Journal of Research in Pharmacy
Research Article
www.jrespharm.com

# Investigation of antiepileptic potentials of usnic acid and some lichen species on the behavioral and biochemical levels in pentylenetetrazole-induced kindling model of epilepsy

**Mehmet BERKÖZ[1]*, Oruç YUNUSOĞLU[2], Ali ASLAN [3,4], Ayşe BOZKURT[3]**

1 Department of Biochemistry, Faculty of Pharmacy, Van Yuzuncu Yil University, Van, Türkiye
2 Department of Medical Pharmacology, Faculty of Pharmacy, Bolu Abant İzzet Baysal University, Bolu, Türkiye
3 Department of Pharmacology, Faculty of Pharmacy, Van Yuzuncu Yil University, Van, Türkiye
4 Department of Biology, Faculty of Science, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan
* Corresponding Author. E-mail: mehmetberkoz@yyu.edu.tr (M.B.); Tel. +90-432-444 50 65/21165

Received: 10 May 2023 / Revised: 17 August 2023 / Accepted: 18 August 2023

**ABSTRACT**: In this study, the effects of various lichen and usnic acid applications on seizure scores and biochemical parameters in brain tissue in rats with epilepsy model was investigated. For this aim, 91 rats were divided into 13 groups, each containing 7 rats, which were: control, pentylenetetrazole (PTZ) (35 mg/kg), PTZ + Valproic acid (100 mg/kg), PTZ + *Dolichousnea longissima* (200 mg/kg), PTZ + *Dolichousnea longissima* (400 mg/kg), PTZ + *Xanthoparmelia somloensis* ( 50 mg/kg), PTZ + *Xanthoparmelia somloensis* (200 mg/kg), PTZ + *Cetraria islandica* (250 mg/kg), PTZ + *Cetraria islandica* (500 mg/kg), PTZ + *Pseudevernia furfuracea* (250 mg/kg), PTZ + *Pseudevernia furfuracea* (500 mg/kg), PTZ + usnic acid (50 mg/kg), and PTZ + usnic acid (200 mg/kg). All items were applied with an interval of 120 minutes for a period of one week. Seizure detection, seizure scores and total seizure duration of each group was recorded. After the applications, oxidative stress parameters and acetylcholinesterase enzyme activity in the brain tissue of rats were measured. There was no difference between the groups in the 1*st*, 2*nd*, and 3*rd* injections ($p$>0.05). Starting from the 4th injection, the seizure score was significantly higher in the PTZ group compared to the control group ($p$<0.05). When the effects on locomotor activity were evaluated, no difference was found between any group ($p$>0.05). In PTZ applied groups, an increase in lipid and protein oxidation as well as a decrease in antioxidant and acetylcholine esterase levels were observed (p<0.05). Valproic acid, high concentration of lichen extract applications and high and low concentration of usnic acid applications were found to reverse this situation ($p$<0.05). As a result, various lichen extracts and usnic acid were shown to reduce behavioral symptoms and oxidative stress of epilepsy, with a preventive effect on the complications of epilepsy.

**KEYWORDS**: Epilepsy; lichen; pentylenetetrazole; oxidative stress; seizure scores; brain

## 1. INTRODUCTION

The World Health Organization (WHO) has stated that epilepsy is one of the most common, most serious brain disorders worldwide. This disease causes very serious physical, psychological, social and economic consequences. Epilepsy is the name given to a disorder in the brain called an epileptic seizure, which is defined as a recurrent and unpredictable interruption of brain functions. This situation reflects brain dysfunction caused by a wide variety of causes, rather than being a disease alone. Epileptic convulsions not only have very important effects on the brain structure, but also cause neuronal death [1].

One of the commonly used methods to study brain excitability is experimental pentylenetetrazole (PTZ) administration, which is widely used in studies to develop antiepileptic drugs. Administration of this substance causes hippocampal atrophy in experimental animals. Selective neuronal loss and astrocytosis are observed in the hippocampus in experimental animals which are treated with PTZ, and magnetic resonance imaging studies on these experimental animals shows a decrease in cerebellum volume [2].

Although the mechanism of action of PTZ is not known exactly, it has been reported that it causes changes in the GABAergic system, glutamergic system and antioxidant defense system. Oxidative stress plays an effective role in many epilepsy models. A strong correlation appears to be existence between PTZ application and oxidative stress. It has been reported that PTZ kindling causes changes in the antioxidant

**How to cite this article:** Berkoz M, Yunusoğlu Y, Aslan A, Bozkurt A. Investigation of antiepileptic potentials of usnic acid and some lichen species on the behavioral and biochemical levels in pentylenetetrazole-induced kindling model of epilepsy. J Res Pharm. 2024; 28(5): 1378-1390.


ISSN: 2630-6344
http://dx.doi.org/10.29228/jrp.816

defense system of the brain [3]. Due to oxidative stress, abnormal structural changes occur in membrane lipids, cellular proteins, DNA and RNA. Oxidative stress in the brain is recognized as a common cause of many acute neurological disorders, including more chronic diseases such as Parkinson's and Alzheimer's diseases [4]. Today, drugs used in the treatment of epilepsy mainly have a palliative effect. In addition, since the approved drugs in epilepsy are mainly used for a life-time, this can cause significant side effects. It also creates serious problems in terms of drug interactions. Therefore, there is a serious need to investigate new pharmacological agents for the treatment of epilepsy. Therefore, the discovery of natural alternatives and/or complementary agents with therapeutic properties is of great importance [4].

Nitric oxide (NOx) appears to play a crucial role in a number of physiological and pathophysiological processes in the brain, including the modulation of neuronal plasticity, cerebral blood-flow, cognitive and behavioral functions, as well as, its involvement in neurological disorders such as ischemia and epilepsy. Some studies have suggested that NOx induces neuronal loss and reactive glial proliferation and therefore may potentially play a role in the pathogenesis of epilepsy. However, the exact role of NOx in epilepsy is still unknown [5].

Acetylcholine esterase (AchE) is one of the brain's highly active enzymes that is widely expressed in different brain regions and has several functions. It is an enzyme that terminates the cholinergic transmission by rapid hydrolysis of the neurotransmitter acetylcholine. The cholinergic system in the brain modulates neuronal excitability, synaptic transmission, and synaptic plasticity, playing a significant role in many physiological functions. Although a direct role of cholinergic pathway has not been established, studies in several models of epilepsy have suggested a link between cholinergic activation and epileptogenesis. However, limited data are available on the causal role of cholinergic neurons in the occurrence, propagation, and control of seizures [6].

Lichens, which are known to have existed since 400 million years, are symbiotic organisms formed as a morphological and physiological integrity as a result of the combination of fungi and algae. In lichens, which possesses different features from the algae and fungi in terms of morphology and function, there are structures called thallus, which are formed as a result of the placement of algae cells between the fungal hyphae [7]. Lichen extracts are a source of a plethora of compounds including pulvinic acid derivatives, terpenes, carotenoids, depsides, depsidones, depsones, anthraquinones, and xanthones. Such a high diversity of compounds, often acting synergistically, makes the extracts very attractive for investigation. Moreover, there is growing evidence that crude lichen extracts often have greater in vitro or/and in vivo activity as compared to pure compounds. Studies have shown that the use of lichen extracts at the appropriate dose and time does not cause a significant toxic effect. On the other hand, conflicting results have been obtained regarding usnic acid. While some studies report that usnic acid is hepatotoxic, some studies report that it has a hepatoprotective effect. For this reason, it is very important to determine the dose applied in studies with lichen extract and lichen secondary metabolites [7,8]. Thanks to the secondary metabolites they have, lichens exhibit properties that include various biological activities such as antioxidant, antimycobacterial, antiviral, analgesic, cytotoxic, antimicrobial, fungicidal, herbicidal and photosystem inhibitor [8]. The effects of lichens and their secondary metabolites on many central nervous system diseases have been investigated, and even positive results have been obtained in some of them [9,10]. Reddy et al. [9] showed that atranorine, perlatolic acid, and physodic acid exhibit significantly higher neurotrophicity, induce up-regulation of neurotrophic gene expressions, inhibit AChE activity, and exhibit neurogenic activity. Evidence also exists for neuroprotective and anti-inflammatory properties of evernic acid and usnic acid, which can especially be important in the context of Parkinson's disease [10]. In this study, it was aimed to investigate the antiepileptic, antioxidant and potential anti-inflammatory effects of lichen species of *Dolichousnea longissima* (Ach.), *Xanthoparmelia somloensis*, *Cetraria islandica*, and *Pseudevernia furfuracea* as well as usnic acid, which is one of the most important secondary metabolites of lichens, in the epileptic seizure model experimentally induced with PTZ in rats.

## 2. RESULTS

### 2.1. Seizure scores for *D. longissima* extract administration

There was no difference between the groups in the 1st, 2nd, and 3rd injections of PTZ ($p$>0.05). Starting from the 4th injection, the seizure score was significantly higher in the PTZ group compared to the control group ($p$<0.05). Compared with the PTZ group, it was determined that *D. longissima* extract reduced seizure scores at a concentration of 400 mg/kg at the 4th injection, at a concentration of 200 and 400 mg/kg at the 5th and 6th injections, and at a concentration of 400 mg/kg at the 8th and 11th injections ($p$<0.05) (Figure 1).

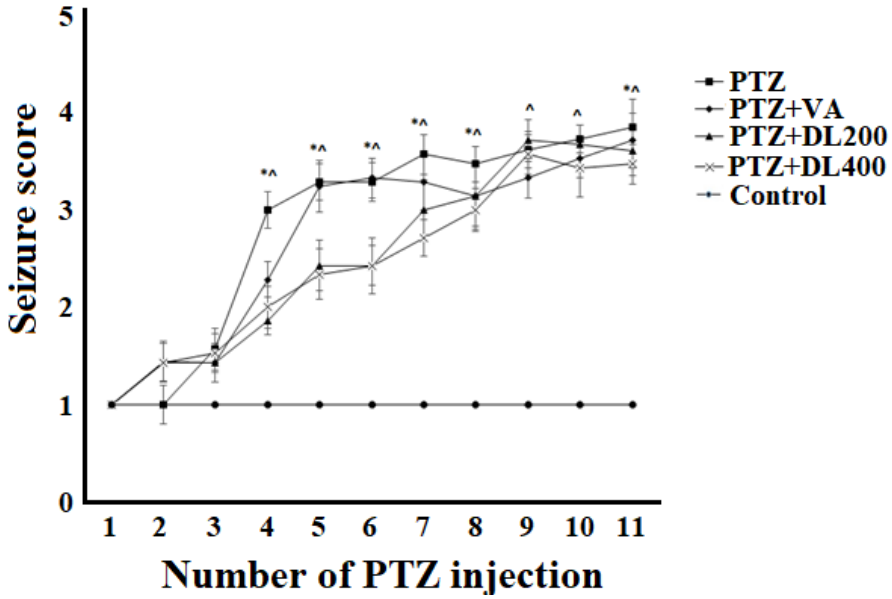


**Figure 1.** Seizure scores of rats treated with Dolichousnea longissima extract
^ There is a statistically significant difference when compared with the control group ($p$<0.05)
* There is a statistically significant difference when compared with the PTZ group ($p$<0.05)

### 2.2. Seizure scores for *X. somloensis* extract administration

There was no difference between the groups in the 1st, 2nd, and 3rd injections of PTZ ($p$>0.05). Starting from the 4th injection, the seizure score was found to be significantly higher in the PTZ group compared to the control group ($p$<0.05). Compared with the PTZ group, 200 mg/kg concentration of *X. somloensis* extract was found to reduce seizure scores in the 4th, 5th, 6th and 7th injections ($p$<0.05) (Figure 2).

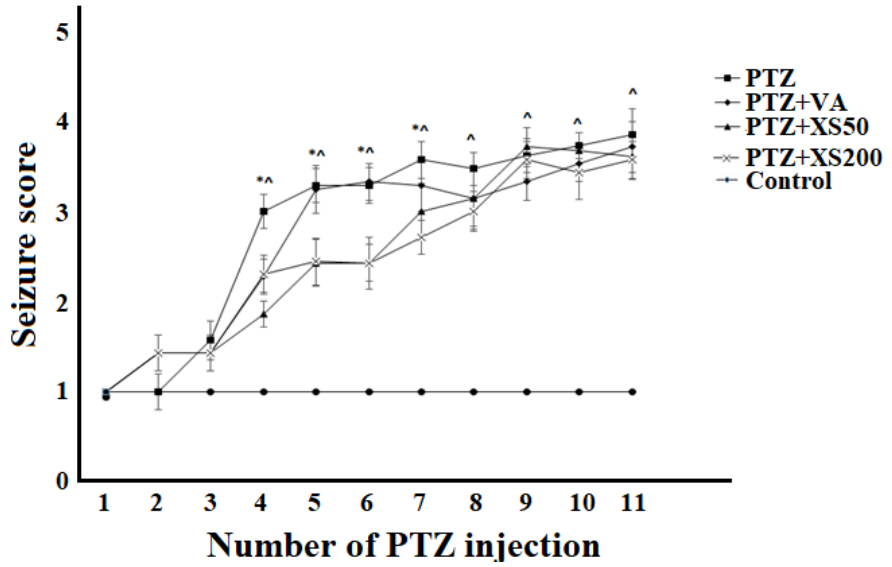


**Figure 2.** Seizure scores of rats treated with *Xanthoparmelia somloensis* extract
^ There is a statistically significant difference when compared with the control group ($p$<0.05)
* There is a statistically significant difference when compared with the PTZ group ($p$<0.05)

### 2.3. Seizure scores for *C. islandica* extract administration

There was no difference between the groups in the 1st, 2nd, and 3rd injections of PTZ ($p$>0.05). Starting from the 4th injection, the seizure score was found to be significantly higher in the PTZ group compared to the control group ($p$<0.05). Compared with the PTZ group, it was determined that *C. islandica* extract reduced seizure scores at 250 mg/kg concentration in the 6th and 7th injections, and 250 and 500 mg/kg concentrations in the 4th, 5th, 8th, and 11th injections ($p$<0.05) (Figure 3).

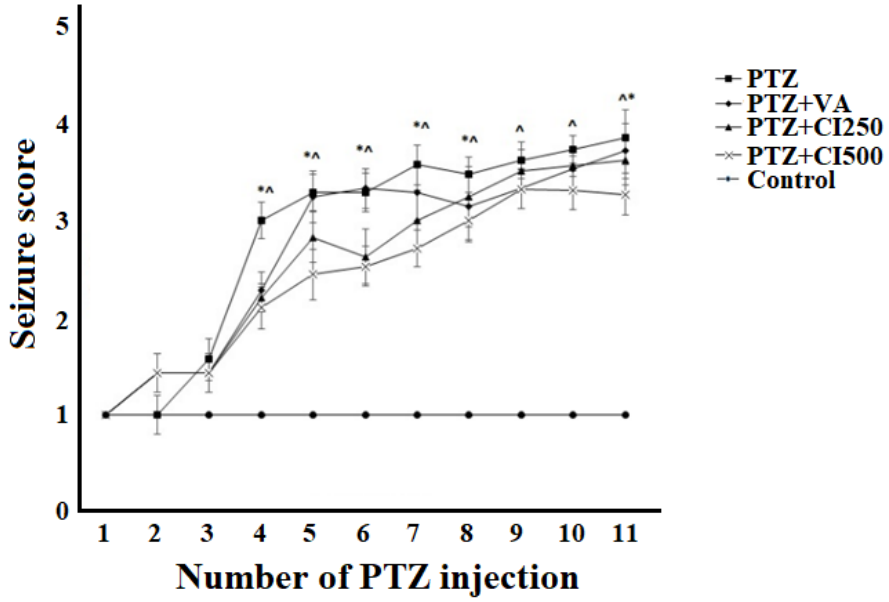


**Figure 3.** Seizure scores of rats treated with *Cetraria islandica* extract
^ There is a statistically significant difference when compared with the control group ($p$<0.05)
* There is a statistically significant difference when compared with the PTZ group ($p$<0.05)

### 2.4. Seizure scores for *P. furfuracea* extract administration

There was no difference between the groups in the 1st, 2nd, and 3rd injections of PTZ ($p$>0.05). Starting from the 4th injection, the seizure score was found to be significantly higher in the PTZ group compared to the control group ($p$<0.05). Compared with the PTZ group, it was determined that *P. furfuracea* extract reduced seizure scores at 250 and 500 mg/kg concentrations in the 5th injections, and 500 mg/kg in the 4th, 6th and 7th injections ($p$<0.05) (Figure 4).

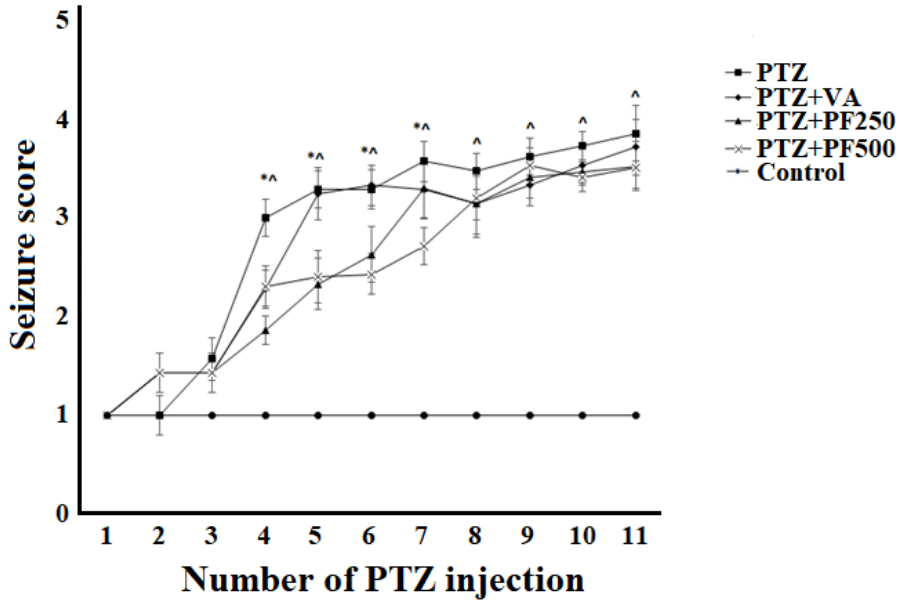


**Figure 4.** Seizure scores of rats treated with *Pseudevernia furfuracea* extract
^ There is a statistically significant difference when compared with the control group ($p$<0.05)
* There is a statistically significant difference when compared with the PTZ group ($p$<0.05)

### 2.5. Seizure scores for usnic acid administration

There was no difference between the groups in the 1st, 2nd, and 3rd injections of PTZ ($p$>0.05). Starting from the 4th injection, the seizure score was found to be significantly higher in the PTZ group compared to the control group ($p$<0.05). Compared with the PTZ group, it was observed that usnic acid administration did not cause a significant change in seizure scores ($p$>0.05) (Figure 5).

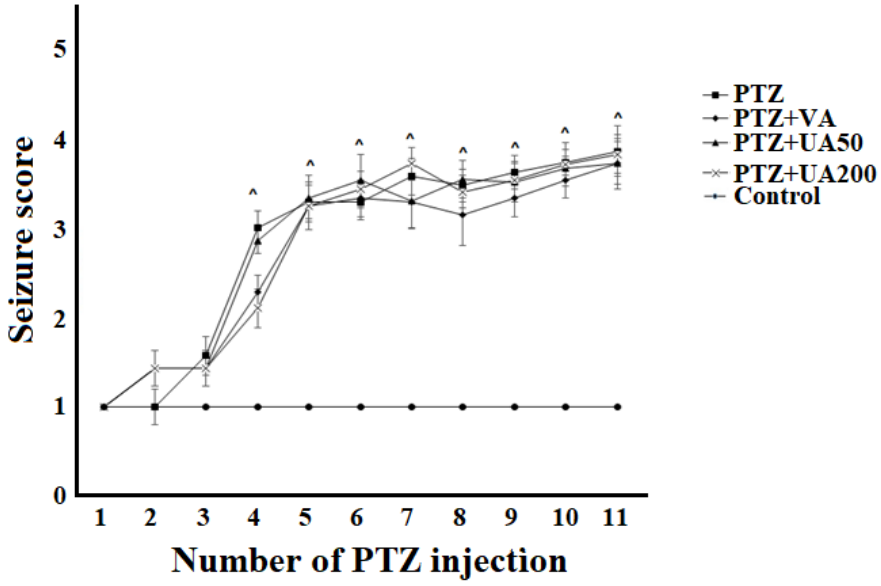


**Figure 5.** Seizure scores of rats treated with usnic acid
^ There is a statistically significant difference when compared with the control group ($p$<0.05)

### 2.6. Locomotor activity findings for lichen extracts and usnic acid administrations

When the effects of lichen extracts and usnic acid applications on locomotor activity were examined, no significant difference was found between the groups in terms of the duration of rats on Rota Rot ($p$>0.05) (Figure 6A-6E).

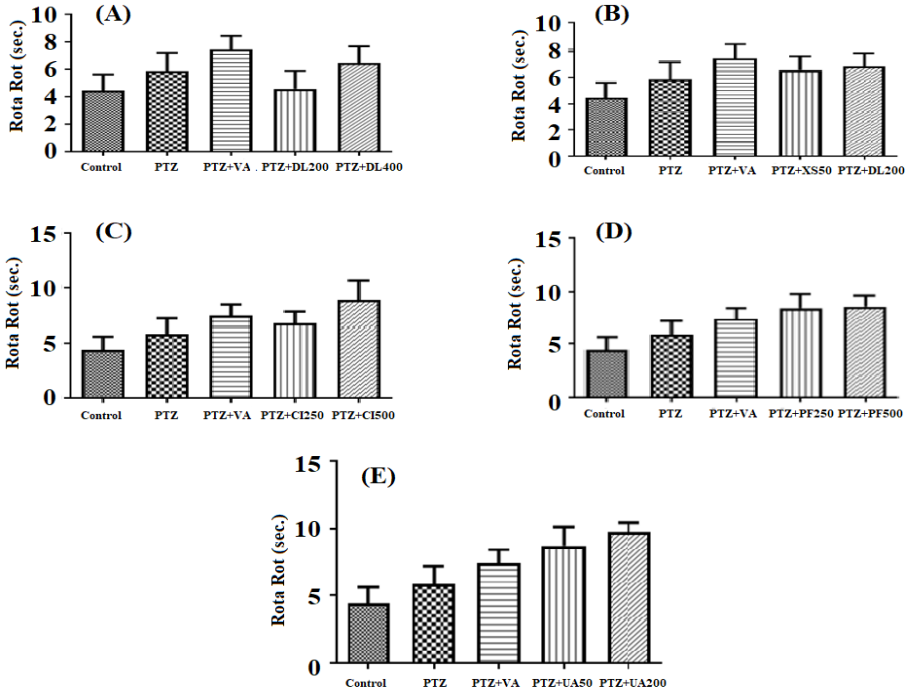


**Figure 6.** Duration of rats treated with several lichen extracts (A: *D. longissima*; B: *X. somloensis*; C: *C. islandica*: D: *P. furfuracea*) and usnic acid (E) on rota rot.

### 2.7. Effects of lichen extracts and usnic acid applications on biochemical parameters

In our study, superoxide dismutase (SOD), catalase, and AchE activities and reduced glutathione (GSH) level in the brain tissue in the PTZ group were found to be lower than the control group ($p$<0.05). Valproic acid, usnic acid and high concentrations of lichen extracts increased the levels of SOD, catalase, and AchE activities and GSH level in the brain tissue ($p$<0.05). It was observed that low-dose lichen applications did not cause a significant change in SOD, catalase, and AchE activities and GSH level ($p$>0.05) (Table 1).

**Table 1.** Catalase, SOD and AchE activities and GSH levels of the study groups

| Groups | Catalase Activity (U/mg prt) | SOD Activity (U/mg prt) | AchE Level (nmol/min/mg prt) | GSH Level (nmol/mg prt) |
|---|---|---|---|---|
| Control | 372.6±31.5 | 3.2±0.3 | 112.2±12.9 | 67.1±7.2 |
| PTZ | 246.8±23.6* | 2.1±0.2* | 64.4±7.3* | 42.5±4.3* |
| PTZ+VA | 315.5±28.6** | 2.9±0.2** | 99.2±8.2** | 59.1±5.1** |
| PTZ+DL200 | 264.8±26.9 | 2.3±0.2 | 76.0±7.1 | 48.6±4.6 |
| PTZ+DL400 | 291.2±21.5** | 2.8±0.2** | 87.3±7.5** | 56.8±5.2** |
| PTZ + XS50 | 257.3±28.3 | 2.4±0.3 | 69.1±7.2 | 49.2±4.8 |
| PTZ+XS200 | 304.9±22.3** | 2.8±0.2** | 84.2±6.3** | 55.9±4.9** |
| PTZ+CI250 | 267.4±27.4 | 2.3±0.2 | 68.5±8.6 | 47.1±4.7 |
| PTZ+CI500 | 316.7±24.3** | 2.7±0.2** | 93.±7.5** | 54.3±4.3** |
| PTZ+PF250 | 243.1±29.1 | 2.2±0.2 | 73.2±7.5 | 48.8±4.6 |
| PTZ+PF500 | 314.9±26.8** | 2.9±0.3** | 81.1±7.4** | 53.1±5.1** |
| PTZ+UA50 | 294.8±31.6** | 2.8±0.2** | 93.8±8.8** | 53.7±5.9** |
| PTZ+UA200 | 305.4±29.4** | 2.9±0.2** | 84.7±6.3** | 55.4±4.8** |

* Statistically significant compared to the control group ($p$<0.05)
** Statistically significant compared to PTZ group ($p$<0.05)

In the current study, malondialdehyde (MDA), NOx, and protein carbonyl (PCO) levels in the brain tissue in the PTZ group were found to be lower than those of the control group ($p$<0.05). It was also observed that valproic acid, usnic acid, and high concentrations of lichen extracts decreased the MDA, NOx, and protein carbonyl levels in the brain tissue ($p$<0.05). Moreover, the low-dose lichen applications did not cause a significant change in these parameters as shown in Table 2 ($p$>0.05).

**Table 2.** MDA, NOx, and PCO activities and GSH levels of the study groups

| Groups | MDA Level (nmol/mg prt) | NOx Level (nmol/mg prt) | PCO Level (nmol/mg prt) |
|---|---|---|---|
| Control | 24.3±2.6 | 17.2±1.8 | 3.2±0.3 |
| PTZ | 32.9±2.8* | 29.2±2.6* | 5.3±0.4* |
| PTZ+VA | 26.7±2.6** | 21.8±2.2** | 3.7±0.3** |
| PTZ+DL200 | 29.5±2.5 | 27.3±2.8 | 4.8±0.5 |
| PTZ+DL400 | 27.6±2.7** | 22.7±2.6** | 4.1±0.4** |
| PTZ + XS50 | 30.7±3.2 | 26.4±2.4 | 4.9±0.5 |
| PTZ+XS200 | 27.3±2.5** | 23.4±2.5** | 4.3±0.4** |
| PTZ+CI250 | 28.7±3.1 | 26.9±2.6 | 5.1±0.4 |
| PTZ+CI500 | 25.4±2.4** | 23.7±2.0** | 4.4±0.4** |
| PTZ+PF250 | 29.4±2.6 | 27.9±2.2 | 4.9±0.4 |
| PTZ+PF500 | 26.8±2.1** | 23.4±1.9** | 3.9±0.3** |
| PTZ+UA50 | 24.9±3.2** | 21.9±3.2** | 3.6±0.4** |
| PTZ+UA200 | 27.2±2.3** | 22.1±2.1** | 4.1±0.4** |

* Statistically significant compared to the control group ($p$<0.05)
** Statistically significant compared to PTZ group ($p$<0.05)

## 3. DISCUSSION

Epilepsy is a chronically progressive brain disease that occurs due to rapid, increased neuronal excitability of nerve cells in the brain, with a prevalance of approximately 0.5-1%. Although approximately 30% of the patients use antiepileptic drugs, epileptic seizures remains to be existent. Mortality and morbidity may occur as a result of these uncontrollable seizures. The prevention or decrease in the number of seizures

is achieved with antiepileptic drugs used by patients. By carrying out studies in this direction, there is strong effort to develop drugs that are suitable for the type of epilepsy and that are pharmacoeconomically accessible [29].

Unexpected and sudden deaths that occur during seizures or seizure free perios in epilepsy patients may also occur due to physiological irregularities that occur in the body as a result of seizures over time. Although many studies have been carried out to understand the mechanism of epilepsy and seizures by creating different epileptic experimental animal models, and many antiepileptic drugs have been investigated, the cellular basis of epilepsy has not been fully understood nor an effective antiepileptic drug has been found for all epilepsy types [29, 30]. In the current study, the PTZ-induced kindling model was used to create the experimental epilepsy model. Studies have suggested that PTZ exerts its effect by blocking GABAA receptors. Chemical kindling seizures induced by PTZ are an animal model of human absence epilepsy and myoclonic, generalized tonic-clonic seizures. This model is a frequently used epilepsy model for drug development [31]. In this study, rats were administered with saline, various lichen extracts, usnic acid and valproic acid as a positive control for a period of three days a week (Monday, Wednesday and Friday). Behavioral signs of animals were monitored for 30 minutes on the study day. When the control group and PTZ group were compared, it was found that the number of seizures was statistically significantly higher in the PTZ group. In addition, a statistical significance was found in the valproic acid, usnic acid and lichen extracts groups when compared with the PTZ group. Our results show that injection of 35 mg/kg PTZ produces seizures lasting 30 minutes or more.

On the test day, various lichen extracts, usnic acid or valproic acid were given to the animals 120 minutes before the administration of PTZ (35 mg/kg, i.p), and the duration of myoclonic seizures was monitored for 30 minutes. Animals were placed in clear plexiglass cages immediately after PTZ application and evaluated according to the modified Racine scale for 30 minutes. When the control group and PTZ group were compared, it was found that the duration of myoclonic seizures was statistically significantly higher in the PTZ group. When the control group and other experimental groups were compared in terms of myoclonic seizure duration, no statistical difference was found between them. These results were in line with the results in the literature in terms of myoclonic seizure duration of 35 mg/kg PTZ injection. We think that these results could occur due to the agonist effects of lichens on the GABA receptor. GABA receptors are known as the most important receptors taking part in epilepsy. The observed effects in this study were probably due to the effects of lichens on the GABA receptor [32]. In addition, we think that the existing antioxidant activities of lichens may contribute to this effect [33-35]. It was observed that valproic acid and lichen extractsprolong the onset of epileptic seizures. It is thought that this result may be caused by the effects of lichens on the GABA receptor. These data are similar to studies conducted with substances with GABA-mimetic and antioxidant properties, which are alsoprobably related to the GABA-mimetic effect and antioxidant effect of lichens [32-35].

In the current study, the biochemical parameters of changes in the acetylcholinesterase enzyme activity, total antioxidant levels, and total oxidant levels of the brain tissue were investigated. AchE is an enzyme playing an important role in cholinergic neuron transmission, which provides rapid hydrolysis of acetylcholine and releaseing into the synapses [36]. In more significant studies, it has been reported that seizures reduce AchE activity in epileptic rat brains, leading to unconsciousness [37]. Decreased AchE levels in the brain are associated with loss of consciousness [38]. The decrease in AchE activity in the hippocampus and midbrain has been reported to occur within 30 minutes of electrically induced crises [39]. Pahuja et al. [40] reported that AchE activity decreased significantly in the epilepsy model, which they created with the PTZ group. This situation is associated with impaired consciousness development in epilepsy. According to the findings of our study, AchE activity decreased in the epilepsy model created with PTZ. It was observed that the decreased AChE activity increased again in the groups treated with valproic acid and lichen extracts. We think that the reason why different types of lichen extracts delay the onset of epileptic seizures in PTZ kindling epilepsy model is resulted from the re-increase of AchE activity in the brain.

In recent years, studies have focused on oxidative stress in epileptic seizures. There is some evidence to suggest that mitochondrial dysfunction and oxidative stress are both causes and consequences of epileptic seizures. Experimental seizures are known to be associated with severe release of reactive oxygen groups. Likewise, PTZ-induced epileptic seizures seem to cause oxidative stress by disrupting the antioxidant defense system and increasing lipid peroxidation in brain tissue [41]. Increasing number of data brings to mind the relationship of oxidative stress in the pathophysiology of epilepsy. Excessive oxidative stress contributes to neuronal degeneration through lipid peroxidation and decreases glutathione concentration in the epileptic focus [42]. İlhan et al. [43] reported that MDA and NOx levels in the brain tissue increased and SOD activity did not change in epileptic seizures induced by PTZ in mice. Obay et al. [41] showed that in the epileptic seizure model which developed with PTZ in rats, the levels of MDA in the brain tissue of those

who had seizures increased compared to the controls, and the levels of SOD, CAT, and GSH decreased. Erakovic et al. [44] showed that there was no significant difference in SOD activity in brain tissue after a single dose of PTZ compared to controls. Akbas et al. [45] reported that in the seizure model they created with PTZ, SOD activity decreased in erythrocyte and liver compared to controls, whereas CAT activity decreased in erythrocyte but remained unchanged in the liver. Some studies report that SOD, CAT and NOx levels increase during epileptic seizures, while some do not change, while others decrease. These differences may be due to the methods used in these studies. The increase in enzyme activity resulting from oxidative stress may decrease after a while due to the degradation of the enzyme. The relationship between epilepsy and NOx has been reported in many studies. However, the nature of this relationship is still unclear. Some researchers report that inhibiting NOx formation prevents seizure formation [46].

In recent years, studies have focused on oxidative stress in seizures. There is evidence that mitochondrial dysfunction and oxidative stress play a role as both a consequence and a cause of epileptic seizures. Experimental seizures are known to be associated with severe release of reactive oxygen groups. In some studies, it has been reported that many antiepileptic drugs increase oxidative stress. Likewise, acute PTZ-induced epileptic seizures cause an increase in oxidative stress in brain tissue. It has been reported that SOD activity in the brain tissue of PTZ-induced epileptic rats decreases [41]. The opposite effect associated with antiepileptic drugs and recurrent seizures limits their use. A growing body of data suggests the relevance of oxidative stress in the pathophysiology of epilepsy. Excessive oxidative stress contributes to neuronal degeneration through lipid peroxidation and decreases glutathione concentrations in the epileptic focus [42]. In an experimental study on TAS values in epilepsy patients, it was stated that while total antioxidant capacity levels were decreased in the valproate-treated group, with a significant decrease in total antioxidant capacity among untreated epilepsy patients [47]. In another study conducted with epileptic patients, it was reported that the antioxidant status in the blood of the patients was lower than that of the control group and improved with anti-epileptic drug treatment [48].

PTZ-induced seizures cause apoptotic neurodegeneration [49]. In fact, both necrotic and apoptotic mechanisms contribute to damage. However, in kindling seizures, it also contacts the formation of free radicals as a molecular pathway that causes neuronal damage [50]. Fernández-Moriano et al. [51] suggested that lichens possess antioxidant effect thanks to the secondary metabolites they contain. Due to their antioxidant properties, lichens play an important role in the prevention of many diseases such as heart diseases and cancer that threaten life [52]. Moreover, it has been determined by experimental studies that these secondary metabolites reduce blood pressure by increasing the permeability in capillaries [53]. Martinc et al. [54] stated that epileptic seizures increase oxidative stress parameters and that antioxidant mechanisms are not sufficient to reduce oxidative damage, and stated that antioxidant components to be added to antiepileptic treatment may be useful strategies in the therapeutic fight against oxidative stress and neurodegeneration caused by epileptic seizures.

In this study, SOD, catalase and GSH levels in the brain tissue of animals in the PTZ group were found to be lower than those of the control group, while MDA, NOx; however, protein carbonyl levels were found to be higher. It is seen that valproic acid, both concentrations of usnic acid and especially high concentrations of D. longissima (Ach.), X. somloensis, C.islandica, and P. furfuracea extracts increase brain tissue acetyl choline esterase, SOD, catalase and GSH levels and decrease MDA, NOx and protein carbonyl levels. On the other hand, no significant difference was observed in low-dose lichen applications.

## 4. CONCLUSION

In conclusion, it was observed that high-dose lichen extracts and both concentrations of usnic acid application were as effective as valproic acid treatment by preventing epileptic seizures and harmful effects of epileptic seizures in the CNS. It was also revealed that lichen and usnic acid applications increased the levels of AchE, SOD, catalase and GSH in the brain tissues of epileptic rats. In the current study, it was determined that the secondary metabolites in the content of lichens, which are used as natural protective agents, have very important functions in the elimination of oxidative stress, and lichen extracts reduce oxidative stress in the epilepsy model and have a protective effect. We believe that the results obtained from the current study will be evaluated from different perspectives, and will shed light on research on the applicability of the nervous system on the biosystem and its applicability in psychological diseases. Moreover, it would be useful to investigate the effects of lichens belonging to different flora on the treatment of different diseases of the nervous system. It is thought that it is also important to use lichen extracts obtained by using different extraction methods for preventive or therapeutic purposes in epileptic rats and to observe possible different effects.

## 5. MATERIALS AND METHODS

### 5.1. Materials

In this study, 91 male rats weighing 250-300 g, obtained from the Animal Experiments Unit of Van Yüzüncü Yıl University, were divided into 13 groups for each group includes 7 rats. The experimental animals were kept in a 12/12 hour light/dark period and at 18-22°C. The rats were quarantined for adaptation for 5 days before the experimental application. All experiments followed NIH rules for the care and use of laboratory animals, and ethical approval of the study was obtained from Van Yuzuncu Yil University Ethical Commission for Animal Experiments (Decision number: 02, Date: 01.03.2018).

In the current study, 4 lichen species were studied, namely *Dolichousnea longissima* (Ach.), *Xanthoparmelia somloensis*, *Cetraria islandica* and *Pseudevernia furfuracea*. Lichen samples were collected by Prof. Dr. Ali ASLAN from Oltu district of Erzurum province on 15 August 2011 and identified by using various flora books [11, 12]. The identified lichen samples were kept in Van Yüzüncü Yıl University Faculty of Pharmacy Herbarium. The usnic acid used in the study was commercially available (Sigma Aldrich, USA) (Catalog No: 329967).

Lichens were dried and ground into powder, the 10 g of each lichen powder was weighed and transferred into a Soxhlet device for extraction (Soxhlet extractor (Isopad, Heidelberg, Germany) with 250 mL of methanol for 72 hours. The mixture was filtered with Whatman filter paper (no: 1) and the solvent was removed in a rotary evaporator (Buchi Labortechnic AG, Flawil, Switzerland) at 40°C. The obtained lichen extracts were stored at +4°C until use [13].

### 5.2. PTZ kindling model

The PTZ kindling model was performed by administering 35 mg/kg PTZ (*i.p.*) to rats every other day [14]. Seizures were recorded, and scored with a camera for 15 minutes after each PTZ injection. Seizure scores were evaluated according to the Racine scale [15] (Table 3). Animals in the SF+PTZ group having seizures with a score of 4 or higher in 3 consecutive injections were considered to be inflamed.

**Table 3.** Behavioral parameters used in seizure scoring

| Seizure Score | Observed Behaviors | Righting Reflex |
|---|---|---|
| 0 | No seizure | Yes |
| 1 | Temporary twitching of the muscle (twitches in the ear and face) | Yes |
| 2 | Myoclonic jerks without rearing | Yes |
| 3 | Myoclonic jerks with rearing | Yes |
| 4 | Rollover on one side with tonic-clonic seizure | No |
| 5 | Rollover on two sides with generalized clonic-tonic seizures | No |

### 5.3. Behavioral analyzes

Rearing, distance traveled, crawling, stereotypical movements and total locomotor activities of the rats were measured in the locomotor activity device for 10 minutes. In order to test the motor coordination, the measurement was carried out by adjusting the speed of the Rotary Rod tool as 10 rpm. A preliminary trial was carried out for the rats for accustoming to the Rota Rot device. The time they were able to stay in the Rotary Rod tool was recorded. The highest value of 300 seconds was recorded for rats which never fell off the instrument.

### 5.4. Experimental groups

The animals used in the experiments were randomly divided into 13 groups and each group included 7 rats, as detailed as follows:

**Group 1 (Control group):** 1 cc saline (SF) (via gavage) + 1 cc SF (*i.p.*) was administered.

**Group 2 (PTZ group):** 1 cc SF (via gavage) + 1 cc PTZ was administered [14].

**Group 3 (Valproic acid group (100 mg/kg) + PTZ Group):** 1 cc valproic acid (100 mg/kg, gavage) [14] + 1 cc PTZ was administered.

**Group 4 (*D. longissima* (Ach.) (200 mg/kg) + PTZ Group):** 1 cc *D. longissima* (Ach.) extract (200 mg/kg, gavage) [16] + 1 cc PTZ was administered.

**Group 5 (*D. longissima* (Ach.) (400 mg/kg)+ PTZ Group):** 1 cc *D. longissima* (Ach.) extract (400 mg/kg, gavage) [16] + 1 cc PTZ was administered.

**Group 6 (*X. somloensis* (50 mg/kg)+ PTZ Group):** 1 cc *X. somloensis* extract (50 mg/kg, gavage) [17] + 1 cc PTZ was administered.
**Group 7 (*X. somloensis* (200 mg/kg)+ PTZ Group):** 1 cc *X. somloensis* extract (200 mg/kg, gavage) [17] + after 120 minutes 1 cc PTZ (35 mg/kg, i.p.) was administered.
**Group 8 (*C. islandica* (250 mg/kg)+ PTZ Group):** 1 cc *C. islandica* extract (250 mg/kg, gavage) [18] + 1 cc PTZ was administered.
**Group 9 (*C. islandica* (500 mg/kg)+ PTZ Group):** 1 cc *C. islandica* extract (500 mg/kg, gavage) [18] + 1 cc PTZ was administered.
**Group 10 (*P. furfuracea* (250 mg/kg)+ PTZ Group):** 1 cc *P. furfuracea* extract (250 mg/kg, gavage) [19] + 1 cc PTZ was administered.
**Group 11 (*P. furfuracea* (500 mg/kg)+ PTZ Group):** 1 cc *P. furfuracea* extract (500 mg/kg, gavage) [19] + 1 cc PTZ was administered.
**Group 12 (Usnic acid (50 mg/kg)+ PTZ Group):** 1 cc usnic acid (50 mg/kg, gavage) [20] + 1 cc PTZ was administered.
**Group 13 (Usnic acid (200 mg/kg)+ PTZ Group):** 1 cc usnic acid (200 mg/kg, gavage) [20] + 1 cc PTZ was administered.

All applications were performed three days a week, as Monday, Wednesday and Friday. A total of 11 PTZ injections were performed over a period of approximately 4 weeks. PTZ applications were performed intraperitoneally. Intraperitoneal applications in all groups were performed 2 hours after gavage applications.

### 5.5. Analysis of oxidative stress parameters

In this study, oxidative stress parameters were examined using total brain tissues. Brain tissues, whose wet weights were adjusted as 50 mg, were hardened with the help of liquid nitrogen and powdered in a porcelain mortar. 1/10 (w/v) Tris/sucrose buffer was added onto the tissue transferred to the glass tube. Tissue samples transferred to glass tubes were numbered and centrifuged at 3000 rpm for 10 minutes at +4°C. After centrifugation, MDA, NOx, PCO, and GSH levels, catalase, SOD, and AchE activities and protein levels of the supernatant were measured.

MDA level was determined by Yagi's [21] tiobarbituric acid reaction method. Catalase activity was determined according to the method reported by Aebi [22]. SOD activity was determined according to the method reported by Sun *et al*. [23]. GSH level was analysed according to the method reported by Beutler *et al*. [24]. Measurement of AchE activity was performed according to the method reported by Ellman *et al*. [25]. The amount of NOx was measured using the "Vanadium-3-chloride-Gries Reaction" method reported by Miranda *et al*. [26]. PCO level was measured according to the method reported by Levine *et al*. [27]. Total protein levels of brain tissues were measured in order to specify enzyme activities as specific activities. Tissue protein levels were measured according to the method described by Lowry *et al*. [28].

### 5.6. Statistical analysis

Statistical analyzes were performed using SPSS for Windows v16.0 package program. ANOVA test was used to test for statistical differences between groups for all parameters, and Tukey's multiple comparison test was used for subgroup comparisons. Mean ± standard deviation values are given as descriptive statistics. A $p<0.05$ value was considered as the difference or relationship is statistically significant.

**Acknowledgements:** This research was financially supported in part by the Office of Scientific Research Projects of Van Yuzuncu Yil University under Grant number (TSA-2019-7396).

**Author contributions:** Concept – M.B., O.Y.; Design – A.A., O.Y., M.B.; Supervision – A.A.; Resources – A.A., M.B.; Materials – A.A., M.B.; Data Collection and/or Processing – M.B., O.Y., A.B.; Analysis and/or Interpretation – M.B., O.Y., A.B.; Literature Search – M.B., O.Y.; Writing – M.B.; Critical Reviews – M.B., O.Y., A.A., A.B.

**Conflict of interest statement:** No conflict of interest was declared by the authors.

## REFERENCES

[1] Fisher RS. Redefining epilepsy. Curr Opin Neurol. 2015; 28(2): 130-135. https://doi.org/10.1097/wco.0000000000000174
[2] Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, Bueno-Junior LS, Leite JP. Animal models of epilepsy: use and limitations. Neuropsychiatr Dis Treat. 2014; 10: 1693-1705. https://doi.org/10.2147/ndt.s50371
[3] Dhir A, Naidu PS, Kulkarni SK. Effect of naproxen, a non-selective cyclo-oxygenase inhibitor, on pentylenetetrazol-induced kindling in mice. Clin Exp Pharmacol Physiol. 2005; 32(7): 574-584. https://doi.org/10.1111/j.1440-1681.2005.04233.x
[4] Ilhan A, Iraz M, Kamisli S, Yigitoglu R. Pentylenetetrazol-induced kindling seizure attenuated by *Ginkgo biloba* extract (EGb 761) in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2006; 30(8): 1504-1510. https://doi.org/10.1016/j.pnpbp.2006.05.013
[5] Arhan E, Serdaroglu A, Ozturk B, Ozturk HS, Ozcelik A, Kurt N, Kutsal E, Sevinc N. Effects of epilepsy and antiepileptic drugs on nitric oxide, lipid peroxidation and xanthine oxidase system in children with idiopathic epilepsy. Seizure. 2011; 20(2): 138-142. https://doi.org/10.1016/j.seizure.2010.11.003
[6] Essawy AE, El-Sayed SA, Tousson E, Abd El-Gawad HS, Alhasani RH, Abd Elkader HAE. Anti-kindling effect of *Ginkgo biloba* leaf extract and L-carnitine in the pentylenetetrazol model of epilepsy. Environ Sci Pollut Res Int. 2022; 29(32): 48573-48587. https://doi.org/10.1007/s11356-022-19251-6
[7] Adenubi OT, Famuyide IM, McGaw LJ, Eloff JN. Lichens: An update on their ethnopharmacological uses and potential as sources of drug leads. J Ethnopharmacol. 2022; 298: 115657. https://doi.org/10.1016/j.jep.2022.115657
[8] Xu M, Heidmarsson S, Olafsdottir ES, Buonfiglio R, Kogej T, Omarsdottir S. Secondary metabolites from cetrarioid lichens: Chemotaxonomy, biological activities and pharmaceutical potential. Phytomedicine. 2016; 23(5): 441-459. https://doi.org/10.1016/j.phymed.2016.02.012
[9] Reddy RG, Veeraval L, Maitra S, Chollet-Krugler M, Tomasi S, Dévéhat FL, Boustie J, Chakravarty S. Lichen-derived compounds show potential for central nervous system therapeutics. Phytomedicine. 2016; 23(12): 1527-1534. https://doi.org/10.1016/j.phymed.2016.08.010
[10] Fernández-Moriano C, Divakar PK, Crespo A, Gómez-Serranillos MP. Protective effects of lichen metabolites evernic and usnic acids against redox impairment-mediated cytotoxicity in central nervous system-like cells. Food Chem Toxicol. 2017; 105: 262-277. https://doi.org/10.1016/j.fct.2017.04.030
[11] Dobson FS, Lichens. An illustrated guide to the British and Irish species. first ed., Richmond Publishing, Slough, 2000.
[12] Brodo IM, Sharnoff SD, Sharnoff S, Lichens of North America, first ed., Yale Univ Press, New Haven, London, 2001.
[13] Aslan A, Güllüce M, Sökmen M, Adıgüzel A, Şahin F, Özkan, H. Antioxidant and antimicrabial properties of the lichens *Cladonia foliacea*, *Dermatocarpon miniatum*, *Evernia divaricata*, *Evernia prunastri* and *Neofuscella pulla*. Pharm Biol. 2006; 44(4): 247-252. https://doi.org/10.1080/13880200600713808
[14] Golmohammadi R, Pejhan A, Azhdari-Zarmehri H, Mohammad-Zadeh M. The role of ethanol on the anticonvulsant effect of valproic acid and cortical microvascular changes after epileptogenesis in mice. Neurol Sci. 2013; 34(7): 1125-1131. https://doi.org/10.1007/s10072-012-1190-y
[15] Racine RJ, Gartner JG, Burnham WM. Epileptiform activity and neural plasticity in limbic structures. Brain Res. 1972; 47(1): 262-268. https://doi.org/10.1016/0006-8993(72)90268-5
[16] Verma P, Paswan SK, Raj A, Nath V, Gupta RK, Verma S, Srivastava S, Rao CV. Hematological, antioxidant and protective performance of *Usnea longissima* on chemical induced hepatotoxicity in experimental animals. J Coast Life Med. 2017; 5(5): 224-232. http://dx.doi.org/10.12980/jclm.5.2017J6-250
[17] Halıcı MB. PhD Thesis. Investigation of the antiulcer mechanisms of substances isolated from some lichens in the indomethacin-induced ulcer model in rats. Department of Chemistry, Institute of Natural and Applied Sciences, Ataturk University, Erzurum, Turkiye, 2008.
[18] Gülçin I, Oktay M, Küfrevioğlu OI, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol. 2002; 79(3): 325-329. https://doi.org/10.1016/s0378-8741(01)00396-8
[19] Bakır TÖ, Geyikoglu F, Çolak S, Türkez H, Aslan A, Bakır M. The effects of *Cetraria islandica* and *Pseudevernia furfuracea* extracts in normal and diabetic rats. Toxicol Ind Health. 2015; 31(12): 1304-1317. https://doi.org/10.1177/0748233713475521
[20] Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, Kazaz C. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. J Ethnopharmacol. 2006; 103(1): 59-65. https://doi.org/10.1016/j.jep.2005.06.043
[21] Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol. 1998; 108: 101-106. https://doi.org/10.1385/0-89603-472-0:101
[22] Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105: 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3
[23] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988; 34(3): 497-500. https://doi.org/10.1093/clinchem/34.3.497
[24] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61: 882-888.
[25] Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7(2): 88-90. https://doi.org/10.1016/0006-2952(61)90145-9

**[26]** Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 2001; 5(1): 62-71. https://doi.org/10.1006/niox.2000.0319
**[27]** Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990; 186: 464-478. https://doi.org/10.1016/0076-6879(90)86141-h
**[28]** Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1): 265-275.
**[29]** Allahverdiyev O, Dzhafar S, Berköz M, Yıldırım M. Advances in current medication and new therapeutic approaches in epilepsy. East J Med. 2018;23(1): 48-59. http://dx.doi.org/10.5505/ejm.2018.62534
**[30]** Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J Jr. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia. 2005; 46(4): 470-472. https://doi.org/10.1111/j.0013-9580.2005.66104.x
**[31]** Wu XH, Ding MP, Zhu-Ge ZB, Zhu YY, Jin CL, Chen Z. Carnosine, a precursor of histidine, ameliorates pentylenetetrazole-induced kindled seizures in rat. Neurosci Lett. 2006; 400(1-2): 146-149. https://doi.org/10.1016/j.neulet.2006.02.031
**[32]** Seher Y, Filiz O, Melike B. Gamma-amino butyric acid, glutamate dehydrogenase and glutamate decarboxylase levels in phylogenetically divergent plants. Plant Syst Evol. 2013; 299(2): 403-412. https://doi.org/10.1007/s00606-012-0730-5
**[33]** Ergul Erkec O, Arihan O, Kara M, Karatas E, Erten R, Demir H, Meral I, Mukemre M, Ozgokce F. Effects of *Leontice leontopetalum* and *Bongardia chrysogonum* on oxidative stress and neuroprotection in PTZ kindling epilepsy in rats. Cell Mol Biol (Noisy-le-grand). 2018; 64(15): 71-77. http://dx.doi.org/10.14715/cmb/2017.64.15.12
**[34]** Calcott MJ, Ackerley DF, Knight A, Keyzers RA, Owen JG. Secondary metabolism in the lichen symbiosis. Chem Soc Rev. 2018; 47(5): 1730-1760. https://doi.org/10.1039/C7CS00431A
**[35]** Munguía-Martínez MF, Nava-Ruíz C, Ruíz-Díaz A, Díaz-Ruíz A, Yescas-Gómez P, Méndez-Armenta M. Immunohistochemical study of antioxidant enzymes regulated by Nrf2 in the models of epileptic seizures (KA and PTZ). Oxid Med Cell Longev. 2019; 2019: 1327986. https://doi.org/10.1155/2019/1327986
**[36]** Turski L, Ikonomidou C, Turski WA, Bortolotto ZA, Cavalheiro EA. Review: Cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. Synapse. 1989; 3(2): 154-171. https://doi.org/10.1002/syn.890030207
**[37]** Sales IM, Freitas RL, Saldanha GB, Souza GF, Freitas RM. Choline acetyltransferase and acetylcholinesterase activities are reduced in rat striatum and frontal cortex after pilocarpine-induced seizures. Neurosci Lett. 2010; 469(1): 81-83. https://doi.org/10.1016/j.neulet.2009.11.048
**[38]** Giacobini E. Cholinesterase inhibitors stabilize Alzheimer disease. Neurochem Res. 2000; 25(9-10): 1185-1190. https://doi.org/10.1023/a:1007679709322
**[39]** Visweswari G, Prasad KS, Chetan PS, Lokanatha V, Rajendra W. Evaluation of the anticonvulsant effect of *Centella asiatica* (gotu kola) in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission. Epilepsy Behav. 2010; 17(3): 332-335. https://doi.org/10.1016/j.yebeh.2010.01.002
**[40]** Pahuja M, Mehla J, Reeta KH, Joshi S, Gupta YK. Hydroalcoholic extract of *Zizyphus jujuba* ameliorates seizures, oxidative stress, and cognitive impairment in experimental models of epilepsy in rats. Epilepsy Behav. 2011; 21(4): 356-363. https://doi.org/10.1016/j.yebeh.2011.05.013
**[41]** Obay BD, Taşdemir E, Tümer C, Bilgin HM, Atmaca M. Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. Peptides. 2008; 29(3): 448-455. https://doi.org/10.1016/j.peptides.2007.11.020
**[42]** Agarwal NB, Jain S, Agarwal NK, Mediratta PK, Sharma KK. Modulation of pentylenetetrazole-induced kindling and oxidative stress by curcumin in mice. Phytomedicine. 2011; 18(8-9): 756-759. https://doi.org/10.1016/j.phymed.2010.11.007
**[43]** Ilhan A, Aladag MA, Kocer A, Boluk A, Gurel A, Armutcu F. Erdosteine ameliorates PTZ-induced oxidative stress in mice seizure model. Brain Res Bull. 2005; 65(6): 495-499. https://doi.org/10.1016/j.brainresbull.2005.02.027
**[44]** Eraković V, Zupan G, Varljen J, Simonić A. Pentylenetetrazol-induced seizures and kindling: changes in free fatty acids, superoxide dismutase, and glutathione peroxidase activity. Neurochem Int. 2003; 42(2): 173-178. https://doi.org/10.1016/s0197-0186(02)00070-0
**[45]** Akbas SH, Yegin A, Ozben T. Effect of pentylenetetrazol-induced epileptic seizure on the antioxidant enzyme activities, glutathione and lipid peroxidation levels in rat erythrocytes and liver tissues. Clin Biochem. 2005; 38(11): 1009-1014. https://doi.org/10.1016/j.clinbiochem.2005.07.012
**[46]** De Luca G, Di Giorgio RM, Macaione S, Calpona PR, Di Paola ED, Costa N, Cuzzocrea S, Citraro R, Russo E, De Sarro G. Amino acid levels in some brain areas of inducible nitric oxide synthase knock out mouse (iNOS-/-) before and after pentylenetetrazole kindling. Pharmacol Biochem Behav. 2006; 85(4): 804-812. https://doi.org/10.1016/j.pbb.2006.11.016
**[47]** Hamed SA, Abdellah MM, El-Melegy N. Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients. J Pharmacol Sci. 2004; 96(4): 465-473. https://doi.org/10.1254/jphs.fpj04032x
**[48]** Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta. 2001; 303(1-2): 19-24. https://doi.org/10.1016/s0009-8981(00)00337-5

**[49]** Naseer MI, Shupeng L, Kim MO. Maternal epileptic seizure induced by pentylenetetrazol: apoptotic neurodegeneration and decreased GABAB1 receptor expression in prenatal rat brain. Mol Brain. 2009; 2: 20. https://doi.org/10.1186/1756-6606-2-20

**[50]** Pitkänen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol. 2002; 1(3): 173-181. https://doi.org/10.1016/s1474-4422(02)00073-x

**[51]** Fernández-Moriano C, Gómez-Serranillos MP, Crespo A. Antioxidant potential of lichen species and their secondary metabolites. A systematic review. Pharm Biol. 2016; 54(1): 1-17. https://doi.org/10.3109/13880209.2014.1003354

**[52]** Paluszczak J, Kleszcz R, Studzińska-Sroka E, Krajka-Kuźniak V. Lichen-derived caperatic acid and physodic acid inhibit Wnt signaling in colorectal cancer cells. Mol Cell Biochem. 2018; 441(1-2): 109-124. https://doi.org/10.1007/s11010-017-3178-7

**[53]** Schinkovitz A, Le Pogam P, Derbré S, Roy-Vessieres E, Blanchard P, Thirumaran SL, Breard D, Aumond MC, Zehl M, Urban E, Kaur A, Jäger N, Hofer S, Kopp B, Stuppner H, Baglin I, Seraphin D, Tomasi S, Henrion D, Boustie J, Richomme P. Secondary metabolites from lichen as potent inhibitors of advanced glycation end products and vasodilative agents. Fitoterapia. 2018; 131: 182-188. https://doi.org/10.1016/j.fitote.2018.10.015

**[54]** Martinc B, Grabnar I, Vovk T. Antioxidants as a preventive treatment for epileptic process: a review of the current status. Curr Neuropharmacol. 2014; 12(6): 527-550. https://doi.org/10.2174/1570159x12666140923205715