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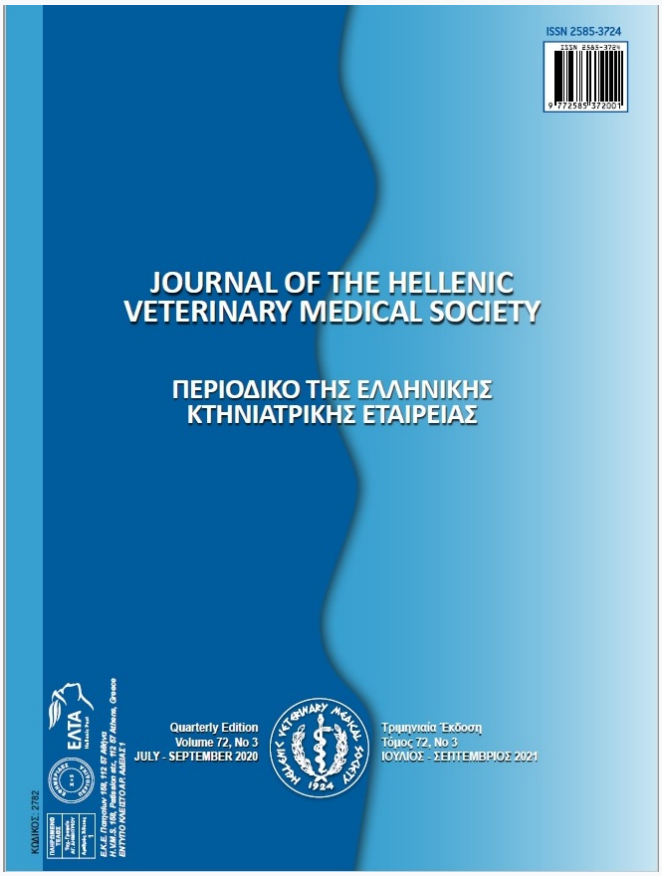

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V AZIZI, F ALLAHYARI, A HOSSEINI

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Research article
Ερευνητικό άρθρο

The anxiolytic and antidepressant effect of *Buxus hyrcana* in the pentylenetetrazole kindled rat

V. Azizi, F. Allahyari, A. Hosseini*

Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

ABSTRACT: Pentylenetetrazole (PTZ) is a chemical substance which largely used for induction of seizure and epilepsy in the animal model, and it can also, disrupts free radicals balance and causes oxidative stress in the body with a negative impact on behavioral statuses like anxiety and depression. In this study, the medicinal plant *Buxus hyrcana*, was used to evaluate its effect on oxidative stress, anxiety and depression caused by PTZ in the rat. Twenty-four male rats were randomly allocated to 4 groups: control negative under treatment with PTZ (sub-threshold dose 35 mg/kg for one month), control positive under treatment with phenobarbital (PB-30 mg/kg), and two PTZ groups under treatment with *B. hyrcana* extract (BHE-300, and -600 mg/kg). For anxiety parameters, the elevated plus maze (EPM) was used. The forced swim test (FST) and rotarod test were employed to assess the antidepressant and balance potential, respectively. After behavioral evaluation, rats were anesthetized, brains were removed, and following preparation of brain homogenates, oxidative stress was evaluated using specified methods. BHE administered at the doses of 300, and 600 mg/kg, reduced immobility time in the FST exerting antidepressant-like activity. In the EPM test, BHE at the same doses, produced the anxiolytic-like effect. Also, the rats which received BHE had a significant improvement in rotarod test in contrast to control groups. In addition, brain catalase activity and superoxide dismutase level were significantly greater versus PTZ group BHE-300 treated PTZ group was significantly lower and. BHE could prevent anxiety and depression and ameliorate oxidative stress in PTZ-kindled rats.

***Keywords*:** Elevated Plus Maze; Epilepsy; Forced Swim Tests; Kindling; *Buxus hyrcana;* Pentylenetetrazole

Corresponding Author:
Abdolkarim Hosseini, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
E-mail address: ab_hosseini@sbu.ac.ir

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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 (Kowski et al. 2016; Saha and Chakrabarti 2014). Oxidative stress and free radicals are among the most important mechanisms that can contribute to disorders such as epileptic seizures (Waldbaum and Patel 2010). Epileptic seizures have also been shown to increase the amount of reactive oxygen species (ROS) and the production of superoxide anion (O_2-) in the brain (Costello and Delanty 2004). Oxidative stress is the result of an imbalance between the antioxidant defense system and the formation of ROS (Stadtman 2001). Among the antioxidant defense system there are two key enzymes which is very important in the first line of defense against production of ROS. These enzymes are catalase and superoxide dismutase (SOD) which they are very fast in neutralizing any molecule with the potential of developing into a free radical or any free radical with the ability to induce the production of other radicals (Ighodaro and Akinloye 2018).

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 familiarized for the dealing 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 (Kowski et al. 2016; Saha and Chakrabarti 2014). In effect, to discover and develop sufficient AEDs have been a desideratum. To this end, kindling has been posited as an efficacious model for the clinical facets of epilepsy at biochemical, electrophysiological and behavioral levels (Dhir 2012). Also, one of the common approaches in the discovery and development of AEDs - which is the backbone for the cure of patients with seizures and epilepsy - is the utilization of natural substances with medicinal properties (Pahuja et al. 2012). One of the herbs that has evinced beneficial medicinal properties and has been studied in traditional medicine is *Buxus hyrcana* (Buxaceae family), which grows in various part of the world (Babar et al. 2006; Choudhary et al. 2006). Previous studies have demonstrated many medicinal properties for *B. hyrcana* such as being antimalaria, anticancer, immunosuppressive due to its active ingredients, antifungal and antileishmania; it, further, has anti-inflammatory and antioxidant characteristic because of its triterpenoids and alkaloids (Ata et al. 2010; Babar et al. 2006; Choudhary et al. 2006; Ebrahimzadeh et al. 2010; Esmaeili et al. 2009; Mesaik et al. 2010). Moreover, it has recently been documented that it has anticonvulsive and neuroprotective properties in experimental animal models (Azizi et al. 2018).

Given the behavioral manifestations associated with epilepsy and the beneficial effects of *B. hyrcana* on above mentioned disorders, it was hypothesized that the *B. hyrcana* extract (BHE) would have positive effect on behavioral parameters, related to stress, depression, and balance in pentylenetetrazole (PTZ)-kindled rats. The aim of this study, therefore, was to evaluate the effects of BHE on behavioral test (related to stress, depression and balance), in order to assess behavioral disruptions resulted from epilepsy and seizures in adult male rats.

MATERIALS AND METHODS

Animals and ethics

This experiment was conducted at Shahid Beheshti University (Tehran, Iran) on twenty-four male adult Wistar rats (200 ± 20 g, 8 weeks old) obtained 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 (42×27×15 cm; Tajhiz Gostar-e Omid Iranian Co.) made from polycarbonate in order for them to adapt to a new environment. The animals had free access to water and feed for rats (Pars Animal Feed, Iran) throughout the experiment. Animals were randomly (simple randomization) given to experimental groups (each group containing 6 rats) after one week of adaptation to the laboratory environments. Each animal was used only once through the trial, 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. All conducted experiments pertaining to animal rights and conservation in this study were in accordance with the standard ethical guidelines (European Communities Directive 2010/63/EU) and were approved by Local Ethics Committee at the Shahid Beheshti University (ethical code: IR.REC.SBU.1397.156).

Obtaining the plant and preparation of the extract

The *B. hyrcana* plant was obtained from the Insti-

tute of Medicinal Plants of Shahid Beheshti University. The extract was prepared according to the references and previous work (Azizi et al. 2018). Thus, 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 drenched in 1L of ethanol 80% for 72 h, then the resulting mixture was filtered and concentrated in vacuum at 45°C using a rotary apparatus (EYEL A, Japan). The resultant extract was 37.8% dry and stored in the refrigerator at 4°C until the experiment. The dose required for intraperitoneal (i.p.) injection was based on animal weight.

Medications and treatments

Pentylenetetrazole from Sigma Company (USA), phenobarbital sodium (PB) 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 current experiment as a conventional anticonvulsant drug. Phenobarbital sodium was also dissolved in the physiological saline solution and administrated intraperitoneally in a dose of 30 mg kg^{-1} of rat weight to the animals. BHE was continued in normal saline solution via ip in doses of 150 and 300 mg kg^{-1} with PTZ during the experiment until complete animal kindling. Animals were randomly alienated into four groups of six: (1) negative control group receiving normal saline (0.5 mL/rat), (2) positive control group receiving PB (30 mg kg^{-1}), (3 and 4) groups receiving BHE (300 and 600 mg kg^{-1}, respectively). Thirty min after vehicle injection, PB and BHE (300 and 600 mg kg^{-1}) animals were challenged with the sub-threshold dose of PTZ (35 mg kg^{-1} of body weight). All injections were administered in the form of fresh solutions in a constant volume of 0.5 mL/rat throughout the study (Azizi et al. 2018).

PTZ-induced kindling test

The study was conducted in a double-blind manner (the experimenter was unaware of which animal belonged to which group). Pentylenetetrazole was injected in a sub-threshold dose of 35 mg kg^{-1} every 48 h interval 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 one hour after PTZ administration and recorded by a computer-connected camera. Seizure threshold was measured on a 6-step scale (Table 1). Animals were considered kindled if they showed stages 4 or 5 in two consecutive trials. Animals were given the PTZ challenge (70 mg kg^{-1}) 7 days after the kindling development. Meanwhile, the animals underwent the anxiety, depression and rotarod test. Anxiety, depression and rotarod test were carried out after the PTZ challenge (Pahuja et al. 2013).The experimental schedule and intervals for the estimation of various parameters is shown in Figure 1.

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

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

Elevated plus maze (EPM) test

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 and behavior was scored using "EthoVision XT" software. 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 (Iwamoto et al. 2007).

Forced swim test (FST)

The modified forced-swim test was performed according to the modified method described earlier (Cryan et al. 2005). All testing was carried out in a 20-min test with no preswim session in order to negate any confounding aspect of an induction procedure.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). The immobility time during the last 5 min of a 20-min swim test was defined as the absence of active/escape directed movements. After the test, animals were removed from the water, dried with a towel then carried back to their home cages.

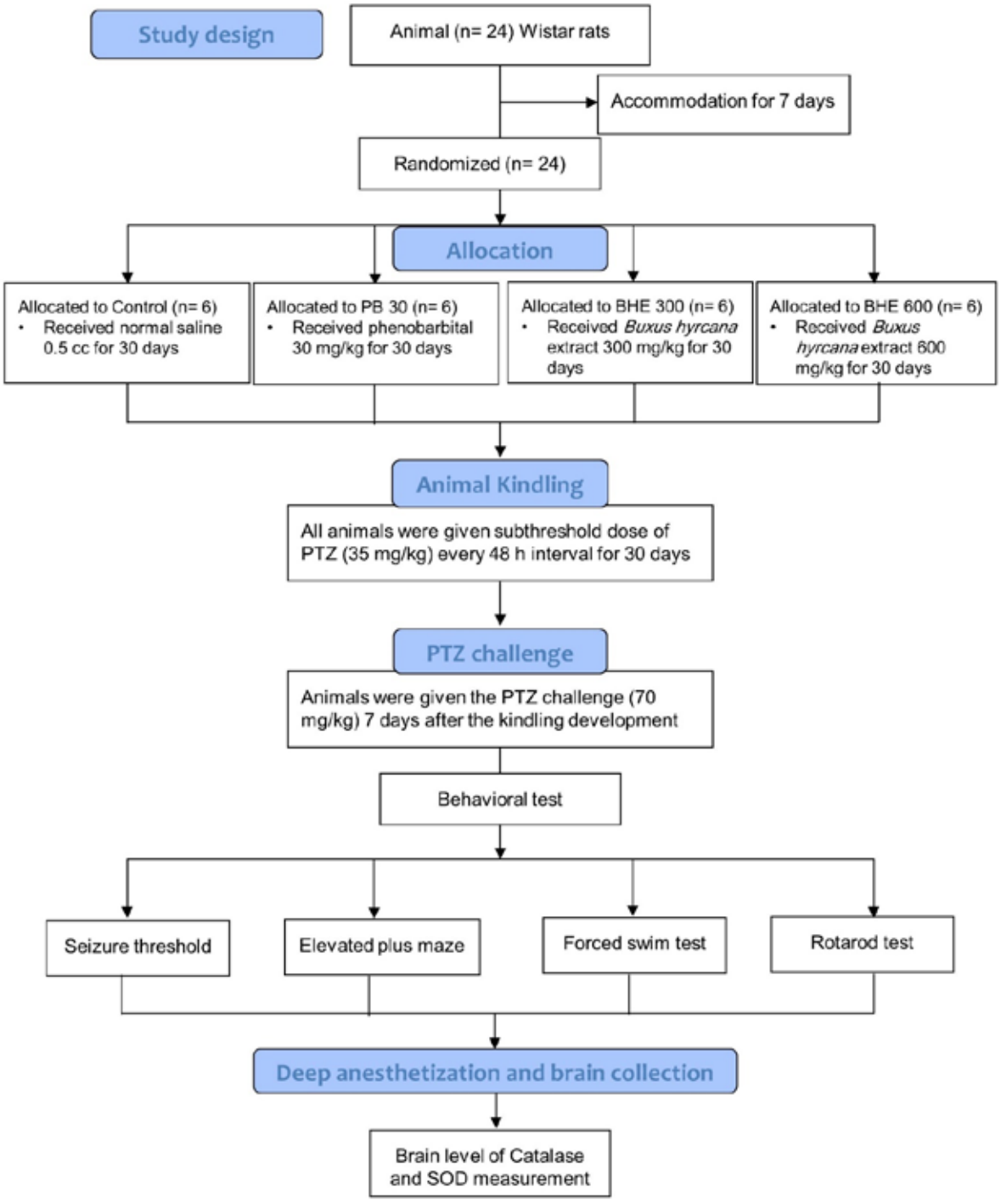

Figure 1. Flow chart of the experimental design. Schematic timeline representation for PTZ kindling, administration of drugs and behavioral test in experimental groups.

Rotarod test

The rotarod test is a widely used test to measure coordinated motor skills. It requires animals to balance and walk on a rotating cylinder. The rotarod (49 cm diameter and 45 cm width; Borj Sanat Azma Co.) unit consisted of a rotating rod, which divided into four parts by compartmentalization, which allows examining four rats at a time. When the rats fell down from rotating rod, the time automatically stopped. In this study, 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 is directly dependent on the movement and balance skill of the animal. Twice daily training for two consecutive days was done before the test day (Khan et al. 2013).

Biochemical measurement

After the behavioral test, the rats were quickly beheaded under deep anesthesia by a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), and their brains were removed, washed with ice saline, and kept at minus 80°C for subsequent analyses.

Measurement of brain catalase (CAT) activity

Catalase activity of brain tissue was performed according to the previous work (Milanizadeh et al. 2018). In short, 50 mL of phosphate buffer is removed and 0.05 mL is H_2O_2 is added. To study the changes in the optical density of the CAT enzyme in the samples of different groups, after combining them, the optical density of the CAT enzyme activity was measured at 240 nm wavelength for 2 min by the spectrophotometric device.

2.8.2. Measurement of brain superoxide dismutase (SOD) activity

In this section, the previous reference was also used to measure SOD activity of brain tissue (Milanizadeh et al. 2018). Briefly, 0.43 g of Na_2HPO_4 is dissolved in 61 mL of distilled water and 0.3 g of $NaHPO_4$ is dissolved in 39 mL of distilled water. The two solutions were poured together in a beaker and the pH of the solution is brought to 7. Fifty mL from previous solution was mixed with ethylenediaminetetraacetic acid (EDTA) (0.0018 g), and pyrogallol (0.003 g). For comparison purposes, the optical density changes of the SOD enzyme in the sample were calculated at 420 nm wavelength for 4 min by the spectrophotometric device.

Statistical Analysis

All statistical evaluations and graphs were run using the GraphPad Prism software (version 8). All behavioral and biochemical tests were articulated as mean ± standard error from the mean (SEM) or median (min, max). The normality test was carried out to show the data distribution is normal. All data met ANOVA assumptions for normality and homogeneity of variance. One-or two-way ANOVA was done to compare the means of the statistics. 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.

RESULTS

The effect of BHE on seizures in PTZ-induced kindled rats

Analysis of variance showed a significant increase in the mean of seizure threshold [$F(3,20) = 15.65$, $P<0.001$] between the experimental groups. Further analysis with Post *hoc* test showed a significant increase ($P = 0.001$) in the seizure threshold mean in the BHE treatment group in a dose of 600 mg kg^{-1} compared to control group. However, BHE-treated groups in dose of 300 failed to show significant changes when compared to the control group. Also, a significant increase in the mean of the seizure threshold was observed in the PB 30-treated group in comparison with control group ($P < 0.001$) (Fig.2).

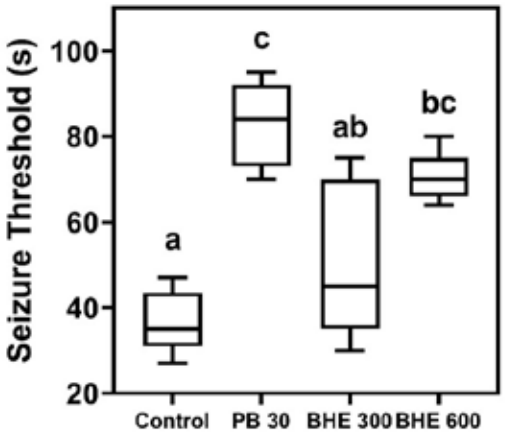

Figure 2. Effect of BHE on seizure threshold in PTZ-induced kindled rats. The box plots showing the median (min, max) is related to six male Wistar rats. Different small letters in the box indicate the significance of differences at $P<0.05$.

Effect of BHE on EPM in PTZ-induced kindled rats

As shown in Figure 2. a-e, and confirmed by analysis of variance, there were a significant differenc-

es between the experimental groups with respect to the time spent in the open arms [F (3,20) = 13.90, P <0.001], the time spent in the closed arms [F (3, 20) = 26.86, P <0.001], the number of open arm entries [F (3,20) = 10.64, P <0.001], number of closed arm entries [F (3,20) = 26.54, P <0.001] and latency to enter open arms [F (3,20) = 4.84, P =0.013] in the EPM test.

The post-test indicated that the PB 30 receiving group caused a significant increase in the mean of the time spent in the open arms (P <0.001; Fig.3.a) and the number of open arm entries (P <0.001; Fig.3.c) and a significant decrease in the time spent in the closed arms (P <0.001; Fig.3.b), number of closed arm entries (P <0.001; Fig.3.d), and latency to enter open arms (P =0.011; Fig.3.e) in comparison to the PTZ group. There was also a significant increase in the group receiving BHE in the doses of 300 and 600 compared to the control group in the time spent in the open arms (P =0.006 and P <0.001, respectively; Fig.3.a) and the number of open arms entries (P =0.063 and P =0.006, respectively; Fig.3.c) and a significant decrease in the time spent in the closed arms (P <0.001; Fig.3.b), number of closed arm entries (P <0.001; Fig.3.d). However, in concern to latency to enter open arms parameter there is no significant changes in the group receiving BHE in a dose of 300 compared to the control group (Fig.3.e)

Effect of BHE on FST in PTZ-induced kindled rats

Significant differences were observed in the mean of immobility [F (3,20) = 54.92, P<0.001] and swimming [F (3,20) = 24.36, P<0.001] factors between the different groups. The post *hoc* analysis suggested that the immobility time mean was significantly increased in the BHE-treated groups in doses of 300 and 600 compared to the control group (P<0.001) (Fig.4.a). Also, there was a significant increase in the mean of swimming time in the BHE group in doses of 300 and 600 (P =0.029 and P =0.002, respectively; Fig.4.b) compared to the control group.

The Effect of BHE on Rotarod test in PTZ-induced kindled rats

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) = 6.37, P=0.004], 60 min [F (3,20) = 17.07, P<0.001] and 90 min [F (3,20) = 33.38, P<0.001] after treatments. The mean latency to fall in the BHE group in 300 and 600 doses at 30 min (P=0.175 and P=0.020, respectively), 60 min (P=0.021 and P<0.001, respectively) and 90 min (P=0.001 and P<0.001, respectively) after treatments was significantly higher than that of the control group (Fig.5).

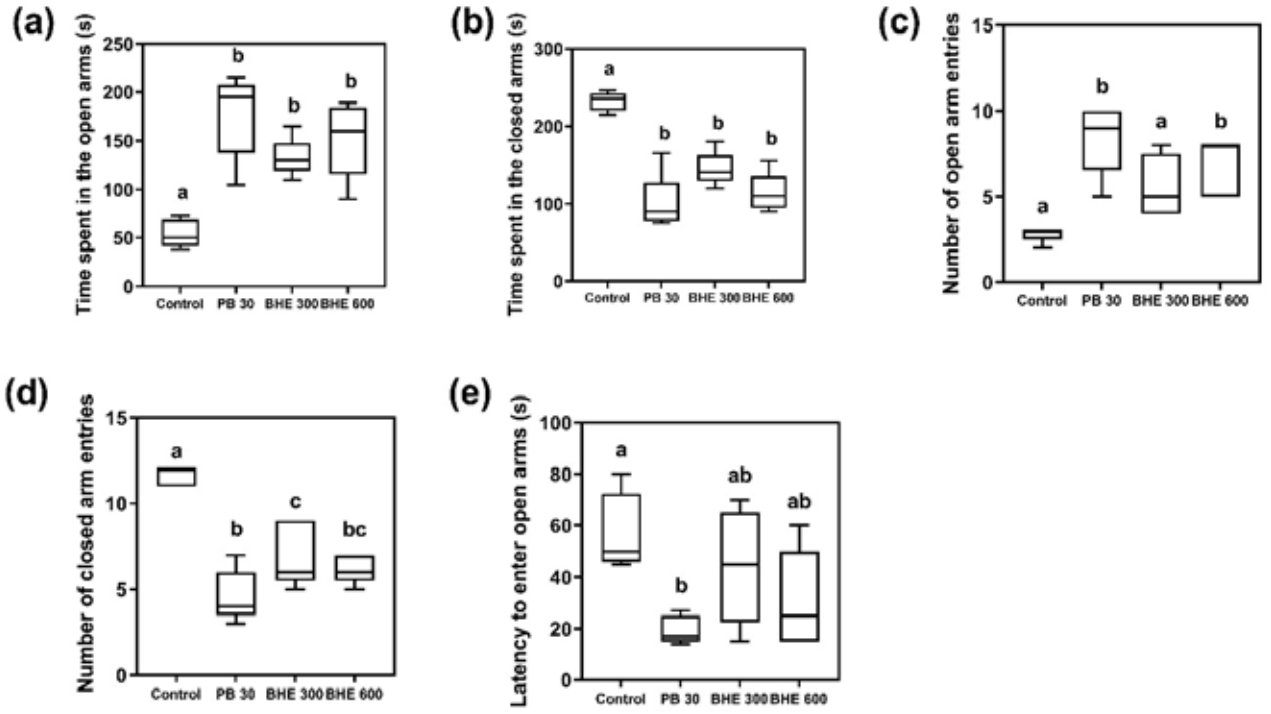

Figure 3. Effect of BHE on time spent in the open arms (a), time spent in closed arms (b), number of open arm entries (c), number of closed arm entries (d), and latency to enter open arms (e) in PTZ-induced kindled rats. The box plots showing the median (min, max) is related to six male Wistar rats. Different small letters in the box indicate the significance of differences at P<0.05.

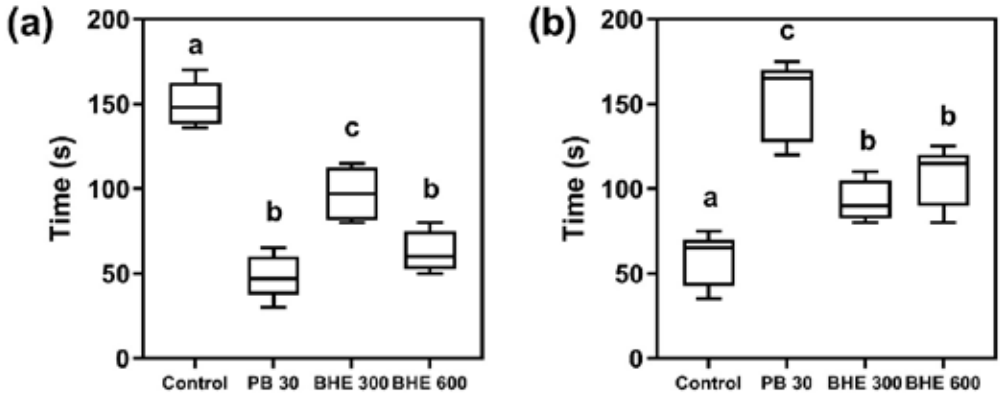

Figure 4. Effect of BHE on immobility (a), and swimming (b) in PTZ-induced kindled rats. The box plots showing the median (min, max) is related to six male Wistar rats. Different small letters in the box indicate the significance of differences at P<0.05.

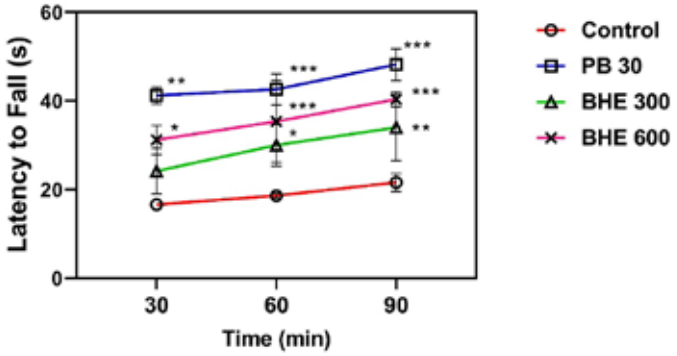

Fig.5. Effect of BHE on latency to fall in rotarod test in PTZ-induced kindled rats. Data represents mean ± SEM, * *P* <0.05, ** *P* <0.01, *** *P* <0.001, as compared to control group.

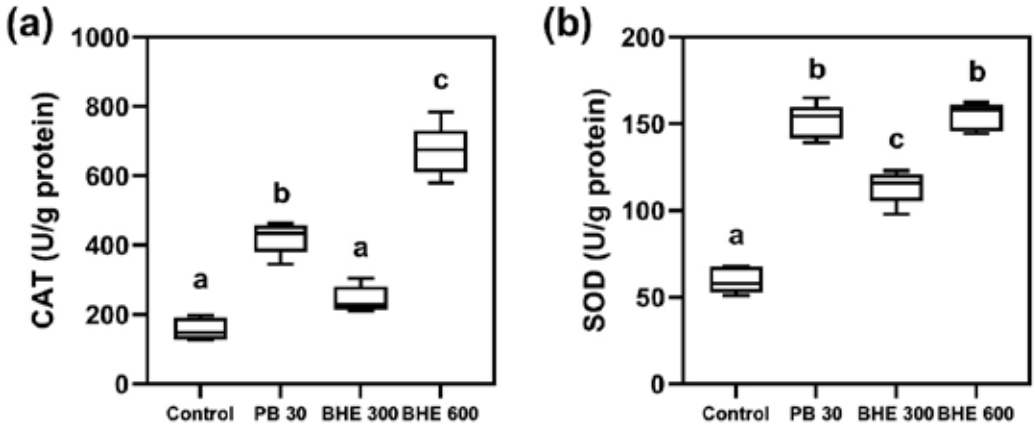

Figure 6. Effect of BHE on CAT (a), and SOD (b) levels in PTZ induced kindling in rats. The box plots showing the median (min, max) is related to six male Wistar rats. Different small letters in the box indicate the significance of differences at P<0.05.

Effect of BHE on oxidative stress parameters

Effect of BHE on brain CAT levels

Significant differences were observed in the mean of CAT levels in the brains of the rats between the different groups [F (3,20) = 101.6, P<0.001]. The *post-hoc* analysis suggested that the CAT level mean was significantly increased in the BHE-treated groups in dose of 600 compared to the control group(P<0.001) (Fig.6.a). However, there is no significant change in the group receiving BHE in a dose of 300 compared to the control group.

The effect of BHE on brain SOD levels

Analysis of variance showed a significant difference in the mean of SOD total content of the brain between the different groups [F (3,20) = 123.3,

P<0.001]. The SOD level mean in the BHE group in 300 and 600 doses was significantly (P<0.001) higher than that of the control group (Fig.6.b).

DISCUSSION

This study identified the positive effect of BHE on stress and depression in the experimental model of kindling induced by PTZ. The data showed that pretreatment with BHE raises seizure threshold in PTZ-induced kindling. In addition, our findings revealed a significant decrease in epileptic stress and depression in pre-treated BHE rats. In the present study, as the results revealed, BHE at doses of 300 and 600 mg kg^{-1} significantly elevated seizure threshold in kindled rats *vis-à-vis* the control group. In line with this study, Azizi et al. (2018) observed that *B. hyrcana* exhibits neuroprotective and anticonvulsant characteristicin Wistar rats in the PTZ-induced seizure model. They have also documented that the dose of 600 mg kg^{-1} of the extract has the greatest effect, which is in harmony with the present study (Azizi et al. 2018).

In the current work, the EPM and FST tests were adopted to evaluate the anti-anxiety and antidepressant properties. The outcomeof the study confirmedthat doses of 300 and 600 mg kg^{-1}BHE increased the frequency and duration of entry into open arms and reduced it in the closed arms compared to the control group. It also significantly reduced the delay in open arm entry in the EPM test, indicating a decrease in anxiety in BHE-receiving rats. Furthermore, the BHE 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 the recent studies on *B. hyrcana*, it has been observed that this planthaving steroidal alkaloids, inhibits acetylcholinesterase activity (AChE) -an enzyme which degrade acetylcholine by hydrolytic cleavage- and by this means can be used to cure Alzheimer's disease, which is a neurological progressive deficit illness(Babar et al. 2006; Choudhary et al. 2003; Choudhary et al. 2006). In the most recent experiment in mouse model, it has been shown that nicotinic acetylcholine receptor can excite the release of the gamma-aminobutyric acid (GABA) (Aracri et al. 2017). Although the most conventional models of seizure studies are induction by PTZ, the mechanism underling of this procedure was not completely acknowledged. Nevertheless, there is a public agreement that one of the mechanisms of the PTZ can cause the seizure is inhibition of the ion channel GABA type A complex and disruption in the neural inhibitory pathway (Mandhane et al. 2007). Consequently, according to the above-mentioned reason, at least in part the anti-seizure properties and consequently anxiety and depression amelioration observed in this study are due to the presence of the steroidal alkaloids in the BHE. These are due to the anti-AChE activity as well as the rise in the levels of ACh and subsequently rise in the release of the GABA, the competition with the PTZ on the active site of the GABA receptors and increase in the inhibitory activity in the neural cells.

In this study, to measure the motor activity, rotarod performance test was used. As the results show, BHE at the doses of 300 and 600 mg kg^{-1} had the highest anticonvulsant effect, as this effect rate was also evident in the locomotor activity of animals treated with BHE and was significantly increased compared to the control group. Since this study examined the positive effects of BHE in the rotarod motor testing for the first time, therefore, it is not possible to compare it with previous works. That said, to confirm this hypothesis cogently, further research is warranted.

In this study, we examine the antioxidant activity of BHE, therefor the total brain level of the CAT and SOD were evaluated. As the results show, BHE at the doses of 300 and 600 mg kg^{-1} significantly increase the CAT and SOD level, as compared to the control group. Pentylenetetrazole also disrupts the blood-brain barrier (BBB) and disrupts brain function by creating free radicals (Choudhary et al. 2013). The brain is very sensitive to the damage of free radicals, because it contains a lot of fatty acids and it has a high rate of oxidative metabolism (Mariani et al. 2005). Reactive oxygen species are involved in the pathogenesis of various types of neurodegenerative diseases (Perry et al. 2002). Oxidative stress may play an important role in causing neurological damage due to seizures (Sudha et al. 2001). Prolonged administration of PTZ induces free radicals, leading to seizure activity in animals. The oxidative stress induced by PTZ leads to tonic-clonic seizures and subsequent neurological death (Zhao et al. 2014). Inhibition of brain neurons and production of free radicals by PTZ is one of the major causes of epilepsy in animal models of epilepsy. In epilepsy, seizure activity is always associated with increased levels of reactive oxygen species (Rauca et al. 1999). Studies have focused on elucidating whether prolonged seizure activity in animals leads to increased ROS production and whether

oxidative damage leads to seizure-induced brain injury. Therefore, this study is consistent with the theory that in animals induced by PTZ, oxidative stress is probably one of the parameters involved in the pathophysiology of epilepsy (Nassiri-Asl et al. 2013).

Chemically, flavonoids and iso-flavonoids destroy the free radicals and reduce oxidative stress by single electron transfer. The accumulation of free radicals by flavonoids can be one of the reasons for the protective effect of this substance on nerve cells (Bors et al. 1990). There is also ample evidence that flavonoids are involved in preventing the destruction of nerve cells caused by oxidative stress (Ishige et al. 2001). It has been shown that in the epileptic condition the neural cells the level of oxidative stress markers rise up and the levels of antioxidants will decrease (Cárdenas-Rodríguez et al. 2014). Based on studies on the active ingredients of the *B. hyrcana* have confirmed that it has characteristic to block the oxidative stress. As noted above, BHE has numerous flavonoids, which exert anti-inflammatory and antioxidant properties (Wollenweber and Rustaiyan 1991); therefore, at least partially, the antiepileptic and ultimately the improvement in anxiety and depression observed in this study may be due to the presence of flavonoids present in BHE, as well as the control of CAT and SOD status in the brain.

Our study has some limitations that should be considered. As limitations, our study did not evaluate the other biochemical and molecular parameters which very important such as MDA, nitric oxide, inflammatory cytokines, gene expression of enzymes or receptors responsible for epilepsy or stress pathway due to limited funding, and sample size. Therefore, supplementary studies need to justify the result and beneficial effect of BHE on the epilepsy or stress conditions. It is also suggested that in order to determine the role of the inflammatory cytokines and the effects of BHE in the pathogenesis of epilepsy and stress, other pathways such as the inflammation pathway and the expression of genes in the relevant pathways should be examined.

Overall, the results of this study indicated that the BHE has anticonvulsant properties in PTZ-kindled rats in that it increases 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. These effects can be attributed to the improvement of oxidative stress status, including increased in CAT and SOD activity.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The Authors declare that they have no conflicts of interest to disclose.

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