Published online 2020 August 31 **Original Article**

Protective Effects of *Biarum carduchrum* Ethyl Acetate Extract on Seizure Severity, Depression, Memory, and Learning in Pentylenetetrazole-Induced Kindling Rats

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Received 2020 April 23; **Revised** 2020 July 01; **Accepted** 2020 August 29.

Abstract

Background: Based on traditional beliefs, *Biarum carduchrum* (family Araceae) can strengthen the nervous system and prevent from sleep disorder and agitation.

Objectives: This study was performed to investigate the protective effects of the ethyl acetate extract of *Biarum carduchrum* leaves against pentylenetetrazol (PTZ)-induced seizure in rats.

Methods: The present experimental research was conducted on 50 male Wistar rats in Iran during 2019. The animals were examined in five groups of control, PTZ, intervention (PTZ with 100 and 200 mg/kg ethyl acetate extract for 10 days), diazepam (PTZ, 200 mg/kg extract, and diazepam). The research groups were compared in terms of behaviors. Furthermore, total phenol and flavonoid levels in the extract were determined using high-performance liquid chromatography.

Results: According to the results, the intervention and diazepam groups had a significantly lower number of the whole body, tonic, head, and upper organ seizures (P<0.05), and jumps (P<0.05), compared to the PTZ group. Both doses of the extract also significantly decreased immobility time in tail suspension and secondary latency time and significantly enhanced spatial memory in Morris water maze test (P<0.05). This treatment also significantly reduced the levels of nitric oxide and malondialdehyde in the brain and serum (P<0.05) and showed antioxidant activity (IC_{50}=200 µg/ml). The extract contained 42.63±0.7494 and 85.16±6.499 µg/mg phenol and flavonoid, respectively. The HPLC analysis also revealed the presence of quercetin (30 µg/g) in the extract.

Conclusion: Based on the results, *Biarum carduchrum* extract can be used for depression control and improvement of learning and memory impairments in seizure patients after complementary testing.

Keywords: Biarum carduchrum, Ethyl acetate extract, Learning, PTZ-kindled rats, Seizure

1. Background

Epilepsy is the most prevalent chronic and debilitating neurological disease that affects 0.5-1% of people globally. Despite the available treatments, 30% of patients still suffer from epileptic seizures. Although this disease might be controlled in about 60-70% of patients via antiepileptic drugs, the treated patients may suffer from the unwanted side effects of the drugs (1). The hippocampal system is one of the major areas of the brain involved in epilepsy. Moreover, the hippocampus plays an important role in learning process and memory, particularly in spatial memory, as lesions in the CA1 region of the hippocampus cause memory impairments (2). The frequent epileptic seizures markedly undermine memory and learning (3). It has been found that electrical and chemical kindling following the application of PTZ can cause learning impairment in laboratory animals (4-6).

The hippocampus is involved not only in memory and learning but also in seizure onset, progression, and termination. Brain neuronal degeneration, especially in the hippocampus CA1 area, alters the function of variable synapses that reserve the information, thereby inducing learning impairment as observed in folding the kindling (7). The PTZ has selective harmful effects on neuronal membrane characteristics since it blockades chloride channels by coupling with a gamma-aminobutyric acid A ($GABA_A$) receptor complex and reduces the neurotransmitter-induced chloride induction. This drug also affects potassium and calcium channels and releases intracellular calcium ion reserves. It is also applied as a chemical seizure inducer that is used for the evaluation of antiepileptic drugs in animal models (8). According to a study, PTZ elevates the concentration of intracellular calcium ion through increasing N-methyl-D-aspartate receptors. The increased calcium in the cell protects against the inhibitory effects caused by GABA (9).

Oxidative stress is a condition in which the balance between antioxidants and oxidants is interrupted as the consequence of inconsonance between the production and exhaustion of reactive oxygen and nitrogen species (ROS and RNS, respectively). This process is accompanied by the elevation of ROS and RNS levels and the reduction of biological nitric oxide (NO). The oxidative and nitrosylation stress pathways lead to inflammatory

responses and metabolic mitochondrial process, as well as the activation of free radical production (10). The ROS and RNS molecules (e.g., dismutase anion, hydroxyl radical, and proxy nitrites) play roles in the immune system and physiological conditions of the body. However, they react with fatty acids, proteins, and DNA in the pathologic conditions (accompanied by an increase in these factors) and cause cell damage. Oxidative stress and nitrosylation are involved especially in the diseases of the nervous system, such as ischemia, Alzheimer's, and epilepsy (11, 12).

Biarum carduchrum, belonging to Arecaceae family, is considered one of the most valuable herbs. This wild growing plant is found in the Zagros Mountains located in Fars and Kohgiluyeh-Boyer-Ahmad provinces, Iran, as well as in some areas of Iraq, Syria, and Turkey (13). The presence of flavonoids and anthocyanins in Araceae was first reported by Williams et al. (1981). Alkaloids, amines, saponins, cyanotic acids, and flavonoids are also found in the members of this family (14).

2. Objectives

The current research was aimed to determine the protective effects of the ethyl acetate extract of *Biarum carduchrum* leaves on PTZ-induced seizures in rats.

3. Methods

3.1. Materials

This experimental study was conducted in Kurdestan Province, Sanandaj, located in Iran, in 2019, using Wistar rats. In order to establish a chronic epileptic rat model, the animals were induced with epileptic kindling using PTZ (intraperitoneal [IP] injection of 35 mg/kg/day; CAS:54-95-5; Sigma, St. Louis, MO, USA) or diazepam (Caspian Tamin, Iran). Thiobarbituric acid (TBA) and sodium dodecyl sulfate (SDS) were kindly provided by the Saba Co., Ltd. (Sigma, USA). Before the experiments, PTZ and diazepam were dissolved in physiological saline. Drug administration was performed between 08:30 and 10:30 a.m. to mitigate the impact of circadian rhythms; in addition, all equipment was calibrated in this study.

3.2. Extract preparation

Biarum carduchrum was obtained from the local market of Izeh and confirmed by an herbalist (Dr. Rafieian). The reference sample was restored in the herbarium site of the Islamic Azad University of Izeh under the voucher No. 80145. For the purpose of the study, the leaves were washed and dried at 40°C under vacuum and then milled and sieved for extraction. In the next stage, they were added with ethyl acetate and restored for 72 h at room temperature. The extracts were then concentrated by filtration and rotation under vacuum at 40°C. Finally, they were dried at the same temperature in an oven under vacuum (15).

3.3. Measurement of total phenol compounds

The assessment of phenolic compounds was accomplished using the Folin Ciocalteu method. To this end, 0.5 mL of the extract before condensation and drying was diluted 10 times with 2.5 mL Folin Ciocalteu reagent and then well mixed by distilled water and 2 mL sodium carbonate solution (7.5%). The mixture was then inserted in a hot bath (45°C) for 15 min. Subsequently, the absorbance was read by a spectrophotometer at a wavelength of 760 nm. Methanol solution (80%), in addition to reagents without the extract, was used as the control sample. The calculated total phenol content (mg in each gram of the dried extract) was finally compared with a standard calibration curve established based on various concentrations of gallic acid (16).

3.4. Measurement of flavonoids compounds

In brief, 0.5 mL of the extract solution (0.01 g of dried extract per 10 mL of methanol 60%) was mixed with 0.5 mL of 2% aluminum chloride and 3 ml of 5% potassium acetate. After storing at room temperature for 40 min, the absorption of the mixture was determined as compared to that of distilled water at a wavelength of 415 nm. The absorbance value of the mixture was compared with the standard curve that was prepared based on the absorbance values of different concentrations of quercetin. In addition, the level of flavonoids in the extract was calculated as flavonoids content (mg) in each gram of dried extract (16).

3.5. Diphenyl-β-picrylhydrazyl radical scavenging activity

After the preparation of the extract at various concentrations in distilled water, 1 mL of each concentration was added at the equal amount of DPPH solution (0.1 mM, in 95% ethanol). The mixture was then stored for 15 min at room temperature. In the next stage, the mixture absorbance was determined at a wavelength of 517 nm using a spectrophotometer. In the control sample, distilled water (1 mL) was used instead of the extract. The scavenging activity (%) of diphenyl-β-picrylhydrazyl (DPPH) radicals was calculated using the following formula:

DPPH radical scavenging activity (%)=[(control absorbance – sample absorbance)/control absorbance]×100

Furthermore, IC_{50} amount was assessed by plotting the graph of concentration (X-axis) versus the inhibition percentage (Y-axis) (16).

3.6. High-performance liquid chromatography analysis

The HPLC analysis was conducted using the

chromatography apparatus, including Waters 2695 separations module and Waters PDA detector 996 (USA). The injection was performed by the autosampler injector equipment. A 150×4.6-mm column was utilized for chromatographic assay (Eurospher 100-5 C18 analytical column). In addition, elution was carried out using a gradient system by methanol (organic phase; solvent A) and distilled water (solvent B) at a flow rate of 1 mL/min. The peaks were monitored at the wavelengths ranging from 195 to 400 nm. The volume of 20 µL was injected, and the temperature was adjusted to 25°C. The data were analyzed using Millennium software (version 32).

3.7. Laboratory animals and grouping

The experiments were conducted at the Islamic Azad University of Sanandaj Kurdestan, Iran. Prior to the study, research approval was obtained from the Ethics Committee of the university. The animals investigated in this study included 50 male Wistar rats within a weight range of 150-200 g, supplied from the Center of Animal Breeding Facility in Pasteur Institute, Karaj, Iran. The animals were kept under appropriate temperature (21±2°C) and 12 h light/dark cycle with water and food ad libitum. For the purpose of the study, the animals were allocated into five groups, each containing 10 cases. In this regard, the control and negative control groups were respectively subjected to IP injection of normal saline and PTZ every 48 h for 10 days. Additionally, the positive control group was injected with PTZ every 48 h for 10 days and *Biarum carduchrum* ethyl acetate extract (200 mg/kg, daily), followed by diazepam (2 mg/kg) on the 10th day, about 30 min before PTZ injection. The intervention group was also administered with the IP injection of *Biarum carduchrum* ethyl acetate extract at the doses of 100 and 200 mg/kg on a daily basis, 30 min before PTZ injection performed every 48 h for 10 days. In order to establish the epilepsy model, PTZ (35 mg/kg) was administered for 9 days intraperitoneally every 48 h. On day 10, PTZ (60 mg/kg) was injected, and the severity and degree of seizure were recorded for 30 min. Finally, after obtaining blood samples from the rats under deep anesthesia, their brains were removed and restored at -80°C for the following biochemical tests.

3.8. Passive avoidance memory testing by shuttle box

The measurement of passive avoidance memory was accomplished using the shuttle box. This apparatus contains an electrical shocker, two chambers (one dark and one bright, connected by a guillotine door), and a 15-watt bulb. The floor of this device is composed of a conductive metal grid. The animals were subjected to this fear-based experiment over 4 consecutive days. The performed trials on the first 2 days were targeted toward training the rats and their acclimatization to the instrument. The first day of the experimentation was initiated by leaving the rat in the bright chamber for 5 min. After opening the door separating the light and dark chambers, the animal voluntarily departed to the dark side and left the place after 5 min. On day 3 of the experiment, the barrier between the two chambers was removed 20 sec later than the time the rat had previously left the bright chamber. At this stage, the latency of entering the dark chamber was calculated and regarded as initial latency (t1). After the rat entrance to the dark chamber, it was subjected to an electrical shock (1 mA/s); as a result, it left the device through only paddling. On the 4th day, the rat remained in the first chamber. After turning on the lamp and opening the door, the interval time between the entrance to the bright chamber and dark chamber (up to 300 sec) was calculated and recorded as delay through passing (t2) (17).

3.9. Tail suspension test

The tail suspension test was performed using metal bars with a height of 70 cm connecting through a 50-cm rope stretched longitudinally. The rat was hanged up from its tail, tied by a rope. The test was first begun by jerking the rat. The total experiment time duration was 6 min with the first 2 min being used for animal adaptation to the device. The immobility time (sec) was evaluated in the next 4 min by means of a chronometer. All measurements were investigated for each rat individually (16).

3.10. Spatial memory testing

The evaluation of learning, memory, and motor function of the rats was performed using the Moriss water maze. The device included a water pond (136-cm in diameter and 60 cm in height), which was filled up with water (20±1°C) up to a height of 25 cm. A 10-cm diameter Plexiglass platform was inserted in the center of the southwest quadrant, about 1 cm below the water surface. Each rat was trained four times a day for 4 days. On the 5th day, the test was performed one time without a platform (18).

3.11. Measurement of serum nitrite and nitrate

Nitrite and nitrate measurements were performed based on the rate of nitrate to nitrite reduction by cadmium and the Griess1 and Griess2 reagents (4).

3.12. Measurement of lipid peroxide levels in the serum and brain

In order to measure lipid peroxide level, 1.5 ml acetic acid (20%), 1.5 ml TBA (0.8%), and 200 µl SDS (8.1%) were mixed with 200 µl tissue homogenate or serum. The mixture volume was adjusted to 4 mL by distilled water and warmed 60 min in boiling water. After cooling, the reaction mixtures were added with 5 mL n-butanol/pyridine and 1 mL distilled water and then subjected to vigorous shaking. The obtained

solution was centrifuged for 10 min at 4,000 rpm, and the optical density of the supernatant was detected at a wavelength of 532 nm. A comparison was made between the estimated lipid peroxide levels and standard calibration curve (presented in µmol of MDA) (17).

3.13. Serum and brain total anti-oxidant capacity assay

After the completion of the behavioral tests, heart blood samples were obtained from anesthetized rats before the removal of their brain. Ferric reducing capacity was detected based on the serum ability to restore ferric ions in the presence of tripyridyl-s-triazine using the colorimetric method (17).

3.14. Statistical analysis

Data analysis was performed in SPSS software (version 20). The normality of data was tested using the Kolmogorov-Smirnov test. In addition, the comparison of the groups was established based on the ANOVA and Tukey's test. All data were presented as mean±SD, and the significance of differences was calculated at 95% confidence level. Given the function of standard deviation (measuring inter-sample variability), such a value is required for quantitative variables. The calculation of the sample size was performed using the freely downloadable G Power software (Faul, Erdfelder, Lang and Buchner, 2007). Based on this software, the sample size was determined as 10 animals per group. Considering 20% attrition, this size was divided by 0.8 as presented in the following formula:

Corrected sample size=sample size/(1−[% attrition/100])

4. Results

4.1. Phytochemical analysis of Biarum carduchrum ethyl acetate extract

The total levels of phenolic compounds and flavonoids in the dried extract were estimated at 42.63±0.7494 µg/mg and 85.16±6.499 mg/g, respectively. The results of HPLC analysis revealed the presence of quercetin in *Biarum carduchrum* ethyl acetate extract at a concentration of 30 µg/g (Figure 1). Furthermore, the IC_{50} level of the extract was obtained as 200 µg/mL based on the results of the DPPH inhibitory activity (Figure 1).

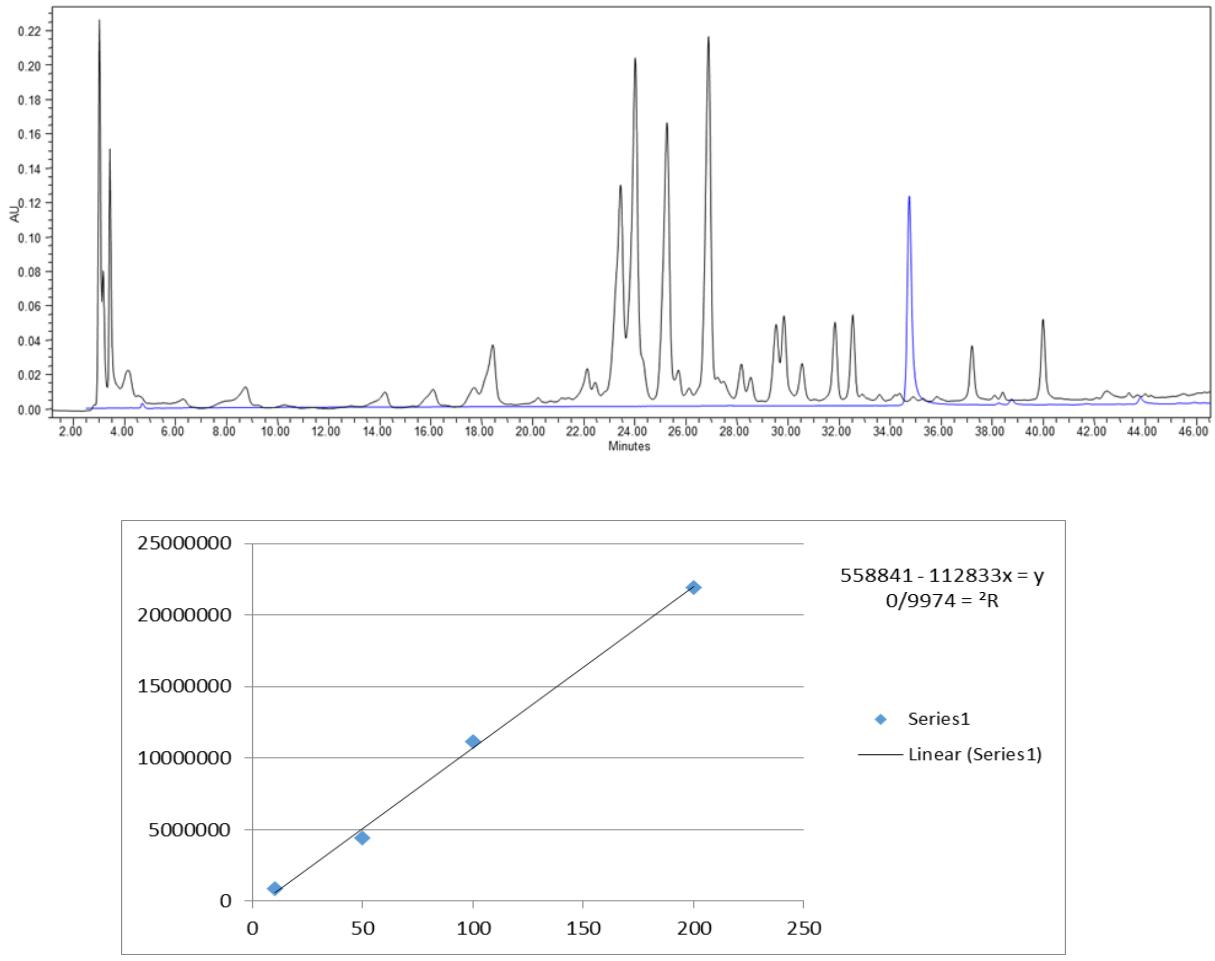


Figure 1. Results of high-performance liquid chromatography analysis and calibration of *Biarum carduchrum* extract

4.2. Behavioral tests

The frequencies of death rate, tonic, whole body, head, and upper organ seizures, head tick, and jumping and rotation in the research groups are depicted in Figure 2 (A-F). The treatment of rats with *Biarum carduchrum* ethyl acetate extract at the doses of 100 and 200 mg/kg led to a significant decrease in the rate of tonic, total body, head, and upper organ seizures, as well as the number of rotations and jumps, compared to those in the PTZ group. However, the numbers of tonic seizures, total body seizures, and rotation and jumping were significantly lower in the group receiving diazepam than in the PTZ group.

Based on the results, the number of head ticks underwent a significant decrease in the group injected with 100 mg/kg *Biarum carduchrum* ethyl acetate extract as compared with that in the PTZ group. According to the results of the tail suspension

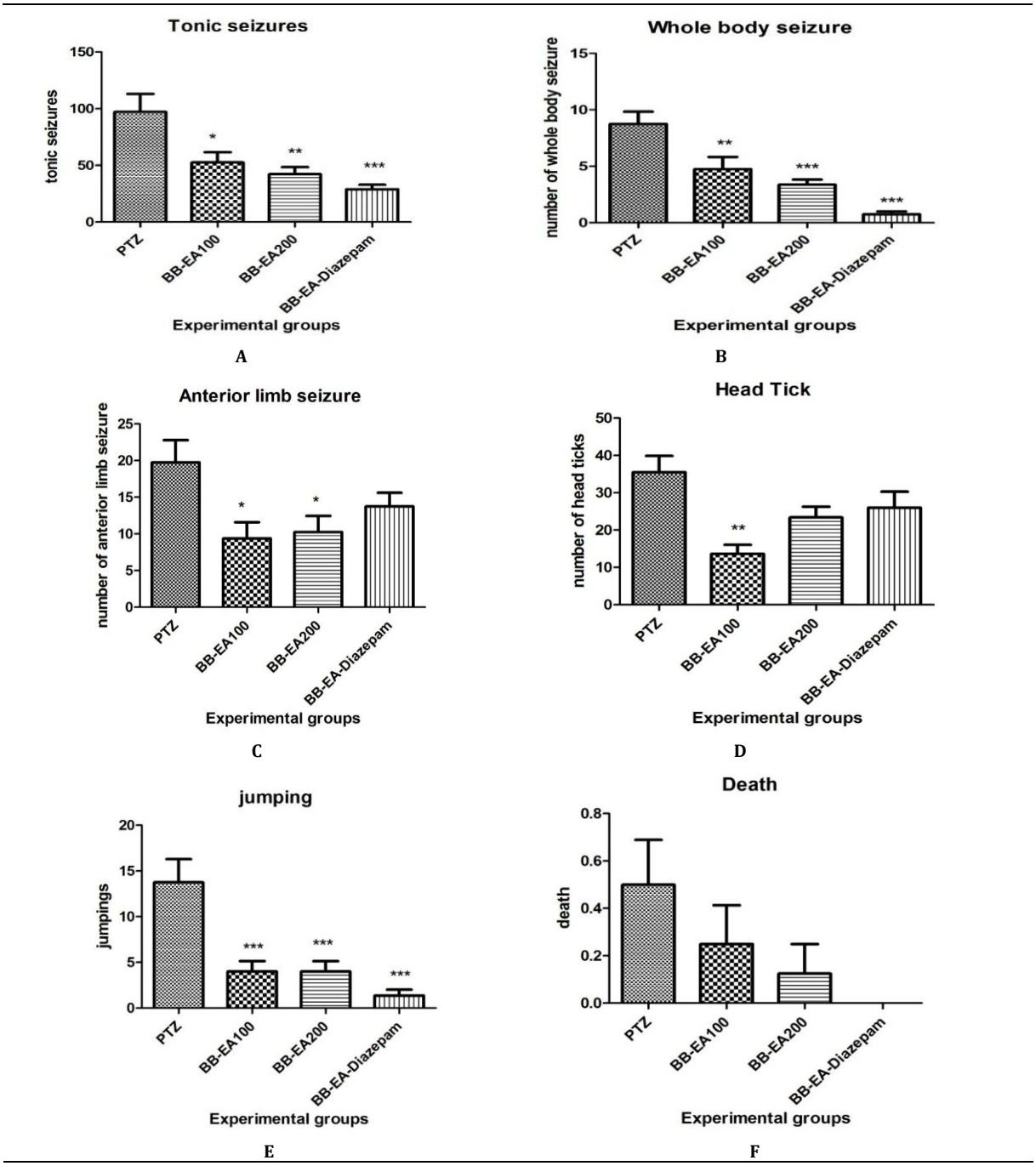


Figure 2. Comparison of the frequency of tonic seizures (A), total body seizures (B), seizures of the head and upper limbs (C), head tick (D), frequent rotation and jumping (E), and death (F) among the research groups (* shows a significant difference with the group administered with PTZ [*: P<0.05, **: P<0.01, and ***: P<0.001])
BB-EA=*Biarum carduchrum*-ethyl acetate

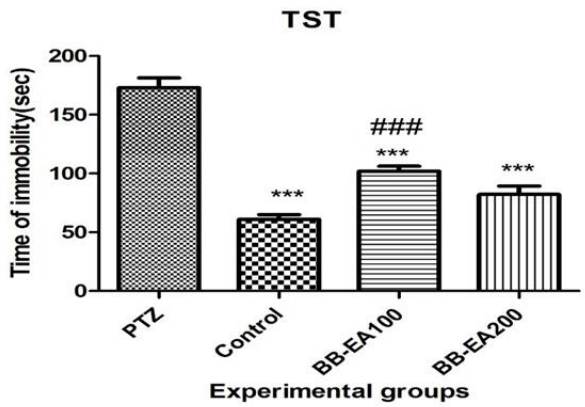


Figure 3. Comparison of research groups in terms of immobility time based on the tail suspension test (* shows a significant difference with the PTZ group [***: P<0.001]; # shows a significant difference with the control group [###: P<0.001])
BB-EA=*Biarum carduchrum*-ethyl acetate

test (Figure 3), the duration of immobility in the PTZ-receiving rats showed a significant increase, compared to that in the control group. Furthermore, the treatment of the rats with *Biarum carduchrum* ethyl acetate extract at the doses of 100 and 200 mg/kg resulted in a significant decrease in the duration of immobility, compared to the PTZ treatment.

Based on the results, the sequential injection of PTZ resulted in the significant inhibition of the secondary delay (t2) in the PTZ group in comparison to that in the control group. However, the group administered with the two doses (100 and 200 mg/kg) of *Biarum carduchrum* ethyl acetate extract showed a significant increase in secondary delay, compared to the PTZ group (Figure 4 A-B).

As the results indicated, the delay time in reaching the platform in the Morris water maze test was significantly higher during the 3rd and 4th days in the PTZ group than in the control group (Figure 5 A, B). On the first day, this delay time was significantly lower in the group injected with 200 mg/kg *Biarum carduchrum* extract than in the PTZ group. According to the results, the delay in reaching the platform significantly decreased in the groups receiving the doses of 100 and 200 mg/kg of *Biarum carduchrum* extract during the 2nd, 3rd, and 4th days in comparison to that in the PTZ group. Furthermore, during the 1st, 2nd, and 4th days, the frequency of swimming in the target quadrant was significantly higher in the group administered with 200 mg/kg dose of *Biarum carduchrum* extract than in the PTZ group.

According to the results of the Morris water maze test (Figure 6 A, B), on the examination day, the control group had a significantly longer swimming duration and higher swimming frequency in the target quartile than the PTZ group.

As illustrated in Figure 7 (A-D), the sequential infusion of PTZ into rats was associated with a significant elevation in the MDA level and a significant decrease in the brain and serum antioxidant capacity. However, the treatment of rats by 200 mg/kg of *Biarum carduchrum* extract resulted in a significant increase in the serum antioxidant capacity, compared to PTZ treatment. Moreover, the groups receiving the doses of 100 and 200 mg/kg of the extract showed a significant elevation in the antioxidant activity of the brain tissue and a significant decrease in the MDA levels of the serum and brain tissue.

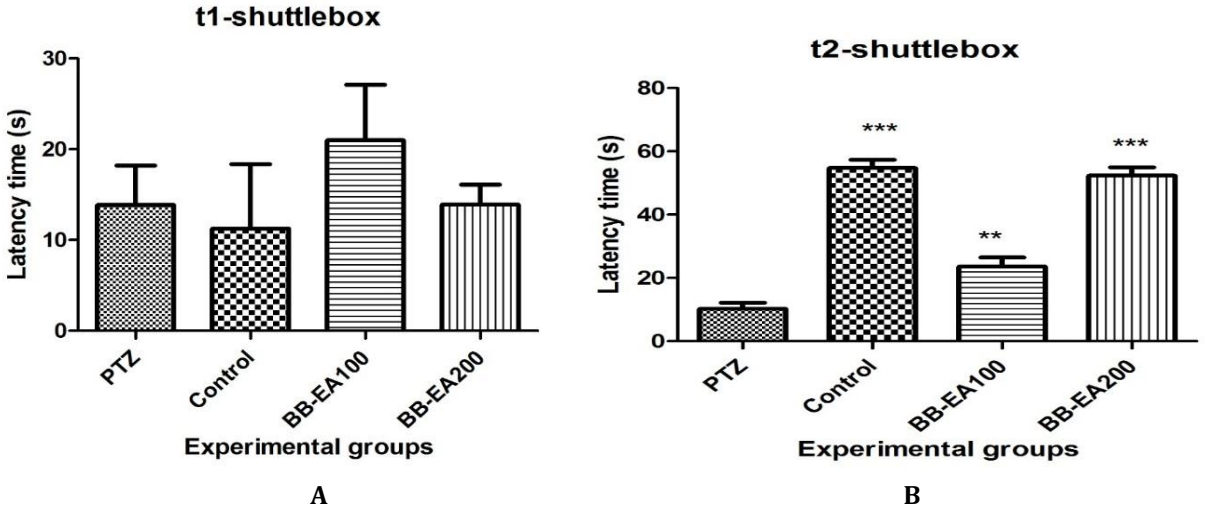
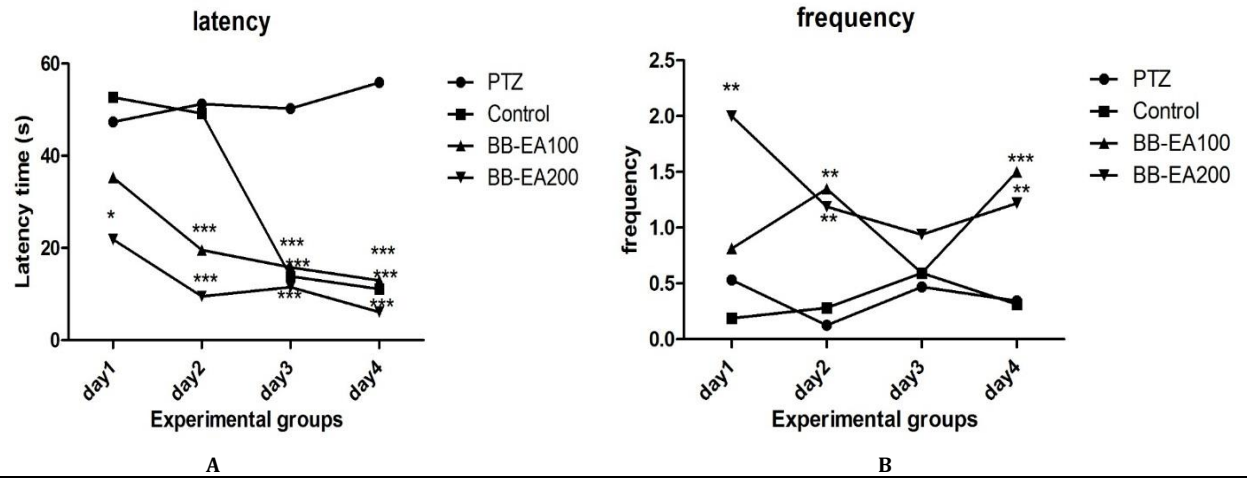
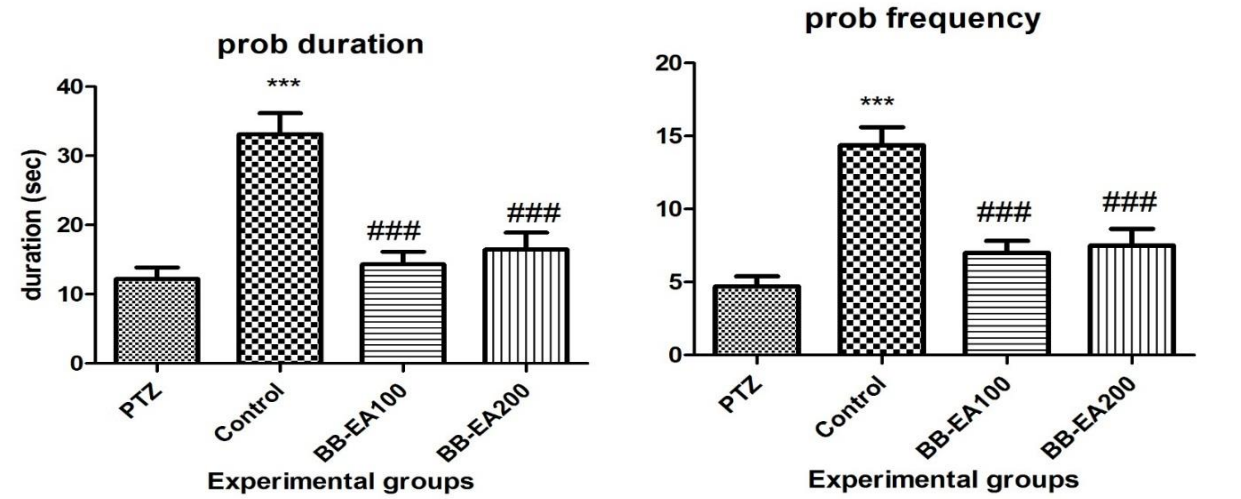


Figure 4. Comparison of secondary delay among the research groups based on the shuttle box test (* shows a significant difference with the PTZ group [**: P<0.01 and ***: P<0.001]).
BB-EA=*Biarum carduchrum*-ethyl acetate



A B

Figure 5 (A, B). Comparison of delay in reaching the hidden platform (A) and the swimming frequency in the target quartile (B) during the test days among the research groups (* indicates significant difference with the PTZ group [*: P<0.05, **: P<0.01, ***: P<0.001])
BB-EA=*Biarum carduchrum*-ethyl acetate



A B

Figure 6 (A, B). Comparison of swimming time (A) and swimming frequency in the target quartile (B) during the examination day among the research groups (* shows a significant difference with the PTZ group [***: P<0.001]. # shows a significant difference with the control group [###: P<0.001])
BB-EA=*Biarum carduchrum*-ethyl acetate

The results revealed no significant changes in the serum NO levels among the research groups (Figure 8 A, B). According to the results of this study, the PTZ groups had a significantly higher level of NO level in the brain tissue than the control group. In addition, the groups receiving 100 and 200 mg/kg of the extract showed significantly lower levels of NO in the brain tissue as compared to the PTZ group.

5. Discussion

The present research evaluated the protective impacts of *Biarum carduchrum* ethylacetate extract against PTZ-induced seizures in rats. The results revealed that the rats treated with 100 and 200 mg/kg doses of ethyl acetate extract had a significant decrease in the number of tonic seizure, total body seizures, seizures of the head and upper organs, and rotation and jumping. The highest anticonvulsant activity was observed in the seizure-induced rats administered with extract (200 mg/kg) and diazepam as a GABA agonist. It is proposed that the GABAergic system has an important role in the epilepsy process. The GABA is a main inhibitor neurotransmitter of the central nervous system, and more than 25% of the inhibitory neurons are GABAergic. The GABA plays a key role in the modulation of neuronal activity. This neurotransmitter applies its effects through various receptors. There are two types of GABA receptors, namely $GABA_A$ and $GABA_B$. The $GABA_A$ receptors are

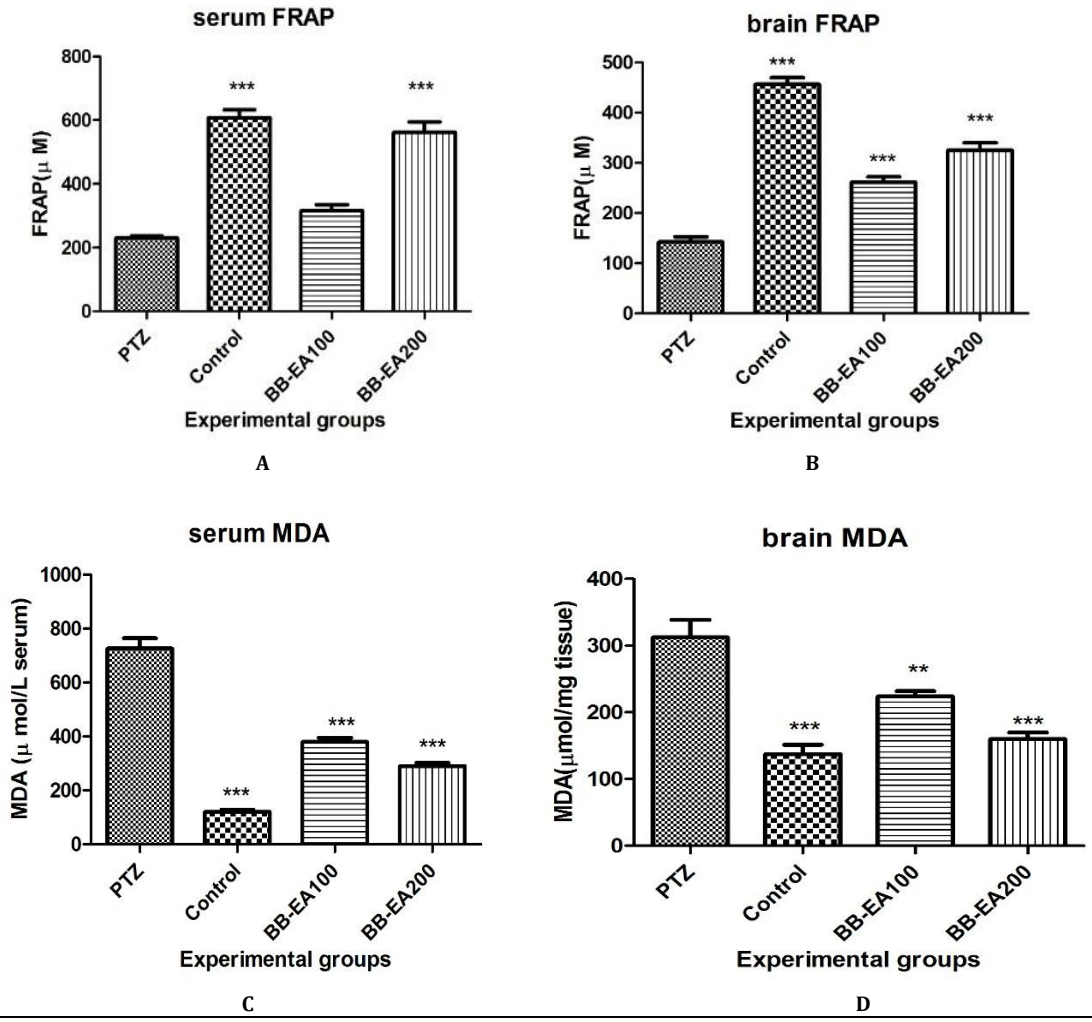


Figure 7. Comparison of antioxidant activity in the serum (A) and brain (B) and malondialdehyde levels of the serum (C) and brain (D) among the research groups (* shows a significant difference with the PTZ group (**: P<0.01 and ***: P<0.001)
BB-EA=*Biarum carduchrum*-ethyl acetate

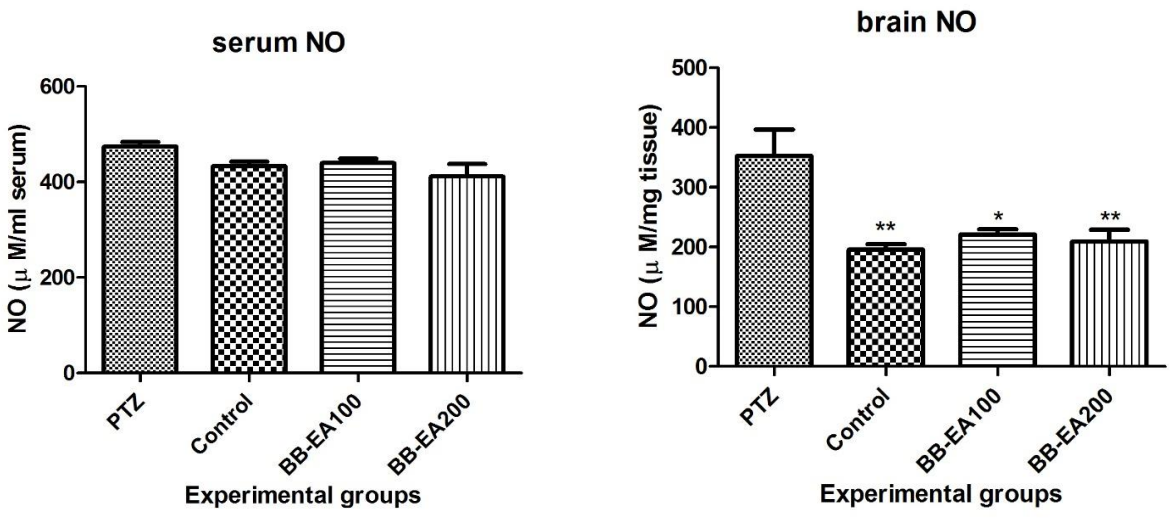


Figure 8. Comparison of nitric oxide levels in the serum (A) and brain (B) among the research groups (* indicates a significant difference with the PTZ group (**: P<0.01 and ***: P<0.05)
BB-EA=*Biarum carduchrum*-ethyl acetate

ionotropic and embed chlorine flow; on the other hand, the $GABA_B$ receptors change the function of the neurons by binding to and activating G proteins and the intracellular messenger system.

According to the literature, the drugs increasing the synaptic rates of GABA by blocking GABA catabolism or elevating GABA re-absorption are categorized among effective antiepileptics. Benzodiazepines are among these drugs that elevate the binding rate of GABA to its receptors, and result in the increase of the permeability of chloride channels. The results of HPLC analysis of *Biarum carduchrum* ethyl acetate extract were indicative of the high levels of quercetin flavonoid in the extract. Quercetin is a flavonoid that is found in different fruits and vegetables, including apple, citrus fruits, berries, onion, cereals, legumes, and tea. Several pharmacological properties, including antioxidant, anti-inflammatory, and hepatic protective effects, have been reported for quercetin.

In a study performed by Nassiri-Asl et al. (2013), quercetin at a dose of 50 mg/kg mitigated seizure severity and enhanced avoidance memory. In addition, pre-treatment with quercetin at the doses of 50 and 100 mg/kg inhibited the increasing amount of mRNA transcription for the production of GABAA receptor β1 and β3 subunits, 2 h after kainic acid injection (19). In terms of innovation, very few studies have investigated this plant and its anti-epileptic effects. One of the weaknesses of our research was the non-investigation of the molecular mechanism of this plant.

In the current research, diazepam strengthened the anticonvulsant effects of *Biarum carduchrum* extract, which could be ascribed to the positive effects of diazepam on the GABAergic system.

Recent studies indicated that oxidative stress and mitochondrial dysfunction can make the brain susceptible to epileptic seizures. On the other hand, seizure attacks lead to free radicals production and oxidative damage to the proteins, fats, and nucleic acids in the cells. Therefore, oxidative stress and the production of free radicals are currently known as the outcomes of seizure attacks (20). Reactive oxygen species, like superoxide radical, hydrogen peroxide, hydroxyl radical, and oxygen radical, are generated during normal cell metabolism. The physiological levels of ROS can be neutralized by enzymes, including superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and peroxiredoxin, or reduced by non-enzymatic molecules (e.g., vitamins C and E) and the antioxidant defense system (GSH).

However, excessive ROS production reduces the ability of the antioxidant defense system and leads to oxidative stress. Furthermore, additional ROS reacts with NO and results in the production of RNS, such as peroxynitrite (4). Seizure induction with PTZ can result in a significant decrease in the levels of GSH, GSSG, cysteine, and thiol proteins. In addition, there is a report regarding the elevation of carbonyl and D-sulfide proteins in the cerebral cortex of the mice (6). The seizure caused by acute PTZ significantly increased NO (five times) in the cerebral cortex (4). The adjustment of oxidative stress using herbal and chemical drugs is seen as a tool to reduce the damages caused by epilepsy and the onset of seizure.

In the present study, the results revealed a significant elevation in the peroxidation of lipids and NO production in the brain and a significant decrease in antioxidant capacity in the rats receiving PTZ injections. Moreover, the treatment of the seizure-induced rats with *Biarum carduchrum* ethyl acetate extract was found to enhance the brain antioxidant capacity and decrease the levels of NO and MDA in the brain. Regarding this, the *Biarum carduchrum* extract seems to show its protective effects against the damage caused by PTZ through dealing with oxidative damage to the nerve cells and strengthening the antioxidant defense.

Based on our results, *Biarum carduchrum* ethyl acetate extract had a high ability to neutralize DPPH radicals, indicating the powerful antioxidant activity of the extract. In addition, the evaluation of the total phenol and flavonoid levels of the *Biarum carduchrum* extract revealed high levels of these compounds. The results of Hosseini et al. (2014) showed that *Biarum carduchrum* extract had a higher capacity of inhibiting DPPH and reducing its activity than BHT and alpha-tocopherol, respectively (15). Moreover, the results of the mentioned study were suggestive of the increase of antioxidant activity with the elevation of the hydromethanol extract concentration, which is due to the entry of more phenolic compounds into the reaction environment. The increased phenolic compound concentration and hydroxyl group number in the reaction environment increase the possibility of free radical hydrogenation, thereby leading to the enhancement of antioxidant activity (15).

Cognitive disorders are mainly seen in patients with epilepsy, which itself can disrupt the cognitive processes. The common antiepileptic drugs also cause disruptions in the cognitive processes, which is associated with a significant decline in the quality of life. Epileptic seizures are accompanied by the destruction of nerve cells in the limbic areas, including CA3, CAL, hippocampus dentate gyrus, amygdala, and entorhinal cortex. The damage to the neural cells in the hippocampus can lead to memory and learning impairments. The hippocampus acts as a significant structure in memory processes, and damages to this area of the brain could lead to severe amnesia (21). According to the reports, the induction of epilepsy in rats by PTZ causes disruption in passive avoidance memory and reduces the secondary latency time of entering the dark room in the shuttle box test, which is consistent with our

results (5).

In our study, *Biarum carduchrum* extract significantly improved spatial memory and passive avoidance memory in the rats receiving PTZ. It can be concluded that *Biarum carduchrum* extract prevents memory destruction by preventing damage to the tissues involved in the memory and learning processes. Quercetin as the major flavonoid component of *Biarum carduchrum* ethyl acetate extract can improve learning and spatial memory by reducing oxidative stress and increasing GSH in the mice (22). In addition, a single dose of quercetin injected 1 h before receiving scopolamine was reported to improve memory and learning in zebrafish (23).

Based on the evidence, patients with epilepsy have a significantly higher prevalence of depression than the general population. Accordingly, depression is one of the serious medical and social problems in epileptic patients, which affects the quality of life in these patients. In a study, depression was reported to affect almost half of the patients treated in the epilepsy centers (24).

The administration of *Biarum carduchrum* extract significantly decreased the immobilization time in the tail suspension test. Pre-treatment with quercetin as a bioflavonoid has increased the time of social interaction and reduced the immobility time in mice, indicating its anti-anxiety and anti-depressant effects (25). Quercetin also dose-dependently shortened the duration of immobilization in the forced swim test in diabetic mice (26). According to these studies, the antidepressant effects of *Biarum carduchrum* could be due to its high quercetin content.

6. Conclusion

The results of this study revealed that the administration of *Biarum carduchrum* extract to PTZ-treated rats significantly decreases the frequency of seizures in the entire body, as well as repeated rotation and jumping. Additionally, the results of the shuttle box test indicated a significant increase in passive avoidance memory and spatial memory in the treated rats. The results of the tail suspension test were also indicative of the shortening of the immobilization time in this group. Therefore, it can be stated that the protective effects of *Biarum carduchrum* extract against PTZ-induced seizure are probably obtained by the modulation of GABA receptors and reduction of the brain oxidative stress markers.

Acknowledgements

Herein, the authors of the current study extend their gratitude to the staff of the Laboratory of Islamic Azad University of Sanandaj, Iran, for their technical support. The present research was derived from a thesis submitted in partial fulfillment of the requirement for a Ph.D. degree.

Footnotes

Authors' Contribution: Youness Teymorivand conceived and extracted the data, Zahra Hooshmandi performed the study design, data analysis, and manuscript drafting. Mahbubeh Setorki carried out the paper revision with complete access to all research data, and Sabrieh Amini checked the integrity of the data and the accuracy of data analysis, therefore acting as a guarantor.

Conflict of Interests: The authors declare that there is no conflict of interest.

Ethical Approval: The research protocol was approved in 2019 by the Ethics Committee of Sanandaj Branch, Islamic Azad University, Sanandaj, Iran (Code number: (110483732395782162273123).

Funding/Support: The authors have received no financial support from any agency for this study.

Informed consent: Not applicable.

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