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Research Article

Investigation of the Effect of Propolis on Penicillin Induced Epileptiform Activity in Rats

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Abstract

Aim: The aim of this study was to investigate the effects of propolis (PP), which has antioxidant and neuroprotective effects, on penicillin-induced epileptiform activity in rats.

Material and Methods: Forty-two adult male Wistar rats were divided into 6 groups as control (CONT), penicillin (PEN), diazepam (DZM), only propolis (OPP), 50 mg/kg propolis (PP50), and 100 mg/kg propolis (PP100). ECoG recording was taken from rats. At the end of the experiment, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels were determined from serum samples. Moreover, the latency of the first epileptiform activity, spike-wave frequency (SWF), and spike-wave amplitude (SWA) of the epileptiform activity were analyzed.

Results: The latency of the DZM and PP100 groups was found to be longer than the CONT groups. The time-dependent SWF and total SWF of the PP50 and PP100 groups were lower than the CONT group. No significant difference was found between the groups in terms of SWA. SOD, CAT, and GPx levels were found to be higher, but the MDA level was lower in PP50 and PP100.

Conclusion: As a result, propolis may be a potential antiepileptic drug candidate in the future with its antioxidant activity as well as prolonging latency and reducing SWF in epilepsy models.

Keywords: Epilepsy, propolis, oxidative stress, penicillin, electrocorticography

INTRODUCTION

Epilepsy is one of the most common and heterogeneous neurological conditions, and also a brain disorder characterized by a persistent predisposition to generate seizures and the neurobiological, cognitive, psychological, and social consequences of seizure recurrence. Although an etiological agent can be identified, the cause of approximately 50% of cases is still unknown (1). Currently, there are around 70 million people with active epilepsy who require treatment and have ongoing seizures. Of these patients, 30% are resistant to all known antiepileptic drugs (2,3). In the past thirty years, studies on various diseases of the nervous system have focused on the balance between the oxidant and antioxidant systems. The first experimental findings that described the relationship between oxidative stress and epilepsy were presented by Armstead et al. in 1989 (4). The relationship between oxidative stress and epilepsy has been demonstrated in many studies in different experimental models and in epileptic patients (5). In animals, it has been shown that increased reactive oxygen species (ROS) levels lead to a decrease in GABA levels in the brain, which occurs parallel to the onset of convulsions. One of the main reasons for this is that ROS production inactivates the glutamate decarboxylase enzyme (6).

Approximately 70% of epilepsy patients are controlled with monotherapy using current antiepileptic drugs. Herbal products play an important role in the development of new antiepileptic drugs. It is known that many plants have anticonvulsant effects. Various phytochemical, pharmacological, and electrophysiological studies have been carried out on these anticonvulsant plants, and these studies are increasing day by day (7).

Propolis (PP) is a natural, non-toxic, resinous substance collected by bees from various plants to maintain hive homeostasis and provide physical and biochemical protection (8,9).

CITATION

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The bioactive components of PP vary according to geographical origins, plant sources, and bee species. The chemical composition of PP can vary considerably both qualitatively and quantitatively (10). Nevertheless, most unprocessed PP consists of approximately 50-70% resin, 30-50% oils and waxes, 5-10% pollen, along with various minor chemical constituents like amino acids, sugars, vitamins B, C, and E, minerals, as well as flavonoids, phenols, and aromatic compounds such as caffeic acid (CA), caffeic acid 1,1-dimethylallyl ester, and caffeic acid phenethyl ester (CAPE) (11).

The therapeutic effects of PP's bioactive compounds have been extensively researched and continue to be investigated in many fields. Previous studies have shown that PP has antioxidant, antiproliferative, anti-inflammatory, antiparasitic, cytotoxic, and antibacterial properties (12,13). Additionally, it exhibits cardioprotective, hepatoprotective, and neuroprotective activity (14). However, to date, no studies have investigated the antiepileptic or anticonvulsant effects of PP. Only some studies have examined the effectiveness of PP in reducing the side effects of the antiepileptic drug valproate (15).

Based on the information provided, it has been shown that oxidative stress triggers the occurrence and frequency of epileptic seizures, and also increases neuronal loss and cognitive impairments after seizures. The aim of this study is to investigate the effects of PP, which has antioxidant and neuroprotective effects, on epileptiform activity induced by penicillin in rats.

MATERIAL AND METHOD

Animals

Fourty two male Wistar rats (250±30 g) were used. Rats were acquired from Duzce University Experimental Animals Application and Research Center. The rooms where the animals were housed were maintained under optimal conditions (23°C room temperature, 60±5% humidity, and a 12/12 light-dark cycle). Ethical approval was taken from the Animal Research Local Ethics Committee of Duzce University with the code number 2021/3/03. Animal maintenance and applications were conducted following the Health Guide for Animals, as approved by Animal Experiments Center Ethics Committee. Rats were divided into 6 groups control (CONT), Penicillin (PEN), Diazepam (DZM), Only Propolis (OPP), Propolis 50 mg/kg (PP50), and Propolis 100 mg/kg (PP100).

Preparation of Propolis Extraction

PP samples were obtained from honey bee colonies of Düzce University. Raw PP was harvested during the summer season. In previous studies, ethanol extraction of PP was used because the strongest antioxidant activity of PP was in the extractions obtained with ethanol solvent (16,17). Therefore, we have used ethanol as a solvent of the PP. The content of PP used had been determined in previous study (16).

Drugs and Doses

PP was administered orally at the doses of 50 and 100 mg/kg. 5 mg/kg DZM (DIAZEM, DEVA Holding A.Ş. Istanbul, Turkey) was administered intraperitoneally. As an anesthetic, 1.25 g/kg urethane (Sigma-Aldrich Missouri, USA) was applied intraperitoneally to the rats. Penicillin G (IE Ulagay Turkey Pharmaceuticals Inc., Istanbul, Turkey) used to induce epilepsy was applied as 500 IU intracortical (i.c.) in 2 µl volume.

Surgical Procedure and Electrophysiological Recordings

The surgical procedure and ECoG recording were performed as in previous studies (3,18). Briefly, urethane was used to anesthetize animals in all groups. Then rats were fixed in the stereotaxic frame (Harvard Instruments, MA, USA) after being placed in the prone position. After the head area was shaved, the scalp was cut with a scalpel from front to back along the midline. The bone portion over the left cerebral cortex was then carefully removed by slenderized with a drill (FST, KF Technology, Rome, Italy). Two Ag-AgCl ball electrodes were placed in the somatomotor cortex area opened lateral to the bregma line on the left hemisphere. After the electrodes were placed, ECoG recordings were taken with the PowerLab system (PoweLab/8 SP ADInstruments, Australia) throughout the experiment. A five-minute baseline activity recording was taken. PP or DZM application were applied 30 mins before PEN injection. Epileptiform activity was performed as in previous studies (3,18). Briefly, epileptic activity was created by intracortical administration of 500 IU/2 µl PEN with a micro injector (Hamilton Co., USA) to 2 mm laterally, 1 mm in front of the bregma line and 1.2 mm in the cortex depth. A total of 125 minutes of ECoG recording was obtained from each animal. The records were digitized with the help of the PowerLab Chart v.7.0 software program. ECoG recordings from each animal were divided into 5-minute periods. The data obtained were analyzed in terms of the time of onset of epileptiform activity, SWF and SWA, and total SWF.

After the ECoG recording of the animals in the groups, blood was taken from the heart by cardiac puncture method under anesthesia. At the end of the experiment rats were sacrificed with cervical dislocation under urethane anesthesia. The blood samples taken were centrifuged at 4000 rpm for 15 minutes, and serum was removed and stored at -80ºC until analysis. In the study, CAT, GPx, SOD, and MDA levels were measured with ELK (ELK Biotechnology CO., Ltd., Hubei, P.R.C) ELISA kits.

Statistical Analysis

From recordings obtained for each animal, the onset of epileptiform activity, SWF, and SWA were calculated automatically using software (Chart v.7.3.8, ADInstruments Pty Ltd, Australia). Epileptiform activity recordings were analyzed after dividing into five-minute periods. Differences between groups in terms of the onset of epileptiform activity and SWF and SWA measurements in each period were examined with the Kruskal-Wallis

test, and different groups were determined by the post hoc Dunn multiple comparison test. P<0.05 was accepted as the statistical significance level. GraphPad Prism 9 program was used in the analysis.

RESULTS

It was tested whether PP alone had an effect on ongoing basal activity. Accordingly, no epileptiform activity effect was observed on basal activity of only PP application (Figure 1). Similarly, no epileptiform activity was detected in the CONT group that underwent only surgery (Figure 1).

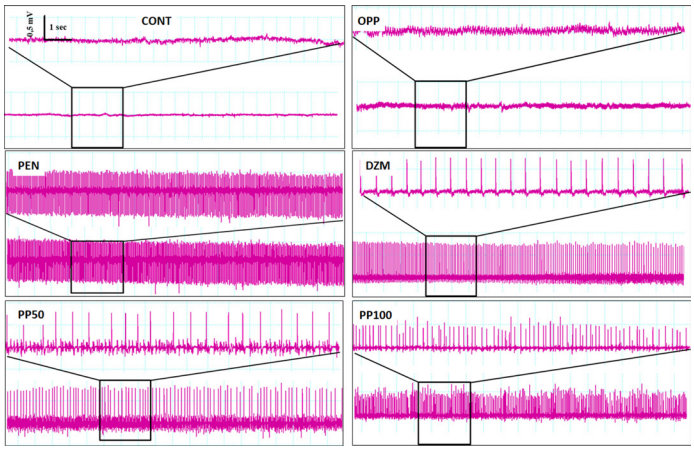

Figure 1. Representative samples of ECoG records from groups

Time of Onset of First Epileptiform Activity

Spike waves of epileptiform activity after PEN administration were observed between 360 and 1200 seconds (Figure 2). A statistically significant difference was identified among the groups when comparing based on the onset time of the first epileptiform activity (P=0.0012). When the groups were analyzed in more detail, it was observed that the means of onset times of the first epileptiform activity of the DZM and PP100 groups were statistically longer than the PEN group (p=0.0126 and p=0.0183, respectively). In addition, the means of onset times of the first epileptiform activity of the DZM and PP100 groups were found to be statistically longer than the PP50 group (p=0.0484 and p=0.0486, respectively).

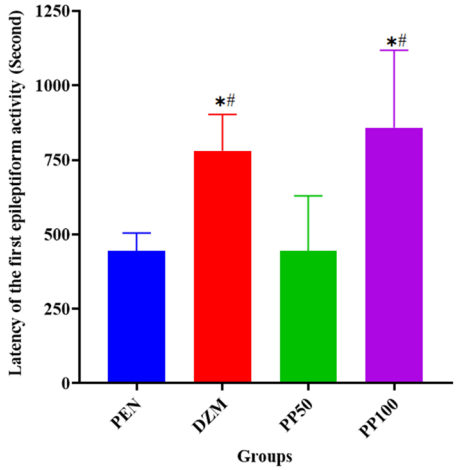

Figure 2. Latency of first epileptiform activity (*significant according to PEN group, #significant according to PP50 group)

The Effect of Propolis on the Time-Dependent Spike-Wave Frequency of Epileptiform Activity

During the 5-minute baseline activity recordings from the groups, any epileptiform activity was not detected in the ECoG recording measurements. SWF values were determined in 24 different measurements taken in five-minute periods after PEN application. Between 0-50th and 96th-120th minutes following PEN administration, a statistically significant difference was observed in the mean spike-wave numbers among all groups (p<0.05). Nevertheless, there was no difference between the groups between 51st-95th minutes (p>0.05). In the periods between 6th-60th and 96th-120th minutes, SWF of the PP50 group was lower than the PEN group (p<0.05). Similarly, in the time periods between 6th-50th minutes (except for the 21-25 time periods) the SWF of the DZM group was less than PEN group (p<0.05). In addition, the spike-wave frequency of the of the PP100 group was statistically less than the PEN group in the time periods between 6th-30th minutes (p<0.05). Data on the spike-wave frequency of epileptiform activity obtained from 120-minute ECoG recordings from the groups were given in Figure 3.

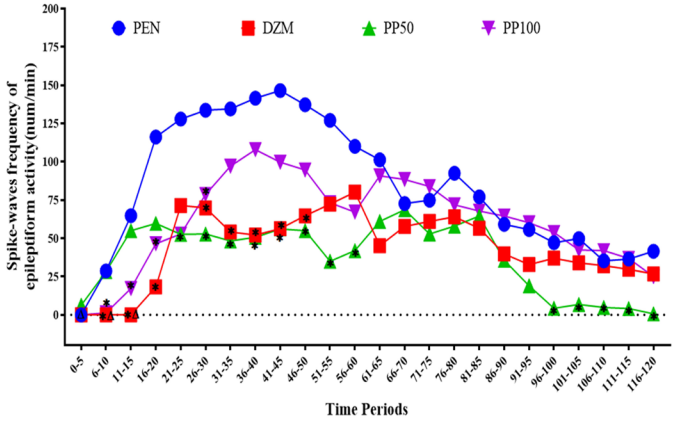

Figure 3. Mean of the time-dependent spike-wave frequency of epileptiform activity (number/min) obtained from recording after penicillin. *Significance compared with the PEN group; Δsignificance compared with the PP50 group)

Effect of Propolis on the Total Spike-Wave Frequency of Epileptiform Activity

After penicillin application in the groups, the mean of the total SWF that occurred during the 120-minute ECoG recording was evaluated. According to the results of the comparison of the means of the total spike wave frequency of the groups, it was found that statistical differences between the groups (P<0.0001), (Figure 4). In terms of total spike wave frequency of epileptiform activity, the means of the DZM, PP50, and PP100 groups were found to be statistically less than the PEN group (p<0.0001). In addition, it was determined that the mean of the DZM and PP50 groups were lower than those of the PP100 groups (p=0.0022 and p<0.0001).

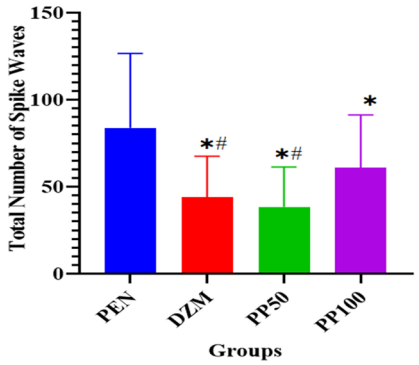

Figure 4. Display of total spike-wave number averages obtained from 120-minute recordings after penicillin (*significant according to PEN group, significant according to #PP100 group)

The Effect of Propolis on the Spike-Wave Amplitude of Epileptiform Activity

There was no statistical difference between the groups in terms of mean SWA obtained from all groups between 0-5th and 21st-120th minutes after penicillin administration (p>0.05) (Figure 5). However, there was a statistically difference in SWA of the groups between 6th-20th minutes (p<0.05). It was found that the SWA mean of the PP100 group was statistically less than the PEN group in the 6th-10th, 11st-15th and 16th-20th time periods (p=0.0389, p=0.0135 and p=0.0497). In the same time period, although the SWA means of the DZM and PP50 groups were less than those of the PEN group, it was not statistically significant (P>0.050).

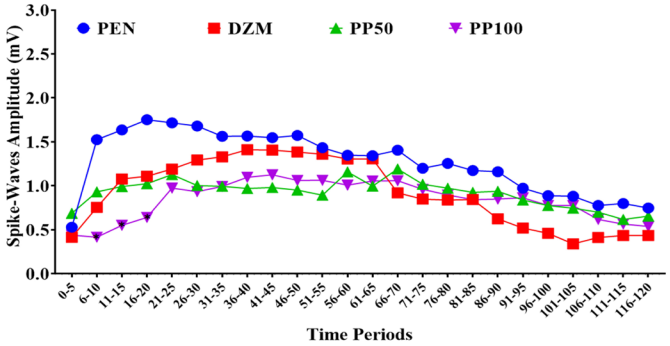

Figure 5. Mean of the time-dependent spike-wave amplitude of epileptiform activity (mV) obtained from recording after penicillin. *Significance compared with the PEN group)

Evaluation of Antioxidant Activity of Propolis

When the groups were compared in terms of SOD level, a statistical difference was found between the groups (P<0.0001) (Figure 6A). As the results were examined in more detail, the mean SOD level of the DZM group was found to be higher than the CONT and PEN groups (p<0.0001 and p<0.0001). Similarly, the mean SOD level of the OPP group was determined to be higher than the CONT and PEN groups (p<0.0015 and p<0.0001). It was determined that the mean SOD level of the PP50 group was higher than the CONT and PEN groups (p=0.0002 and p<0.0001, respectively). In addition, the mean SOD level of the PP100 group was found to be higher than the CONT and PEN groups (p=0.0008 and p=0.0002, respectively). It was no difference between OPP, DZM, PP50, and PP100 groups (p>0.05).

The groups were compared for CAT level, and a statistical difference was found between the groups (P<0.0001) (Figure 6B). Similarly, the mean CAT level of the OPP group was determined to be higher than the CONT and PEN groups (p=0.0002 and p<0.0001). It was determined that the mean CAT level of the PP50 group was higher than the CONT and PEN groups (p=0.0119 and p=0.0041). In addition, the mean CAT level of the PP100 group was found to be higher than the CONT and PEN groups (p=0.0353 and p=0.0127).

A significant difference was found between the groups in terms of GPx levels (P<0.0001) (Figure 6C). While the groups were examined in detail, it was determined that the mean GPx level of the OPP group was higher than the CONT and PEN groups (p=0.0041 and p=0.0041, respectively). The mean GPx level in the DZM group was determined to be elevated compared to the CONT and PEN groups (p=0.0178 and p=0.0178, respectively). The mean GPx level of the PP50 group was determined to be higher than the CONT, PEN and DZM groups (p<0.0001, p<0.0001 and p=0.0214). Likewise, the mean GPx level of the PP100 group was found to be higher than the CONT and PEN groups (p=0.0034 and p=0.0033).

A significant difference was found between the groups in terms of MDA levels (P<0.0001) (Figure 6D). When the groups were examined in detail, it was determined that the mean MDA levels of the PEN, PP50 and PP100 groups were lower than the CONT group (p<0.0001, p=0.0464 and p=0.0138, respectively). Similarly, the mean MDA levels of the PEN, PP50 and PP100 groups were determined to be lower than the OPP group (p<0.0001, p=0.0464 and p=0.0138, respectively). The mean MDA level of the PP50 group was found to be less than the PEN group (p<0.0001). Similarly, the mean MDA level of the PP100 group was found to be lower than the PEN group (p<0.0001).

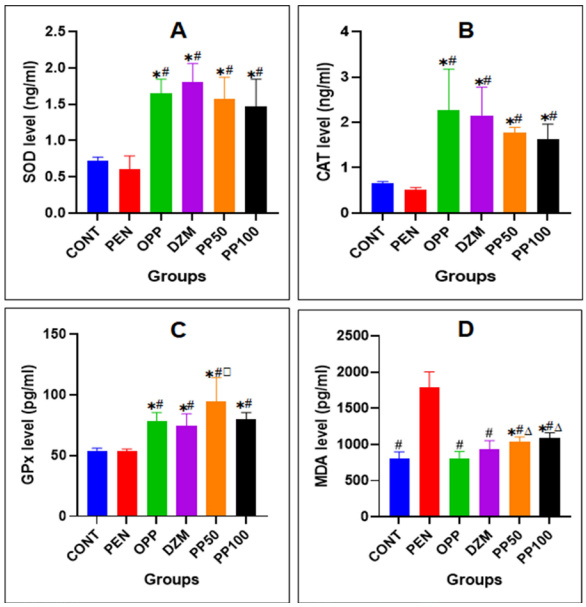

Figure 6. Effect of propolis on SOD (A), CAT (B), GPx (C) and MDA (D) levels (*Significant compared to CONT group; #Significant according to PEN group; □Significant compared to the DZM group; ΔSignificant compared to the OPP group)

DISCUSSION

This study is the first study to examine the effects of PP on PEN-induced epileptiform activity electrophysiologically. In the study, the effect of PP in the doses of 50 and 100 mg/kg, which was acutely applied, on experimentally generated PEN-induced epileptiform activity was investigated in male Wistar rats. The epileptiform activity shapes and patterns spied out in the ECoG recordings are compatible with the literature (3,18).

In the current study there was found no evidence of epileptiform activity during the baseline recording period in any of the groups prior to administration of PEN, and no spike-wave discharges were observed during the entire recording period in the CONT and only PP groups. These data cannot be compared to those of other electrophysiological studies in the literature due to the lack of such studies on the effects of PP. However, the data from the groups suggest that the use of PP does not lead to convulsions. This finding is significant as there are no electrophysiological studies on the effects of PP on epilepsy in the literature.

Intracortical administration of PEN to anesthetized rats generated an epileptiform activity within 360-536 seconds in the PEN group, this value was found within 360-1200 seconds in DZM, PP50, and PP100 groups. It was determined that the onset time of the first epileptiform activity in the PP100 group was grown approximately twice compared to the PEN group. On the contrary, the first epileptiform activity onset time of the PP50 group was found to be similar to the PEN group. In this case, it shows that the efficacy of PP may be dose dependent. Only PP which was administered group without inducing PEN epilepsy did not cause any epileptiform activity in any animal. These data suggest that the use of PP in epileptic or healthy rats will not cause any epileptic effect.

After the application of PEN, a significant difference was found in the mean SWF between all groups during the 0-50th and 96-120th minute periods. This suggests that PP reduces SWF over time. As PP' effects on epilepsy have not been studied electrophysiologically in the literature, this finding is important.

In the present study, the total spike wave frequencies during the 120-minute ECoG recording period after PEN administration were compared between groups, and it was found that both PP50 and PP100 groups had significantly lower mean spike wave frequencies compared to the PEN group. This suggests that PP reduces spike wave activity. As there is no electro-physiological study on the effect of PP on epilepsy in the literature, this finding is important.

In terms of mean epileptiform activity spike wave amplitude obtained from PP groups, similarity was found between the groups at 0-120 time periods (except 06-20 time periods). In addition, since there is no study in the literature that shows changes in epileptiform activity spike wave amplitude over time, the obtained data could not be compared.

There have been many studies on the mechanism of action of PP (19,20). However, due to the fact that PP contains 300 different substances, such as flavonoids and phenolic acids, its mechanism of action has not yet been fully understood. Hence, the potential protective impact of PP may arise through diverse mechanisms. For instance, it might involve the elimination of free oxygen radicals from the surroundings or the mitigation of free oxygen radicals' generation.

In a status epilepticus (SE) model induced by lithium+pilocarpine, it has been reported that PP repairs neuronal damage (19). The researchers emphasized that PP works by clearing free radicals or reducing the production of free radicals that can damage surrounding neuronal cells. The study in question indicated that PP could hold utility in managing SE, demonstrating potential to ameliorate neurological harm both as an antiepileptic medication on its own and when employed alongside an antiepileptic agent.

Epileptic rats induced by pilocarpine were treated with fish liver oil and PP in combination with valproate (21). After pilocarpine administration, it was reported that the lipid peroxidation levels and lactate dehydrogenase activity significantly increased in the hippocampus while the total antioxidant capacity significantly decreased compared to the control group. However, the combined application of PP and valproate improved the effect of lipid peroxidation toward normal levels. This finding is supported by other studies (20). In the present study, both doses of PP (50 and 100 mg/kg) were shown to reduce MDA levels.

Several investigations have highlighted the extensive pharmacological spectrum of CAPE, a key constituent within PP. Its manifold properties encompass antioxidative, anti-inflammatory, antiviral, antifungal, antiproliferative, and antineoplastic effects (22-24). CAPE has been reported to increase SOD, CAT, and GSH-Px levels in brain, while decreasing MDA levels (25). In the current study, it was shown that PP increased serum SOD, CAT, and GPx levels compared to the PEN group.

Cortical pyramidal cells play an active role in the epileptiform activity induced by PEN. In the epilepsy model induced with PEN, the potentials dependent on GABAA and GABAB receptors contribute to the sudden depolarization shifts observed in cells (26). Direct application of PEN to the cortex causes inhibition of GABA receptors, disrupting the brain's inhibitory system and initiating locally but generalized continuing epileptiform activity. Studies show that PEN binds to subunits of GABAA receptors, reducing the intracellular Cl- influx in cells (27). In addition, it has been reported in other studies that PEN also binds to the chloride receptor and blocks the opening of the chloride channel (28). In another study, it was suggested that PEN binds to the binding site of benzodiazepines, causing convulsions (29). The primary target of PEN is the β-subunit of the GABAA receptor to which GABA binds. It is believed that PEN binds to the GABA binding site with its β-lactam ring, preventing GABA from binding to this site.

This finding demonstrates a possible mechanism of PEN in epileptogenesis.

In recent years, many studies have been conducted or planned to clarify the role and impact of oxidative stress in epilepsy. In the early 2000s, oxidative stress was studied in epileptic conditions. The findings showed that oxidative stress is important in the neurological pathology of epilepsy. Particularly, in animal models such as lithium-pilocarpine, kainic acid, PEN, bicuculline, pentylenetetrazole (PTZ), sleep deprivation, and cocaine, an increase in ROS, nitrite levels, and lipid peroxidation products was observed. Since the 2000s, extensive studies have been conducted on the use of antioxidants in the treatment of epilepsy. In various studies, the use of various antioxidants such as plant extracts or flavonoids used in the treatment of epilepsy has been shown to reduce lipid oxidation in the hippocampus, striatum, and cortex and improve SOD, CAT, GSH, and GPx activity (30). In the current study, it was shown that PP increases CAT, SOD, and GPx levels while decreasing MDA levels. The increase in MDA levels in the PEN group in the experimental epilepsy model induced with PEN suggests that PEN triggers the production of reactive oxygen species. The data obtained from the groups are consistent with the literature.

In seizures induced by (PTZ), activation of glutamate receptors and inhibition of GABA are observed. Activation of glutamate receptors increases ROS levels. However, it has been reported that CAPE administration prolongs latency and reduces seizure duration in PTZ-treated mice (31). By virtue of its capability to eliminate ROS, diminish MDA concentration, and elevate antioxidant SOD levels, CAPE has demonstrated its capacity to safeguard brain tissue against oxidative harm.

It has been reported that administration of bee PP (30 and 60 mg/kg/day) to male Wistar rats significantly prolonged the latency of both clonic and tonic seizures induced by PTZ, reduced the duration and frequency of seizures, and decreased mortality (32). In a study on the effects of PP application on oxidative stress in rats with PTZ-induced epilepsy model, it was reported that total antioxidant capacity levels in the PP groups were significantly higher than in other groups and total oxidant status levels were lower (33).

PP has been shown to have agonist effects on the GABA receptor in different studies (34). It is known that GABA receptors are the most important receptors in epilepsy. PP contains flavonoids and their derivatives, and it is known that these substances have antioxidant effects (35). In different studies, it has been found that substances with antioxidant properties reduce epileptic seizures (36).

It has been reported that MDA and NO levels in brain tissue increase in epileptic seizures induced by PTZ in mice, while SOD activity remains unchanged (31,37). In another study, it was shown that in rats with the PTZ seizure model, the MDA level in the brain tissue of seizure animals increased compared to controls, while SOD, CAT, and GSH levels decreased (38). However, there are also studies in the literature that show no significant difference in SOD activity in brain tissue compared to controls after a single dose of PTZ application (39,40).

The current study's findings are consistent with the literature in terms of PP extending latency, reducing seizure frequency, lowering MDA levels, and increasing SOD, CAT, and GPx levels.

CONCLUSION

In conclusion, the protective and reducing effects of PP have been demonstrated in experimental epilepsy models. In the present study, only the electrophysiological response of PP to epileptiform activity and oxidative stress was investigated. Future longer-term and multidisciplinary studies in this field will shed light on the subject.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Ethical approval was taken from the Animal Research Local Ethics Committee of Düzce University with the code number 2021/3/03.*

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