The effect of acute and chronic harmaline administration on penicillin-induced epileptiform activity in rats

Sıçanlarda akut ve kronik uygulanan harmalinin penisilin ile oluşturulmuş epileptiform aktivite üzerine etkisi

Kayhan Ozkan[1], Serif Demir[1]

[1] Department of Physiology, Faculty of Medicine, Düzce University

Abstract

Aim: Harmaline (HR) is a monoamine oxidase inhibitor (MAOi) and antioxidant alkaloid obtained from Banisteriopsis caapi and Peganum harmala, where experimental studies have been conducted to support modern medicine. The electrophysiological impact of short-term and long-term HR treatment on the penicillin G-induced epileptic model in rats was examined in this study.

Methods: Eighty-four adult male Wistar rats were randomly assigned to two groups: one received a single dose/day of HR, and the other received repeated doses/days of HR. Each group was further divided into six subgroups based on the dose of HR (10, 50, and 100 mg/kg). Epileptiform activity (EA) was triggered in the experimental groups with intracortical penicillin administration. Electrophysiological data were collected and analyzed using electrocorticography (ECoG). The serum levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) were measured using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method to assess the free radical scavenger effects of HR. The latency, frequency, and amplitude of EA waves and serum antioxidant marker levels were analyzed statistically.

Results: There was no observed EA in the sham group. Nevertheless, the results showed that both acute and chronic HR treatment increased the seizure threshold dose-dependently ($p<0.05$). It was observed that the acute HR group reduced the frequency and amplitude of spike-wave discharges up to the 10th period, compared to the control and other groups, and did not affect these parameters in the remaining periods. No significant difference was observed in the chronic groups in terms of spike wave frequency and spike-wave amplitude, except for some time periods. In addition, while there was a significant increase in antioxidant enzyme levels of the chronic HR group compared to the control and other groups ($P<0.05$), there was no significant difference in the acute groups.

Conclusion: It was observed that HR did not affect spike wave frequencies and amplitudes in all acute groups, except for the 10th period and in chronic HR groups. HR prolonged the latency to first EA onset in acute and chronic groups and may have an antioxidant effect with long-term use.

Keywords: Antioxidants; electrocorticography; epilepsy; harmaline; monoamine oxidase

Öz

Amaç: Harmalin (HR), modern tıbbı desteklemek amacıyla deneysel çalışmaların yapıldığı Banisteriopsis caapi ve Peganum harmala bitkilerinden elde edilen, monoamin oksidaz inhibitörü (MAOi) ve antioksidan bir alkaloiddir. Bu çalışmada kısa süreli ve uzun süreli HR tedavisinin sıçanlarda penisilin G ile indüklenen epileptik model üzerindeki elektrofizyolojik etkisi incelenmiştir.

Yöntemler: Seksen dört yetişkin erkek Wistar sıçan rastgele iki gruba ayrıldı. Akut gruba tek doz/gün HR, kronik gruba tekrarlayan doz/gün HR verildi. Her grup ayrıca HR (10, 50 ve100 mg/kg) dozuna göre altı alt gruba ayrıldı. İntrakortikal penisilin uygulamasıyla deney gruplarında epileptiform aktivite (EA) tetiklendi. Elektrofizyolojik veriler, elektrokortikografi (ECoG) kullanılarak izlendi ve analiz edildi. HR'nin serbest radikal temizleyici etkilerini değerlendirmek için süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx) ve glutatyon redüktazın (GR) serum seviyeleri Enzim Bağlı İmmüno-Sorbent testi (ELISA) yöntemi kullanılarak ölçüldü. EA dalgalarının latensi, frekansı ve amplitüdü ile serum antioksidan belirteç düzeyleri istatistiksel olarak analiz edildi.

Bulgular: Sham grubunda EA görülmedi. Ancak sonuçlar hem akut hem de kronik HR tedavisinin nöbet eşiğini doza bağlı olarak arttırdığını gösterdi ($p<0.05$). Akut HR grubu kontrol ve diğer gruplarla karşılaştırıldığında diken dalga deşarjlarının sıklığı ve genliğinde 10. periyoda kadar düşürürken, kalan periyotlarda bu parametreleri etkilemediği gözlendi. Kronik gruplarda diken dalga frekansı ve diken dalga genliği açısından bazı zaman dilimleri dışında anlamlı fark görülmedi. Ayrıca kronik HR grubunun antioksidan enzim düzeylerinde kontrol ve diğer gruplara göre anlamlı bir artış görülürken ($P<0.05$) akut gruplarda anlamlı fark oluşmadı.

Sonuç: Harmalinin Kronik HR gruplarında ve akut HR gruplarının 10. periyotundan sonra diken dalga frekanslarını ve genliklerini etkilemediği görüldü. HR akut ve kronik gruplarda ilk EA başlama latensini uzattı ve uzun süreli kullanımda antioksidan etkiye sahip olabilir.

Anahtar Sözcükler: Antioksidanlar; elektrokortikografi; epilepsi; harmalin; monoamin oksidaz

Received/*Geliş* : 07.02.2024
Accepted/*Kabul*: 15.04.2024

DOI: 10.21673/anadoluklin.1433359

Corresponding author/*Yazışma yazarı*

Kayhan Özkan
Düzce Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, Düzce, Türkiye
E-mail: kayhanozkan @hotmail.com

ORCID
Kayhan Özkan: 0000-0002-5956-093X
Şerif Demir: 0000-0002-0305-5758

INTRODUCTION

Epilepsy, distinguished by recurring and sudden seizures, ranks as the fourth most prevalent neurological disorder, following stroke, migraine, and Alzheimer's disease. Despite encompassing diverse structural alterations such as genetic factors, traumatic brain injury, central nervous system infections, or brain tumors, approximately 65% of patients exhibit no discernible cause (1). Currently, around 65 million people worldwide receive treatment for epilepsy (2). Despite the treatment with classical, second, or third-generation antiepileptic drugs targeting the underlying pathophysiological processes of epileptogenesis, approximately 30% of epilepsy patients still exhibit resistance to these drugs (3). Additionally, the existing antiepileptic medications used in treatment often come with significant side effects, necessitating the use of two or more antiepileptic drugs for some patients. Epileptic seizures develop due to an increase in excitatory neurotransmitters such as glutamate or a decrease in inhibitory neurotransmitters like gamma-aminobutyric acid (GABA). Hence, antiepileptic drugs either augment GABA activity in the brain or manifest their impact through the inhibition of glutamate receptors (4). Therefore, these substances acting on neurons may generally cause a delay in epileptiform activity. For this purpose, the harmaline we used in the study may affect the pathways involved in the formation of epilepsy (Hypothesis 1, H_1).

- **H_1: Harmaline prolongs the latency of epileptiform activity.**

Abnormal discharges and spike waves appear in the electroencephalography (EEG) during epileptogenesis. Several epilepsy models using experimental animals, such as cats and rats, have been developed to understand the pathways in epilepsy. In these studies, epilepsy models were created by administering penicillin G intraperitoneally, and electroencephalographic recordings were obtained. Spike wave discharges were recorded, and various drug efficacies were evaluated. Furthermore, local application of penicillin to the cortex surface or interior also reported the induction of epileptiform activity. Recent research has turned to medicinal plants intending to suppress epileptic seizures (Hypothesis 2, H_2).

- **H_2: Harmaline reduces the EA frequency and amplitude of spike waves.**

The antioxidant properties of various experimental models have demonstrated a reducing effect on the severity and frequency of seizures. Many plants are known to have anticonvulsant effects, and various electrophysiological studies are being conducted with these anticonvulsant and antioxidant plants. One such medicinal plant is Peganum harmala (5). These alkaloids were first isolated from Peganum harmala and Banisteriopsis caapi in 1847 (6). The seeds of the plant have been employed in the treatment of various ailments such as fever, diarrhea, subcutaneous tumors, joint diseases, cough, diabetes, hypertension, and asthma (7-8). However, the traditional use of Peganum harmala has increased in the past two decades. Studies on the use of Peganum harmala seeds date back to 1980, while research on harmaline began in 2000. These studies predominantly focus on revealing the analgesic, antitumor, anti-inflammatory, bronchodilator, anticonvulsant, and antiepileptic properties of harmaline. There are also studies investigating the anticonvulsant properties of Peganum harmala and the effect of harmaline on seizure thresholds (9).

The dry seeds of the plant contain approximately 5.6% harmaline, 4.3% harmin, 0.6% harmalol, and 0.1% tetrahydroharmine, which are known as human monoamine oxidase (MAO) inhibitors (10). Moreover, the beta-carboline harmaline, with a molecular formula of C13H14N2O, has been reported to exhibit antidepressant, anxiolytic, anticonvulsant, sedative, hallucinogenic stimulant, excitatory stimulant, and antitumor effects. Studies by Rahimian (2023) reported that intraperitoneal harmaline administration reduced seizure threshold in mice. Hence, the study demonstrates that Peganum harmala, through its prevention of complex neuronal damage resulting from neurodegeneration caused by oxidative stress, is effective against various neurodegenerative diseases, including epilepsy (11). An imbalance between oxidative and antioxidant systems can lead to the detrimental effects of free radicals. Flavonoids such as harmaline have been shown to mitigate the potential harmful effects of free radicals in diseases related to neuronal degeneration, including epilepsy. Experimental evidence clearly indicates that flavonoids, structurally

similar to benzodiazepines, modulate the GABAA-Cl channel complex, exhibiting antiepileptic activity (12, 13). Therefore, due to their phenolic structures, flavonoids may play a modulating role in the treatment of neurodegenerative diseases by disrupting cellular oxidative processes in the central nervous system (14, 15). Oxidative stress originates from an overabundance of reactive oxygen species, encompassing hydroxyl radicals (HO), superoxide anion radicals (O2), hydrogen peroxide (H2O2), peroxyl radicals (HOO), and elevated levels of nitric oxide. The brain is particularly susceptible to harm owing to its heightened oxygen demand and extensive mitochondrial activity. Additionally, seizures increase the production of reactive oxygen molecules, leading to oxidative damage to biomolecules (16).

Therefore, the following hypotheses were investigated and their compatibility with the literature was examined (Hypothesis 3, H_3).

- **H_3: Due to its antioxidant properties, harmaline is neuroprotective, has monoamine oxidase inhibitor (MAOi) properties, and protects against epileptiform activity.**

Peganum harmala, containing beta-carboline group alkaloids such as harmaline, norharman, harman, harmol, and harmin, has been the subject of recent research into its antidepressant, antitumor, antidiabetic, analgesic, anti-addictive, antihypertensive, anticoagulant, antimicrobial, antioxidant, anti-inflammatory, and anticonvulsant effects (17). In this research, we examined the antioxidant impact of intraperitoneal harmaline applications in a dose-dependent manner, alongside their effects on EA, in order to explore the hypotheses formulated. The primary objective of this study is to investigate the effects of harmaline on EA and its potential neuroprotective role attributed to its antioxidant properties, in alignment with the formulated hypotheses. Through experimental models involving intraperitoneal administration of harmaline at varying doses, the study aims to elucidate whether harmaline prolongs the latency of epileptiform activity, reduces the frequency and amplitude of spike waves associated with EA, and exhibits neuroprotective effects against epileptiform activity by acting as a monoamine oxidase inhibitor (MAOi).

MATERIALS AND METHODS

Animals

Wistar male rats, aged 2-3 months and weighing 270±30 grams, were accommodated in optimal conditions, maintaining a room temperature of 23 °C, 60±5% humidity, and a 12:12 light-dark cycle (N=84). The experimental animals employed in this research were sourced from the Duzce University Experimental Animals Research and Application Center (Düzce, Türkiye). They were provided ad libitum access to both feed and water. The study received ethical approval from the Duzce University Animal Research Local Ethics Committee (date: 16.04.2018, decision no: 2018/1/1).

Inclusion Criteria

- Male Wistar Breed Rats: Only male Wistar breed rats were included in the study to maintain uniformity and minimize potential confounding factors related to gender differences.
- Controlled Feeding: Rats received controlled feeding from birth until they reached the age of 2-3 months to ensure consistent nutritional intake and minimize variability in physiological parameters.
- Regular Weight Monitoring: Animal weight was measured every other day to track growth patterns and ensure that rats were within the specified weight range for inclusion in the study.
- Content Analysis of Feeds: The feeds consumed by the animals underwent content analysis to ensure that they met the daily nutritional requirements without exceeding the recommended amounts.
- Controlled Water Consumption: Water consumption of the animals was controlled to maintain hydration levels and minimize potential confounding factors related to dehydration.
- Monitoring of Physiological Parameters: Urination rates, body temperatures, and body mass indexes were recorded regularly to monitor the overall health and well-being of the animals throughout the study period.
- Anesthesia Suitability Assessment: Prior to the experiment, suitability for anesthesia was assessed by measuring blood glucose levels to ensure that animals could safely undergo experimental procedures without complications.

Table. 1: Experimental groups, substances doses and administration route

Group	Substance	Dose	Route	Acute group (n)	Chronic group (n)
Sham (SG)	Saline	1 ml/kg/day	I.P.	7	7
Only Harmalin (OHG)	Harmaline	100 mg/kg/day	I.P.	7	7
Control (Penicillin G, CG)	Saline	1 ml/kg/day	I.P.	7	7
10 mg/kg Harmaline (HR10)	Harmaline	10 mg/kg/day	I.P.	7	7
50 mg/kg Harmaline (HR50)	Harmaline	50 mg/kg/day	I.P.	7	7
100 mg/kg Harmaline (HR100)	Harmaline	100 mg/kg/day	I.P.	7	7

SG: Sham group, CG: Control group, HR10: 10 mg/kg Harmaline, HR50: 50 mg/kg Harmaline, HR100: 100 mg/kg Harmaline, I.P.: Intraperitoneal, n: Number of animals, min: Minute

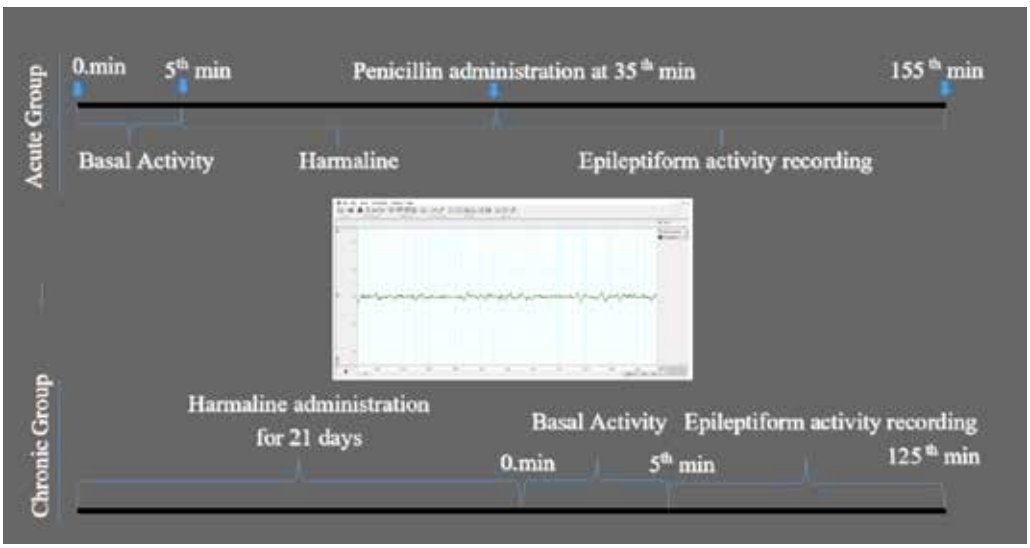

Figure 1. Duration of administration of substances in acute and chronic harmaline groups (min:Minute)

- Supervised Monitoring: Daily monitoring and examination of animals under the supervision of a veterinarian ensured their health and welfare throughout the study.
- Minimum Weight Requirement: Animals with a live weight of 270±30 grams or less on the day of the study were excluded from the study to ensure consistency and reliability of experimental results.

Exclusion Criteria

- Less than 2% of animals cared for at the experimental animal center were excluded from the study if they did not meet the specified inclusion criteria. The study incorporated an adequate sample size, determined through power analysis, to guarantee statistical power and the reliability of the experimental outcomes.

Substances and doses

Harmaline (Chemical abstract service number: 304-21-2) was administered intraperitoneally (I.P.) to the rats at doses of 10, 50, and 100 mg/kg in our study (Sigma-Aldrich, Missouri, USA). It was dissolved in dimethyl sulfoxide (DMSO) mixture and diluted with saline (Merk, Darmstadt, Germany). The induction of epilepsy was achieved by intracortical (I.C.) administration of Penicillin G potassium salt (İ.E. Ulagay İlaç, Istanbul, Türkiye) at a volume of 2 µl, containing 500 IU. Anesthesia was induced using urethane (Sigma-Aldrich, Missouri, USA) at a dosage of 1.25 g/kg administered intraperitoneally (I.P.).

Surgical procedure

The animals were anesthetized with urethane and positioned in a stereotaxic frame (Harvard Instruments,

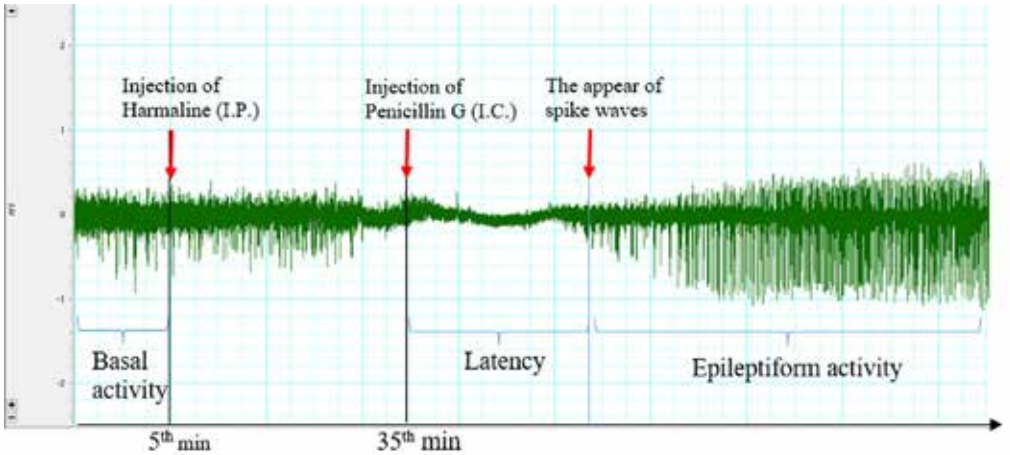

Figure 2. Epileptiform activity recording obtained by intracortical penicillin injection (I.P.:Intraperitoneal, I.C.:Intracortical, min: Minute)

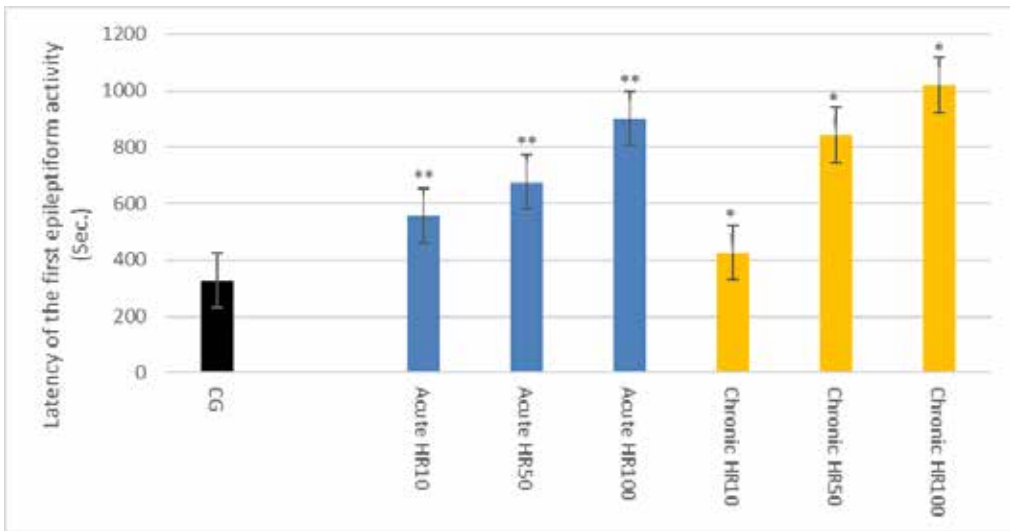

Figure 3. (CG; Control Group, Acute HR10; Acute 10mg/kg harmaline, Acute HR50; Acute 50mg/kg harmaline, Acute HR100; Acute 100mg/kg harmaline, Chronic HR10; Chronic 10mg/kg harmaline, Chronic HR50; Chronic 50mg/kg harmaline, Chronic HR100; Chronic 100mg/kg harmaline, sec: Second (*; p=0.003, **; p=0.009).

MA, USA) under the supervision of a veterinarian, ensuring vital signs were evaluated and electrophysiological observations were conducted in accordance with literature studies (16). After shaving the head region, the scalp was incised midline from front to back with a scalpel. Subsequently, the bony portion over the left cerebral cortex was delicately thinned using a microdrill (FST Rechargeable Microdrill, KF Technology, Rome, Italy) and carefully removed.

Experimental groups

Before commencing the experiment, rats were categorically divided into two primary groups: chronic administration (n=42) and acute administration (n=42) (Table 1). In the SG group, only surgical procedures were performed. OHG group, which only received 100 mg/kg of harmaline. CG group, which only received Penicillin G (500IU/2 µl, i.c.). HR10, HR50, and HR100 groups, which received HR+Penicillin G. Except for penicillin, all of the substances used in

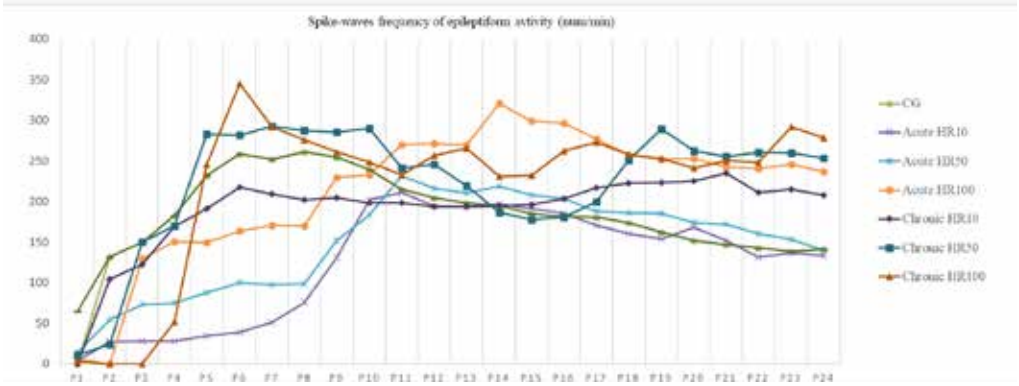

Figure 4. CG; Control Group, Acute HR10; Acute 10mg/kg harmaline, Acute HR50; Acute 50mg/kg harmaline, Acute HR100; Acute 100mg/kg harmaline, Chronic HR10; Chronic 10mg/kg harmaline, Chronic HR50; Chronic 50mg/kg harmaline, Chronic HR100; Chronic 100mg/kg harmaline; P. 5 minute period

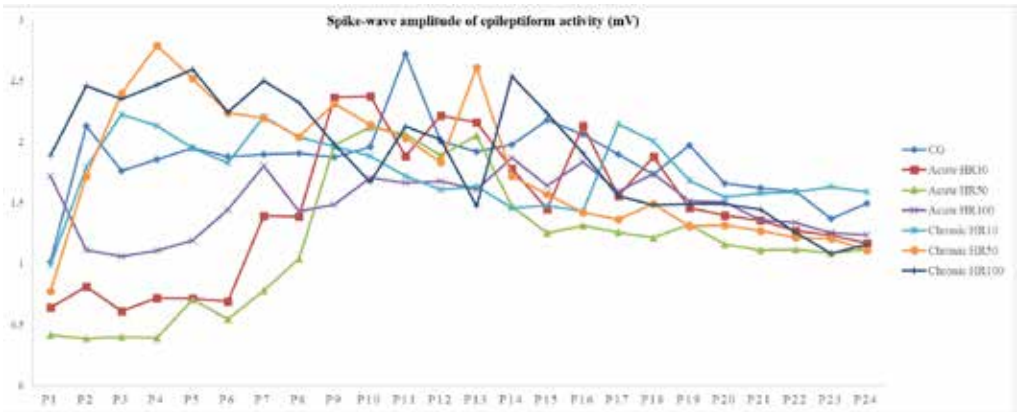

Figure 5. CG; Control Group, Acute HR10; Acute 10mg/kg harmaline, Acute HR50; Acute 50mg/kg harmaline, Acute HR100; Acute 100mg/kg harmaline, Chronic HR10; Chronic 10mg/kg harmaline, Chronic HR50; Chronic 50mg/kg harmaline, Chronic HR100; Chronic 100mg/kg harmaline; P. 5 minute period

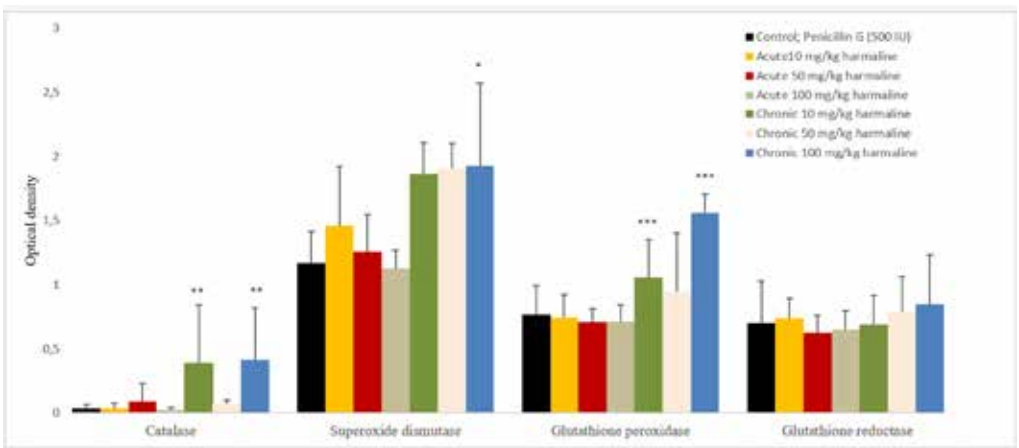

Figure 6. Effect on antioxidant optic density levels after harmaline administration in acute and chronic harmaline groups (* ; p=0.001, **; p=0.003, ***; p=0.002)).

the study were administered intraperitoneally for 21 days in the chronic group. The harmaline-only group (OHG) was given only harmaline. The substances were administered to the animals in the acute group during the experiment (Figure 1).

Creation of penicillin-induced epileptiform activity

Intracortical penicillin administration triggered the initiation of epileptiform activity. The induction of epileptiform activity through penicillin administration involved the intracortical injection of 500 IU/2 µl of penicillin. The administration of penicillin to the somatomotor cortex was conducted using a microinjector (Hamilton, Reno, USA). The injection coordinates were set at 2 mm lateral, 1 mm anterior and 1.2 mm deep to the Bregma line (18).

Electrophysiological records

Rats in the chronic group were prepared for surgery at the end of the 21st day, and rats in the acute group were prepared for surgery on the same day that harmaline was administered. First, animals were administered 1.25 g/kg urethane i.p. Anesthesia was applied. The left portion of the skull bone was then removed and two silver/silver chloride electrodes were placed in the somatomotor cortex area. After the electrodes were placed, electrocorticographic (ECoG, PowerLab/8SP, ADInstruments, NSW, Australia) recordings were obtained during the experiment. Rats were appropriately anesthetized, and after the removal of the skull region (blue area) as illustrated in Figure 1, the injection was performed at the point indicated by the black dot located 2 mm lateral to and 1 mm anterior to the bregma. Five-minute basal activity records were taken before epileptiform activity was initiated with penicillin. Intracortical administration of penicillin (500 IU / 2 ul) resulted in epileptiform activity (EA). Data analysis was conducted utilizing the PowerLab Chart v.8.0 software package (ADInstruments Pty Ltd., CO, US). Each animal underwent ECoG recordings lasting 125 minutes. Bipolar spike and spike-wave complexes, indicative of EA, were scrutinized. Additionally, within 120-minute intervals of ECoG recordings for each animal, the mean, median, minimum, and maximum values of spike-wave frequency and amplitudes per 5 minutes were quantified and utilized as data.

Determination of antioxidant activity

Subsequent to the ECoG procedure, rat blood samples were collected into tubes with yellow caps and subjected to centrifugation at 4,000 rpm for 15 minutes. (Heraeus labofuge 400, Thermo Scientific, Waltham, Massachusetts, USA). The serum obtained after centrifugation was stored at -20 °C until the day of testing. The enzyme-linked immunosorbent assay (ELISA) kits for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GSR) levels in the serum were used (Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China). Optical density values were obtained by performing the ELISA test according to the procedure in the kit manual.

Statistical analysis

The sample size was calculated by considering the correlation coefficient of 0.40, which is stated in the literature as the limit for a moderate relationship in the variables whose clinical relationships were examined. The G*Power (G*Power ver. 3.0.10, Franz Faul, Universität Kiel, Germany) package program was used to determine the required sample size for the study. As a result of the analysis (with 0,85 power, α=0.05 type I error, β=0.10 type II error, critical F value= 2,267), a correlation coefficient of at least 0.40 was found to be significant (19). The total required minimum sample size in each large group was determined to be 42 animals.

The initiation time of the initial epileptiform activity, spike-wave frequency, and spike-wave amplitude were automatically computed from the records acquired for each animal using computer software (Lab Chart 8, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). Epileptiform activity records were segmented into five-minute intervals for analysis. Group differences in the onset time of the first epileptiform activity, spike-wave frequency, and amplitude measurements within each period were evaluated through the one-way ANOVA test, and homogenous subgroups were identified using the multiple comparison method. To compare groups regarding ELISA SOD, CAT, GR, and GPx values, the ANOVA test was employed, and homogenous sub-

groups were identified for distinct groups using the multiple comparison method. Statistical Package for the Social Sciences package program version 23.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses. One-way ANOVA was employed as a statistical analysis to assess differences in antioxidant activity within groups periodically. Differences with a P value below 0.05 were considered statistically significant.

Potential primary outcomes from the study

Delay in the Time of Onset of Epileptiform Activity: Both acute and chronic administration of harmaline can cause a dose-dependent delay in the time of onset of penicillin-induced EA. This delay may reveal the potential antiepileptic effect of harmaline.

Decrease in Spike-Wave Frequency: Acute harmaline administration can lead to a statistically significant decrease in spike-wave frequency at certain intervals; such a finding may indicate a potential modulation of epileptiform activity. However, in chronic harmaline groups, this effect may not occur due to some metabolic dysregulation.

Increase in Antioxidant Enzyme Levels: Chronic administration of harmaline can significantly increase the levels of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). This demonstrates the potential neuroprotective effect of harmaline thanks to its antioxidant properties.

Secondary outcomes from the study

Effect on Spike Wave Amplitude: While harmaline showed some statistically significant effects on spike-wave amplitude during acute administration, these effects were not consistent across all time periods. Overall, harmaline did not significantly alter spike wave amplitude during epileptiform activity.

Antiepileptic and Neuroprotective Effects: The study confirms previous findings suggesting that harmaline has antiepileptic properties, as evidenced by its ability to delay the onset time of EA and modulate spike-wave frequency. In addition, the increase in antioxidant enzyme levels in chronic harmaline groups supports the hypothesis that harmaline may have a neuroprotective effect thanks to its antioxidant properties.

The findings imply that harmaline warrants exploration as a promising candidate for epilepsy therapy, potentially owing to its ability to modulate monoamine transmission and exert antioxidant properties. However, further investigation is imperative to unravel the precise molecular mechanisms driving its antiepileptic and neuroprotective actions, as well as to assess its viability as a novel therapeutic intervention for epilepsy.

RESULTS

Electrocorticographic results

The substance harmaline was exclusively examined in the harmaline groups (OHG) to assess its potential impact on the basal brain activities of rats. The investigation revealed that doses of 10, 50, and 100 mg/kg of harmaline administered in both acute and chronic groups did not elicit any discernible effect on basal activity. Additionally, it is noteworthy to mention that no epileptic activity was observed in the Sham groups throughout the entirety of the experiment.

Analysis of penicillin-induced epileptiform activity

The induction of epileptic activity was achieved through intracortical administration of 500 IU/2 µl of penicillin at a depth of 1.2 mm, utilizing a Hamilton microinjector. Epileptic discharges, characterized by spike-wave formations, emerged 3-8 minutes post-penicillin injection, as depicted in Figure 2. According to the electrocorticographic (ECoG) recordings obtained within the initial five minutes following penicillin injection in the control groups, an average amplitude of 1.01 mV was observed. The amplitude generated waves of approximately 2 mV until the 30th minute. Subsequently, an increase was noted at the 50th minute, reaching its maximum value between the 50th and 55th minutes, followed by a declining trend throughout the recording period.

In the first five minutes after penicillin injection, 132 spike-wave complexes were identified in the ECoG records of the control groups. The intervals with the highest number of spike-wave complexes were observed between the 21st and 25th minutes, totaling 258 spike-wave complexes. Although the values remained relatively consistent thereafter, a decrease in

the number of spike-wave complexes was observed after the 50th minute. By the 120th minute, the number of spike-wave complexes had decreased to 130.

Onset Latency of Initial Epileptiform Activity

The appearance of spike waves indicative of epileptiform activity commenced between the 5th and 10th minutes following penicillin administration. Upon comparison of the short and long-term harmaline groups with the control group (penicillin), it was observed that the acute harmaline application groups exhibited a dose-dependent increase in seizure onset time ($p = 0.003$). Similarly, in the chronic groups, harmaline prolonged the onset time of epileptiform activity in a dose-dependent manner, mirroring the trend observed in the acute groups ($p = 0.009$) (see Figure 3). These findings confirm that harmaline prolongs the latency of epileptiform activity in both acute and chronic groups, thereby supporting our H1 hypothesis.

Time-dependent effect of harmaline on the Spike-Wave Frequency of Epileptiform Activity

The acute administration of harmaline at doses of 10 mg/kg, 50 mg/kg, and 100 mg/kg resulted in notable differences in epileptiform activity spike wave counts compared to the control group. Specifically, at the 10 mg/kg dose, significant differences were observed up to the 40th minute, while at the 50 mg/kg dose, this effect extended to the 45th minute, and at the 100 mg/kg dose, it persisted until the 35th minute ($p=0.01$). However, beyond these time points, although the spike-wave counts remained lower than the control group from the 40th to the 120th minute, statistical significance was not reached except for specific intervals.

In the chronic harmaline groups, a similar pattern was observed. For instance, the chronic administration of 10 mg/kg harmaline resulted in a non-statistically significant decrease in spike wave counts up to the 30th minute compared to the control group. Subsequent intervals showed statistically significant differences, particularly between the 36th and 45th minutes and from the 96th to 120th minutes.

Likewise, the chronic administration of 50 mg/kg harmaline demonstrated significant differences in spike-wave counts during specific intervals, notably in the initial 5-minute period and from the 80th to 120th minutes. Moreover, the chronic administration of 100 mg/kg harmaline led to a statistically significant decrease in spike wave counts up to the 15th minute and subsequent intervals from the 21st to the 30th minute. However, no statistical differences were found during certain intervals despite higher spike wave counts compared to the control group. Overall, it was observed that harmaline did not significantly affect spike wave frequencies throughout the recording period. Thus, despite partial decreases in spike-wave frequencies, our H2 hypothesis was rejected.

Harmaline Effect on Spike-Wave Amplitude

In our investigation, animals receiving an acute dose of 10 mg/kg harmaline exhibited statistically significant differences in spike-wave amplitudes compared to the control group in every 5-minute interval up to the 40th minute ($p=0.01$). While between the 41st and 65th minutes, with the exception of the interval between 51-55 minutes where significance was maintained compared to the control group, spike wave amplitudes were higher without statistical significance. Subsequently, from the 66th to the 110th minute (excluding intervals between 76-80 and 86-90 minutes where amplitudes were higher but not statistically significant), each 5-minute period showed significantly lower spike wave amplitudes compared to the control group ($p=0.02$). However, no statistical significance was observed between the 111th and 120th minutes, despite lower amplitudes compared to the control group.

In the acute harmaline group receiving a dosage of 50 mg/kg, statistically significant differences in spike-wave amplitudes compared to the control group were observed at every 5-minute interval up to the 40th minute ($p=0.01$). Despite higher amplitude values compared to the control group between the 41st and 50th minutes, statistical significance was not reached. Similarly, during each 5-minute period between the 51st and 70th minutes, although spike wave values were lower than the control group, no statistical difference was observed. However, from the 71st to the 120th minutes (excluding the 61-65 minute interval), significant differences in spike-wave amplitudes compared to the control group were noted ($p=0.015$).

In the acute harmaline group administered a dosage of 100 mg/kg, statistically significant differences in spike wave amplitudes compared to the control group were observed in every 5-minute interval up to the 30th minute (excluding the 0-5 minute interval) (p=0.01). Although amplitude values were lower than the control group between the 31st and 35th minutes and between the 36th and 40th minutes, no statistical difference was observed. From the 41st to the 60th minutes, significant differences in spike-wave amplitudes compared to the control group were noted in each 5-minute interval. However, between the 61st and 120th minutes (excluding intervals between 71-75 and 76-80 minutes), although spike wave amplitudes were lower than the control group, no statistical significance was observed.

In the chronic harmaline group receiving a dosage of 10 mg/kg, although spike wave amplitude was lower than the control group up to the 10th minute, statistical significance was not reached. Subsequent intervals between the 11th and 20th minutes showed higher amplitude values than the control group and were statistically significant. Intervals between 21-25, 36-40, 41-45, 81-85, 86-90, and 51-55, 56-60, 66-70, 71-75, 76-80 minutes exhibited lower spike wave amplitudes compared to the control group and were statistically significant. However, between the 91st and 120th minutes (excluding intervals between 111-115 minutes), no statistical significance was observed despite lower spike wave amplitudes compared to the control group.

In the chronic harmaline group administered a dosage of 50 mg/kg, spike wave amplitude was significantly lower than the control group up to the 10th minute. Between the 11th and 30th minutes, amplitude values were higher than the control group and statistically significant. Although spike wave amplitudes were higher than the control group up to the 50th minute, no statistical significance was observed. However, from the 51st to the 120th minutes (excluding the 61-65 minute interval), spike-wave amplitudes were lower than the control group and statistically significant.

In the chronic harmaline group administered a dosage of 100 mg/kg, spike-wave amplitude was higher than the control group and statistically significant up to the 25th minute. Although amplitude values were higher than the control group between the 26th and 45th minutes, no statistical significance was observed. Intervals between 46-55, 61-65, and 81-120 minutes exhibited lower spike wave amplitudes compared to the control group and were statistically significant (p=0.01). Despite the statistically significant decreases in certain intervals during EA recordings induced by harmaline, no significant impact on spike-wave amplitudes was observed. Thus, our H2 hypothesis was rejected.

Antioxidant effect of harmaline

Upon analyzing the CAT optical density levels obtained from the ELISA test, the chronic HR10 and HR100 groups exhibited statistically significant differences compared to both the control and other groups ($p = 0.003$). Similarly, SOD levels in animals from the chronic HR100 group showed statistical significance compared to both the control and other groups (p=0.01). Furthermore, GPx levels in the chronic HR10 and HR100 groups were statistically significant compared to the control and other groups (p=0.02). Conversely, when comparing the GR levels among the groups, no statistically significant difference was observed (p=0.06) (Figure 6). These results suggest that long-term administration of harmaline may possess neuroprotective properties, thus confirming our H3 hypothesis.

DISCUSSION AND CONCLUSION

The beta-carboline alkaloids present in nature have been reported in various plants, including Banisteriopsis caapi and Peganum harmala (20). These compounds are known to bind to benzodiazepine, serotonin, opioid, and imidazoline receptors in the human body and brain. Additionally, they interact with enzymes such as cytochrome P450 and MAO (21). Due to these properties, they exhibit a wide range of psychopharmacological effects (22). The present study investigated the effects of harmaline administration on electrocorticographic (ECoG) activity and penicillin-induced epileptiform activity in rats, as well as its potential antioxidant properties. Our findings contribute to understanding harmaline's impact on epileptiform activity and its neuroprotective potential. Harmaline derived from Peganum harmala seeds demonstrated

negligible impact on seizures in a mouse model of maximal electroshock. These findings confirm the prolongation of EA latency with H_1. However, the co-administration of 3 mg/kg diazepam alongside doses of 50 mg/kg and 100 mg/kg harmaline revealed an antiepileptic effect in both groups. In the presented study, the impact of chronic and acute doses of harmaline, administered intraperitoneally (I.P.), on the onset time, frequency, and amplitude of penicillin G-induced epileptiform activity was investigated in male Wistar rats. Additionally, the potential protective effect of harmaline on the biochemical mechanisms of epilepsy was examined based on its antioxidant properties (11). Harmaline, administered in doses of 10, 50, and 100 mg/kg in both acute and chronic settings, did not demonstrate any discernible effect on basal brain activities. This suggests that harmaline does not significantly alter normal brain function under the conditions tested. Additionally, no epileptic activity was observed in the Sham groups throughout the experiment, indicating the specificity of harmaline's effects on epileptiform activity induced by penicillin. This finding showed that harmaline used for long or short periods does not cause epileptic seizures. Nevertheless, both the acute and chronic harmaline groups exhibited a delayed onset time of epileptiform activity in comparison to the control group, and this delay was dose-dependent. Assessment of spike-wave frequency and amplitude in the acute harmaline group revealed a statistically significant reduction compared to the control group. Conversely, in the chronic groups, no notable differences were observed in spike-wave frequency and amplitude of epileptiform activity compared to the control group. Previous studies have elucidated the neuroprotective effects of harmala alkaloids, specifically their modulation of calcium channels (23). However, these findings do not mean that harmaline has a reducing effect on spike-wave amplitudes and frequencies (H_2). Penicillin-induced epileptiform activity, characterized by spike-wave formations, emerged within 3-8 minutes post-administration, as expected. Our analysis of ECoG recordings revealed a time-dependent increase in spike-wave amplitude following penicillin injection, peaking between the 50th and 55th minutes before declining. Spike-wave complexes were most prevalent between the 21st and 25th minutes, reflecting the dynamic nature of epileptiform activity induced by penicillin.

Regarding the onset latency of initial epileptiform activity, harmaline administration prolonged the seizure onset time in a dose-dependent manner, both acutely and chronically. This supports the hypothesis that harmaline extends the latency of epileptiform activity, suggesting a potential antiepileptic effect.

The time-dependent effect of harmaline on spike-wave frequency demonstrated significant differences compared to the control group, particularly at lower doses and earlier time points. However, beyond certain intervals, harmaline did not significantly affect spike-wave frequencies, indicating a limited impact on the overall frequency of epileptiform activity over time. In terms of spike-wave amplitude, harmaline administration led to significant differences compared to the control group, especially in the acute phase and at lower doses. However, while statistically significant differences were observed during specific intervals, harmaline did not exert a consistent effect on spike-wave amplitudes throughout the recording period. Harmaline, in particular, blocks voltage-gated calcium channels, reducing calcium entry into cells. The neuroprotective effect of harmaline was more pronounced in the chronic groups in our study. Another study in rat dorsal root ganglia showed that harmaline dose-dependently inhibited voltage-gated calcium, sodium, and potassium channels, mainly acting on L and N types of calcium channels. The interaction of harmala alkaloids with the imidazoline contained in Peganum harmala was also shown to have antiepileptic effects (24).

Monoamine oxidase catalyzes the oxidative deamination of amines and neurotransmitters, playing a role in mood disorders, depression, and oxidative stress. The investigation into harmaline's antioxidant properties revealed significant differences in catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels in the chronic harmaline groups compared to controls. This suggests that long-term administration of harmaline may confer neuroprotective benefits, possibly through its antioxidant effects. Studies on Peganum harmala have shown that these plants suppress the MAO enzyme, improving mood and mental perception. Considering epilepsy treatment mechanisms, studies have suggested that thera-

peutic options targeting the NMDA receptor complex, which our study used harmaline for, are more effective. Harmaline, used in our study, is believed to block the NMDA receptor by binding to the glycine site, thereby reducing intracellular calcium entry and exhibiting antiepileptic effects. Chemicals acting as glycine antagonists have been shown to block NMDA and exert antiepileptic effects in previous studies (25).

When considering epilepsy treatment mechanisms, alongside new genetic models, gaining more knowledge about different aspects of monoaminergic neurotransmission using human genetic biomarkers can elucidate the complex roles of monoamines in the pathophysiology of epilepsy (26). It is thought that the adrenergic neuron-modulating property of harmaline reduces the development of epileptiform activity (27-28). The effects of harmaline, which has an MAOi property, on brain monoamine transmission have been investigated. The role of monoamines in epilepsy mechanisms was first demonstrated in a study using the adrenergic neuron blocker, reserpine. After reserpine administration, it was observed that the onset time of convulsions was prolonged. Another study in MAO-deficient mice showed slower development of seizures compared to the control group, attributed to elevated levels of serotonin and norepinephrine neurotransmitters (29). In the study conducted by Abdulrahman and Alsahrani (2016), harmaline was shown to be anticonvulsant (18, 30).

Furthermore, in addition to the obtained electrocorticogram (ECoG) recordings, the effects of harmaline on blood levels of free radicals were investigated. Free radical levels were examined in all the same groups in this study. Superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase levels were assessed to determine free radical levels. Only in the group receiving chronic harmaline application, CAT, SOD, GPx, and GR were significantly increased. The results of the study indicate that harmaline has antiepileptic and free radical-scavenging effects (31, 32). To support this effect, further studies can explore glutamate and GABA levels and receptor distribution in the same groups (33,34,35).

In the chronic harmaline application groups, the levels of antioxidant enzymes were found to be statistically significant compared to the control group. Long-term use of harmaline reduces the levels of free radicals (H_3). Recent studies have implicated increased levels of free radicals in the pathophysiology of epilepsy (36,37,38). Oxidative stress is involved in the pathogenesis of many neurodegenerative diseases, including Alzheimer's, Parkinson's, and epilepsy (39,40,41,42,43).

In the presented study, harmaline was found to have an experimental anti-epileptic effect when applied in rats with induced epilepsy models, suggesting its antioxidant properties. Therefore, it is considered to be antiepileptic. Additionally, further studies may reveal that drugs developed from harmaline could play a significant role in the treatment of epilepsy through monoamine transmission and glutamate blockade. One of the primary limitations of this study is the absence of molecular analysis to determine the negative or positive feedback mechanisms of the molecules involved in the Monoamine Oxidase Inhibitor (MAOi) pathways within the brain tissues of rats with epileptiform activity (EA). This limitation hinders a comprehensive understanding of the intricate biochemical interactions underlying harmaline's effects on epileptiform activity and its antioxidant properties. The translational relevance of the findings may be limited due to the use of animal models, specifically rats, in this study. While animal models provide valuable insights into the biological mechanisms underlying disease processes, the extrapolation of these findings to human epilepsy treatment requires careful consideration of species-specific differences and potential variations in drug responses. However, this is the first study to electrophysiologically examine the effects of acute and chronic HR in a penicillin-induced epileptiform activity model. We can say that 10, 50, and 100 mg/kg doses of harmaline in acute groups have an effect in short periods. We can also see its antioxidant properties in long-term use. Future studies can investigate the molecular connection pathways of MAOi mechanism and antioxidant effect.

In conclusion, our study provides insights into the effects of harmaline on epileptiform activity and its potential antioxidant properties. Harmaline prolonged the latency of epileptiform activity, particularly in the acute and chronic phases, supporting its potential antiepileptic properties. While harmaline exerted significant effects on spike-wave frequency and ampli-

tude during specific intervals, its overall impact was limited, suggesting a nuanced relationship between harmaline and epileptiform activity. Additionally, the observed alterations in antioxidant enzyme levels indicate a potential neuroprotective role for harmaline, which warrants further investigation. Overall, our findings contribute to understanding harmaline's pharmacological effects and its therapeutic potential in epilepsy and neuroprotection.

Conflict-of-Interest and Financial Disclosure

The authors declared that there is no conflict of interest. This study was supported by the Scientific Research Project Fund of Duzce University with the Project number BAP-2018.04.01.851.

Acknowledgments

This study was derived from the Ph.D thesis of the first author.

REFERENCES

1. Lolk K, Lange T, Elwert F, Dreier JW, Christensen J. Traumatic brain injury, stroke, and epilepsy: A mediation study in a Danish nationwide cohort. Epilepsia. 2023;64(3):718-27.
2. Czuczwar SJ. Epilepsy [Internet]. Brisbane (AU): Exon Publications; 2022. pp. 50-71.
3. Löscher W, Potschka H, Sisodiya SM, Vezzani A. Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options. Pharmacol Rev. 2020;72(3):606-38.
4. Chen TS, Huang TH, Lai MC, Huang CW. The Role of Glutamate Receptors in Epilepsy. Biomedicines. 2023;11(3):783.
5. de Melo AD, Freire VAF, Diogo ÍL, Santos HL, Barbosa LA, de Carvalho LED. Antioxidant Therapy Reduces Oxidative Stress, Restores Na,K-ATPase Function and Induces Neuroprotection in Rodent Models of Seizure and Epilepsy: A Systematic Review and Meta-Analysis. Antioxidants (Basel). 2023;12(7):1397.
6. Marangoz C, Bağirici F. Effects of L-arginine on penicillin-induced epileptiform activity in rats. Jpn J Pharmacol. 2001;86(3):297-301.
7. Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475-82.
8. Im JH, Jin YR, Lee JJ, et al. Antiplatelet activity of beta-carboline alkaloids from Perganum harmala: a possible mechanism through inhibiting PLCgamma2 phosphorylation. Vascul Pharmacol. 2009;50(5-6):147-52.
9. Kaur J, Famta P, Famta M, et al. Potential anti-epileptic phytoconstituents: An updated review. J Ethnopharmacol. 2021;268:113565.
10. Song Y, Kesuma D, Wang J, et al. Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine. Biochem Biophys Res Commun. 2004;317(1):128-32.
11. Rahimian Z, Sadrian S, Shahisavandi M, et al. Antiseizure Effects of Peganum harmala L. and Lavandula angustifolia. Biomed Res Int. 2023;2023:4121998.
12. Castel-Branco MM, Alves GL, Figueiredo IV, Falcão AC, Caramona MM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol. 2009;31(2):101-6.
13. Goldschen-Ohm MP. Benzodiazepine Modulation of GABAA Receptors: A Mechanistic Perspective. Biomolecules. 2022;12(12):1784.
14. Herraiz T, Guillén H. Monoamine Oxidase-A Inhibition and Associated Antioxidant Activity in Plant Extracts with Potential Antidepressant Actions. Biomed Res Int. 2018;2018:4810394.
15. Maan G, Sikdar B, Kumar A, Shukla R, Mishra A. Role of Flavonoids in Neurodegenerative Diseases: Limitations and Future Perspectives. Curr Top Med Chem. 2020;20(13):1169-94.
16. Łukawski K, Czuczwar SJ. Oxidative Stress and Neurodegeneration in Animal Models of Seizures and Epilepsy. Antioxidants (Basel). 2023;12(5):1049.
17. Shahrajabian MH, Sun W, Cheng Q. Improving health benefits with considering traditional and modern health benefits of Peganum harmala. Clin Phytosci. 2021;7(1):18-32.
18. Aricioglu F, Yillar O, Korcegez E, Berkman K. Effect of harmane on the convulsive threshold in epilepsy models in mice. Ann N Y Acad Sci. 2003;1009:190-5.
19. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ: Erlbaum; 1988.
20. Larit F, León F. Therapeutics to Treat Psychiatric and Neurological Disorders: A Promising Perspective from Algerian Traditional Medicine. Plants (Basel). 2023;12(22):3860.
21. Nafisi S, Bonsaii M, Maali P, Khalilzadeh MA, Manouchehri F. Beta-carboline alkaloids bind DNA. J Photochem Photobiol B. 2010;100(2):84-91.

22. McDaniel SS, Wong M. Therapeutic role of mammalian target of rapamycin (mTOR) inhibition in preventing epileptogenesis. Neurosci Lett. 2011;497(3):231-9.
23. Vrabec R, Blunden G, Cahlíková L. Natural Alkaloids as Multi-Target Compounds towards Factors Implicated in Alzheimer's Disease. Int J Mol Sci. 2023;24(5):4399.
24. Doskaliyev A, Seidakhmetova R, Tutai DS, Goldaeva K, Surov V, Adekenov S. Alkaloids of Peganum harmala L. and their Pharmacological Activity. Open Access Maced J Med Sci. 2021;9(A):766-75.
25. Jewett BE, Thapa B. Physiology, NMDA Receptor. Treasure Island (FL): StatPearls Publishing; 2022. pp. 20-56.
26. Golub VM, Reddy DS. Post-Traumatic Epilepsy and Comorbidities: Advanced Models, Molecular Mechanisms, Biomarkers, and Novel Therapeutic Interventions. Pharmacol Rev. 2022;74(2):387-438.
27. Rodríguez L, López A, Moyna G, et al. New Insights into the Chemical Composition of Ayahuasca. ACS Omega. 2022;7(14):12307-17.
28. Baru Venkata R, Prasanth DSNBK, Pasala PK, et al. Utilizing Andrographis paniculata leaves and roots by effective usage of the bioactive andrographolide and its nanodelivery: investigation of antikindling and antioxidant activities through in silico and in vivo studies. Front Nutr. 2023;10:1185236.
29. Trindade-Filho EM, de Castro-Neto EF, de A Carvalho R, et al. Serotonin depletion effects on the pilocarpine model of epilepsy. Epilepsy Res. 2008;82(2-3):194-9.
30. Abdulrahman M, Alshahrani M. Antiepileptic effect of ethanolic extract of seeds of Peganum harmala on maximal electroshock seizure model in albino mice. Int J Allied Med Sci Clin Res. 2016;4(3):529-35.
31. Tsabang N, Guedje NM, Fokunang C, et al. Ethnopharmacological and Ethnomedical Study of Anti-Epileptic Plants used in Traditional Medicine in Yaounde and its Surroundings Areas (Cameroon). J Biol Chem Res. 2016;33(1):496-509.
32. Marwat SK, Rehman F. Medicinal and Pharmacological Potential of Harmala (Peganum harmala L.) Seeds. Nuts and Seeds in Health and Disease Prevention. Academic Press: San Diego, USA; 2011. pp. 585-599.
33. Thurman DJ, Beghi E, Begley CE, et al. Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia. 2011;52 Suppl 7:2-26.
34. Grosso C, Santos M, Barroso MF. From Plants to Psycho-Neurology: Unravelling the Therapeutic Benefits of Bioactive Compounds in Brain Disorders. Antioxidants (Basel). 2023;12(8):1603.
35. Dewanjee S, Sohel M, Hossain MS, et al. A comprehensive review on clinically proven natural products in the management of nerve pain, with mechanistic insights. Heliyon. 2023;9(5):e15346.
36. Ethemoglu MS, Kutlu S, Seker FB, Erdogan CS, Bingol CA, Yilmaz B. Effects of agomelatine on electrocorticogram activity on penicillin-induced seizure model of rats. Neurosci Lett. 2019;690:120-125.
37. Ghasemi M, Schachter SC. The NMDA receptor complex as a therapeutic target in epilepsy: a review. Epilepsy Behav. 2011;22(4):617-40.
38. Handforth A. Harmaline tremor: underlying mechanisms in a potential animal model of essential tremor. Tremor Other Hyperkinet Mov (N Y). 2012;2:02-92-769-1.
39. Marangoz C, Bağirici F. Effects of L-arginine on penicillin-induced epileptiform activity in rats. Jpn J Pharmacol. 2001;86(3):297-301.
40. de Melo AD, Freire VAF, Diogo ÍL, Santos HL, Barbosa LA, de Carvalho LED. Antioxidant Therapy Reduces Oxidative Stress, Restores Na,K-ATPase Function and Induces Neuroprotection in Rodent Models of Seizure and Epilepsy: A Systematic Review and Meta-Analysis. Antioxidants (Basel). 2023;12(7):1397.
41. Ngugi AK, Kariuki SM, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Incidence of epilepsy: a systematic review and meta-analysis. Neurology. 2011;77(10):1005-12.
42. Pimpinella G, Palmery M. Interaction of beta-carbolines with central dopaminergic transmission in mice: structure-activity relationships. Neurosci Lett. 1995;189(2):121-4.
43. Yildirim M, Marangoz AH, Ayyildiz M, Ankarali S, Marangoz C. The interactions of nitric oxide and adenosine on penicillin-induced epileptiform activity in rats. Acta Neurobiol Exp (Wars). 2011;71(2):208-19.