Anticonvulsant Profile of *Valeriana officinalis* on Pentylentetrazole-Induced Seizure Threshold in Mice

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**Abstract:** In this study the anticonvulsant effects of hydro-alcoholic extract of *Valeriana officinalis* were evaluated by using pentylenetetrazole model (PTZ) on mice. The extract of underground parts of *Valeriana officinalis* was prepared as a basic method to produce a solution for injection administration. *Valeriana officinalis* and normal saline were injected intraperitoneally at the doses (100, 300, 600 and 1200 mg/kg, i.p) and (10 ml/kg i.p) respectively 30 minutes before PTZ (90 mg/kg, i.p). For measurement of anticonvulsant activity in animals convulsive symptoms were divided into 3 level (scores 1-3) which were C1, C2 and C3. The time taken before C1, C2 and C3 and the percentage of seizure and mortality protection were recorded. Statistical analyses of all treatment and control groups show that *Valeriana officinalis* significantly increases onset of convulsion symptoms in all 3 levels of seizure in the PTZ model. On the other hand, onset time of convulsive symptoms increases with increasing doses which may indicate the dose-dependence effect of *Valeriana officinalis*. In conclusion, *Valeriana officinalis* is a medicinal herb which may have dose dependent anticonvulsant value by reducing and delaying onset of symptoms. It seems that it could be useful for treatment of seizure and these effects may be related to effect of it on GABAergic system.

**Key word:** Anticonvulsant activity · *Valeriana officinalis* · Convulsion · Herbal extract

**INTRODUCTION**

*Valeriana officinalis* is a perennial herb comprised of grooved hollow stems and saw-toothed green leaves. White, pale pink or reddish flowers appear from June to August. *Valeriana officinalis* grows to heights of 3-5 feet in the temperate climates of North America, western Asia and Europe, often in moist soil along riverbanks [1, 2]. The vertical rhizome and attached roots of *Valeriana officinalis* are parts used medicinally [3-5].

Traditional uses include treatment of insomnia, migraine headache, anxiety, fatigue and seizures [6]. *Valeriana officinalis* is the medicinal herb which widely used as anxiolytic and sleep-promoting.

It contains sesquiterpenes such as valerenic acid, which appear to inhibit γ-Aminobutyric acid (GABA) breakdown, thus causing a net increase of CNS-depressant neurotransmitter [7]; the monoterpenes known as valepotriates which relax smooth muscle [8]; large amounts of free GABA bind to the GABA receptors [9]; lignans, e.g. 1-hydroxypinoresinol, which inhibit binding of serotonin to its receptors [10]; and flavonoids, e.g. 6-methylapigenin, which binds to benzodiazepine receptors [11]. All of these effects contribute to depression of CNS activity and to overall relaxation, thus aiding reduction in anxiety and promoting the onset of sleep [12, 13].

*Valeriana officinalis* extracts are associated with markedly less negative side-effects than benzodiazepines, with levels similar to placebo across clinical trials [12]. In animal experiments, the interaction of the various constituents of *Valeriana officinalis* are centrally depressive, sedative, anxiolytic, spasmolytic, muscle relaxing and anti-ulcerogenic. The pharmacological efficacy is heavily dependent on the quality of the extract used. The main effect in humans is to reduce sleep induction time [14]. In vitro the valerenic acid components have been shown to decrease the degradation of γ-Aminobutyric acid (GABA.) Animal experiments have demonstrated an increase of GABA at the synaptic cleft via inhibition of re-uptake and an increase in secretion of
the neurotransmitter. The increase of available GABA is one factor that may be responsible for the sedative and anticonvulsant properties of *Valeriana officinalis* root [4, 15, 16].

**MATERIALS AND METHODS**

**Herb:** *Valeriana officinalis* was used for the present study. First of all, *Valeriana officinalis* plant was collected freshly from cultivation farm of medicinal plants of Islamic Azad university of Ardashil. It was done in the July of 2009. Afterward the underground parts of plant which were the roots and rhizomes were separated immediately and then were sent to department of chemistry for decoction.

**Decoction of Valeriana officinalis:** At first step, the roots and rhizomes of *Valeriana officinalis* were dehumidified at room temperature followed by grinding was done of 50 gram of dried sample. This powder was soaked in 400° methanol (60%) -water (40%) mixture for 72 hours. Afterward it was filtrated by gossamer and remaining slag also was washed with mentioned solvent to produce a semi clear solution. This solution was percolated with Buchner funnel and vaporized and condensed by rotary in low pressure to produce the final extract. The last stage was the dehumidification of extract by an oven in 60°C for at least 24 hours. We got finally a waxy brown to black net solid extract.

**Preparing of Injectable Solutions of Extract:** To prepare the injectable solution from the extract, 2.5 gram of extract was solved in 10ml distilled water to produce 250 mg/ml injectable solution of extract.

**Dose Determination of Extract:** The initial dosage of solution of *Valeriana officinalis* extract was 100 mg/kg. Dose selection should have rational like 1/10th of lethal dose (LD50 dose).

**Animals:** Albino male BALB/c mice (25-30 g) were obtained from the Razi Institute (Kuraj, Iran). The animals were housed in colony rooms with 12/12 h light/dark cycle at 22 ± 2°C and had free access to food and water. There are should follow CPCSEA rules which had free access to tap water and food pellet mention the manufacturer. All animals 45 minute before examination were transferred to individual small cage and also were used only one time [17].

**Materials:** Drugs used as follows: Pentylentetrazole (PTZ) [Sigma], extract of *Valeriana officinalis*, Normal saline [Samen, Iran]. PTZ was dissolved in normal saline. All compounds were prepared fresh each time and administered intraperitoneally.

**Anticonvulsant Effects of Herbal Extract:** In this study, animals (N=60) were divided to 5 groups of treatment (*Valeriana officinalis*) and 1 group of control (The mice were divided into groups of ten animals each). In the five treatment groups, the mice were given *Valeriana officinalis* solution at the doses (100, 300, 600, 900 and 1200 mg/kg i.p.) 30 min before the administration of PTZ (90 mg/kg i.p) and one group was injected normal saline 30 min before the administration of PTZ (90 mg/kg i.p.) [17, 18].

**Response Measurement Method:** Each animal is placed into an individual plastic cage for observation lasting 1 h. Convulsion symptoms of animals were divided into 3 levels (score 1-3) which are called "C1", "C2", "C3". C1 was the onset of a general clonus which was characterized by forelimb clonus. C2 was the onset of myoclonic convulsion and C3 was the onset of the tonic-clonic convulsion. The time taken before the onset of each level of convulsions and the percentage of seizure and mortality protection were recorded [17, 18].

**Statistical Analysis:** All statistical analyses were performed with using SPSS 11.5 (Statistical Packages for Social Science) and One-way repeated measures ANOVA was used to determine the association of each factor and to be significant was considered at p <0.05.

**RESULTS**

In this survey, the main purpose was to investigate of anticonvulsant activity of *Valeriana officinalis* in three levels (C1, C2, and C3) of a PTZ model of convulsion.

C1 level of convulsion (Slight strain of forelimbs): *Valeriana officinalis* at the all dose prolonged the C1 symptoms of convulsion (Fig. 1). Moreover it prolonged the onset time of seizure compared to saline group (p <0.001) (Table 1).

As it is shown in Table 1, *Valeriana officinalis* exhibited its protection against seizure in a dosedependent manner. Furthermore, the onset time of C1 level of convulsion prolonged with increasing of administrative dosages.
Table 1: Statistical analysis of treatment and control groups at C₁ level of convulsion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>C T</td>
<td>-1316.9</td>
<td>13.61808</td>
<td>.000</td>
<td>-1344.5188</td>
<td>-1289.2812</td>
</tr>
<tr>
<td>T C</td>
<td>1316.9*</td>
<td>13.61808</td>
<td>.000</td>
<td>1289.2812</td>
<td>1344.5188</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level.

The error term is Mean Square (Error) = 927.261
(C) Control, (T) treatment (Valeriana officinalis) groups

Table 2: Statistical analysis of treatment and control groups at C₂ level of convulsion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>C T</td>
<td>-1282.3*</td>
<td>15.26932</td>
<td>.000</td>
<td>-1313.2676</td>
<td>-1251.3324</td>
</tr>
<tr>
<td>T C</td>
<td>1282.3*</td>
<td>15.26932</td>
<td>.000</td>
<td>1251.3324</td>
<td>1313.2676</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level.

The error term is Mean Square (Error) = 1165.761
(C) Control, (T) treatment (Valeriana officinalis) groups

Table 3: Statistical analysis of treatment and control groups at C₃ level of convulsion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>C T</td>
<td>-1470.9*</td>
<td>20.68588</td>
<td>.000</td>
<td>-1512.8529</td>
<td>-1428.9471</td>
</tr>
<tr>
<td>T C</td>
<td>1470.9*</td>
<td>20.68588</td>
<td>.000</td>
<td>1428.9471</td>
<td>1512.8529</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level.

The error term is Mean Square (Error) = 2139.528
(C) Control, (T) treatment (Valeriana officinalis) groups.

**Fig. 1:** Effect of administration of Valeriana officinalis in C₁ stage of convulsion (Depending on the time) in mice. The number of mice used for each group was 10.

**C₁ Level of Convulsion (Myoclonic Convulsion):** In the second stage, Valeriana officinalis at the all dose prolonged the C₂ symptoms of convulsion (Fig. 2).

Moreover it prolonged the onset time of seizure compared to saline group (p < 0.001) (Table 2).
Table 4: Effects of *Valeriana officinalis* extract on Seizure and Mortality protection on PTZ-induced convulsion in mice

<table>
<thead>
<tr>
<th>Doses Mg/kg</th>
<th>S (%) (C1)</th>
<th>S (%) (C2)</th>
<th>S (%) (C3)</th>
<th>M (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>20</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>600</td>
<td>10</td>
<td>40</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>900</td>
<td>20</td>
<td>40</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>1200</td>
<td>20</td>
<td>50</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Normal saline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(S) Seizure protection; (M) Mortality protection

DISCUSSION

The main goal of this study is to evaluate the anticonvulsant effect of *Valeriana officinalis* using the PTZ-model. The some herbs like *Valeriana officinalis* with antiepileptic activity are identified and developed as a result of their ability to block induced acute convulsions in animal models of epilepsy. *Valeriana officinalis* has been used medicinally for at least 2000 years. It was first used as a treatment for epilepsy in the late 16th century reportedly by Fabius Columna, who related a personal cure, but subsequently was also reported to have relapsed. Fifty years later, additional reports of its effectiveness in three cases of epilepsy were reported by Dominicus Panarolus. Numerous reports by a wide variety of writers followed and *Valeriana officinalis* subsequently became routinely used for the treatment of various nervous disorders [19]. John Finley Ellingwood in Systematic Treatise on Materia Medica and Therapeutics considered *Valeriana officinalis* to be a nervine and sedative for the treatment of hysteria, epilepsy and menopausal nervous anxiety [20]. It was included in many editions of the United States Dispensatory which reported on its effect on the nervous system and its ability to produce drowsiness and sleep [21].

The roots of *Valeriana officinalis* contain several compounds with demonstrable pharmacological activity. These include the essential oil and its sesquiterpenoids (valeric acid), epoxy iridoid esters (valepotriates) and their decomposition products such as baldrinal and homobaldrinal, amino acids (arginine, GABA, glutamine, tyrosine) and alkaloids. *Valeriana officinalis* also possesses small amounts of phenolic acids and flavonoids, valerosidatum, chlorogenic acid, caffeic acid, choline, $\beta$-sitosterol, fatty acids and various minerals [22].

Extracts from the roots and rhizomes of *Valeriana officinalis* have a long, cross-cultural tradition of medicinal usage, mainly as mild sedatives and anxiolytics [23], but they also demonstrate spasmolytic properties [24]. Root extracts also contain appreciable levels of GABA [25], constituents which bind to a variety of
neurotransmitter receptors [26] including GABA_A [27], where they perform as an allosteric modulator of subunit specific GABA_A channels [28], adenosine A1 receptors where they also exert a range of allosteric effects [29] and act on the 5HT_1A [30] and the 5-HT_3 receptors [31], which are implicated in circadian rhythms and anxiety. _Valeriana officinalis_ has also been reported to have a similar effect to benzodiazepines, with similar positive effects [32].

One other mechanism that may contribute to the sedative properties of _Valeriana officinalis_ could be the high levels of glutamine present in the extract. Unlike GABA, glutamine more effectively crosses the blood-brain barrier where it can be taken up by the nerve terminals and converted to GABA [16]. In general, _Valeriana officinalis_ is used for restlessness, sleeping disorders based on nervous conditions, mental strain, lack of concentration, excitability, stress, headache, neuralgia, fainting, nervous stomach cramps, colic, uterine spasticity and states of anxiety [33]. No health hazards are known in conjunction with the proper administration of designated therapeutic dosages. Gastrointestinal complaints can occur in rare cases, contact allergies in very rare ones. With long-term administration, the following can occasionally appear: headache, restless states, sleeplessness, mydriasis, disorders of cardiac function [12].

The only pharmacokinetic data available for _Valeriana officinalis_ are regarding the valpatriates which administered orally to mice are reported to be poorly absorbed, having an efficiency of 0.19% of the administered dose. Valpatriates were found in the stomach lining and in the intestines and uncharged valpatriates were reported in the stomach contents 15 hours after administration. Small amounts of valpatriates and their decomposition products were reported to be found in the blood, liver, kidneys, heart, lungs and brain [34]. For these reasons, we decided to administrate parenterally by IP route. Research into the pharmacological activity of _Valeriana officinalis_ has almost exclusively focused on its sedative and spasmylytic properties and it has been shown that the sedative and spasmylytic effects attributed to _Valeriana officinalis_ are due to the activity of multiple constituents [35-39].

In a study the essential oil of _Valeriana officinalis_ and the isolated components were screened for central nervous system effects on mice upon i.p. administration. The essential oil showed sedative and/or muscle relaxant activity with the oxygenated components exhibiting more activity than the hydrocarbon fraction. Valerenic acid also produced a dose-related increase in pentobarbital-induced sleep with 50 and 100 mg/kg i.p. [40]. It shows that valerenic acid may be a general central depressant rather than a neuroleptic or muscle relaxant which may be a key factor in treatment of convulsion. An anabolic extract of _Valeriana officinalis_ given to male mice (dose equivalent to 400 mg root/kg ip body weight) did not produce overt sedation or tranquilization, as was observed in mice treated with benzodiazepine and diazepam (a group of sedative-hypnotics) at 2 mg/kg. However, at the same dose the extract prolonged thiopental induced anesthesia and was anticonvulsant against picrotoxin, but not pentetrazol or harmar (ED50 of 24 mg root equivalent/kg body weight). So we can conclude that _Valeriana officinalis_ does not exert the same type of anti-anxiety effect as diazepam, although they did suggest an interaction with the GABA-A-benzodiazepine receptor complex. This indicates that although _Valeriana officinalis_ has not anti-anxiety effect as benzodiazepines, it may have anticonvulsant effect for its interaction with the GABA-A receptor which is one of the main factors in convulsion [41].

Several researchers have linked the effects of _Valeriana officinalis_ extracts and/or its components with an effect on the inhibitory neurotransmitter GABA [35]. GABA mediates sedation in the central nervous system. Benzodiazepines exert their actions via this system. Valerenic acid and acetyvalerenic acid have been reported to inhibit GABA transaminase, thereby prolonging the inhibitory effect of GABA [36]. Analysis of the content of the extract for amino acids revealed that the extract itself contained GABA in sufficient quantity (4.6 mM) to account for the displacement activity [42].

In another study, _Valeriana officinalis_ extracts showed no binding to benzodiazepine receptors isolated from rat brains. They did show an approximately 30% to 70% displacement of radiolabeled cyclohexyladenosine from adenosine receptors with high concentrations (0.01 to 1 mg/mL) of the hydroalcoholic extract [43].

**CONCLUSION**

In brief, the present study provides evidence for anticonvulsant activity of _Valeriana officinalis_ in the tonic seizure of PTZ model. Our experimental results show that _Valeriana officinalis_ extract increases the onset time of the classic tonic-clonic convulsion. On the other hand, there is a significant difference between each administrated dosage which may indicate the dosedependent effects of _Valeriana officinalis_. Totally, it seems that _Valeriana officinalis_
could be useful for treatment seizure alone or with other
classic drugs. Although the anticonvulsant activity of
*Valeriana officinalis* is an interesting finding more
studies are needed in order to investigate its exact
mechanism.

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