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Research Article

Effects of *Thymus vulgaris* L. in Acute and Chronic Epilepsy Models in Rats Induced by Pentylenetetrazole

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Abstract

The aim of this study was to investigate the effects of *Thymus vulgaris* L. (TVL) on oxidative stress, motor coordination and learning/memory in acute and chronic epilepsy models in rats induced by Pentylenetetrazole (PTZ). To this end, 64 male Wistar-albino rats were randomly divided into eight groups with 8 rats each: (1) acute control (AC), (2) acute PTZ (APTZ), (3) acute PTZ + sodium valproate (APTZ+VPA), (4) acute PTZ + TVL (APTZ+TVL), (5) chronic control (CC), (6) PTZ kindling (PTZk), (7) PTZ kindling + VPA (PTZk+VPA) and (8) PTZ kindling + TVL (PTZk+TVL) . Seizures were observed for 30 min after each PTZ injection and were scored. Acute PTZ-induced seizures were created by injecting a single convulsive dose of PTZ (60 mg/kg, ip) in acute groups. PTZ kindling was produced by injecting a subconvulsant dose of PTZ (35 mg/kg, ip) every other day, with 14 injections in total. No significant difference was found among the PTZk + VPA, PTZk, and PTZk + TVL groups with regard to seizure scores. No significant difference was found among all the 8 groups in the learning/memory tests conducted using the Morris Water Maze (MWM) test and the motor activity tests conducted using the rotarod test (P>0.05). The analysis of total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) indicated that the administration of PTZ decreased the antioxidant capacity and increased the oxidant capacity. Moreover, the TVL administration established the oxidant/antioxidant balance, particularly in the chronic groups. Further studies are needed to investigate whether high doses of TVL have an effect on PTZ-induced seizure scores.

Keywords: *Behavior, Epilepsy, Kindling, Seizure, Pentylenetetrazole, Thymus vulgaris* L.

Sıçanlarda Pentilentetrazol İle İndüklenen Akut ve Kronik Epilepsi Modellerinde *Thymus vulgaris* L.'nin Etkileri

Öz

Bu çalışmanın amacı pentilentetrazol ile indüklenen akut ve kronik epilepsi modellerinde *Thymus vulgaris* L. (TVL)'in oksidatif stres, motor koordinasyon ve öğrenme/bellek üzerine etkilerini araştırmaktır. Bu amaçla 64 adet Wistar albino sıçan randomize olarak sekiz gruba ayrıldı. (1) akut kontrol (AC), (2) akut pentilentetrazol (APTZ) (3) akut PTZ + sodyum valproat (APTZ+VPA), (4) akut PTZ + TVL (APTZ+TVL), (5) kronik kontrol (CC), (6) PTZ tutuşma (PTZk), (7) PTZ tutuşma + VPA (PTZk+VPA) ve (8) PTZ tutuşma + TVL (PTZk + TVL). PTZ enjeksiyonundan sonra 30 dk boyunca nöbetler gözlenerek skorlandı. Çalışmada tek doz PTZ (60 mg/kg, ip) enjeksiyonu ile akut PTZ indüklü nöbet modeli oluşturuldu. Tutuşma ise gün aşırı subkonvulsan dozda PTZ (35 mg/kg, ip) uygulanarak toplam 14 enjeksiyonla oluşturuldu. PTZk+VPA, PTZk ve PTZk+TVL grupları arasında nöbet skorları bakımından anlamlı bir fark bulunamadı. Kronik çalışma gruplarında Morris water maze cihazı ile yapılan öğrenme ve bellek testleri yanında rota rod cihazı ile yapılan motor aktivite testlerinde gruplar arasında farklılık görülmedi (P>0.05). Total antioksidan statüsü (TAS), total oksidan statüsü (TOS) ve oksidatif stres indeksi (OSİ) sonuçları, PTZ uygulaması neticesinde antioksidan kapasitenin azaldığı, oksidan kapasitenin ise arttığı belirlendi. *T. vulgaris*'in özellikle uzun süre uygulanması ile total oksidan-antioksidan dengenin sağlandığı görüldü. Sonuç olarak gelecekte, daha yüksek dozlarda *T. vulgaris* L.'nin PTZ indüklü nöbet skorlarını değiştirip değiştirmeyeceğini belirlenmesi için ek çalışmaların yapılabileceği düşünülmektedir.

Anahtar sözcükler: *Davranış, Epilepsi, Tutuşma, Nöbet, Pentilentetrazol, Thymus vulgaris* L.

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INTRODUCTION

Epilepsy is one of the most common and serious brain disorders in the world (WHO, 2004). About 1/3 of the patients undergoing antiepileptic therapy continue to experience seizures and in some of these patients, progressive conditions such as increasing seizure frequency and cognitive decline can also be seen [1]. Although the underlying cause of epilepsy remains unclear, a long-standing hypothesis posits that the immune system plays a role in the pathogenesis of epilepsy. Additionally, this hypothesis has also been supported by the recent reports that implicate the use of steroids or immunoglobulins in the treatment of certain types of epilepsy and those documenting the role of inflammation and antibodies in the clinical course of certain types of epilepsy [2]. Pentylenetetrazole (PTZ) is a widely used behavioral approach in antiepileptic drug discovery studies [3]. Development of novel anticonvulsant drugs and disease-modifying antiepileptogenic agents constitute the next generation of therapeutic approaches for epilepsy. Moreover, accumulating evidence suggests that inflammatory events play a role in the etiopathogenesis of various types of epilepsy [4,5].

Experimental and clinical studies in the past decade have revealed that some specific inflammatory mediators and their cognate receptors are upregulated in epileptic brain tissue. Some of these drugs have already been shown to have a therapeutic effect on chronic peripheral inflammation and to have the potential to facilitate the hyperexcitability processes by reducing inflammation in epileptic brain tissue. Moreover, development of drugs that interfere with the mechanisms involved in the etiopathogenesis of seizures could lead to disease-modifying and curative effects in addition to symptomatic effects [6]. Therefore, drugs with anti-inflammatory or antioxidant properties are commonly studied in experimental epilepsy models for their potential therapeutic effects.

Thyme belongs to the Lamiaceae family and is a well-known therapeutic agent and is commonly used as folk medicine. In particular, the composition of the essential oils from *Thymus vulgaris* L. (TVL) are grown in the Mediterranean parts of Turkey [7,8]. This composition consists of thirty main elements of essential oil that constitute 100% of this composition. Some of these elements include thymol (46.2%), carvacrol (2.44%), linalool (4.0%), gamma-terpinene (14.1%), p-cymene (9.9%), myrcene (3.5%), α-pinene (3.0%), and flavonoids [8,9]. Of these, carvacrol has been shown to have antifungal, antibacterial, anthelmintic, analgesic, insecticidal, cholesterol-reducer, liver protective, antioxidant and anti-cancerogenic effects [10-13]. Its been shown to have anti-diabetic effects and to improve the learning functions of the brain [14].

Epilepsy, mediated by oxidative stress, results in abnormal structural changes in cellular proteins, membrane lipids, DNA, and RNA. Oxidative stress in brain has been implicated as the widespread cause of numerous acute neurological disorders including Parkinson's disease and Alzheimer's disease [15]. Moreover, oxidative stress has been reported to play a key role in various epilepsy models and PTZ is known to cause oxidative stress [16]. The biochemical changes induced by oxidative stress implicate the role of free radicals during seizures. Since antioxidants have a potential to reduce the seizures, though partially, they may be used as additional agents to antiepileptic drugs. Therefore, in the present study, we aimed to investigate the antiepileptic activity of TVL in acute and chronic epilepsy models to evaluate the protective and antioxidant effects of TVL on memory.

MATERIAL and METHODS

First of all, fresh leaves of thyme were collected in May 2017 from the area between the Aydın and İzmir provinces in the Aegean Region of Turkey. Following identification, a voucher specimen was prepared with a code number VAN YYU VANF-164096.

The animal care protocol and the experimental study were approved by the Animal Care and Use Committee of the Experimental Animal Unit and Ethics Committee (2018/05).

Preparation of Extract

The ground leaves were dried in shade, sheltered from direct sunlight, milled to a homogeneous powder, and then sifted through a 1-cm mesh. The powdered plant material (20 g) was extracted in 150 mL of distilled water with decantation method for 5 min. The extract was concentrated to dryness using a rotary evaporator followed by a lyophilizer and then the yield percentage of the extract was calculated. Water extraction of TVL was performed using a modified version of the decoction method used by Eddouks et al.[17].

Experimental Design

Sixty-four male Wistar Albino rats were randomly divided into eight groups with 8 rats each: (1) acute control (AC), (2) acute PTZ (APTZ), (3) acute PTZ+sodium valproate (APTZ+VPA), (4) acute PTZ+TVL (APTZ+TVL), (5) chronic control (CC), (6) PTZ kindling (PTZk), (7) PTZ kindling+VPA (PTZk+VPA) and (8) PTZ kindling + TVL (PTZk+TVL). PTZ (Sigma) was dissolved in isotonic saline (0.9% NaCl) and was administrated by intraperitoneal injection. VPA and TVL extract were administered orally by intragastric gavage daily.

Induction of Acute Seizures and PTZ Kindling

Tonic-clonic seizures were induced by injecting a single convulsive dose of PTZ (60 mg/kg, 0.1 mL/100 g) [18] in the acute groups (APTZ, APTZ+VPA, APTZ+TVL). The rats in the APTZ+VPA group received VPA (100 mg/kg by gavage, 0.1 mL/100 g) [19] and the rats in the APTZ+TVL group received TVL (200 mg/kg by gavage, 0.1 mL/100 g) [14] 2 h before

the acute PTZ injection. The rats in the AC group received saline solution only (0.1 mL/100 g by gavage).

PTZ kindling was produced by injecting a subconvulsant dose of PTZ (35 mg/kg) every other day (three times a week; every Monday, Wednesday and Friday; 14 treatments in total).

The rats in the chronic control group received isotonic saline (0.9% NaCl) TVL by gavage every day. In the PTZk+VPA and PTZk+TVL groups all PTZ injections were administrated 2 h after oral treatments (VPA or TVL). TVL extract was prepared freshly (in isotonic saline, 0.9% NaCl) and administrated daily to the rats (by gavage at dose 0.1 mL/100 g) in the PTZk+TVL group. Blood and tissue samples were collected under ketamine (90 mg/kg ip), and xylazine (10 mg/kg s.c) anesthesia.

Seizures were observed for 30 min after each PTZ injection. Seizure scores were evaluated using the following scoring system: stage 0: no response; stage 1: ear and facial twitching; stage 2: myoclonic jerks without upright position; stage 3: myoclonic jerks, upright position with bilateral forelimb clonus; stage 4; tonic-clonic seizures; stage 5: generalized tonic-clonic seizures, loss of postural control [20,21]. The rats demonstrating at least three consecutive stage 4 or 5 seizures were considered to be kindled.

Statistical Analysis

Statistical Analysis for Seizures, Spatial Learning-Spatial Memory and Motor Activity Tests: Data were analyzed using SPSS for Windows version 13.0 (SPSS Inc. Co., Chicago, IL, USA). Descriptive statistics were expressed as median, mean, standard deviation (SD), minimum, and maximum. The groups were compared using Kruskal-Wallis test. Friedman tests was used to compare days for Morris Water Maze (MWM) data. A P value of $P<0.05$ was considered significant.

Statistical Analysis for TAS-TOS: Results are expressed as Mean±Standart Deviation (SD). Analysis of variance (ANOVA) was performed, and the statistical comparisons among the groups were carried out with post hoc Tukey's test for normally distributed variables, or with nonparametric Bonferroni test for non-normally distributed data using a statistical package program (SPSS 23.0 for Windows).

RESULTS

No significant difference $P>0.05$ was found among the seizure scores in the acute groups *(Table 1)*.

In the PTZk group, the experiment was finalized when kindling was achieved after the 14th injection *(Table 2)*. In the same group, no rat died throughout the experiment.

Table 1. *Comparison of seizure scores in the acute groups*

Group		Median	Mean	SD	Min.	Max.	P
Score	APTZ	5.00	5.00	.00	5.00	5.00	0.329
	APTZ+TVL	5.00	4.71	.76	3.00	5.00	
	APTZ+VPA	5.00	4.43	.98	3.00	5.00	

Table 2. *Effects of VPA and TVL treatment on the development of PTZ-kindled seizures*

Injection Number	PTZk + VPA					PTZk					PTZk + TVL					*P Values for Groups
	Med.	M	SD	Min.	Max.	Med.	M	SD	Min.	Max.	Med.	M	SD	Min.	Max.	
#1	1.0 c	1.3	.5	1.0	2.0	1.0 d	1.3	.5	1.0	2.0	1.0 d	1.4	.7	1.0	3.0	.981
#2	1.0 c	1.9	1.1	1.0	3.0	1.5 cd	1.8	1.0	1.0	3.0	1.0 d	1.4	.7	1.0	3.0	.549
#3	3.0 b	2.3	1.3	1.0	4.0	3.0 c	2.3	1.0	1.0	3.0	1.0 d	1.6	1.2	1.0	4.0	.432
#4	3.0 b	3.0	1.6	1.0	5.0	3.0 c	3.0	1.3	1.0	5.0	2.0 c	2.1	1.2	1.0	4.0	.433
#5	3.0 b	2.7	1.4	1.0	5.0	3.0 c	3.3	.8	3.0	5.0	3.0 b	2.3	1.0	1.0	3.0	.213
#6	3.0 b	2.7	1.4	1.0	5.0	3.0 c	3.0	.9	2.0	4.0	3.0 b	2.4	1.2	1.0	4.0	.649
#7	4.0 a	3.9	.9	3.0	5.0	3.5 bc	3.7	.8	3.0	5.0	3.0 b	3.2	.4	3.0	4.0	.266
#8	3.0 b	3.1	.9	2.0	5.0	4.0 b	3.7	1.0	2.0	5.0	3.0 b	3.0	1.1	2.0	5.0	.379
#9	3.0 b	3.1	1.5	1.0	5.0	4.0 b	3.7	.5	3.0	4.0	3.0 b	2.6	1.3	.0	4.0	.248
#10	4.0 a	3.7	1.0	2.0	5.0	4.0 b	3.8	.4	3.0	4.0	3.0 b	3.4	.9	2.0	5.0	.437
#11	3.0 b	3.3	.8	2.0	4.0	4.0 b	3.8	.4	3.0	4.0	3.0 b	3.1	.8	2.0	4.0	.184
#12	3.0 b	3.3	.8	2.0	4.0	4.0 b	4.0	.0	4.0	4.0	3.0 b	3.4	1.2	2.0	5.0	.210
#13	4.0 a	3.9	1.2	2.0	5.0	5.0 a	4.7	.5	4.0	5.0	3.5 ab	3.8	.9	3.0	5.0	.178
#14	4.0 a	4.1	.9	3.0	5.0	5.0 a	4.7	.5	4.0	5.0	4.0 a	3.6	.9	2.0	5.0	.091
** *P values for injection*	.001					.001					.001					

** Kruskal-Wallis Test→; ** Friedman test ↓; Variables with more than one letter indicate statistical significance between injection numbers (the column)*

On the other hand, no significant difference was found among the PTZk, PTZk+VPA, and PTZk+TVL groups in terms of seizure scores. However, seizure frequency increased progressively in all the three groups (P<0.001, *Table 2*).

Spatial learning/memory was tested in the rats in the chronic groups using the Morris Water Maze (MWM) [22,23]. MWM was performed using the spatial version of the MWM test used by Tuzcu and Baydaş [24]. For pretraining orientation, the rats were made to swim in the platform-free maze for 2 min. Before the testing, environmental cues that aid the rats in spatial learning including high-contrast geometric patterns were placed on the walls visible to the animal from the water and platform throughout the duration of the experiment. Using a computer equipped with Noldus EthoVision Tracking System, the maze was divided into 4 equal quadrants. Care was taken to position the platform in the same quadrant throughout the test. Each rat swam for 4 times over a period of four consecutive days. The time spent for locating the hidden platform (reaction time) and the time spent in the target quadrant were recorded for each rat.

After the completion of these tests, total anti-oxidant status (TAS) and total oxidant status (TOS) were measured using a commercially available kit [25]. Oxidative stress index (OSI) was defined as the ratio of TOS to TAS level [25]. For the calculation of OSI, the resulting unit of TAS (mmol Trolox equivalent/L) was converted to micromole equivalent/L. The OSI value was defined as 'arbitrary unit' (AU) and was calculated using the following formula:

$$OSI = \frac{(TOS,\ \mu mol\ H_2O_2\ Eq./lt)}{(TAS,\ mmol\ Trolox\ Eq./lt\ X\ 10)}$$

The rotarod test consists of a circular rod turning at a constant or increasing speed and is used for estimating the duration a rat can remain on the rod rather than fall onto a platform below. Prior to the test, the rats were trained to remain on the rod rotating at a speed of 6 rpm for a total duration of 3 min. For testing, each rat was placed on the rod turning at 16 rpm for three sessions of 60 sec each. The integral of time spent on the rod over three sessions (maximum, 180 sec) was accepted as the rotarod performance for each rat [26].

Spatial Learning Test

The spatial learning test on MWM indicated that the PTZk+TVL group had a greater decrease in the time spent for locating the hidden platform compared to other chronic groups, although no significant difference was established among the groups (P>0.05) *(Fig. 1)*.

Spatial Memory Test

The spatial memory test on MWM indicated that the time spent in the target quadrant was longer in the PTZk + TVL group compared to other chronic groups, although no

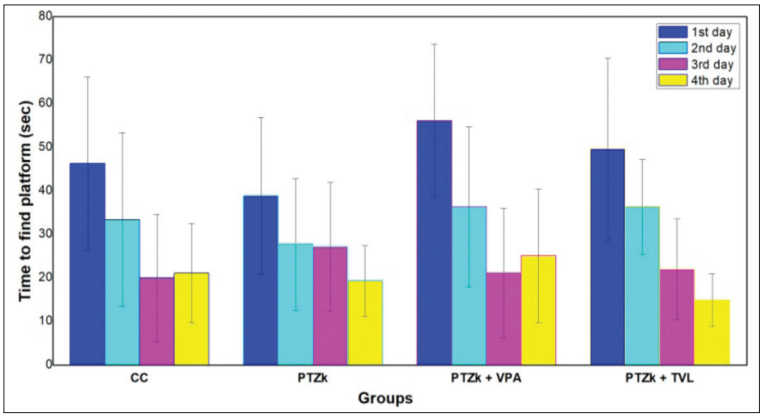

Fig 1. Time spent for locating the hidden platform on the MWM test in the chronic groups *(Friedman* Test was applied there was no significant difference among the groups, P>0.05)

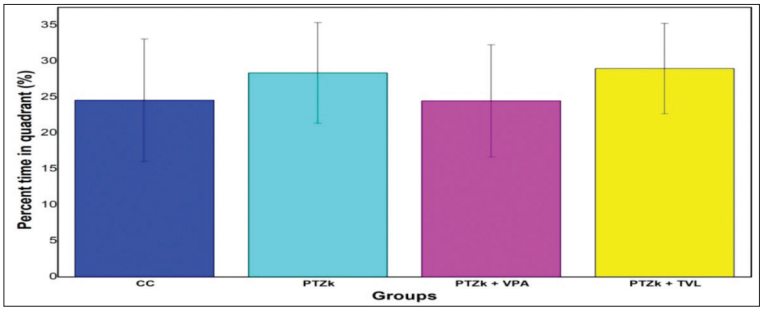

Fig 2. Comparison of the time spent in the target quadrant among the chronic groups *(Friedman* Test was applied there was no significant difference among the groups, P>0.05)

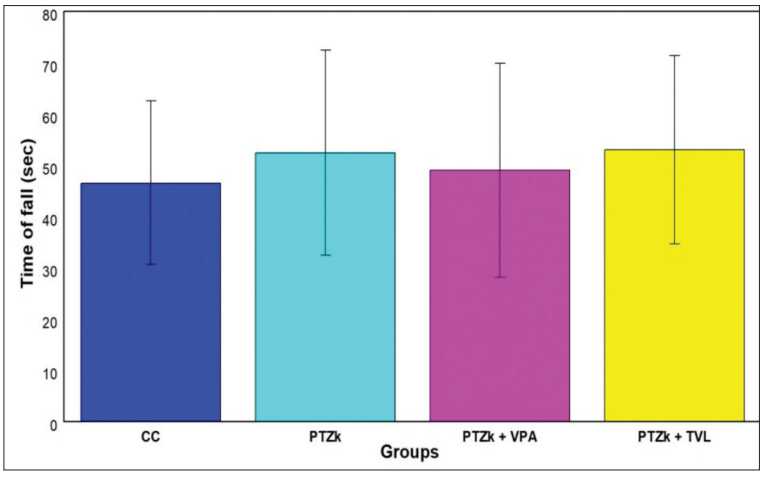

Fig 3. Comparison of the time spent on the rotating rod among the chronic groups *(Friedman* Test was applied there was no significant difference among the groups, *P>0.05)*

Table 3. *Serum TAS, TOS, and OSI levels*

Group	TAS Mean±SD	TOS Mean±SD	OSI Mean±SD
AC	1109±47.65[a]	8.3±0.46[a,c]	0.76±0.05[a,a1]
APTZ	909.2±87.51[c]	11.76±0.50[a1,b,b1,c,c1]	1.32±0.07[a2]
APTZ+VPA	710.2±39.07[b]	7.71±0.49[a2,c1]	1.10±0.09[a3,c]
APTZ+TVL	996.9±104.8[b1]	34.91±1.51[a,a1,a2,a3,a4,a5,a6]	3.74±0.49[a,a2,a3,a4,a5,a6,a7]
CC	1223±146.6[a1,b,c2]	7.67±0.65[a3,b,]	0.69±0.11[a4,a8]
PTZk	434.7±28.36[a,a1,b1,b2, c]	9.36±0.49[a4]	2.17±0.07[a1,a5,a8,a9,c]
PTZk+VPA	781.6±101.1[c2]	10.29±0.83[a5]	1.42±0.20[a6]
PTZk+TVL	1004±90.76[b2]	7.02±0.43[a6,b1]	0.75±0.10[a7,a9]

[a,a1-a9] P<0.001, [b,b1, b2] P<0.01, [c, c1, c2] P<0.05 (Groups with the same letter indicate the presence of a significant difference)

significant difference P>0.05 was established among the groups *(Fig. 2)*.

Motor Activity Test

The Rotarod performance test indicated no significant difference P>0.05 among the chronic groups with regard to motor activity *(Fig. 3)*.

TAS and TOS Measurement

The analysis of serum samples indicated that the TAS levels decreased in the APTZ group compared to the control group and increased, though insignificantly, in the APTZ+TVL group compared to the APTZ group (P>0.05). Moreover, TAS levels decreased significantly (P<0.001) in the PTZk group compared to the control group and increased significantly (P<0.001) in the PTZk+TVL group compared to the PTZk group *(Table 3)*.

In acute groups, the TOS levels were significantly higher in the APTZ and APTZ + TVL groups compared to the control group (P<0.05, P<0.001, respectively). However, no significant different was found among the chronic groups with regard to TOS levels, a significant (P<0.001) difference was found between the APTZ+TVL and PTZk+TVL groups *(Table 3)*.

In acute groups, OSI was increased significantly in the APTZ+TVL group compared to the other groups (P<0.001). In chronic groups, OSI increased significantly (P<0.001) in the PTZk group compared to control group and decreased significantly (P<0.001) in the PTZk+TVL group compared to the PTZk group *(Table 3)*.

DISCUSSION

The present study evaluated the antiepileptic effects of a variant of thyme and its effects on spatial learning/memory, and motor activity and also examined the oxidant and antioxidant capacity levels of TVL in experimental epilepsy models induced by acute or chronic PTZ administrations.

Epilepsy affects more than 65 million people worldwide [27]. Epilepsy is a highly prevalent, serious brain disorder and oxidative stress is regarded as a possible mechanism involved in epileptogenesis. Inflammation and oxidative stress are known as critical factors in the pathophysiology of epilepsy [28,29]. However, it has been suggested that there may also be an association between seizure generation and the disruption of the balance between oxidants and antioxidants [30].

Experimental studies suggest that oxidative stress is a factor contributing to the onset and evolution of epilepsy. Oxidative stress is regarded as a result of the disruption of the balance between the formation and the extermination of reactive oxygen species (ROS) [28]. In addition, excessive ROS formation may alter DNA and thereby may lead to lipid modification and formation of pro- and anti-inflammatory cytokines [31]. Increasing data suggest that there is an association between the immune system and the pathophysiology of epilepsy [32]. The inflammatory responses are also considered to contribute to epileptogenesis [32]. Moreover, it has been shown that there is an association among IL-1β, IL-6, TNFα and epilepsy and it has also been reported that seizures increase both the level of IL-1β and its mRNA and this increase is associated with oxidative stress [32].

Oxidative stress is an important factor in numerous epilepsy models [33]. Moreover, researchers have recently focused on a possible association between oxidative stress and epilepsy, contending that the abnormal Ca^{+2} signaling resulting from oxidative stress may lead to excessive free radical formation, mitochondrial dysfunction, cell injury, and ultimately to epilepsy [34]. Oxidative stress and mitochondrial dysfunction are involved in neuronal death and seizures. However, oxidative damage occurred in proteins, lipids, and mitochondrial DNA after seizure activity [35]. These findings implicate that RNA oxidation is a significant factor contributing to seizure-induced neuronal degeneration and to epileptogenesis [35,36].

Accumulating evidence suggests that antioxidant therapy may reduce lesions induced by oxidative free radicals in

some animal seizure models. Recent studies have also shown that the association between mitochondrial dysfunction and chronic oxidative stress may play an important role in epileptogenesis [28]. Therefore, there is a growing interest into antioxidants that decrease oxidative stress in the treatment of epilepsy [30]. Induced seizures may be partially prevented with the treatment methods that are based on antioxidants such as superoxide dismutase (SOD) mimetics, vitamin E, melatonin, spin traps, vitamin C and coenzyme Q10 [37,38].

Literature indicates that agents with anti-inflammatory or antioxidant properties are commonly studied in experimental epilepsy models for their potential therapeutic effects. Oxidative stress is considered to play a role in epileptogenesis and in PTZ-induced acute seizures. Moreover, herbs with antioxidant properties have also been shown to have therapeutic effects in experimental epilepsy models [39,40]. In the present study, we found that TVL, particularly its chronic administration, strengthened the antioxidant system. Also, it was reported that chronic administration of *Aloe vera* leaf (extract) powder prevented the progression of kindling in PTZ-kindled mice and also reduced brain levels of malondialdehyde (MDA) and increased the glutathione (GSH) levels compared to the PTZ-kindled non-treated group. Depending on these findings, the authors concluded that *Aloe vera* leaf (extract) powder has significant anticonvulsant and antioxidant activity [41]. On the other hand, the phytochemical results presented by the epilepsy studies conducted with plant extracts have indicated the presence of three flavonoids and four additional compounds belonging to the steroid, terpenoid, phenol, alkaloids, saponins, tannins or sugar classes of compounds in jasmine flowers and *P. daemia* roots [42,43]. Flavonoids (luteolin) have been shown to provide protection against some biological epilepsy models such as the seizures induced by PTZ [44].

Skalicka-Wozniak et al.[45] performed efficient purification of single constituents from *Thymus vulgaris* essential oil (EO) (borneol, thymol and carvacrol) and investigated anticonvulsant activities of single constituents from *Thymus vulgaris* EO. The authors indicated that *Thymus vulgaris* EO provided protection against maximal electroshock (MES)-induced seizures. Accordingly, preclinical research on *Thymus vulgaris* EO, as well as on isolated terpenoids, provides evidence suggesting that the essential oil has partial protective activity against seizures.

Sodium valproate (VPA), an antiepileptic drug used in the treatment of epilepsy due to its therapeutic effect, shows gamma-aminobutyric acid (GABA)-mimetic activity and elevates brain GABA levels by inhibiting some enzymes that are responsible for the synthesis and destruction of GABA. Moreover, VPA also enhances the glutamic acid decarboxylase activity that plays a key role in GABA synthesis and weakly inhibits the GABA-aminotransferase that plays a part in GABA metabolism [46]. Accordingly, it is reported that VPA, when used with ethanolic extract of TVL, has no effect on MES- and PTZ-induced convulsions. However, combination treatments such as Thyme+VPA have been shown to provide full protection (100% and 33%, respectively) against convulsions induced by PTZ and MES [47]. In our study, however, we did not use TVL and VPA in combination and we found that the sole application of TVL extract seems to have no effective constituents that can battle these mechanisms. Therefore, it is tempting to consider that TVL extract could not enhance GABA-ergic neurotransmission.

Hippocampus is known to have a crucial role in learning and memory in mammals. Accordingly, any neuronal damage or dysfunction in the hippocampus leads to cognitive impairment [48]. Previous studies indicated that the PTZ-induced seizures resulted in hippocampal neuronal damage, subsequently leading to spatial learning and memory impairment [48,49]. Additionally, it has also been reported that antiepileptic drugs (AEDs), which are known to have limited effectivity even in drug-controlled seizures, have little or no effect on the cognitive deficits in epilepsy [50].

Most AEDs interfere with cognitive functions and thus there is an urgent need for AEDs that are effective but do not show this side effect. Of note, some of the plants that have been investigated in the search for alternative drugs against the neuronal damage caused by epilepsy have been shown to have favorable effects on epilepsy and cognitive functions [48].

For instance, some researchers have shown anti-inflammatory and neuroprotective effects of Sinomenine (SN) in nervous system diseases. Moreover, the researchers also determined the effect of SN on epilepsy in PTZ-induced chronic epilepsy models by assessing spatial learning and memory using MWM and reported that different doses of SN blocked the hippocampal neuronal damage, minimized the impairment of spatial learning and memory in PTZ-kindled rats, and also provided neuroprotection both *in vitro* and *in vivo* [48].

Morteza-Semnani et al.[51] investigated the effect of methanol extracts and essential oils from Thymus species *(Thymus fallax, T.kotschyanus* Boiss. and *T. pubescen* Boiss.) on swimming performance in mice. This finding implicates that the extracts and oils shortened the immobility period during the forced swimming test in the experimental group compared with the control group and exhibited a dose-dependent antidepressant activity.

On the other hand, Zhen et al.[44] investigated the anticonvulsant effects of a natural flavonoid, luteolin (LU), on PTZ-induced cognitive impairment in rats. The researchers revealed that pretreatment with LU suppressed the seizure induction, duration, and severity following PTZ injection, reversed cognitive impairment, reduced neuronal and oxidative stress damage.

Similarly, given that TVL has favorable effects on spatial learning and memory, the present study investigated the protective effects of TVL on neuronal damage. However, since the cognitive functions of the rats were not affected in our experimental epilepsy model, no evaluation could be made regarding the effect of TVL on cognitive functions.

Our results indicated that although there was a decrease in the scores of the PTZk+TVL group compared to the PTZk group, a satisfactory decrease was not obtained probably due to the inadequate dose (200 mg/kg) of TVL. From these findings then, it can be thought that TVL does not involve an effective substance to enhance inhibitory GABA-ergic neurotransmission in the central nervous system. However, favorable effects of TVL on cognitive functions were demonstrated in one of our previous studies. Our results showed that thyme extract improves the cognitive learning functions that are impaired by diabetes [52]. Depending on these successful outcomes, in the present study, we administered TVL at dose of 200 mg/kg.

The performance of a rat on the rotarod test is a valuable measure that can be used for evaluating the aspects of motor function such as balance and coordination [53]. In the present study, we used the accelerating rotarod test to investigate whether epilepsy and the administration of TVL extract have any effect on the motor functions of the rats. The test indicated no significant difference among the chronic groups with regard to motor activity. This finding implicates that the motor activity is not affected in PTZ-kindling epilepsy model and the administration of TVL 200 mg/kg does not affect the motor coordination center in the rat brain. Meaningfully, future studies to be conducted with higher doses of TVL may elucidate whether motor functions are affected at higher doses. Similarly, Addae et al.[42] examined the neuropsychiatric effects of Jasmine leaf extract that is extensively used in folk medicine. The researchers concluded that Jasmine leaf extract provided favorable outcomes in an animal model of acute partial complex epilepsy and also had a significant anxiolytic effect at a dose that does not affect motor coordination. Additionally, it has also been shown that essential oil and any single compound of *Thymus vulgaris* has no significant effect on motor performance of the experimental animals assessed by the chimney test [45].

In our study, the evaluation of TAS, TOS, and OSI levels indicated that the PTZ administration decreased the anti-oxidant capacity and increased the oxidant capacity while the TVL administration established the oxidant/antioxidant balance in the chronic groups. These findings implicate that TVL may protect the organism against oxidative stress in chronic PTZ-induced epilepsy model rather than in acute PTZ-induced seizure model and also suggest that a better understanding of these effects could be obtained by further studies to be conducted dose-dependent. Based on the other studies, it can be considered that these biological and neuroprotective effects are associated with the components of the essential oil such as borneol, thymol, eugenol, carvacrol, flavonoids, and terpenoids. The compounds of *Thymus vulgaris* can be further investigated in acute and chronic PTZ-induced epilepsy models.

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Conflicts of Interest

There are no conflicts of interest.

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