Effects of chronic administration of thymoquinone on penicillin induced epileptiform activity in rats

Sıçanlarda kronik uygulanan timokinonun penisilin ile oluşturulmuş epileptiform aktivite üzerine etkileri

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Abstract

Aim: Thymoquinone (TQ) is derived from Nigella Sativa (NS) which is a traditional medicinal plant used as a spice, and medication in traditional medicine. This study aims to investigate the chronic effects of TQ, which has been shown anticancer, antioxidant, and neuroprotective effects, on experimental penicillin-induced epilepsy models in rats.

Methods: Forty-nine adult male Wistar rats were used in this study. The rats were divided into 7 groups as; sham (S), control (penicillin, CONT), diazepam (DZM), 10 mg/kg TQ (TQ10), 50 mg/kg TQ (TQ50), 10 mg/kg TQ+DZM (TQ10+DZM) and 50 mg/kg TQ+DZM (TQ50+DZM). TQ was administered intraperitoneally for 21 days. Epileptiform activity (EA) was initiated by intracortical administration of penicillin. Electrocorticographic (ECoG) data obtained from the recordings were analyzed. The latency, frequency, and amplitude of EA waves were analyzed statistically.

Results: There was no EA in the sham group. However, it was observed that the first spike-wave latency increased significantly in TQ10 and TQ50 groups compared to the CONT group. Except for some time periods, there was no significant difference between the groups according to spike-wave frequency, and spike-wave amplitude.

Conclusions: It was observed that the application of chronic thymoquinone on the experimental penicillin-induced EA model in rats did not affect the frequency and amplitude of spike-wave representing epileptic seizures. However, TQ was found to prolong the latency of the first EA.

Keywords: Electrocorticography; epilepsy; nigella sativa; rat; thymoquinone

Öz

Amaç: Timokinon (TQ), geleneksel bir tıbbi bitki olan ve geleneksel tıpta baharat ve ilaç olarak kullanılan Nigella sativa (NS)'den elde edilmiştir. Bu çalışmanın amacı, antikanser, antioksidan ve nöroprotektif etkileri bilinen TQ'nun sıçanlarda deneysel penisilin kaynaklı epilepsi modelinde kronik etkilerini araştırmaktır.

Yöntemler: Bu çalışmada kırk dokuz adet erişkin erkek Wistar sıçan kullanıldı. Sıçanlar sham (S), kontrol (penisilin, CONT), diazepam (DZM), 10 mg/kg TQ (TQ10), 50 mg/kg TQ (TQ50), 10 mg/kg TQ+DZM (TQ10+DZM) ve 50 mg/kg TQ+DZM (TQ50+DZM) olmak üzere 7 gruba ayrıldı. TQ, 21 gün süresince intraperitoneal olarak uygulandı. Penisilin intrakortikal uygulanması ile epileptiform aktivite (EA) oluşturuldu. Kayıtlardan elde edilen elektrokortikografik veriler analiz edildi. EA dalgalarının latansı, frekansı ve amplitüdü istatistiksel olarak analiz edildi.

Bulgular: Sham grubunda EA gözlemlenmedi. Ancak TQ10 ve TQ50 gruplarının ilk diken-dalga latansının CONT grubuna göre anlamlı olarak arttığı gözlendi. Diken-dalga frekansı ve diken-dalga amplitüdü bakımından bazı zaman periyotları dışında gruplar arasında istatistiksel olarak anlamlı fark yoktu.

Sonuç: Sıçanlarda deneysel penisilinle oluşturulmuş EA modelinde kronik TQ uygulamasının, epileptik nöbetleri temsil eden herhangi bir diken dalga frekansını ve amplitüdünü etkilemediği gözlendi. Ancak, TQ›nun ilk EA başlama süresini uzattığı belirlendi.

Anahtar Sözcükler: Elektrokortikografi; epilepsi; nigella sativa; sıçan; timokinon;

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INTRODUCTION

Epilepsy is one of the most common neurological disorders Even though epilepsy is not a disease, but a symptomatic condition caused by various factors (such as genetic factors, traumatic brain injury, central nervous system infections, stroke, or structural brain lesions including brain tumors), and yet, any cause is not found in nearly 65% of the patients (1).

Nowadays, there are about 70 million patients with active epilepsy who need treatment and have continuous seizures. It has been found that thirty percent of these people are resistant to all antiepileptic drugs (2). Besides that, the adverse impact profile of current antiepileptic drugs (AED) used in epileptic treatment is quite wide. Therefore, efforts to find more effective AED with low adverse impact profiles and inexpensive, ones and to shed light up the mechanisms of epilepsy are still intensely continued today. 85% of epilepsy cases are seen in developing countries. 60-90% of epilepsy patients living in these countries cannot be treated due to insufficient healthcare resources and financial difficulties (3).

Approximately 70% of epilepsy patients are treated by monotherapy of existing antiepileptic drugs. Herbal products play a prominent role in the progress of the new AED. Many herbs are known to have anticonvulsant effects. Numerous phytochemical, pharmacological, and electrophysiological studies have been performed on these anticonvulsive plants, which are increasing daily.

The chemical contents of NS seeds, which are used as a medication in traditional medicine and as a spice in kitchens, vary slightly depending on the geographical region and climate in which the plant is grown. The seeds include on average 37% fixed oil, protein, alkaloid, saponin, and 1.45% essential oil. TQ, which is an essential oil and makes up the largest part of the bioactive essential oil of black seed ($C_{10}H_{10}O_2$; the molecular weight is 164.2 g/mol), has been used as a medicine for more than 2000 years. Studies have shown that TQ, which has antioxidant, anti-inflammatory, and antineoplastic effects, has a suppressor effect on cell proliferation in a large number of cancer types (4). On the other hand, it is a substance with very few known side effects (5). At the same time, Al-Naggar *et al.* demonstrated the analgesic and central nervous system (CNS) suppressive activity of NS oil (4). It has been reported that TQ performed its antinociceptive effect through opioid receptors (5). Recently, some neuropharmacological effects of TQ have been demonstrated. Among these neuropharmacological effects of TQ like anticonvulsant effects, muscle relaxants, and hypnotic effects, it is effects on motor coordination and locomotor activity were also investigated(6,7).

The fact that the effects of chronic TQ use on penicillin-induced experimental epilepsy model have not been demonstrated, and that this model constitutes the prototype of focal motor and generalized seizures in humans constituted the main factor in our preference for this model in our study. Another factor is the mechanism of action of penicillin. TQ can increase GABAergic transmission through opioid kappa receptors, which generally affect Ca^{2+} channels and block cellular Ca^{2+} influx (8–10). These results suggest that the anticonvulsant effect of TQ is mediated by opioid kappa receptors.

This study aims to look into the effects of chronic administration of TQ on penicillin-induced epileptiform activity in rats.

MATERIALS AND METHODS

Animals

Wistar male rats (230±30 g, aged 12 weeks, N=49) were used in the study. All animals were obtained from the Duzce University, Experimental Animals Research Center (Duzce, Turkiye). They were hosted as five animals per cage and kept at room temperature (21±2°C). Rats fed *ad libitum*. All studies were applied between 08:00-10:00 a.m. Ethical approval was got from the Animal Research Local Ethics Committee of Duzce University (date: 07.05.2018, decision no: 2018/4/4).

Drugs and doses

Thymoquinone was administered intraperitoneally (i.p.) to the rats at 10 and 50 mg/kg (Sigma, Missouri, US) in our study. It was thawed in dimethyl sulfoxide (DMSO, Loba Chemie, Mumbai, India). Then it was diluted with saline (1:4, v/v). For positive control, it was used 2 mg/kg diazepam, and for anesthetic 1.25 g/kg dose of Urethane (Sigma, Missouri, USA) was injected into the animals.

Surgical procedure

The methods which are used in this study for the surgical and electrophysiological recording were the same as those defined in previous publications (11,12)the influence of nitric oxide (NO. A stereotaxic frame (Harvard Instruments, MA, USA) were used to fix urethane anesthetized animals. Before placing rats in the stereotaxic frame, they were shaved from the head to clear the scalp of feathers. Then scalp of the rats was opened up through the midline, from front to back using a scalpel. Before the bone, which is above the left cerebral cortex, was removed, it was thinned with a drill.

Experimental groups

In the study, all animals have been divided into seven groups (Table 1). S group, which only underwent surgical procedures. DZM group, which only received 2 mg/kg diazepam. CONT group, which only received Penicillin G (500IU/2 µl, i.c.). TQ10 group, which received TQ+Penicillin G. TQ50 group, which received TQ+Penicillin G. TQ10+DZM group, which received TQ+diazepam and Penicillin G. TQ10+DZM group, which received TQ+diazepam and Penicillin G.

It was reported in previous studies that TQ and DMSO did not show any EA. Therefore, groups of only TQ and DMSO were not used in the current study (13,14)and a large part of these patients are resistant to antiepileptic drugs discovered so far. In addition, side effect profiles of these drugs are very wide. Rapamycin that is an inhibitor of mammalian target of rapamycin (mTOR. Except for penicillin, all of the substances used in the study were administered intraperitoneally for 21 days (Figure 1).

Creation of penicillin-induced epileptiform activity

Application of intracortical penicillin caused to start EA. Administration of penicillin to induce EA was performed by injecting 500 IU/2 µl penicillin intracortically. Penicillin was applied to the somatomotor cortex with a microinjector (701N, Hamilton Co., Reno, NV, USA). The coordinates of injection were 2 mm lateral, 1 mm anterior, and 1.2 mm depth of the Bregma line.

Electrophysiological records

Rats were prepared for the surgical operation at the end of the 21st day. Firstly animals were anesthetized with 1.25 g/kg of urethane i.p. Then the left part of the bone was removed, and two silver/silver chloride ball electrodes were superimposed on the somatomotor cortex area. Electrocorticographic (ECoG, PowerLab/8SP, ADInstruments Pty Ltd, NSW, Australia) was recorded in the courses of the experiment after the electrodes were placed. Before the penicillin administration, five minutes of basal activity recording was taken. The administration of intracortical penicillin (500 IU / 2 ul) induced EA. Analysis of the data records was performed by using the PowerLab Chart v.8.0 software package (ADInstruments Pty Ltd., CO, US). The duration of the ECoG recordings for each animal was 125 mins. Bipolar spike and spike-wave complexes, which are EA markers, were examined. Moreover, in the 120 minutes-periods of ECoG recordings of each animal the mean, median, minimum, and maximum values of spike-wave frequency and amplitudes per 5 minutes were measured and used as data.

Statistical analysis

All data were digitized and calculated from the records with Chart software. For measuring differences between groups in latency, frequency, and amplitude of spike-wave data Kruskal-Wallis test was used. It was used to examine the differences between the groups in terms of latency, the frequency of spike-wave, and spike-wave amplitude in each period. Also, different groups were determined by the post hoc Dunn test. $p<0.05$ was accepted as the statistical significance level, and the Statistical Package for the Social Sciences package program version 22.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical computations.

RESULTS

Electrocorticographic results

All the substances used in the study were tested on at least seven animals to investigate whether they affected the ongoing basal activity. Accordingly, it was determined that 10 and 50 mg/kg doses of TQ used did not affect basal activity. No epileptic activity discharge was observed in the S group.

Determination of penicillin-induced epileptiform activity

Penicillin causes epileptic discharges in the cortex, and this can be clearly recorded with ECoG records. In the

Table 1. Experimental groups

Group	N	Group name	Substance	Dose
1	7	Sham (S)	Saline	1 ml/kg/day
2	7	Control (penicillin, CONT)	Saline	1 ml/kg/day
3	7	Diazepam (DZM)	Diazepam	2 mg/kg/day
4	7	10 mg/kg TQ (TQ10)	TQ+ Penicillin	10 mg/kg/day
5	7	50 mg/kg TQ (TQ50)	TQ+ Penicillin	50 mg/kg/day
6	7	10 mg/kg TQ +Diazepam (TQ10+DZM)	TQ+ Diazepam + Penicillin	10 mg/kg/day+2mg/kg/day
7	7	50 mg/kg TQ+Diazepam (TQ50+DZM)	TQ+ Diazepam + Penicillin	50 mg/kg/day+2mg/kg/day

S: Sham, CONT: Control, DZM: Diazepam, TQ: Thymoquinone, TQ10:10 mg/kg Thymoquinone, TQ50: 50 mg/kg Thymoquinone

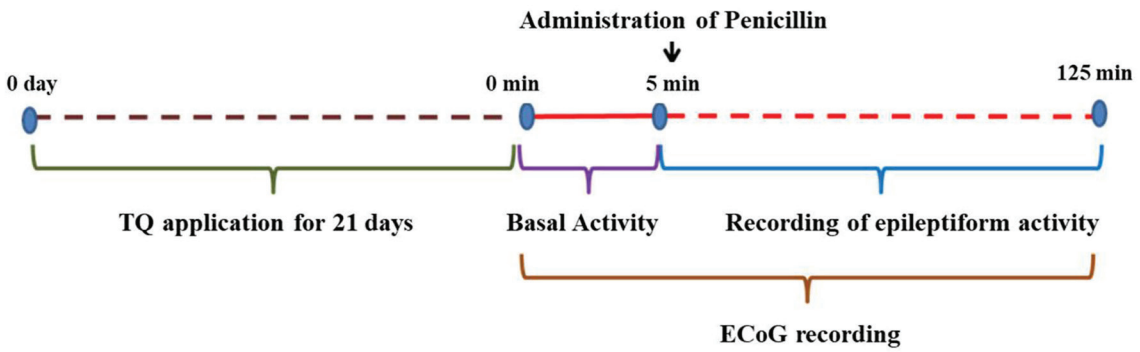

Figure 1. Schematic representation of the experiment procedure (TQ: Thymoquinone, ECoG : Electrocorticographic, min: minute)

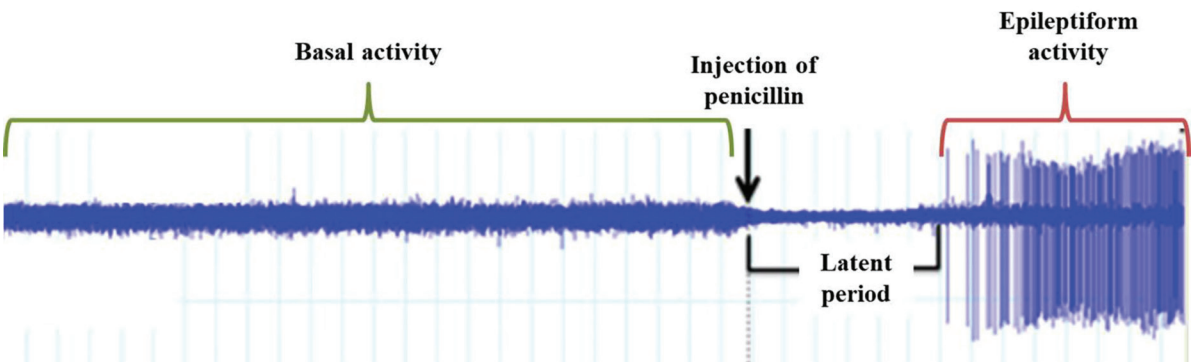

Figure 2. Observed changes in ECoG waves after penicillin G injection (ECoG : Electrocorticographic)

CONT group, EA manifested itself in the emergence of spike-wave patterns in ECoG recordings 5-10 minutes after penicillin was injected 1.2 mm below the cortex surface (Figure 2). These epileptic discharges, which started approximately 5-10 minutes after penicillin administration, ended after 6-7 hours of administration of it.

In the CONT group, the average spike-wave count was 1.28 and spike-wave amplitude was found as 2,689 mV between the 6^{th}-10^{th} mins, the first period after penicillin administration. The period in which the average spike-wave frequency is highest is found between 86^{th} -90^{th} mins and counts as 155, while the period with the highest spike-wave amplitude was found to be between 16^{th}-20^{th} mins and is as 3.323 mV. In the CONT group, the average number of spike waves between 116^{th}-120^{th} mins, which is the last period, was found to be 84.57, while the spike-wave amplitude was found to be 1.720 mV.

The latency period started instantly after the penicillin injection. The latency period manifested itself

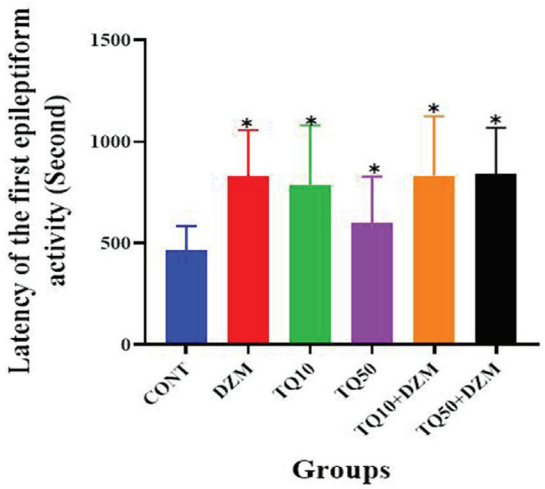

Figure 3. Latency time of the first EA (CONT; Control, DZM; Diazepam, TQ; Thymoquinone, TQ10; 10 mg/kg Thymoquinone, TQ50; 50 mg/kg Thymoquinone, *Significance compared to CONT group, p<0.05)

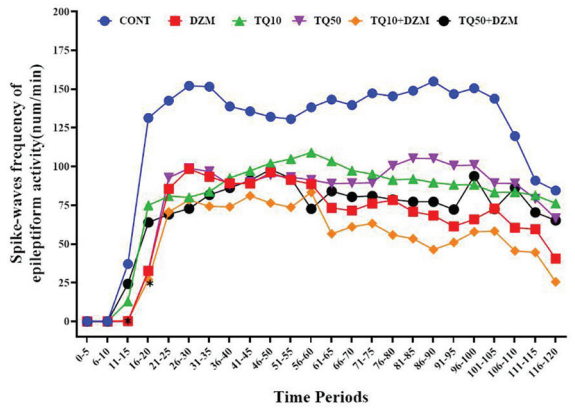

Figure 4. Median values of spike-wave frequency of EA (number/min) obtained from recording after penicillin (CONT; Control, DZM; Diazepam, min: minute, TQ; Thymoquinone, TQ10; 10 mg/kg Thymoquinone, TQ50; 50 mg/kg Thymoquinone, *Significance compared to CONT group, p<0.05)

with waves of lower amplitude than basal activity, lasting an average of 442.28 seconds in the CONT group. After this period, instantaneous, irregular spike-wave beginnings were monitored, and the epileptic process began, usually without a significant transition period.

Latency of the first epileptiform activity

Spike waves of EA after penicillin administration began to be seen between the 5th-14th mins (Figure 3). When the groups were compared according to the starting time of the first EA, a statistically significant difference was found between the groups (p=0.007) (Figure 3). It was seen that the mean EA onset time of all groups was statistically higher than the CONT group when the groups were examined in more detail (p<0.05).

Time-dependent effect of chronic thymoquinone administration on the spike-wave frequency of epileptiform activity

EA was found in the ECoG recordings of the 5-minutes basal activity recordings from the chronic groups. After the penicillin administration, a certain number of spike-wave frequency values were obtained in 24 different measurements taken in 5-minute periods (Figure 4) for 120 mins. The results of spike-wave frequency values measured at different times are given below. When Figure 4 is examined, no statistically significant difference was found between the average number of spike waves of all groups during 120 minutes (except between the 11th -15th and 16th -20th minutes) recording after penicillin administration (p>0.05). However, a statistically significant difference was found between the groups in the spike-wave frequency recordings between the 11th -15th and 16th -20th minutes (p=0.001 and p=0.015, respectively).

The effect of chronic thymoquinone administration on total epileptiform activity spike wave frequency

The average number of spike waves that occurred during 120-minute ECoG recording after penicillin administration in the groups was evaluated. As a result of the comparison of the total spike-wave numbers according to their average, no statistically significant difference was found between the groups (p=0.147) (Figure 5).

The effect of chronic thymoquinone administration on the spike-wave amplitude of epileptiform activity

The findings including the effects of the applied substances on EA amplitude were shown in Figure 6.

There was no statistically significant difference between the groups in terms of the average spike wave amplitudes taken from all groups between 0-95th and 116th -120th minutes after the penicillin administration

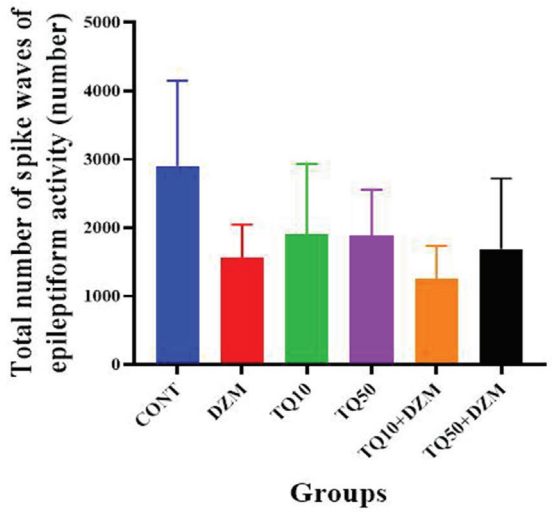

Figure 5. Total of spike-wave frequency of EA (number/min) obtained from recording after penicillin (CONT; Control, DZM; Diazepam, TQ; Thymoquinone, TQ10; 10 mg/kg Thymoquinone, TQ50; 50 mg/kg Thymoquinone, *Significance compared to CONT group, p<0.05)

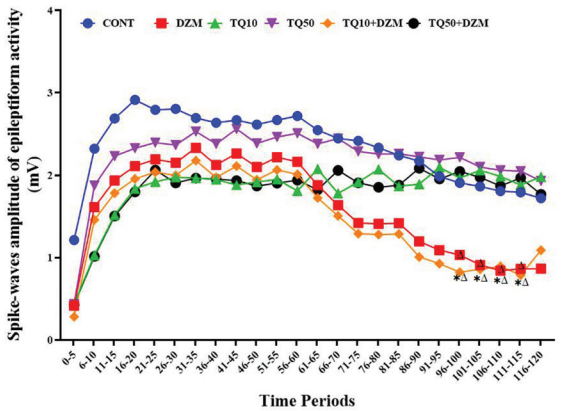

Figure 6. Spike-wave amplitude of EA (mV) median values obtained from recording after penicillin (CONT; Control, DZM; Diazepam, EA; epileptiform activity, TQ; Thymoquinone, TQ10; 10 mg/kg Thymoquinone, TQ50; 50 mg/kg Thymoquinone, *Significance compared to others group, p<0.01, ΔSignificance compared to others group, p<0.05)

(p>0.05). On the contrary, it was shown that there was a statistically significant difference in spike-wave amplitudes of the groups between 96th -115th minutes (p values p=0.037, p=0.015, p=0.020, and p=0.012, respectively).

In the results of the groups between 96th-100th minutes, it was revealed that the average EA spike-wave amplitude of the TQ10+DZM group was statistically lower than the TQ10, TQ50+DZM and TQ50 groups (p values, respectively, p=0.025, p=0.016, and p=0.012). In addition, the averages of the spike-wave amplitude of the DZM group were found to be statistically lower than the TQ50+DZM and TQ50 groups (p values p=0.046 and p=0.036, respectively).

The results of the groups between 101st -105th minutes were shown that the average of the EA spike-wave amplitude of the TQ10+DZM was statistically lower than the CONT, TQ10, TQ50+DZM and TQ50 groups (p values, respectively, p=0.022, p=0.007, p=0.020, and p=0.009). In addition, the averages of the spike-wave amplitude of the DZM group were determined that statistically lower than the TQ10 and TQ50 groups (p values p=0.024 and p=0.029, respectively).

DISCUSSION AND CONCLUSION

In the present study, the effect of 10 and 50 mg/kg doses of TQ, administered to the rats intraperitoneally for 21 days, on penicillin-induced EA was investigated. The EA records, which were taken from ECoG recordings, were found to be compatible with the literature (15) the interaction between these systems remain elusive. Therefore, the present study was initiated to evaluate the possible interactions between cannabinoid compounds and NMDA receptor antagonist in the penicillin-induced epileptiform activity in rat. In the first set of experiments, 30min after intracortical injection of penicillin, five different doses of memantine (3,5-dimethyl-1-adamantanamine hydrochloride, 1, 2.5, 5, 10 or 20mg/kg. Intracortical administration of 500 IU penicillin G to rats under urethane anesthesia caused an EA which was recorded in the form of spike-wave components from the cortex surface within 3rd-8th minutes, and the activity reached its maximum frequency and amplitude at approximately 30 minutes. Based on previous literature information, DMSO and TQ alone were not applied in this study. (13,14)and a large part of these patients are resistant to antiepileptic drugs discovered so far. In addition, side effect profiles of these drugs are very wide. Rapamycin that is an inhibitor of mammalian target of rapamycin (mTOR. It has been reported in the literature that TQ and DMSO which is used as a solvent do not cause any EA (13,14)and a large part of these patients are resistant to antiepileptic drugs discovered so far. In addition, side effect profiles of these

drugs are very wide. Rapamycin that is an inhibitor of mammalian target of rapamycin (mTOR. This suggests that TQ consumption will not cause any EA either in patients with epilepsy or in healthy individuals

In the ECoG records obtained from rats induced with penicillin, in which EA was induced with penicillin, the first EA initiation times after penicillin administration were evaluated. The first EA onset times of all groups were found statistically longer than the CONT group. Although the initial EA onset times of the TQ10+DZM and TQ50+DZM groups in which TQ and DZM were administered in combination were longer than the TQ10 and TQ50 groups treated with only TQ, however, it was not statistically significant. Similarly, the onset time of the first EA in the TQ10 group was longer than in the TQ50 group, but it was not statistically significant. In addition, there was no significant difference between TQ administration and DZM groups used in epilepsy treatment. According to these results, chronic administration of TQ caused a twofold delay in the onset time of penicillin-induced EA, compared with the CONT. The results of the CONT group are consistent with other studies in the literature (16,17). Latency results for TQ are consistent with different epilepsy models such as pentylenetetrazole (PTZ) and maximal electroshock seizure (MES) (16).

According to the EA spike-wave frequency average values in the recordings of different doses of TQ, there was no significant difference in all time intervals of 2 hours except between the first 11^{th} -20^{th} minutes. Although the spike-wave frequencies of the groups decreased compared to the CONT group from the beginning of the first EA, however, it was observed that it was not statistically significant. Our findings show some differences between with literature, but they have similar results with some literature (18,19).

According to the EA spike-wave amplitude median values in the recordings of different doses of TQ; there was no significant difference in all time intervals of 2 hours except between the 96^{th} -115^{th} minutes. In the time interval of 96^{th} -115^{th} minutes; TQ caused a decrease in spike-wave amplitude. According to these results; it can be said that TQ has a reducing effect on the EA spike wave amplitude, and thus reducing effect occurs 90 minutes after the application of the TQ. These findings are consistent with the literature (16,20).

Hosseinzadeh *et al*. reported that the PTZ-induced status epilepticus (SE) model, intracerebroventricular administration of 200 μM and 400 μM TQ prolonged the onset of the first EA in the PTZ-induced status epilepticus (SE) model(16). In the same study, it was reported that the administration of naloxone (10 μM icv) with a 200 μM dose of TQ, which is used in the treatment of epilepsy, prolongs the onset of the first EA and reduces the seizure duration (16). The antiepileptic effect of TQ is likely due to an opioid receptor-mediated enhancement of GABAergic stimulation. In addition, another study reported the antiepileptic effect of TQ in mice using PTZ and MES-induced epilepsy models: Indeed, it has been reported that 50 and 100 mg/kg doses of TQ increase the effect of sodium valproate in both PTZ and MES models (20). In addition, the administration of the combination of phenobarbital and TQ in the PTZ-induced rat model showed a stronger anticonvulsant effect compared to the administration of phenobarbital alone (21). In another study, it was reported that TQ and vitamin C showed anticonvulsant effects by activating the GABAB1R/CaMKII/CREB pathway (22). In a penicillin-induced epilepsy model, TQ has been reported to have antiepileptic activity in rats. In the study, it was reported that 10, 50, and 100 mg/kg TQ administration prolonged the onset of the first EA and decreased the spike-wave frequency and amplitude of the EA (14)Medical School, Duzce University, Duzce, Turkey, between October 2013 and December 2014. Animals were divided into the following 7 groups: sham, control, only thymoquinone, vehicle (Dimethylsulfoxide. Another epilepsy model is the SE model created by bilateral administration of 0.5 μg icv kainic acid to rats. Rats were cured with TQ for 4 days. It has been reported that TQ significantly reduces spontaneous motor seizures, and decreased neuronal degeneration in the brain. (23). In another study, TQ reduced brain injury in the lithium-pilocarpine rat model. It has been shown that TQ prolongs the initiation latency of the first EA (24).

Experimental epilepsy models are used to elucidate the mechanisms underlying epilepsy and to investigate the effects of many different substances thought to have antiepileptic effects on epilepsy. Thus, it is trying to develop a permanent and effective treatment method for epilepsy, which is an important health problem.

$GABA_A$ receptors are considered the foremost inhibitory control system in the brain. It is thought that the underlying cause of convulsant activities is the weakening or disappearance of this inhibitory activity (17). Decreased pause activity disrupts the inhibitory-excitatory imbalance in the excitatory direction, leading to the formation of a neural environment suitable for the initiation and propagation of EA (25)3-dione (CNQX. Sometimes excessive activation of N-methyl-D aspartate (NMDA) receptors, a type of glutamate receptor, causes epileptiform activities (26)kainate and N-methyl-d-aspartate (NMDA. In both cases, EA can be reduced by using competitive or non-competitive NMDA channel antagonists (blockers) and other non-NMDA glutamate receptors (15)the interaction between these systems remain elusive. Therefore, the present study was initiated to evaluate the possible interactions between cannabinoid compounds and NMDA receptor antagonist in the penicillin-induced epileptiform activity in rat. In the first set of experiments, 30min after intracortical injection of penicillin, five different doses of memantine (3,5-dimethyl-1-adamantanamine hydrochloride, 1, 2.5, 5, 10 or 20mg/kg.

GABA receptors in the CNS, $GABA_{A,}$ and $GABA_B$, are suppressed when exposed to picrotoxin or competitive antagonist bicuculline, resulting in the formation of EA (27)modulation of GABA(A. Epileptiform field potentials and paroxysmal depolarization shifts can be created by the administration of the $GABA_A$ receptor antagonist bicuculline or the $GABA_A$ channel blocker picrotoxin, and this activity can be blocked by various antagonists. Therefore, the relative increase in excitatory neurotransmitter (especially glutamate) release due to the elimination or weakening of the inhibitory effect of GABA in the brain will cause excessive stimulation in the brain.

Cortical pyramidal cells also play an active role in the EA created by penicillin. In the epilepsy model created by penicillin; potentials linked to $GABA_A$ and $GABA_B$ receptors contribute to the sudden depolarization shifts observed in cells (14)Medical School, Duzce University, Duzce, Turkey, between October 2013 and December 2014. Animals were divided into the following 7 groups: sham, control, only thymoquinone, vehicle (Dimethylsulfoxide. Penicillin applied directly to the cortex causes inhibition of GABA receptors by acting similarly to bicuculline and thus suppressed GABA activity initiates local EA by disrupting the inhibitory system of the brain (28).

Marangoz *et al.* suggested that 500 IU cortical penicillin injection may cause EA to become dependent on increased excitatory activity by suppressing the effect of the cortical GABA system and disrupting the inhibition balance (29). Another similar study has done by Chen *et al.* (30). In the study, rats were given 2.5-5 million IU / kg i.p. penicillin administration caused the emergence of spike activity after 45 ± 31 minutes and the emergence of mature seizure activity after 71 ± 38 minutes.

Consequently, we showed that TQ has a protective effect on the penicillin model epilepsy, as in other experimental epilepsy models. The limitation of this study, we did not conduct molecular and biochemical analyzes. However, it has the potential to be the first research to study the effects of chronic thymoquinone electrophysiologically in a penicillin-induced epileptiform activity model. The present study examined the protective effect of TQ before penicillin. Conducting molecular and biochemical studies with a multidisciplinary approach on the long-term protective effect of TQ during seizures will shed light on the issue. As a result; as in experimental epilepsy models, we can say that 10 mg/kg and 50 mg/kg doses of TQ's protective effect can be beneficial in epileptic patients.

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Conflict-of-interest and financial disclosure

The authors declares that they have no conflict of interest to disclose. The authors also declare that they did not receive any financial support for the study.

Author Contributions

EB and OB conceived and designed research; EB DAA and OB performed experiments; EB, DAA and OB analyzed data; EB, DAA and OB interpreted results of experiments; EB, DAA and OB prepared figures; EB and OB drafted manuscript; EB and OB edited and revised manuscript; EB, DAA and OB approved final version of manuscript.

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