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Evaluation of antidepressant-like effect of hydroalcoholic extract of *Passiflora incarnata* in animal models of depression in male mice

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ABSTRACT

Introduction: *Passiflora incarnata* (PI) is one of the commonest herbal anti-anxiety and sedative agents. The aim of the present study was to investigate the antidepressant effect of hydroalcoholic extract of PI in forced swim test (FST) and tail suspension test (TST) in male mice.
Methods: In this experimental study, 48 male mice were randomly divided into 6 groups of 8: Negative and positive control groups received normal saline (10 ml/kg), fluoxetine (20 mg/kg) and imipramine (30 mg/kg), respectively and treatment groups received extracts of PI (200, 400 and 800 mg/kg). Immobility, swimming and climbing behaviors were recorded during 6-min.
Results: All doses of PI extract compared to control group significantly reduced the duration of immobility time in both of two tests ($p<0.001$). Also, these extracts increased swimming time ($p<0.001$) without significant change of climbing time.
Conclusion: PI has considerable antidepressant-like effect in animal models of depression. However, further studies are needed to determine its exact mechanism of action.

Implication for health policy/practice/research/medical education:
considerable antidepressant-like effect in animal models of depression and its usage might be useful in depressive patients.

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Introduction

Depression is a prevalent psychiatric dysfunction that is identified with depressed mood, loss of interest or pleasure, feelings of guilt or low value, disturbance of sleep and appetite, low power and poor centralization, interfering with normal function, and often has problems at work, community and family. According to the World Health Organization (WHO), depression will become the second factor contributing to disability of disease in 2020 (1,2). It has been shown that various factors such as psychological-social, genetic and biological factors play a role as causing depression. However, one of the major biological causes of depression is reduction in the amount of neurotransmitters such as serotonin (5-hydroxytriptamine, 5HT), dopamine (DA) and noradrenalin (NA) (3,4).

Nowadays, various pharmacological treatments, including tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors and selective serotonin-noradrenalin inhibitors are used (5). These drugs exert their effects by increasing the amount of brain monoamines (6). However, most of these drugs cause some unwanted symptoms and their mechanism of action has not been very satisfactory. Therefore, it is essential to search new, strong and especially healthy drugs (7).

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Passionflower is an herbaceous plant with the scientific name of *Passiflora incarnate* (PI). This plant is a perennial herb and has a climbing or trailing stem with alternate and lobed leaves. It shows individual blue flowers. Fruits are green and after ripening will be orange. This plant is cultivated in different countries and even in northern Iran as an ornamental plant. It has been shown that passion flower has a number of compounds including flavonoids, the cyanogenic glycosides, indole alkaloids and other alkaloids, such as the passiflorine, harmaline, harmine and harmalol in its building. Studies have reported that passion flower has different effects such as analgesic, anticonvulsant, anti-bacterial, anti-anxiety, hypnotics, sedatives, etc. (8,9). Considering that no study already has been done on anti- depression effects of passionflower; the purpose of the present study was to investigate the effect of passionflower hydroalcoholic extract in the forced swim test and the tail suspension test as two animal models of depression in male mice.

Materials and Methods

In this experimental study, 48 male albino mice NMRI (weighing 20 to 30 g) were randomly divided into 6 groups of 8. Animals were housed in cages of 5 at 22 ± 1 ºC in a 12-h light/dark cycle, and had free access to water and food. Each animal was evaluated only once. Animals were transferred laboratory to adapt to the lab environment for 48 hours before testing. All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Committee on Animal Research; Urmia Medical University.

In this study, fluoxetine hydrochloride (Arya Pharmaceutical Co., Iran), imipramine hydrochloride (medicine Pars Co., Iran) and PI hydroalcoholic extract (Iran Darouk Pharmaceutical Co., Iran); all in powder form were used. All drugs and extracts were dissolved in normal saline (NS 0.9%) and administered intraperitoneally (i.p.) at a constant volume of 10 ml/kg.

The negative control group or normal saline group received normal saline (10 ml/kg, i.p). Positive control groups received fluoxetine (20 ml/kg, i.p) and imipramine (30 ml/kg, i.p). The other three groups were treated with different doses of 200, 400 and 800 mg/kg of PI hydroalcoholic extract, respectively.

In the forced swim test (FST), mice were separately placed in cylindrical containers, with dimensions of 8 × 12 × 25 cm, containing water at 25 °C, 30 min after injection of extracts or drugs. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The whole test was 6 minutes, 2 minutes to match the animal to the environment, and next 4 minutes were recorded as immobility time, swimming time and climbing time in seconds by the chronometer (10). In the forced swim test, immobility time and its reduction were recorded as depression and anti-depression effect, respectively. Swimming is equivalent to active movements of the hands and feet of the animal and the rotation around the cylinder, and climbing is also equivalent to active movements of animal hands on the walls of the cylinder (11).

Tail suspension test (TST) is also an additional common animal model for estimating depression in animals. In this test, the metal legs with a height of 70 cm were used and a string of 50 cm was longitudinally stretched between two metal legs. Mice tail was closed by a clause and the animal was hung on the tail. In this part also the test began with a rush mice, 30 min after drug or extract administration. The immobility time was considered when the animal was completely immobile, disabled and had no response. The period of this test, the same as the previous method, was 6 minutes, again first 2 minutes to match the animal to the environment and next 4 minutes were recorded as immobility time in seconds by the chronometer. In both tests, all the samples were recorded by a person who did not know which sample belonged to which group. The test was performed 30 min after drug or extract injection (12).

In this study, one-way analysis of variance (one-way ANOVA) and Tukey test were used. The statistical analysis was performed using SPSS, version 19, and in each case the $p<0.05$ was considered as the significance level.

Results

The results of this study showed that normal saline injection caused no significant change in the immobility, swimming and climbing times compared with the situation before the injection. Therefore, all experimental groups were compared with saline as a negative control.

Results showed that all three doses of hydroalcoholic extract of PI in the forced swim test (128.83±9.34, 114.14±6.64 and 99.73±9.15 respectively; $p<0.001$) and tail suspension test (120.03±10.49, 97.98± 8.51 and 76.72± 6.05 respectively; $p<0.001$) significantly reduced the immobility time compared with control groups (189.11±7.25 and 197.55±3.86 respectively) (Figure 1 and 2).

Fluoxetine and imipramine in comparison with the control group significantly decreased the immobility time (73.55± 7.03 and 23.79±5.36 respectively; $p<0.001$) (Figure 1). Both drugs also reduced immobility time in the tail-supension test (128.6± 6.02 and 27.08±3.73 respectively; $p<0.001$) (Figure 2).

Doses of hydroalcoholic extract of passion flower (107.67±9.67, 120.84±5.69 and 138.21±10.41 respectively; $p<0.001$) compared with the control group (44.56±6.84) significantly increased the swimming time (Figure 1). Climbing behavior did not increase significantly by any of the doses of extract (Figure 1). In this section, fluoxetine increased swimming behavior compared to the control group (153.85±11.3; $p<0.001$), without significant change of climbing behavior ($p>0.05$) (Figure 1). But conversely, imipramine increased the climbing behavior (105.66±11.39; $p=0.000$), but was not observed a

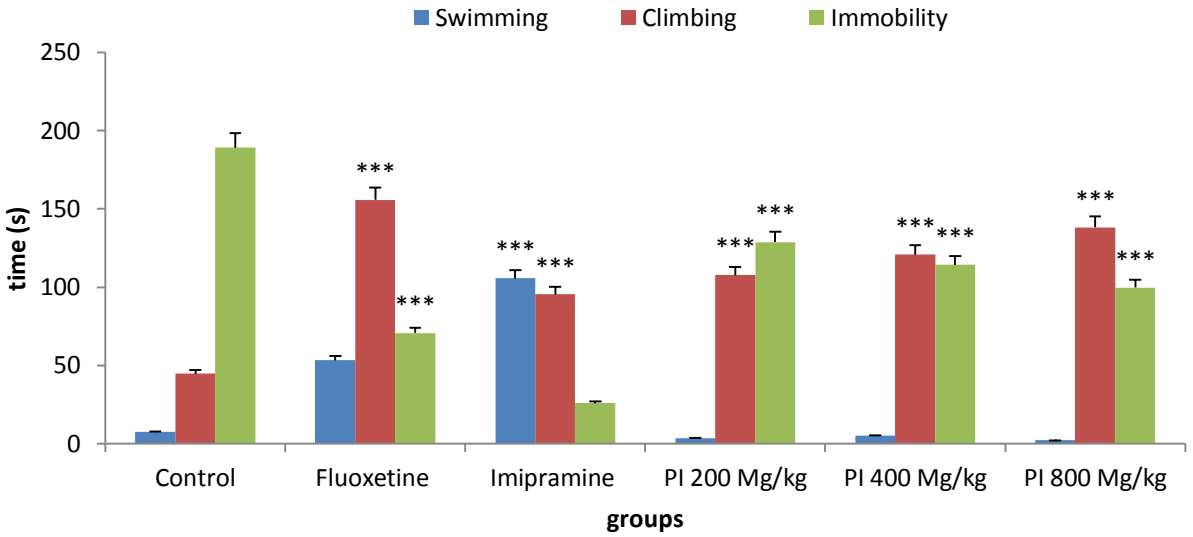

Figure 1. The effect of different doses of hydroalcoholic extract of *Passiflora incarnata* (PI; 200, 400, and 800 mg/kg; i.p), fluoxetine (20 mg/kg; i.p), and imipramine (30 mg/kg; i.p) on immobility, swimming and climbing in the forced swimming test in mice. The data are shown as Mean±SEM; ***significant at p<0.05 compared with control group.

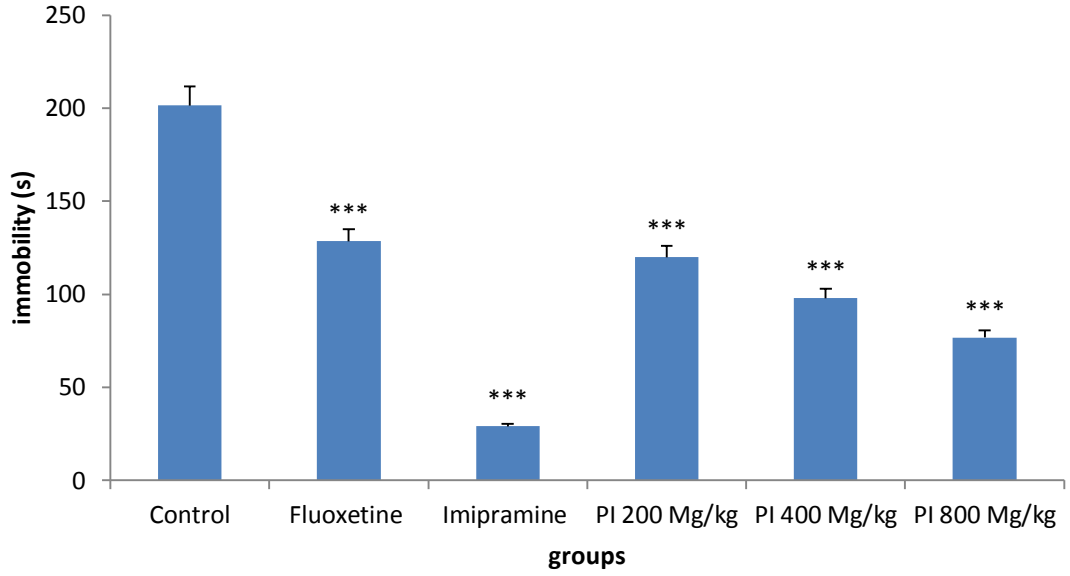

Figure 2. The effect of different doses of hydroalcoholic extract of *Passiflora incarnata* (PI; 200, 400, and 800 mg/kg; i.p), fluoxetine (20 mg/kg; i.p), and imipramine (30 mg/kg; i.p) on immobility time in the tail-supension test in male mice. The data are shown as Mean±SEM; ***significant at p<0.05 comparison with control group.

significant increase in the swimming behavior (p<0.05) (Figure 1).

Discussion

In this study, the antidepression effects of hydroalcoholic extract of PI were studied in mice by using FST and TST as an animal models of depression. Forced swim test is one of the most commonly used animal models for estimating the anti-depression effects of chemical and herbal agents in mice and rats. This model is sensitive to the effects of all medications and extracts (13). Tail suspension test is also among other animal models to estimate the effect of antidepressants. This test unlike the FST, does not lead to a stress and reduction in body temperature that usually is caused by the forced swim test (14). The results of this study demonstrated that different doses of PI extract compared with the control group decreased immobility time in both the FST and TST. On the other hand, the extract increased swimming time, but climbing behavior did not significantly enhance. The results of the present in accordance with other findings. The effects of fluoxetine and imipramine were demonstrated in the FST and TST (15,16). In other words, previous studies have reported that the drugs with serotonergic mechanism

(e.g. fluoxetine) reduce the time immobility, increase the swimming time, without any significant change of climbing time. On the hand, drugs with noradrenergic mechanisms reduce the immobility time, increase the climbing time and the changes are not significant in the swimming time (17,18). Although the exact mechanism of antidepression effect of PI has not been established, but consistent with other findings and conclusions of this study, the effects of the PI extract is similar to fluoxetine. In support of these findings, studies have shown that PI has several compounds in its building, but among them, the beta-carboline alkaloids such as harmaline, harmine and harmalol have anti-depression properties (19). In this regard, it has been shown that these alkaloids are irreversible monoamine oxidase-A inhibitors (20). There are two isoforms (MAO-A and MAO-B) for the monoamine oxidase enzyme. MAO-A inhibits reduce the degeneration of adrenaline and serotonin in the brain, but MAO-B inhibits reduce the degeneration of dopamine. Thus, MAO-A inhibitors are used in depression and MAO-B inhibitors are applied in diseases such as Parkinson's (21,22). Therefore, inhibition of MAO-A by the passion flower alkaloids increases the amount of adrenaline and serotonin in the brain of animals treated with it (19). In agreement with these findings, it has been shown that the harmine binds to MAO-A and many cell surface receptors, including the serotonin receptor 2A (5HT2A) that are involved in pharmacotherapy for depression (23-25). On the other hand, other studies have reported that harman and other beta-carbolines can cause a wide range of antagonistic effects against benzodiazepines via binding to inverse agonist sites of GABA-A receptors. Unlike benzodiazepines that bind to benzodiazepine receptor sites (BZ1 and BZ2) and reduce norepinephrine and serotonin release, beta-carbolines bind to inverse agonist sites and increase catecholamines and serotonin release (26,27). In support of these findings, flumazenil (antagonist of benzodiazepine receptors and an inverse agonist) antagonized the beta-carboline effect (28). Thus, it seems that serotonergic and GABAergic systems involved in PI antidepressant effects.

Conclusion

According to the results of this study, PI has considerable antidepressant-like effect in animal models of depression. However, concomitant use of serotonin and GABA antagonists along with the PI extract, and isolation of each of its components and evaluation of them on depression is recommended in order to determine the exact mechanism of PI antidepression effects.

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Authors' contributions

All the authors wrote the manuscript equally.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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