The Aqueous Extract of *Ziziphora persica* Cause Positive Mood on Depressive Rat

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**ABSTRACT**

*Ziziphora persica* dried leaves are used in Iranian traditional medicine as an anxiolytic and mood enhancer, but its contributory mechanisms are unknown. In this research, efficacy of aqueous extract of this plant was investigated on depressed male rats measuring changes in levels of CSF serotonin and dopamine as mood indicators. Four groups of male Wistar rats, seven rats in each, were enrolled in this study. Swimming test was used to assure that depression was induced by injection intraperitoneal of reserpine (5mg/kg). After 14 days oral intake of plant aqueous extract of plant (2mg/10ml), CSF serotonin and dopamine levels of rats were measured using ELISA Kit. Our finding indicated that aqueous extract of *Ziziphora persica* significantly increased concentration of CSF serotonin and dopamine in depressed group who were treated by this plant. Concentration of CSF serotonin in control group receiving extract was increased as compared to the control group who didn’t receive any medicine. These results showed serious changes of neurotransmitters due to *Ziziphora persica* which can be translated as mood enhancer. It can be concluded that *Ziziphora persica* induces positive mood in depressive and control groups.

**Key words:** *Ziziphora persica*, Depression, Serotonin, Dopamine, ELISA, Rat.

**INTRODUCTION**

*Ziziphora persica*, a member of Labiatae family has chemical components including pulegone, sis-isopulegone, cineol, thymol, α and β pinen, piperitenone, terpenoids and flavonoids (Zargari, 1997; Naghibi et al., 2005; Sonboli et al., 2006; Salehi et al., 2005; Behravan et al., 2007; Meral et al., 2002; Belyaev and Demoeubaeva, 1999; zargari, 1995). In Iranian folk medicine, *Ziziphora* species have been used as infusion, decoction and maceration for various purposes such as sedative, stomach tonic, heart disorders, common cold, inflammation, diarrhea, expectorant, coughing, antiseptic, migraine, fever and carminative (Naghibi et al., 2005). In Iranian folklore, the dried aerial parts of aforementioned species have been frequently used as culinary and spice in food. However, there are no experimental reports on the effects of this plant on mood. This study sought to find possible effects of aqueous extract of *Ziziphora persica* on mood. We investigated the antidepressant activities of aqueous extracts of *Ziziphora persica* with depression model in rats by measurement changes in level of CSF...
serotonin and dopamine as mood indicators using Elisa kite.

MATERIAL AND METHODS

Animals
The experiment was conducted using four groups (seven rats each) of Wistar rats weighing 250-300 g. The animals were kept in a 25 ± 2 °C temperature with a 12 hr light/dark cycle and were fed with standard diet and tap drinking water. Rats were purchased from the animal house of Ilam University. The rats were distributed into groups of seven as controls and depression groups. The rats were acclimatized to the laboratory for at least 1 h before oral administrating of *Ziziphora persica* and were performed extraction CSF sample after two weeks. The experiments were carried out between 9.00 and 14.00 h. The experimental protocol was approved by of Animal house and Ethical Committee at Ilam University of Medical Sciences (IUMS).

Porsolt swim test for assessment of depression in rats
The procedure for the Porsolt forced swim test was as previously reported (Porsolt, LePichon, and Jalfre, 1977) was used to assure that depression was induced by injection intrapritonal of reserpine (5mg/kg) (Gaylord and Curzon, 1972). Briefly, rats were placed in a cylindrical container (40 cm deep, 27 cm in diameter) filled with 30 cm of 30°C water. The period of time the rats spent swimming or immobile was recorded in a 10-min test. Swimming was defined as movement of the forelimbs and hind limbs without the front paws breaking the surface of the water. Immobility was recorded when there was an absence of any movement other than that necessary to keep the head and nose above the water (e.g., when rats were floating in a vertical position).

Preparation of plant materials
The plants were collected in Zagros Mountains located in Ilam Province at west part of Iran. The leaves (dry weight = 10 g) were subjected to an aqueous extraction (900 ml) by shaking for 24 h at room temperature to obtain extract, the extract was dissolved in normal saline for final suitable concentrations to give the desired concentration (2mg/10 ml).

CSF sampling
The rats were anesthetized with 40 mg of sodium thiopental, then intrapritonal and CSF was drawn (40–60 micro liter per rat) by direct puncture of the cisterna magna with an insulin syringe (27 gauge 31/20 length). CSF was collected from each rat using estriutax system (Rafael et al., 2004).

Neurotransmitters analysis
The concentrates of CSF serotonin and dopamine samples were measured using the IBL international GMBH ELISA kit (Germany) and LDN GMBH Kit according to the manufacturer instruction. The procedure for each neurotransmitter is summarized as follows:

Serotonin ELISA
First 20 micro liter sample of each group was pipetted into glass test tubes, then 100 microliter of diluted Assay Buffer was added to each tube. Vortex. By pipetting, 25 microliter of Acylation Reagent 1 (3%) was added into each tube. Vortex, and the tubes were immediately covered and incubated for 15 min at 37°C in bath water. Then 4 mL of diluted Assay Buffer was pipetted into each tube. All tubes were centrifuged for 10 min at 1500 x g. The prepared samples were immediately assayed. The supernatant was stabilzed for only 1 h at 18-25°C.

Dopamine ELISA
To measure level of CSF dopamine by dopamine ELISA, at first 25 microliter enzyme Solution was pipetted into all wells of the Dopamine Microtiter Strips. Then 100 microliter of the [what?] standards, controls and samples added into the appropriate wells and incubated for 30 min at RT on a shaker (approx. 600 rpm). Itwas continued by adding 50 microliter Dopamine antiserum to the wells and then the plate was covered with adhesive Foil and was incubated for 2 hours at RT on a shaker. The content of the wells was discarded followed by 3 times washing using 300 microliter washing buffer. Enzyme conjugate was added (100 microliter) into all wells incubating 30 min at RT on a shaker. After washing, 100 microliter of the substrate was added into all wells and incubated for 20-30 min at RT followed by adding 100 microliter of the stop solution to each well and reading the absorbance of the solution in the wells within 10 minutes, using a micro plate reader set to 450 nm and a reference

**Statistical analysis**

ANOVA was used to assess the effects of Ziziphora persica on changing levels of serotonin and dopamine in each group of 7 rats. Normality of data in each group was checked using one sample kolmogoror- simirnov test; and Dunnett-test was used as a post hoc multiple comparison analysis, P-value < 0.05 was considered statistically significant.

**RESULTS**

**Impact of aqueous extract Ziziphora persica on changes level CSF serotonin**

As shown in Figure 1, the effect of aqueous extract Ziziphora persica on changing level of CSF serotonin in different groups, ANOVA indicated that the difference between various groups was significant. The following analysis with Dunnett-test as a post hoc multiple comparisons revealed that the different levels of CSF serotonin in the depression group and control group was statically significant (p < 0.05). On the other hand, the different level of CSF serotonin in the depression group and depression group receiving Ziziphora persica was statically significant (p < 0.05), (Table 1). The different level of CSF serotonin in the control group and control group receiving Ziziphora persica was statically significant (p < 0.05), too. In tables and figures Zizi is equal Ziziphora persica.

**Impact of aqueous extract of Ziziphora persica on changing level of CSF dopamine**

As shown in Figure 2, aqueous extract of Ziziphora persica changes level of CSF dopamine in different groups. ANOVA analysis indicated that the differences between various groups were significant (p < 0.05). Also, following analysis with Dunnett-test showed that changes in dopamine level in the control group was statically significant (p < 0.05) as compared with the depression group. However, the difference between control group and depression groups receiving Ziziphora persica was statically significant (p < 0.05), whereas for other cases, it was not (Table 2).

**DISCUSSION**

The monoamine hypothesis claims that changes in some monoamine neurotransmitters such as serotonin and dopamine (DA) can cause some mood disorders (Ruhe et al., 2007). Some researchers has developed techniques for inducing low mood using monoamine depletion in MDD (Ruhe et al., 2007; Booij et al., 2003). Niklasson and Agren have revealed some association between the diminished brain monoaminergic function and reduced cerebral metabolism (Niklasson, 1984). Many studies reported that dopamine dysfunction can be complicate in mania and types of depression such as bipolar and retarded depressions, as dopamine-active treatments can cause some therapeutic impact on mood disorders (Diehl and Gershon, 1992). Preliminary phytochemical screening of the Ziziphora persica has demonstrated that the aqueous extract contains pulegone, sis-isopulegone, cineol, thymol, ± and δ pinen, piperitenone, terpenoides and flavonoids (Zargari, 1997; Naghibi et al., 2005; Sonboli et al., 2006; Salehi et al., 2005; Behravan et al., 2007; Meral et al., 2002; Belyaev and Demeubaeva, 1999; Zargari, 1995). The effects of essential oils obtained from Ziziphora species decreased the swimming time suggesting a central nervous system depressant activity (Öztürk et al., 1995). Our finding showed that Ziziphora persica significantly increased level of serotonin in depression group which were treated by this plant while it increased level of serotonin of CSF in control group which received Ziziphora persica as well. It can be concluded that using Ziziphora persica can increase serotonin level and induce positive mood (Figure 1). Moreover, findings showed that level of CSF dopamine was increased in depression group treated by Ziziphora persica. These results are suggestive that Ziziphora persica has an antidepressant and/or enhancer mood effect, which is possibly part of antidepressant mechanism of Ziziphora persica and is related to changes in level of serotonin and dopamine as indicators of depression. We offer investigating effect of this plant on changing level of norepinephrine, GABA and other neurotransmitters of mood indicator for finding complicated mechanisms of this plant on mood changes.
REFERENCES


