Effects of ethanolic extract of pine needles (*Pinus eldarica* Medw.) on reserpine-induced depression-like behavior in male Wistar rats

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ABSTRACT

Background: In this study, the antidepressant activity of ethanolic extract of *Pinus eldarica* Medw needles was assessed using forced swimming test (FST) in rats. **Materials and Methods:** Male Wistar rats were randomly divided into six Groups and treated as follows: first group was received only reserpine (6 mg/kg, i.p.), second group was received reserpine (6 mg/kg, i.p.) and imipramine (10 mg/kg, i.p.), three experimental groups received reserpine (6 mg/kg, i.p.) and three doses of pine needle extract (100, 300, and 500 mg/kg, p.o.) respectively and the final group (control group) received only vehicle (5% DMSO, i.p.). **Results:** Acute oral administration of ethanolic extract of *P. eldarica* Medw needles at a dosage of 300 mg/kg reduced reserpine-induced increase in immobility time in the FST, demonstrating an antidepressant effect in the FST. Additionally, extract treatment did not modify the ambulation and rearing evaluated in open field test, indicating that antidepressant effect found in the forced swimming test was not based on the stimulation of locomotor activity. **Conclusion:** These results indicate that ethanolic extract of *Pinus eldarica* needles possesses an antidepressant activity.

Key words: Antidepressant activity, depression, forced swimming test, open-field test, *Pinus eldarica* Medw., reserpine

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INTRODUCTION

In recent years, depression has become recognized as a major public health problem. It is estimated that in the U.S., approximately 20% of the population have some symptoms of depression, and around 2–5% are thought to suffer from severe forms of depression.[1] Understanding how to prevent and treat depression is therefore an urgent subject. Although the mechanism provoking depression has not been clearly elucidated, one main trigger is known to be exposure to chronic stress.[2,3] Many types of antidepressant drugs, such as tricyclic antidepressants and selective serotonin-reuptake inhibitors (SSRIs), as well as antidepressant herbal medicines like St. John's wort, are used to treat depression. However, most of the synthetic drugs are not without side effects.[4] Furthermore, disturbances of the drug-metabolizing enzyme systems were revealed with St. John's wort,[5] and thus, the search for new herbal antidepressants with fewer side effects is important.

Pinus eldarica Medw. is an evergreen tree that naturally occurs in the Transcaucasian region between Europe and Asia, and grows also in Iran, Afghanistan and Pakistan.[6] Various parts of this tree (e.g., needles, buds, resin and tar) have been widely used in traditional medicine for the treatment of bronchial asthma,[7] skin wounds, skin irritations, allergic rashes and dermatitis[8] in Russia and the Central Asian countries. Pine needles are rich in terpenoids, polyphenols and tannins.[9] Extracts of pine needles have diverse physiological and pharmacological actions. Experimental data pertaining to the beneficial properties of *Pinus* in general relate to their anti-inflammatory,[10] antioxidant,[11,12] antineoplastic[13,14] and immuno-modulatory properties.[13,10] These properties are related to the effects of pine extracts on cyclo-oxygenase activity,[14] prostaglandin E2 production,[15,12] nitric oxide synthesis[15-17] and regulation of cancer-related proteins.[13] In this study, we evaluated the

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antidepressant activity of ethanolic extract of *P. eldarica* needles using forced swimming test (FST) in rats.

MATERIALS AND METHODS

Plant material

P. eldarica Medw needles were collected in March 2009 in the city of Mashhad, Iran, and identified by Ms. Soozani (School of Pharmacy, Mashhad University of Medical Sciences). A voucher speciment (No.11945) was deposited at the herbarium of School of Pharmacy, Mashhad University of Medical Sciences.

Prepration of alcoholic extract

The pine needles were air dried and powdered. Five hundred grams of powder was separated and extracted with 70% ethanol at room temperature for 48 h. Then, the solution was filtered. This procedure was repeated three times and the resulting extracts were combined. The combined extract was concentrated *in vacuo* (40ºC) and the residue was dried at 40ºC in incubator. This dried sample was stored at 4ºC until use.

Animals

Male adult Wistar rats, weighing 250–300 g were purchased from Pasteur Institute of Iran and were maintained on a 12 h light/dark cycle in a temperature-controlled (22 ± 1°C) colony with access to water and food *ad libitum*. Before the beginning of the experiment, animals were allowed to adapt to the environment for 2 week. All experiments were carried out between 09:00 a.m. and 4:00 p.m. The experimental protocols were approved by the local institutional committee for animal ethics.

Drug treatment

Reserpine (Sigma-Aldrich, USA) at a dose of 6 mg/kg was dissolved in 5% dimethyl sulfoxide (DMSO) and imipramine (Marham Darou co., Iran) at a dose of 10 mg/kg was dissolved in 0.9% normal saline. Both drugs were administered intraperitonealy (i.p.) in a constant volume of 1 ml/kg. Different doses of ethanolic extract of pine needles (100, 300 and 500 mg/kg, respectively) were dissolved in 5% DMSO (10 ml) and administered by oral gavage (p.o.). Control animals received only 5% DMSO (1 ml/kg, i.p.) as vehicle.

Forced swimming test

The procedure used has been previously described by Porsolt *et al*,[18] Detke and Lucki.[19] Briefly, the rats were randomly divided into six groups (ten in each group) and treated as follows: first group was received only reserpine (6 mg/kg, i.p.), second group was received reserpine (6 mg/kg, i.p.) and imipramine (10 mg/kg, i.p.), three experimental groups received reserpine (6 mg/kg, i.p.) and three doses of pine needle extract (100, 300 and 500 mg/kg, p.o.), respectively and the final group (control group) received only vehicle (5% DMSO, i.p.).

The rats were placed individually in vertical Plexiglass cylinder (50×20 cm) containing water (25°C) at a depth of 30 cm for a 15-min period (pre-test session). At the end of this pre-test session, each rat was removed from the water, partially dried with a towel and placed in a warm enclosure, and afterwards returned back to their home cages. Twenty-four hours later, the animals were exposed to the same experimental conditions outlined above for only a 5-min period (test session) and their behavior was videotaped with videorecorder placed above the cylinder. The total duration of immobility in each rat was measured during the test session. Immobility was assigned when no additional activity was observed other than that required to keep the rat's head above the water. The behavioral assessments were performed by observers blind to experimental treatment. Reserpine and vehicle were administered 24 h before the test session. Imipramine and different doses of pine needle extract were administered 24, 5 and 1 h before the test session.

Open-field test

Locomotor activity was measured in the open-field test. Twenty-four hours after the last treatment, all subjects were tested in the open-field apparatus. The apparatus consisted of a square arena (100×100 cm), with a 40-cm high, opaque, white wall. The floor was divided into 25 equal square. The rats were individually placed in the center of the arena and the following behavioral parameters were measured over 5 min: Locomotion (number of squares crossed) and rearing frequencies (number of times that the rat stands on its hind legs). A square crossed was defined as the rat placing its four paws into the quadrant and going to the adjacent quadrant. The open field was cleaned with a water-alcohol (10%) solution before behavioral testing to avoid possible bias due to odors and/or residues left by rats tested earlier. All experiments were carried out in a quiet room under controlled light conditions between 10:00 a.m. and 12:00 a.m.

Statistical analysis

All the data were analyzed using one way analysis of variance (ANOVA) followed by Tukey- Kramer's multiple comparison test. *P*-values <0.05 were considered significant.

RESULTS

Forced swimming test

Reserpine (6 mg/kg, i.p.) significantly ($P<0.01$) increased immobility time in FST as compared to the control

group [Figure 1]. imipramine (10 mg/kg, i.p.) significantly (P<0.001) antagonized reserpine-induced increase in mean immobility time in FST [Figure 1].

The oral administration of pine needle ethanolic extract at dosage of 300 mg/kg significantly (P<0.001) decreased mean immobility time in rats pretreated with reserpine as compared to the animals received reserpine only. The same effect was not observed at doses of 100 mg/kg and 500 mg/kg of pine needle extract (P>0.05) [Figure 1].

Moreover statistical significant increase (P<0.05) in mean immobility time was observed with dose of 100 mg/kg pine needle extract as compared to imipramine-treated group [Figure 2]. There was no significant difference (P>0.05) in mean immobility time between imipramine and other doses of pine needle extract-treated groups [Figure 2].

There was no significant difference (P>0.05) in mean immobility time between control group, and different doses of pine needle extract-treated groups [Figure 3].

Open-field test

Significant differences in the number of crossed squares, and the number of rearing times between groups had not been found (P>0.05) [Figures 4 and 5].

DISCUSSION AND CONCLUSION

In the present study, we investigated the antidepressant effect of ethanolic extract of *P. eldarica* Medw needles using FST in rats. This test is the most widely used tool for assessing antidepressant activity preclinically. Immobility in this model is reduced by a variety of treatments which are therapeutically effective in depression.[18]

In this study, the oral administration of ethanolic extract of *P. eldarica* Medw at the dose of 300 mg/kg reversed reserpine-induced increase in immobility time in the FST, demonstrating an antidepressant effect in this model. Reserpine is a vesicular monoamines re-uptake blocker which depletes monoamines in the brain, and produces depression-like syndrome in animals.[20] Since reserpine-induced immobility is found to significantly reverse by extract, it is tempting to suggest the involvement of biogenic amines in antidepressant action of extract.

Decrease of mean immobility time produced by dose of 300 mg/kg extract was comparable to that produced by imipramine (10 mg/kg), the standard antidepressant drug. However, this effect was not observed at doses 100 mg/kg and 500mg/kg of extract, indicating these doses of extract were not able to antagonize reserpine effect in FST. Thus the antidepressant-like effect of extract appeared not to be in a dose-dependent manner.

Psychostimulants also reduce the time of immobility in the FST.[18] In order to prove that the reduction of immobility time was not caused by the stimulation of motor activity, all groups were submitted to the open-field field test.[21] The

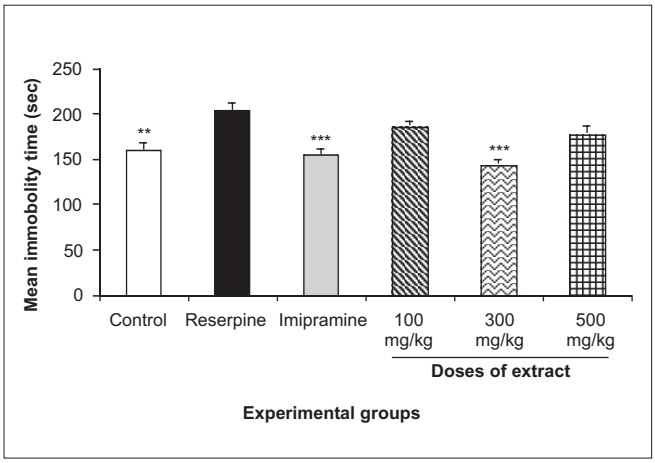

Figure 1: Effects of drug treatments on the immobility time in forced swimming test in rats. Columns represent Mean ± S.E.M. Reserpine (6 mg/kg, i.p.) and vehicle (5% DMSO, i.p.) were administered 24 h before the test session. Imipramine (10 mg/kg, i.p.), and different doses of pine needle extract (100, 300 and 500 mg/kg, p.o.) were administered 24, 5 and 1 h before the test session. **P<0.01, ***P<0.001 as compared to reserpine group using ANOVA and Tukey-Kramer's multiple comparison test. (n=8)

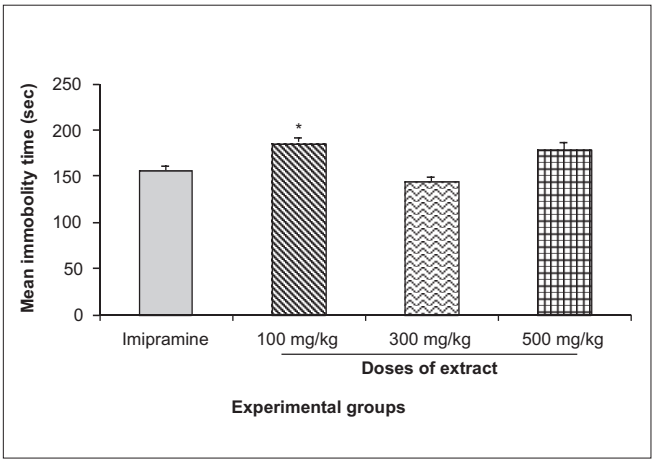

Figure 2: Effects of different doses of extract and imipramine on the immobility time in forced swimming test in rats. Columns represent Mean ± SEM. Imipramine (10 mg/kg, i.p.), and different doses of pine needle extract (100, 300 and 500 mg/kg, p.o.) were administered 24, 5 and 1 h before the test session. *P<0.05 as compared to imipramine-treated group using ANOVA and Tukey-Kramer's multiple comparison test. (n=8)

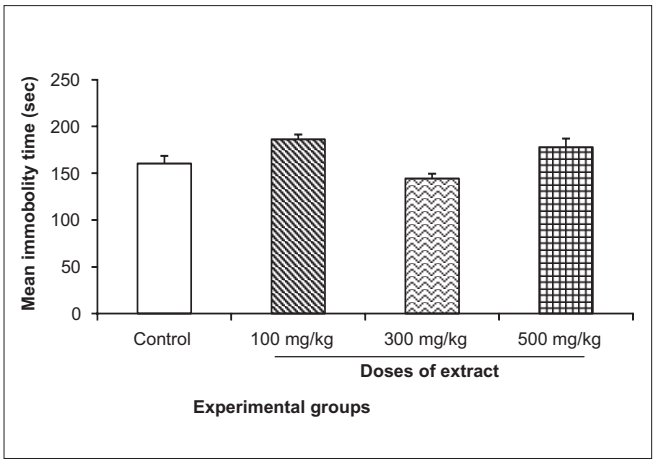

Figure 3: Effects of different doses of extract and vehicle on the immobility time in FST in rats. Columns represent Mean ± SEM. Vehicle (5% DMSO, i.p.), and different doses of pine needle extract (100, 300 and 500 mg/kg, p.o.) were administered 24, 5 and 1 h before the test session. All groups were compared to control group using ANOVA and Tukey-Kramer's multiple comparison test. (n=8)

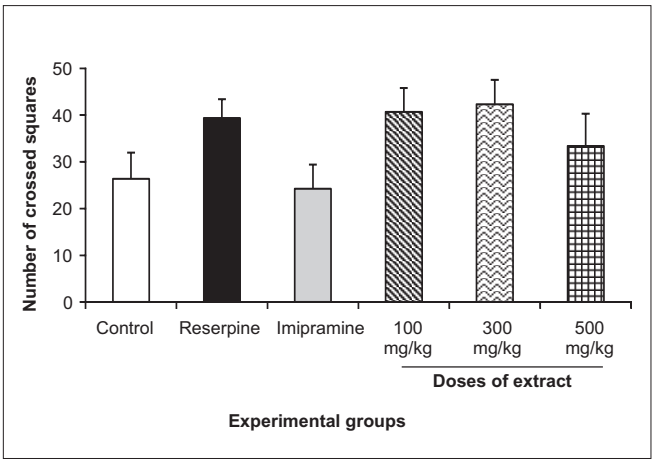

Figure 4: Effects of drug treatments on the number of squares crossed in the open-field test in rats. Columns represent Mean ± SEM. All drugs were administered 24 h before open field test. All groups were compared to reserpine group using ANOVA and Tukey-Kramer's multiple comparison test. (n=8)

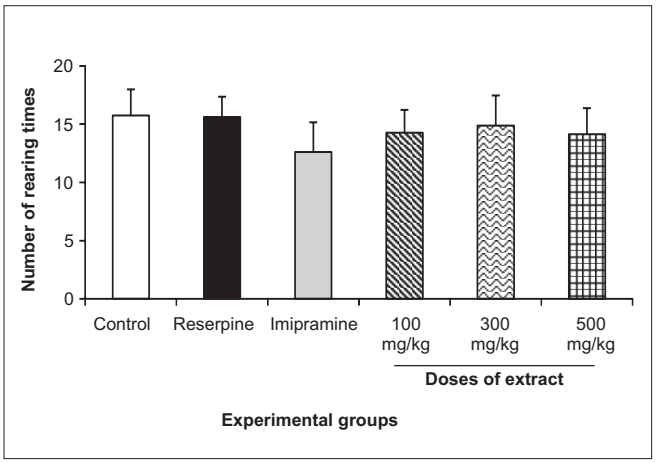

Figure 5: Effects of drug treatments on the number of rearing times in the open-field test in rats. Columns represent Mean ± SEM. All drugs were administered 24 h before open field test. All groups were compared to reserpine group using ANOVA and Tukey-Kramer's multiple comparison test (n=8)

drugs administration resulted in any significant changes in locomotor function, indicating that antidepressant effect found in the FST was not bases on the stimulation of locomotor activity. However, in our study, reserpine-induced increase in locomotion (number of squares crossed) in open-field test as compared to control group. This data are contrary to the previous studies that have shown reserpine to decrease locomotion in rats and other animals.[22,23] However, a few studies have reported increased locomotor activity due to reserpine in mammals.[24,25]

The extract of *P. eldarica* needles contains flavonoids, including proanthocyanins and flavonols. Isorhamnetin, quercetin and kaempferol are the most abundant flavonols in *P. eldarica* needles.[26] It can be speculated that antidepressant-like effect of extract might be related to the flavonoids quercetin, kaempferol and isorhamnetin. Quercetin may exert beneficial actions on the central nervous system (CNS) such as neuroprotective, anxiolytic and cognitive-enhancing effects. It should be noted that recent studies have demonstrated the permeability of quercetin across the blood-brain barrier *in situ* and *in vivo*.[27,28] Thus, this evidence provides the possibility that quercetin may exert modulatory effects on the central nervous system. However, it is important to note that the effects of quercetin may be mediated substantially by its metabolites such as isorhamnetin.[29] Quercetin and kaempferol, as both flavonoids recently showed antidepressant actvity in the FST.[30-32] Quercetin, a flavonol found in St. John's wort (*Hypericum perforatum*) extract, which is a herbal medicine used in the treatment of depression.[33] EGb 761 is a standardized extract from the leaves of the herbal medicine *Ginkgo biloba* that contains a high amount of quercetin, kaempferol and isorhamnetin. It was reported that ginkgo flavonols, quercetin, kaempferol and isorhamnetin exerted antidepressant-like effects in tail suspension test after 7 days of intraperitoneal injection.[34]

In another study, EGb761® concentration dependently inhibited synaptosomal uptake of dopamine, serotonin (5-HT) and norepinephrine (NE) and MAO activity *in vitro,* although rather high concentrations are required for inhibition of MAO-A and MAO-B activity. However, after 14 days of daily oral treatment with 100 mg/kg EGb761® only NE uptake is significantly decreased in mice, while 5-HT uptake and MAO activity are not affected. The results

of this study showed flavonoid fraction had the strongest effect on *in vitro* 5-HT uptake and NE uptake.[35]

Quercetin and kaempferol have shown MAO-A inhibitory capabilities.[36-38] MAO-A preferentially deaminates serotonin and norepinephrine.[39] Thus, inhibition of MAO-A may alleviate symptoms of depression.[40] In one study, quercetin has been shown to influence the electropharmacogram of adult rats in the same manner as moclobemide, a reversible inhibitor of MAO-A. Furthermore, a certain similarity of the electrical effects of quercetin is also observed to those produced by imipramine.[33]

Therefore, the antidepressant mechanisms of ethanolic extract of *P. eldarica* needles is thought to involve extract flavonoids, which inhibited uptake of monoamines or MAO activity or both in the brain. However, further studies dealing with the effects of extract on the reuptake of monoamines and MAO activity in the brain are necessary to clarify this issue.

In conclusion, our study is the first study to demonstrate the antidepressant like activity of ethanolic extract of *P. eldarica* needles in rats. However, further investigations are essential for the isolation of the active principles of *P. eldarica* and its mechanism of action.

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