

Effects of Thymoquinone on Plasma Leptin, Insulin, Thyroid Hormones and Lipid Profile in Rats Fed A Fatty Diet ^[1] ^[2] ^[3]

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Summary

The purpose of this study was to investigate the effects of thymoquinone (TQ) on plasma hormone levels, which regulate energy metabolism, and on the lipid profile of rats fed a standard and fatty diets. The study was performed on four experimental groups for 6 weeks using Wistar Albino rats weighing 200-260 g, aged 5-6 months, with 10 rats in each group. 1) The control group: Rats were fed a standard diet and given 1 ml 0.9% saline /kg body weight/day by gastric gavage. 2) The TQ group: Rats were fed a standard diet and given 50 mg TQ/kg body weight/day dissolved in 1 ml 0.9% saline, by gastric gavage. 3) The fatty diet (FD) group: Rats were fed an experimental diet containing 50% animal fat and given 1 ml 0.9% saline/kg body weight/day by gastric gavage. 4) The FD+TQ group: Rats were fed an experimental diet containing 50% animal fat and given 50 mg TQ/kg body weight/day dissolved in 1 ml 0.9% saline by gastric gavage. At the end of the experimental period, plasma insulin, thyroxine (T₄), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were significantly decreased in the TQ group compared with the control group while the plasma glucose level was increased (P<0.01). In the FD group compared to the control group, HDL and carnitine levels were significantly decreased while plasma leptin, glucose, total triiodothyronine (T₃), T₄, free triiodothyronine (FT₃), free thyroxine (FT₄), triglyceride (TG), LDL and very low-density lipoprotein (VLDL) levels were significantly increased. Compared to the FD group, in particular, leptin, T₃, FT₄, TG, LDL and VLDL levels were significantly decreased in the FD+TQ group except for increased glucose. The study concludes that TQ may be a natural source for the prevention of the harmful effects of a fatty diet, due to the dropped levels of leptin, TG, LDL, VLDL and could be considered for use in the field of preventive medicine.

Keywords: Fatty diet, Insulin, Leptin, Thymoquinone, Thyroid hormones

Yağlı Diyet ile Beslenen Ratlarda Timokinonun Plazma Leptin, İnsulin, Tiroid Hormonları ve Lipid Profiline Etkileri

Özet

Bu çalışmanın amacı standart ve yağlı diyetle beslenen ratlarda Timokinon (TQ)'nun, enerji metabolizmasını düzenleyen plazma hormon seviyeleri ve lipid profiline etkilerini araştırmaktır. Çalışma 5-6 aylık, 200-260 g Wistar Albino rat kullanılarak 6 haftalık sürede 4 deneysel gruba ve her bir grupta 10 rat ile gerçekleştirildi. 1) Kontrol Grubu: Ratlar standart yem ile beslendi ve gastrik gavaj ile 1 ml 0.9% NaCl/kg canlı ağırlık/gün olarak verildi 2) TQ grubu: Ratlar standart yem ile beslendi ve gastrik gavaj ile 1 ml 0.9% NaCl'de çözülmüş 50 mg TQ/kg canlı ağırlık/gün olarak verildi 3) Yağlı Yem (YY) grubu: Ratlar kg'na %50 hayvansal yağ ilavesi yapılmış deneysel diyetle beslendi ve gastrik gavaj ile 1 ml 0.9% NaCl'de çözülmüş 50 mg TQ/kg canlı ağırlık/gün olarak verildi. Deneme süresi sonunda kontrol gruba göre TQ grubunda plazma insülin, T₄, HDL ve LDL seviyeleri azalırken, plazma glikoz seviyesi arttı (P<0,01). Kontrol grubu ile karşılaştırıldığında YY grubunda, plazma leptin, glikoz, T₃, T₄, FT₃, FT₄, TG, VLDL ve LDL seviyeleri önemli düzeyde artmasına rağmen, HDL ve karnitin düşüktü. Grup YY ile karşılaştırıldığında, glikozun artışı dışında özellikle leptin, T₃, FT₄, TG, VLDL ve LDL seviyeleri YY+TQ grubunda önemli düzeyde düşüktü. Çalışmada plazma leptin, TG, LDL ve VLDL düzeylerini düşürmesi nedeniyle, TQ'nun yağlı diyetin neden olduğu zararlı etkilerin önlenmesinde doğal bir kaynak olabileceği ve koruyucu hekimlik alanında kullanımının düşünülebileceği sonucuna varıldı.

Anahtar sözcükler: İnsulin, Leptin, Timokinon, Tiroid hormonları, Yağlı diyet



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INTRODUCTION

Nigella sativa L.(*Ranunculaceae*) has long been used in traditional herbal medicine as a natural remedy in many countries around the world, especially in the Middle East [1]. The seeds include 36%-38% fixed oils, proteins, carbohydrates, alkaloids, saponin and 0.4%-2.5% essential oils [2]. In research of recent years, several beneficial therapeutic effects have been attributed to thymoquinone (TQ). Thymoquinone, the main bioactive constituent of the volatile oil extracted from *Nigella sativa* (NS), has many pharmaceutical uses, such as acting as an antioxidant, carminative, anticancerogenic, diuretic, lactagogue, anti-hyperlipidemic, hypocholesterolemic and anti-inflammatory agent [1,3]. Moreover, recent research has reported that TQ extracted from NS seeds and commercial TQ caused inhibition of the synthesis of cholesterol [4]. Currently, investigations have indicated that dietary fat intake can lead to obesity and lead to the development of many metabolic disorders, such as cardiovascular diseases, diabetes and hypertension [5,6]. In spite of widespread recognition of NS seeds in traditional medicines, the in vivo effects of its seeds or TQ on plasma leptin, insulin, thyroid hormones, carnitine, paraoxanase (PON) and lipid profile levels in standard and fatty diets have not been investigated until now.

Therefore, the present study was designed to investigate the effects of TQ on hormone levels which regulate energy metabolism, and on the lipid profile of rats fed standard and fatty diets, in order to reveal and elucidate the effect of TQ scientifically.

MATERIAL and METHODS

Thymoquinone

The TQ (2-isopropyl-5-methyl-1,4-benzoquinone, C₁₀H₁₂O₂) was purchased from Sigma-Aldrich Chemical Company. It was administered at a dose of 50 mg/kg body weight once daily by gastric gavages for 6 weeks. TQ was dissolved by the addition of a 0.9% serum physiologic (SP).

Animals, Diets and Experimental Protocols

Forty male Wistar Albino rats (5-6 months, 200-260 g body weight) were used. The study was approved by the Institutional Ethics Committee of Afyon Kocatepe University (No; 2008/09-08), and performed according to international standards for animal experiments. The animals were left for two days to acclimate to their room, where the researches maintained a 12 h light/12 h dark cycle at room temperature (22±2°C), and provides a standard pellet diet and water *ad libitum*. The fatty diet used for the groups was prepared to be enough for 2-3 days. The standard diet, which was in pellet form, was ground in a blender, and then mixed with minced animal

fat, such that each kilogram of the diet contained 50% animal fat. This mixture was then converted to pellet form and kept at +4°C until it was given to the animals.

The study was performed on four experimental groups for 6 weeks using Wistar Albino rats weighting 200-260 g and aged 5-6 months. Ten animals were used in each group. The groups were as follows: 1) The control group: Rats were fed a standard diet and given 1 ml 0.9% saline/kg body weight/day by gastric gavage. 2) The TQ group: Rats were fed a standard diet and given 50 mg TQ/kg body weight/day dissolved in 1 ml 0.9% saline, by gastric gavage. 3) The fatty diet (FD) group: Rats were fed an experimental diet containing 50% animal fat and given 1 ml 0.9% saline/kg body weight/day by gastric gavage. 4) The FD+TQ group: Rats were fed an experimental diet containing 50% animal fat and given 50 mg TQ/kg body weight/day dissolved in 1 ml 0.9% saline by gastric gavage. The nutrient contents and metabolizable energy levels of the standard and fatty diets were determined by The Province Control Laboratory of Afyonkarahisar (Table 1).

The body weight of all animals was recorded weekly during a 6- week period. At the end of the period, blood samples were collected after the animals were fasted overnight and killed under anaesthesia with a combination of ketamine and xylazine HCl. Their plasma and serum was obtained by blood centrifugation at 3.000 rpm (+4°C) for 10 min. and stored at -20°C until used.

Biochemical Analysis

Serum FT₃ (Roche/Cobas/code no: 03051986 190), FT₄ (Roche/ Cobas/ code no: 11731297 122), T₃ (Roche/Cobas/ code no: 11731360 122) and T₄ (Roche/Cobas/code no:12017709 122) concentrations were determined by the chemiluminescence method on a Moduler E170 analyzer. Plasma insulin and leptin levels were measured using a commercial enzyme-linked immunosorbent assays kit (Biovender) according to the manufacturer's instructions (Trinity Biotech ELISA reader). Glucose, serum PON, plasma carnitine, total cholesterol (TC), TG, HDL and LDL concentrations were determined by using commercial kits (a Dimension RL Max, Hitachi Moduler analyzer and a Rel assay analyzer). The VLDL level was calculated using

Table 1. Nutrient contents (%) and metabolizable energy levels (kcal/kg) of the standard and fatty diets (dry matter basis)

Tablo 1. Standard ve yağlı diyetin besin madde içeriği (%) ve metabolik enerji değeri (kcal/kg)

| Chemical Composition | Standard Diet | Fatty Diet |
|----------------------|---------------|------------|
| Metabolizable energy | 2600 | 4345 |
| Crude fat | 1.52 | 40.68 |
| Crude protein | 15.22 | 11.15 |
| Crude cellulose | 8.0 | 8.80 |
| Crude ash | 9.10 | 5.06 |
| Humidity | 12.0 | 7.34 |

the Friedwald equation. The amount of saturated and unsaturated fatty acids in the beef tallow used in this study was determined by TUBITAK-MAM (Table 2).

Statistical Analysis

Data obtained from experiment animals is expressed with mean standard error (\pm S.E.M.). The statistical differences between the control and experimental groups were evaluated by one-way ANOVA and Duncan post hoc tests [7]. A difference in the mean values with $P < 0.05$ was considered to be significant.

RESULTS

At the end of the experimental period, an analysis of the fatty acids in the animal fat, the rats body weights and the rats' biochemical parameters was conducted. The results are shown Table 2, 3 and 4, respectively.

Table 2. Saturated and unsaturated fatty acid compounds in the animal fat
Tablo 2. Hayvansal yağdaki doymuş ve doymamış yağ asitleri kompozisyonu

| Analysis | Fatty Acids (%) | Method |
|--------------------------------------|-----------------|--------------|
| Myristic acid (C14:0) | 2.46 | IUPAC II D19 |
| Arachidic acid (C20:0) | 0.24 | IUPAC II D19 |
| Gadoleic/ekiekosenoic acid (C20:1) | 0.39 | IUPAC II D19 |
| Palmitic acid (C16:0) | 22.50 | IUPAC II D19 |
| Palmitoleic acid (C16:1) | 0.37 | IUPAC II D19 |
| Heptadekanoic/margaric acid (C17:0) | 2.95 | IUPAC II D19 |
| Heptadesenoic/margoleic acid (C17:1) | 0.70 | IUPAC II D19 |
| Stearic acid (C18:0) | 32.10 | IUPAC II D19 |
| Oleic acid (C18:1) | 29.65 | IUPAC II D19 |
| Linoleic acid (C18:2) | 3.12 | IUPAC II D19 |
| Linolenic acid (C18:3) | 0.20 | IUPAC II D19 |
| Saturated fatty acids | 60.25 | |
| Unsaturated fatty acids * | 34.43 | |
| Unidentified compounds | 5.32 | |

*The unsaturated fatty acids in the animal fat were calculated as 36.36%; this percentage includes unidentified compounds in the animal fat

At the end of the experimental period, body weight had decreased ($P < 0.05$) in the TQ group compared to the control group. In the FD group, as compared with the control group, body weight had insignificantly increased. Body weight had significantly decreased ($P < 0.05$) in the FD+TQ group, compared to the FD group.

Plasma insulin ($P < 0.05$), T_4 ($P < 0.01$), HDL ($P < 0.05$) and LDL ($P < 0.01$) was significantly decreased in the TQ group compared to the control group, while the plasma glucose level was increased ($P < 0.01$). In the FD group, compared to the control group, HDL ($P < 0.05$) and carnitine ($P < 0.01$) were significantly decreased while plasma leptin, glucose, T_3 , T_4 , FT_3 , FT_4 , TG, LDL and VLDL levels were significantly increased ($P < 0.01$). Compared to the FD group, in particular, leptin, T_3 , FT_4 , TG, LDL and VLDL were significantly decreased ($P < 0.01$) in the FD+TQ group except for increased ($P < 0.01$) glucose. The changes in PON levels were found insignificant in all groups compared to their control groups respective. Carnitine levels were significantly lowered ($P < 0.01$) in the FD group in compared to the control group (Table 4).

DISCUSSION

As shown in Table 3, body weight decreased ($P < 0.05$) in the TQ group compared to the control group. Previous studies on rats reported that rats which received a daily administration of 1 mg/kg of NS oil had significantly lower body weight than a control group [8]. This finding shows that reduced body weight may be associated with TQ-induced body weight loss in this group in the present study.

A high fat diet leads to increases in body weight, mainly due to excessive energy intake compared to a standard diet [9]. In this study, however, the numerical increases in body weight were found to be statistically insignificant in the FD group compared to the control group at the end of the 6th week. Several studies reported that body weights in high fat diet groups were heavier than standard diet groups during 6-12 week periods [9,10]. The reason for this result may be due to the short experimental period of

Table 3. Mean body weight (g) of the groups during the experimental period ($X \pm$ SEM)

Tablo 3. Deneme süresi boyunca grupların ortalama canlı ağırlıkları (g) ($X \pm$ SEM)

| Period | Control Group | Group TQ | Group FD | Group FD+TQ | P |
|----------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|-------|
| 0 day | 247.00 \pm 6.80 | 256.50 \pm 4.17 | 258.00 \pm 5.81 | 242.40 \pm 5.81 | 0.201 |
| 1 st week | 275.70 \pm 6.05 ^b | 242.50 \pm 7.04 ^a | 271.40 \pm 5.72 ^b | 261.60 \pm 7.09 ^b | 0.005 |
| 2 nd week | 277.00 \pm 6.49 ^b | 249.30 \pm 5.96 ^a | 286.20 \pm 6.05 ^b | 262.70 \pm 8.70 ^{ab} | 0.004 |
| 3 rd week | 283.50 \pm 6.18 ^{bc} | 252.50 \pm 6.20 ^a | 292.60 \pm 6.13 ^c | 270.90 \pm 8.33 ^{ab} | 0.001 |
| 4 th week | 282.80 \pm 5.94 ^{bc} | 261.60 \pm 7.40 ^a | 299.20 \pm 5.22 ^c | 276.10 \pm 8.18 ^{ab} | 0.004 |
| 5 th week | 292.70 \pm 6.05 ^{ab} | 274.00 \pm 6.80 ^a | 297.60 \pm 4.69 ^b | 280.00 \pm 7.27 ^{ab} | 0.041 |
| 6 th week | 297.30 \pm 6.13 ^{bc} | 278.90 \pm 6.50 ^a | 305.80 \pm 3.81 ^c | 287.70 \pm 6.76 ^{ab} | 0.017 |

S.E.M: mean standard error, Different superscripts ^{a, b, c} in the same line indicate a significant difference between groups ($P < 0.05$)

Table 4. Results of the analysis of biochemical parameters in all groups ($X \pm SEM$)**Tablo 4.** Tüm gruplardaki biyokimyasal parametrelerin analiz sonuçları ($X \pm SEM$)

| Parameters | Group Control | Group TQ | Group FD | Group FD +TQ | P |
|-------------------------|----------------------------|---------------------------|----------------------------|----------------------------|-------|
| Leptin (pg/ml) | 434.99±52.83 ^{ab} | 283.19±32.53 ^a | 995.51±163.56 ^c | 623.86±111.92 ^b | 0.000 |
| Insulin (ng/ml) | 3.23±0.25 ^b | 2.38±0.32 ^a | 3.75±0.32 ^b | 3.36±0.27 ^b | 0.015 |
| Glucose (mg/dl) | 143.50±5.27 ^a | 170.60±6.74 ^b | 171.40±8.48 ^b | 197.10±7.31 ^c | 0.000 |
| T ₃ (ng/ml) | 1.02±0.87 ^{ab} | 0.98±0.87 ^a | 1.40±1.24 ^c | 1.15±1.08 ^b | 0.000 |
| T ₄ (µg/dl) | 5.40±4.92 ^b | 4.76±4.39 ^a | 6.39±5.60 ^c | 5.62±4.90 ^{bc} | 0.002 |
| FT ₃ (pg/ml) | 2.85±2.58 ^a | 2.55±2.18 ^a | 4.53±3.97 ^b | 4.04±3.76 ^b | 0.000 |
| FT ₄ (ng/dl) | 2.52±2.26 ^a | 2.32±2.02 ^a | 3.01±2.66 ^b | 2.61±2.34 ^a | 0.006 |
| PON(U/L) | 130.61±32.20 ^{ab} | 174.61±16.54 ^b | 93.18±18.38 ^a | 114.64±14.68 ^{ab} | 0.071 |
| Carnitine (µmol/L) | 35.33±2.06 ^{bc} | 42.83±3.39 ^c | 23.00±2.55 ^a | 29.00±2.38 ^{ab} | 0.000 |
| TC(mg/dl) | 72.60±4.16 ^{ab} | 66.90±1.93 ^a | 79.70±3.03 ^b | 77.70±2.71 ^b | 0.025 |
| TG(mg/dl) | 131.30±13.55 ^a | 127.50±10.72 ^a | 254.50±31.04 ^b | 176.60±14.01 ^a | 0.000 |
| HDL(mg/dl) | 51.60±3.86 ^b | 40.70±2.34 ^a | 38.50±3.29 ^a | 39.60±2.04 ^a | 0.013 |
| LDL(mg/dl) | 41.40±1.81 ^b | 29.17±2.19 ^a | 79.89±4.84 ^d | 56.62±3.23 ^c | 0.000 |
| VLDL(mg/dl) | 26.26±2.71 ^a | 25.50±2.14 ^a | 50.90±6.21 ^b | 35.32±2.80 ^a | 0.000 |

Different superscripts ^{a, b, c} in the same line indicate a significant difference between groups ($P < 0.05$)

this study. Body weight significantly decreased ($P < 0.05$) in both the TQ group and the FD+ TQ groups compared to the control and FD groups, respectively. This result is in agreement with previous studies [4,11]. There isn't any meaningful difference when the FD+TQ group compared to the control group. According to the literature [8], this result may be related to the effect of TQ which prevents weight gain.

Previous studies reported that reduced body weight results in dramatically decrease in plasma leptin concentration [12,13]. Le et al. [11] stated that NS treated rats had lower fasting plasma levels of insulin at the end of a 4-week period. Despite intensive research on TQ, its effects on leptin or insulin are still not very clear. As shown in Table 4, plasma leptin (though not statistically significant) and insulin levels decreased ($P < 0.05$) in the TQ group. This finding shown that reduced plasma insulin levels may be associated with TQ-induced body weight loss as TQ reduced food intake [8]. Additionally, other research has stated that during food deprivation, levels of insulin are decreased by low plasma leptin [14], which is consistent with the report of Seeley and Schwartz [13]. Research has recently indicated that leptin, insulin and thyroid hormones are the most conspicuous hormones in the regulation of energy metabolism [15]. Leptin, an adipocyte-derived hormone, stimulates signals from adipose tissue for energy expenditure and increases in the basal metabolic rate and energy expenditure [13]. Insulin regulates lipid metabolism by stimulating lipogenesis and so inhibiting fatty acid oxidation. Plasma leptin and insulin levels are increased by a positive energy balance, and thereby activate catabolic pathways and inhibit anabolic pathways. Thus, these changes in the central nervous system changes lead to

decreased food intake and increased energy expenditure, resulting in the loss of excess calories stored in the form of adipose tissue [16].

In this study, when the plasma leptin level significantly increased ($P < 0.01$), an enhanced insulin level was not found statistically significant in the FD group with respect to control group. Woods et al. [10] indicated that plasma leptin levels increased in rats with fed a high fat diet, due to increasing fat mass. Additionally, the 36.36% unsaturated fatty acids in the animal fat used in this study could increase the plasma leptin level, consistent with the report of Yıldız et al. [17]. In this study, however, parallel to the increase in the plasma leptin level in the other study, variations in the insulin of the group eating a fatty diet were found insignificant compared to the control group, which was not consistent with the findings of Woods et al. [10]. This difference may be attributed to the unchanged body weight of the rats or the short experimental period of this study. As seen in Table 4, the plasma leptin level significantly decreased ($P < 0.01$) in the FD+TQ group compared to the FD group while the insulin level was not significantly changed. These results show that decreased plasma levels of leptin may be related to reduced body weight caused by TQ in the same group.

Despite intensive study of the effects of TQ on diabetes, the effects of neither NS oil nor TQ on insulin or leptin levels in animals fed with a fatty diet have been investigated until now. However, several studies reported that TQ increased insulin levels while decreasing blood glucose levels in diabetic rats [18]. Additionally, Kanter et al. [19] demonstrated that NS treatment caused a sharp decrease in the elevated serum glucose and a slight increase in the lowered serum insulin concentrations in diabetic rats. In this study,

however, plasma glucose levels increased ($P < 0.01$) in all experimental groups compared to the control group. The increased glucose, however, was not at hyperglycemic levels (300 mg/dl) [20]. This study thus did not replicate the hypoglycemia obtained by administering the fixed oil of *NS* [8] to normal rats. However, recent studies have demonstrated that the plasma glucose level remained stable [11] or slightly increased [21] in normal rats treated with *NS* or *TQ*. This study's result is thus in line with the findings of previous research. The results show that increased plasma glucose may be due to a decreased insulin level in the *TQ* group. Furthermore, there was a statistically significant increase ($P < 0.01$) in the plasma glucose level in both the *FD* group and the *FD+TQ* group as compared with their control groups respectively. The reason for this may be that the high fat diet induced hyperglycemia, hyperinsulinemia and insulin resistance [9]. However, the short experimental period of this study may not have been long enough to observe hyperinsulinemia and insulin resistance.

Thyroid hormones are potent stimulants of basal metabolism, leading to loss of body weight and reduced circulating leptin and insulin [15]. Several studies have investigated the relationship between leptin and thyroid hormones; some found a negative correlation [22], while others found a positive correlation [23]. The effects of *TQ* on thyroid hormones in rats have not yet been sufficiently investigated. In this study, T_4 concentration and insulin levels significantly decreased ($P < 0.05$) in the *TQ* group as compared to the control group, while the other thyroid hormones did not significantly change. Given the restrictive effect of *NS* or *TQ*-induced food intake reduction [11], these results may be associated with *TQ*-induced body weight loss and/or a reduced leptin level in the same group. In addition, thyroid hormones change during the transition from the fed to the starved state, since starvation rapidly inhibits T_4 and T_3 levels.

Leptin is involved in maintaining TRH (Thyrotrophin-releasing hormone), which is necessary for the synthesis of TSH (thyroid stimulation hormone) and other thyroid hormones [22]. Brito et al. [24] reported that a high fat and low-protein diet could have a direct effect on TSH secretion or influence pituitary T_4 uptake or deiodination. In this study, all thyroid hormones, in parallel with leptin levels, significantly increased in the *FD* group as compared to the control group. Additionally, Avci et al. [25] reported that plasma leptin, insulin and FT_3 levels increased in mice fed a high fat diet while the FT_4 level was decreased. Similar findings were reported elsewhere [24,26]. Compared with the *FD* group, in particular, T_3 and FT_4 levels in parallel with leptin levels significantly decreased ($P < 0.01$) in the *FD+TQ* group. Given that leptin stimulates the conversion of T_4 to T_3 [24], changes in thyroid hormones may be associated with a reduced leptin level in this group.

Paraoxonases (PON_1 , PON_2 , PON_3) are a group of enzymes

involved in the hydrolysis of several organophosphates and aromatic carboxylic acid esters of fatty acids. In addition, PON_1 is an esterase closely associated with HDL, and may contribute to HDL's antiatherogenicity by preventing the oxidation of LDL [27]. In this study, serum PON levels did not change in any of the experimental groups compared to the control group. Previous studies have shown that the PON level decreased in rats and mice fed a high fat diet for several weeks, due to a reduction of PON_1 mRNA levels in males and to a reduced stability and/or number of HDL particles responsible for PON_1 transport in females [27,28]. In the *TQ* group compared with the control group, HDL and LDL levels were significantly lowered, while the remaining lipid parameters did not decrease significantly. In the *FD* group, compared to the control group, a decreased HDL ($P < 0.05$) level and increased TG, LDL and VLDL ($P < 0.01$) levels were found significant. However, TG, LDL and VLDL levels were significantly decreased ($P < 0.01$) in the *FD+TQ* group as compared with the *FD* group. These results may be associated with a *TQ*-induced hypocholesterolemic effect by decreasing the LDL level significantly. Al-Naqeep et al. [4], state that *TQ* and *TQ*-rich fractions extracted from *NS* seeds caused a hypocholesterolemic effect by inhibiting hepatic HMG-CoAR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) and reducing the expression mRNA level of LDL receptors. Ahmad and Beg [29], reported that thymoquinone phenolic compound common to the methanolic extract (2.64% contained *TQ*) and volatile oil extracted (13.53% contained *TQ*) from *NS* seed oil extracts ameliorated all the cardiovascular risk parameters via reduction in hepatic HMG-CoAR activity, levels of LDL receptor and antioxidant mechanisms in atherogenic suspension fed rats.

Carnitine is an important bio-molecule for the transport of long-chain fatty acids from the cytosol into the mitochondria during beta-oxidation [9]. Normal plasma carnitine concentration may be regulated by dietary sources, excretion from the kidney and endogenous biosynthesis. A few studies have reported that the plasma carnitine level was neither reduced [30] or increased [31,32] in rats or mice fed a high fat diet. In the *TQ* and the *FD+TQ* groups, compared with the control group, carnitine levels did not change while they significantly lowered ($P < 0.01$) in the *FD* group in comparison with control group. The decrease of plasma carnitine level in the *FD* group seems consistent with the results of the previous study [30]. There is no information to explain the relationship between *TQ* and carnitine. Thus, further research on this subject is needed.

In conclusion, the observed leptin, TG, LDL, VLDL-reducer effects of *TQ* may have medicinal value and explain its ethnomedical use. This study suggests that *TQ* may be a natural source for the prevention of the harmful effects of a fatty diet, but more advanced research is needed to explain the relationship between *TQ* and energy metabolism.

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