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# Effects of Thymoquinone (TQ) on the Molecular Structure and Total Antioxidant Capacity of Cerebellum Tissue of Healthy Rats

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## ABSTRACT

**Objective:** The neuroprotective effects of thymoquinone (TQ), the active compound of *Nigella sativa*, have been reported in accordance with the anti-inflammatory and antioxidant features of this drug. It has been suggested that the cerebellum plays a considerable role in neurodegenerative processes. In the current study, the possible effects of TQ on the structure and composition of cerebellum tissues and its total antioxidant capacity were studied dose-dependently.

**Materials and Methods:** Fifteen adult Long Evans female rats were divided into groups as follows: G1: Control, G2: 10 mg/kg TQ treatment, G3: 20 mg/kg TQ treatment. TQ was injected into the rats intraperitoneally for two weeks. The control group only received corn oil used for the dissolving of TQ. Fourier-transform infrared spectroscopy (FTIR) studies and total protein, and antioxidant capacity measurements were carried out with cerebellum tissues which were removed following the decapitation of rats.

**Results and Conclusion:** 10 mg/kg TQ treatment improved the saturated and unsaturated lipid and protein content in addition to decreasing nucleic acid content and lipid peroxidation and increasing the total antioxidant capacity of cerebellum tissues. However, 20 mg/kg TQ treatment did not have any significant effect.

**Keywords:** Thymoquinone, cerebellum, total antioxidant capacity, FTIR spectroscopy

## INTRODUCTION

In recent years, many neurodegenerative disorders have been treated by plants commonly used in traditional medicine. These plants are preferred for easy access, collection and minimal side effects among the public (1). *Nigella sativa* (NS) which is a member of *Ranunculaceae* family, is known as black seed or cumin. NS has been proven to have anti-inflammatory and antioxidative effects besides its neuroprotective role (2), and its active compound thymoquinone (TQ) has been indicated to play an important role on oxidative stress and inflammation processes in the body as well (1).

In previous studies, the neuroprotective effects of TQ on neurological disorders were reported in both cell culture and animal studies (3-7). This neuroprotective effect results from its anti-inflammatory and antioxidant properties. One possible mechanism is its inhibitory effect on acetylcholinesterase enzyme which breaks down the neurotransmitter acetylcholine in the neuromuscular junction (6). The other mechanisms include the reduction of pro-inflammatory cytokines and chemokines besides the inhibition of nitric oxide synthase enzyme (3). TQ's antioxidant effect includes the reduction of reactive oxygen species (ROS) produced in oxidative stress. In neurodegenerative diseases, it has also been reported that an abundant value of ROS was produced by the activation of microglial cells as in Alzheimer's disease (8).

The cerebellum, which is located near the brainstem, is a primary component of the hindbrain. The main function of the

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cerebellum is maintaining motor coordination for movement and balance of the body (9). Structural and functional alterations in cerebellum tissue were reported in different neurodegenerative disorders including Alzheimer's disease, (10), Parkinson disease (11), and multiple system atrophies (12). Although the role of the cerebellum in the clinical condition of neurodegenerative diseases is still unclear, disruption in the structure and function of the brain, including the cerebellum, is well documented. A recent study showed that the cerebellum shares the same pathology with the cerebral cortex in neurodegenerative disorders (9).

In a study conducted by Ismail et al. (13), the protective effect of TQ for the β-amyloid peptide$_{1-40}$-induced neurotoxicity was investigated by *in vitro* studies using neuron cells. According to the results of this study, TQ has a neuroprotective effect on the apoptosis of cerebellar granule neurons (CGNs) due to β-amyloid peptide$_{1-40}$ (13). In another recent study, the antioxidant effect of TQ was investigated in the cerebellum of rats who had consumed a high-fat diet (HFD) (1). Since HFD is accepted as a major problem for neuronal damage, oxidative stress markers were measured in the serum of those rats to investigate the meliorative effect of TQ after 4 weeks of HFD supplementation. Alrafiah reported that the treatment with TQ reduced the inflammation and ameliorated the antioxidant enzymes implying an improvement in the HFD-induced neuronal damage in cerebellum tissues (1).

The cerebellum plays a considerable role in neurodegenerative processes, and revealing the molecular and antioxidant effects of TQ in the structure, composition and function of the cerebellum is promising for the prevention of neuronal damage. In the light of previous studies, the aim of the current study was to investigate the dose-dependent effects of TQ on the structure and composition of cerebellum tissues. Besides the structural changes, the effects of TQ in the antioxidant levels of the cerebellum were also studied in the current study.

In recent years, the biomolecular alterations in different parts of brain tissue (14, 15) and other hard (16-18) and soft tissues (19, 20) were studied by our group with attenuated total reflectance attenuated total reflection-fourier-transform infrared (ATR-FTIR) spectroscopy and FTIR microspectroscopy techniques. The TQ-induced variations on the content and confirmation of the cerebellum proteins, lipids and nucleic acids were also studied by ATR-FTIR spectroscopy in the current study.

## MATERIALS AND METHODS

### Animal Studies

In the current study, adult Long Evans female rats (400-450 g) were divided into 3 groups:

G1: Control group (n=5)

G2: 10 mg/kg TQ treatment group (n=5)

G3: 20 mg/kg TQ treatment group (n=5)

*Ad libitum* standard lab chow diet besides tap water were provided for the rats. The rats were kept in a 12-h light/12-h dark cycle at room temperature (21°C). TQ dissolved in corn oil (2-2,5 mL) was given to the rats by intraperitoneal (ip) injection every day for 14 days. The control group was only given corn oil with ip injection for the same period.

The doses used in the current study were selected according to previous *in vivo* studies in the literature which investigated the antioxidant effects of TQ in different tissues (21-23). Those effects were commonly seen in TQ doses of between 10 and 20 mg/kg.

Throughout the experiment process guidelines provided by "The Guide for the Care and Use of Laboratory Animals" were followed. All the experimental processes of the study were confirmed by the Scientific Ethical Committee of Bezmialem Vakif University (2016/317).

After two weeks, the rats were decapitated, and the cerebellum tissues were collected from the rats. They were kept at -80°C until ATR-FTIR spectroscopy and biochemical studies.

### ATR-FTIR Spectroscopy Studies

The cerebellum tissues were first washed with PBS (0.01M, pH 7.4) to remove any blood surrounding the tissue. Tissue the size of a needle head was put on a crystal plate made up of diamond/zinc selenide (Di/ZnSe) on ATR attachment. The rest of the tissues were homogenized for biochemical studies given in the next sections. The spectra of the samples were recorded using 4 $cm^{-1}$ resolution with 128 scan number between 4000-650 $cm^{-1}$ by Bruker Alpha II 100 FTIR spectrometer (Bruker, Berlin, Germany). Three spectra were recorded from each sample as replicates. The average of those three spectra was calculated to be used in the following spectral analysis.

For the detailed spectral analysis, band wavenumber, band area's ratio and band's width values of the target bands were measured using OPUS v8.5 software (Bruker Optics GmbH Co., Ettlingen, Germany). In this way, the molecular variations in the proteins, lipids and nucleic acids of the cerebellum tissue due to TQ treatment were investigated in the current study.

### Total Protein Measurement

After removing the surrounding blood from the cerebellum tissue by washing with PBS solution, 250 mg of tissue were homogenized with lysis buffer for the isolation of cerebellum proteins. For the lysis buffer, the buffer found in ReadyPrep™ Protein Extraction Kit (Bio-Rad Laboratories, California, USA) was used. For the homogenization 2.5 µL lysis buffer was used for every 1 mg of tissue. After mixing the tissue with an appropriate amount of lysis buffer, the homogenization was done with a homogeniser (VELP O5, IKA Process Technology, Germany). Homogenized tissue was centrifuged at 4°C for 10 minutes at 14000g (Eppendorf 5810R, Hamburg, Germany). After the centrifugation, the supernatants were collected.

The total protein of the cerebellum tissue was calculated using Bradford assay with coomassie plus reagent and as a standard bovine serum albumin (Pierce, Thermo Fisher Scientific, Roskilde, Denmark). Optimization for the dilution of samples were done and it was decided that a 1/50 dilution for the supernatants would be the amount of dilution for the measurement.

Five microliters of each diluted supernatant were mixed with 245 µL of Bradford Reagent in 98-well microplate. Seven standards with different protein concentrations (125, 250, 500, 750, 1000, 1200, 1500 µg/mL) were used in the colorimetric measurement with microplate reader (Biochrome EZ Read 400, Biochrome LTD., Cambridge, UK) at 595 nm. The total protein concentrations of the samples were calculated using the standard curve plotted after the measurement. Three replicates for each sample were measured, the average of which was used for the evaluation of the results and the statistical analysis.

### Total Antioxidant Capacity Measurement

Hundred mg of cerebellum tissues were homogenized with PBS (0.01M, pH 7.4) at a ratio of 1:9 (the weight of the tissue in milligrams: the volume of homogenized medium in microliters) using homogenizer (VELP O5, IKA Process Technology, Germany). Homogenized tissue was centrifuged at 4°C for 10 minutes at 10000g (Eppendorf 5810R, Hamburg, Germany). Supernatans were used to carry out the total antioxidant capacity measurement with a colorimetric kit (T-AOC Colorimetric Assay Kit, E-BC-K136S) according to recommendations of the manufacturer. Samples were studied in two replicates and the average of these two values were used for the evaluation of the results and the statistical analysis.

### Statistical Analyses

The GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, California, USA) was used to test the significance of the variation between the groups. After testing for normal distribution with Kolmogorov Smirnov test, One-way ANOVA with the addition of Tukey's post-hoc test was chosen for the comparison of the groups. The mean values and the standard deviation values were calculated and used to summarize the data for each of the groups. p values less or equal to 0.05 were accepted as statistically significant in the comparison of the rat groups.

## RESULTS

In the current study, dose-dependent effects of TQ on the molecular content and antioxidant capacity of cerebellum tissues of rats were investigated.

Figure 1 shows the representative average spectra of cerebellum tissue of the healthy group. As can be seen from the figure, there are various bands belonging to the several functional groups as a part of tissue proteins, saturated besides unsaturated lipids, phospholipids and nucleic acids. The band assignment table for the labelled bands in Figure 1 is given in Table 1. The band due to

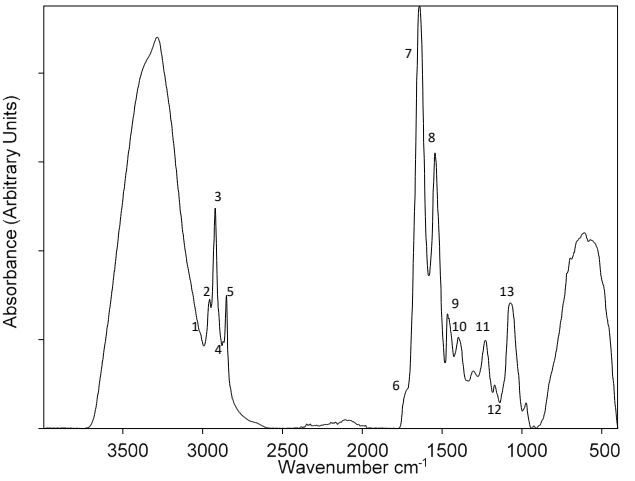


**Figure 1.** The representative spectrum of control cerebellum tissue at 3800-450 $cm^{-1}$. (*p≤0.05; **p≤0.01, ***p≤0.001, ****p≤0.0001)

**Table 1.** General band assignment of a cerebellum tissue between 3700-900 $cm^{-1}$ wavenumber region (15).

| Band # | Wavenumber value ($cm^{-1}$) | Band assignment |
|---|---|---|
| 1 | 3012 | Olefinic HC=CH stretch, due to unsaturated lipids |
| 2 | 2963 | $CH_3$ antisym stretch, due to proteins and lipids |
| 3 | 2927 | $CH_2$ antisym stretch, due to primarily lipids |
| 4 | 2873 | $CH_3$ sym stretch, due to primarily proteins |
| 5 | 2854 | $CH_2$ sym stretch, due to primarily lipids |
| 6 | 1742 | Ester C=O stretching due to triglyceride and cholesterol esters |
| 7 | 1638 | Amide I primarily C=O stretch, vibrations of amide groups of proteins |
| 8 | 1545 | Amide II primarily N-H bending with C-N stretch, vibrations of amide groups of proteins |
| 9 | 1454 | $CH_2$ bending due to lipids |
| 10 | 1398 | Fatty acids and amino acids |
| 11 | 1235 | $PO_2^-$ asym stretch, due to primarily nucleic acid with slight contribution of phospholipids |
| 12 | 1152 | Glycogen |
| 13 | 1080 | $PO_2^-$ sym stretch, due to phospholipids |

olefinic only appears in the second derivative spectra.

The spectral band area/intensity values supply information on the amounts of the functional groups related to the relative molecules; for example, a rise in the band area values correlate to higher concentrations of the molecules allotted to the spectral bands (24). Band area ratios are used instead of separate band area values in order not to be affected by any difference coming from the changes in tissue thickness during the experiment (19). Band area and intensity ratios were used to get information about total lipid and protein contents, saturated and unsaturated lipid contents besides carbonyl and nucleic acid contents.

The value of the band area/intensity ratios of the calculated bands is given in Table 2.

The band area/intensity ratios of various lipid bands located in CH region and the C=O stretching vibrations of carbonyl ester groups were used to analyse the variations in lipid structure and composition. The calculated ratios for analysing the variations in lipid's structure and composition were as follows: olefinic =CH/total lipid; $CH_2$ antisym /$CH_3$ antisym; $CH_2$ antisym / total lipid and carbonyl /total lipid.

Total lipid amounts were determined using lipid bands in the CH stretching wavenumber region (3030–2800 $cm^{-1}$) namely $CH_2$ antisym and $CH_2$ sym (15). The total lipid content which is obtained by getting the band area ratio of $CH_2$ antisym (2927 $cm^{-1}$) to $CH_2$ antisym + $CH_2$ sym (2854 $cm^{-1}$) bands, was significantly higher in the 10 mg/kg TQ treatment group compared with the control, while there was no significant change in the 20 mg/kg TQ treatment group (Table 2 and Figure 2A). This increase was also confirmed by the increase in $CH_2$ symmetric stretching band, while taking the normalization to $CH_2$ antisymmetric band seen in Figure 3.

The olefinic and carbonyl ester contents of cerebellum tissues were calculated from the band intensity ratios from second derivative spectra since these bands can be clearly seen in the second derivative of the spectrum but are difficult to see in the first derivative (Figure 4). The band intensity ratio of olefinic band located at 3014$cm^{-1}$ to the total of $CH_2$ antisym + $CH_2$ sym bands was significantly increased only in the 10 mg/kg TQ treatment group in comparison to the control (Table 2 and Figure 2B). Since this ratio gives information related to the unsaturation degree of lipids, the result referred to an increase in unsaturated lipid content in treated groups especially in the 10 mg/kg TQ treatment group (19). In addition, the band area ratio of carbonyl band at 1743 $cm^{-1}$ to the total of $CH_2$ antisym + $CH_2$ sym bands was slightly increased in the 10 mg/kg treated group implying a higher carbonyl content in the cerebellum of this group compared with the control group (Table 2 and Figure 2C) (20).

The major protein bands: Amide I (at 1640 $cm^{-1}$) and amide II (at 1545 $cm^{-1}$) were used for the calculation of total protein content by taking the band area's ratios of amide I to amide I + amide II (25). There was a significant and a slight increase in protein content of the 10 mg/kg and 20 mg/kg TQ treatment groups, respectively, in comparison to the control (Table 2 and Figure 2D). This result was also confirmed by the increase in these bands seen in Figure 5.

The total protein content of the cerebellum tissues were also calculated by Bradford assay. This result supported the results of ATR-FTIR spectroscopy studies by showing an increase of

**Table 2.** The band area/intensity ratios of the control and treatment groups.

| Band area/intensity ratio | Control | TQ 10mg/kg | TQ 20mg/kg |
|---|---|---|---|
| **Total Lipid Content** Band area ratio of $CH_2$ antisym stretch. to $CH_2$ antisym stretch. + $CH_2$ sym stretch. | 0.605±0.03 | 0.613±0.07 ↑* | 0.605±0.03 |
| **Unsaturated Lipid Content** Band intensity ratio of Olefinic HC=CH stretch. to $CH_2$ antisym stretch. + $CH_2$ sym stretch. | 0.313±0.053 | 0.421±0.060 ↑** | 0.314±0.043 |
| **Carbonyl Content** Band area ratio of Ester C=O stretch. to $CH_2$ antisym stretch. + $CH_2$ sym stretch. | 0.183±0.027 | 0.217±0.028 ↑ | 0.182±0.017 |
| **Total Protein Content** Band area ratio of Amide I to Amide I + Amide II | 0.568±0.004 | 0.602±0.008 ↑**** | 0.574±0.010 ↑ |
| **Nucleic Acid Content** Band area ratio of $PO_2^-$ antisym stretch. + $PO_2^-$ sym stretch. to Amide I + Amide II | 0.591±0.073 | 0.583±0.044 ↓ | 0.632±0.025 ↑ |

The arrows pointing upward and downward represent a relative increase and decrease, respectively, in the treatment groups compared to the control group.
*: p≤0.05; **: p≤0.01; ****: p≤0.0001

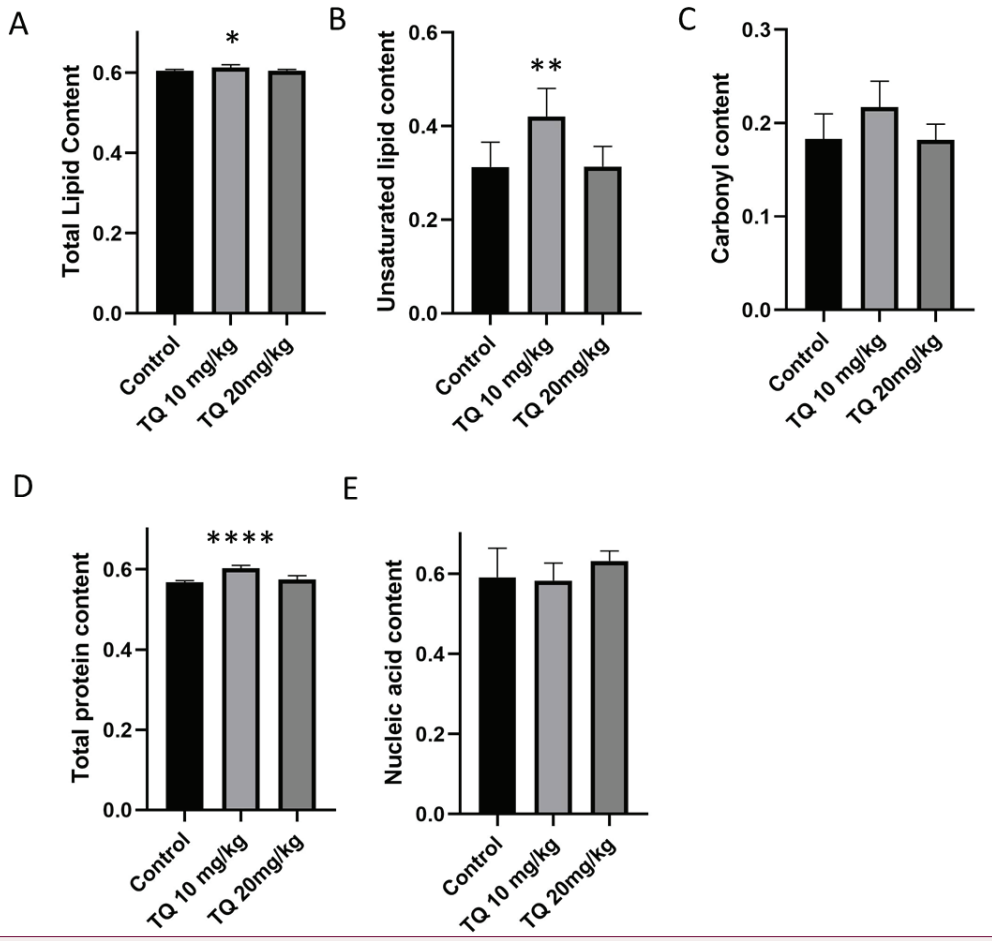


**Figure 2.** The band area/intensity ratio of different bands for the control, 10 mg/kg TQ treatment and 20 mg/kg TQ treatment groups. The significance compared to the control is indicated with a star symbol (*). *: p≤0.05; **: p≤0.01; ****: p≤0.0001

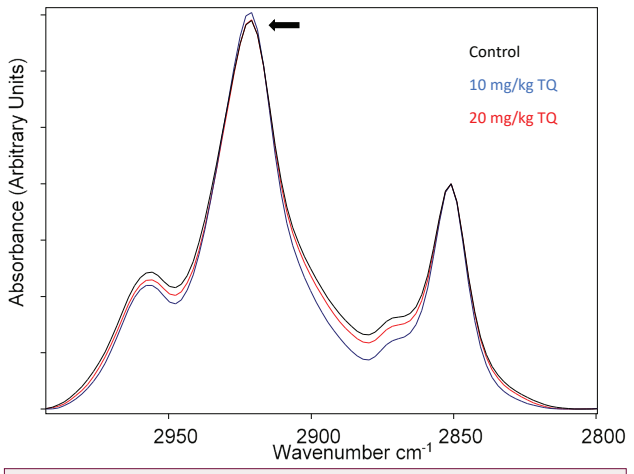


**Figure 3.** Average ATR-FTIR spectra of cerebellum tissues belonging to control (black), 10 mg/kg TQ treatment (blue) and 20 mg/kg TQ treatment (red) rat groups in 3800-2800 $cm^{-1}$ wavenumber region (The spectrum was normalized to $CH_2$ sym stretch, band at 2852 $cm^{-1}$).

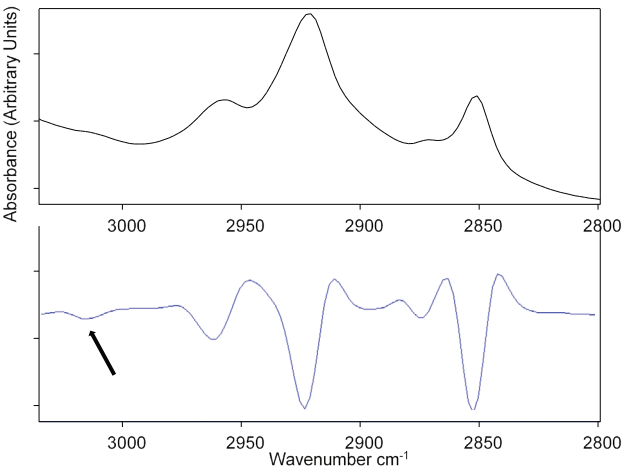


**Figure 4.** ATR-FTIR first and second derivative spectra of the control cerebellum tissue between 3100-2800 $cm^{-1}$ spectral region, showing olefinic band at 3011 $cm^{-1}$ labelled with an arrow.

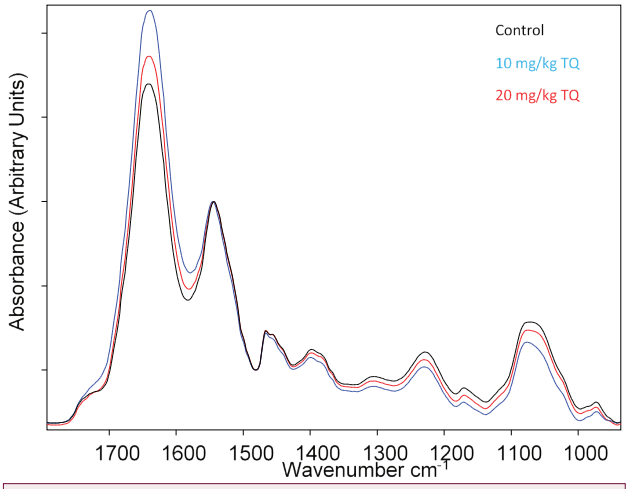


**Figure 5.** Average ATR-FTIR spectra of cerebellum tissues belonging to control (black), 10 mg/kg TQ treatment (blue) and 20 mg/kg TQ treatment (red) rat groups in 1800-900 $cm^{-1}$ wavenumber region (The spectrum was normalized to amide II at 1544 $cm^{-1}$).

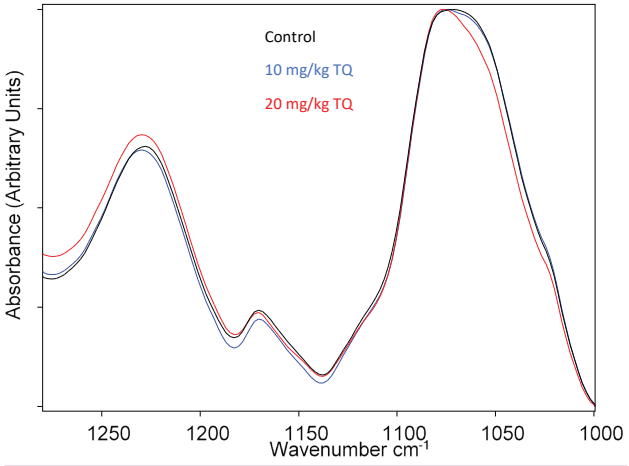


**Figure 7.** Average ATR-FTIR spectra of the cerebellum tissues belonging to control (black), 10 mg/kg TQ treatment (blue) and 20 mg/kg TQ treatment (red) rat groups in 1300-1000 $cm^{-1}$ wavenumber region (The spectrum was normalized to phosphate sym stretch. band at 1081 $cm^{-1}$).

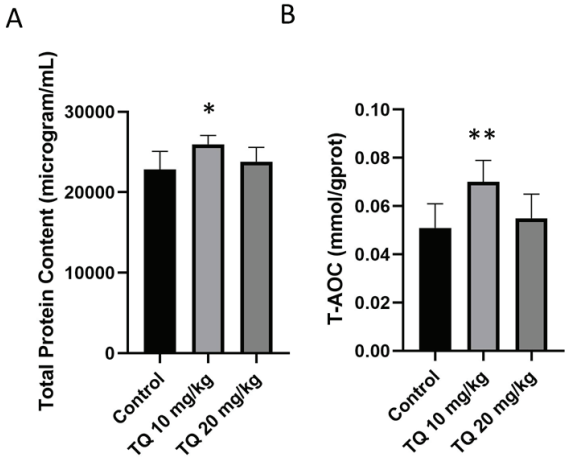


**Figure 6.** The total protein content (A) and total antioxidant capacity (B) of control, 10 mg/kg TQ treatment and 20 mg/kg TQ treatment groups. The significance compared to the control is indicated with a star symbol (*). *: p≤0.05; **: p≤0.01

total protein amount in the TQ-treated groups compared with the control group as can be seen from Figure 6A.

Bands at 1238 $cm^{-1}$ and 1080 $cm^{-1}$ in the spectra of cerebellum tissues come from the absorbance of P=O bonds of phosphate ($PO_2^-$) groups in nucleic acids/phospholipids (25). The nucleic acid contents were measured by taking the band area's ratio of phosphate anti-sym stretching (1238 $cm^{-1}$) + phosphate sym stretching (1081 $cm^{-1}$) to amide I + amide II (19). The nucleic acid content was decreased in the 10 mg/kg TQ treatment group, while increased in the 20 mg/kg TQ treatment group in comparison with the control (Table 2, Figure 2E and Figure 7).

The effects of two different doses of TQ treatment on the total antioxidant capacity of cerebellum tissues were also evaluated in the present study. The total antioxidant capacity was found to be significantly increased in the cerebellum tissues of the 10 mg/kg TQ treatment group with a slight increase in the 20 mg/kg TQ treatment group compared to the control (Figure 6B).

In summary , the 10 mg/kg TQ treatment caused significant alteration in the composition of cerebellum tissue lipids, proteins and nucleic acids besides increasing the total antioxidant capacity of the tissue. Moreover, the 20 mg/kg TQ treatment did not cause any significant changes in the biomolecular composition of cerebellum tissue and its antioxidant capacity.

## DISCUSSION

The cerebellum, which is responsible for the motor coordination and the balance of the body, was also found to be significant in the formation of neurodegenerative diseases by an alteration in its structure and function (10). TQ was reported to have neuroprotective effects in neurological disorders by its role in oxidative stress and inflammation processes (1). However, dose-dependent effects of TQ on the molecular structure and function of cerebellum tissue is still unclear. In the current study, the possible effects of two distinct doses of TQ treatment on the molecular composition, structure and the total antioxidant capacity of cerebellum tissue were studied by ATR-FTIR spectroscopy and biochemical techniques.

Protein, lipid and nucleic acid composition of tissues with the degree of saturation/unsaturation of tissue lipids might be accepted as the basic parameters for the analysis of structure

and function of the tissues. Since they are related to the proper functioning of the tissues, they could indirectly show the disorder/disease state of the tissue.

The total lipid content of cerebellum tissue belonging to the 10 mg/kg rat group was significantly increased compared with the control group, though there was no change in the 20 mg/kg treated group. Elevated serum levels of total cholesterol, low density lipoprotein (LDL) and triglyceride (TG) with less high density lipoprotein (HDL) level is known as hyperlipidemia. The condition of hyperlipidemia is found to be related to neurodegenerative diseases including Alzheimer's disease (26). In our study, the increase in total lipid content in the 10 mg/kg treated group might be mainly due to the increase levels of HDL which has antioxidant properties besides anti-inflammatory features. It was reported that HDL induce the production of M2 polarized macrophages reducing inflammation in the brain tissue (27). The increase in HDL level was also associated with the improvement of cognitive function in aging and also in neurodegenerative diseases (28).

The band area's ratio of the olefinic to total lipid which provides information about unsaturated lipid content, is utilized as an indicator of lipid peroxidation (19). A significant increase in unsaturated lipid content in the 10 mg/kg TQ group compared to the control group was observed in the present study. The study showed that unsaturated fatty acids decrease inflammatory responses, saturated fatty acid-induced cytotoxicity, and ROS production in various models of brain damage and neurodegenerative diseases (29). Moreover, total antioxidant capacity of cerebellum tissues of rats treated with 10 mg/kg TQ was significantly increased when compared with the control according to the present study's results. This implies that 10 mg/kg TQ improves cerebellar antioxidant capacity which is important for protection against the oxidative stress, accumulation of ROS in the brain and, as a result, formation of neurodegenerative diseases. In previous studies in the literature, it was reported that 15 mg/kg TQ treatment for 3 days increased heart and brain total antioxidant capacity in prilocaine (a local anaesthetic)-treated rats with a decrease in ROS formation in different parts of the brain including cerebellum and cerebral cortex (30). In the study of Alrafiah (1), rats were fed with HFD with the supplementation of 300 mg/kg for 4 weeks duration. It was reported that malondialdehyde as an indicator of lipid peroxidation was elevated in the HFD group while it was reduced when it was supported with TQ. The improvement in the levels of antioxidant enzymes with the supplementation of TQ was also reported in the same study (1). It was concluded that TQ treatment can reduce the neural damage with the inflammation induced by HFD by increasing the antioxidant enzyme levels in those rats. The other studies in the literature also reported neuroprotective and neuromodulatory effects of TQ between 2.5-10 mg/kg doses in the brain tissue (5, 31, 32).

According to the results of the current study, 10 mg/kg TQ treatment induces significant changes in the saturated and unsaturated lipid content with an increased total antioxidant capacity of cerebellum tissue while 20 mg/kg TQ treatment did not cause a significant change in those parameters.

Total protein content which was calculated in both FTIR study and Bradford assay, was significantly and slightly increased in the 10 mg/kg and 20 mg/kg TQ treatment groups compared to the control, respectively. This increase may be due to the induction of the levels of antioxidant enzymes and other proteins that have antioxidant property in the cerebellum tissue since increased total antioxidant capacity can arise from an increase into the system of any molecule with antioxidant properties. Increased levels of brain glutathione and superoxide dismutase with the treatment of TQ were reported in the study of Alrafiah (1).

The nucleic acid content of cerebellum tissue was affected in opposite ways in different doses of TQ treatment. There was a slight decrease in the 10 mg/kg TQ treatment group conversely with a slight increase in the 20 mg/kg TQ treatment rat group in comparison to the control. High levels of nucleic acids in the blood were reported in different states including ageing and age-related neurodegenerative disorders, inflammatory conditions, and autoimmune diseases. This high level of nucleic acids can interact with the misfolded proteins causing protein misfolding disorders (PMDs) e.g. Alzheimer's disease, prion diseases or Parkinson disease (33). Although the decrease in nucleic acid content of cerebellum in the 10 mg/kg TQ treatment rat group may be the part of the antioxidant system induced by TQ, both the increase and decrease of this content were not much to boast about.

According to the previous study, 20 mg/kg TQ can be accepted as a high dose for female rats when given by intraperitoneal injection (34). In the study of Abukhader, it was reported that males and females can tolerate 22.5 mg/kg and 15 mg/kg respectively (34). However, both can tolerate 250 mg/kg TQ when given by intragastric intubation because of the elimination of it in the digestive tract (34). Since 20 mg/kg TQ dose is a higher dose for female rats to be tolerated, the improvement effect of 10 mg/kg could not be seen in those rat groups. Besides that, it is not a toxic dose and there was no toxic effect on the cerebellum tissues of rats.

In conclusion, 10 mg/kg TQ treatment improves the saturated and unsaturated lipid, protein and nucleic acid content besides decreasing lipid peroxidation and increasing the total antioxidant capacity of cerebellum tissues. However, 20 mg/kg TQ treatment did not show any significant effect on the molecular composition or the antioxidant capacity of cerebellum tissues. Moreover, ATR-FTIR spectroscopy is a rapid, sensitive, non-invasive method to detect the molecular and compositional alterations in the tissues in disease states and/or supplementation as a part of traditional medicine.

**Ethics Committee Approval:** All the experimental processes of the study were confirmed by the Scientific Ethical Committee of Bezmi-

alem Vakif University (2016/317).

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study - S.G.U., B.E.; Data Acquisition - S.G.U.; Data Analysis/Interpretation - S.G.U.; Drafting Manuscript - S.G.U.; Critical Revision of Manuscript - S.G.U., B.E.; Final Approval and Accountability - S.G.U., B.E.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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