Introduction

Diabetes mellitus (DM) is a metabolic disorder with different etiologies. It is characterized by insufficiency to regulate blood glucose level caused by relative or absolute deficiency in insulin. Diabetes mellitus may occur as a result of pancreatic β-cells dysfunction, which causes reduction in insulin secretion. The disease could also occur due to the insulin receptors resistant to the functions of circulating insulin (ADA 2010). Repetitive or obstinate hyperglycemia during diabetes causes formation glycated body proteins. This effect leads to secondary complications affecting eyes, kidneys, nerves and arteries (Sharma 1993, Aladodo et al. 2013). Erythrocytes (RBC) are seriously affected by hyperglycemia. It was reported these effects of hyperglycemia such as altered membrane lipid composition, reduced filterability, glycated hemoglobin, and the accumulation of advanced glycosylation end-products on the membrane (Schmid-Schönbein and Volger 1976, Wautier et al. 1994, Labrouche et al. 1996, Manodori and Kuypers 2002). In diabetes, reduced hemoglobin has been reported (Mansi 2006) and it may be associated with the decrease in the red blood cell count and packed cell volume (Moss 1999, Muhammad and Oloyede 2009, Aladodo et al. 2013).

Coenzyme Q10 (CoQ10) has been focused as a dietary supplement capable of influencing cellular bioenergetics and counteracting on some damage caused by free radicals (Linnane et al. 2002, Butler et al. 2003, Rosenfeldt et al. 2003, Zhou et al. 2005). CoQ10 is known as a vitamin like and fat-soluble substance existing in all cells (Crane 2001). It is strictly related with various activities including the transferring of electrons within the mitochondrial oxidative respiratory chain and ATP production. Further, it acts as [1] an essential antioxidant and supporting the regeneration of other antioxidants, [2] affecting the stability, fluidity and permeability of membranes, [3] stimulating cell growth and inhibiting cell death (Niki 1997, Crane 2001, Cooke et al. 2008).

Hence, the objective of the present study was to investigate hematological effects of CoQ10 in streptozotocin-induced diabetic rats.

Materials and Methods

We used 38 healthy, adult male Wistar Albino rats. The animals were kept in individual cages during the four weeks experiment and allowed free access to water and standard pellets. Diabetes was induced by SC injection of streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 40 mg/kg daily in 0.1 M citrate buffer (pH 4.5) single daily dose for two days. To prevent the streptozotocin-induced hypoglycemia, rats received 5% dextrose solution after 6 h of streptozotocin administration for next 3 days. After 1 week, induction of diabetes was verified by measuring blood glucose level with strips using glucometer (PlusMED Accuro, Taiwan) via the tail vein. Animals whose blood glucose level higher than 250 mg/dL were considered diabetic and included in the experiments. The mean weights of all groups were similar. The rats were divided into five groups. Group 1 (n=6) was fed standard rat pellets for four weeks, group 2 (n=6) was administered at 0,3 ml corn oil IP daily for four weeks, group 3 (n=6) was injected IP with 10 mg/kg CoQ10 (Sigma-Aldrich, St. Louis, MO, USA) daily for four weeks, group 4 (n=7) was made diabetic by SC injections of streptozotocin at dose of 40 mg/kg in 0.1 M citrate buffer (pH 4.5) single daily dose for two days, group 5 (n=9) was made diabetic by SC injections of streptozotocin in the same way and then was injected IP with 10 mg/kg CoQ10 daily for four weeks. During the experiment, three animals from group 4 and one animal from group 5 were died due to streptozotocin-induced hypoglycemia.

At the end of the study, blood samples were taken from all animals. In these blood samples, red blood cells (RBC) and white blood cells (WBC) counts, hemoglobin amount, hematocrit value (PCV), differential leucocyte counts and mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined using an automated cell counter (Abbott Cell Dyn 3700, Chicago, USA). The Ethical Committee of Selcuk University Experimental Medicine Research and Application Center (Report no. 2015-50) approved the study protocol.

The data were analyzed using 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 streptozotocin induced diabetic rats, RBC, hemoglobin and hematocrit values were determined to be significantly lower (Table 1, $p<0.05$) than the control group. There were no any changes in respect to the same parameters with CoQ10 treatment alone compared to the control group. CoQ10 application for four weeks to diabetic animals alleviated the reducing in some hematologic parameters compared with the diabetic group levels. But the changes in hematocrit value was important (Table 1, $p<0.05$) in the diabetic group with CoQ10 treatment. MCV, MCH and MCHC levels didn't show significant differences in all groups. On the other hand, experimentally induced diabetes caused significant (Table 2, $p<0.05$) increments in WBC count and neutrophil percentage, while lymphocyte percentage was relatively decreased (Table 2, $p<0.05$) compared to control group. CoQ10 treatment to the diabetic group markedly (Table 2, $p<0.05$) decreased the neutrophil percentage and increased relative lymphocyte percentage compared to diabetic group (Table 2, $p<0.05$). In the same group, WBC count tended to decline but this was not important compared to diabetic group. In oil and CoQ10 group, WBC count and differential leucocyte values weren't

Table 1. Mean RBC, Hb, PCV, MCV, MCH and MCHC values (Mean ± SE)

Groups	RBC (x 10^6/mm^3)	Hb (g/dl)	PCV (%)	MCV (μm^3)	MCH (pg)	MCHC (%)
Group 1	7,40±0,37^{a}	14,78±0,63^{a}	41,23±0,99^{a}	56,46±3,15	20,06±0,49	35,95±1,67
Group 2	7,25±0,43^{ab}	14,05±0,59^{ab}	40,57±1,61^{a}	56,71±3,21	19,73±1,37	34,69±1,02
Group 3	7,61±0,55^{a}	15,25±0,40^{a}	42,87±1,16^{a}	57,48±3,48	20,46±1,23	35,65±0,99
Group 4	6,28±0,26^{b}	11,71±0,54^{c}	34,40±0,96^{b}	55,14±2,22	18,79±1,09	34,13±1,61
Group 5	7,11±0,14^{ab}	13,07±0,37^{bc}	39,68±0,74^{a}	55,97±1,57	18,47±0,78	33,03±1,12

The difference between mean values with different superscripts in the same column is significant for each parameter, p<0.05.
Group 1, control; group 2, oil; group 3, CoQ10; group 4, diabetes; group 5, CoQ10 and diabetes.

Table 2. Mean WBC and differential leukocyte counts (Mean ± SE)

Groups	WBC (x 10^3/mm^3)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophiles (%)
Group 1	9,75±1,55^{b}	21,43±2,48^{b}	72,42±2,26^{a}	4,17±0,65	1,53±0,60	0,45±0,21
Group 2	10,78±0,43^{ab}	20,25±0,95^{b}	74,25±1,75^{a}	3,83±0,87	1,33±0,49	0,33±0,21
Group 3	10,52±0,70^{ab}	19,77±0,77^{b}	74,55±0,96^{a}	3,63±0,80	1,67±0,42	0,38±0,24
Group 4	13,18±0,98^{a}	31,96±3,26^{a}	61,16±3,72^{b}	4,71±0,87	1,75±0,29	0,41±0,20
Group 5	11,13±0,54^{ab}	25,18±1,40^{b}	68,40±1,94^{a}	4,31±0,53	1,62±0,37	0,49±0,17

The difference between mean values with different superscripts in the same column is significant for each parameter, p<0.05.
Group 1, control; group 2, oil; group 3, CoQ10; group 4, diabetes; group 5, CoQ10 and diabetes.

different from the control group levels.

Discussion

The reductions in RBC, hemoglobin and hematocrit levels of the diabetic group when compared to the control group were seem to consistent with other studies which used similar experimental diabetes (Mansi 2006, Aladodo et al. 2013). The reasons of these changes are attributed to oxidative stress, decreasing of erythrocyte lifespan, bone marrow suppression. It has been suggested that anemia resulting from diabetes mellitus are due to peroxidation of membrane lipids, decreasing of membrane fluidity, oxidation of glycosylated membrane proteins and hemolysis of RBC (Kennedy and Baynes 1984, Bakan et al. 2006, Mansi 2006).

In this study, CoQ10 treatment to diabetic animals alleviated the reducing in some hematologic parameters compared with the diabetic group levels. The changes in hematocrit value was important (Table 1, p<0.05) in the diabetic group treated with CoQ10. It has been well known that elevated glycose causes oxidative stress as a result of increased production of reactive oxygen species (ROS), nonenzymatic glycation of proteins and glycose autoxidation in diabetes mellitus (Brownlee 2001, Modi et al. 2006). The positive effect of CoQ10 on hematocrit value of diabetic animals could be based on its some antioxidant properties. CoQ10 is recognized as a powerful systemic radical scavenger (Prosek et al. 2008). Moreover, there are many findings related to antioxidant effects of CoQ10 in diabetes mellitus. Niklowitz et al. (2004) found that CoQ10 is greater antioxidant than Vitamin E. In another research, it has been suggested that CoQ10 enhanced the availability of other antioxidants such as Vitamin E, Vitamin C and beta-carotene (Shekelle et al.2003).

The beneficial effect of CoQ10 on hematological parameters may be because of decreasing lipid peroxidation and inhibiting certain enzymes involved the formation of free radicals, blocking oxidative injuries to DNA and reducing glycation of membrane proteins (Modi et al. 2006, Prosek et al. 2008). Further, Ahmadvand et al. (2012) reported that CoQ10 reduced the lipid peroxidation and enhanced antioxidant enzyme activities (SOD, GSH, CAT) in experimentally diabetic rats. Quinzii et al. (2010) stated that there is strictly correlation reactive oxygen species, oxidative stress and cell death with CoQ10 deficiency.

On the other hand, experimentally induced diabetes lead to significantly (Table 2, p<0.05) increments in WBC count and neutrophil percentage compared to control group. Also, lymphocyte percentage was relatively decreased (Table 2, p<0.05) compared to control group. In diabetic rats treated

with CoQ10, neutrophil percentage and relative lymphocyte percentage were found to be lower and higher (Table 2, $p<0.05$) than that of diabetic group, respectively. In the same group, WBC count tended to decline but this changing was not important compared to diabetic group.

A positive association between increased levels of inflammatory markers (WBC count, CRP, and inflammatory cytokines) and diabetes incidence were determined in several studies (Gkrania-Klotsas et al. 2010, Twig et al. 2013). Tong et al. (2004) reported that elevated WBC count, even within the normal range, is associated with macro- and microvascular complications in diabetes. It has been noted that higher WBC count in diabetes reflects an inflammation and the other tissue complications (Tong et al. 2004, Twig et al. 2013). The increase neutrophil percentage by diabetes in this study is important regarding to show immune response against causal effects of diabetes. This result seems in coherent with above studies that noted about the same data obtained from diabetic subject. Although the decrease in WBC count is not important, significantly decrease in neutrophil percentage may be evaluated as a result of CoQ10 treatment's beneficial effect. There are many acknowledgements about anti-inflammatory properties of CoQ10. It was reported that CoQ10 administration in several doses decreased WBC count and inflammatory cytokines in human and animals (Ahmadvand et al. 2012, Abdollahzad et al. 2015). In the study conducted in animals, it has been suggested that CRP level were reduced with CoQ10 application (Devadasu et al. 2011). Above findings have supported our results obtained from CoQ10 administration in diabetic animals.

Conclusion

In the light of our results, it can be said that CoQ10 supplementation has some ameliorative effects in respect to at least hematological parameters in streptozotocin-induced diabetic rats.

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