Evaluation of Anxiolytic Effects of Plantago Major Leaf Extract in Male Albino Mice

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ABSTRACT

The present study aimed to investigate the effect of Plantago Major leaf extract (PMLE) on the anxiety level of laboratory mice. Forty male albino mice were divided into four groups three experimental groups and one control group each of 10 mice. The mice of the experimental groups were injected with three doses of 100, 200 and 300 mg/kg, and normal saline solution was injected intraperitoneally to the mice of the control group. To assess the level of anxiety, an elevated plus maze (EPM) device was used for 5 min. The standard indicators of anxiety assessment (number of entries and time spent in the open arm) were checked and recorded through observation. The results showed that PMLE in three doses of 100, 200 and 300 mg/kg, significantly increased the number of entries into the open arm (4.63±0.32, 5.37±0.41, 6.68±0.48) and the time spent in the open arm (53.1±3.88, 62.4±2.61, 69.58±2.02) compared to the control group (P<0.05). This study showed that PMLE can reduce the level of anxiety in mice.

INTRODUCTION

Anxiety is a type of internal response to the threatening conditions of people’s material and spiritual situations (Karim et al., 2015). Natural anxiety is an adaptive emotional response to physiological, psychological and social stressors that every person experiences in life (Sadaf et al., 2020). Pathological anxiety is the most common mental disorder that causes disruption in daily life and human suffering. Benzodiazepines and barbiturates are used to treat it (Finn et al., 2003). Anxiety is a diffuse, unpleasant and vague feeling, panic and anxiety of unknown origin and includes uncertainty, helplessness and physiological arousal, which is accompanied by one or more physical sensations such as nausea, chest tightness, heart palpitations, sweating and headache (Johnston et al., 2003). Several receptors such as serotonin, GABA, catecholamines and sex hormones are involved in the process of modulating this phenomenon (Enoch, 2008). GABA is the most important inhibitory neurotransmitter in the brain. The binding of GABA to the receptor opens the chloride ion channel and inhibits neuron hybridization (Kittler and Moss, 2003). On the other hand, glutamate is the most important excitatory neurotransmitter in the central nervous system and its effects are exerted through receptors in the membrane called ionotropic receptors and metabotropic receptors. The concentration of glutamate in the nervous system is much higher than in other tissues of the body, and it is involved in synaptic transmission, long-term changes in the excitability of nerve cells, and the development of nerve cells. Despite the great effects of glutamate on the physiological function of nerve cells, this compound is also a strong neurotoxin and is involved in many disorders of the central nervous system, including neurodegenerative disorders, ischemia, and trauma (Shahraki, 2011).

Oxidative stress is caused by an imbalance between the production of free radicals and reactive oxygen species on the one hand and the antioxidant defense system on the other hand. In order to deal with free radicals and reactive oxygen species, antioxidant defense mechanisms have been designed to neutralize or minimize the harmful effects of these aggressive agents. Some of the components of this defense system are superoxide dismutase, glutathione peroxidase and catalase enzymes. Some are also supplied...
through different foods. Oxidative stress is an effective factor in causing various diseases in the central nervous system, which can accelerate the aging process and anxiety-related behaviors (Miladi Gorgi et al., 2010).

In the past, drugs from the benzodiazepine family such as Diazepam (Valium), Alprazolam (Xanax), Lorazepam (Ativan) and Buspirone were used in the treatment of anxiety disorders, and now antidepressants such as Sertaline (Zoloft), Paroxetine (Paxil), Fluoxetine (Prozac) and Venlafaxine are more commonly used. The prescription of anti-anxiety drugs is a well-known phenomenon all over the world. It is estimated that between 10 and 20% of people in the Western Hemisphere use these drugs almost regularly. According to the official statement of FDA (American Food and Drug Administration) in 1978, ten million Americans have used benzodiazepines. Some believe that these sedatives are an effective and inexpensive method compared to other treatment methods. Some also emphasize the effective effects of non-drug treatments. Non-pharmacological treatment includes several techniques. In all these methods, the patient is taught the classical techniques of obtaining relaxation, and in this way, the body itself rises to deal effectively and directly with stress-causing factors.

The use of herbal therapy has been common in ancient civilizations since ancient times, and today herbal therapy in various forms is common all over the world, including the use of herbal products or their whole extracts, and special attention has been paid to herbal therapy. Most aromatic plants are edible and have been used for thousands of years. Since there is a positive trend and public attention for the use of medicinal plants that form a major part of traditional Chinese medicine, it will be useful to identify and investigate their effects and reduce the complications caused by chemical drugs and invasive procedures (Vafaei et al, 2011; Miladi Gorgi et al, 2010; Rezaie et al., 2010). Vafaei et al (2011) showed that fruit Cassia fistula aqueous extract with doses of 250 and 500 mg/kg of body weight reduce the anxiety level in small laboratory mice. Miladi Gorji et al. (2010) in a study used hydroalcoholic extract of water spinach leaf in doses of 0.05, 0.10 and 0.15 mg/kg of body weight and their test results showed that this extract has anti-anxiety effects in higher doses. In the study of Rezaie et al. (2011), Nardostachys Jatamansi extract with the highest response at a dose of 200 mg/kg of body weight showed better sedative and anti-anxiety effects compared to diazepam in female Wistar rats.

Plantago is one of the medicinal plants that is widely used in traditional medicine. Plantago major and Plantago lanceolate are used. Plantago major contains biologically active compounds such as alkaloids, polysaccharides, lipids, caffeic acid derivatives, flavonoids, glycosides, iridoid, terpenoids, fatty acids, mucilage, organic acids, polyaccharide, plantagin substance, carotenoid, equine, catalpine, saponin, invertin, epigenin, sorbitol, minerals, vitamins, tannin, resin and gum, and flavonoid compounds are one of the most characteristic compounds in Plantago (Genc et al., 2020). Plantago major has also been used in traditional medicine as an anesthetic, antiviral, anti-inflammatory, astringent, antiparasitic, pain reliever, mental stimulant, antihistamine, anti-rheumatic, anti-tumor, anti-ulcer, diuretic, expectorant and blood pressure lowering. The seeds of this plant have been used for a long time for disinfection, immune system modulator, anti-inflammatory, pain reliever, antimicrobial, anti-ulcer, antioxidant, anti-cancer agent and also for wound healing purposes (Mohamed et al., 2011).

The studies conducted on the chemical compounds found in Plantago plant show that Plantago leaf contains phenolic groups, organic acid groups, flavonoids and terpenoids. Also, Plantago plant leaf is a rich source of essential fatty acids omega-6, omega-3, carotenes and vitamin C (Schmitt-Schilling et al., 2005). The presence of such compounds has led to the emergence of numerous therapeutic properties. Among these effects, it can be mentioned to increase the efficiency of the immune system, liver protective effects, anti-diarrheal effects, analgesic effects, antioxidant effects, anti-cancer effects, cytotoxic activity, wound healing effects, anti-inflammatory effects, and fatigue reducing effects, the effects of detoxification and elimination of external toxins, antibacterial effects, anti-yeast and recently anticonvulsant effects of Plantago (Barua et al., 2011; Beara et al., 2010; Nilsson and Sterner, 2011). Since the anti-anxiety effects of Plantago plant have not been investigated in mice, the present study aims to determine the effect of Plantago Major leaf extract (PMLE) on the level of anxiety in mice.

MATERIALS AND METHODS

Animals
In the present experimental study, 40 male albino mice weighing 25-30 g were used. The mice were kept in special cages under optimal environmental conditions and temperature of approximately 22 degrees celsius and 12 h of light and 12 h of darkness, and they had free access to food and water all the time.

Preparation of herbal extract
To prepare the extract, first the dry leaves of Plantago major plant were ground. Then, for every 10 g of powder, 100 ml of 70% ethyl alcohol was added and soaked for 72 h in a dark environment. After passing through Whatman
No. 1 filter paper, the extract was spread on a plate for evaporation at room temperature. Normal saline was used to dissolve the dried extract powder (Nhu et al., 2019). After the intraperitoneal injection of the extract to different groups, the animals were monitored for 48 h and the result of 48-h mortality was checked.

**Anxiety assessment**

To evaluate the level of anxiety, a device called Elevated Plus Maze (EPM) was used, which is a standard model for evaluating the level of anxiety in rodents. This device is made of wood and includes two open arms (each 5 x 50 cm) and two closed arms (each 40 x 50 x 50 cm) and a central shelf (5 x 5 cm); so that the open arms are facing each other and the closed arms are opposite each other and are placed about 50 cm above the floor of the room. This experimental model of anxiety assessment is unconditional and does not require the training and learning of the animal. In this experiment, three groups (n=10 each group) received different doses of PMLE (100, 200 and 300 mg/kg of body weight) intraperitoneally. The animals of the fourth group (control group of 10 mice) were also injected with the same volume of normal saline. Thirty min after the injection of the extract and 5 min before the experiment, each mouse was placed separately inside a box with black walls made of Plexiglas with dimensions of 30x40x40 cm to increase the explorative activity of the animal. Then, to assess the level of anxiety, the animal was placed in a EPM (in the palm part and facing the open arm), and for 5 min during the day, explorative activities in the time spent on open arms and the number of entries into open arms were directly evaluated and recorded. It is worth mentioning that the increase in the number of entries into open arms and the time spent on them is considered as an indicator of reducing anxiety in mice. Also, the judgment about the significant difference in the level of anxiety is that if at the same time both indicators (the time spent on open arms and the number of entries into open arms) decrease or increase in the same direction and at least one of them has a significant difference from the control group, it is considered as a significant change in the level of anxiety.

**Statistical analysis**

The data were analyzed using SPSS software (version 20) and one-way analysis of variance (ANOVA) and Tukey’s post hoc tests. The significance level was defined as P<0.05.

**RESULTS AND DISCUSSION**

There was no significant statistical difference between the three experimental groups and one control group in the number of entering into open arms and the percentage of time spent on open arms. Comparison of the average of number of entering into open arms and time spent on open arms, in three intervention groups compared to the control group are given in Table 1 (P<0.05). Mice receiving doses of 100, 200, and 300 mg/kg of PMLE did not show statistically significant differences compared to the control group. In all three experimental doses, the number of entries into open arms and the percentage of time spent in on open arms increased significantly compared to the control group; in such a way that in doses of 100, 200 and 300 mg/kg, the average of the number of entering into open arms was 4.63±0.32, 5.37±0.41, 6.68±0.48 respectively and the percentage of time spent on open arms was 53.1±3.88, 62.4±2.61, 69.58±2.02, respectively.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Control group</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of entries into open arms</td>
<td>2.12±0.46</td>
<td>4.63±0.32</td>
<td>5.37±0.41</td>
<td>6.68±0.48</td>
</tr>
<tr>
<td>Time spent on open arms</td>
<td>19.83±0.33</td>
<td>53.1±3.88</td>
<td>62.4±2.61</td>
<td>69.58±2.02</td>
</tr>
</tbody>
</table>

Group 1, 100mg/kg; Group 2, 200mg/kg; Group 3, 300mg/kg.

This study was conducted to investigate the anti-anxiety effect of PMLE in a laboratory environment. PMLE in doses of 100, 200 and 300 mg/kg led to a significant decrease in anxiety compared to the control group. This study showed that PMLE increases the time spent on high open arm and also increases the number of entering into open arms. Previously, Caro et al. (2018) in a similar study pointed out phenolic compounds such as flavonoids and tannins with pentobarbital sodium-induced hypnosis using the sodium pentobarbital-induced hypnosis method and showed that these compounds have a calming effect. Kaviani-fard et al. (2016) in their study investigating the effect of hydroalcoholic extract of Plantago major on the seizure threshold in mice, pointed to penteline tetrazole and showed that epileptic seizures caused by the effect of penteline tetrazole reduced GABA receptors and probably has antiepileptic effects through the effect on the GABA system. In an experimental study, Mojtabahedin (2016) has investigated the anxiolytic effects of hydro-alcoholic PMLE and the possible role of the GABA adrenergic system in rats. The results of its findings showed that the
hydroalcoholic extract of Plantago major leaf has anti-anxiety effects due to the presence of flavonoid compounds through the GABAergic system. Therefore, it can be said that, as the soothing effects of Plantago major have been mentioned in traditional medicine, the compounds in the extract of this plant have anti-anxiety effects.

Researches have shown that the ingredients of Plantago major include compounds such as phenolics, terpenoids, vitamin C and various flavonoids (Mojtahedin, 2016). Many plant extracts with the similar compounds showed sedative effects (Chan et al., 2010). Studies show that these compounds activate the GABAergic system (Santos et al., 1994). GABA is known as an inhibitory neurotransmitter in brain synapses and is mostly secreted by nerve terminals in the spinal cord, cerebellum or other basal ganglia in the brain. GABA is one of the most important inhibitory chemical mediators that acts by increasing the permeability of the nerve membrane to chloride ions (Yuan et al., 2004).

Research shows that there are many flavonoid derivatives that are ligands for the GABAa receptor in the central nervous system and by binding to the binding points of drugs such as benzodiazepines, they lead to suppressive activities in the central nervous system of mice (Huang et al., 2001). For this reason, flavonoids, which like diazepam have the ability to interact with GABAA receptors in the central nervous system (Shen et al., 2002), are known as plant benzodiazepines (Hanrahan et al., 2003; Aslam and Sultana, 2016; Atack, 2010; Campbell et al., 2004; Gupta et al., 2016; Jamilah et al., 2012). In addition, flavonoids such as coumarin and limonene are able to exert their anti-anxiety and sleep-inducing effects by binding to the GABAA receptor and releasing GABA.

**CONCLUSION**

This study showed that PMLE reduces the level of anxiety in mice, and the anti-anxiety effects of Plantago major can be attributed to the activation of the Adrenergic GABA system.

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**IRB approval**

This study was approved by the Advanced Studies Research Board of the Fourth Affiliated Hospital of Harbin Medical University, Harbin, China.

**Ethical approval**

In the present study, the use of laboratory animals was in accordance with international guidelines for the care and use of laboratory animals. Also, this study was conducted based on the ethical protocol of working with laboratory animals of the Fourth Affiliated Hospital of Harbin Medical University, China.

**Statement of conflict of interest**

The authors have declared no conflict of interest.
Effects of Plantago Major Leaf Extract on Anxiety in Mice


