats were induced to become diabetic then they received 75 mg/kg Q10 by gavage. According to the obtained morphological results in the present study it can be said that the Q10 administration in diabetic groups can't be effective as a hypoglycemic, but on the other hand the morphologic results of renal capsule thickness suggest that the fibroblastic cells have diabetic reactions following the effects of free radicals; so, by the administration of Q10 the pathogenic reaction can be prevented. Also according to the obtained results about renal corpuscle diameter it was revealed that Q10 can prevent meaningfully from the renal corpuscle diameter decrease resulted by free radicals following the diabetes disease. The effects of Q10 on a damaged kidney suggest its high effects of anti oxidant factors which can resist against free radicals created by diabetes disease. Totally, the positive effect of Q10 in a partial recovery from diabetic nephropathy is consistent with the results of our study, such that there is a meaningful difference between control and treatment groups in terms of resulted damages, like: glomerulonephritis, tubular necrosis, and nephrosis. So, it can be concluded that the administration of Q10 can be considered as a preventive procedure of diabetes side effects on kidney.

Keywords: *Alloxan, Q10, Nephropathy, Rat*

INTRODUCTION
Being the most common type of diabetes, type I Diabete has been always as an increasing problem in worldwide health. Although the pathogenesis of type I diabetes is not clear perfectly, glucose and fat metabolism are involved in the problem (McGarry, 1992). There are most evidences suggesting its oxidative stress role and following it the production of free radicals in diabetic people and their involvement in diabetes pathogenesis (Kaneto *et al.*, 2007). It has been cleared that Hyperglycemia causes increased active oxygen and leads to sever oxidative tensions in cells (Signorini *et al.*, 2002). The researches have been demonstrated that free radicals removing enzymatic and non-enzymatic defensive systems are attenuated in diabetic people and the lipid peroxidation rate increases in cells (Wohaieb *et al.*, 1987). Accordingly, several and sever damages occur in different organs of diabetic people; such that, renal failure has been known as a main factor of diabetics mortality (Pickup *et al.*, 1997). Significant improvement have been obtained in the area of diabetes control using synthetic drugs, but the diabetic people demand to use natural products with anti diabetes characteristics are increasing continuously due to adverse effects of insulin and hypoglycemic medicines (Rao *et al.*, 2007). So, attempts to find natural agents to deal with the disease have an important clinical value. Plants have been used widely used and it has been demonstrated that some plants can decrease complications of diabetes with or without

decreasing the blood sugar (Neef *et al.,* 1995). There are more than several hundreds of plant species that have anti diabetic effects. However, only a few of them have been studied (Noel *et al.,* 1997).

Q10 was discovered in 1957 by Fredrick L. Crane. Q10 is considered as a vitamin or a vitamin-like substance and is found in food resources naturally as other vitamins, but its amount is very in food resources (Dhanasekaran *et al.,* 2005).

Q10 is synthesized in all tissues. It biosynthesis is a multistage process that needs at least eight vitamins and several rare minerals. Q10 is soluble in fat and found in all cells of the body. It acts also as a coenzyme in most of important enzymatic stages to produce intra cell energy. The maximum amount of it is found in liver, kidney, heart, muscle, and brain.

Another function of Q10 is as an antioxidant. Internal synthesis of vitamin and also its absorption through food caused normal rate maintenance of Q10 in a healthy person. The positive effects of Q10 in the treatment of Aids, cancer, gastric ulcers, obesity, muscular dystrophy, sensitivity, immune system function, and body physical strength have been studied (Dhanasekaran *et al.,* 2005). The researchers' studies demonstrate that Q10 decreases in heart disease, muscular dystrophies, Parkinson, cancer, diabetes, and Aids. Furthermore, it protects membrane proteins from oxidative damages. In diabetes, cancer and heart diseases the decreased Q10 is observed (Dhanasekaran *et al.,* 2005). Considering the various effects of Q10 especially hypoglycemic and antioxidant characteristics, it is assumed that Q10 can decrease diabetes nephropathy complications. Due to the antioxidant effects of Q10, it is assumed that it can decrease the nephropathy side effects of diabetes. In any case, considering that no study has been conducted so far about the Q10 effects on renal tissue damages resulted of diabetes, the present study was conducted in order to evaluate the protective effects of Q10 on serum levels of renal function indicators in diabetic rats induced by alloxan.

MATERIALS AND METHODS

The experimental model of diabetes type A in rats was induced by intraperitoneally injection of 120 mg Alloxan monohydrate per kg/bw and physiology serum was used as Alloxan solvent (Ugbenye *et al.,* 2009). 72 hours after injection of Alloxan, glucometer was used to measure the animal FBS using glucometer (Lazos, 1986). FBS within 120-250 mg/dl was considered as diabetic in the present study (Gupta *et al.,* 2005). Ziest Chem glucometer kit made by Iran zist chimi was used.

Control group rats received buffer citrate 0.05 M with pH 4.5 intraperitoneally. Q10 Treatment group received 75 mg/kg Q10 via gavage for one month (Dhanasekaran *et al.,* 2005). The fourth group (combined experimental group) at first the rats were induced to become diabetic then they received 75 mg/kg Q10 by gavage.

The keeping condition in other cases was considered equal for all groups. At the end of experiment period, following to 12 hour diet, 20 blood samples were obtained from the back of the eyeball. The data were expressed as Mean±Sem. ANOVA statistical analysis method was used for data analysis and Tukey tests were used to compare the difference between groups. $P<0.05$ was used to determine the significance level.

RESULTS AND DISCUSSION

Results

Histological Results

Based on tissue samples in diabetic group, sever tissue damages were observed. The damages were as acute tubular necrosis, interstitial tubular nephritis, vacuolar nephrosis, fat changes and vascular arteriolosclerosis, which were observed both in cortex and medulla.

The coagulative necrosis was mainly observed in proximal tubules in wide range of tissue. The tubules' cytoplasm inflammation and the decrease of tonality power of the cells along with tubular cells flux were other obtained results of the study.

Furthermore, outstanding increase of the Mesangial matrix, Dilation of the urinary space, as well as visceral and wall adhesions of Bowman's capsule were observed. Hyaline cysts were seen in medullar

part of Alloxan-affected rats' kidneys. Other side effects were renal vascular arteriosclerosis which was observed as hyalination of vessels' wall along with stenosis. Mononuclear infiltration in interstitial renal tissue, glomerular congestion, and hemorrhage in interstitial spaces of tubules were visible in diabetic rats. Hypertrophy in renal tubules' epithelial cells in proximal and distal parts followed by fatty changes of the cells cytoplasm were observed as fine and transparent vacuoles around nuclear (vacuolar nephrosis). In the kidneys of Q10- administrated rats all signs were seen as mild. The occurrence of coagulation necrosis of renal cortex and medulla was lower in experimental group compared with diabetics. Glomerular congestion in Q10 experimental was as diabetics, but the congestion has been decreased in tubules interstitial spaces. Hyaline cysts dispersion in Q10-experimental group was very low. The rate of arteriosclerosis was lower in Q10-experimental group compared with diabetics, such that there was no stenosis. Visceral and wall adhesions of Bowman's capsule as well as mononuclear cells infiltration and interstitial space edema and also glomerular capillaries' microtrombosis were the same in both groups.

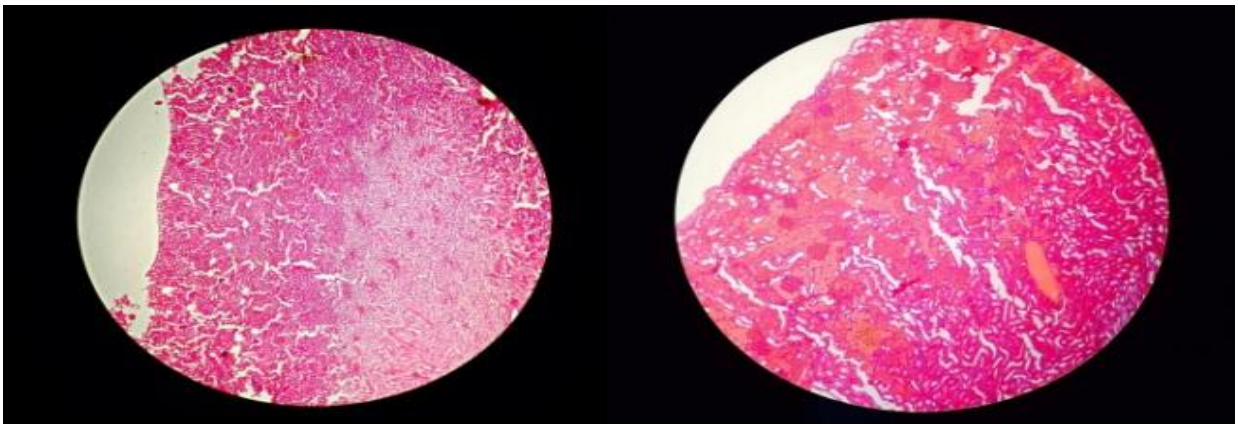

Figure 1: Microscopic view of a rat's renal cortex. The observation of a wide range of tubular acute necrosis and glomerular hemorrhage along with edema in renal interstitial spaces in diabetic groups kidneys (right side) and the comparison of the mild status in Q10-experimental group kidneys (left side) (H&E staining, magnification of 100×)

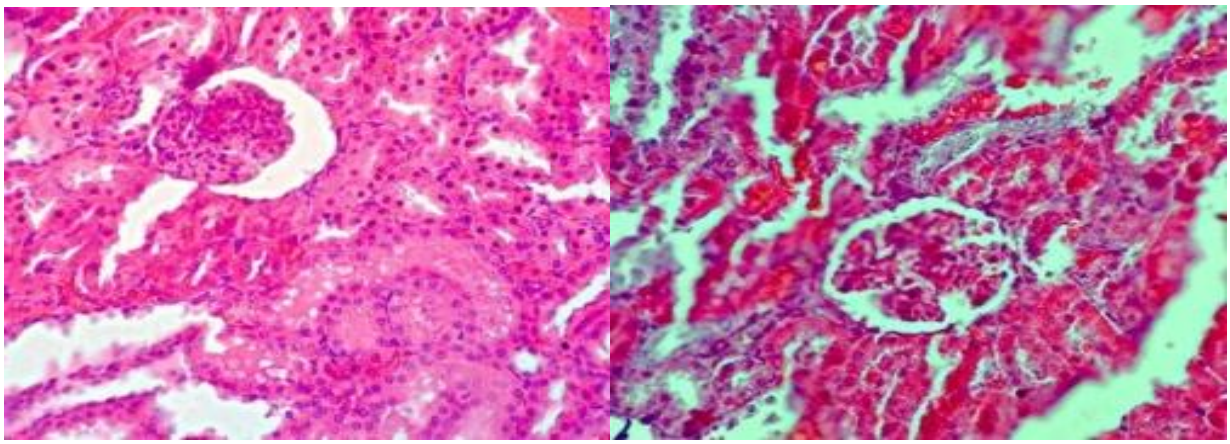

Figure 2: Microscopic view of a rat's renal cortex. The increased Mesangial matrix, tubular necrosis and congested regions as well as hemorrhage along with edema in renal interstitial spaces in diabetes-induced rats (right side) compared with mid congestion and tubular necrosis in Q10-experimental group (left side), along with the fatty changes in tubular epithelial cells' cytoplasm which are seen as fine and transparent vacuoles in experimental group (tip of the arrow) (H&E staining, magnification of 400×)

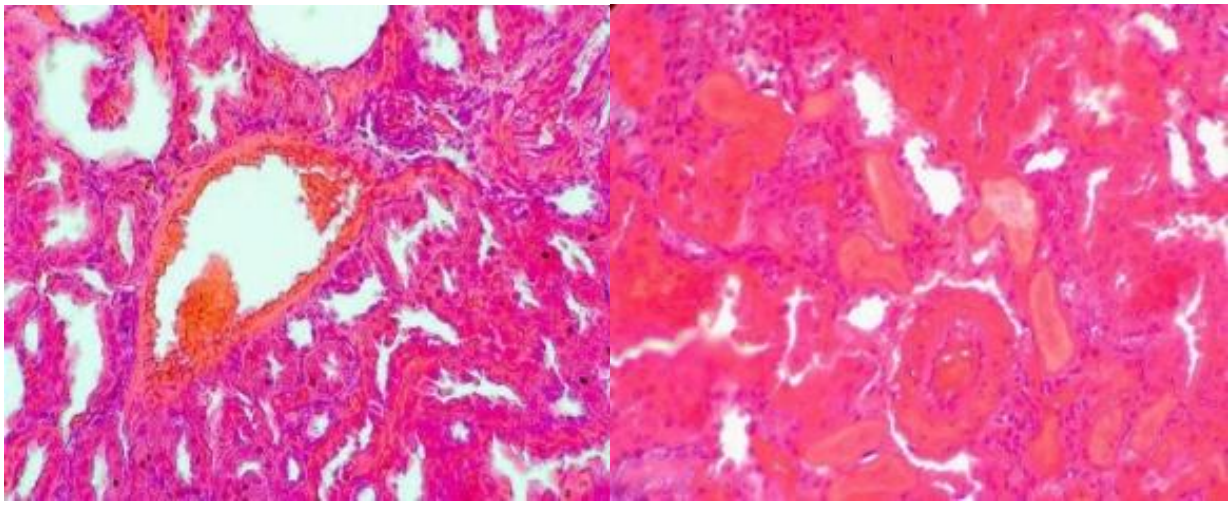

Figure 3: Microscopic view of a rat's renal cortex. occurrence of renal vascular arteriosclerosis (large arrow) and vascular hyalination along with fibrination of its wall. The occurrence of tubular acute coagulant necrosis (small arrow) and mononuclears' infiltration (tip of the arrow) in the renal interstitial tissue of the diabetic group (right side) and comparison with the mild status in Q10-experimental group (left side) (H&E staining, magnification of 400×)

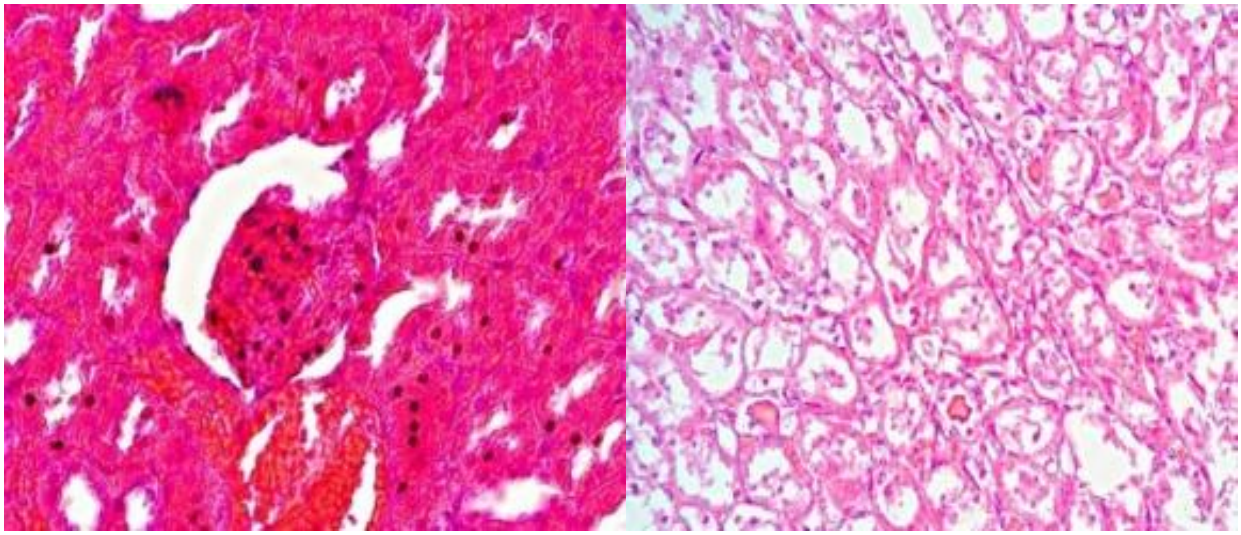

Figure 4: The right side fig.: microscopic view of the diabetic rats' renal medulla and the observation of hyalinated cysts in urinary collecting duct lumen (H&E staining, magnification of 400×). The left side fig.: Microscopic view of a diabetic rat's renal cortex. Occurrence of tubular acute coagulant necrosis, increased mesangial matrix, visceral and wall adhesion of Bowman's capsule, as well as urinary space dilation (H&E staining, magnification of 400×)

Table 1: Comparison of the parameters mean in the control and experimental groups. Dissimilar letters in each vertical column indicate a significant difference in Mean + SD, (P<0.05)

Groups variables	Control	Q10	Alloxan	Q10+Alx
Renal capsule thickness	3.87±0.175a	4.33±0.183a	16.57 ±0.849c	8 ±0.527 b
Renal corpuscle diameter	108.37±3.77a	108.12±3.47 a	59.16±2.1 a	73.95±2.5 a
Blood sugar	94.8 ±3.88 a	94.2 ±3.45 a	245.1 ±20.9 b	240.5 ±9.26 b
Renal weight	1±0.121 a	1±0.145 a	0.74±0.09 b	0.77±0.11 a

Effect on Blood Sugar

The mean blood sugar in normal, Q10, Alloxan, and Q10+Alloxan groups were 94.8±3.88, 94.2±3.4, 245.1±2.1, and 240.5±9.26 mg/dl, respectively. The obtained results suggest no meaningful difference between Alloxan and Q10± Alloxan groups (P>0.05).

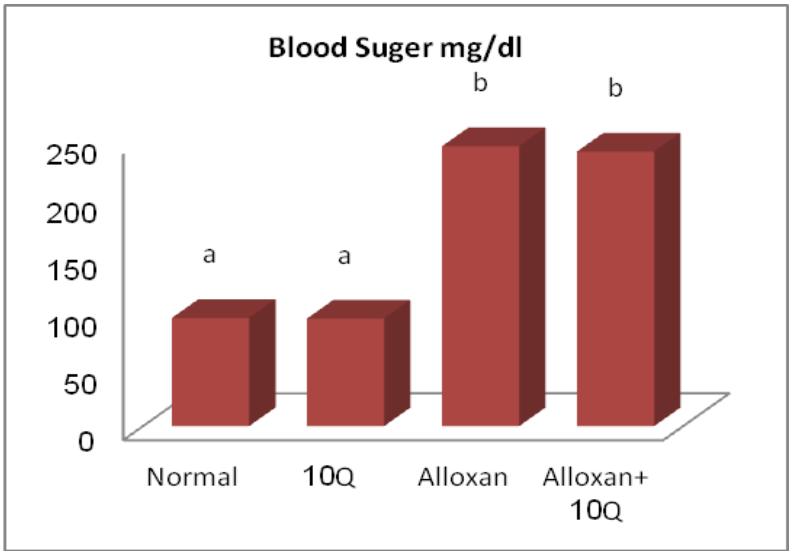

Figure 5: Comparison of Mean±SEM of blood sugar, followed by administration of Q10, Alloxan, and both of which coincidentally in rats in a one-month period. Dissimilar letters show a meaningful difference of mean among groups (P<0.01)

Effect on Renal Capsule Thickness

The mean Renal corpuscle thickness in normal, Q10, Alloxan, and Q10+Alloxan groups were 3.78±0.175, 4.33±0.183, 16.57±0.849, and 8±0.527 µm, respectively. The obtained results suggest a meaningful difference between Alloxan and Q10± Alloxan groups (P<0.05).

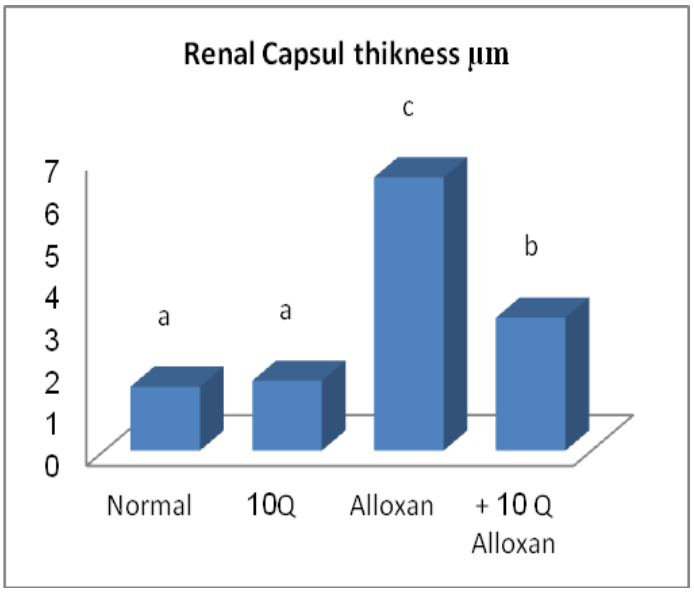

Figure 6: Comparison of Mean ±SEM of renal capsule thickness followed by administration of Q10, Alloxan, and both of which coincidentally in rats in a one-month period. Dissimilar letters show a meaningful difference of mean among groups (P<0.05)

Effect on Renal Corpuscle Diameter

The mean renal corpuscle diameter in normal, Q10, Alloxan, and Q10+Alloxan groups were 108.37±3.77, 10.12±3.47, 59.16±2.1, and 73.95±2.5 µm, respectively. The obtained results suggest a meaningful difference between Alloxan and Q10± Alloxan groups (P<0.05).

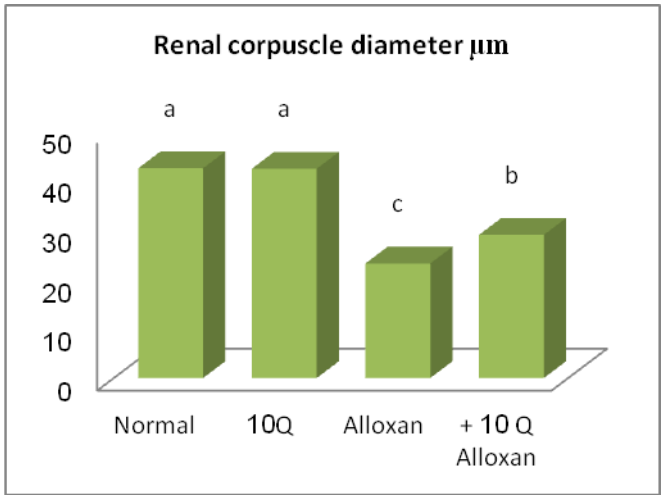

Figure 7: comparison of Mean ±SEM of renal corpuscle diameter followed by administration of Q10, Alloxan, and both of which coincidentally in rats in a one-month period. Dissimilar letters show a meaningful difference of mean among groups (P<0.05)

Discussion

Diabetic nephropathy has been studied by several researchers as a very important damage, and it has been always tried to decrease the damages on renal tissue in diabetics all over the world. Different drugs have been used in this area, but they have failed so far to decrease the side effects of diabetes on renal tissue.

Several methods have been suggested in order to renal toxicity identification such as urea and serum creatinine levels, as well as glomerular infiltration (Lau, 1999). In the present study the renal toxicity was examined by histopathologic and morphologic studies on renal tissue such as tubular nephritis, tubular cells flux, and protein casts aggregation especially in medulla.

In a study conducted by Tabrizi et al., the increased amount of Malondialdehyde in the renal tissue of diabetic rats induced by Alloxan revealed that oxidative stress caused by free radicals is one of the mechanisms involved in diabetic nephropathy (Tabrizi *et al.,* 2011).

Oxidative stresses along with increased production have a main role in renal pathologic damages. In a study, the diabetes-induced rats with Alloxan had different cell damages along with cell membrane damage which were may be due to oxidative stresses resulted by hyperglycemia. Early renal damage in this study is consistent with the results obtained by Liu *et al.,* (2008) and Tabrizi *et al.,* (2011) on diabetic nephropathy. Oxidative stress resulted by super oxide anions are involved in diabetic nephropathy pathophysiology (Vural *et al.,* 2002).

The causes of the tangled tubes following diabetes have not been known yet, but several factors such as semi-insulin growth factor are involved (Flyvbjerg *et al.,* 1990).

The important observed damage in the present study in diabetes-induced rats' kidney by Alloxan was in Mesangial matrix. It has been revealed that the increased Mesangial matrix in diabetic nephropathy has a relationship with the changes in extracellular matrix (Yamanea *et al.,* 2004). When the blood sugar is very high, the mesangial cell proliferation is stimulated and a high rate of collagen 1 and 4, as well as TGF-β are produced.

According to the obtained morphological results in the present study it can be said that the Q10 administration in diabetic groups can't be effective as a hypoglycemic, but on the other hand the morphologic results of renal capsule thickness suggest that the fibroblastic cells have diabetic reactions

following the effects of free radicals; so, by the administration of Q10 the pathogenic reaction can be prevented.
Also according to the obtained results about renal corpuscle diameter it was revealed that Q10 can prevent meaningfully from the renal corpuscle diameter decrease resulted by free radicals following the diabetes disease.
The effects of Q10 on a damaged kidney suggest its high effects of anti oxidant factors which can resist against free radicals created by diabetes disease.
The damages which were observed in renal tissue as a result of diabetes disease in the present study are as follows: tubular acute necrosis, interstitial tubular nephrosis, vacuolar nephrosis, fatty changes, and vascular arteriosclerosis; and the administration of Q10 had a mild effect on these damages. Necrosis occurrence in Q10-administrated groups was observed in a lower range. Furthermore, interstitial spaces congestion was decreased by Q10 administration which suggested the affection of Q10 antioxidants against free radicals. In the present study, it was observed that Q10 administration had not any successful effect on some pathologic cases such as glomerular congestion, capillary thrombosis, and interstitial spaces edema.
Having several anti oxidant factors, Q10 can fight against free radicals resulted of diabetes which have irreversible pathogenic effects on the renal tissue.
It is well known that diabetic nephropathy is caused by several factors which are not preventable by hyperglycemia and hypertension control. Although in the early stages of the disease the diabetic nephropathy changes are induced by hyperglycemia, the later injuries have no relationship to hyperglycemia (Liu *et al.*, 2008). Then, blood glucose control is not enough by itself to postpone the process of diabetic nephropathy.

Conclusion

Totally, the positive effect of Q10 in a partial recovery from diabetic nephropathy is consistent with the results of our study, such that there is a meaningful difference between control and treatment groups in terms of resulted damages, like: glomerulonephritis, tubular necrosis, and nephrosis. So, it can be concluded that the administration of Q10 can be considered as a preventive procedure of diabetes side effects on kidney.

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