

Full Length Research Paper

Gabaergic system role in aqueous extract of *Valeriana officinalis* L. root on PTZ-induced clonic seizure threshold in mice

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Pharmacognosy studies have shown that *Valeriana officinalis* L. roots containing significant amounts of free amino acids such as GABA and other substances like valeric acid and isovalerate. In this study, anticonvulsant effects of *V. officinalis* (VO) roots aqueous extract, using standard methods of chemical seizure caused by pentylenetetrazole (PTZ) in mice was assessed. To evaluate mechanisms involved in VO anticonvulsant effects, diazepam and flumazenil were used. After determining the seizure threshold in the control group, different doses of VO (0.25, 0.5 and 1 g/kg) were intraperitoneally (IP) administered and their effects on the threshold of PTZ-induced seizure were studied. Diazepam (1, 2 and 3 mg/kg) and flumazenil (1, 2 and 4 mg/kg) were injected intraperitoneally and the seizure threshold was determined, after which the different doses of the VO drugs were evaluated. The results showed that PTZ-induced seizure threshold in control mice was 35.52 ± 0.87 mg/kg. VO and diazepam were increased and flumazenil were decreased the threshold of PTZ-induced seizure. Also results showed that diazepam and flumazenil, respectively increase and decrease the effect of VO on PTZ-induced seizure. Changes in seizure threshold in this study indicate that probably the mechanism of VO anticonvulsant effects is due to the inhibitory GABA system, but this issue needs further investigations.

Key words: *Valeriana officinalis*, root, diazepam, flumazenil, PTZ, clonic seizure threshold.

INTRODUCTION

Epilepsy is a chronic disease with a heterogeneous set of symptoms that is characterized by recurrent seizure. The seizure event is limited brain function, caused by abnormal neurons discharge. Clinical symptoms of epilepsy, including sudden temporary abnormal phenomenon such as: changes in the level of consciousness, motor, sensory, autonomic or psychological (Lott, 2001). To date, the mechanism of epilepsy and the factors affecting the incidence precisely and completely is not identified yet. Causes of seizure are numerous, such as neurological diseases, infections, tumors and brain injuries. About 30% of the seizures are because of central nervous system disorders. In total the reason of

epileptic seizure disturbs between balance of stimulatory and inhibitory neurons, and the most important role of glutamate and GABA neurotransmitters are responsible (Coulter, 2001; Faingold, 2004; Lott, 2001).

Epileptic drug therapy in most patients is based on experimental seizure classification, because diversity causes the seizure drugs to be less specific for each of these effects. About 1% of people are born with epilepsy and approximately 10% of the population will experience a seizure. Although, by standard treatment in 80% of the seizure can be controlled, nevertheless the millions of people have uncontrolled epilepsy (Engel, 2001). Despite the many advances in the field of medicine and pharmacy, patients and epileptic seizure disorders have always been challenging to physicians and researchers. Today, in the treatment of epilepsy, combinations of the three mechanisms are: strengthening Gabaergic inhibitory currents, typically reduction of glutamatergic drive current and balanced ionic currents, particularly sodium

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ions, calcium and chlorine. In some cases, with recurrence, toxicity and side effects of the drugs increased and the patient should have a long period of treatment (Gale, 1992; Roger, 2001).

Valeriana officinalis L. is one of the Valerianaceae family members, dried roots and rhizomes were used as a medical herb science thousand years ago. *V. officinalis* were used widely in different countries and Iran due to the effects of sedation to treat insomnia and anxiety, the effect of CNS depressant is proved (Hadley and Petry, 2003; Rangahau, 2001), in Turkey it is used to treat rheumatic pain and wound healing (Cakilcioglu et al., 2011; Cakilcioglu and Turkoglu, 2010) and the root extract of this plant, alone or in combination as with other herbal drugs are used for central nervous system depression (Hadley and Petry, 2003; Kennedy et al., 2006). VO extracts containing free amino acids such as Gaba aminobutyric acid and other substances such as isovalerate (Eadie, 2004). Several studies have shown that the effects of this plant with other drugs to treat various CNS diseases, and most do in relation to interference, such as GABA neurotransmitter (Eadie, 2004; Hadley and Petry, 2003; Krystal and Ressler, 2001; Ortiz et al., 1999). Before potassium bromide the extract of VO was used to treat seizure (Eadie, 2004).

Diazepam is a member of benzodiazepines family and is used to treat anxiety and insomnia (Garfinkel et al., 1999; Ugale et al., 2004). Diazepam also has muscle relaxant and anticonvulsant effects. Many previous studies on the anticonvulsant effects of diazepam in several animal models have been done (Krystal and Ressler, 2001). Anticonvulsant effects of this drug are obtained by increasing the effect of GABA neurotransmitter, mediated through the inhibitory chemicals in the brain and which acts through GABA_A receptors. Researchers have shown that diazepam increases sensitivity of GABA neurotransmitter to GABA_A receptors (Garfinkel et al., 1999; Silva et al., 2009; Ugale et al., 2004). Therefore, probably anticonvulsant effects of VO root extract is increased by this drug. Flumazenil is benzodiazepine receptor antagonist, and is used to evaluate the effects of other drugs (Silva et al., 2009; Ugale et al., 2004). It is probably that anticonvulsant effect of VO is reduced by this drug.

Seizure caused by GABA receptor antagonist known as, pentylenetetrazole (PTZ), is usually used in rodent seizure models, due to its repeatability, and providing the situation for comparison of different chemical compounds anticonvulsants effects, under standard conditions. The substance causing the seizure, probably work through interaction with GABA_A receptors, and antagonized chloride ions flow caused by the GABA (Huang et al., 2001). This study investigated the effect of VO root extracts on PTZ-induced seizure threshold and the effect of diazepam and flumazenil on this herbal drug was compared in order to evaluate mechanisms involved in VO effects.

MATERIALS AND METHODS

Animals

Male mice of Naval Medical Research Institute (NMRI), age 8 to 10 weeks and weight between 25 to 30 g of Razi Vaccine Institute purchased and kept in a room that was constant temperature, light and humidity. Animals access to food and water *ad libitum*. The National Institutes of health guidelines for care and use of animals and guidelines on ethical standards for investigation of experiments in animals were followed.

Chemicals

PTZ, diazepam and flumazenil were purchased from Sigma-Aldrich Company. Diazepam dissolved with polyethylene glycol 400 and by normal saline was brought to the required volume. Flumazenil and PTZ were resolved in normal saline 0.9%.

Extracting method

Spring *V. officinalis* roots with their stems were collected from east Azerbaijan state located in North West of Iran. Collected roots were identified by Dr Ebrahm Mirshkri Assistant Professor of Agriculture and Specialist in Herbal Plants in Islamic Azad University of Tabriz. Roots were dried in the shade, after collection. Root extract prepared according to Grainger Bisset (2001) were crushed, then milled and added into a hot water (20 min), and separately purified the solution until finally a yellowish brown extract was obtained, concentrated extract was dried under vacuum to obtain powder.

Administration of test agent

Animals were randomly divided into treatment groups (for each group n = 8), VO root extract, diazepam, flumazenil and the vehicle was injected as intraperitoneal with constant volume and based on weight of each animal. Seizure threshold in animals receiving saline was evaluated. Then the effect of VO vehicle on the seizure threshold was evaluated for 30 min before administration of PTZ, and the threshold of the two other drugs' vehicle was evaluated for 15 min before the seizure test was determined. Then, animals received VO in times of 15, 30, 60 and 90 min before the seizure threshold test, until the maximum anticonvulsant activity was obtained. In the next experiment, different doses of VO (0.25, 0.5 and 1 g/kg) were administered 30 min before seizure threshold test. Then animals received flumazenil (2 mg/kg) in times of 15, 30, 45 and 60 min before the seizure threshold test until the maximum seizure activity was obtained. After that, different doses of diazepam (1, 2 and 3 mg/kg) and flumazenil (1, 2 and 4 mg/kg) 15 min before the seizure threshold test were injected. To evaluate the effect of diazepam and flumazenil on anticonvulsant of VO, these two drugs were administered in 2 mg/kg dose, 15 min after VO (0.25, 0.5 and 1 g/kg) injection and after 15 min, the seizure threshold was measured.

To determine seizure threshold, PTZ solution (5 mg/ml) was infused in a constant rate of 0.5 ml/min into the lateral tail veins of mice. Infusion continued until the occurrence of upper limb clonic seizure and followed by full body tonic seizure. Minimum dose of PTZ (mg/kg of mice body weight) needed to create clonic seizure as an index of clonic seizure threshold was considered. The National Institutes of Health guidelines for care and use of animals and guidelines on ethical standards experiments in animals were followed (Zimmermann, 1983). All efforts were made to minimize the number of animals which were used and their suffering degree.

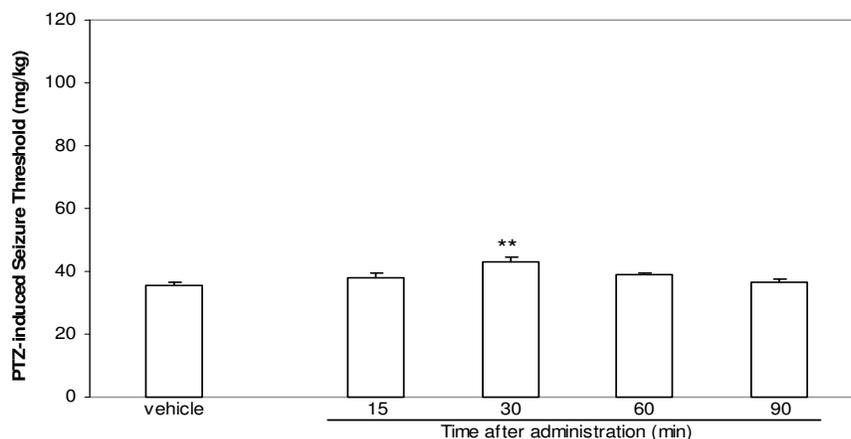


Figure 1. Effect of VO root extract (0.25 g/kg) on PTZ-induced clonic seizure threshold in mice at different times. Each graph is presented as mean \pm SEM. ** $P < 0.01$ is compared with the vehicle.

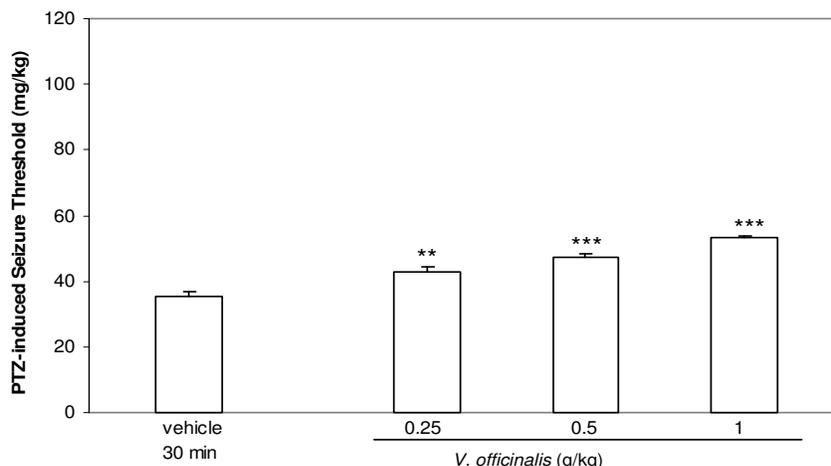


Figure 2. Effect of different doses of VO root extract on PTZ-induced clonic seizure threshold in mice. Each graph is presented as mean \pm SEM. ** $P < 0.01$ and **** $P < 0.001$ are compared with the vehicle.

Statistical analysis

SPSS software package version 13 was used to analyze the data. Group data are presented as mean \pm SEM and analyzed statistically using one-way ANOVA test followed by Tukey multiple comparison tests. The level for statistical significance was set at $P < 0.05$ and * used for $P < 0.05$, ** used for $P < 0.01$ and *** used for $P < 0.001$ used for multiple comparison.

RESULTS

Seizure threshold obtained in mice that received normal saline as control was 35.52 ± 0.87 mg/kg. Vehicles effect on seizure threshold showed that the vehicles used in this study did not have significant effect on seizure threshold.

VO dose of (0.25 g/kg) at the time 15, 30, 60 and 90 min before intravenous injection of PTZ, showed that maximum anticonvulsant effects was achieved after 30 min. Anticonvulsant effects 30 min after injection was significantly ($P < 0.01$) higher when compared with control and the other groups. Therefore, in the next stage of testing time, 30 min was used (Figure 1).

Different doses of VO (0.25, 0.5 and 1 g/kg) administered to the animals and their effect on PTZ-induced seizure threshold was determined. Results showed a significant dose dependent manner, increasing the threshold of PTZ seizure. This significance was $P < 0.001$ in groups that received 1 g/kg when compared with control group (Figure 2).

Flumazenil injection (2 mg/kg) in times of 15, 30, 45 and 60 min before PTZ IV injection indicated that the

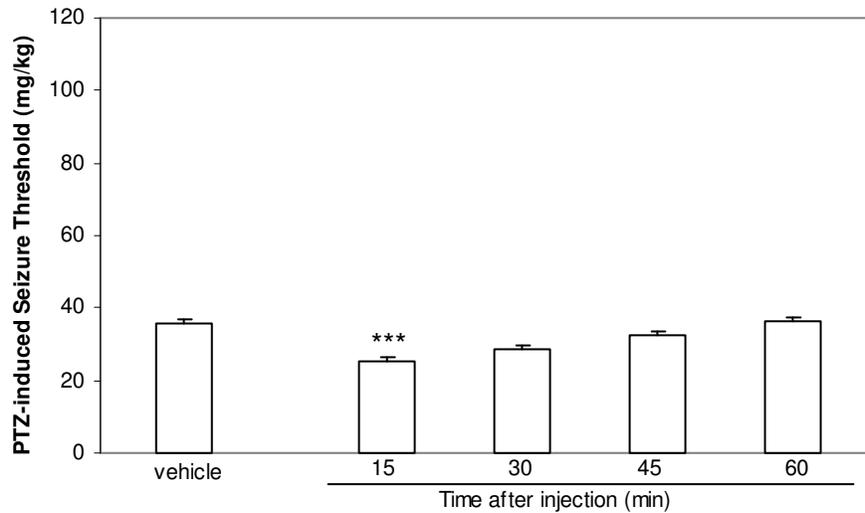


Figure 3. Flumazenil (2 mg/kg) effect on the threshold of clonic seizure induced by PTZ in mice at different times. Each graph is presented as mean \pm SEM. *** $P < 0.001$ is compared with the vehicle.

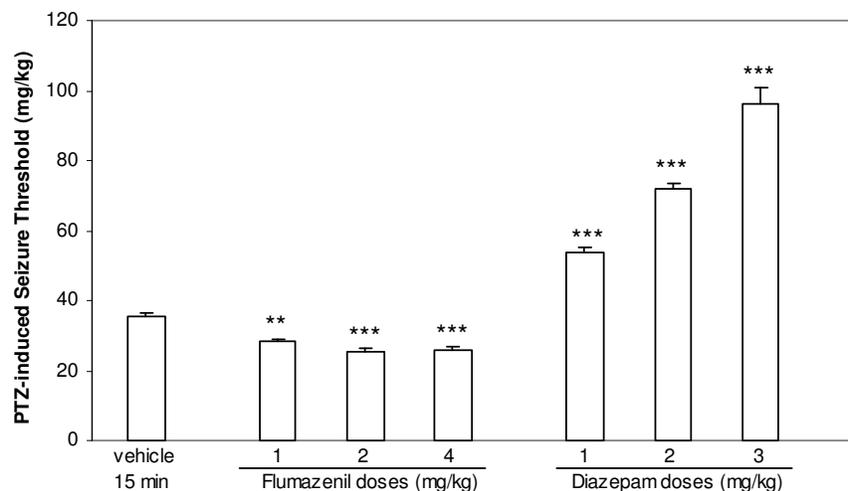


Figure 4. Effect of different doses of diazepam and flumazenil on PTZ-induced clonic seizure threshold in mice. Each graph is presented as mean \pm SEM. ** $P < 0.01$ and *** $P < 0.001$ are compared with the vehicle.

maximum decrease in threshold was achieved after 15 min from flumazenil injection. This decrease was observed for 15 min after flumazenil injection was statistically significant ($P < 0.001$) when compared with the control group. Therefore, in the next stages, the previous injection time (15 min) was used (Figure 3).

Diazepam dose dependently increased convulsions and flumazenil dose dependently decreased PTZ-induced seizure threshold. The increased and decreased by diazepam and flumazenil was statistically significant (Figure 4).

Simultaneous administration of diazepam and flumazenil with VO respectively increased and decreased

PTZ seizure threshold. Likewise, the simultaneous administration of diazepam increased the seizure threshold when compared to that of VO when used alone ($P < 0.001$). Simultaneous administration of flumazenil reduced seizure threshold when compared to the case when VO was used alone ($P < 0.05$) (Figure 5).

DISCUSSION

In this study, VO root extract on PTZ-induced seizure threshold and the effects of diazepam and flumazenil on VO and PTZ seizure were evaluated. GABA_A receptor is

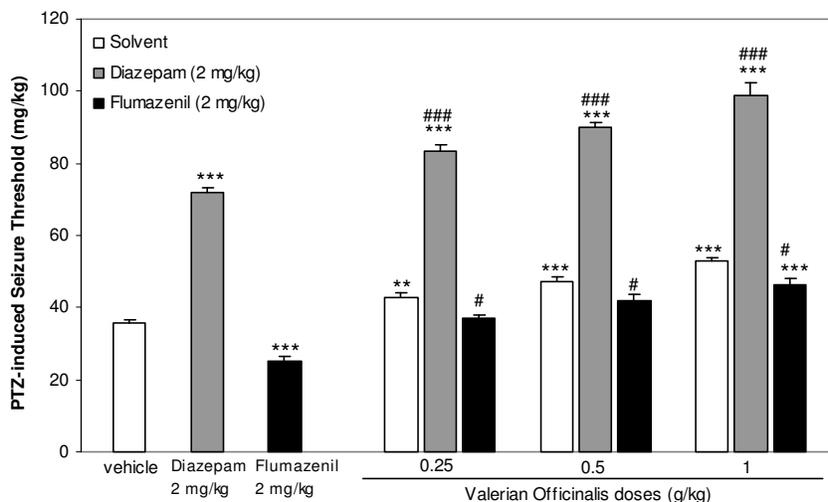


Figure 5. Effect of simultaneous administration of diazepam (2 mg/kg) and flumazenil (2 mg/kg) with different doses of VO on PTZ-induced clonic seizure in mice. Each graph is presented as mean \pm SEM. ** $P < 0.01$ and *** $P < 0.001$ are compared with the vehicle. # $P < 0.05$ and ### $P < 0.001$ are compared with VO group.

prominent inhibitory neurotransmitter receptors in vertebrate central nervous system. When this receptor is activated, receptor's chloride channels open, leading to the flow of chloride ions and nervous hyperpolarization (Huang et al., 2001). The receptor has a multiple connection positions through which different drugs can adjust GABA, by chloride ions. Benzodiazepines and barbiturates are known as current amplifiers of GABA-induced chloride ions (Hevers and Luddens, 1998). Drugs such as picrotoxin and several other drugs are known to suppress the chloride flow that is mediated by GABA. It is well marked that PTZ acts on the position of picrotoxin action complex on GABA receptor (Huang et al., 2001). The advantages of this standard method, is due to high repeatability capability and provide the underlying model to compare the anticonvulsant nature of chemicals, and PTZ are used to induce seizure in animal models.

In this study, VO increased PTZ-induced seizure threshold dose-dependently. Since PTZ acts via GABA_A receptor, it seems that an anticonvulsant effect of VO is through GABAergic system. Other researchers showed with biochemical studies that VO root extract contains amino acids such as GABA (Eadie, 2004) and also increased the concentration of GABA in the brain (Barnes, 2002a); because GABA is an inhibitory neurotransmitter in brain, it decreases activity in central nervous system (Barnes et al., 2002b). Other studies have shown that the root extract of this plant has valeric acid, which is a GABA degrading enzyme inhibitor. In addition, VO root extract inhibits reuptake (Houghton, 1999) and increases GABA release in synaptosomes isolated from rat brain cortex (Homayoun et al., 2002;

Ortiz et al., 1999). Also, suggested that VO root extracts has affinity to connect to benzodiazepines place in its receptor (Barnes et al., 2002b). In a similar study, it was shown that the VO root extract contain isovalerate, which is structurally similar to valproic acid substance that had potent anticonvulsant activity in humans and experimental animals (Isoherranen et al., 2003). Valproic acid mechanisms are being through inhibition of reuptake or prevent the GABA neurotransmitter analysis (Eadie, 2004; Isoherranen et al., 2003), so, it is possible that VO have anticonvulsant similar effects as the mechanism of valproic acid.

In the present study, diazepam as positive modulators of GABA_A receptor complex, facilitate GABA effect on GABA_A receptor (Macdonald and Olsen, 1994) and showed anticonvulsant effects on PTZ-induced seizure. This issue is because, diazepam bind to its position on GABA_A receptors, and antagonize PTZ effects, by so doing increases the seizure threshold. Anticonvulsant effects of diazepam also increased the threshold of seizure in VO extract received groups. In conclusion diazepam increases the effects of VO anticonvulsant, which is in agreement with previous studies that activity of GABA can be increased by diazepam and other benzodiazepine (Ortiz et al., 1999) and barbiturates (Kennedy et al., 2006; Ortiz et al., 1999), and VO pretreatment can facilitate other anticonvulsants agents (Eadie, 2004; Krystal and Ressler, 2001). These effects have used to treat insomnia, anxiety, stress and behavioral disorders (Andreatini et al., 2002; Malva et al., 2004; Poyares et al., 2002). These findings show that this herbal drug may interfere with the GABA_A receptor, and also increase GABA neurotransmitter, that could regulate

its function and therefore opening chloride channels that lead to increase in the seizure threshold.

For further investigation of the interactions involved in the modulation of a benzodiazepines receptor antagonist, flumazenil (Silva et al., 2009; Ugale et al., 2004) was used. Flumazenil epileptic effect was mediated through GABA_A receptors, because flumazenil has an affinity with the benzodiazepine receptor and there is close relationship with the power of convulsant effects (Silva et al., 2009; Ugale et al., 2004). Moreover, simultaneous administration of VO and flumazenil caused the reduction of the VO effects on PTZ-induced seizure threshold. This can probably confirm that VO anticonvulsant effect occurs through the benzodiazepine receptor involvement. On the one hand, flumazenil as benzodiazepine receptor antagonist inhibits the effects of the VO anticonvulsant and on the other hand, flumazenil alone decreases the PTZ-induced clonic seizure threshold.

Conclusion

In summary, results of this study show that the main mechanism of increasing the threshold of PTZ-induced seizure due to VO root extracts, is probably through Gabaergic inhibitory neurotransmitter system. Further investigations are needed to find the other mechanism involved in the anticonvulsant activity of the extract.

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