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Effects of hydro-alcoholic extract of Ziziphoratenuior L. on serum biochemical and antioxidant in PTZ seizure model in male mice

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Abstract

Background: Seizure is due to abnormal electrical discharge of neurons in the brain. The possible involvement of superoxide dismutase (SOD), is investigated in anticonvulsant and antioxidant effects of hydro-alcoholic extract of Ziziphoratenuior L. on seizure in male mice model.

Methods: In this study, 40 male mice (25-30 g) divided into five groups of 8 that included a control group (receiving saline and PTZ) and 4 treatment groups (receiving the extract doses of 150, 300, 600, 900 mg/kg). Thirty minutes after IP administration with saline (in control group) and different doses of extract (in treatment groups), PTZ (80 mg/kg) were injected and were transferred to a special cage immediately and convulsive behaviors were recorded by a camera during the 20 minutes. Then, the different phases of seizure were evaluated. At last, the mice were exsanguinated and their biochemical factor levels including SOD were determined.

Results: The results showed that *Ziziphoratenuior L.* extract has the ability to increase seizure onset time and reduce its duration time dose-dependently. It also has increased the level of SOD in serum. Our study indicated that, the hydro-alcoholic extract of *Ziziphoratenuior L.* had an appropriate dose-dependently anticonvulsant effect through its antioxidant activity.

Conclusions: It seems the further studies will be necessary to separate its ingredients and understand its mechanism of action.

Keywords: Anticonvulsant, Antioxidant, Seizure, Superoxide Dismutase, Ziziphoratenuior L.

1. Background

Epilepsy, is characterized by recurrent highly synchronized discharges of neurons and leads to mortality and morbidity in patients. Epileptic seizures are transient symptoms of abnormal neuronal activity. Glutamate and γ-aminobutyric acid (GABA) have an important role in eplepsy (1;2). Most available treatments, suppress seizure only symptomatically, due to lack of a clear understanding of the underlying mechanisms. Most patients have to use antiepileptic drugs (AEDs) for many years of life. These result in severe side effects such as cognitive impairment and psychiatric problems. Accordingly, new AEDs with fewer adverse effects and higher efficacy are needed (1;2). Herbal mixtures prevent cellular oxidation and can slow down the mitochondrial dysfunction (5-7). A relationship between epilepsy and oxidative stress has recently begun to be recognized both in animal models and in patients (8-12). Superoxide dismutase (SOD) enzyme catalyzes reducing the peroxides to hydrogen peroxide (H_2O_2) in order to protect the cell from radical agents and oxidative stress. The serum level of SOD changes in the most disorders (3). The alteration in the balance of oxidant/antioxidant or oxidative stress can decrease the cellular functions and leads to cellular death. Oxidative stress because of its role in mitochondrial dysfunctions can cooperate in epilepsy incidence (4). Disturbances in mitoe chondrial functions influence the production of reactive oxygen and this will affect macromolecular function, glutamate excitotoxicity and can increase the risk of seizure occurrence (14). Herbal mixtures with their antioxidant and anti-inflammatory features can face with oxidative stress and inflammation. *Ziziphoratenuior* L. is a genus of annual or perennial herbs and subshrubs in the Lamiaceae family, which naturally and widely distributed in Iran. Ziziphora species herb were used somewhat as culinary veget in Iran (5). In Iranian traditional medicine, Ziziphoc ra species have been used for various purposes including stomach tonic, heart disorders, inflammation, expectorant, diarrhea, fever, migraine, antiseptic and depression (6). So, in this study we investigate relationship between pentylenetetrazol induced seizure in mice and anticonvulsant activity of hydro-alcoholic extract of *Ziziphoratenuior* L. through its antioxidant activity. To evaluate whether the hydro-alcoholic extract of *Ziziphoratenuior* L. has any protective effect on PTZ-induced seizure in mice;

we treated mice with hydro-alcoholic extract of *Ziziphoratenuior* L. before PTZ administration and assess the anticonvulsant effects through its antioxidant properties.

2. Methods

2.1. Animals

In the present study 40 male mice (25-30 g) were purchased from Razi institute (Karaj, Iran) and randomly divided in five groups, eight rats in each including; a control group (receiving PTZ 80 mg/kg i.p.) and 4 treatment groups (receiving 150, 300, 600 and 900 mg/kg hydro alcoholic extract of *Ziziphoratenuior L.* i.p. and PTZ 80 mg/kg i.p.).

Then mice per cage were housed at temperature-controlled room 21±2°C and under a 12 h light–dark cycle with lights on at 7:00 to 19:00. The mice had free access to standard food and tap water ad libitum. All experiments involving animals were conducted according to the policy of Iranian Convention for the Protection of Vertebrate Animals used for Experimental Purposes, and the protocol was approved by the Ethics Committee of the Medical School, Qazvin University of Medical Sciences, Qazvin, Iran.

2.2. Preparation of Plant

The aerial parts of *Ziziphoratenuior L.* were collected and were dried, protected from sunlight, and then were powdered. The powdered material (50 g) was extracted 3 times with an absolute ethanol and distilled water mixture (1:1) at room temperature overnight. The obtained extracts were compeletely mixed and then concentrated under decreased pressure on an evaporator, filtered and finally lyophilized. The extract was stored in vials at 4 °C until analysis time.

2.3. Preparation of Hydro Alcoholic Extract of Plant

100 gram of the *Ziziphoratenuior L.* plant in 500 ml of distilled water and poured into 500 ml of ethanol (96%) and it was placed in a 2000 ml balloon for 72 hours at laboratory temperature (22 ° C). It is necessary to shake the solution several times in 12 hours by Shaker. After 72 hours, the solution was clear by pass through a filter paper and we pour the solution in a sterile balloon with a short pipe (outlet). After purifying the solution, the resulting solution is filtered and it placed in the water bath (boiling water bath at 50 °C). When the solution was condensed, we put them in to the sterile plates and placed them in to the incubator with 50°C in order to dry up.

2.4. PTZ-induced seizure

The experimental model in this study was the PTZ epilepsy model. PTZ (Sigma) were dissolved in 0.9% saline solution and with dose of 80 mg/kg intraperitoneally (IP) were injected to the animals, and gradually was causing seizures. Seizure severity in this model was evaluated accordance with the following phases:

1. Tonic phase: Severe muscle stiffness and stretching arms and legs to the sides.
2. Clonic phase: A short period of seizure with neck cramps, arms movements, jumping and turning around.
3. Tonic-Clonic: Generalized seizures with sudden tonic contractions and status epilepticus state associated with a very short time jumping.
4. And also jumping (sudden jumping higher than 20 cm from the ground level), Imbalances (falling of animals to the sides during seizure), and death (death of animals during 20 min of seizure observation).

Thirty minutes after i.p. administration of 0.9 percentage normal saline in control group, PHT 50 mg/kg and PHB 40 mg/kg in positive control groups, and different doses of hydro alcoholic extract of *Ziziphoratenuior L.* in treatment groups, PTZ 80 mg/kg was injected to the animals and immediately transferred to a special cage, and the convulsive behaviors were recorded by a camera during 20 minutes. Then, different phases of seizure were observed and evaluated by a researcher. The efficacy of hydro alcoholic extract of *Ziziphoratenuior L.* in prevention of seizure attacks was scaled according to the following factors: The delay time of each seizure phases (Latency), the duration time of tonic and tonic-clonic phase (Duration time), the generalized seizure total time, and the number of jumping, imbalances and death during seizure attacks in each group.

2.5. Serum superoxide dismutase (SOD) assay

The SOD activity was done, according to the chemical calorimetrically method (1). In this method, xanthine oxidase-xanthine system was used to produce a superoxide radicals, a kind of reactive oxygen species (ROS), and nitroblue tetrazolium (NBT) was used as an indication of ROS production upon reduction with superoxide anion. SOD activity was then measured by the rate of inhibition of the reaction unit of enzyme provides 50% inhibition of NBT reduction. The unit of SOD enzyme activity in serum was stated as units of SOD per milliliter (U/mL) of serum (7).

2.6. Statistical analysis

The results were expressed as mean ± SD and Statistical comparison between groups was tested using one-way ANOVA by using SPSS 16 software program. In order to investigate the statistical differences of means, ANOVA, Tukey and LSD tests were used. When $P<0.05$, it was considered as statistical significance.

3. Results

3.1. Effect of Ziziphoratenuior L. on PTZ-induced seizure onset times

Statistical analysis in Figure 1 showed that there are

significant differences between treatment groups and control group. As it shown, administration of hydro-alcoholic extract of *Ziziphoratenuior L.* at dose of 600 mg/kg were able to increase tonic-clonic, clonic seizures and tonic latencies significantly (p<0.01), it also decreased latencies of tonic-clonic, clonic and tonic seizures significantly at dose of 900 mg/kg compared to control group (p<0.05)

3.2. Effect of Ziziphoratenuior L. on PTZ-induced seizure duration time

According to the analysis shown in Figure 2, pretreatment of animals with hydro-alcoholic extract of *Ziziphoratenuior L.* decreased tonic-clonic seizure duration time at doses of 150, 300, 600 and 900 mg/kg significantly compared to the control group (p<0.01, p<0.001 respectively).

3.3. Effect of Ziziphoratenuior L. on mortality

Results indicated that pretreatment of extract prevent animals from death in treatment groups. The percentage of animal mortality in Control, Zt 150, Zt 300, Zt 600 and Zt 900 were 75, 75, 50, 0 and 0 respectively. It can concluded that mortality rates in animals treated with extract at dose of 300 mg/kg decreased significantly compared to the control group (p<0.05). Also 600 and 900 mg/kg dosages of extract prevent animals from death significantly compared to the control group (p<0.01).

3.4. Effect of Ziziphoratenuior L. on SOD Serum levels

Figure 3 indicates the serum level of SOD as an index of oxidative stress. The SOD serum level in treatment groups at doses of 300 and 600 mg/kg of hydro-alcoholic extract was increased compared to the control group.

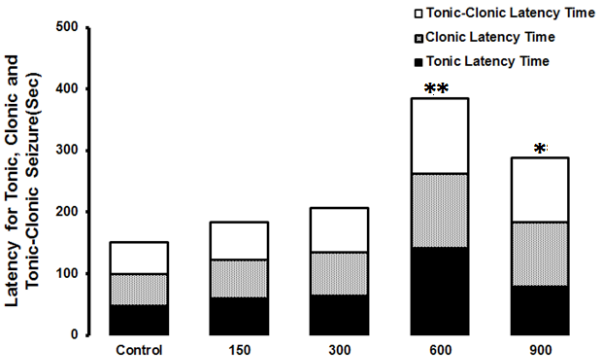

Figure 1. Effect of *Ziziphoratenuior L.* on PTZ-induced seizure latencies in mice. Values are shown as Mean+ SEM of eight mice in each group. * P < 0.05; ** P < 0.01 compared to control group (Saline).

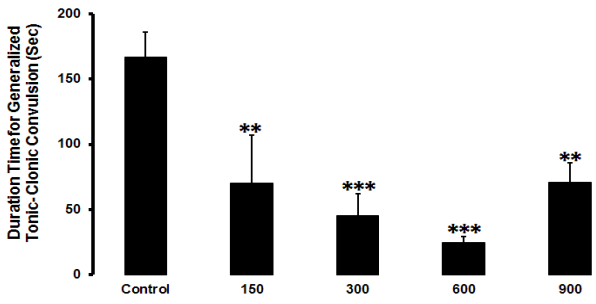

Figure 2. Effect of *Ziziphoratenuior L.* on PTZ-induced seizure duration time in mice. Values are shown as Mean+ SEM of eight mice in each group. ** P < 0.01, *** P < 0.001 compared to control group (Saline).

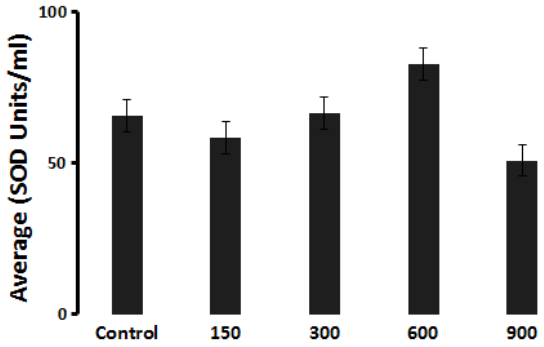

Figure 3. Effect of *Ziziphoratenuior L.* on SOD Serum levels (Units/ml). Values are shown as Mean+SEM of eight mice in each group

4. Discussion

In this study, we found that Extract injection in all doses delayed the clonic, tonic and tonic-clonic seizures onset times dose-dependently, but doses of 600 and 900 mg/kg had showed the greatest effect on onset times. The duration time of clonic, tonic and tonic-clonic seizures in animals that received extract decreased. Extract injection also dose-dependently decreased the rate of mortality and the number of suddenly jumping during seizures, these effects in doses of 600 and 900 mg/kg was significant. Also, pretreatment with hydro-alcoholic extract of *Ziziphoratenuior L.* increased SOD serum levels, indicating that hydro-alcoholic extract of *Ziziphoratenuior L.* have an anti-oxidative effect. The brain has a collection of antioxidant defense enzyme system, including SOD, glutathione (GSH), and catalase (CAT), to prevent from oxidative stress damage. However, there is controversy about the change in endogenous antioxidant reagents after SE. It has been reported that the activities of SOD and CAT are increased 48 h and 5 d after KA-induced SE (8), respectively. In a pie locarpine model, SOD and CAT activities were increased at 2 h and 60 d after SE, respectively, indicating that en-

dogenous antioxidant reagents are up-regulated due to increased oxidant radicals (9). However, other reports have demonstrated no change or even a decrease in SOD activity (10;11). This disparity may be result from differences in type of animal models, selected time points and /or methods of detection and severity of injury. To reveal the details of the change in SOD activity after SE, we evaluated the activity at multiple time points in this study.

The SOD activity was obviously decreased immediately after SE (1 h), indicating increased oxidative stress and privation of SOD enzyme shortly after beginning of SE. SOD enzyme activity then substantially increased both in the acute seizure (6 h) and chronic periods (3 to 28 d), which may represent over-compensation results from persistence of oxidative stress. As well as, MDA value was increased at 3, 7, and 28 d after KA seizure. These results are slightly different from those reported previously (8).

The results of the present experiments indicate that single PTZ treatment did not change SOD activity, which has correlation by before study (12), which might describing by simultaneous increased of SOD and enzyme degradation (13;14). In this study neuroprotective effects of anticonvulsant activity of hydro-alcoholic extract of Ziziphoratenuior L. in 600 mg/kg on pentylenetetrazol induced seizure might happen through their antioxidant properties which has consistent with anti-epileptic effects of allopurinol have been correlated with increases in SOD activities (15) and seizure related behavior decrease. Also other study indicated that SOD administration in amygdala blocked seizures (16). bezafibrate decreased the kindling prevalence and recovered seizures related behaviors. In the present study bezafibrate increased the catalase and superoxide dismutase activity in the brain (17, 18). LFs have protective effects on seizure through their anti-oxidative effects (19). Evaluation of anti-convulsant of extract of Glycyrrhiza glabra and its action on SOD of rat brain tissue indicated that SOD was decreased in PTZ-induced seizure, whereas administration of extract elevated SOD activity (20). Also, Diazoxide decreased oxidative stress injury by upregulating superoxide dismutase (SOD) activity and wortmannin, attenuated the changes SOD levels after seizures (21). Inconsistent with this study, PTZ administration decreased activities of SOD (22) and SOD activity were increased in epileptic subjects compared to control groups, but only SOD activity was significantly higher in patients with generalized tonic-clonic seizure than in those with partial seizure (23). superoxide dismutase activity were decreased in rats with seizures than in those without seizures (24). Junior et al, suggest that there is a direct relationship between the lipid peroxidation and nitrite values during epilepsy condition that may be responsible for the SOD and catalase enzymatic activity changes observed during the establishment of seizures and SE induced by pilocarpine. SOD activity in male rats with epileptic status was significantly higher in compared to the control group and also to females with epileptic status. Obay et al, indicates that PTZ at a convulsive dose induces an oxidative stress response by depleting the antioxidant defense systems and increasing lipid peroxidation in the brain, liver and RBC of rats. Ghrelin pretreatment reduced the oxidative stress and prevented the decrease in antioxidant enzyme activities, and thus may reduce brain neuronal death during the seizures. However, more studies are needed in order to confirm mentioned hypothesis (25). superoxide dismutase decreased in cerebrospinal fluid (CSF) of patients with epilepsy which can be a predictive of anticonvulsant drug resistance in patients with seizure (26). Kindling caused distinctive change of antioxidative defense in the frontal cortex, hippocampus, and pons-medulla region (27). Adaptive mechanisms, as the induction of Mn-SOD, may be taken into consideration to counteract oxidative stress-mediated free radical formation. Evidence of peroxide free radical production during epileptic status that may be responsible for neuronal damage in the rats hippocampus, during the establishment of PILO model of seizure (10) and mitochondrial dysfunction is accompanied by chronic oxidative stress and may have an important role in the epileptogenesis process; however, few studies have shown an established link between oxidative stress, seizures, and age (4). Antioxidant therapies aimed at reducing oxidative stress have received considerable attention in epilepsy treatment. However, much evidence suggests that oxidative stress does not always have the same pattern in all seizures models (28). Results of the present study showed that Ziziphoratenuior L. extract has the ability to increase seizure onset time and reduce its duration time dose-dependently. It also has increased the level of SOD in serum. Our study also indicated that, the hydro-alcoholic extract of Ziziphoratenuior L. had an appropriate dose-dependently anticonvulsant effect through its antioxidant activity. It seems the further studies will be necessary to separate its ingredients and understand its mechanism of action.

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Footnote

Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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