Antidepressant effects of *Ziziphora tenuior* L. hydroalcoholic extract in animal models of depression

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**ABSTRACT:** The antidepressant effects of *Ziziphora tenuior* L. (*Z. tenuior*) hydroalcoholic extract in animal models of depression was investigated. The effect of extract (50, 75 and 100 mg/kg) on immobility, swimming and climbing behaviours were evaluated in forced swim test (FST) and tail suspension test (TST). Imipramine (30mg/kg) and fluoxetine (20mg/kg) were used as a reference drugs. Results showed that different doses of extract significantly reduced immobility time in FST (p<0.05 and p<0.001, respectively) and TST (p<0.001). All doses of extract significantly and dose dependently increased swimming time (p<0.01 and p<0.001, respectively), without increasing climbing time (p>0.05). Thus, it is concluded that *Ziziphora tenuior* may possess an antidepressant-like effect and its effect similar to fluoxetine. However, further studies suggested of characterize the exact mechanism of antidepressant effects of *Z. tenuior*.

**Keywords:** Antidepressant effect, *Ziziphora tenuior* L., Forced swim test, Tail suspension test, Mice

**INTRODUCTION**

Depression is one of most significant major public health problems, and is a serious and common medical condition affecting physical health, mood, and thoughts (Lijian, 2011). Recent epidemiological surveys showed that the lifetime incidence of depression is up to 10% to 15% (Lepine, 2011). In other hand, according to the data from the World Health Organization (WHO), depression will become the second leading cause of disease-related disability by the year 2020 (Rybnikova, 2007; Kumar, 2010). The dysfunction of brain neurotransmitters is an important mechanism in depressed patients (Nash, 2004; Meyers, 2000; Daws, 2009). These neurotransmitters include dopamine, epinephrine, norepinephrine, and serotonin. Among the various pharmacological agents selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) are most commonly used but have a lot of distressing adverse effects as they are often used for very long period of time (Gaynes, 2008). Thus, there remains a pressing need for new, potent and safe therapeutic agents. On the other hand, drugs obtained from natural sources have good efficacy, least risk and low side effects profile. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly. Therefore, herbal therapies should be considered as alternative or complementary medicines (Jintanaporn, 2007).

*Ziziphora tenuior* L. (*Z. tenuior*) is an aromatic herb of Labiatae family, which naturally and widely distributed in Iran. *Ziziphora tenuior* L. (named as Kakoti in Persian) is one of this genus member which widely distributed in the country. *Ziziphora* species were used as culinary herb in Iran (Talebi, 2012). In Iranian folk medicine, *Ziziphora*...
species have been used as infusion, decoction and maceration for various purposes such as sedative, stomachic, heart disorders, common cold, inflammation, carminative, diarrhea, expectorant, coughing, antiseptic, migraine, fever and depression (Zargari, 1995; Naghibi, 2005). These findings promoted us to study its antidepressant activity. Thus, the aim of the present study was to evaluate possible antidepressant effects of hydroalcoholic extract of Z. tenuior by using forced swim test and tail suspension test in male mice.

MATERIALS AND METHODS

The whole plant material of Z. tenuior was shade dried ground and extracted with ethanol (80% V/V). 50 ml of solvent was added to five grams of the powder and the resultant mixture was mixed for two hours at the ambient temperature using a magnetic mixer. The solid part was then removed by using ordinary filter paper and the extraction process was repeated as before using fresh solvent. The extract obtained from these two steps was combined, concentrated at 40 °C by using a rotary evaporator and were finally dried at the ambient temperature. In this experiment, male NMRI mice (n=48), 25-35 g were obtained from the Animals House, Urmia University of Medical Sciences. Mice were housed in cages of 5 at 22 ± 1°C in a 12-h light/dark cycle, and had free access to water and food. 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. The animals were grouped into 6 different groups, each containing 8 animals, according to different tests of antidepressant activity as follows: Group 1: Normal saline 0.9 % (10 ml/kg, i.p.), negative control for FST and TST; Group 2: Fluoxetine hydrochloride (20 mg/kg, i.p.) (Arya Pharmaceutical Co, Tehran, Iran), positive control for FST and TST; Group 3: Imipramine hydrochloride (30 mg/kg, i.p.) (Pars Daru, Tehran, Iran), positive control for FST and TST; Group 4: Hydroalcoholic extract of Z. tenuior (50, 75 and 100 mg/kg, i.p.), treatment group in FST and TST. All solutions were dissolved in normal saline 0.9 % and administered interaperitoneally (i.p.) at a constant volume of 10ml/kg. The forced swim test (FST) was carried out on mice individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 15 cm of water at 25±1 °C; the total duration of immobility (flouting in water without swimming), climbing (active movements of forelimbs on the container wall) and swimming (active movements of extremities and circling in the container) behaviors during the 6-min test was scored. 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 duration of immobility was recorded. Increases in climbing or swimming and reduction in immobility were considered as behavioral responses consistent with an antidepressant-like action (Porsolt, 1977; Zomkowski, 2005). The total duration of immobility induced by tail suspension test (TST) was measured during the 6-min test. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period (Steru, 1985). All the results are expressed as the mean ± standard error (SEM). Data were analyzed using One-way analysis of variance (ANOVA), followed by Tukey's test to determine statistical significance of various groups as compared to control group. Statistical significance was set at p < 0.05. All the analyses were done using SPSS Statistics (17.0).

RESULTS AND DISCUSSION

Our pre-clinical experiments demonstrate the antidepressant effects of Z. tenuior hydroalcoholic extract in the FST and TST. Both of these models commonly used as animal models of depression. These tests are quite sensitive and widely employed in rodents (e.g. mice) to predict antidepressant potential by decrease of immobility time produced by severe stress or emotional disturbances. Forced swimming test (FST) is a sensitive and widely employed in rodents (e.g. mice) to predict antidepressant potential by decrease of immobility time. Both of these tests commonly used as animal models of depression. These tests are quite sensitive and widely employed in rodents (e.g. mice) to predict antidepressant potential by decrease of immobility time produced by severe stress or emotional disturbances. Our preliminary results demonstrated that all doses of extract signif-
p=0.000, respectively) compared to the control group (Figure 1). None of the administered doses influenced the climbing behavior of mice in the FST (p>0.05) (Figures 1). Although all antidepressant drugs reduce immobility in the FST, two distinct active behavioral patterns are produced by pharmacologically selective antidepressant drugs (Detke, 1995). It has been demonstrated that swimming is sensitive to serotonergic compounds such as fluoxetine (a serotonin reuptake inhibitor), and that climbing is sensitive to tricyclic antidepressants and drug with selective effects on noradrenergic transmission (Detke, 1995; Page, 1999). In this study, in agreement with previous report (Page, 1999), the decrease in immobility (73.55±7.03 sec vs 178.23±8.76 sec and 128.6±6.02 sec vs 202.59±5.73 sec in FST and TST, p=0.000, respectively) induced by fluoxetine was accompanied by an increase in swimming (153.85±11.3 sec vs 43.46±7.24 sec, p=0.000) whereas climbing was not affected by this drug. On the other hand, imipramine reduced immobility (23.79±5.36 sec vs 178.23±8.76 sec and 27.08±3.73 sec vs 202.59±5.73 sec in FST and TST, p=0.000, respectively, p=0.000) and increased climbing (105.66±11.39 sec vs 11.89±2.18 sec, p=0.000) without modifying swimming (Detke, 1995). Although the exact mechanism of antidepressant effect of Z. tenuior extract is unknown, however according to our results, the pattern of behaviors exerted by the extract in the FST is similar to those of fluoxetine which suggests that this plant extract acts probably by enhancement of serotonergic neurotransmission as it is related to swimming behavior in the FST.

The Ziziphora species (e.g. Z. tenuior) are source of essential oils, flavonoids, caffeoyl derivatives, fatty acids and sterols (Aghajani, 2008; Ozturk, 2007). Phytochemical investigations on the Ziziphora genus mainly focused on its essential oil composition. Many literature surveys indicated that the major compounds of Z. tenuior essential oil were Pulegone, limonene and thymol (Ghasemi, 2013; Najafi, 2011). The responsible compound for antidepressant effect of Z. tenuior cannot be determined by the results of the current study but flavonoids, monoterpenes(e.g. Pulegone), terpenes(e.g. limonene) showed antidepressant effect in previous studies (Moallem, 2007; Sousa, 2006; Carvalho-Freitas, 2002). Therefore, these compounds may be responsible for the antidepressant effect.

Figure 1. The effects of Z. tenuior L. hydroalcoholic extract (ZT-mg/kg i.p.), fluoxetine (Flu-20mg/kg) and imipramine (Imip- 30 mg/kg i.p.) on active behaviors in the forced swim test in mice. Data represent means±S.E.M. of the duration of climbing, swimming and immobility during the 6-min test session (n = 8 animals per group). Comparisons were made by using a one-way ANOVA followed by tukey's test: ***p < 0.001 compared with control group.
The effects of Z. tenuior L. hydroalcoholic extract (ZT-mg/kg i.p.), fluoxetine (20mg/kg) and imipramine (Imip- 30 mg/kg i.p.) on immobility time in the tail suspension test in mice. Data represent means±S.E.M. of the duration of immobility during the 6-min test session (n = 8 animals per group). Comparisons were made by using a one-way ANOVA followed by tukey's test:

***p < 0.001 compared with control group

CONCLUSION

The results of this pre-clinical study on mice after an acute administration of various doses of Z. tenuior hydroalcoholic extract show that Z. tenuior displays an antidepressant effect in two animal models of depression. The antidepressant effect was dose-dependent. However, further studies suggested of characterize the exact mechanism of antidepressant effect of Z. tenuior.

REFERENCES


