Research Journal of Pharmacognosy (RJP) 7(2), 2020: 13-20
Received: 14 Dec 2019
Accepted: 18 Feb 2020
Published online: 27 Feb 2020
DOI: 10.22127/rjp.2020.211810.1541

Original article

The Anxiolytic and Antidepressant Effects of *Tanacetum polycephalum* in the Pentylenetetrazole Kindled Rats

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Abstract

Background and objective: Epilepsy is one the most prevalent neurological illnesses which affects behavioral statuses like anxiety, depression and balance. Based on the anti-inflammatory and antioxidant properties of *Tanacetum polycephalum*, its effect on anxiety and depression caused by pentylenetetrazole (PTZ)-kindling in rats was investigated. **Methods:** In order to prepare the required extract, aerial part of *Tanacetum polycephalum* was powdered (100 g) and macerated in 1 L of ethanol (80%) for 24 h. Twenty-four rats were randomly assigned to four groups: PTZ (sub-threshold dose 35 mg/kg for one month, intraperitoneal (i.p)), PTZ + phenobarbital (30 mg/kg, i.p), PTZ + plant extract (300 mg/kg, i.p), and PTZ + plant extract (600 mg/kg, i.p). Elevated plus maze (EPM), forced swim test (FST) and rotarod test were employed to assess the anxiety parameters, antidepressant and balance potential, respectively. **Results:** *Tanacetum polycephalum* extract administered at the doses of 300, and 600 mg/kg, exert antidepressant-like activity in the FST test and reduced the immobility time. In the EPM test, the extract at the same doses produced anxiolytic-like effects. Also, the rats which received the extract showed a significant improvement in the rotarod test in contrast to the PTZ group. **Conclusion:** The findings from current study showed that *Tanacetum polycephalum* could ameliorate neurobehavioral parameters of anxiety and depression in the PTZ-kindled rats.

Keywords: anxiety; depression; epilepsy; pentylenetetrazole; *Tanacetum polycephalum*

Citation: Azizi V, Allahyari F, Rezaali F, Hosseini A. The anxiolytic and antidepressant effect of *Tanacetum polycephalum* in the pentylenetetrazole kindled rats. Res J Pharmacogn. 2020; 7(2): 13-20.

Introduction

One of the most prevalent chronic neurological diseases is epilepsy which is associated with reversible seizures and can affect the patients both psychologically and behaviorally. Nowadays, antiepileptic drugs (AEDs) such as barbiturates and a sundry of sedatives are adopted to ward off or treat epilepsy. Although in recent decades, myriad drugs have been introduced for the treatment of epilepsy, they continue to show adverse and even deleterious effects that in most cases even with a sufficient dosage, fail to properly stave off the seizures; therefore, they would pose a great negative impact on one's quality of life both psychologically and behaviorally [1,2]. In effect, to discover and develop sufficient AEDs have been a desideratum. Kindling has been posited as an efficacious model for the clinical facets of epilepsy at biochemical, electrophysiological and behavioral levels [3]. Also, one of the common approaches in the discovery and development of AEDs - which is the mainstay for the treatment of patients with

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seizures and epilepsy - is the utilization of natural substances with medicinal properties [4].

One of the herbs that has evinced beneficial medicinal properties is *Tanacetum polycephalum*, which grows in several areas of the Northern Hemisphere, including Iran [5,6]. Previous studies have listed many therapeutic properties and benefits for *T. polycephalum* running the gamut from being anti-allergic, anti-irritant, anticancer, antiseptic, analgesic and antihypertensive to being anti-inflammatory and antioxidant due to the presence of sesquiterpene lactones and volatile oils [5-9]. Moreover, it has recently been documented that the plant has anticonvulsant and neuroprotective properties in experimental animal models [10].

Given the behavioral manifestations disorder associated with epilepsy and the effects of *T. polycephalum* on behavioral disorders, it was hypothesized that the *T. polycephalum* extract would have positive effect on the behavioral parameters of stress, depression, and balance in pentylenetetrazole (PTZ)-kindled rats. The aim of this study, therefore, was to evaluate the effects on behavioral disorders (i.e. stress, depression and balance) resulted from epilepsy and seizures in adult male rats.

Materials and Methods

Ethical considerations

All experiments pertaining to animal rights and conservation in this study were in accordance with the standard ethical guidelines (NIH, publication no. 85-23, revised 1985; European Communities Directive 2010/63/EU) and were approved by Local Ethics Committee at the Shahid Beheshti University (ethical code: IR.SBU.97.154).

Chemicals

Pentylenetetrazole from Sigma Company (USA), phenobarbital sodium from Chemidarou Pharmaceutical Company (Iran), ketamine and xylazine from Alfasan Company (the Netherlands) were obtained. PTZ was prepared as a 1% v/w solution in saline. Phenobarbital sodium was used in this study to make a comparison with a conventional anticonvulsant drug. Phenobarbital sodium was also dissolved in the physiological saline solution and injected intraperitoneally (i.p) in a dose of 30 mg/kg of rat weight to the animals [10].

Plant material

Tanacetum polycephalum was obtained in 2018 from the Institute of Medicinal Plants of Shahid Beheshti University (Voucher no. MPH-1212), Tehran, Iran. The extract was prepared according to the references and previous work [10]. About 100 g of the aerial part of the plant was prepared, cleaned, dried in the shade, and powdered by mechanical shredder. To prepare the hydroalcoholic extract, the plant powder was soaked in 1 L of ethanol 80% for 24 h; then, the resulting mixture was filtered and concentrated in vacuum at 45 °C using a rotary apparatus (EYELA, Japan**).** The resultant extract was dried and stored in the refrigerator at 4 °C until the experiment.

Animals

This experiment was conducted at Shahid Beheshti University (Tehran, Iran) on adult Wistar rats (200±20 g, 8 weeks old) purchased from Shahid Beheshti University of Medical Sciences (Tehran, Iran). Animals were kept for 1 week in a room with constant conditions (12 h light/dark period with lighting starting at 7 a.m., 22±2 °C, 55 ± 5% relative humidity) in standard cages made from polycarbonate in order to adapt to the new environment. The animals had free access to water and special feed for rats (Pars animal feed, Iran) throughout the experiment. Animals were randomly assigned to experimental groups (each group containing 6 rats) after one week of adaptation to the laboratory conditions. Each animal was used only once during the experiment, and efforts were made to reduce the animal suffering and at the same time to obtain reliable scientific data. All experiments were carried out between 09:00 and 15:00.

Experimental design and grouping

The animals were randomly divided into four groups of six: (1) negative control group receiving normal saline (0.3 mL per rat), (2) positive control group receiving phenobarbital (30 mgkg), (3 and 4) groups receiving *Tanacetum polycephalum* extract (300 and 600 mg/kg, respectively). *Tanacetum polycephalum* extract was continued in normal saline solution in doses of 300 and 600 mg/kg with PTZ during the experiment until complete animal kindling. Thirty min after vehicle injection, PB and TPE (300 and 600 mg/kg) animals were challenged with the sub-threshold dose of PTZ (35 mgkg of body weight). All injections were administered in the form of fresh

solutions in a constant volume of 0.3 mL per rat throughout the study [10].

PTZ-induced kindling test

In this study, the experimenter was unaware of which animal belonged to which group. Pentylenetetrazole was injected in a sub-threshold dose of 35 mg/kg with 24 h interval time for a period of one month. In order to record and measure seizure behavior, the animals were transferred individually to transparent plastic boxes and were immediately observed for 1 h after PTZ injection and recorded by a computer-connected camera. Seizure threshold was measured on a 6-step scale (table 1). The animals were considered kindled if they showed stages 4 or 5 in two consecutive trials. The animals were given the PTZ challenge (70 mg/kg) 7 days after the kindling development. Meanwhile, they underwent anxiety, depression and rotarod test. The rate of anxiety, depression and rotarod were evaluated after the PTZ challenge [11].

Table 1. Modified Racine's scale for pentylenetetrazole (PTZ) induced seizure in rats

Stage	Seizure intensity
0	No behavioral changes
1	Ear and facial twitching
2	Nodding of the head, head clonus and myoclonic jerks
3	Unilateral forelimb clonus with lorditic posture
4	Bilateral forelimb clonus with rearing and falling
5	Generalized tonic-clonic seizure (GTCS) with loss of postural tone, usually causing death

Elevated plus maze (EPM) Test

After PTZ challenge, EPM test was used to determine the anxiety-like behavior in rats. The EPM consists of two open arms without walls (50×10 cm) and two enclosed arms with high walls (50×10×40 cm) extending from a common central platform (10×10 cm). Each rat was individually placed in the center of the maze, its head facing an open arm and was allowed for five min of free exploration. All sessions were videotaped. After each test, the floor was cleaned with ethanol (10%) and dried. Measurements were made from the frequencies of total open and closed arm entries (arm entry = all four paws into an arm) and the time spent in open, closed and central parts of the maze. The latency to open arm entries as the standard index of anxiety-like behaviors were calculated [12].

Forced swimming test (FST)

The forced-swim test was performed according to standard published procedures with minor modifications. The rats were placed in a glass cylinder (45 cm diameter and 50 cm high; Borj Sanat Azma Co.) filled to a depth of 30 cm with water (23 °C). A 6 min test session was conducted and videotaped. The immobility time during the last 5 min of a 6-min swim test was defined as the absence of active/escape directed movements. After the test, the animals were removed from water, dried with a towel then carried back to their home cages [13].

Rotarod test

The rotarod test (49 cm diameter and 45 cm width; Borj Sanat Azma Co.) is a widely used test to measure coordinated motor skills. It requires animals to balance and walk on a rotating cylinder. The rotarod unit consisted of a rotating rod, which was divided into four parts by compartmentalization, which allowed examining four rats at a time. When the rats fell down from rotating rod, the time automatically stopped. The rotating speed of rotarod was constant (15 rpm). After training, the time for each rat to remain on the rotating rod (rotarod latency) was recorded for three trials at 30 min intervals. The maximum time for each trial was 90 s. The rotarod latency was directly dependent on the movement and balance skill of the animal. Twice daily training for two consecutive days was done before the test day [14].

Statistical analysis

All statistical analyses were run using the SPSS software (version 26, SPSS Inc., Chicago, IL). All behavioral tests were expressed as mean ± standard error from the mean (SEM). The Kolmogorov-Smirnov test was used to show the normality of the data distribution. One-way ANOVA was employed to compare the means of the data. The Tukey post hoc test was used where data were significant to compare the groups by pairs. The significance level was considered $p < 0.05$ for all the study groups. Graphs were created using the GraphPad Prism software.

Results and Discussion

Analysis of variance showed a significant increase in the mean of seizure threshold [$F(3,20) = 32.02$, $p<0.001$] between the experimental groups. Further analysis with Post *hoc* test showed a significant increase ($p<0.001$) in the seizure threshold mean in the *Tanacetum polycephalum* extract treatment group in the doses of 300 and 600 mg/kg compared to PTZ. Also, a significant

increase in the mean of the seizure threshold was observed in the phenobarbital treated group in comparison with PTZ (p<0.001) (figure 1).

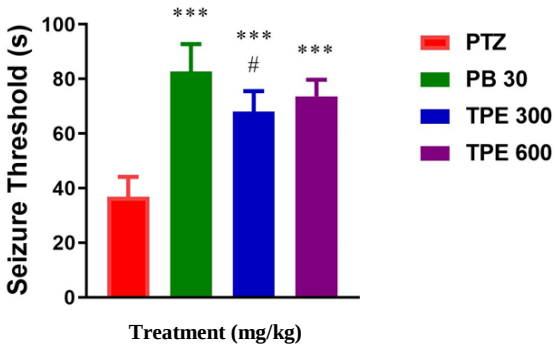

Figure 1. Effect of *Tanacetum polycephalum* extract on seizure threshold in PTZ-induced kindled rats. Data represent mean±SEM. Each group consisted of n = 6 rat. *** *p*<0.001, as compared to PTZ group; # *p*<0.05, as compared to PB 30 group; TPE: *Tanacetum polycephalum* extract; PB: phenobarbital sodium

As shown in figure 2. A-E, and confirmed by analysis of variance, there were a significant differences between the experimental groups with respect to the time spent on open armed indexes [F (3,20) = 16.61, p<0.001], the time spent on close armed [F (3, 20) = 27.47, p<0.001], the number of open armed entries [F (3,20) = 15.63, p <0.001], number of close armed entries [F (3,20) = 37.48, p <0.001] and latency to enter open armed [F (3,20) = 12.20, p <0.001] indices in the EPM test.

The Tukey post hoc test indicated that the phenobarbital receiving group caused a significant increase in the mean of the time spent on open armed (p <0.001; figure 2. A) and the number of open armed entries (p <0.001; figure 2. C) and a significant decrease in the time spent on close armed (p <0.001; figure 2. B), number of close armed entries (p <0.001; figure 2. D), and latency to enter open armed (p <0.001; figure 2. E) indices in comparison to the PTZ group. There was also a significant increase in the group receiving *Tanacetum polycephalum* extract in a dose of 300 and 600 compared to the PTZ group in the time spent on open armed (p <0.01 and p <0.001, respectively; figure 2. A) and the number of open armed entries (p <0.01 and p <0.001, respectively; figure 2. C) and a significant decrease in the time spent on close armed (p <0.001; figure 2. B), number of close armed entries (p <0.001; figure 2. D), and latency to enter open armed (p <0.01; figure 2. E) indices.

Significant differences were observed in the mean of immobility [F (3,20) = 88.64, p<0.001] and swimming [F (3,20) = 25.75, p<0.001] factors between the different groups. The post hoc analysis suggested that the immobility time mean was significantly increased in the plant-treated groups in doses of 300 and 600 compared to the PTZ group (p<0.001) (figure 3. A). Also, there was a significant increase in the mean of swimming time in the *Tanacetum polycephalum* extract group in doses of 300 and 600 (p <0.01 and p <0.001, respectively; figure 3. B) compared to the PTZ group.

Analysis of variance showed a significant difference in the mean of latency to fall in rotarod test between the different groups in 30 min [F (3,20) = 19.55, p<0.001], 60 min [F (3,20) = 35.50, P<0.001] and 90 min [F (3,20) = 52.52, p<0.001] after treatments. The mean latency to fall in the *Tanacetum polycephalum* extract group in 300 and 600 doses at 30 min (p<0.01 and p<0.001, respectively), 60 min (p<0.001) and 90 min (p<0.001) after treatments was significantly higher than that of the PTZ group (figure 4).

The present study for the first time revealed the positive effects of *Tanacetum polycephalum* extract on stress and depression in the experimental model of kindling induced by PTZ. The data showed that pretreatment with the plant extract raised seizure threshold in PTZ-induced kindling. Besides, the findings revealed a significant decrease in epileptic stress and depression in pre-treated *Tanacetum polycephalum* extract rats. In the present study, as the results revealed, TPE at doses of 300 and 600 mg/kg significantly elevated seizure threshold in kindled rats vis-à-vis the control group. In line with this study, Allahyari et al. observed that *T. polycephalum* exhibited neuroprotective and anticonvulsant properties in Wistar rats in the PTZ-induced seizure model. They have also documented that the dose of 600 mg/kg of the extract showed the greatest effect, which is in harmony with the present study [10].

The EPM and FST tests were adopted to evaluate the anti-anxiety and antidepressant properties. The results of the study demonstrated that doses of 300 and 600 mg/kg *T. polycephalum* extract increased the frequency and duration of entry into open arms and reduced it in the closed arms compared to the control group.

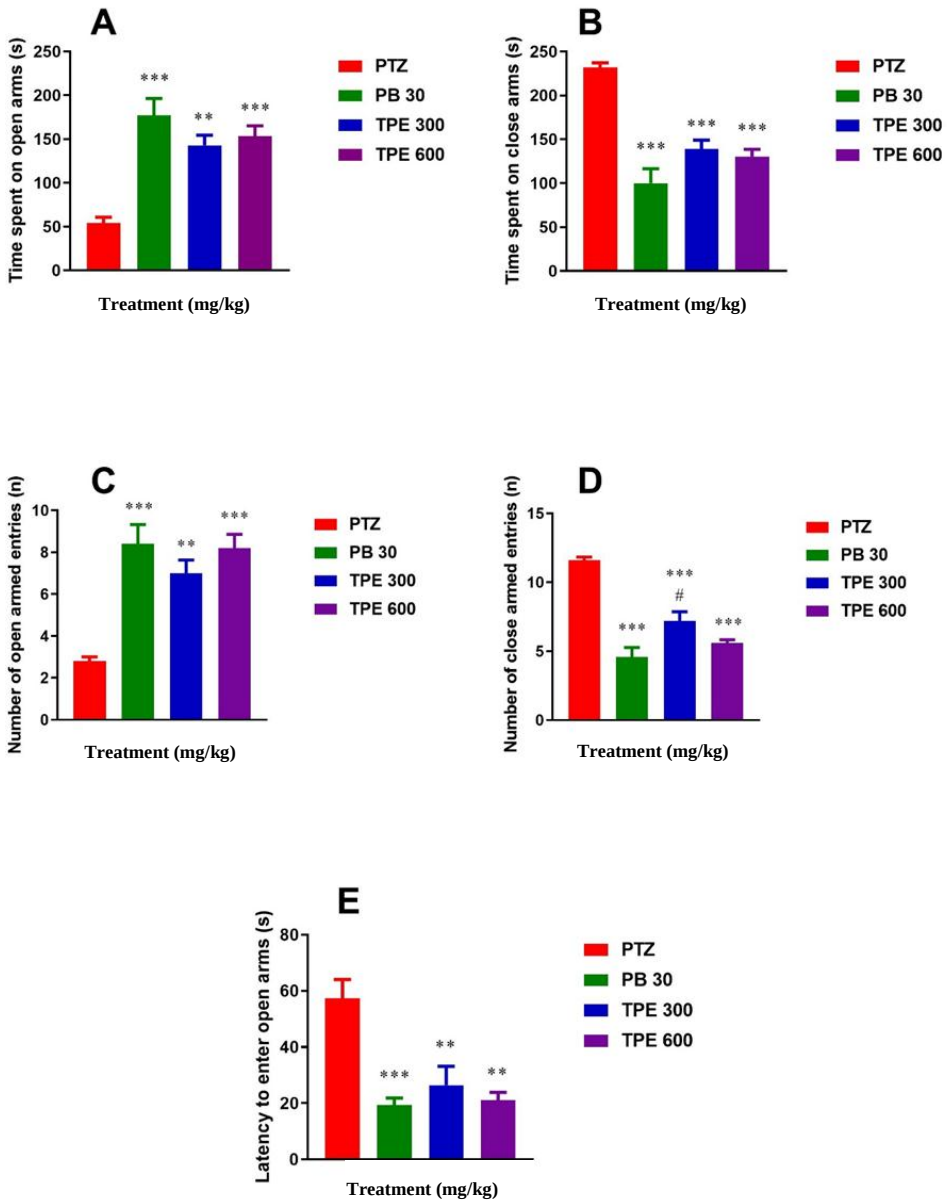

Figure 2. Effect of *Tanacetum polycephalum* extract on (**A**) time spent on open arms, (**B**) time spent on close arms, (**C**) number of open arms entries, (**D**) number of close arms entries, (**E**) latency to enter open arms in PTZ-induced kindled rats. Each column and bar represent mean ± SEM. Each group consisted of n=6 rat. ** $p<0.01$, *** $p<0.001$, as compared to PTZ group; # $p<0.05$, as compared to PB 30 group; TPE: *Tanacetum polycephalum* extract; PB: phenobarbital sodium

It also significantly reduced the delay in open arm entry in the EPM test, indicating a decrease in anxiety in plant extract-receiving rats. Furthermore, the *T. polycephalum* extract treatment significantly reduced the period of non-movement and significantly increased the time of swimming compared to the control group in the FST-treated rats. In line with our results, in a recent study on *T. parthenium*, it has been observed that *T. parthenium* showed anti-anxiety and antidepressant properties in mice; in this study, it was also shown that *T. parthenium*, having flavonoids such as apigenin, could interact with benzodiazepine receptors, including gamma-amino butyric acid (GABA) receptors and increase its activity [15]. Another study also clearly demonstrated the presence of apigenin with benzodiazepine-GABA activity from *T. parthenium* [16]. Studies on the active chemical compounds present in *T. polycephalum* extract have demonstrated that this plant, like *T. parthenium*, contains high levels of flavonoids,

including apigenin and luteolin [17]. Although PTZ-induced seizures are one of the common models for seizure studies, the mechanisms by which PTZ leads to seizures are not entirely known. It is widely accepted, however, that one of the mechanisms of PTZ seizures is the interaction with the chloride ion channel and its control in the GABA complex type A [18].

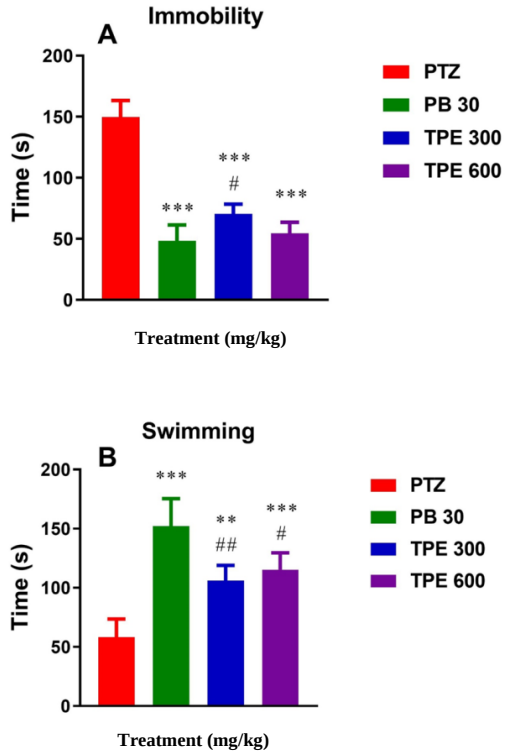

Figure 3. Effect of *Tanacetum polycephalum* extract on (**A**) immobility, (**B**) swimming in PTZ-induced kindled rats. Data represents mean ± SEM. Each group consisted of n= 6 rat; ** p<0.01, *** p<0.001, as compared to PTZ group; # P<0.05, ## P<0.01, as compared to PB 30 group; TPE: *Tanacetum polycephalum* extract; PB: phenobarbital sodium

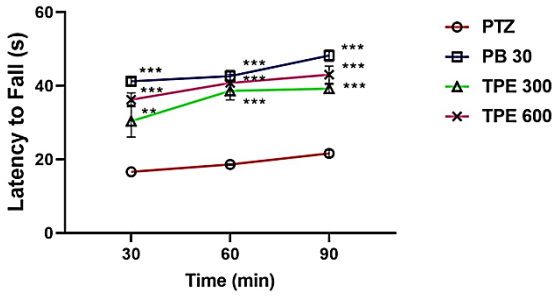

Figure 4. Effect of *Tanacetum polycephalum* extract on latency to fall in rotarod test in PTZ-induced kindled rats. Data represents mean±SEM. Each group consist of n = 6 rat. ** p<0.01, *** p<0.001, as compared to PTZ group; TPE: *Tanacetum polycephalum* extract; PB: phenobarbital sodium

Therefore, at least in part, the anticonvulsant properties and consequently the anti-anxiety and antidepressant properties observed in this study may be attributed to the presence of flavonoids in *T. polycephalum* extract, especially apigenin, competition with PTZ on the level of active position of GABA receptors and the increase in the inhibitory activity in the central nervous system.

Increase in the glutamate release and cellular excitability followed by increased intracellular calcium levels are other pivotal PTZ mechanisms in the pathology of epilepsy and seizures that lead to cell death [19,20]. Recent studies have documented that apigenin and luteolin, which are natural flavonoids in many plants, inhibit the release of glutamate in the hippocampus of rats by reducing calcium entry through blocking calcium channels [21,22]. As noted above, *T. polycephalum* extract has numerous flavonoids, including apigenin and luteolin [17]; therefore, at least partially, the antidepressant and anti-anxiety properties observed in this study may be due to the presence of flavonoids present in the extract, especially apigenin and luteolin as well as the control of glutamate release from the synaptic terminal.

In this study, to measure the motor activity, Rotarod performance test was used. As the results show, *T. polycephalum* extract at the doses of 300 and 600 mg/kg showed the highest anticonvulsant effect, while this effect rate was also evident in the locomotor activity of animals treated with the extract and was significantly increased compared to the control group.

Overall, the results of this study indicated that *T. polycephalum* extract showed anticonvulsant properties in PTZ-kindled rats and it increased seizure threshold in the groups receiving the extract. Moreover, given the improvement of seizure symptoms, it was observed that the behavioral markers of the rats (such as anxiety, depression and movement) receiving the extract was improved compared to the PTZ group.

Acknowledgments

This paper was supported by the Shahid Beheshti University, Tehran, Iran.

Author contributions

Vahid Azizi, Farzin Allahyari, Farnoosh Rezaali

and Abdolkarim Hosseini participated in study design, data collection and evaluation, drafting and statistical analysis; Vahid Azizi, and Abdolkarim Hosseini contributed extensively in interpretation of the data and the conclusion; Farzin Allahyari, and Farnoosh Rezaali; conducted experiments and analyses. All authors performed editing and approving the final version of the paper and also participated in the finalization of the manuscript and approved the final draft.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

AEDs: antiepileptic drugs; PTZ: pentylenetetrazole; i.p: intraperitoneal; EPM: elevated plus-maze; FST: forced swimming test; GABA: gamma-amino butyric acid