Antidepressant effect of *Melissa officinalis* in the forced swimming test

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ABSTRACT

Background: In Iranian and other traditional medicines, an antidepressant effect has been indicated for *Melissa officinalis* (Lamiaceae). However, studies showing its antidepressant effect is lacking. Therefore, the present study was undertaken to examine whether the aqueous extract and essential oil from leaves of *Melissa officinalis* have an antidepressant-like activity in mice.

Materials and Methods: The effect of subchronic administration of different doses of the aqueous extract (25, 75, 150, 300 mg/kg or water; n=9-10) and the essential oil (10, 25, 75, 150, 300 mg/kg or almond oil; n=9-10) on immobility, climbing, and swimming behaviors were evaluated in the forced swimming test. Fluoxetine (20mg/kg) and imipramine (15 mg/kg) were used as reference drugs. Additionally, the effect of both plant preparations on spontaneous activity was examined.

Results: All doses of the aqueous extract, used in this study, produced a significant reduction in immobility along with an increase in climbing behavior which is similar to those which have been observed with imipramine. Essential oil caused a dose-dependent reduction in immobility and an increase in climbing at all studied doses, compared to control group. Only the highest dose (300mg/kg) of essential oil showed a significant increase in swimming behavior. The aqueous extract, but not the essential oil, decreased spontaneous activity in a dose dependent manner.

Conclusion: The results of this study suggests that the *Melissa officinalis* possess an antidepressant-like activity similar to imipramine which may have a potential clinical value for treatment of depression.

Keywords: *Melissa officinalis*; Forced swimming test; Antidepressant; Spontaneous activity

INTRODUCTION

Melissa officinalis (Lamiaceae) or lemon balm is an herbal medicine native to the eastern Mediterranean region and western Asia. This plant is known as "Badranjboyeh" in Iran, and grows widely in provinces of Tehran, Golestan, Azarbayjan, Lorestan and Kermanshah (1). Dried or fresh leaves and top aerial section of the plant are the parts which are used as medicine (1). Lemon balm has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic, strengthening the memory, and relief of stress-induced headache(2). It is currently used for the relief of stress-induced headache, as a mild sedative-hypnotic, and as an antiviral to improve healing of herpes simplex cold sores (3). In Iranian traditional medicine, lemon balm has also been used in treatment of irritability and nervousness in young girls and women, lack of interest and energy, and depression (1, 4, 5) and usually 20-50 grams (up to 100 grams) of the dried leaves are infused in 1000 ml of boiled water for 5-15 minutes and 3-4 cups of this tea is taken daily, (1, 4, 5). The distillate (or hydrosol) of lemon balm (known as Arrack) is commonly used as an antidepressant, Ibn Sina (Avicenna), the well-known Iranian scientist, recommended *Melissa officinalis* for above indications. In addition, other traditional medicines have indicated that lemon balm is useful for seasonal affective disorder when mixed with St. John's wort (3). Furthermore, it is stated that the essential oil of lemon balm which is, used in aromatherapy, may be beneficial for mild depression (3). Despite of all this reports no pharmacological study showing antidepressant effect of this plant has been reported. Therefore, the present study was undertaken to evaluate the antidepressant activity of the aqueous extract and essential oil of this plant in the forced swimming test in mice.

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MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Melissa officinalis* were collected from the Eram botanical garden at Shiraz, and air-dried in the shade. It was authenticated by Dr. A. Khosravi (Department of biology, College of Science, Shiraz University). A voucher specimen (#3654) was deposited at the herbarium of Eram garden.

Preparation of aqueous extract

Dried leaves were grounded to a fine powder. The powdered leaves (50g) were macerated in distilled water (500ml) at room temperature for 24 hrs. Subsequently, the mixture was filtered using Watman filter paper. The filtrate was concentrated over the vapor of the water bath and dried under vacuum. The yield of extract was 31.6% (w/w).

Preparation of essential oil

The essential oil (1% v/w) was obtained from dried powdered leaves of *Melissa officinalis* by steam distillation for 4 hrs, using a Clevenger type apparatus.

Animals

Male albino mice (n=129), 25-35 g were obtained from the Animals House, Shiraz University of Medical Sciences. Mice were housed in cages of 5 at 22 ± 1^{o}C in a 12-h light/dark cycle, and had free access to water and food. All experiments were conducted in accordance with European Union Regulations for handling and use of laboratory animals (86/609 EEC Council Directives).

Drugs

Imipramine hydrochloride and fluoxetine hydrochloride (Pars Daru, Tehran, Iran) were used as reference drugs (positive controls).

Treatments

Imipramine (15 mg/kg) and fluoxetine (20 mg/kg) were dissolved in distilled water and administered intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight. Different concentrations of the aqueous extracts of *Melissa officinalis* were prepared by serial dilution from a stock solution of 300 mg/10ml of the extract in distilled water. Negative control group received distilled water. Different concentrations of the essential oil were prepared by serial dilution of a stock solution (300mg/10 ml) in almond oil. Negative control group received almond oil. All solutions were prepared freshly on the test days and administrated i.p. in a volume of 0.1ml per 10g of the body weight of mice.

Preliminary phytochemical screening

Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer reagent, 3-glycosidated flavonoids by the use of Zn and HCl; tannins by the use of ferric chloride solution; anthraquinones by the use of born-traeger reaction and saponins by the ability to produce suds (7).

Spontaneous motor activity

Activity of individual mice was recorded using Animex Activity Meter (AB FARAD model, Sweden). Activity counts were cumulated 30 minutes after administration of water, almond oil, the aqueous extract (25, 75, 150, 300 or 900 mg/kg, i.p, n=5) or essential oil (10, 25, 75, 150, or 300 mg/kg, i.p., n=6-7) at 5-min intervals for 20 minutes.

Forced swimming test (FST)

The test was performed according to a modification (8) of the traditional method (9). Mice were placed individually in a transparent Pyrex cylinder (25 cm height x 18cm in diameter) filled with 25^{o}C water to a 15-cm depth. Two swim sessions were conducted between 13:00 and 18:00 hrs: an initial 15-min pre-test followed 24 hrs later by a 5-min test. Following swim sessions, the mice were removed from the water, dried and placed in their home cages. Different doses of the aqueous extract (25, 75, 150 or 300 mg/kg, n=9-10) and essential oil (10, 25, 75, 150 or 300mg/kg, n=9-10) of the *Melissa officinalis*, fluoxetine (20 mg/kg), imipramine (15mg/kg), distilled water or almond oil were administered i.p. three times at: 23.5, 5 and 0.5 hr prior to the FST. During the 5-min test, the climbing, swimming and immobility behaviors of the mice were recorded at 5 second intervals. Increases in climbing or swimming and reduction in immobility were considered as behavioral responses consistent with an antidepressant-like action (8).

Statistical analysis

All values were expressed as mean + SEM. The differences in the mean of activity counts per 5 minutes and the mean of immobility, climbing and swimming counts among different treated groups were statistically analyzed by one-way ANOVA followed by Dunnett *t* test. Dose dependency of the effects of the extract and essential oil on different behavioral responses was determined by linear regression analysis. SPSS 11.5 was used for statistical analyses and $p < 0.05$ was considered as a significant level.

RESULTS

Phytochemical screening of the aqueous extract

The phytochemical screening showed that the aqueous extract of *Melissa officinalis* leaves contains tannins and saponins.

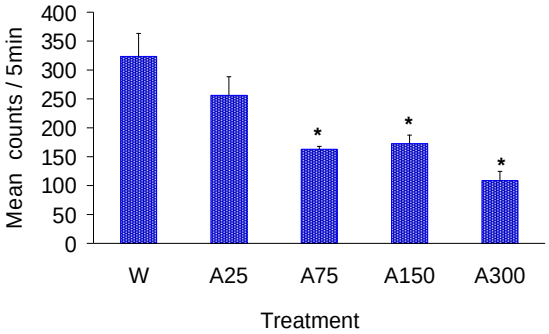

Figure 1. Effects of the aqueous extract of *Melissa officinalis* (A25-300 mg/kg; i.p) on spontaneous motor activity in mice (n=5). Data represents mean + SEM of the mean activity counts per 5 min during 20-min test period. Comparisons were made using one-way ANOVA followed by post hoc Dunnett t test.
* p< 0.01 compared with control group (W)

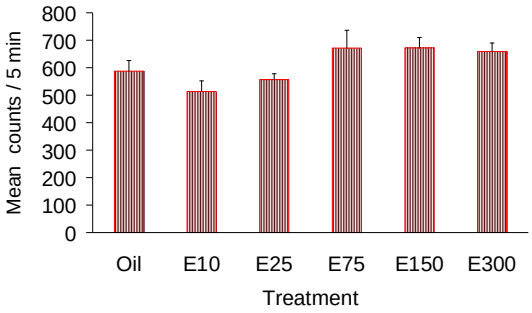

Figure 2. Effects of the essential oil of *Melissa officinalis* (E10-300 mg/kg; i.p) on spontaneous motor activity. Data represents mean + SEM of the mean activity counts per 5 min during 20-min test period (n=6-7 mice per group). Comparisons were made using one-way ANOVA followed by post hoc Dunnett t test.

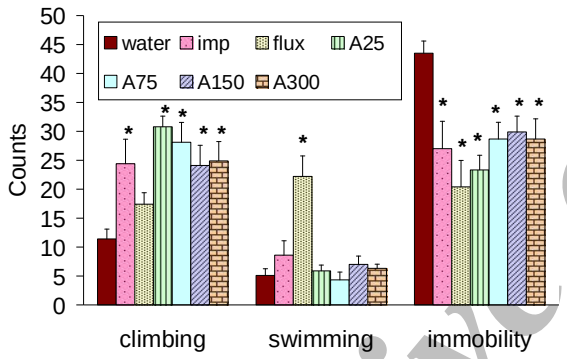

Figure 3. Effects of the aqueous extract of *Melissa officinalis* (A 25-300 mg/kg i.p.), fluoxetine (flux 20 mg/kg i.p.), and imipramine (imp 15 mg/kg i.p.) on active behaviors in the forced swimming test. Data represents means + SEM of the climbing, swimming and immobility counts during the 5 min test session (n=9-10 mice per group). Comparisons were made using one-way ANOVA followed by post hoc Dunnett t test.
* p< 0.01 compared with control group (W)

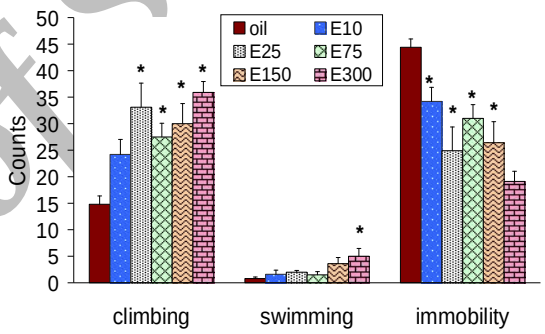

Figure 4. Effects of essential oil of *Melissa officinalis* (E 10-300 mg/kg i.p.) on active behaviors in the forced swimming test. Data represents means + SEM of the climbing, swimming and immobility counts during the 5 min test session (n=9-10 mice per group). Comparisons were made using one-way ANOVA followed by post hoc Dunnett t test.
* p< 0.01 compared with control group (oil)

Spontaneous motor activity

One-way ANOVA showed a significant effect of the aqueous extract on motor activity counts [F(4,19)=10.5, p<0.001]. Dunnett t test indicated a significant reduction in motor activity at doses of 75 (49.6%), 150 (46.6%) and 300 mg/kg (66.4%) of the aqueous extract, compared to control group (p<0.01) (Fig1). Tukey *post hoc* comparison indicated that the effect of 300mg/kg of the extract on reduction of spontaneous activity was significantly different from that of 25 mg/kg (p<0.01). Linear regression showed that the effect of extract on reduction of motor activity was dose dependent (r= 0.71, p<0.001). All studied doses of essential oil did not cause any significant effect on motor activity in comparison to oil-treated group (Fig 2).

Forced swimming test (FST)

Effect of the aqueous extract on FST

One way ANOVA showed a significant effect on immobility [F(4,42)=7.71, p<0.001] and climbing [F(4,42)=6.95, p<0.001] behaviors, but not swimming [F(4,42)=0.79, p>0.05), among different treatment groups. Dunnett t test indicated that doses of 25, 75, 150 and 300 mg/kg of the extract significantly caused a reduction in immobility (46.4, 34.1, 31.3, 34.1%, respectively) and an increase in climbing (170, 147, 111, 118%, respectively), compared to control group (p<0.01)

(Fig. 3). Linear regression analysis did not show a dose-dependent effects of the extract on immobility (r=0.14, p>0.05), swimming (r= 0.14, p>0.05) and climbing (r=0.17, p>0.05) behaviors. Imipramine and fluoxetine significantly increased climbing and swimming behaviors, respectively, and both reduced immobility, compared to control group (p<0.01) (Fig. 3).

Effect of the essential oil on FST

A significant effect was observed on immobility [F(5,53)=7.96, p<0.001], climbing [F(5,53)=5.79, p<0.001] and swimming [F(5,53)=3.22, p=0.01] behaviors among different treatment groups. The essential oil significantly reduced immobility (p≤0.01) and increased climbing (p<0.01) at doses of 25, 75, 150 and 300 mg/kg, compared to control group (Fig 4). The essential oil at the dose of 10 mg/kg reduced immobility (23%) in comparison to oil-treated group which was not significant (p=0.086). Only the dose of 300 mg/kg of the essential oil increased swimming behavior significantly (525%, p<0.01), compared to control group. The essential oil at the dose of 150 mg/kg increased swimming (350%) in comparison to oil treated group but it was not significant (p=0.09) (Fig 4). The essential oil showed a dose–dependent reduction in immobility (r=0.49, p<0.001) and increases in climbing (r=0.4, p<0.01) and swimming (r=0.46, p<0.001) behaviors. In addition, Tukey *post hoc* comparison between different doses showed a significant effect of 300 mg/kg of essential oil on immobility, compared to the dose of 10 mg/kg (p=0.01); and also a trend toward significant effect on immobility, in comparison to the dose of 75 mg/kg (p=0.09).

DISCUSSION

The present study showed that the aqueous and essential oil of *Melissa officinalis* exerted antidepressant-like activity in the FST. The FST is the most widely used pharmacological model for assessment of potential antidepressant activity in rodents following acute or short-term treatment (9, 10). This test is sensitive and relatively specific to all major classes of antidepressants including tricyclics, serotonin selective reuptake inhibitors, and MAO inhibitors (8, 11). Although all antidepressant drugs reduce immobility in the FST, two distinct active behavioral patterns are produced by pharmacologically selective antidepressant drugs (8). 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 (8, 12).

In this study, in agreement with previous report (12), the decrease in immobility induced by fluoxetine was accompanied by an increase in swimming, whereas climbing was not affected by this drug. On the other hand, imipramine increased climbing without modifying swimming. The present study showed that the aqueous extract of *Melissa officinalis* had significant antidepressant effects in mice. Different doses of the extract which were used in this study, were able to reduce immobility and to enhance active behaviors, i.e. climbing, simultaneously. However, the effect of the extract on immobility and climbing decreased by increasing the dose. These effects might reflect the sedative effect of the extract shown by a reduction in general motor activity. This may also be a reason for the lack of dose-dependent effect of the extract on immobility and climbing behaviors. On the other hand, the doses used in this study might not be in the linear portion of dose-responsive curve, or the metabolites of the phytoconstituents present in the extract might interfere with its antidepressant effect. Nevertheless, the antidepressant activity of the extract was close to that observed for IMP in the current study. As IMP is a clinically used antidepressant drug, thus, the extract may have potential therapeutic value for the management of depressive disorders. In addition, the extract of lemon balm showed a sedative effect which was manifested by a dose-dependent reduction in spontaneous motor activity. This is in agreement with the use of this plant in folk medicine as a sedative-hypnotic. Several studies have also shown its mild sedative effects (13). This sedative effect of the extract ensures that the increased active behaviors in the FST were not caused by the possible CNS stimulating effect of the extract i.e. increased general motor activity and confirms the specificity of antidepressant effect of the extract.

The mechanism of antidepressant effect of the aqueous extract of *Melissa officinalis* is unknown. However, the pattern of behaviors exerted by the extract in the FST is similar to those of imipramine which suggests that this plant preparation acts probably by enhancement of norepinephrine neurotransmission as it is related to climbing behavior in the FST (8).

Sub-chronic administration of the essential oil of lemon balm also showed an antidepressant activity. Since the essential oil did not affect locomotor activity of mice, the antidepressant effect of essential oil could not be related to any motor effects. The pattern of behaviors observed by the essential oil in the FST resemble to imipramine at low doses, and to fluoxetine at high dose. This implies that the essential oil

preparation may contain active chemical constituents that may act through both NE and 5-HT systems, however, activation of these systems may depend to the concentration of the essential oil.

Comparing the aqueous extract and the essential oil, both plant preparation reduced immobility, however, the effect of essential oil was greater and dose dependent. In contrast to the aqueous extract, the essential oil did not show sedative effects and increased swimming behavior at high doses. These differences can be explained by the fact that the extract and essential oil have different combinations of constituents. Obviously the aqueous extract consisted mainly water-soluble compounds such as phenolic acids (rosmarinic acid), whereas essential oil contains mainly lipophylic principles (citronella, citral and other monoterpenes and sesquiterpenes). At present the phytoconstituents responsible for the antidepressant effects of *Melissa officinalis* is unknown. The leaf of *Melissa officnalis* contains monoterpenoid aldehyde, flavonoids (quercitrin, rhamnocitrin), polyphenolic compounds (rosmarinic acid), triterpenes (ursolic and oleanolic acids), tannins and monoterpene glycosides (2). Any of these components may underlie the effects seen in this study. Regarding the aqueous extract, as the preliminary phytochemical results indicated, it could be suggested that its antidepressant effect may be due to the content of tannins and saponins. However, there is also a possibility that rosmarinic acid may contribute to some of the antidepressant effects of *Melissa officinalis,* as an antidepressant activity for rosmarinic acid and its metabolite i.e. caffeic acid has been previously reported (14). Furthermore, citral is one of the main components of lemon odor found in the leaves of *Melissa officinalis*. Interestingly, lemon odor has been shown to reduce total immobility time in the forced swimming test significantly and also to potentiate the imipramine-induced reduction of total immobility time in the test (15). Thus, citral may be another phytoconstituent responsible for the antidepressant effect of *Melissa officinalis*. Certainly, further studies are required for isolation and identification of the active compound(s) from leaves of *Melissa officinalis*.

CONCLUSION

This study has shown that the aqueous extract and essential oil of leaves of *Melissa officinalis* possess antidepressant effects. The present findings support the use of *Melissa officinalis* as an antidepressant as recommended in traditional medicine and gives a clue to development of new antidepressant agents from well-known folk remedies.

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