Kocatepe Veterinary Journal

Kocatepe Vet J (2020) 13(2):192-202
DOI: 10.30607/kvj.702306

RESEARCH ARTICLE

Effects of Coenzyme Q_{10} on Some Blood Antioxidant System Parameters and Histological Changes in the Pancreas and Aorta of Streptozotocin-induced Diabetic Rats

Ercan KESKIN[1], Deniz ULUIŞIK[1*], Yasemin ÖZNURLU[2], Tuğba ÖZAYDIN[2]

[1]Selcuk University, Faculty of Veterinary Medicine, Department of Physiology, Konya, Turkey
[2]Selcuk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Konya, Turkey

ABSTRACT

The purpose of the present study was to determine the effects of Coenzyme Q_{10} on antioxidant enzymes in diabetic rats. Group I was not exposed any application. Group II was intraperitoneally administrated 0.3 ml corn oil in daily for four weeks. Group III received 10 mg/kg CoQ_{10} in 0.3 ml corn oil intraperitoneally daily for four weeks. Diabetes was induced by subcutaneous injections of streptozotocin in group IV. Group V was made diabetic in the same way and then these animals were intraperitoneally injected with 10 mg/kg CoQ_{10} in 0.3 ml cornoil daily for four weeks. In blood samples, GSH, TBARS, SOD, NO levels and GPx, CAT activities were determined. Pancreas and aorta tissue samples were examined using histological and immunohistochemical methods. Plasma SOD, GPx, CAT and GSH levels in diabetic group were significantly lower than control group. These parameters significantly increased with CoQ_{10} application to diabetic rats when compared to diabetic group. The increased plasma TBARS level with diabetes reduced with CoQ_{10} treatment. The histological findings of the study support the changes in enzyme levels as a result of CoQ_{10} application. In conclusion, CoQ10 application to diabetic rats may have beneficial effects on some negative changes caused by diabetes.
Keywords: Antioxidants, Coenzyme Q_{10}, diabetes, pancreas, rats

***

Streptozotosin ile Diyabet Oluşturulan Ratlarda Koenzim Q_{10}'un Bazı Kan Antioksidan Sistem Parametreleri ile Pankreas ve Aorttaki Histolojik Değişiklikler Üzerine Etkileri

ÖZ

Bu çalışmanın amacı diyabet oluşturulan ratlarda koenzim Q10'un antioksidan parametreleri üzerine etkilerini değerlendirmektir. Çalışmada 38 yetişkin, erkek Wistar Abino rat (250-300 gr) kullanıldı. Grup I'deki hayvanlara herhangi bir uygulama yapılmadı. Grup II'deki hayvanlara günde 0.3 ml mısır yağı dört hafta boyunca intraperitoneal olarak uygulandı. Grup III'deki hayvanlara günde 0.3 ml mısır yağında çözdürülen 10 mg/kg CoQ10 dört hafta boyunca intraperitoneal olarak uygulandı. Grup IV'deki hayvanlarda, 40 mg/kg streptozotosinin günde tek doz olmak üzere iki subkutan enjeksiyonu ile diyabet oluşturuldu. Grup V'deki hayvanlarda aynı protokolle diyabet oluşturuldu ve daha sonra bu hayvanlara dört hafta boyunca 0.3 ml mısır yağında çözdürülen 10 mg/kg CoQ10 intraperitoneal olarak uygulandı. Kan örneklerinde GSH, TBARS, SOD, NO seviyeleri ile GPx, CAT aktiviteleri belirlendi. Pankreas ve aorta doku örnekleri histolojik ve immünohistokimyasal yöntemler kullanılarak incelendi. Diyabetik grupta plazma SOD, GPx, CAT ve GSH seviyeleri control grubuna gore önemli oranda düşüktü. Diyabetik ratlara CoQ10 uygulaması ile bu parametreler diyabetik gruba göre önemli oranda arttı. Diyabetle birlikte artan plazma TBARS seviyesi, CoQ10 uygulamasıyla azaldı. Çalışmanın histolojik bulguları CoQ10 uygulaması sonucunda enzim düzeylerindeki değişiklikleri desteklemektedir. Sonuç olarak, diyabetik ratlara CoQ10 uygulaması diyabetin neden olduğu bazı olumsuz değişiklikler üzerine faydalı etkilere sahip olabilir.
Anahtar Kelimeler: Antioksidan, Koenzim Q_{10}, diyabet, pankreas, rat

To cite this article: Keskin E. Uluışık D. Öznurlu Y. Özaydın T. Effects of Coenzyme Q_{10} on Some Blood Antioxidant System Parameters and Histological Changes in the Pancreas and Aorta of Streptozotocin-induced Diabetic Rats. Kocatepe Vet J. (2020) 13(2):192-202

Submission: 02.03.2020 **Accepted**: 20.05.2020 **Published Online**: 24.05.2020
ORCID ID; EK: 0000-0003-3839-0414, DU: 0000-0003-1462-0836, YÖ: 0000-0002-6296-3107, TÖ: 0000-0002-4552-5658
*Corresponding author e-mail: denizfedai@selcuk.edu.tr

INTRODUCTION

Diabetes, which is a chronic metabolic disease, continues to be a major health problem in worldwide (Rahimi et al. 2005). Diabetes causes many complications arising from imbalances in the metabolism of carbohydrates, lipids and proteins. The high glucose level causes oxidative stress by leading glucose autoxidation, nonenzymatic glycation of proteins, and increased production of mitochondrial reactive oxygen species (ROS) (Brownlee 2001). The elevation in the amount of free fatty acids in diabetes increases β-oxidation and it results in high ROS production and hence the oxidative stress. There are a number of findings suggesting that the mechanisms of antioxidant defense against oxidative stress are low in diabetes. Oxidative stress resulting from the increased production and the inability of adequately removing of ROS plays a crucial role in the pathogenesis of diabetic complications (Brownlee 2000, Brownlee 2001). Free radicals similar to protein kinase C (PKC), nuclear factor κB (NFκB), NH_2-terminal Jun kinase/stress-activated protein kinases (JNK/SAPK) and p38 mitogen-activated protein (MAP) kinase can function as signals patways causing cellular stress (Giuglian et al. 1995, Mohamed et al. 1999, Rosen et al. 2001, Stehouwer and Schaper 1996, West 2000, Yaqoob et al. 1993). Activation of these pathways is also associated with insulin resistance and β-cell dysfunction, including complications observed in the late stages of the disease (Brownlee 2000, Brownlee 2001, Modi et al. 2006, Rosen et al. 2001).

It was reported that the decreased in plasma/serum total antioxidant capacity or free radical scavenging activity increase the tendency to oxidative stress in type 2 diabetes. However, it was also reported that the levels of specific antioxidants such as ascorbic acid and vitamin E have decreased (Aguirre et al. 1998, Ashour et al. 1999, Ceriello et al. 1997a, b, Ceriello et al. 1998, Haffner et al. 1995, Maxwell et al. 1997, Paolisso et al. 1994). The decreases in antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase and catalase) were stated in diabetes (Ashour et al. 1999, Mohan and Das 1997, Tüzün et al. 1999). Also, there was also observed a decrease in endothelial NO synthesis together with decrease in vascular antioxidant defense in type 2 diabetes (Laight et al. 2000, Makimattila et al. 1999).

A study conducted in patients with type 1 diabetes showed that plasma total antioxidant capacity was 16% lower than healthy humans (Vessby et al. 2002). The reduction in antioxidant enzyme activities (SOD ve CAT) in kidney tissue was determined in diabetic rats (Kedziora-Kornatowska et al. 2000). On the other hand, it was suggested that people with high levels of other serum antioxidant, especially serum tocopherol levels, had lower risk of Type 2 diabetes (Reunanen et al. 1998). In contrast, there are also studies in which there is no difference with regard to antioxidant capacity between healthy and diabetic individuals (Feillet et al. 1998, Rahimi et al. 2005, Willems et al. 1998).

There are many studies using exogenous and endogenous antioxidants to reduce or prevent oxidative stress (Kucharská et al. 2000, Meghana et al. 2007, Modi et al. 2006). Endogenous antioxidants play a crucial role in protecting the balance between oxidants and antioxidants (Kucharská et al. 2000). Although Coenzyme Q_{10} plays an important role in mitochondrial energy systems, it also has antioxidant properties. It functions as a dehydrogenase cofactor in the transport of electrons and protons like ATP production (Crane and Navas 1997). On the other hand, this enzyme has been regarded as an important antioxidant since it was determined the decline of biosynthesis and tissues level due to degenerative changes with age (Beyer et al. 1985, Kalén et al. 1989, Kucharská et al. 2000). CoQ_{10} described as a powerful systemic radical scavenger is reported to have the ability to function synergistically with other antioxidants as well as prevent oxidative damage of lipids, DNA, proteins and other important molecules (Lass et al. 1999, Prosek et al. 2008). While tissue lipid peroxidation and SOD levels were found to be significantly higher in diabetic animals, CoQ_{10} supplementation to these animals significantly decreased lipid peroxidation and increased SOD enzyme level. Reduced levels of tissue catalase and glutathione in diabetic animals are significantly increased with CoQ_{10} application (Modi et al. 2006). SOD and GSH levels were determined significantly lower in diabetic rats and its levels significantly increased with CoQ_{10} application paralelly with dose. It was stated that the significant increase of MDA levels in diabetic rats is reduced by the addition of CoQ_{10} (Visnagri et al. 2012).

According to mentioned information above, this study aimed to evaluate the histological changes in the pancreas and aorta with some antioxidant enzymes of CoQ_{10} application in diabetic rats.

MATERIAL and METHOD

In this study, 38 adult, male, healthy Wistar Abino rats (250-300 gr) were used. The animals were divided into five groups and fed ad libitum with standard rat pellet for four weeks. While animals in group I (n=6) was not exposed any application, 0.3 ml corn oil was intraperitoneally administrated at animals in group II (n=6) daily for four weeks. Animals in group III (n=6) received 10 mg/kg CoQ_{10} (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally daily for four weeks. At the beginning of the study, diabetes was induced by subcutaneous injections of streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) at dose of 40 mg/kg in 0.1 M citrate buffer (pH 4.5) for two days as a single daily dose in group IV (n=7) animals. Animals in group V (n=9) was made diabetic by subcutaneous injections of streptozotocin in the same

way and then was injected intraperitoneally with 10 mg/kg CoQ_{10} daily for four weeks. To prevent the streptozotocin-induced hypoglycemia, rats received 5% dextrose solution after 6 h of streptozotocin administration for next 3 days. After one week from streptozotocin injections, diabetes was verified by measuring blood glucose level strips using glucometer (PlusMED Accuro, Taiwan) via the tail vein. Animals having a blood glucose level higher than 250 mg/dl were accepted as diabetic and were included in the experiment. During the experiment, three animals from group IV and one animal from group V were died due to streptozotocin-induced hypoglycemia. Blood samples were taken from all animals at the end of the study. In plasma samples, SOD (Cayman), GSH (Cayman), TBARS (Oxis), NO (Cayman) levels and GPx (Cayman), CAT (Cayman) activities were determined with ELISA (Biotek ELx800, Biotek Instrumentations, Inc, Winooski, VT, USA) using sandwich enzyme-linked immunosorbent method via commercial kits.

All animals were sacrificed via cervical dislocation. Tissue samples were taken from pancreas and aorta of animals. These samples fixed in 10% neutral-buffered formalin and embedded in paraffin blocks. The sections (6 μm thickness) were taken from blocks and were stained with Crossman's triple stain for histological examination (Crossman 1937). Insulin and eNOS was stained immunohistochemically using a sensitive peroxidase-labelled streptavidin-biotin detection system (Ultra Tek HRP Anti-Polyvalent Lab Pack, ScyTek Laboratories, Inc., Logan, UT). Insulin antibody (ISL-8J) (Genetex GTX11163) and eNOS antibody (Genetex GTX50892) was used with 1:100 dilution. Negative control slides were stained by incubating tissue sections with PBS instead of primer antibody. All specimens were examined under light microscope (Leica DM2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed by digital camera (Leica DFC 320). In pancreas sections, four different Langerhans islets' areas which were randomly chosen were measured and the percentages of insulin immunreactive cells (the number of immunreactive cells/the number of total islet cells X 100) were determined using a IM-50 image analysis program (AG CH-9435, Leica Microsystems, Heerbrugg Switzerland). eNOS immunreactivity in aorta sections was assessed semi-quantitatively. In addition, aortic wall thickness was measured from five different regions of each aorta using a IM-50 image analysis program.

The data obtained from the study were analyzed by one-way ANOVA (SPSS 17). Differences among the groups were determined by Duncan's multiple range test. Differences were considered significant at $p<0.05$.

RESULTS

In this study, the effect of CoQ_{10} on plasma oxidant status markers in experimentally induced diabetic rats were summarized Tablo 1. Plasma GSH and CAT levels in diabetic group were found to be significantly lower than control group (Table 1, $p<0.05$). Plasma GSH and CAT levels with CoQ_{10} application to diabetic rats statistically increased to diabetic group (Table 1, $p<0.05$). Experimentally induced diabetes resulted in significantly increments in plasma TBARS level, while this parameter statistically reduced with CoQ_{10} treatment to diabetic rats when compared to diabetic group (Table 1, $p<0.05$). Plasma SOD and GPx levels were importantly diminished depend on diabetes (Table 1, $p<0.05$) and the changes in both parameters of CoQ_{10} application to diabetic rats compared to diabetic group were not important (Table 1). In diabetic group, plasma NO level statistically decreased compared to control group (Table 1, $p<0.05$). This parameter slightly increased with CoQ_{10} application to diabetic rats but the increment was not important when compared to diabetic group. The CoQ_{10} application to the rats did not affect the plasma GSH, TBARS, SOD, GPx, CAT and NO levels compared to the control group.

It was observed that the normal histological structure was preserved in pancreatic tissue of control, corn oil and CoQ_{10} groups. The contours of Langerhans islets in these groups were clearly seen (Figures 1A, 1B) and the insulin immunreactive cells were intensively stained at the center of the islets (Figures 2A, 2B). In the diabetic group, irregularity in the contours of Langerhans islets, vacuolization and atrophy in the islet cells (Figure 1C) and significant decrease in the percentage of the insulin immunreactive cells (Figure 2C, Table 2) were observed ($p<0.05$). It was seen that the CoQ_{10} application to diabetic rats led to partially improve in pancreatic tissues (Figure 1D). Also, a statistically significant increase in the percentage of the insulin immunreactive cells was noted in this group when compared to the diabetes group ($p<0.05$) (Figure 2D, Table 2). No immunohistochemical staining was observed in the negative control preparation (Figure 3). There was no statistically significant difference between the groups in terms of the area of Langerhans islets in the pancreas (Table 2).

There was no statistically significant difference in terms of aortic wall thickness among the groups ($p>0.05$, Figures 4A, 4B, Table 2). In all groups, eNOS immunreactivity was seen in the endothelium. There was no difference in eNOS immunostaining intensity among the groups (Figures 5A, 5B). No immunohistochemical staining was observed in the negative control preparation (Figure 5C).

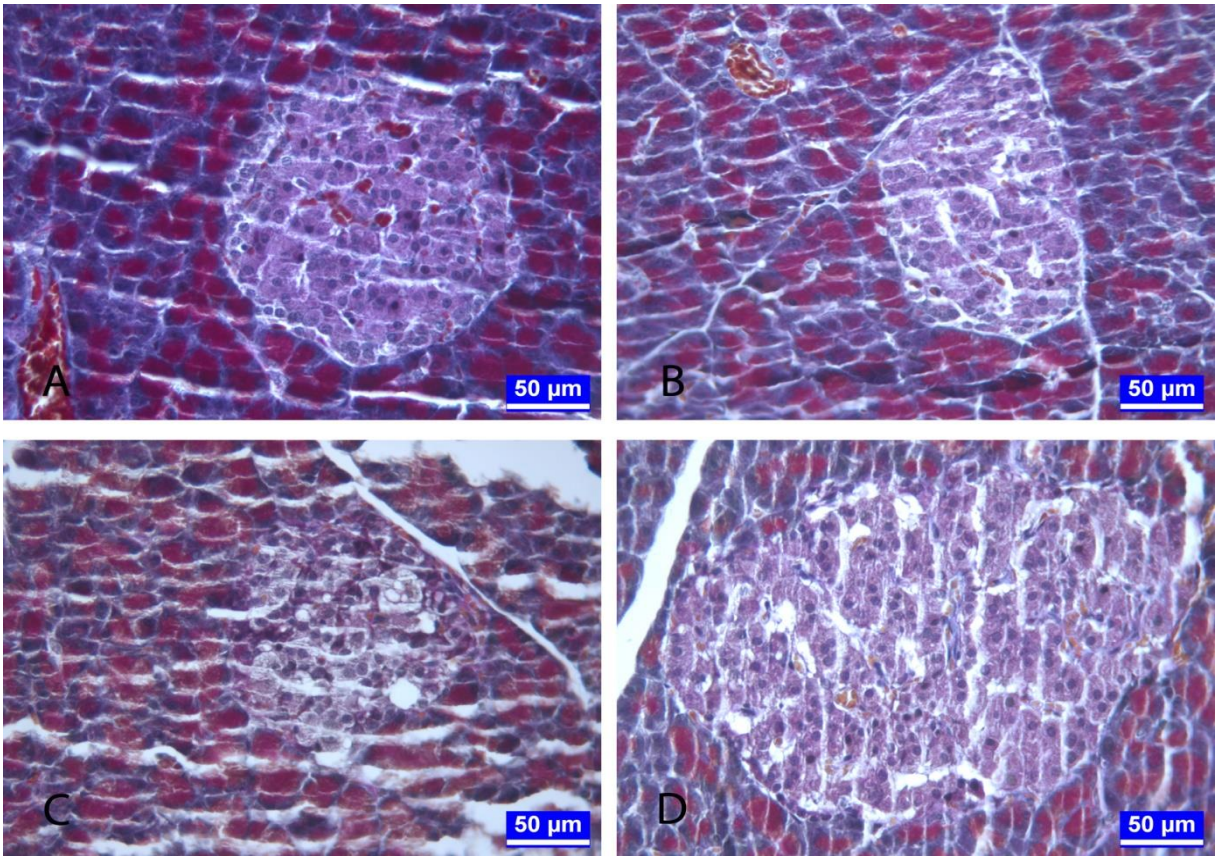

Figure 1. A: Pancreatic tissue of control group rat. **B:** Pancreatic tissue of CoQ_{10} group rat. **C.** Pancreatic tissue of diabetes group rat. **D.** Pancreatic tissue of CoQ_{10} and diabetes group rat. Crosmans's Triple stain.

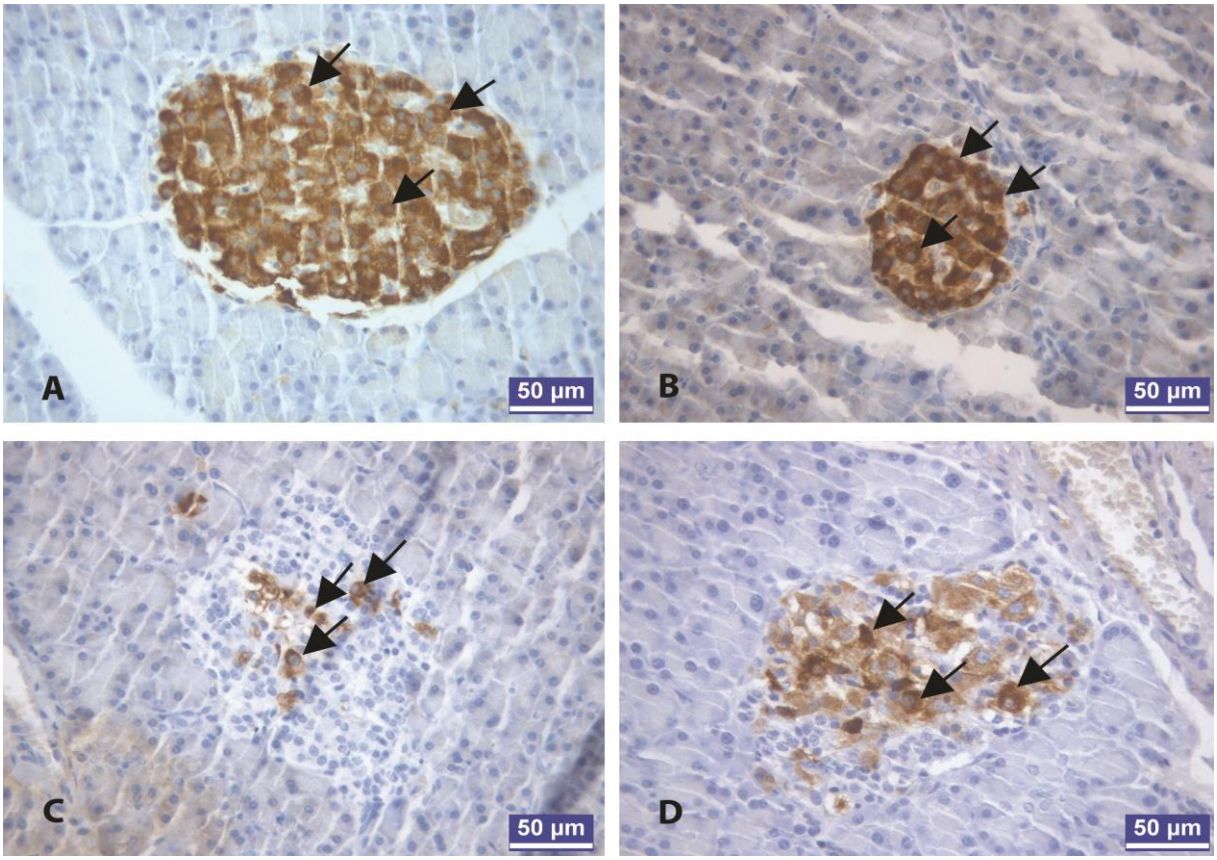

Figure 2. A: Pancreatic tissue of control group rat. **B:** Pancreatic tissue of CoQ_{10} group rat. **C.** Pancreatic tissue of diabetes group rat. **D.** Pancreatic tissue of CoQ_{10} and diabetes group rat. Insulin immunohistochemical staining. **Arrows:** Insulin immunreactive cells.

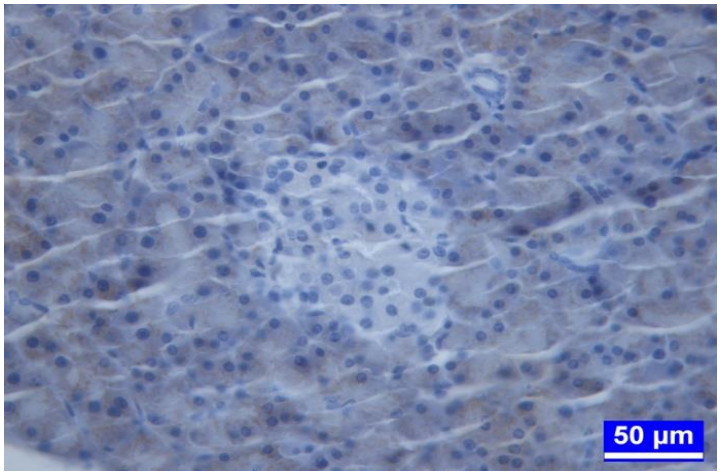

Figure 3. Negative control insulin immunohistochemical staining.

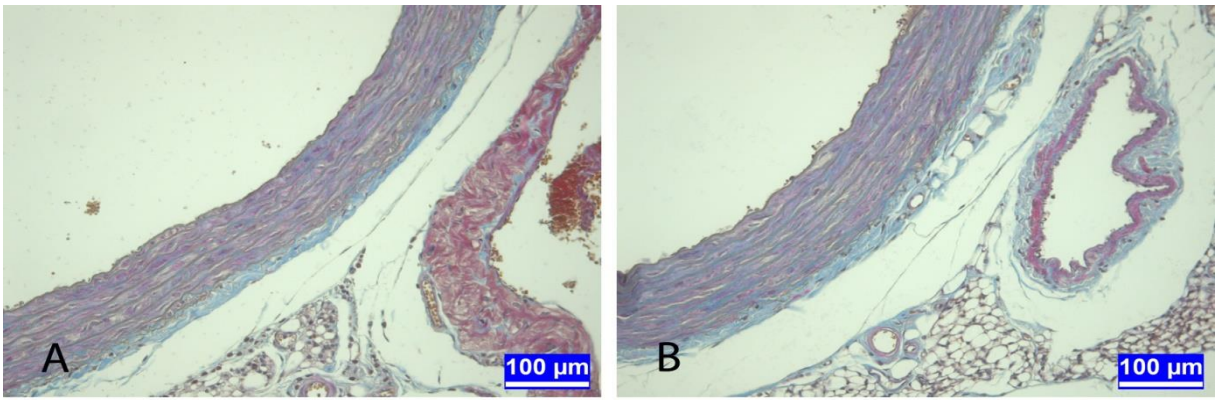

Figure 4. A: Aorta section of control group rat. **B:** Aorta section of diabetes group rat. Crosmans's Triple stain.

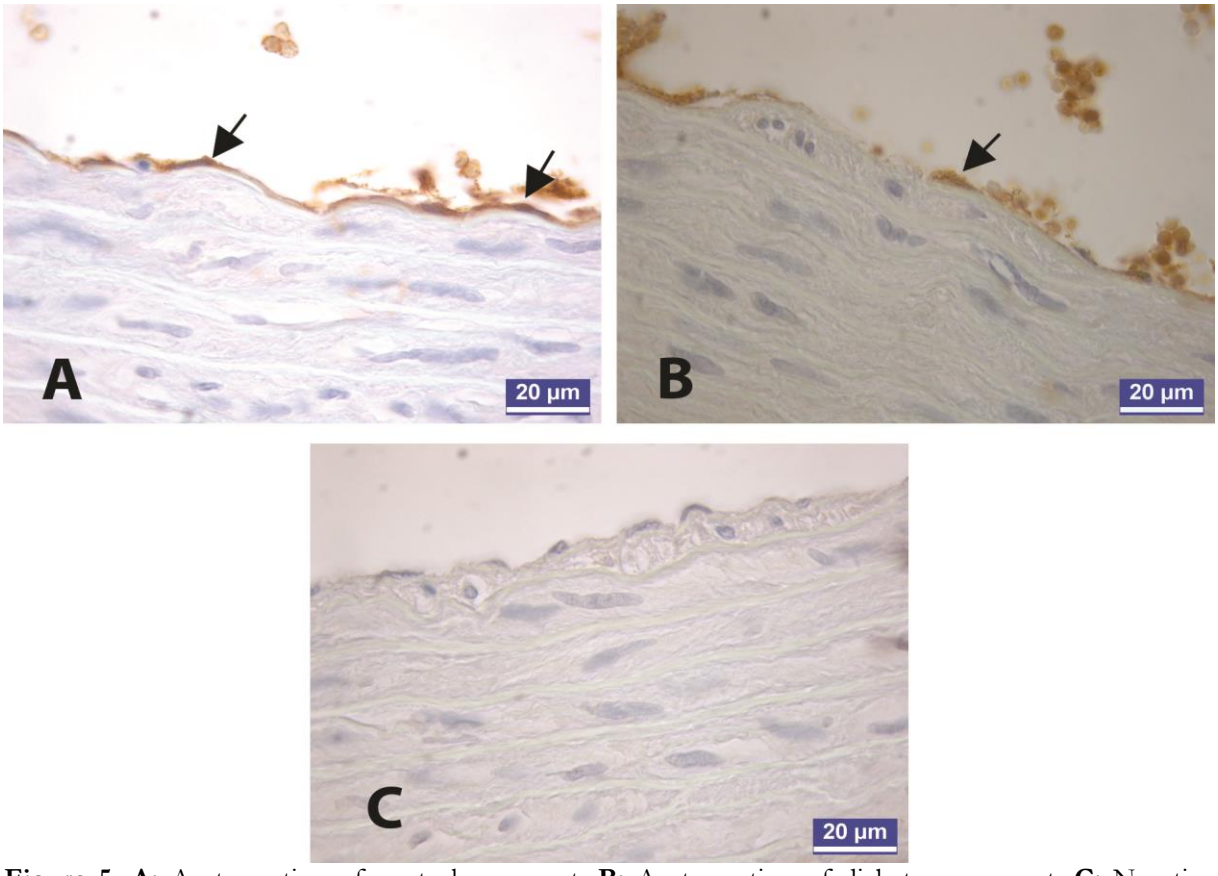

Figure 5. A: Aorta section of control group rat. **B:** Aorta section of diabetes group rat. **C:** Negative control. eNOS immunohistochemical staining. **Arrows:** eNOS immunreactive cells.

Table 1. Effect of CoQ_{10} on plasma GSH, TBARS, SOD, GPx, CAT and NO levels in streptozotocin-induced diabetic rats (Mean±SE).

	GSH (µM)	TBARS (µM)	SOD (U/ml)	GPx (nmol/min/ml)	CAT (nmol/min/ml)	NO (µM)
Grup 1	24,26±1,13[a]	7,46±0,58[c]	26,27±1,68[a]	44,74±2,58[a]	51,11±2,64[a]	16,81±1,76[a]
Grup 2	22,47±2,73[a]	8,72±0,84[bc]	23,93±2,09[a]	42,67±3,78[a]	54,73±2,56[a]	17,78±1,38[a]
Grup 3	23,02±1,85[a]	8,33±0,81[bc]	25,62±2,80[a]	45,43±3,88[a]	50,82±2,68[a]	16,35±2,36[a]
Grup 4	11,63±1,19[b]	15,74±1,21[a]	13,66±1,05[b]	29,57±1,57[b]	33,69±2,50[c]	11,17±1,47[b]
Grup 5	19,76±0,85[a]	10,52±0,79[b]	17,89±1,29[b]	32,39±1,88[b]	41,54±1,61[b]	13,68±1,07[ab]

[a-c]The difference between mean values with different superscripts in the same column is significant at the $p<0.05$ level. Group 1, Control, group 2, Corn oil, group 3, CoQ_{10}, group 4, Diabetesis, group 5, CoQ_{10} and Diabetesis.

Table 2. Langerhans islets' areas, percentages of insulin immunreactive cells in pancreatic tissue and aortic wall thickness (Mean±SE).

	Langerhans islets' areas (µm²)	Percentages of insulin immunreactive cells (%)	Aortic wall thickness (µm)
Grup 1	15816.53±2487.89	66.62±2.19[a]	157.28±5.52
Grup 2	13411.13±2551.95	67.82±1.73[a]	156.97±8.39
Grup 3	10299,69±1993.64	69.21±3.52[a]	152.16±3.47
Grup 4	8134.84±1884.00	17.66±3.22[c]	153.61±6.08
Grup 5	11733.91±2022.48	26.14±2.67[b]	154.57±2.49

[a-c] Differences between mean values with different superscripts in the same column is significant ($p<0.05$) for each parameter. Group 1, Control, group 2, Corn oil, group 3, CoQ_{10}, group 4, Diabetesis, group 5, CoQ_{10} and Diabetesis.

DISCUSSION

It is suggested that the increases in tissue oxidants and oxidative stress play a critical role in the etiology of many diseases (Dlugosz et al. 2004). It is reported that oxidative stress caused by the increase of free radicals is also effective in complications of diabetes such as atherosclerosis, retinopathy, nephropathy and neuropathy (Hussein et al. 2012, Hussein et al. 2013). Free radicals are produced continuously in the body during environmental stimuli and normal metabolic processes (Halliwell and Gutteridge 1989). Under normal physiological conditions, there is a broad antioxidant defense system against the adverse effects of free radical production in vivo. However, oxidative stress occurs the result of the increases in free radical production and decreases in antioxidant defense capacity or both (Baynes 1991, Mullarkey et al. 1990). In addition to the free radical formation in diabetes that accelerates lipid peroxidation, there is also a question of reductions in SOD, catalase and reduced glutathione levels in many tissues (Shih et al. 1999). The increase in free radicals due to protein glycation and glucose autoxidation contributes to lipid peroxidation in diabetes (Baynes 1991, Feillet-Coudray et al. 1999, Mullarkey et al. 1990).

In the study, reduced glutathione, which is a major defense factor of cells against oxidants, decreased significantly with diabetes (Table 1, $p<0.05$), while CoQ_{10} administration to diabetic rats significantly increased this parameter compared to the diabetes group (Table 1, $p<0.05$). Glutathione peroxidase activity, which inhibits lipid peroxidation as one of the organism's antioxidant components, significantly decreased in diabetic animals (Table 1, $p<0.05$). Although glutathione peroxidase activity showed a certain increase depend on CoQ_{10} application to diabetic animals, the difference was not significant. The increase in the reduced glutathione level in diabetic animals with CoQ_{10} administration supports the findings reported by various researchers in plasma (Ahmadvand et al. 2012) and various tissues (Coldiron et al. 2002, Modi et al. 2006, Sena et al. 2008).

SOD and CAT enzymes, which are important in term of the determination of serum antioxidant capacity, decreased significantly with experimental diabetes (Table 1, $p<0.05$). The obtained findings from diabetic animals are consistent with the data identified in streptozotocin-induced diabetic rats by Kedziora-Kornatowska et al. (2000) and with reductions achieved at these enzyme levels in diabetic patients by Vessby et al. (2002). CoQ_{10} administration to diabetic rats significantly increased CAT level compared to diabetic animals (Table 1, $p<0.05$). The differences in SOD level with CoQ_{10} administration to diabetic rats were not significant. The changing determined at the CAT level by CoQ_{10} application coincides with the results of Song et al. (2009) and Lee et al. (2012).

NO formation and oxidative stress play an important role in the development of diabetic complications. Particularly, it was reported that nitric oxide reduction is effective in disorders in the vascular system in diabetes, while it has been suggested that other vasodilatators and hyperlipidemia also contribute to vascular disorders (Cohen 2005). It has been reported that insulin stimulates NO production by specific signaling pathways (phophotidyl-inositol 3-(PI3)-kinase and protein kinase B), high glucose levels reduce NO in diabetes and NO release and production also deteriorate due to loss of positive effect of insülin (Balletshofer et al. 2000, Tuck 2003, Zeng et al. 2000). In this study, significant reductions (Table 1, $p<0.05$) in NO levels with experimental diabetes compared to control group seems consistent with the above notifications. It was observed that the increase in NO level with CoQ_{10} application to diabetic animals removed the difference with the control group level. It was observed significantly increase in thiobarbituric acid reactive substances (TBARS) as lipid peroxidation marker of lipoproteins and membranes in diabetic group compared to the control group (Table 1, $p<0.05$). This increase seems to be in line with the notifications that TBARS levels increase in diabetes (Griesmacher et al. 1995, Kakkar et al. 1998, Sundaram et al. 1996). On the other hand, there was a significant decline in TBARS level with CoQ_{10} application to diabetic animals compared to the diabetes group (Table 1, $p<0.05$). Unimportant changes determined in these parameters with only CoQ_{10} application compared to control group may be due to the absence of oxidative damage in healthy animals.

It has been proposed various mechanisms in related to the positive effects of CoQ_{10} on the antioxidant system in diabetes (Prakash et al. 2010, Song et al. 2009). It has been reported that CoQ_{10} can directly eliminate free radicals such as lipid peroxyl, peroxyl or alkoxyl radicals (Roginsky et al. 2009, Sohal and Forster 2007). Forsmark-Andree and Ernster (1994) have been reported that CoQ_{10} may indirectly act as an antioxidant via providing α-tocopherol regeneration from α-tocoperoxyl radicals formed by a reaction between α-tocopherol and lipid peroxyl radicals. Tiano et al. (2007) have been suggested that nitric oxide is an active antioxidant against free radical-mediated lipid peroxidation and that CoQ_{10} can reduce free radical and superoxide formation via improving nitric oxide bioactivity. Abdin and Hamouda (2008) have been also stated that CoQ_{10} showed in vivo antiapoptotic effects by increasing the expression and activation of mitochondrial proteins and consequently, it may function as an antioxidant by reduce the formation of free radicals.
Streptozotocin is a substance that causes decrease of insulin-producing capacity by creating damaged via oxidative stress in insulin-producing β-cells. Studies have shown that the most common organs affected oxidative stress related organ damage following diabetes is liver, kidney, pancreas, and eye. Severe metabolic changes and oxidative disturbances in the pancreas also play an important role in the pathogenesis of diabetes (Baynes and Thorpe 1999, Ihara et al. 1999). In experimental diabetes, it has been stated that there is an increase in oxidative stress markers in pancreatic islet cells and it has been determined damage and dysfunctions in β cells of rats (Ihara et al. 1999, Meghana et al. 2007). In this study, irregularity in the contours of Langerhans islets, vacuolization and atrophy in the islet cells were observed in streptozotocin induced diabetic groups. These histological findings were found to be consistent with datas of the previous conducted studies (Kamalakkannan and Prince 2005, Hassan et al. 2015, Yaman et al. 2017). In recent studies, it has been reported that antioxidants and plant extracts improved functional state of pancreatic β cells and partially reversed the damage caused by streptozotocin to β-cells of the pancreatic islets (Kanter et al. 2004, Kamalakkannan and Prince 2005, Omar and Atia 2012, Abunasef et al. 2014, Niture et al. 2014, Hassan et al. 2015, Yaman et al. 2017). Luo et al. (2019) suggested that CoQ_{10} has beneficial effects for reducing mitochondrial injury via its antioxidative properties and is effective in ameliorating pancreatic β cell dysfunction by tacrolimus. Similarly our study, it was seen that the CoQ_{10} application to diabetic rats led to partially improve in pancreatic tissues. Also, a statistically significant increase in the percentage of the insulin immun reactive cells was noted in this group when compared to the diabetes group.

Nitric oxide occurs during conversion of L-arginine to L-citrulline. This enzymatic pathway is managed by nitric oxide synthase (NOS). Nitric oxide synthase has at least 3 isoenzymes: Inducible NOS (iNOS) is found in macrophages and it is involved in pathological events. Structural neuronal NOS (nNOS) are found in the brain. Structural endothelial NOS (eNOS) are synthesized from endothelial cells, endocardial cells, ventricular myocytes and other myocardial cells and its activation is dependent on Ca and calmodulin (Felaco et al. 2001). It is stated that endothelial damage in diabetes may be a consequence of glucometabolic and peroxidative stress. It was reported that even if high amounts of NO produced by iNOS have been seen to be toxic and damaging, structurally optimum of NO produced by eNOS is required for the protection of endothelial function. Therefore, eNOS is important determining of the microvascular complications of diabetes and the susceptibility to cardiovascular disease (İnan 2015, Kröncke et al. 1995, Özgün et al. 2014). In this study conducted by Felaco et al. (2001) related with eNOS localization and expression in myocardial tissue in healthy and diabetic rats, it was reported that there was no difference between the diabetic group and the

control group in terms of eNOS immunreactivity in myocytes and there was less immunreactivity in the diabetic group compared to the control group in the vascular endothelium. In this study, there was no difference among the groups in terms of eNOS immunreactivity in aortic endothelium. Long-term complications of diabetes are retinopathy, nephropathy and cardiovascular disease. In fact, the duration of this study is thought to be insufficient for the development of cardiovascular complications.

As a result, if it regards to the significant changes particularly in the levels of GSH, CAT, TBARS and positively changes in NO level with CoQ_{10} administration to experimental diabetic rats, it was concluded that CoQ_{10} may alleviate the negative effects of diabetes on these parameters and that it may be useful due to protective properties in diabetes. The histological findings of the study support partially the changes in some plasma enzyme levels as a result of CoQ_{10} application. It has been thought that the determined data may contribute to the studies be conducted the same subject in the future and further studies are required in different dose and duration.

ACKNOWLEDGEMENTS

This study was supported by The Scientific Research Projects Coordination Unit of Selcuk University (Project no. 15401074).

This study was presented in 1st International & Xth National Veterinary Anatomy Congress of Turkey.

The study protocol was approved by The Ethical Committee of Selcuk University Experimental Medicine Research and Application Center (Report no. 2015-22).

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Abdin AA, Hamouda HE. Mechanism of the neuroprotective role of coenzyme Q_{10} with or without L-dopa in rotenone-induced parkinsonism. Neuropharmacology. 2008; 55(8):1340-1346. doi: 10.1016/j.neuropharm.2008.08.033.

Abunasef SK, Amin HA, Abdel-Hamid GA. A histological and immunohistochemical study of beta cells in streptozotocin diabetic rats treated with caffeine. Folia Histochem Cytobiol. 2014;52(1):42-50. doi: 10.5603/FHC.2014.0005.

Aguirre F, Martin I, Grinspon D, Ruiz M, Hager A, De Paoli T et al. Oxidative damage, plasma antioxidant capacity and glucemic control in elderly NIDDM patients. Free Radic Biol Med. 1998;24(4):580-585. doi: 10.1016/S0891-5849(97)00293-1.

Ahmadvand H, Tavafi M, Khosrowbeygi A. Amelioration of altered antioxidant enzymes activity and glomerulosclerosis by coenzyme Q_{10} in alloxan-induced diabetic rats. J Diabetes Complications. 2012;26(6):476-482. doi: 10.1016/j.jdiacomp.2012.06.004.

Ashour M, Salem S, Hassaneen H, El-Gadban H, Elwan N, Awad A et al. Antioxidant status and insulin-dependent diabetes mellitus (IDDM). J Clin Biochem Nutr. 1999; 26(2):99-107. doi: https://doi.org/10.3164/jcbn.26.99.

Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, Matthaei S, Rett K, Häring HU. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. Circulation. 2000;101(15):1780-1784. doi: https://www.ahajournals.org/doi/full/10.1161/01.CIR.101.15.1780.

Baynes JW. Role of oxidative stress in the development of complications in diabetes. Diabetes. 1991;40(4):405-412. doi: https://doi.org/10.2337/diab.40.4.405.

Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes. 1999;48(1):1-9. doi: https://doi.org/10.2337/diabetes.48.1.1.

Beyer RE, Burnett BA, Cartwright KJ, Edington DW, Falzon MJ, Kreitman KR, Kuhn TW, Ramp BJ, Rhee SYS, Rosenwasser MJ, Stein M, An LC. Tissue coenzyme Q (ubiquinone) and protein concentrations over the life span of the laboratory rat. Mech Ageing Dev. 1985;32(2-3):267-281. doi: https://doi.org/10.1016/0047-6374(85)90085-5.

Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813-820. doi: 10.1038/414813a.

Brownlee M. Negative consequence of glycation. Metabolism. 2000;49(2 Suppl 1):9-13. doi: https://doi.org/10.1016/S0026-0495(00)80078-5.

Ceriello A, Bortolotti N, Pirisi M, Crescentini A, Tonutti L, Motz E, Russo A, Giacomello R, Stel G, Taboga C. Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. Diabetes Care. 1997b;20(10):1589-1593. doi: https://doi.org/10.2337/diacare.20.10.1589.

Ceriello A, Bortolotti N, Crescentini A, Motz E, Lizzio S, Russo A, Ezsol Z, Tonutti L, Taboga C. Antioxidant defences are reduced during the oral glucose tolerance test in normal and non-insulin-dependent diabetic subjects. Eur J Clin Invest. 1998;28(4):329-333. doi: https://doi.org/10.1046/j.1365-2362.1998.00295.x.

Ceriello A, Bortolotti N, Falleti E, Taboga C, Tonutti L, Crescentini A, Motz E, Lizzio S, Russo A, Bartoli E. Total radical-trapping antioxidant parameter in NIDDM patients. Diabetes Care. 1997a;20(2):194-197. doi: https://doi.org/10.2337/diacare.20.2.194.

Cohen RA. Role of nitric oxide in diabetic complications. Am J Ther. 2005;12(6):499-502. doi: 10.1097/01.mjt.0000178776.77267.19.

Coldiron AD Jr, Sanders RA, Watkins JB 3rd. Effects of combined quercetin and coenzyme Q(10) treatment on oxidative stress in normal and diabetic rats. J Biochem Mol Toxicol. 2002;16(4):197-202. doi: 10.1002/jbt.10035.

Crane FL, Navas P. The diversity of coenzyme Q function. Mol Aspects Med. 1997;18:1-6. doi: https://doi.org/10.1016/S0098-2997(97)00016-2.

Crossmon G. A modification of Mallory's connective tissue stain with a discussion of the principles involved. Anat Rec. 1937;69:33-38. doi: https://doi.org/10.1002/ar.1090690105.

Dlugosz A, Kuzniar J, Sawicka E, Marchewka Z, Lembas-

Bogaczyk J, Sajewicz W, Boratynska M. Oxidative stress and coenzyme Q_{10} supplementation in renal transplant recipients. Int Urol Nephrol. 2004;36(2):253-258. doi: https://doi.org/10.1023/B:UROL.0000034652.88578.a8.

Felaco M, Grilli A, De Lutiis MA, Patruno A, Libertini N, Taccardi AA, Di Napoli P, Di Giulio C, Barbacane R, Conti P. Endothelial nitric oxide synthase (eNOS) expression and localization in healthy and diabetic rat hearts. Ann Clin Lab Sci. 2001;31(2):179-186. doi: http://www.annclinlabsci.org/content/31/2/179.long.

Feillet C, Roche B, Tauveron I, Bayle D, Rock E, Borel P, Rayssiguier Y, Thieblot P, Mazur A. Susceptibility to oxidation and physicochemical properties of LDL in insulin-dependent diabetics. Atherosclerosis. 1998;136:405-407. doi: https://doi.org/10.1016/S0021-9150(97)00246-3.

Feillet-Coudray C, Rock E, Coudray C, Grzelkowska K, Azais-Braesco V, Dardevet D, Mazur A. Lipid peroxidation and antioxidant status in experimental diabetes. Clin Chim Acta. 1999;284(1):31-43. doi: https://doi.org/10.1016/S0009-8981(99)00046-7.

Forsmark-Andree P, Ernster L. Evidence for a protective effect of endogenous ubiquinol against oxidative damage to mitochondrial protein and DNA during lipid peroxidation. Mol Aspects Med. 1994;15(1):73-81. doi: https://doi.org/10.1016/0098-2997(94)90015-9.

Giuglian D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension and cardiovascular disease. Which role for oxidative stress?. Metabolism. 1995;44(3):363-368. doi: https://doi.org/10.1016/0026-0495(95)90167-1.

Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, Pietschmann P, Prager R, Schnack C, Schernthaner G, Mueller MM. Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. Am J Med. 1995;98(5):469-475. doi: https://doi.org/10.1016/S0002-9343(99)80347-7.

Haffner SM, Agil A, Mykkanen L, Stern MP, Jialal I. Plasma oxidizability in subjects with normal glucose tolerance, impaired glucose tolerance and NIDDM. Diabetes Care. 1995;18(5):646-653. doi: https://doi.org/10.2337/diacare.18.5.646.

Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 2nd ed. Oxford: Clarendon Press, 1989.

Hassan SK, El-Sammad NM, Mousa AM, Mohammed MH, Farrag ARH, Hashim ANE, Werner V, Lindequist U, Nawwar MAE. Hypoglycemic and antioxidant activities of Caesalpinia ferrea Martius leaf extract in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed. 2015;5(6):462-471. https://doi.org/10.1016/j.apjtb.2015.03.004.

Hernández-Camacho JD, Bernier M, López-Lluch G, Navas P. Coenzyme Q_{10} Supplementation in Aging and Disease. Front Physiol. 2018;9:44. doi: 10.3389/fphys.2018.00044.

Hussein J, Abo El-Matty D, El-Khayat Z, Abdel-Latif Y. Brain neurotransmitters in diabetic rats treated with coenzyme Q_{10}. Int J Pharm Pharm Sci. 2012;4(4):554-556.

Hussein J, Abo El-Matty D, El-Khayat Z, Abdel-Latif Y. Therapeutic role of coenzyme Q_{10} in brain injury during experimental diabetes. J App Pharm Sci. 2013;3(06):213-217. doi: 10.7324/JAPS.2013.3636.

Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y. Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. Diabetes. 1999;48(4):927-932. doi: https://doi.org/10.2337/diabetes.48.4.927.

İnan S. Diyabetik retinopative nitric oksit. Kocatepe Tıp Derg. 2015;16:57-66. doi: http://hdl.handle.net/123456789/8489.

Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clin Sci (Lond). 1998;94(6):623-632. doi: 10.1042/cs0940623.

Kalén A, Appelkvist EL, Dallner G. Age-related changes in the lipid compositions of rat and human tissues. Lipids. 1989;24(7):579-584. doi: https://doi.org/10.1007/BF02535072.

Kamalakkannan N, Stanely Mainzen Prince P. The Effect of Aegle Marmelos Fruit Extract in Streptozotocin Diabetes: A Histopathological Study. J Herb Pharmacother. 2005;5(3):87-96.

Kanter M, Coskun O, Korkmaz A, Oter S. Effects of Nigella sativa on oxidative stress and β-cell damage in streptozotocin-induced diabetic rats. Anat Rec A Discov Mol Cell Evol Biol. 2004;279(1):685-691. doi: 10.1002/ar.a.20056.

Kedziora-Kornatowska KZ, Luciak M, Paszkowski J. Lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney: effect of treatment with angiotensin convertase inhibitors. IUBMB Life. 2000;49(4):303-307. doi: 10.1080/15216540050033177.

Kröncke KD, Feshel K, Kolb-Bachofen V. Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. Biol Chem Hoppe Seyler. 1995;376(6):327-343. doi: https://doi.org/10.1515/bchm3.1995.376.6.327.

Kucharská J, Braunová Z, Ulicná O, Zlatos L, Gvozdjáková A. Deficit of coenzyme Q in heart and liver mitochondria of rats with streptozotocin-induced diabetes. Physiol Res. 2000;49(4):411-418.

Laight DW, Carrier MJ, Anggard EE. Antioxidants, diabetes and endothelial dysfunction. Cardiovascular Res. 2000;47(3):457-464. doi: https://doi.org/10.1016/S0008-6363(00)00054-7.

Lass A, Forster MJ, Sohal RS. Effects of coenzyme Q_{10} and alpha-tocopherol administration on their tissue levels in the mouse: elevation of mitochondrial alpha-tocopherol by coenzyme Q_{10}. Free Radic Biol Med. 1999;26(11-12):1375-1382. doi: https://doi.org/10.1016/S0891-5849(98)00330-X.

Lee BJ, Huang YC, Chen SJ, Lin PT. Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme activity in patients with coronary artery disease. Nutrition. 2012;28(3):250-255. doi: 10.1016/j.nut.2011.06.004.

Luo K, Yu JH, Quan Y, Shin YJ, Lee KE, Kim HL, Ko EJ, Chung BH, Lim SW, Yang CW. Therapeutic potential of coenzymeQ_{10} in mitochondrial dysfunction during tacrolimus-induced beta cell injury. Sci Rep. 2019;9(1):7995. doi: 10.1038/s41598-019-44475-x.

Mäkimattila S, Liu ML, Vakkilainen J, Schlenzka A, Lahdenperä S, Syvänne M, Mäntysaari M, Summanen P, Bergholm R, Taskinen MR, Yki-Järvinen H. Impaired endothelium-dependent vasodilation in type 2 diabetes. Relation to LDL size, oxidized LDL, and antioxidants. Diabetes Care. 1999;22(6):973-981. doi: https://doi.org/10.2337/diacare.22.6.973.

Maxwell SR, Thomason H, Sandler D, LeGuen C, Baxter MA, Thorpe GH, Jones AF, Barnett AH. Poor

glycaemic control is associated with reduced serum free radical scavenging (antioxidant) activity in Non-Insulin-Dependent Diabetes Mellitus. Ann Clin Biochem. 1997;34(6):638-644. doi: 10.1177/000456329703400607.

Meghana K, Sanjeev G, Ramesh B. Curcumin prevents streptozotocin-induced islet damage by scavenging free radicals: A prophylactic and protective role. Eur J Pharmacol. 2007;577(1-3):183-191. doi: 10.1016/j.ejphar.2007.09.002.

Modi K, Santani DD, Goyal RK, Bhatt PA. Effect of coenzyme Q_{10} on catalase activity and other antioxidant parameters in streptozotocin-induced diabetic rats. Biol Trace Elem Res. 2006;109(1):25-34. doi: 10.1385/BTER:109:1:025.

Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP. The role of oxidative stress and NFκB activation in late diabetic complications. Biofactors. 1999;10(2-3):157-167. doi: https://doi.org/10.1002/biof.5520100211.

Mohan IK, Das UN. Oxidant stress, antioxidants and nitric oxide in non-insulin dependent diabetes mellitus. Med Sci Res. 1997;25(1):55-57.

Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun. 1990;173(3):932-939. doi: https://doi.org/10.1016/S0006-291X(05)80875-7.

Niture NT, Ansari AA, Naik SR. Anti-hyperglycemic activity of rutin in streptozotocin-induced diabetic rats: An effect mediated through cytokines, antioxidants and lipid biomarkers. Indian J Exp Biol. 2014;52(7):720-727.

Omar NM, Atia GM. Effect of Nigellasativa on pancreatic β-cell damage in streptozotocin-induced diabetic rats: histological and immunohistochemical study. The Egyptian J Histol. 2012;35(1):106-116. doi: 10.1097/01.EHX.0000411475.79484.90.

Özgün GS, Özgün E, Eskiocak S, Süt N. Streptozotosin ile diyabet oluşturulmuş sıçanlarda L-karnitinin nitrikoksit metabolizması üzerine etkisi. Turkish J Biochem. 2014;39(4):416-421. doi: 10.5505/tjb.2014.04934.

Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, Sgambato S, Varricchio M, D'Onofrio F. Plasma vitamin C affects glucose homeostasis in healthy subjects and in Non-Insulin-Dependent Diabetics. Am J Physiol. 1994;266(2):261-268. doi: 10.1152/ajpendo.1994.266.2.E261.

Prakash S, Sunitha J, Hans M. Role of coenzyme Q_{10} as an antioxidant and bioenergizer in periodontal diseases. Indian J Pharmacol. 2010;42(6):334-337. doi: 10.4103/0253-7613.71884.

Prosek M, Butinar J, Lukanc B, Fir MM, Milivojevic L, Krizman M, Smidovnik A. Bioavailability of water-soluble CoQ_{10} in beagle dogs. J Pharm Biomed Anal. 2008;47(4-5):918-922. doi: 10.1016/j.jpba.2008.04.007.

Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother. 2005;59(7):365-373. doi: 10.1016/j.biopha.2005.07.002.

Reunanen A, Knekt P, Aaran RK, Aromaa A. Serum antioxidants and risk of non-insulin dependent diabetes mellitus. Eur J Clin Nutr. 1998;52(2):89-93.

Roginsky VA, Tashlitsky VN, Skulachev VP. Chain-breaking antioxidant activity of reduced forms of mitochondria-targeted quinones, a novel type of geroprotectors. Aging (Albany NY). 2009;1(5):481-489. doi: 10.18632/aging.100049.

Rosen P, Nawroth PP, King G, Möller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a congress series sponsored by UNESCO–MCBN, the American Diabetic Association and the German diabetic society. Diabetes Metab Res Rev. 2001;17(3):189-212. doi: https://doi.org/10.1002/dmrr.196.

Sena CM, Nunes E, Gomes A, Santos MS, Proença T, Martins MI, Seiça RM. Supplementation of coenzyme Q_{10} and alpha-tocopherol lowers glycated hemoglobin level and lipid peroxidation in pancreas of diabetic rats. Nutr Res. 2008;28(2):113-121. doi: 10.1016/j.nutres.2007.12.005.

Shih PT, Elices MJ, Fang ZT, Ugarova TP, Strahl D, Territo MC, Frank JS, Kovach NL, Cabanas C, Berliner JA, Vora DK. Minimally modified low-density lipoprotein induces monocyte adhesion to endothelial connecting segment-1 by activating beta1 integrin. J Clin Invest. 1999;103(5):613-625. doi: 10.1172/JCI5710.

Sohal RS, Forster MJ. Coenzyme Q, oxidative stress and aging. Mitochondrion. 2007;7:103-111. doi: 10.1016/j.mito.2007.03.006.

Song Y, Cook NR, Albert CM, Van Denburgh M, Manson JE. Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr. 2009;90(2):429-437. doi: 10.3945/ajcn.2009.27491.

Stehouwer CD, Schaper NC. The pathogenesis of vascular complications of diabetes mellitus: one voice or many?. Eur J Clin Invest. 1996;26(7):535-543. doi: https://doi.org/10.1046/j.1365-362.1996.1780527.x.

Sundaram RK, Bhaskar A, Vijayalingam S, Viswanatthan M, Moha R, Shaninugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond). 1996;90(4):255-260. doi: 10.1042/cs0900255.

Tiano L, Belardinelli R, Carnevali P, Principi F, Seddaiu G, Littarru GP. Effect of coenzyme Q10 administration on endothelial function and extracellular superoxide dismutase in patients with ischaemic heart disease: a double-blind, randomized controlled study. Eur Heart J. 2007;28(18):2249-2255. doi: 10.1093/eurheartj/ehm267.

Tuck ML. Nitric oxide in diabetes mellitus. J Hypertens. 2003;21(6):1081-1083. doi: 10.1097/01.hjh.0000059042.65882.5c.

Tüzün S, Girgin FK, Sözmen EY, Menteş G, Ersöz B. Antioxidant status in experimental type 2 diabetes mellitus: effects of glibenclamide and glipizide on various rat tissues. Exp Toxicol Pathol. 1999;51(4-5):436-441. doi: 10.1016/S0940-2993(99)80036-0.

Uusitupa MIJ, Niskanen LK, Salonen JT, Nyyssonen K. Plasma lipid peroxidation and hyperglycemia: a connection through hyperinsulinaemia?. Diabet Med. 1995;12(9):802-808. doi: https://doi.org/10.1111/j.1464-5491.1995.tb02083.x.

Vessby J, Basu S, Mohsen R, Berne C, Vessby B. Oxidative stress and antioxidant status in type 1 diabetes mellitus. J Intern Med. 2002;251(1):69-76. doi: https://doi.org/10.1046/j.1365-2796.2002.00927.x.

Visnagri A, Kandhare AD, Kumar VS, Rajmane AR, Mohammad A, Ghosh P, Ghule AE, Bodhankar SL. Elucidation of ameliorative effect of Co-enzyme Q_{10} in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and

behavioral markers. Biomed Aging Pathol. 2012;2(4):157-172. doi: https://doi.org/10.1016/j.biomag.2012.10.006.

Yaman T, Uyar A, Celik İ, Alkan EE, Keles ÖF, Yener Z. Histopathological and immunohistochemical study of antidiabetic effects of heracleum persicum extract in experimentally diabetic rats. Indian J Pharm Educ Res. 2017;51(3):450-457. doi:10.5530/ijper.51.3s.66.

Yaqoob M, Patrick AW, McClelland P, Stevenson A, Mason H, White MC et al. Relationship between markers of endothelial dysfunction, oxidant injury and tubular damage in patients with insulin-dependent diabetes mellitus. Clin Sci (Lond). 1993;85(5):557-562.

West IC. Radicals and oxidative stress in diabetes. Diabet Med. 2000;17(3):171-180. doi: https://doi.org/10.1046/j.1464-5491.2000.00259.x.

Willems D, Dorchy H, Dufrasne D. Serum antioxidant status and oxidized LDL in well-controlled young type 1 diabetic patients with and without subclinical complications. Atherosclerosis. 1998;137:61-64. doi: https://doi.org/10.1016/S0021-9150(97)00320-1.

Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ. Roles for insulin receptor, PI3-kinase, and Akt in insulin signaling pathways related to production of nitric oxide in human vascular endothelial cells. Circulation. 2000;101(13):1539-1545. doi: https://www.ahajournals.org/doi/abs/10.1161/01.cir.101.13.1539.