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Study of anxiolytic effect of hydro-alcoholic leaf extract of *plantago major* L. in rats and interaction with epinephrine: Role of Adrenergic system

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ABSTRACT

In this study, the effects of hydro alcoholic extract of plantago major in rats were examined on the level of anxiety. In this experimental study, 30 male Wistar rats were used. Animals in groups of six rats including the control group received normal saline, group receiving epinephrine 1 mg/kg, groups receiving different doses of the extract (100 and 200 mg/kg), the group receiving pretreatment with epinephrine 1 mg/kg and then 100 mg/kg of extract. Elevated Plus-Maze test was used to assess anxiety. The results showed that plantago leaf extract at doses of 100 and 200 mg/kg significantly ($p<0.05$) created the anxiolytic effects and epinephrine prevented the anxiolytic effects of plantago. According to the results of this study, it seems that flavonoid compounds in plantago major leaf extract such as apigenin and its interaction with the adrenergic system is able to create anxiolytic effects.

Key words: *Plantago major* L., Anxiety, Adrenergic system, Elevated plus Maze, Rat.

INTRODUCTION

Anxiety is the result of complex interactions that are looking to make changes in brain function and neuroendocrine processes begin [1]. Hypothalamic-pituitary-adrenal axis is associated with anxiety disorders. Several studies show neurotransmitters systems and neuronal circuits in different parts of the brain such as the limbic system components play an important role in mediating anxiety [2, 3]. It was found that the incidence of anxiety disorders, nervous and hormonal numerous factors such as GABA, serotonin, norepinephrine, dopamine, cholecystokinin and centers such as the amygdala and hippocampus are involved [4]. More studies on the mechanism of action of neurotransmitters involved in anxiety focused on serotonin, norepinephrine and GABA, and drugs can change the levels of these neurotransmitters, or interfere with the action of their receptors, create anxiolytic effects [5]. Typically anxiolytic drugs include benzodiazepines that facilitate the inhibitory action of GABA and other categories involved serotonin reuptake inhibitors and beta-adrenergic blockers that this blockers reduce the effects of adrenaline and noradrenaline [6]. The noradrenergic system is of great importance in the modulation of anxiety. Anxiety caused a significant increase in the release of norepinephrine in different brain areas such as the locus coeruleus, hypothalamus, hippocampus and amygdala [1]. On the other hand the use of medicinal plants in reducing anxiety and improving sleep disorder also traditionally has been used [7]. One of the medicinal plants that are used in traditional medicine is *plantago major* L. (Plantaginaceae). Seeds and leaves of the plant contain phenolic compounds (Caffeic acid derivatives), flavonoids, alkaloids, terpenoids and vitamin C [8]. The presence of such compounds has been caused numerous health benefits. Some of these effects can be used 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, anti-inflammatory effect, reducing fatigue, detoxification effects, anti-bacterial and anti-yeast and anti-convulsant effects noted [9, 10]. Given that in the literature review the anxiolytic effects of *plantago major* was not observed; therefor, in this study the effects of *plantago major* leaf extract on anxiety level and its interaction with the adrenergic system with elevated plus maze in animal models of rats was performed.

MATERIALS AND METHODS

Animals

In this experimental study, 30 healthy male Wistar rats weighing 220–250g were used. Rats were maintained in groups of six per cage in 12-hours light-dark cycle (light on at 07:00) at controlled ambient temperature (20–23 °C) with *ad libitum* food and water. All experiments on animals were performed with observing registered and international ethics for working with laboratory animals and according to the guideline of National Institute of Health. A week before the test, all rats were stroking daily for 5 min to prevent additional stress during the test.

Preparation of Extract

The leaves of the plants were air dried in shade. These were then powdered and ethanol extracts were prepared using 70% ethanol percolation method [1] followed by evaporation in a rotator evaporator under controlled temperature and reduced pressure. Normal saline was used to prepare different amounts of the extract in mg/kg.

Experimental groups

Group (I): received normal saline. Group (II): received epinephrine 1 mg/kg. Group (III): received different amounts of the extract (100 and 200 mg/kg). Group (IV): received pre-treatment with epinephrine 1 mg/kg and then 100 mg/kg of extract. In all groupsintraperitoneal (i.p) injection 30 min before the test was performed.

Assessment of anxiety

To assess the level of anxiety Elevated Plus Maze that the standard model to assess the level of anxiety in rodents was used. The device is made of wood consists of two open arms (10 × 50 cm) and two closed arms (40 × 10 × 50 cm) and elevated to a height of 50 cm. Rats were placed individually in the centre of the plus maze, facing the open arm. The times spent in closed and open arms and the numbers of entry into each of the arms during 5 min test period were recorded visually [11].

Statistical Analysis

The data obtained in this study using SPSS 19 software and one-way analysis of variance (ANOVA) and followed by Duncan test were analyzed. $P<0.05$ were considered statistically significant.

RESULTS

The results obtained from the present study indicated that ethanol extracts of *plantago major* at doses of 100 and 200 mg/kg increased significantly ($p<0.05$) in the number of entries in open arm compared to the control group. So that the 100 mg/kg showed further increase in the number of entries in open arm compared to the 200 mg/kg. This indicator was significantly reduced anxiety in animals. Epinephrine 1 mg/kg alone compared to control group showed no significant effect. While the administration of epinephrine before extract compared to extract alone significantly ($p<0.05$) prevented from entering the open arms (Fig. 1).

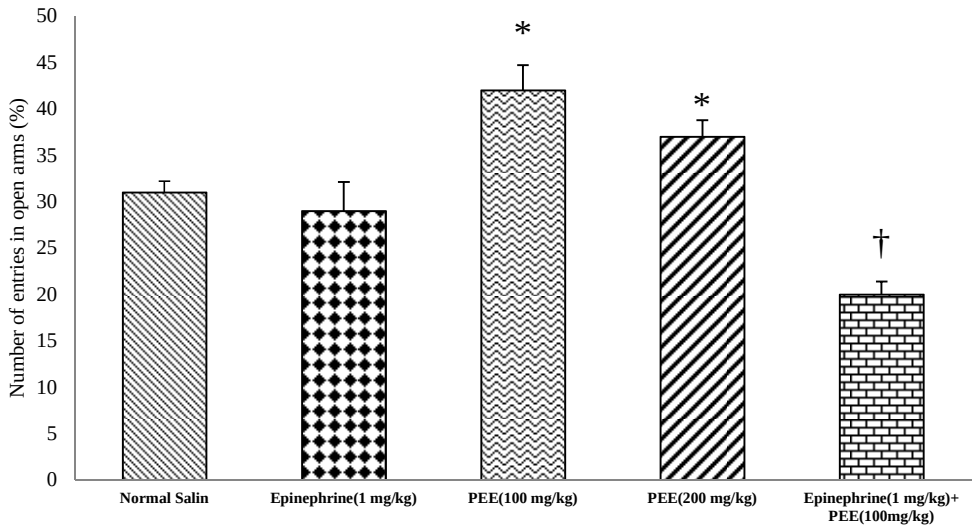

Fig.1. Effects of the *Plantago major* L. leaf extract (100 and 200mg/kg, i.p) and epinephrine (1mg/kg, i.p) on number of entries in open arms in the elevated plus maze test in rats. Data expressed as Mean ± SEM. * p<0.05 compared to the control group, † p<0.05 compared to 100mg/kg of extract alone. (n=6)

Also doses of 100 and 200 mg/kg of extract significantly increased (p<0.05) in the time spent in open arms compared to the control group. Doses of 100 mg/kg, showed highest increase in the time spent in the open arms. Epinephrine alone did not significant effect on the time spent in open arms compared to the control group. While co-administration epinephrine with 100 mg/kg of extract compared to the extract alone significantly (p<0.05) reduced the time spent in the open arms (Fig. 2).

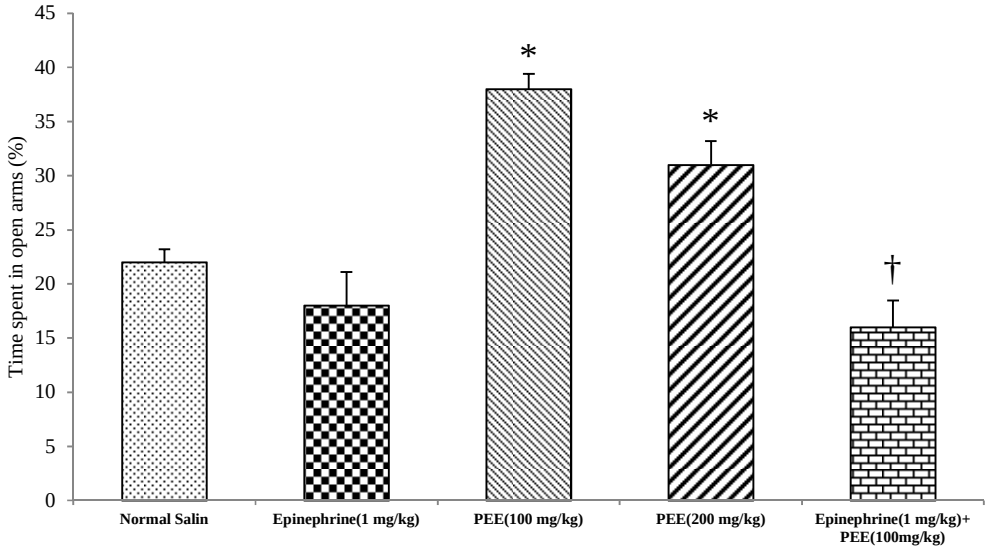

Fig.2. Effects of the *Plantago major* L. leaf extract (100 and 200mg/kg, i.p) and epinephrine (1mg/kg, i.p) on time spent in open arms in the elevated plus maze test in rats. Data expressed as Mean ± SEM. * p<0.05 compared to the control group, † p<0.05 compared to 100mg/kg of extract alone. (n=6)

DISCUSSION

The results obtained from the present study indicated that intraperitoneal injection of 100 mg/kg of *plantago major* ethanol extract significantly the percentage of time spent and the number of entries in open arms compared to the control group. Due to the increased presence in the open arms and the time spent in it, an indicator of anxiety in animals; therefor the extract showed anxiolytic effect. Although no study has been done in relation to the anxiolytic effects of *plantago major*, but researches suggests that this plant has many health benefits which can be somewhat explain the anxiolytic effect. Studies on the chemical composition of the *plantago major* shows leaves containing phenolic groups, organic acids, flavonoids and terpenoids as well as a rich source of ω-6 and ω-3 essential fatty acid, carotene and vitamin C. Ferulic acid (FA) in the seeds of *plantgo major* is also a natural phenol as an antioxidant, has shown anti-tumor effects in cancer of breast and liver [10, 12]. Also FA due to antioxidant effect decreased cell death in neurons of the hippocampus [13].

On the other hand, in a study FA reduced the duration of immobility in the forced swimming tests and tail immersion test in mice and has shown antidepressant effect. FA by inhibiting the activity of monoamine oxidase-A (MAO-A) enzyme can be increased monoamines. Given that the MAO major role in the pathogenesis of psychiatric disorders, especially depression and anxiety [14] so because of MAO-A inhibitor activity of FA in *plantago major*, antidepressants and anxiolytic effects in this way can be created. The role of vitamin C in the prevention of depression and anxiety has been noted in several studies. So that the systemic administration (intraperitoneal and oral) and central (ICV) infusion of vitamin C in the tail immersion test has produced antidepressant effects in mice [15, 16, 17]. Therefore, it seems that part of the anxiolytic and antidepressant effects of *plantago major* can be related to the presence of vitamin C.

Studies show that brain tissue is very vulnerable to oxidizing agents due to high consumption of oxygen. Increased oxygen consumption may lead to oxidative stress. Normal cells have numerous mechanisms to protect against these attacks. In addition glutathione and vitamins C and E, the main cellular defense is done through oxidative enzymes and flavonoids [18]. Consuming foods rich in flavonoids protect humans against diseases associated with oxidative stress such as heart disease and cancer [8] .GABA, an inhibitory neurotransmitter in the central nervous system is very important and has a variety of receptors including type A. Several studies in medicinal plants have shown that flavonoids bind to $GABA_A$ receptors and increase the inhibitory effects of GABAergic system, and create sedative, anticonvulsant and anxiolytic effects [6, 19, 20]. Of course, flavonoids such as quercetine and apigenin are doing their anxiolytic effects on GABA receptors with different mechanism. Apigenin is able to cross the blood-brain barrier and as a positive allosteric regulator, enhance the effects of GABA on the GABA A receptor, and unlike benzodiazepines do not cause dependency [21]. Recent studies have shown a protective effect of apigenin in amyloid-β induced toxicity and therefore could be useful in the treatment of Alzheimer's disease.

On the other hand apigenin has shown antioxidant effects in kainate model of excitotoxicity by removing oxygen metabolites in the hippocampal neurons and anxiolytic and antidepressant effects in behavioral models. Also the role of adrenergic, dopaminergic and serotonergic system in moderating activity of apigenin has been proved [22, 23]. It is well known that depression is related to the deficit of monoamines such as epinephrine, norepinephrine, dopamine and serotonin at critical synapses [24]. MAO is a flavin enzyme located in the outer membrane of mitochondria in all cells in the body. This enzyme catalyzes the oxidative deamination of biogenic and xenobiotic amines. MAO has a crucial role in the metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues. MAO is classified into types A and B.

MAO-A oxidizes the serotonin, epinephrine and norepinephrine and MAO-B metabolize dopamine. Several studies show that increased activity of mitochondrial MAO-A throughout the brain during major depression [25]. Recent studies have suggested an essential role of MAO-A inhibitors in the treatment of depression and anxiety disorders, because it increases the level of monoamine such as norepinephrine, serotonin and dopamine in neuron synaptosome, and significantly reduce symptoms of depression and anxiety [26, 27]. Studies suggest that some natural phenolic compounds such as xanthons and isocumarins and flavonoids to have considerable inhibitory activity against mouse brain MAO [24]. Flavonoids such as apigenin, chrysin, luteolin, kaempferol and epicatechine demonstrate their antidepressant and anxiolytic effects, mainly through the inhibition of MAO and increase bioamines [26]. Several studies show apigenin more effective in inhibiting MAO-A as compared with MAO-B activity and increase the production of catecholamines and norepinephrine activity [23, 28].

CONCLUSION

In the present study, epinephrine alone had no significant effect on the number of entries and time spent in open arms, while co-administration with extract, reduced anxiolytic effects of extract. Given that the *plantago major* also contains numerous flavonoids such as apigenin [12], therefore, it seems that anxiolytic and sedative properties of *plantago major* was applied with influence on the adrenergic system. Further studies are in progress in order to enable us to understand the precise action mechanisms of anxiolytic effect of *Plantago major*.

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