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Research Article

The effect of thymoquinone on serum antioxidant vitamin levels in diabetic rats

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ABSTRACT

Objectives: Thymoquinone, the basic bioactive phytochemical constituent of seeds oil of *Nigella sativa*, is one of these herbal drugs with known antidiabetic effects. In this study, effects of thymoquinone (TQ) on antioxidant vitamin levels in experimental diabetic rats were investigated. Materials and Methods: Twenty-eight male Wistar-Albino rats (200-250 g) were used as material. The rats were divided into four groups of control (C), thymoquinone (T), diabetes (D) and diabetes + thymoquinone (DT). D and DT groups were treated with 45 mg/kg streptozotocin (STZ) (i.p). TQ was administered as 30 mg/kg/21 days by oral gavage in DT and T groups.

Results: The retinol concentrations were observed to have significantly decreased in the diabetes and diabetes + TQ groups ($p \leq 0.05$). There was no difference between the group delivered TQ and the control group. It was identified that the tocopherol concentrations were lower in all study groups than in the control group, and it was identified that the reduction in the diabetes and diabetes + TQ groups was significant when compared to the control group ($p \leq 0.05$). It was identified that the vitamin D3 levels were significantly lower in the study groups when compared to the control group ($p \leq 0.05$).

Conclusion: It was identified that the serum antioxidant vitamin levels decreased in the group with experimental diabetes and that TQ administration did not significantly affect the antioxidant vitamin levels significantly in the group with experimental diabetes.

Keywords: experimental diabetes, antioxidant vitamins, thymoquinone

INTRODUCTION

Diabetes is a syndrome characterized by hyperglycemia and high glucagon levels, accompanied by impaired carbohydrate, protein and lipid metabolisms, and results in acute metabolic and chronic degenerative complications. This chronic disease leads to a negative impact on many systems and organs in the human body due to its complications (1). High blood glucose levels result in functional disorders, especially in the eyes, kidneys, liver, blood vessels and various organs (2). Hyperglycemia increases lipid peroxidation, DNA damage, sulfhydryl oxidation and production of superoxide radicals- the oxidative stress indicators- which has a basic role in the pathogenesis of these complications (3). In diabetes, the inhibition of apoptosis and degradation within the beta cells by antioxidants, which fight against oxidative stress that cause a further impairment in pancreatic beta cell function, have suggested the

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possible beneficial effects of these molecules (4, 5).

Vitamin replacement has been provided for diabetic patients in order to protect them from long-term complications, and diets including antioxidant properties are recommended. Thymoquinone (TQ), which is a hypoglycemic agent and is the basic bioactive component of the seeds of *Nigella sati*va, is a volatile monoterpene quinone with dark yellow crystals (6). With known antidiabetic activity (7), TQ has been reported to have higher antioxidant activity than the other components of the black cumin seed (8). TQ, which provides cellular protection against endogeneous DNA damage, and has been reported to have clinical benefits in the treatement of diabetic patients and in the protection of β-cells against oxidative stress (9-11).

Carotenoids, flavonoids, ascorbic acid and alpha tocopherol are the most important herbal antioxidant molecules (12). Oxidants that cannot be deactivated by the enzymes, first affect the lipids within the cell membrane and start "lipid peroxidation". During lipid peroxidation, such cellular damages may be prevented in the presence of sufficient amounts of antioxidant vitamins such as Vitamin E and Vitamin C. Vitamins transfer a hydrogen molecule to the oxidants and suppress their effects by inactivation (13).

Vitamin A has a potential antihyperglycemic effect by playing a regenerative role in the pancreas and preventing the oxidative damage within the tissues (14). There are studies that support the relationship between Vitamin D deficiency, and Type 1 and Type 2 diabetes. Vitamin D levels have been measured as decreased amounts in diabetic patients (15) and a relationship has been defined between vitamin D and pancreatic cell dysfunction (16, 17). The antioxidant property of vitamin E is the lipid peroxidation inhibitory effect started begun by the free radicals (18); it has a chain-breaking antioxidant function as well.

The aim of this study was to investigate the effect of TQ with known antioxidant and antidiabetic properties on serum antioxidants vitamin A, vitamin E and vitamin D levels in rats with experimentally induced diabetes.

MATERIALS AND METHODS

Animals

For this study, 28 male Wistar-Albino rats weighing 200-250 g, which were acquired from the Experimental Animal Unit of Yüzüncü Yıl University, Faculty of Medicine were used. Subjects were divided into four experimental groups, control (K), thymoquinone given (T), diabetes generated (D), diabetes induced and thymoquinone given (DT). The rats were accommodated in cages, in which the feed and fresh water were present all the time, in the rooms adjusted for a temperature of 22 ± 2 ° C, applied for 12 hours in darkness / light during the 21 days trial period. Experiments conducted according to ethical rules were carried out under the supervision of Yüzüncü Yıl University Animal Experiments Local Ethics Committee.

Preparation of the Trial Groups

D and DL group rats to be diabetized were administered intraperitoneally (i.p.) 45 mg / kg single dose streptozotocin (STZ) (Sigma, USA) by dissolving them in citrate buffer at pH : 4.5 (19).

Control group	Thymoquinone group	Diabetes group	Diabetes+ Thymoquinone group
Salin	Thymoquinone (30mg/kg/day)	STZ (45mg/kg)	STZ+Thymoquinone (30mg/kg/day)

Table 1 Preparation of the trial groups

Control group (C): Randomly selected 7 rats were separated as the control group. 45 mg / kg single dose of physiological serum was injected intraperitoneally.

Thymoquinone group (T): Thymoquinone (Aldrich) was dissolved in corn oil and seven rats were applied orally by gavage for 21 days as 30 mg / kg / day.

Diabetes group (D): 45 mg/kg single-dose STZ were administered i.p. to seven rats; 72 h later, the glucose levels in blood samples taken from the tail vein were determined by PlusMED Accuro brand glucometer equipment and through its strips. Those rats with blood glucose 200 mg/dl and above were regarded as diabetic and were included in the study.

Diabetes+ thymoquinone group (DT): STZ was administered to the group of seven rats i.p.; 72 h later, the glucose levels in blood samples taken from the tail vein were determined by PlusMED Accuro brand biosensors glucometer. TQ, dissolved in corn oil was applied orally (by gavage) to those rats with blood glucose levels of 200 mg/dl and above, at 30 mg/kg/day for 21 days.

Collection of samples

After the 21-day trial period, the blood samples were obtained from the left ventricle, of the animals' hearts under ketamine anesthesia and transferred into tubes with anticoagulant and gel. Vitamin A, vitamin D and vitamin E levels were determined in the obtained serum samples. HbA1c analyzes were performed in all blood samples.

Biochemical analysis

Blood glucose determination in serum samples was performed on a Hitachi / P800 modular auto analyzer (Roche, Germany) using a commercial kit from Roche.

HbA1c levels were determined in whole blood on a Roche brand Cobas Integra 800 autoanalyzer using the Roche brand kit on the same day.

The quantitative analysis of serum vitamins was determined by high performance liquid chromatography (HPLC, Termo Sentific Pinligan Surveyor) according to the procedure modified from the relevant literatures (20-23). For the quantitative determination of the vitamins (A, E and D), DAD (diode-array detector) was employed in 325, 290, 265 and nm wavelengths respectively. As the mobile phase, methanol-water (98:2) was used in 1.5 ml/min flow rate. The separation of vitamins were used from C18 column (4.6 mmx25 cm). Calculations vitamins A, E and D were made according to standard peak area and concentration.

Statistical Analysis

At the end of the study, the obtained data from control, thymoquinone, diabetes and diabetes+thymoquinone groups were evaluated by multiple comparison test using SPSS 22.0 package program. The raw values obtained from all analyzes were presented as the averages of groups ± standard error.

RESULTS

The results of the glucose and HbA1c values obtained from the groups in our thesis study are summarized in Table 2 below (23).

Parameters	Control	Thymoquinone	Diabetes	DT
Glucose (mg/dl)	174.14±4.99b	167.71±6.93c	511.00±45.83a	204.71±10.08b
HbA1c (%)	3.597±0.07b	3.633±0.14b	5.843±0.32a	3.783±0.09b

Table 2 Results of glucose and HbA1c values

It was determined that glucose levels were increased significantly in the diabetic group, decreased significantly and approached to control group in DT group and decreased in the group in which thymoquinone was implemented compared to the control group. HbA1c levels were significantly increased only in the diabetic group and decreased significantly in DT group and approached to the control group (23).

The results of vitamins A, E and D obtained from the serum samples in the study groups have been summarized in Table 3.

Parameters	Control	Thymoquinone	Diabetes	DT
Retinol (µg/ml)	503.71±49.93	505.00±72.31	319.00±41.18	290.14±23.24
α-Tocopherol (µg/ml)	1333.14±268.57	850.33±170.47	829.71±69.32	595.71±70.10
Vitamin D3 (µg/ml)	9.57±1.84	4.33±1.45	2.86±0.51	4.14±1.22

Table 3 Retinol, a-tocopherol and vitamin D3 levels in working groups

It was observed that the retinol concentrations significantly decreased in the diabetes and diabetes + TQ groups ($p \leq 0.05$). There was no difference between the group delivered TQ and the control group (Figure 1).

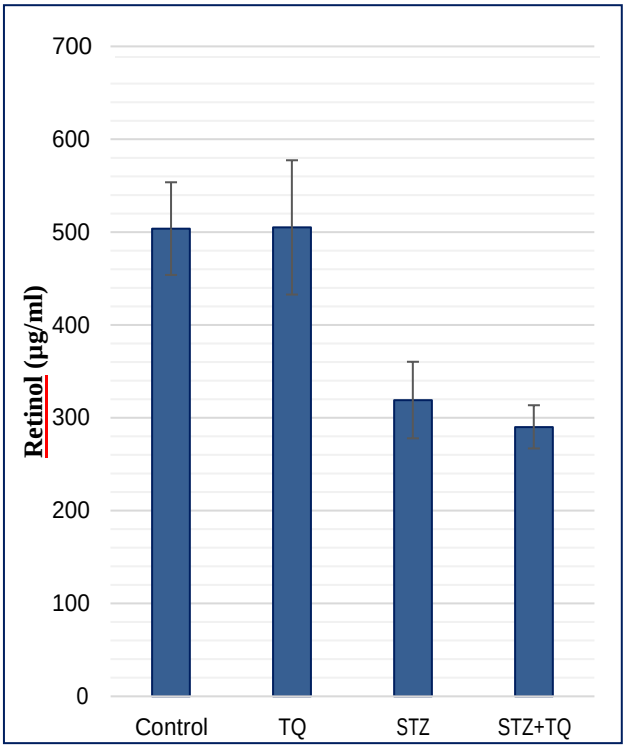

Figure 1 Retinol levels

It was identified that the tocopherol concentrations were lower in all study groups than in the control group, and it was identified that the reduction in the diabetes and diabetes + TQ groups was significant when compared to the control group ($p \leq 0.05$) (Figure 2).

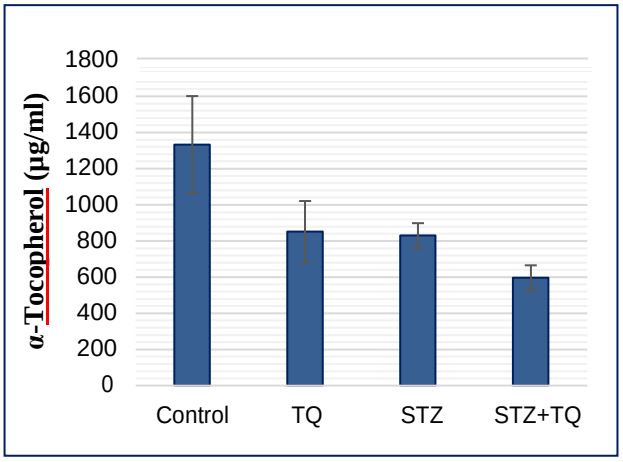

Figure 2 a-Tocopherol levels

It was identified that the vitamin D3 levels were significantly lower in the study groups when compared to the control group ($p \leq 0.05$) (Figure 3).

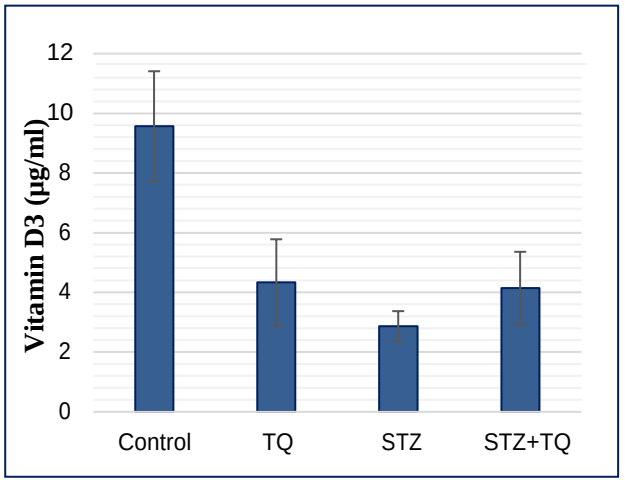

Figure 3 Vitamin D3 levels

It was identified that the serum antioxidant vitamin levels decreased in the group with experimental diabetes and that TQ administration did not significantly affect the antioxidant vitamin levels significantly in the group with experimental diabetes.

DISCUSSION

TQ has a therapeutic effect in diabetes via reducing the oxidative stress caused by diabetes and in protection of β-cell integrity (10). TQ suppresses the synthesis of the enzymes act in glyconeogenesis, reduces hepatic gluconeogenesis, increases insülin secretion from pancreatic β-cells and activates glucose consumption in the cytosol of extrahepatic tissues (24).

It was concluded in a study investigating the effect of TQ on the vascular damage in streptozotocin (STZ)-induced type I diabetes that TQ administration may have a preventive effect in the progressive increase in blood glucose, and that this effect may be related to a process including the CD34 cells which are the indicators of progenitor cells (25).

TQ application in diabetic rats have reduced the oxidative stress to an important extent, the increased endothelial nitric oxide synthase has inhibited protein expression, and the increase in the level of cyclooxygenase-2 has been suppressed. Furthermore, it has significantly reduced the increase in the levels of tumor necrosis factor-α and interleukin-6. Additionally, it has significantly reduced the increase in the levels of phosphorylated protein kinase B (Akt) protein expression and caspase-3 activity. These results have suggested protective effects of TQ by diminishing apoptosis, oxidative stress and inflammation via mediating phosphatidylinositol 3 kinase (PI3K)/Akt pathway and improving cardiovascular function in diabetic rats (26).

TQ has been demonstrated to significantly protect lens tissues against the changes induced by diabetes via its antioxidant, anti-inflammatory and anti-diabetic effects in diabetic rats (27).

Another study on a hepatocellular carcinoma cell line in rats (HepG2) demonstrated that TQ diminished the mechanism of insulin resistance in rats with experimentally induced insulin resistance, and contributed to glucose uptake by affecting the expression levels of critical genes related to the insulin signaling pathway (28). These wide-range medical applications support the use of TQ as a natural supplement.

Again, in a previous thesis study of ours, glucose levels were demonstrated to increase to an important extent in the diabetes group as expected, and TQ application resulted in a significant reduction in glucose levels in diabetic rats, approximating that of the control group. In the group with TQ application only, glucose levels were shown to be reduced, indicating its antiglycemic effects. The HbA1c level, which is a long-term indicator of diabetes, was shown to significantly increase in the diabetic group, parallel to the increase in the glucose level, and to decrease to an important extent in the TQ group, approximating that of the control group (23). Likewise, Pari and Sankaranarayanan (6) reported an important reduction in the glucose concentrations and HbA1c levels in the TQ group and a significant increase in insulin levels.

Oxidative stress formed in diabetes may be overcome by increasing the antioxidant capacity. Antioxidants have been shown to be useful in preventing diabetic complications. Mild exercise and daily vitamin E and

C supplement have been recommended in diabetes since they reduce the level of blood glucose and produce free radicals (29).

Reduced amounts of various antioxidant parameters such as vitamin E, vitamin C, glutathion, superoxide dismutase, catalase and glutathion peroxidase observed in diabetic patients suggest an important role of oxidative stress in the pathogenesis of chronic complications of diabetes (30, 31).

In the study of Sankaranarayanan and Pari (32), lipid peroxidation levels were increased in the hepatic and renal tissues of diabetic rats compared to the control group, whereas the low molecular weight antioxidants vitamin C, vitamin E and reduced glutathion (GSH) levels were decreased.

Increased glutathion, glutathion peroxidase, superoxide dismutase and catalase activity observed in the hepatic tissues following vitamin E, C and D indicate a protective role of vitamin C, E and D against oxidative stress and inflammation in diabetics with STZ (33).

Rağbetli et al. investigated the effect of different doses of streptozotocine on serum micro-nutritions (Cu, Zn, Mg, Fe, vitamin D, E and C) and oxidative stress (MDA) on diabetic rat models. The retinol level was significantly decreased in the STZ groups, and the α-tocopherol amount was low in the 55 mg/kg STZ group (34).

Vitamin A has been included as a basic micro-nutrient in the regulation of immune function. Patients with type 1 diabetes are at risk for vitamin A and carotenoid deficiency (35).

Vitamin A and retinoic acid are important immune tolerence sources by inhibiting the progression of diabetes and islet inflammation (35). Retinoids act in the expression of glucose 4 transporter (GLUT 4) and beta cell renewal (14).

Retinol concentrations have been observed to significantly decrease in the experimentally formed diabetes group and to significantly decrease in the timoquinone application group after diabetes was induced experimentally ($p \leq 0.05$). No difference was observed compared to the control group in the timoquinone-only group.

Studies have demonstrated a decrease in pancreatic insulin secretion in animals fed with diets poor in vitamin D in the 2nd month of the diet. Development of glucose intolerence in these laboratory animals has indicated that vitamin D is essential for the endocrine pancreas (36).

With the direct effect of vitamin D in increasing the risk of type 2 diabetes, various mechanisms may be implicated such as reduction in vitamin D levels, reduction in calcium concentration, increase in parathyroid hormone or deterioration in insulin resistance (37). Vitamin D concentration was found to be lower in patients with type 2 diabetes compared to that of the control group (15). In this study, vitamin D3 levels were observed to be significantly lower than that of the control group in all study groups ($p \leq 0.05$).

Oxidative stress was observed to have decreased through vitamin E administration in patients with diabetes, suggesting that vitamin E could facilitate a reduction in free radical-related oxidant damage (38).

The beneficial effects of vitamin E have been reported in the protection against type 2 diabetes and in the treatment of diabetes. Vitamin E replacement in patients with type 2 diabetes has improved the serum vitamin E level, paraoxonase-1 activity, total antioxidant situation and the fasting blood glucose (39). In a study investigating the role of vitamin E therapy in the prevention and controlling the development of diabetic complications (primary prophylaxis), it was demonstrated to have reduced the risk of cardiovascular disease development (40).

Tocopherol concentrations were shown to have decreased in all study groups compared to the control group, whereas the decrease in the group including rats with Streptozotocin (STZ)-induced diabetes and timoquinone administration was significant compared to the control group ($p \leq 0.05$).

It was concluded that serum antioxidant vitamin levels decreased in the experimentally-induced diabetes group, and TQ administration had no significant effect on the levels of antioxidant vitamins in the experimentally induced diabetes group. In future studies, the application of TQ dependent on diffirent times and concentration may be more helpful in understanding the effect of TQ on vitamin metabolism.

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Ethical approval:

The study approved by judgement with 2014/12 reference number of of Yüzüncü Yıl University Animal Experiments Local Ethics Committee.

Conflict of Interest: No conflict of interest was declared by the authors.

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