Evaluation of the Antioxidative and Protective Effects of Thymoquinone in a Pentylenetetrazole-induced Epilepsy Model

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Abstract

Objective: Epilepsy is a common neurological disorder that leads to neuronal excitability and provokes various forms of cellular reorganization in the brain. We investigated the antioxidative and protective effects of thymoquinone (TQ) from the perspective of biomarkers in serum samples of rats using a pentylenetetrazol (PTZ)-induced epilepsy model.

Methods: Twenty-one adult, male Wistar albino rats were randomly assigned to three groups. (1) Control (n=6); 0.5 mL saline (i.p.). (2) PTZ (n=7); 35 mg/kg PTZ (i.p.). (3) TQ+PTZ (n=8); 20 mg/kg TQ orally (p.o.)+ 35 mg/kg PTZ (i.p.). To induce kindling, PTZ was injected at a subconvulsive dose (35 mg/kg, i.p.) every other day for 24 days. Then, on the 26th day of the study, a single loading dose of PTZ (75 mg/kg) was injected into the animals. Seizure severity was evaluated with the Racine scale. Blood samples were taken from rats under anesthesia by the cardiac puncture method. The serum levels of myeloperoxidase (MPO), ischemia-modified albumin, total oxidant status (TOS), total antioxidant status (TAS), advanced oxidation protein products (AOPP), total sulfhydryl (T. sulfhydryl), and paraoxonase-1 (PON-1) were evaluated colorimetrically by the ELISA method, using a spectrophotometer.

Results: A significant relationship was found between PTZ and TQ+PTZ groups for TAS (p=0.020), TOS (p=0.006), AOPP (p=0.015), and T. sulfhydryl (p=0.009). MPO and PON-1 were not significant (p>0.05).

Conclusion: TQ may be used as an adjuvant agent in the regulation of epileptic seizures with its antioxidative and protective functions in the PTZ-induced epilepsy model. At the same time, serum parameters can potentially be diagnostic tools for the effective managing of treatment.

Keywords: Pentylenetetrazole (PTZ)-kindling model, thymoquinone, antioxidative effect, protective effect

INTRODUCTION

Epilepsy is a multifactorial disease that causes recurrent epileptic seizures, which are characterized by abnormal neuronal activities.[1] It can result from a range of acquired and genetic causes, including traumatic brain injury, stroke, tumors, central nervous system infection, and various medical conditions. In some cases, it may also be linked to specific gene mutations.[2] Seizures are caused by inhibiting γ-aminobutyric acid (GABA) receptors and the excitation of glutamate receptors.[1] Many researchers are working on epilepsy, which includes a kindling model. Kindling is commonly used in modeling epilepsy and its seizures. Experimental animal kindling models are advantageous for investigating seizures, which are the dominant phenotype of the disease.[3] The most common agent used for this purpose is pentylenetetrazol (PTZ). PTZ functions by interacting with the receptor[4] of the GABA type A (GABAA) chloride ionophore complex. Repeated doses of PTZ (20-35 mg/kg) trigger seizures by causing increased excitatory activity or decreased inhibitory effects.[5] PTZ causes changes in GABAergic and glutamatergic systems, leading to receptor blockade by binding to GABAA receptors. Thus, neurons become depolarized.[6] Additionally, PTZ increases the density and sensitivity of receptors for glutamate, an excitatory neurotransmitter.[6]

Natural agents, which are among the options in the treatment of epilepsy, although their efficacy has not yet been fully established, are preferred because they have low side effects and are mostly non-toxic compared to other chemical agents. However, while searching for the action mechanisms of these agents in deep metabolic pathways, their positive or negative effects on known, easily sampled parameters that can be used by almost every service provider are ignored. The use of natural compounds to treat various neurological diseases has recently been an important area of research. Thymoquinone (TQ), the active component of Nigella sativa (NS), is a substance with antioxidant, antihyperlipidemic, antidiabetic, anti-inflammatory, gastroprotective, hepatoprotective, antihypercholesterolemic, anticarcinogenic, anxiolytic, antidepressant, antipsychotic, and analgesic properties.[7] TQ has been shown to have anticonvulsant activity in rats by causing an increase in GABAergic tone via an opioid receptor.[8] In a study, TQ pretreatment (10 mg/kg) reduced oxidative stress (OS) indices such as malondialdehyde and nitrate in hippocampal tissue. Additionally, it decreased severe seizure activity in a rat model of temporal lobe epilepsy (TLE) induced by intrahippocampal injection. Additionally, it has been shown that TQ can reduce hippocampal neuron loss, astrogliosis, and lipid peroxidation in the cornu ammonis-1 (CA-1), CA-3, and hilar regions.[9] The treatment of mice with TQ (5, 10, and 20 mg/kg i.p.) and a subconvulsive PTZ dose on alternate days provided dose-dependent protection against PTZ-induced exacerbation, as well as learning and memory impairments. Additionally, treatment of mice with TQ (20 mg/kg) inhibited PTZ-induced biochemical changes in the brain and increased the brain glutamate level. These results suggest that glutamate and subsequent OS and excess NO production via inducible NO synthase play an important role in the pathophysiology of PTZ-induced kindling and cognitive impairments in mice. TQ may provide dose-dependent protection against PTZ-induced inflammation and cognitive impairment. The inhibition of PTZ-induced brain OS and NO overproduction, alongside an increase in the expression and activity of inducible NO synthase, may play a significant role in the protective effect of TQ against brain injury.[10]

During seizure activity, the antioxidant defense mechanism in the brain decreases, and the amount of free radicals increases. This situation triggers OS.[11] Reactive oxygen species (ROS) are produced during cellular metabolism. Excessive production of ROS causes OS.[11] Total oxidative status (TOS) and total antioxidant status (TAS) are OS parameters.[12] Advanced oxidation protein products (AOPPs) are novel markers of OS. AOPP occurs during OS and performs various biological activities, such as the induction of proinflammatory cytokines and adhesive molecules.[13] The sulfhydryl group (SH) is a thiol group that reliably reflects OS. Thiol groups are oxidized by ROS.[14] Paraoxonase (PON) is a 43-45 kDa glycoprotein mainly synthesized by the liver. PON-1 is a Ca^{2+}-dependent enzyme transported by circulating high-density lipoproteins (HDLs).[15] The antioxidant action of PON-1 and PON-3 protects HDL and low-density lipoproteins from oxidation.[15] The activation of antioxidant activity depends on the hydrolysis of phospholipids or lipid peroxide products.[15] Ischemia in cerebral tissue has been identified as a potential factor influencing the development of epilepsy.[16] Albumin is converted to ischemia-modified albumin (IMA) during ischemic events. IMA has a low binding capacity for metals such as copper, nickel, and cobalt.[17] Increasing evidence points to the role of inflammation during epileptic activity.[18] Gene array studies have demonstrated that immune response-related genes are upregulated during epileptic activity.[19] Leukocytes play a pivotal role during epileptic activity, and depletion of neutrophils in animal models prevents both seizure induction and epilepsy development.[20] Myeloperoxidase (MPO) is found in the azurophilic granules of neutrophils and macrophages. It is released when neutrophils and macrophages are activated. MPO leads to the destabilization of atherosclerotic plaques by activating metalloproteinases.[21]

MAIN POINTS

- Evaluation of the anti-oxidative effect of thymoquinone (TQ).
- Demonstration of the neuroprotective effect of TQ.
- Evaluation of the usability of TQ as an adjuvant agent in the treatment of epilepsy.
- Demonstration of its preventive effect on cognitive impairment caused by seizures.

To evaluate the antioxidant and protective effects of TQ, we measured MPO, IMA, TOS, TAS, AOPP, total sulfhydryl (T. sulfhydryl), and PON-1 serum levels in the epilepsy model induced by PTZ. In this study, we searched for descriptive evidence of the therapeutic nature of TQ in the serum parameters of TQ-treated epilepsy animals.

Additionally, we demonstrated for the first time the antioxidative and protective properties of different doses of TQ in the PTZ-induced epilepsy model. Therefore, TQ may be usable as an adjuvant agent in epilepsy treatment.

METHODS

Animal Treatment

Twenty-one male Wistar albino rats were used. Animal numbers were determined by power analysis (G*Power). Rats were housed with ad libitum access to food and water on a 12-h light/12-h dark cycle at a temperature of 25±2 °C. Rats were randomly divided into 3 groups: (1) The control group (n=6) was administered 0.5 ml of saline (i.p.); (2) The PTZ group (n=7) was administered 35 mg/kg PTZ injection (i.p.). (3) The TQ+PTZ group (n=8) was administered 20 mg/kg TQ orally (p.o.) 2 hours before each PTZ (35 mg/kg) injection. The PTZ (35 mg/kg) injection was administered to the PTZ and TQ+PTZ groups on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24 of the study. Consequently, the kindling model was generated. Following the generation of the kindling model, a convulsive final dose of PTZ (75 mg/kg) was administered (i.p.) to induce seizures.[22] In addition, TQ was not administered before the administration of the final dose of PTZ (75 mg/kg).

In addition to 6 animals per group calculated by power analysis, +2 animals were considered in the PTZ and TQ+PTZ groups for experimental losses. One animal was lost in the PTZ group at the beginning of the experiment. Following all injections, rats were observed in standard cages of 35x35x35 cm for 30 minutes to assess seizure scores. The seizure behaviors of the rats were scored according to the Racine criteria. These criteria included no change in behavior "0", myoclonic jerks characterized by sudden and repetitive head and neck movements, “1”, unilateral or incomplete clonic seizure, “2”, clonic seizure with forelimb clonus and

rearing, "3", tonic-clonic seizure, "4", tonic-clonic seizure with full extension of all four limbs, and "5", falling.[23] Additionally, seizure severity and seizure number were used to evaluate epileptic activity. Seizure severity was considered entering stage 4 or 5, while the number of seizures was considered as three consecutive occurrences at stage 4 or 5.

The latency of a seizure is the time that elapses between the administration of the PTZ dose and the onset of the first clonic jerk or sudden twitch.

Biochemical Analysis

We evaluated the minimum doses in this reference in 4 animals before starting the study. After these doses, to determine whether the animals entered anesthesia, the rats were rolled onto their sides once every minute, starting 1 minute after the injection, to check for loss of righting reflex. Then, toe pinch was applied to each rat to determine whether the withdrawal reflex was present. In the toe pinch response test, a pinch was applied to the metatarsal or metacarpal regions with forceps without causing any damage. In addition, reflexes were measured by pinching one hind leg and one forelimb once every minute. Turning and withdrawal reflexes were not observed in the animals after approximately 5 minutes.[24] Therefore, it was decided that ketamine/xylazine anesthesia was appropriate doses (60/6 mg/kg) (i.p).

Blood samples were taken from rats under anaesthesia by cardiac puncture. We used a needle to draw blood externally, without opening the chest. The needle was inserted into the base of the sternum, inclined upward into the chest cavity at an angle of 15-20 degrees to the left of the midline, and then slowly aspirated. Once the blood started to flow into the syringe, aspiration was continued with constant and equal pressure. Blood samples were taken in EDTA tubes and were centrifuged at 3,000 rpm for 10 min and then serum samples were separated. The serum samples were stored at -20 °C until biochemical analysis was performed.

MPO activity has been measured by spectrophotometry. Then, 290 µL (50 mM, pH: 6) of phosphate buffer, 3 µL (20 mg/mL) of O-dianisidine hydrochloride substrate solution, and 3 µL of hydrogen peroxide (H_2O_2, 20 mM) were added to the 96-well plate. 10 µL of samples were added to each well of the microplate and absorbance was measured at 450 nm. MPO activity was evaluated as the degradation of 1 µmol H_2O_2 per liter per second (U/L) at 25 °C.[25]

The cobalt albumin binding test was used to measure the serum IMA level. A total of 95 µL of serum was mixed with 5 µL of cobalt chloride and then incubated. The IMA concentration was measured spectrophotometrically at 500 nm. IMA levels are shown on the calibration curve with absorbance values in the range of 5-180 U/mL.[26]

Measurements of TAS and TOS were carried out using the spectrophotometric method developed by Erel.[27,28] TAS levels are expressed as millimolar Trolox equivalents per gram (mmol Trolox equivalents). TOS levels are expressed in micromolar hydrogen peroxide equivalents (µmol H_2O_2 equivalents) per liter.

AOPP levels were measured using a spectrophotometric method.[29] The values are expressed as µmol/g protein.

T. sulfhydryl levels were measured via a spectrophotometric method using 5,50-dithiobis-2-nitrobenzoic acid (DTNB). In this method, a thiol-disulfide exchange reaction between DTNB and thiol groups was utilized.[30]

PON activity was measured spectrophotometrically at a wavelength of 412 nm by the method developed by Eckerson et al.[31]

This study was approved by the Bezmialem Vakıf University Experimental Animals Local Ethics Committee (no: 2021/236, date: 21.09.2021).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 24.0 software package was used for statistical analyses. Normality analyses were performed with Kolmogorov-Smirnov, and Shapiro-Wilk tests. ANOVA was used to compare groups and within groups, and the Mann-Whitney U test was used to analyze the differences between them. We identified significant differences using a one-way ANOVA with Bonferroni correction for multiple comparisons at a significance level of $\alpha=0.05$. $P<0.05$ was considered to indicate statistical significance. Results are presented as mean differences±standard error. The data were analyzed using the statistical package SPSS (release 22.0, SPSS Inc, Chicago, IL, USA) for Windows.

RESULTS

Evaluation of the Effect of TQ on Latency and Seizure Duration

In our study, the effects of TQ on the latency period and seizure duration were evaluated. The latent period was longer in the TQ+PTZ group than in the PTZ group (70.6±6.4 and 54.5±2.6), and the seizure duration was shorter in the TQ+PTZ group than in the PTZ group (10.1±0.7 and 11.5±0.9) ($p<0.05$) (Table 1).

The values are presented as the mean±SE of the mean. A comparison was conducted between the PTZ group and the TQ+PTZ group concerning seizure duration and latency ($p<0.05$).

Biochemical Analysis

One-way ANOVA was used to analyze mean differences in the analysis of data within groups. Multiple comparative tests and Bonferroni correction were used in the analysis of differences between groups. One-way ANOVA results; among the TQ+PTZ group, the MPO activity (563±591.256 and 1160.493±510.359 U/mL, p=0.0002), total TOS (18.811±3.763 and 32.354±13.510 µmol/L, p=0.0001), and AOPP (14.714±7.305 and 43.000±30.640 µmol/L, p=0.0022) levels were significantly lower than those in the PTZ. In addition, when the TQ+PTZ group was compared with the PTZ group, the TAS (1.398±0.523 and 1.893±0.0.192 mmol/L;

Table 1. The effects of TQ on seizures

Groups	Latency (sec)	Duration (sec)	p value
PTZ	54.5±2.6	11.5±0.9	**0.0003**
TQ+PTZ	70.6±6.4	10.1±0.7	

TQ: Thymoquinone, PTZ: Pentylenetetrazol

p=0.0004), T. sulfhydryl (1159.245±868.631 and 344.264±90.840 nmol/mg protein; p=0.0013), and PON-1 (1470.601±287.250 and 792.171±211.731 U/L; p=0.0001) levels were significantly greater. The IMA level decreased in the TQ+PTZ group compared to that in the PTZ group, with levels of 23.482±15.654 ng/mL and 28.314±14.928 ng/mL, respectively (p=0.0591). However, this decrease was not statistically significant. Data from multiple comparative analyses expressing the significance between groups are given in Table 2.

DISCUSSION

In our study, we used biochemical analyses to demonstrate that TQ has antioxidative and seizure-protective effects on a PTZ-induced epilepsy model. The results obtained showed that the parameters measured here changed during epileptic seizures. Therefore, the change in parameters other than MPO and PON-1 after TQ treatment, suggests that it may be effective before the seizure.

Table 2. Multiple comparisons analysis of serum parameters and groups

Dependent variable	Groups	Groups	Mean difference	SE	95% CI	p value
Myeloperoxidase (U/mL)	Control	PTZ	-507.822	193.352	-1005.441-(-10.203)	**0.044***
		TQ+PTZ	-913.752	185.563	-1391.324-(-436.179)	**0.0001***
	PTZ	Control	507.822	193.352	10.203-1005.441	**0.044***
		TQ+PTZ	-405.930	210.409	-947.446-135.587	0.197
	TQ+PTZ	Control	913.752	185.563	436.179-1391.324	**0.0001***
		PTZ	405.930	210.409	-135.587-947.446	0.197
TOS (ng/mL)	Control	PTZ	-2.440	3.606	-11.721-6.842	1.000
		TQ+PTZ	-15.982	3.461	-24.890-7.074	**0.0001***
	PTZ	Control	2.440	3.606	-6.842-11.721	1.000
		TQ+PTZ	-13.542	3.924	-23.643-(-3.442)	**0.006***
	TQ+PTZ	Control	15.982	3.461	7.074-24.890	**0.0002***
		PTZ	13.542	3.924	3.442-23.643	**0.006***
TAS (mmol/L)	Control	PTZ	0.188	0.153	-0.205-0.581	0.694
		TQ+PTZ	0.683	0.147	0.306-1.060	**0.0002***
	PTZ	Control	-0.188	0.153	-0.581-0.205	0.694
		TQ+PTZ	0.495	0.166	0.067-0.923	**0.020***
	TQ+PTZ	Control	-0.683	0.147	-1.060-(-0.306)	**0.0002***
		PTZ	-0.495	0.166	-0.923-(-0.067)	**0.020***
AOPP (mmol/L)	Control	PTZ	-1.989	0.153	-23.698-19.719	1.000
		TQ+PTZ	-30.275	0.147	-51.109-(-9.441)	**0.003***
	PTZ	Control	1.990	0.153	-19.719-23.698	1.000
		TQ+PTZ	-28.286	0.167	-51.909-(-4.662)	**0.015***
	TQ+PTZ	Contol	30.275	0.147	9.441-51.109	**0.003***
		PTZ	28.286	0.166	4.662-51.909	**0.015***
Total sulphydryl (nmol/mg protein)	Control	PTZ	-48.282	225.884	-629.628-533.064	1.000
		TQ+PTZ	-863.262	216.785	-1421.189-(-305.336)	**0.002***
	PTZ	Control	48.282	225.884	-533.064-629.628	1.000
		TQ+PTZ	-814.981	245.811	-1447.610-(-182.352)	**0.009***
	TQ+PTZ	Control	863.262	216.785	305.336-1421.189	**0.002***
		PTZ	814.981	245.810	182.352-1447.610	**0.009***
PON-1 (U/L)	Control	PTZ	-456.159	112.561	-745.851-166.467	**0.001***
		TQ+PTZ	-678.430	108.027	-956.452-(-400.409)	**0.0001***
	PTZ	Control	456.159	112.561	166.467-745.851	**0.001***
		TQ+PTZ	-222.271	122.491	-537.518-92.976	0.246
	TQ+PTZ	Control	678.430	108.027	400.409-956.976	**0.0001***
		PTZ	222.271	122.491	-92.976-537.518	0.246

*P<0.05 is significant. Multiple comparison analyses were performed with the Bonferroni post-hoc test.
SE: Standard error, CI: Confidence interval, PTZ: Pentylenetetrazol, TQ: Thymoquinone

In a study evaluating the toxicological effects of TQ, intraperitoneal doses of TQ higher than 50 mg/kg body weight were lethal to mice, the LD50 being 90.3 mg/kg i.p.[32] This study also showed that i.p. injection of 4, 8, 12.5, 25, and 50 mg/kg TQ into mice did not cause any changes in biochemical indices such as serum alanine transaminase and lactate dehydrogenase.[32] Several toxicological studies indicated that oral administration of TQ in the range of 10-100 mg/kg has no toxic or lethal effects in mice.[33-37] In another study, the maximum tolerated dose of TQ when injected ip was 22.5 mg/kg in male rats and 15 mg/kg in female rats, while the dose was 250 mg/kg, after oral administration in both male and female rats.[38] In our study, oral administration of TQ (20 mg/kg) dose is compatible with the literature.

TQ has been shown in several studies to reduce OS and increase antioxidant defense. TLE is a type of epilepsy characterized by neuronal loss, reactive astrogliosis, and increased OS. In a rat model of TLE, TQ treatment was shown to have a protective effect on hippocampal areas. It has been stated that TQ reduces OS and seizure activity.[9] TQ can reduce the OS caused by PTZ. In our study, TQ decreased the TOS level and increased the TAS level. The antioxidant effects of NS and TQ have been suggested.[39,40] Hosseinzadeh et al.[41] found that NS oil and TQ have antioxidant effects during cerebral ischemia-reperfusion injury in the rat hippocampus. The antioxidant effects of NS in other animal models of nervous system disorders have also been reported, which sometimes have been confirmed by human studies.[17,42-44] These findings are compatible with ours. TQ decreased the AOPP levels in our study. Aksu et al.[45] showed that AOPP levels increased in children with generalized-type epilepsy. Additionally, it has been stated that the number of seizures increases with an increase in AOPP. According to these results, TQ can reduce the formation of oxidized protein products. Additionally, in our study, TQ also increased the T. sulfhydryl level. In the PTZ epilepsy model, it was stated that NS extracts administered at 200 mg/kg and 400 mg/kg increased the total thiol amount.[46] Therefore, TQ can increase antioxidant capacity by increasing sulfhydryl levels. we demonstrated that TQ increased the level of PON-1. A study has shown that brain PON-1 activity decreases after PTZ injections. PON-1 has a significant role in neurodegenerative disorders because it has antioxidative and anti-inflammatory properties.[47] OS inactivates PON-1,[48] and the decreased activity of this enzyme increases ROS. This decrease in PON-1 activity will further increase the cell's sensitivity to OS, causing neurodegeneration. This study showed that treatment with Brilliant Blue decreased PON-1 activity. This decrease in activity may be related to either lower levels of OS or a neuroprotective effect.[49] Another study showed that the activity of PON-1 decreases in epilepsy patients, as referenced in citation.[50] Our research results show that TQ may cause an increase in PON-1 activity. Additionally, TQ reduced the serum IMA concentration in our study. However, this decrease was not statistically significant. It was observed that serum IMA levels were significantly greater in epileptic patients than in healthy controls.[17] These findings are compatible with ours. In our study, we demonstrated that TQ decreased the MPO level. In a study investigating the relationship between MPO and seizures in a pilocarpine-induced epilepsy model, MPO activity increased both in the hippocampal regions and in the plasma. Also, this study has shown that an increase in the MPO is associated with epileptogenesis.[51] It was stated that the MPO level increased in a PTZ-induced kindling model in mice.[52] Şimşek et al.[53] showed that serum MPO levels decreased in epilepsy patients using antiepileptic drugs. They suggest that antiepileptics have MPO-inhibiting properties. We suggest that TQ can reduce the level of MPO.

Landucci et al.[54] were the first to demonstrate that TQ has neuroprotective properties in a Kainic acid-induced TLE model. They found that TQ increased the basal level of the key plasticity protein PSD95 and could regulate the endoplasmic reticulum stress pathway. Various studies have shown that TQ has protective effects on brain damage. One of these studies showed that in the status epilepticus model, which causes the production of ROS, TQ treatment attenuates brain injury by modulating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. This study demonstrated that TQ treatment can activate the antioxidant defense system.[55] The severity of seizures was significantly lower in the TQ group. The results of the behavioral experiments performed in this study showed that TQ also positively affects learning and memory. Additionally, TQ has been shown to increase the expression of the Nrf2 and HO-1 proteins, and SOD in the hippocampus.[55] In a lithium-pilocarpine-induced epilepsy model, TQ was shown to prevent epilepsy by reducing the expression of nuclear factor kappa B, which mediates inflammatory reactions.[56] It has been shown that TQ improves electroencephalography profiles, reduces the severity of seizures, and improves learning and memory functions.[56]

In the PTZ-induced epilepsy model, it has been shown that TQ administration causes a prolongation of the latency period and a decrease in the duration of seizures in mice.[57] In our study, we also showed that TQ treatment prolonged the latency period and shortened the seizure duration. Additionally, our findings revealed for the first time, that TQ exerts a protective effect in a model of PTZ-induced epilepsy. These results are in agreement with the literature.

Study Limitations

One limitation of this study is that these results need to be confirmed in humans. Thus, the effectiveness of TQ in the treatment of epilepsy may come to the fore.

CONCLUSION

In conclusion, we evaluated the effect of TQ on MPO, IMA, TOS, TAS, AOPP, T. sulfhydryl, and PON-1 levels in the PTZ-induced epilepsy model. TQ causes antioxidative and protective changes based on these measured parameters. Additionally, TQ prolonged the latency period and shortened the seizure duration. Although experimental studies indicated the beneficial effects of TQ against nervous system problems, better-designed clinical trials in humans are needed to confirm these effects. TQ is considered for use as an adjuvant agent in epilepsy treatment. Additionally, TQ has therapeutic potential against cognitive impairment caused by seizures. As a result, the values of serum biomarkers obtained in the epilepsy model indicate that TQ treatment results in improvements reflected in the serum data, which in turn enhances the related metabolic pathways. Serum biochemistry analyses can measure the effectiveness of treatments in human diseases. They show the effectiveness of TQ treatment in the epilepsy model, and the markers investigated in this study should be evaluated within routine epilepsy screenings.

Ethics

Ethics Committee Approval: This study was approved by the Bezmialem Vakıf University Experimental Animals Local Ethics Committee (no: 2021/236, date: 21.09.2021).

Informed Consent: Animal experiment.

Footnotes

Authorship Contributions

Biochemical Analyses: S.Ö., İ.Ö.K., Surgical and Medical Practices: M.P., Concept: M.P., Design: M.P., Data Collection or Processing: M.P., N.P.A., Analysis or Interpretation: M.P., F.H., İ.Ö.K., S.Ö., Ş.G.Y., Literature Search: M.P., Writing: M.P., İ.M., N.P.A., Ş.G.Y.

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