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Research Article

Investigation of the Antiepileptic Effect of (R)-(-) and (S)-(+) Carvone in Penicillin-Induced Epileptiform Activity Model

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Abstract

Aim: Epilepsy affects approximately 70 million people worldwide. While many drugs can prevent seizures, they have a limited impact on preventing or curing the disease. In this perspective, natural compounds, especially monoterpenes derived from medicinal plants, have been investigated in epilepsy models, such as carvone (CAR). The principal constituent of peppermint oil, (R)-(-)-carvone (R-CAR), and the primary component in cumin and dill seed oils, (S)-(+)-carvone (S-CAR), find diverse applications in cosmetics, food, and pharmaceutical formulations. This study aims to investigate the antiepileptic effects of the natural compounds S-CAR and R-CAR in penicillin (PEN)-induced experimental epilepsy model in rats.

Material and Method: In the research, 91 male Wistar rats were used. The rats were grouped into 3 main groups as common groups, pre-penicillin groups and post-penicillin groups. The main groups were divided into a total of 13 subgroups. Electrocardiogram recording was taken from rats. At the end of the experiment, the latency of the first epileptiform activity (EA), spike-wave frequency (SWF), and spike-wave amplitude (SWA) of the EA were analyzed.

Results: S-CAR and R-CAR administered before penicillin prolonged the latency to the onset of the first EA. S-CAR and R-CAR administered before penicillin decreased SWF. 100 mg/kg doses of S-CAR and R-CAR injected 30 minutes after penicillin administration decreased SWF. While 200 mg/kg dose of R-CAR administered before penicillin decreased SWA in a time-dependent manner, 100 mg/kg dose of S-CAR administered after penicillin decreased SWA.

Conclusion: These findings indicate that carvone could exhibit both protective and therapeutic effects in the management of epilepsy.

Keywords: Carvone, epilepsy, penicillin, rat

INTRODUCTION

Epilepsy stands as one of the prevalent neurological disorders, distinguished not only by recurring unprovoked seizures but also by frequent associated somatic and psychiatric comorbidities (1,2). Epilepsy has negative socioeconomic consequences not only for patients but also for families and society (1,3). Epilepsy affects 70 million people worldwide (4,5). Approximately 20-30% of epilepsy patients are resistant to current antiepileptic drugs (AEDs). Therefore, there demand for drugs that are effective against drug-resistant seizures, have a low negative side effect profile, have favorable side effect profiles, especially in terms of neurological and psychiatric effects, and are cost-effective (6).

Carvone (CAR) is mostly obtained from the essential oils of plants of the Mentha genus. CAR (C10H14O), a ketone monoterpene, is isolated in (R)-(-) and (S)-(+) isomeric forms (7). CAR, which has a wide range of uses, has become popular, especially in the pharmaceutical industry (8). Studies have reported the anti-inflammatory (9), antioxidant (10), antinociceptive (11), antispasmodic (12), and neuroprotective effects of CAR (13).

There is an increasing search for the development of effective drugs with a combined effect, aiming to overcome the problems of polypharmacy that are specific to multiple diseases. In this context, special attention is paid to natural compounds such as terpenoids, which can bind to different pharmacological targets in the body. Moreover, terpenoids and their derivatives enhance penetration by affecting the ordered structure of biological membranes (14,15).

Compounds found in essential oils such as CAR have

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pharmacological effects on molecules involved in the mechanism of epilepsy (NMDA, Glycine, GABA...) (16-18). In addition, they affect resting membrane potential dynamics by modulating voltage-gated sodium and calcium channels (19). In epilepsy treatment, the ability of target molecules to cross the blood-brain barrier (BBB) is taken into consideration. Molecules smaller than 400 Da and lipophilic molecules easily cross the BBB (20). Therefore, CAR in essential oils also meets these criteria. This study aimed to electrophysiologically investigate the acute effects of S-CAR and R-CAR on penicillin-induced experimental epilepsy model in rats.

MATERIAL AND METHOD

Animals

Rats were obtained from Abant Izzet Baysal University (AIBU) Experimental Animal Research and Application Center. Male Wistar rats (n=91) weighing 230±30 g were kept in the laboratory at room temperature of 23°C, 60±5% humidity and 12:12 light-dark cycle, with free food and water intake. Ethical approval for the study was obtained from the AIBU Animal Research Local Ethics Committee with code number 2013/08.

Experimental Groups, Drugs, and Administration Routes

In the study, chemically purchased carvone (Sigma-Aldrich Missouri, USA) was administered intraperitoneally (i.p.) at doses of 100 mg/kg and 200 mg/kg. Urethane (Sigma-Aldrich Missouri, USA) at a dose of 1.25 g/kg i.p. was used as an anesthetic and 99% dimethyl sulfoxide (DMSO; Loba Chemie, India) was used as a solvent. Penicillin G potassium salt (I.E. Ulagay, Türkiye) used to induce epilepsy was administered intracortically (i.c.) at 500 IU in 2 µl volume. All drugs were prepared daily.

Rats were divided into 3 main groups: Common groups, pre-penicillin groups, and post-penicillin groups. The groups were then divided into subgroups as shown in Table 1. Only R-CAR, Only S-CAR, Sham, and DMSO groups were formed to determine whether the substances or surgical procedures caused any epileptic activity.

Table 1. Experimental groups, substances and routes of administration

	Subgroups	Substance	Dose	Route	n
Common groups	Sham	-	-	-	7
	Only R-CAR	(R)-(-) CAR	200 mg/kg	i.p.	7
	Only S-CAR	(S)-(+) CAR	200 mg/kg	i.p.	7
	DMSO (Solvent)	DMSO	1 ml/kg	i.p.	7
	Penicillin (PEN)	PEN	500 IU	i.c.	7
Pre-penicillin groups	Pre-R-CAR100	(R)-(-) CAR+PEN	100 mg/kg +500 IU	i.p.+i.c.	7
	Pre-S-CAR100	(S)-(+) CAR+PEN	100 mg/kg+500 IU	i.p.+i.c.	7
	Pre-R-CAR200	(R)-(-) CAR+PEN	200 mg/kg +500 IU	i.p.+i.c.	7
	Pre-S-CAR200	(S)-(+) CAR+PEN	200 mg/kg+500 IU	i.p.+i.c.	7
Post-penicillin groups	Post-R-CAR100	PEN+(R)-(-) CAR	500 IU+100 mg/kg	i.c.+i.p.	7
	Post-S-CAR100	PEN+(S)-(+) CAR	500 IU+100 mg/kg	i.c.+i.p.	7
	Post-R-CAR200	PEN+ (R)-(-)CAR	500 IU+200 mg/kg	i.c.+i.p.	7
	Post-S-CAR200	PEN+(S)-(+) CAR	500 IU+200 mg/kg	i.c.+i.p.	7

Surgical Procedure and Induction of Epileptiform Activity

In all groups, each animal was anesthetized with 1.25 g/kg urethane. The rats were then fixed to a stereotaxic frame (Harvard Instruments, MA, USA) in the supine position. Following the head shave, an incision was made along the midline of the scalp from front to back. Subsequently, the bony portion over the left cerebral cortex was delicately thinned with a drill and carefully removed. The penicillin-induced EA model was established using a previously described method (20-22). In brief, epileptic activity was induced by administering 500 IU/2 µl of penicillin intracortically at 2 mm lateral to the bregma line, 1 mm anterior, and 1.2 mm cortical depth using a Hamilton microsyringe (701N, Hamilton Co., Reno, NV, USA).

Electrophysiological Recordings

Two silver-silver chloride ball electrodes were placed in the somatomotor cortex area lateral to the Bregma line on the left hemisphere. The reference electrode was fixed to the right ear of the rats. Recording coordinates were set as follows: the first electrode was placed 1 mm anterior to the Bregma line and 2 mm lateral to the sagittal suture, and the second electrode was placed 5 mm posterior to the Bregma line and 2 mm lateral to the sagittal suture. After the electrodes were placed, electrocorticography (ECoG) recordings were obtained using the PowerLab/8SP system (ADInstruments Pty Ltd, NSW, Australia). A 5-minute baseline activity recording was taken after the electrodes were placed. Following the baseline activity recording, substances specified in Table 1 were administered intraperitoneally (i.p.) to all groups except for the post-penicillin groups. In the pre-penicillin groups, 30 minutes after substance administration, intracortical penicillin G application was performed, and ECoG recordings were taken for an additional 120 minutes. For the post-penicillin groups, after placing the electrodes, a 5-minute baseline

activity recording was obtained. Following the baseline activity recording, intracortical penicillin G application was performed. Thirty minutes after penicillin administration, substances specified in Table 1 were administered i.p. and ECoG recordings were taken for another 120 minutes.

Statistical Analysis

Recorded data from each animal were used to automatically calculate the onset latency of the first EA, spike-wave frequency, and spike-wave amplitude using software. Epileptiform activity recordings were analyzed after being segmented into ten-minute intervals. The initial onset time of EA, spike-wave frequency, and spike-wave amplitude measurements were assessed for each interval. Data were presented as mean±SD. GraphPad Prism 8 was utilized for all statistical analyses. The normal distribution of the data was assessed using the Shapiro-Wilk test. Two-way repeated measures ANOVA followed by LSD post-hoc test was employed for the evaluation of other data obtained from the groups. A p-value <0.05 was considered statistically significant.

RESULTS

Carvone Administration did not cause any EA in Groups not Induced Epilepsy with Penicillin

In the study, only (R)-CAR, Only (S)-CAR and DMSO administration did not have any EA effect on basal activity in the groups not stimulated with penicillin. Similarly, no discharge of epileptic activity was observed in the Sham group (Figure 1).

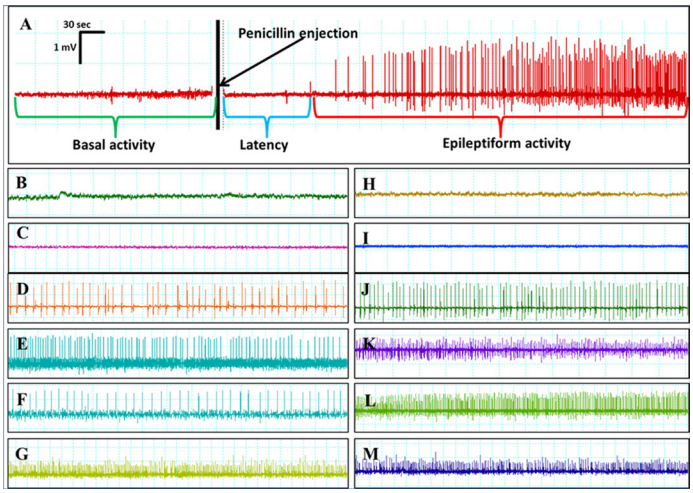

Figure 1. Representative samples of ECoG records from groups. **1A:** PEN group; **1B:** Sham group; **1C:** DMSO groups; **1D:** Pre-R-CAR100 group; **1E:** Pre-S-CAR100 group; **1F:** Pre-R-CAR200 group; **1G:** Pre-S-CAR200 group; **1H:** Only R-CAR200 group; **1I:** Only S-CAR200 groups; **1J:** Post-R-CAR100 group; **1K:** Post-S-CAR100 group; **1L:** Post-R-CAR200 group; **1M:** Post-S-CAR200 group

Carvone Prolongs Time to the Onset of the First EA

When the groups were compared according to the time of onset of the first EA, a statistical difference was detected between the groups (P<0.001) (Figure 2). When the groups were analyzed in more detail, the mean time to onset of the first EA in the Pre-R-CAR100, Pre-S-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups was statistically longer than that in the PEN groups (p<0.001, p=0.007, p<0.001 and p<0.001, respectively). Similarly, the mean time to onset of the first EA in the Pre-R-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups was statistically higher than in the Pre-S-CAR100 group (p<0.001, p<0.001 and p<0.001, respectively). The mean time to onset of the first EA in the Pre-S-CAR200 group was statistically higher than in the Pre-R-CAR100 group (p=0.040).

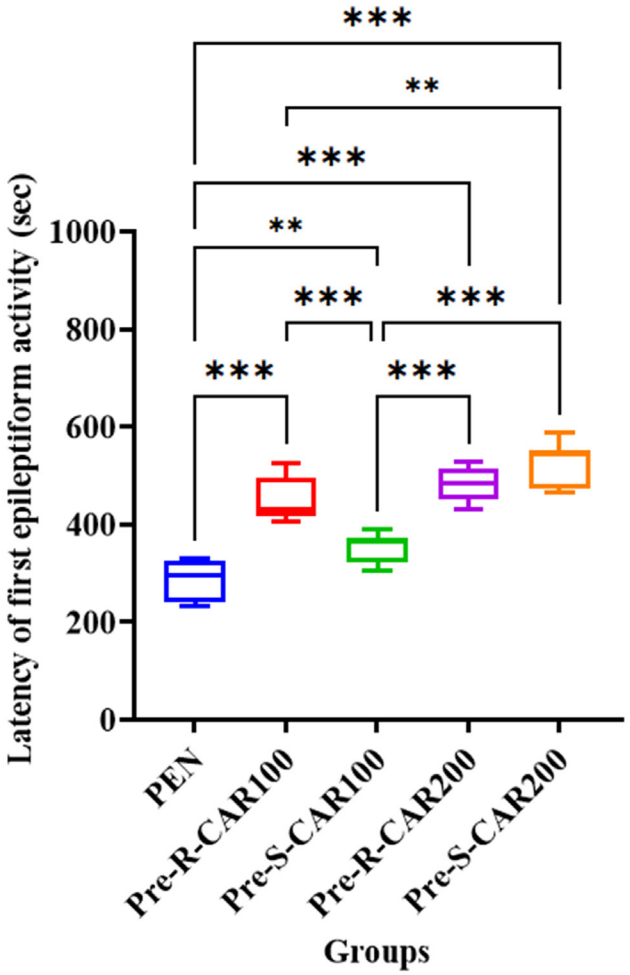

Figure 2. Latency of the first epileptiform activity in pre-penicillin groups (*p<0.05, **p<0.01, ***p<0.001)

Carvone Administration Reduces the SWF

No EA was detected in ECoG recording measurements during the basal activity recordings obtained from the penicillin injection groups. After penicillin administration, a certain number of spike-wave frequency values were obtained in 12 different measurements taken in ten-minute periods (Figure 1A and Figure 3). The mean SWF values of the Pre-R-CAR100 and Pre-R-CAR200 groups were lower than the PEN group in 12 different time periods taken during 0-120 minutes of recording (p<0.05) (Figure 3A). Furthermore, the mean SWF values of the Pre-R-CAR200 group were less than Pre-S-CAR100 group (p<0.01). The mean SWF values of the Pre-S-CAR200 group were lower than the PEN group except for the time periods 11-20, 51-60, and 61-70 (p<0.05). Furthermore, the mean SWF values

of the Pre-S-CAR200 group were lower than the Pre-S-CAR100 group in the measurements taken between 0-80 minutes (p<0.05).

ECoG recordings obtained as a result of carvone administration 30 minutes after penicillin injection were analyzed for 120 minutes. The 120-minute recordings were divided into 12 different time periods of ten minutes. A statistically significant distinction was observed among the groups (p=0.004) (Figure 3B). The mean SWF values of the Post-R-CAR100 and Post-S-CAR100 groups were lower than the PEN group in 12 different time periods taken during 30-150 minutes of the recording (excluding 30-40, 101-110, 131-140, and 141-150 time periods) (p<0.05). In 81-90, 111-120, and 121-130 time periods, the mean SWF values of the Post-R-CAR200 groups were lower than the PEN group (p=0.020, p=0.040, and p=0.040, respectively). Moreover, the mean SWF values of the Post-S-CAR200 group were lower than the PEN group in the 41-50, 61-70, and 71-80 time periods (p=0.020, p=0.020, and p=0.020, respectively). In the 51-60 and 61-70 time periods, the mean SWF values of the Post-R-CAR100 group were lower than the Post-R-CAR200 group (p=0.049 and p=0.030).

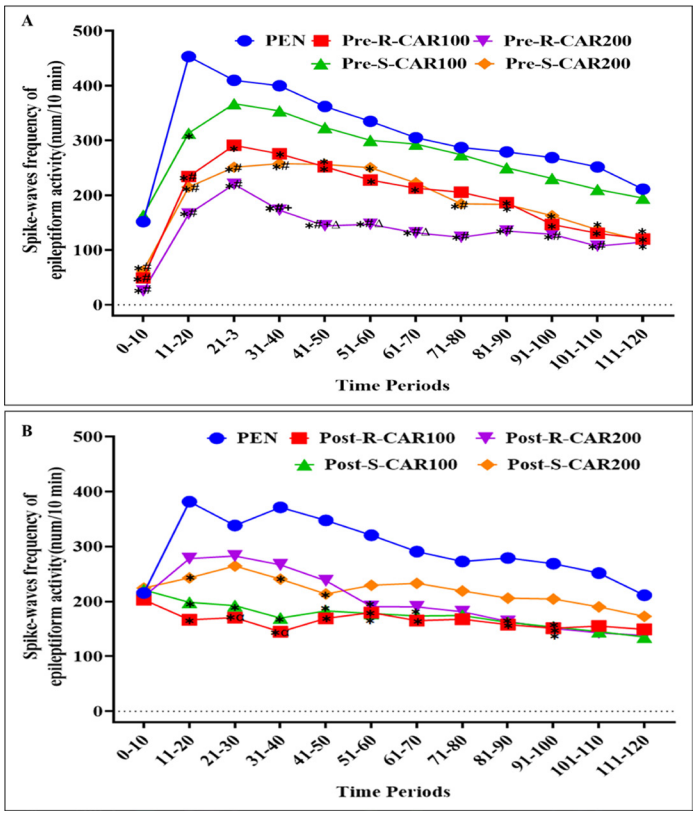

Figure 3. Mean of the time-dependent spike-wave frequency of epileptiform activity (number/min) obtained from the recording of rats. **3A:** pre-penicillin groups and **3B:** post-penicillin groups. (*Significant compared to PEN group; #Significant according to Pre-S-CAR100 group; +Significant compared to the Pre-R-CAR100 group; ΔSignificant compared to Pre-S-CAR200 group)

Effect of Carvone Administration on the Total SWF

The mean total spike wave frequency counts of the groups before penicillin injection were evaluated during 120 minutes of ECoG recording after penicillin administration. According to the results of the comparison of the groups in terms of the mean total SWF counts, a statistically significant distinction was observed among the groups (p<0.001) (Figure 4A). In terms of total SWF, the mean SWF values of the Pre-R-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups were lower than those of the PEN group (p=0.002, p<0.001 and p=0.002, respectively). Likewise, the mean SWF levels of the Pre-R-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups were lower than the Pre-S-CAR100 group (p=0.010, p<0.001 and p=0.009, respectively). However, there was no statistically significant difference between the groups in terms of total SWF value in ECoG recordings obtained as a result of carvone administration 30 minutes after penicillin injection (p=0.230) (Figure 4B).

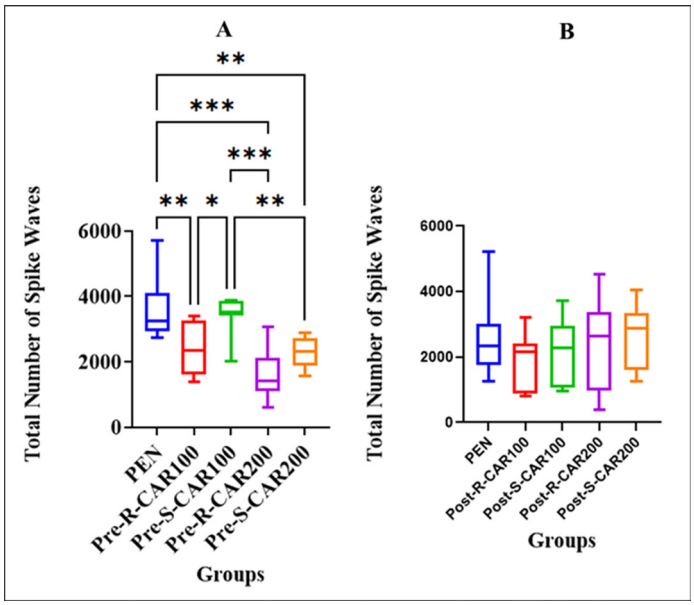

Figure 4. Display of total spike-wave number of pre-penicillin groups (**4A**) and post-penicillin groups (**4B**) (*p<0.05, **p<0.01, ***p<0.001)

Effect of Carvone Administration on SWA

Descriptive statistics of the SWA values measured at different times from ECoG recordings obtained from the groups before penicillin injection and the results of the comparison of the groups are given in Figure 5A. Except for the 0-10 time interval of the recording (p=0.051), the mean SWA values of the Pre-R-CAR200 group were statistically lower than the Pre-S-CAR100 group (p<0.05) in 12 different time intervals taken during 120 minutes of recording. In addition, the mean SWA values of the Pre-S-CAR200 group were statistically lower than the Pre-S-CAR100 group in the 31-40 and 61-70 time periods (p=0.040 and p=0.049). No statistically significant difference was found between the other groups (p>0.050).

ECoG recordings obtained as a result of carvone administration 30 minutes after penicillin injection were analyzed for 120 minutes. The 120-minute recordings were divided into 12 different time periods of ten minutes. A statistically significant distinction was observed among the groups (p<0.001) (Figure 5B). The mean SWA values of the Post-S-CAR100 group were lower than those of the PEN and Post-S-CAR200 groups in 12 different time periods

taken during 30-150 minutes of the recording (p<0.05). The mean SWA values of the Post-S-CAR100 group were lower than the Post-R-CAR100 group in the time periods 61-80, 91-100, and 121-150 (p<0.05). In addition, the mean SWA values of the Post-S-CAR100 group were lower than the Post-R-CAR200 group in the time periods between 51-90 minutes (p<0.05).

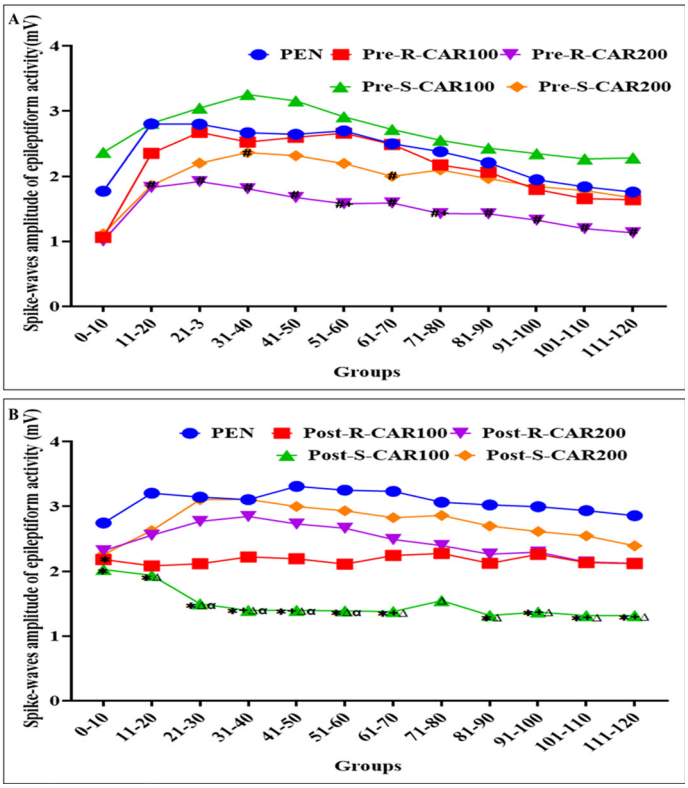

Figure 5. Mean of the time-dependent spike-wave amplitude of epileptiform activity (mV) obtained from the recording of rats. **5A:** pre-penicillin groups and **5B:** post-penicillin groups. (*Significant compared to PEN group; #Significant according to Pre-S-CAR100 group; +Significant compared to the Pre-R-CAR100 group; ΔSignificant compared to Pre-S-CAR200 group; αSignificant compared to Pre-S-CAR200 group)

DISCUSSION

In this study, the antiepileptic effects of 100 and 200 mg/kg doses of S-CAR and R-CAR were investigated in the penicillin-induced EA model. No EA was found in ECoG recordings taken for 120 minutes in the DMSO and Sham groups. These data are consistent with the literature (20-22). Similarly, no EA was observed in the only R-CAR and only S-CAR groups. This finding was not compared due to the lack of data in the literature.

In a study testing the effects of CAR on the latency of the first EA, it was reported that 200 mg/kg S-CAR prolonged the seizure onset time in PTZ and picrotoxin (PTX)-induced seizure models in mice, but 200 mg/kg R-CAR had no effect (23). In studies conducted with cyano-carvone (CC), a synthetic derivative of CAR, the protective effect of CAR was tried to be demonstrated. CC was investigated in the pilocarpine (PILO)-induced epilepsy model in mice. They reported that CC (25, 50, or 75 mg/kg) delayed the onset of the first epileptic seizure (24). In another similar study, the effects of CC against PILO, PTZ, and PTX-induced seizures were investigated (25). They reported that CC prolonged the time to onset of the first EA in all three models. In another study with PTZ, it was reported that CAR prolonged latency (26). In the present study, both doses (100 and 200 mg/kg) of S-CAR and R-CAR administered before penicillin prolonged the latency to onset of the first EA. Especially 200 mg/kg S-CAR and R-CAR doses prolonged the latency to the onset of the first EA approximately two-fold compared to the PEN group.

In a few studies with CC, the effects of carvone on seizure frequency were investigated. Costa et al. reported that CC at doses of 25, 50, or 75 mg/kg reduced seizure frequency in a PILO-induced epilepsy model (24). In another study testing the effect of CAR in the PTZ model, they reported that 10 and 20 mg/kg doses significantly reduced seizure frequency (26). In the present study, S-CAR (200 mg/kg) and R-CAR (100 and 200 mg/kg) administered before penicillin reduced time-dependent SWF. Especially 200 mg/kg R-CAR dose decreased SWF the most. However, SWF values of 100 mg/kg S-CAR dose were similar to the PEN group. Both doses (100 and 200 mg/kg) of R-CAR administered before penicillin decreased total SWF. The 100 mg/kg doses of S-CAR and R-CAR injected 30 minutes after penicillin administration decreased SWF.

In this study, 200 mg/kg dose of R-CAR administered before penicillin decreased SWA over time, while 100 mg/kg dose of S-CAR administered after penicillin decreased SWA. No comparison was made because there were no similar studies in the literature.

The induction of EA by penicillin is actively involved in cortical pyramidal cells. In the penicillin induced epilepsy, potentials relying on both GABAA and GABAB receptors play a role in the abrupt depolarization shifts observed in cells (27). Direct application of penicillin to the cortex inhibits GABA receptors, similar to the effect of bicuculline. Consequently, the suppressed GABA activity initiates EA that begins locally but progresses to generalize by disrupting the brain's inhibitory system. Research indicates that penicillin reduces intracellular Cl- influx by binding to subunits of GABAA receptors (28). Other studies have reported that penicillin binds to the chlorine receptor, preventing the channel from opening (29). Penicillin binds to the benzodiazepine binding site and causes convulsions (21). The primary target of penicillin is the β-subunit of the GABAA receptor, to which GABA binds. It is hypothesized that penicillin binds to the GABA binding site with a β-lactam ring, preventing GABA from binding to this site (30). While GABA levels in the brain were not investigated in this study, it has been demonstrated that CAR, administered at various doses, can reverse penicillin-induced EA. This suggests that CAR may act by increasing GABA levels in the penicillin-induced epilepsy model. It is conceivable that CAR not only affects GABAA receptors but also diminishes the release of excitatory neurotransmitters from excitatory neurons by acting on GABAB receptors. However, there is currently no literature available to corroborate this information.

CONCLUSION

In conclusion, carvone inhibited epileptiform activity, possibly by modulation of the GABAergic system. These results suggest that carvone may have both protective and therapeutic effects in the treatment of epilepsy. However, additional studies are required to determine its clinical use.

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Conflict of interest: The authors have no conflicts of interest to declare.

Ethical approval: Ethical approval for the study was obtained from the AIBU Animal Research Local Ethics Committee with code number 2013/08.

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