Effect of Plantago major L. extract on PTZ-induced seizure threshold in NMRI male mice

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ABSTRACT

Purpose and aim: Epilepsy is a common neurological disorder after stroke. Despite various anticonvulsants current research to discover new drugs with better efficacy and fewer side effects, continue. Herbs with natural variety of materials with different properties, this research are appropriate for field. In the present study Plantago major (P. major) extract is a powerful antioxidant. In the present study the effect of plant extract of Plantago on seizures induced by PTZ injection in male NMRI mouse. Methods: the experiment used 50 NMRI mouse (eight in each group) as follows: the control, control sham, control plus (50, 25, 10, 5 mg / kg) The mice in each group received a single injection of 10 mg/kg of PTZ (60 mg/kg body weight, intraperitoneally, n=10 in each group) a single injection. The animals were injected with (50, 25, 10, 5 mg / kg) P. major extract interperitoneally just 30 min before each PTZ injection. The animals group of control and sham received saline instead of P. major extract after treatment, the behavior of animals was recorded for 20 min. Data were expressed as mean ± standard deviation. In order to compare the groups, analysis of variance (ANOVA) was used. Results: The results of repeated injection of P. major extract (50, 25, 10 mg/kg) with the control group in the seizure threshold compared to the control group, there was a significant increase (p <0.05). Conclusion: These results are probably due to the effect of ethanol on Plantago is a GABAergic system.

KEY WORDS: Pentylentetrazole, Seizure, hydroalcoholic extract plantago major L, Mice

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, causing 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). Up to this day, 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 due to central nervous system (CNS) disorders (Ngugi K. et al., 2010). Principally, the reason of epileptic seizure disturbs between balance of stimulatory and inhibitory neurons. Glutamate and GABA neurotransmitters play the most important role (Coulter, 2001; Faingold, 2004; Lott, 2001). Epileptic drug therapy in most patients is based on experimental seizure classification. Because of diversity causes, the seizure drugs could 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. However, millions of people have uncontrolled epilepsy (Engel, 2001, Fisher and Boas, 2005). Despite the fact that 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 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.
Plantago Major belongs to the genus Plantago and family Plantaginaceae. Flavones are the main flavonoids in P. major (Kawashty et al., 1994; Nishibe et al., 1995). Flavones tend to replace flavonols in Plantago (Harborne & Williams, 1971).

Subgenera Plantago and Coronopus have a tendency to produce flavones, luteolin, and 6-hydroxyluteolin. Attempts have been made to use flavonoids as taxonomic markers in Plantago (Kawashty et al., 1994).

The tensions created by the Antagonist usually are used in rodents as PTZ, GABA receptor model of generalized seizures that due to the very high repeatability and to provide a basis for comparing different anticonvulsant chemical compounds under standard conditions are of the drug’s benefits. This pre-convulsant material has probably antagonized the client through alesertic interactions with the GABAA and it inhibits the Chloride ion currents created by GABA (Huang et al., 2001). According to the numerous reports about the hypnotic, Anexietolytic, analgesic and anti-stress effects of Plantago major, it also has anticonvulsant effects. Plantago major extract (P. major) can have the anticonvulsant effects due to its Witanoid along with GABAergic systems, it means that it can have similar effects as GABA and by opening the chloride channels and GABA hyperpolarizes neurons and blocks the convulsion in mice (Huang et al., 2001). So In this study the anticonvulsant effect of alcoholic P. major extract on pentylentetrazole (PTZ) induced seizure threshold in male mice has been studied.

**MATERIAL AND METHODS**

**Animals and experimental design:**

The experiment used 50 male NMRI mice each weighing approximately 30±5 g that was obtained from Academic Center for Education, Culture and Research (ACECR), Qom, Iran. Animals were divided into five groups in a controlled environment with 12 h light and dark cycles, 22-25°C, and humidity 45-50% humidity. The room was lighted from 06:00 to 18:00 hours.

Experiments were conducted. In the experiment, control PTZ and four treatment 50, 25, 10, 5 mg/kg P. major extract (injected was IP). Then the effect of Plantago major vehicle on the seizure threshold was evaluated for 30 min before administration of PTZ. To determine seizure threshold, PTZ (60 mg/kg) solution in normal saline was injected intraperitoneal.

**Methanolic extraction of P. major:**

P. major were collected in the month of May 2013 from desert of Kashan city (Figure 1). To obtain methanolic extract 2 kg powder P. harmala of ground was immersed in 10 liters 80% (v/v) aqueous methanol at room temperature for five days and filtered through Wattmann Filter Paper (No.42). Extraction by Barij Esans co. (Golkaran Ltd.), Kashan City. The extract was then transferred to a glass bottle and stored in refrigerator before use.

**Fig. 1: Plantago major L**

**Statistical analysis:**

Data were expressed as mean ± standard deviation. In order to compare the groups, analysis of variance (ANOVA) was used. P < 0.05 values were considered to be statistically significant.
**Results:**

Table 1 and figure 2 show that there is significant difference between control PTZ and in seizure threshold. Seizure threshold in mice treated with P.major doses of 50, 25, 10 mg/kg significantly increased (p<0.05).

**Table 1:** Effect of different doses of HydroAlcoholic Extract Plantago major on PTZ-induced Seizures threshold in Male Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>WSRE 50mg/kg</th>
<th>WSRE 25mg/kg</th>
<th>WSRE 10mg/kg</th>
<th>WSRE 5mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizure threshold</td>
<td>21.5±2.8</td>
<td>154.1±7.6*</td>
<td>120.1±6.3*</td>
<td>140.3±2.1*</td>
<td>19.7±4.3</td>
</tr>
<tr>
<td>Phase 1</td>
<td>162±6</td>
<td>43.10±6.3*</td>
<td>26.6±9.1*</td>
<td>43.4±3.8*</td>
<td>163±4.0</td>
</tr>
</tbody>
</table>

*Means followed by different superscript are significantly different (P<0.001)

**Fig. 1:** Seizures threshold (as seconds) in five groups of mice (10 in each): from left to right control (PTZ) and 4 experimental groups receiving 4 different doses of P.major extract (50,25,10 and 5 mg/kg).

**Fig. 2:** phase 1 (as seconds) in five groups of mice (10 in each): from left to right control (PTZ) and 4 experimental groups receiving 4 different doses of P.major extract (50,25,10 and 5 mg/kg).

**Discussion:**

In this study, Plantago major extract on PTZ-induced seizure threshold were evaluated. GABAA receptor is 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). 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( Ebrahimi HR, 2011). In this study, P.major increased PTZ-induced
seizure threshold dose-dependently. Since PTZ acts via GABAergic system (Hosseini, M., et al., 2009). Other researchers showed with biochemical studies that P. major 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 extract of this plant has withanoloid, which is a GABA degrading enzyme inhibitor. In addition, P. major extract inhibits reuptake (Dhuley, 1998) and increases GABA release in synaptosomes isolated from rat brain cortex (Homayoun et al., 2002; Ortiz et al., 1999). Also, suggested that P. major extracts has affinity to connect to benzodiazepines place in its receptor (Barnes et al., 2002b). In a similar study, it was shown that the withania somnifera root extract contain withanoloid and somniferin A, which is structurally similar to withanoloid substance that had potent anticonvulsant activity in humans and experimental animals (Isoherranen et al., 2003). These effects have used to treat insomnia, anxiety, stress and behavioral disorders (Andreatini et al., 2002; Walsh et al., 1984; Purves et al., 1997). These findings show that this herbal drug may interfere with the GABAergic receptor, and also increase GABA neurotransmitter, that could regulate its function and therefore opening chloride channels that lead to increase in the seizure threshold. Some investigators indicated the interactions involved in the modulation of a benzodiazepines receptor antagonist, flumazenil (Silva et al., 2009; Ugale et al., 2004). Of course, it is noteworthy that this plant has a lot of antioxidant compounds and perhaps, one of its anticonvulsant effects is due to these compounds. Antioxidants reduce free radicals in brain cells (Mou X, et al., 2011). As it was mentioned, the P. major extract has a high affinity for binding and stimulating the benzodiazepine receptors. Perhaps, the anticonvulsant mechanism of P. major extract is binding and stimulating Benzodiazepine receptors and boosting the GABAergic system in the brain. This can justify the anticonvulsant effect of this plant against PTZ (Samini et al., 2005). These findings show that this herbal drug may interfere with the GABA receptor, and also increase GABA neurotransmitter, that could regulate its function and therefore opening chloride channels that lead to increase in the seizure threshold. Based on results of this study it can be concluded that intraperitoneal prescription of the P. major extract with doses of 50, 25, 10 mg/kg on male mice dose dependently increased the seizure threshold. The main mechanism of increasing the threshold of PTZ-induced seizure due to P. major extract, is probably through GABAergic inhibitory neurotransmitter system. Further investigations are needed to find the other mechanisms involved in the anticonvulsant activity of the extract.

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