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THE INVESTIGATIONS OF TOTAL ANTIOXIDANT STATUS AND BIOCHEMICAL SERUM PROFILE IN THYMOQUINONE-TREATED RATS

Kurt E[a], Dede S[a]*, Ragbetli C[b]

[a]Biochemistry Department, Veterinary Medicine School, Yuzuncu Yil University, Van, 65080, Turkey.
[b]Microbiology Department, Medical School, Yuzuncu Yil University, Van, 65080, Turkey.
*Corresponding author: Dr. Semiha Dede, mailing address: Yuzuncu Yil University, School of Veterinary Medicine, Biochemistry Department, Van, 65080, Turkey.
***E-mail:** sdede@yyu.edu.tr.

Abstract

Background: This study was planned to determine the dosage and duration of thymoquinone (TQ) at which it demonstrates the optimum effect on the total antioxidant status (TAS) and the biochemical parameters in the blood serum.
Materials and Methods: 48 male rats (Wistar-albino) weighing between 200-250 g were used as material. Group 1 (control) (TQ solution 5 mg/kg/day), Group 2 (15 mg/kg/day), Group 3 (30 mg/kg/day), Group 4 (45 mg/kg/day) and Group 5 (60 mg/kg/day) were designated, each containing 8 rats. Different doses of TQ (oral gavage) were administered for four weeks.
Results: The TAS levels were determined to be considerably low statistically in all TQ-administered groups in comparison with the control group. It was determined that the serum biochemical parameters exerted a significant effect depending on the TQ doses.
Conclusion: As a result, rats administered with TQ orally, at 60 mg/kg dosage, show that the liver and kidney function parameters in particular as different from normal. This brings us to the conclusion that at this dosage, there is reliable biochemical wise but future protective studies in which 30 mg/kg doses can be used safely is encouraged.

Key words: biochemical parameters, rat, thymoquinone, total antioxidant status (TAS)

Introduction

Thymoquinone (TQ) - a type of monoterpene, is a major compound of the *Nigella sativa* seed with promising medicinal herb showing various medicinal effects, and first synthesized in 1959 (Aggarwal et al., 2008). *Nigella sativa* (black cumin) is a medicinal herb that has been used in the treatment of many illnesses for over 2000 years in most countries in the Middle East and the Far East (Medenica et al., 1993).
It has been found that TQ has various positive effects on human health. In addition to its anti-oxidant, anti-carcinogenic, anti-bacterial, anti-inflammatory, anti-neoplastic, anti-ulcer, anti-fungal, antitumor, and anti-allergic characteristics, TQ is renowned for its positive effect on the central nervous system (CNS). Moreover, it is also discovered that it has effects on apoptosis, the cell cycle and the immune system (Al-Naggar et al., 2003; Aljabre et al. 2005; Arslan et al., 2005; Awad, 2005; Gali-Muhtasib et al., 2006).

Under normal physical conditions, the organism embodies a complex antioxidant immune system against free radicals caused by endogenic and exogenic factors and the oxidative stress resultant of these radicals. TAS comprises mainly antioxidant molecules in plasma in which it involves a certain amount of transferrin, ceruloplasmin, albumin, uric acid and ascorbic acid, bilirubin, GSH, flavonoids, α-tocopherol and β-carotene. In recent years, the total antioxidant status (TAS) measurement, rather than separate measurement of individual antioxidants, has become a prominent practice in determining the antioxidative condition of blood (Yao et al., 1998; Prior and Cao, 1999; Ghiselli et al., 2000; Erel, 2004).

Diagnosing an illness and monitoring its course and the response to treatment require the biochemical profiling of blood. Blood parameters such as glucose, urea, creatinine, cholesterol, triglyceride, albumin, globulin, total protein, total lipid, bilirubin; electrolytes such as Ca, Na, K, P; and enzymes such as AST, ALT, ALP, CK are some of the most important biochemical parameters that determine the biochemical profile. The respective parameters have a great importance in the diagnosis, treatment, and prognosis tracking (Karagül et al., 2000).

There are numerous in vivo and in vitro studies conducted regarding the antioxidant characteristics of TQ (Kanter et al., 2003; Gündüz et al., 2002; Şahin et al., 2003; Deger et al., 2004; Khalife and Lupidi, 2007). There are some researches indicating that TQ is an efficient cytoprotective agent against the chemically induced hepatic damages during animal experiments (Al-Gharably et al., 1997; Daba and Abdel-Rahman, 1998; Kanter et al., 2003).

This study was planned to determine the effective dose and time of TQ treatment on the total antioxidant capacity and biochemical parameters.

Material and Methods

Animals

In the study, we used 48 male rats weighing between 200-250 g, acquired from the Yuzuncu Yil University, Faculty of Medicine, Experimental Research Laboratory. The study groups included 1 control (C) and 5 trial groups, each containing 8 rats. During the 4 weeks of the trial, the rats were contained in cages, in which the feed and fresh water were present all the time, located in a room with a temperature of 22 ± 2°C and illumination 12 hours per day.

Design of experiment groups

Control group: A randomly selected group of 8 rats was separated as the control group. They were treated with corn oil per day for 4 weeks.

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For a period of 4 weeks, the TQ solution (**Group 1:** 5 mg/kg/day, **Group 2:** 15 mg/kg/day, **Group 3:** 30 mg/kg/day, **Group 4:** 45 mg/kg/day, **Group 5:** 60mg/kg/day) dissolved in corn oil was treated orally to the eight rats in these groups.

Samples Collection

After 4 weeks, the blood samples were obtained from the left ventricular vein of animals' hearts under Ketalar anesthesia and transferred to glass serum tubes. The blood samples contained in tubes were centrifuged for a period of 10 minutes at +4°C and 3000 rpm. TAS and biochemical parameter analyses were conducted on these samples.

Biochemical analysis

The total antioxidant status was measured in the acquired serum samples through spectrophotometer using the commercial TAS kit (RelAssay, Turkey). The concentrations of total protein, albumin, globulin, total bilirubin, triglyceride, cholesterol, VLDL, HDL, urea, creatinine, uric acid and BUN, and ALP, AST, ALT, and GGT activity determination were conducted using the modular auto-analyzer device (Roche, Germany)

Statistical Analysis

The data from the control and experimental groups were analyzed with One Way Variance analysis and the Duncan test was applied for multiple comparisons. The differences were considered significant when the p-value was lower than 0.05.

Results

The obtained results for this study have been summarized in Table 1.

Table 1: Biochemical parameters and TAS among TQ-applied rats

Parameters	Control Group	Group 1	Group 2	Group 3	Group 4	Group 5
Total protein (g/dl)	6.12±0.31a	6.01±0.26a	5.95±0.22a	6.09±0.34a	6.00±0.11a	7.19±0.36b
Albumin (g/dl)	3.32±0.10a	3.42±0.160a	3.57±0.14a	3.42±0.38a	3.55±0.05a	2.31±0.21b
Globulin (g/dl)	2.79±0.25a	2.60±0.21a	2.37±0.15a	2.680±0.404a	2.625±0.073a	4.900±0.513a
Total bilirubin (mg/dl)	0.116±0.015a	0.090±0.012ab	0.076±0.010b	0.082±0.004ab	0.110±0.008ab	0.270±0.023c
Triglyceride (mg/dl)	77.59±6.77ab	102.41±10.81abc	118.206±19.93bc	79.48±12.07ab	72.03±6.31b	120.97±4.74c
Cholesterol (mg/dl)	70.29±7.71ab	66.38±3.38b	65.14±5.09b	65.00±4.79b	87.50±9.72ab	93.00±9.87c
VLDL (mg/dl)	15.57±1.41ab	20.50±2.14bc	23.71±4.01c	15.80±2.44ab	14.25±1.29a	24.33±0.88c
HDL (mg/dl)	47.29±4.82a	49.50±3.56ab	51.85±4.75ab	52.80±4.16ab	67.75±8.11b	26.67±3.66c
ALP (mg/dl)	149.86±8.88a	254.75±20.92ab	266.83±31.52bc	421.50±74.67c	267.75±31.24ab	460.67±58.86c
AST (mg/dl)	126.14±21.50a	168.00±20.67a	141.71±9.79a	148.00±9.87a	179.00±24.58a	887.33±162.24b
ALT (mg/dl)	36.14±4.28a	48.13±4.58a	45.43±4.59a	45.80±4.83a	37.25±1.88a	146.33±45.18b
GGT (U/L)	1.50±0.08a	1.24±0.17a	0.57±0.19b	1.30±0.60a	1.25±0.17a	1.10±1.34b
Urea (mg/dl)	30.57±1.68a	31.09±1.76a	30.67±0.98a	34.86±2.58a	35.10±2.34a	132.23±67.30b
Creatinine (mg/dl)	0.363±0.025[a]	0.334±0.024a	0.360±0.022a	0.320±0.014a	0.348±0.003a	0.817±0.149b
Uric acid (mg/dl)	1.25±0.11	1.35±0.15	1.66±0.24	1.35±0.11	1.66±0.24	1.83±0.58
BUN (mg/dl)	14.9±0.78a	14.63±0.78ab	14.14±0.51ab	16.20±1.32 ab	16.50±1.09b	28.7±2.73c
TAS (mmoL of Trolox/L)	8.26±0.94a	5.84±0.64b	6.79±0.63bc	5.65±0.49b	6.58±0.85bc	6.81±0.51bc

There was no statistical difference between the same letters in the same sequence. P<0.05

The highest total antioxidant status (TAS) levels were found in the control group. And among the TQ-applied groups, the lowest levels were observed in groups 1 and 3. However, it was observed that the TQ application provided no significant difference among the groups in terms of TAS.

Only in group 5, there was a significant increase present in total protein and globulin levels ($p<0.05$). No considerable change was observed in other groups. While the albumin levels were decreased significantly in group 5 ($p<0.05$), no statistically significant difference was determined between the other groups and the control group.

The total bilirubin levels were found to be highest in group 5 ($p<0.05$), and lowest in group 2 ($p<0.05$). In the other groups, no significant change was observed with regard to the control group. In groups 2 and 5, the triglyceride levels increased significantly with regard to the control group ($p<0.05$). It was found to be lowest in group 4 ($p<0.05$). In the other groups, no significant change was observed with regard to the control group. A significant increase in the cholesterol levels was observed only in group 5 ($p<0.05$), while there was no statistically significant differences between the other groups and the control group. The VLDL levels were observed to be increased significantly in groups 2 and 5 ($p<0.05$), while no statistically significant changes were found between other groups and the control group. The HDL levels were decreased significantly in group 5 ($p<0.05$), while they were increased significantly in the other groups with regard to the control group.

The ALP levels were found to be increased significantly in groups 2, 3 and 5 with regard to the control group, while no statistically significant change was observed between the other groups and the control group. The AST, ALT and CK levels were increased significantly only

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in group 5 ($p<0.05$). The GGT levels were found to be decreased significantly in groups 2 and 5 ($p<0.05$), while no statistically significant changes were found between the other groups and the control group.

Urea and creatine levels increased significantly in group 5 ($p<0.05$), while no statistically significant changes were found between other groups and the control group. Although a slight increase was observed in group 5, no statistically significant difference was found regarding the uric acid levels. The BUN levels were found to be increased significantly in group 5 compared to all other groups ($p<0.05$). Furthermore, there was also an increase in group 4 with regard to the control group. No statistically significant changes were found in the other groups.

Discussion

It is generally known that TQ has a hepatoprotective effect (Daba and Abdel-Rahman, 1998). It has been reported that TQ application does not affect the liver enzymes and therefore safe. An increase in ALT and ALP indicates hepatocellular catabolism and pathological changes in bile flow (Sivaramakrishnan et al., 2008).

TQ application inhibits the increase in hepatic enzymes. Since this effect reduces the efflux of liver enzymes, it is possible that TQ application may result in the stabilization of hepatocyte membranes (Ismail et al., 2010; Sayed-Ahmed et al., 2010). It was observed that liver enzyme (ALT, AST) activities were not affected/changed after the application of TQ at nutritional dosage (10 and 20 mg/kg/day) for 8 weeks (El-Barbry et al., 2012).

In prevention of experimentally induced hepatoxicity, it was reported that TQ application reduced the AST, ALT, ALP (Nili-Ahmadabadi et al., 2011), serum ALT (Nagi et al., 2010) and ALT and AST (Bai et al., 2013), ALT, ALP, (Sayed-Ahmed et al., 2010) ALP and GGT (Helal, 2010) activities, all of which increased due to hepatoxicity, and partially protected against hepatotoxicity (Al-Gharably et al., 1997; Nagi et al., 1999)

While a significant increase in AST, ALT and ALP activities were found only in group 5, ALP activities were found to be significantly increased in groups 2, 3 and 5. GGT activities were significantly decreased only in groups 2 and 5. The fact that liver enzyme activities were increased mostly in group 5 led us to think that the applied TQ dosage (60 mg/kg) contributed to a metabolism in the liver, while other dosages were safe.

It has been reported that TQ application reduces the serum total cholesterol, LDL and triglyceride levels (El-Dakhakhny et al., 2000). It is thought that TQ can reduce the serum lipid and plasma cholesterol levels depending on its antioxidant activities (Swamy and Tan, 2000; Zaoui et al., 2002; Ragheb et al., 2008). TQ is protective against cellular catabolism caused by LDL (Tardy et al., 2011). It was seen that cholesterol, triglyceride, HDL and LDL levels were decreased following TQ application through i.p (Bamosa et al., 2002). However, there is a study indicating that i.p. application increases the triglyceride levels, which is an indicator of hyperlipidemia, whereas oral application induces no changes in the triglyceride levels (Abu Khader, 2012). It was seen that TQ application on rats that had been subjected to experimentally induced hyperlipidemia decreased the blood cholesterol (Badary et al., 2000), total cholesterol and LDL (Ismail et al., 2010), TC, LDL-C (Nader et al., 2010) levels significantly, and decreased the HDL levels. It was concluded that this was a result of TQ's anti-atherosclerotic and antioxidant characteristics (Nader et al., 2010).

The triglyceride, cholesterol, and VLDL levels were observed to be increased significantly in group 5 in comparison to the control group, while the HDL levels were seen to be decreased significantly. In group 2, the triglyceride and VLDL levels had increased significantly. While the triglyceride levels were lowest in group 4, the HDL levels were seen to be increased significantly in all other groups except for group 5 with regard to the control group. These results showed that 60 mg/kg (group 5) of TQ application poses a risk on blood lipids while other dosages presented positive effects on blood lipid.

It was reported that experimentally increased total bilirubin levels decreased to regular levels consequent to TQ application (Sayed-Ahmed et al., 2010; Helal, 2010). In this study, the total bilirubin levels were found to be highest in the group subjected to 60 mg/kg of TQ, whereas the group with 15 mg/kg of TQ application had the lowest levels ($p<0.05$). It can be said that this case was confirmed with the increase in bilirubin that occurred following the high dosage of TQ application, which also led to an increase in liver enzymes.

The increase in serum BUN is a parameter indicating renal tissue damage, whereas an increase in serum creatinine levels indicates renal failure (Erdem et al., 2000). TQ application in treatment of nephrotoxicity formed experimentally depending on various factors was seen to reduce the increased urea (Kanter, 2009), urea and creatinine (Ragheb et al., 2009; Sayed-Ahmed and Nagi, 2007), BUN and creatinine levels (Başarslan et al., 2012) to normal levels. In this study, urea, creatinine and BUN levels in the group subjected to 60 mg/kg of TQ were found to be increased significantly with respect to other groups and the control group, and BUN levels were found to be increased significantly in the group subjected to 45 mg/kg of TQ. In the light of the data given above, we understand that TQ application reduces the levels of urea, creatinine and BUN, which are readily increased. In this study, 5-30 mg/kg of TQ applications did not affect the relevant parameters, which can be interpreted as the confirmation of the study's reliability.

It was seen that the application of TQ at nutritional dosage for 8 weeks had no effect on albumin levels (El-Barbry et al., 2012). It was reported that application of TQ at a dose of 50 mg/kg onto STZ diabetic rats, improved the levels of serum albumin, total protein and albumin removed through urination with regard to renal morphology and functionality. It was reported that the symptoms of experimentally induced nephrotic syndrome, ie. hypoalbuminemia, hypoproteinemia, excretion of albumin and protein through urination, were relieved consequent to the TQ application (10 mg/kg/day) (Badary et al., 2000).

In this study, it has been determined that the albumin levels were decreased significantly in group 5. Symptoms of nephrotic syndrome such as removing albumin through urination (Badary et al., 2000), may lead to a decrease in serum albumin levels. The globulin and total protein levels decreased significantly only in group 5. It was thought that the increase in total protein was due to the increase in globulin. The increase in albumin and the decrease in globulin observed in our study were thought, with reference to the above-given information, to be caused by the changes induced by the metabolism of TQ applied at 60 mg/kg dosage.

The Total antioxidant status (TAS) is an integrated parameter representing the current cumulative level of all antioxidants in the plasma and body fluids, rather than a simple sum of antioxidant levels. It provides insight about known and unknown antioxidants and their synergistic interaction capacity, and thus, the in vivo sensitive balance among oxidants and antioxidants. It is important in understanding the possible variation mechanisms during the formation of homeostatic plasma and oxidative stress and TAS control in tissues. Recently, TAS, has been proposed as a new instrument to investigate the relationships between epidemiological practices, dietary antioxidants, and cancer-risks in demographic studies (Serafini and Del Rio, 2004; Ghisellia et al., 2000).

When TQ was applied as a protective agent following the experimental application of various chemical substances, it was reported that total antioxidant state (El-Saleh et al., 2004), SOD (El-Abhar et al., 2003; Abdelmeguid et al., 2010), glutathione (Ragheb et al., 2009; Helal, 2010; Nili-Ahmadabadi et al., 2011), GSH, GPx and catalase (Sayed-Ahmed and Nagi, 2007), GST, GSH and catalase (Hamdy and Taha, 2009) activities approximated with the control values.

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TQ application increases the plasma and liver antioxidant capacity, and the antioxidant enzyme activities as a result of its regulatory impact on gene expression (Ismail et al., 2010; Sayed-Ahmed et al., 2010).

It was seen that the decrease in TAS levels was statistically significant among all groups. If we take into consideration that TAS is an integrated parameter representing the current cumulative level of all antioxidants in the plasma and body fluids, rather than a simple sum of antioxidant levels, as can be seen through the examples from the literature given above, it has been reported that there are cases where various enzymatic and non-enzymatic antioxidant levels increase and decrease. In this study, it was found that TQ application decreased the general capacity of TAS in the blood serum. This result may arise from the balance between the use of different antioxidants in the current situation or after re-synthesis and entry into the most metabolized tissues such as the liver, muscle, brain, etc.

Although TQ can be safely used at certain dosages, there are a few researches that have focused on its toxicity (Al-Ali et al., 2008; Abu Khader, 2012). It was found that a high dosage of LD50 90.3 mg/kg (77.9-104.7, 95% CL) formed oxidative stress that caused hepatic damage (Mansour et al., 2001). The LD50 for both orally applied and i.p TQ application on mice was investigated and LD50 for i.p was determined as 104.7 mg/kg (89.7-119.7), and 870.9 mg/kg (647.1-1094.8) for the oral applications. For rats, the LD50 for i.p has been determined as 57.5 mg/kg (45.6 - 69.4) and 794.3 mg/kg (469.8-1118.8, %95) for oral applications (Al-Ali et al., 2008). The LD50 levels determined for this study were 10-15 times higher i.p and 100-150 times higher in oral applications than the dosages used in anti-inflammatory, anti-oxidant and anti-cancer experimental treatments. TQ is a relatively safe compound (Al-Ali et al., 2008).

The maximum tolerable TQ levels for Wistar rats in both i.p and oral applications were estimated as follows: 20, 30 and 40 mg/kg (i.p) and 200, 300 and 500 mg/kg. When applied at higher dosages than those levels, acute pancreatitis and acute toxication symptoms were observed in i.p, and bowel obstruction complications were observed in oral applications. (Abu Khader, 2012). Furthermore, it was discovered that applying TQ at high dosages had lethal effects (Mansour et al., 2001). It was seen that in oral application of TQ, the LD50 level for rats was ranging between 90-500 mg/kg. In this study, following the application of TQ at the 60 mg/kg dosage, it was observed that the renal function parameters deviated from the regular values, which indicates that this dosage is problematic in terms of biochemical reliability.

Consequently, with regard to the data acquired in this study, it has been concluded that in further research, where various protective features of TQ will be investigated in our laboratory, the protective dosages should be limited to 30 mg/kg/day and this should be taken into consideration when planning the groups in practices involving varying dosages.

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