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The Antidepressant Activity of *Matricaria chamomilla* and *Melissa officinalis* Ethanolic Extracts in Non-Reserpinized and Reserpinized Balb/C Mice

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Abstract

Background: *Matricaria chamomilla* and *Melissa officinalis* have been used as antidepressants in traditional Iranian medicine.
Objectives: The aim of this study was to determine the effectiveness of *Matricaria chamomilla* and *Melissa officinalis* extracts compared to the classic antidepressant drug, imipramine, in adult non-reserpinized and reserpinized mice through the forced swim test.
Methods: In the current experimental study, 80 mice were divided into 10 groups. The first group received normal saline and the second and third groups received 25 and 50 mg/kg of *Matricaria chamomilla* extract, respectively. The fourth and the fifth groups received 25 and 50 mg/kg of *Melissa officinalis* extract. The sixth group received imipramine at a dose of 15 mg/kg. The seventh group received 5 mg/kg of reserpine and normal saline. The eighth and ninth groups received 25 and 50 mg/kg of *Melissa officinalis* and *Matricaria chamomilla* extracts, respectively. The tenth group was given imipramine through intraperitoneal (I.P) injection. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in SPSS.
Results: *Matricaria chamomilla* (50 mg/kg), *Melissa officinalis* (25 mg/kg), and imipramine (15 mg/kg) in non-reserpinized mice significantly decreased the duration of immobility in the forced swim test compared to the control group ($P < 0.01$). There was a reduction in the duration of immobility in the reserpinized mice administered *Matricaria chamomilla* at a dose of 50 mg/kg compared to the positive control group ($P < 0.01$).
Conclusions: *Matricaria chamomilla* and *Melissa officinalis* have antidepressant effects and may be taken into consideration in treating patients suffering from depression.

Keywords: Depression, Forced Swim Test, *Matricaria chamomilla*, *Melissa officinalis*, Balb/C

1. Background

Depression is an increasingly common affliction that has received growing consideration from the World Health Organization (WHO) (1). From a clinical perspective, it is accompanied by a variety of other disorders such as sexual disabilities, pregnancy complications (2), mental disorders, loss of appetite, and sleep irregularities (3). It affects 25% of women and 12% of men (4-6). According to the WHO, approximately 12.3% of the population are currently suffering from mental and behavioral disorders, and this number is expected to rise to 15% by 2020. However, only a small proportion of those with depression are receiving proper treatment (7). Depression is the second leading cause of disability after cardiovascular diseases in industrialized countries (6, 8). Statistically, the proportion of people diagnosed with depression in Iran is approximately 4.1% (1), which is twice as much as China (2%) (8) and Japan (2.9%) (9), but less than the reports from the US (6.6%) (10) and Ukraine (8.3%) (11). Dramatic social and economic changes have impacted Iranians in recent decades (1) and have resulted in a spike in the consumption of synthetic medications and their subsequent side effects (12). Understanding preventive methods and using herbal treatments can help with the prevention of the undesirable side effects of synthetic drugs (13).

It is believed that the majority of the available antidepressant drugs are associated with increased exposure

to synaptic monoamines such as serotonin (5-ht), norepinephrine (NE), and dopamine (DA) (14, 15). It is worth mentioning that one-third of patients with severe depression do not respond to treatment (4). There are currently three different methods for measuring the effectiveness of antidepressants, namely the Hamilton survey among humans (6, 16) and the forced swim test (3) and tail suspension test among mice (6, 14).

According to the monoamine hypothesis among lab animals, reserpine can block dopamine receptors (17) and subsequently cause dopamine depletion by emptying monoamine resources (14, 17). This can lead to severe depression followed by a drop in body temperature and a lack of movement (14). Throughout the past decade, widespread studies have been conducted on the antidepressant effects of herbal medications on lab animals (18, 19). The current study sought to investigate the effects of herbal antidepressants with fewer side effects.

Melissa officinalis (lemon balm) is an herb of the Lamiaceae family. This plant is known as *Badranj Boya* in Iran. It has been used for more than 2000 years and is found along the Mediterranean and in Europe and Central Asia. In Iran, it is found in several provinces such as Tehran, Golestan, Azerbaijan, Lorestan, and Kermanshah (20). In Iranian traditional medicine, this plant is commonly used as an antidepressant (19), a sedative, and an agent decreasing stress, relieving anxiety, increasing levels of body antioxidants, and treating inflammation and spasms (21). This plant contains several monoterpene aldehydes and flavonoids (e.g., quercitrin and rhamnocitrin) (22). Phenolic compounds found in lemon balm include rosmarinic acid, tannins, and flavonoids (23-25). Recent studies have shown that *Melissa officinalis* extract contains rosmarinic acid, triterpenoids, oleanolic acid, and ursolic acid, which inhibit gamma-aminobutyric acid transaminase (GABA-T) activity (26) and increase GABA levels in the brain, which can cause dysfunction in the GABA-T and lead to an abundance of GABA-T in brain cells (27).

Matricaria chamomile belongs to the Asteraceae family and is one of the herbal medications most commonly used in Iranian traditional medicine. Matricaria chamomile is found in Asia, the Mediterranean, North Africa, North and South America, Australia, and New Zealand (28). Its dried flowers have sedative properties (29) and are effective in treating spasms, nephritis, menstrual cramps, nausea, acne, and mouth ulcers. It also works as a disinfectant (28) as a component of the flowers includes several phenolic compounds, primarily the flavonoids apigenin, quercetin, patuletin, luteolin, glucosides coumarins, and dicycloethers (30).

Flavonoids are regarded as natural polyphenolic compounds and are commonly found in nature. Studies have shown that flavonoids have some biological functions, and with their antioxidant effects, they can help reduce the risks of human immunodeficiency virus (HIV), bacterial infections, cancer, and diabetes, ameliorate the effects of radiation, reduce blood vessel expansion, and prevent atherosclerosis and thrombosis (31, 32).

2. Objectives

Many natural alternatives to antidepressant medications are considered safer in the treatment of depression. Flavonoids have been the focus of recent antidepressant studies as they have been shown to have antidepressant effects. The aim of the current study was to determine the antidepressant-like effects of *Matricaria chamomilla* and *Melissa officinalis* ethanolic extracts on reserpinized mice through the forced swim test.

3. Methods

3.1. Plant Extract Preparation

First, the plant samples of both *Matricaria chamomilla* and *Melissa officinalis* were purchased from a local provider in Shahrekord, and their authenticity was verified in the Medical Plants Research Center, Shahrekord Branch, Islamic Azad University. They were then labeled as 406 and 407. During the preparation phase of the ethanolic extracts, air-dried and powdered flowering branches of the plants were macerated with 70% ethanol (96%, Ghadir Industries, Iran) for 48 h. The macerated powder was then shaken, filtered, and evaporated in a rotary evaporator under reduced pressure until dry (20, 33).

3.2. Animals

In the current experimental study, 80 albino Balb/C female mice (weight: 20 - 25 g) were acquired from Pasteur Institute of Iran. After being sent to the Islamic Azad University of Shahrekord, the mice were kept in poly-carbon cages at 21°C - 25°C in 12 hours of light and 12 hours of darkness. Eight mice were placed in each cage with free access to water and a commercial pellet diet (20, 23). This study was performed in accordance with the guide for the care and use of laboratory animals of the Islamic Azad University of Shahrekord, Iran.

3.3. Choice of Drugs

Imipramine hydrochloride (Pars Daru, Tehran, Iran) at a dose of 15 mg/kg was injected intraperitoneally (I.P) (19). *Matricaria chamomilla* and *Melissa officinalis* extracts were freshly prepared before I.P administration at the doses of 25 and 50 mg/kg, respectively. Imipramine hydrochloride

(15 mg/kg, I.P) was injected 30 minutes before the forced swim test, and immobility time records were documented. Then, in order to induce depression, reserpine (Sigma-Aldrich, MO, USA) was mixed with normal saline (0.9%) and 0.8% acid glacial acetic acid (34) and injected at a dose of 5 mg/kg 18 hours before the forced swim test (35) in order to clear aminergic receptors from amine.

3.4. Arrangement of the Experimental Groups

In order to analyze the antidepressant effects of the hydroalcoholic extracts of *Matricaria chamomilla* and *Melissa officinalis* in nonreserpinized and reserpinized mice, they were divided into 10 groups of 8 (Table 1).

3.5. Forced Swim Test

The forced swim test was performed according to previous protocols (36). First, water containers (height 25 cm and diameter 12 cm) were chosen and filled with 25°C water. Then, the mice were gently put in the container from a height of 20 cm, and as commonly accepted, the lack of hand movement was considered as motionless state. The test time was set to 6 minutes, with the first 2 minutes designated for acclimatization to the new environment and not taken into account. After two minutes, the movements were recorded, documented, and timed (37). The containers were thoroughly cleaned to reduce residue, urine, and soft hair. Each animal was used only once. Immediately after the test, the mice were dried in a room with a temperature of 29°C - 31°C (12).

3.6. Statistical Analysis

All the data are presented as mean ± SEM. Differences between the experimental groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P value less than 0.01 was considered significant.

4. Results

4.1. Findings Regarding the Common Antidepressant Effects of the Hydroalcoholic Extracts of Melissa officinalis and Matricaria chamomilla and Imipramine Among Adult Non-Reserpinized Mice

According to our data, injection of the hydroalcoholic extracts of *Matricaria chamomilla* (50 mg/kg, I.P) and *Melissa officinalis* (25 mg/kg) and imipramine (15 mg/kg) significantly reduced immobility time during the forced swim test ($P < 0.01$; Table 2). However, the hydroalcoholic extracts of *Matricaria chamomilla* and *Melissa officinalis* at the at the doses of 25 and 50 mg/kg respectively had no effects on immobility time compared to the saline group ($P > 0.05$; Table 2).

4.2. The Effects of the Hydroalcoholic Extracts of Matricaria chamomilla and Melissa officinalis and Imipramine on Immobility Duration of the Reserpinized Mice

The most active compound in the forced swim test was Matricaria chamomile (50 mg/kg), which significantly antagonized the reserpine action and decreased the immobility time ($P < 0.01$), whereas the I.P injection of the hydroalcoholic extract of *Melissa officinalis* (25 mg/kg) and imipramine (15 mg/kg) 18 hours after the injection of reserpine (5 mg/kg) had no effects on reserpine-induced immobility time and did not antagonize reserpine ($P > 0.1$; Table 3).

5. Discussion

In the present study, we used the forced swim test to compare the effects of the hydroalcoholic extracts of *Matricaria chamomilla* and *Melissa officinalis* and imipramine (standard antidepressant) on behaviors, including immobility, in reserpinized and non-reserpinized mice. The forced swim test, as described in detail by Abelaira et al. (37), was used to evaluate depressive behavior.

As previously mentioned, many neurotransmitters are involved in the pathophysiology of depression. According to the monoamine hypothesis, the increase of neurochemicals during depression might be a result of serotonin, norepinephrine, and/or dopamine depletion in the central nervous system (38). Antidepressant drugs can immediately increase synaptic concentrations of these monoamines (39) and are able to reduce the immobility time in rodents (3).

Reserpine-induced depression in animal models is based on the monoamine hypothesis of depression with reductions of brain levels of both serotonin and noradrenaline and declined locomotion (14, 17). In the current study, the antidepressant effects of *Melissa officinalis* and *Matricaria chamomilla* extracts at the doses of 25 and 50 mg/kg, respectively, were evaluated through the forced swim test. There was a significant decrease in the immobility time following the intraperitoneal injection of *Matricaria chamomilla* at the dose of 50 mg/kg and *Melissa officinalis* at the of 25 mg/kg compared to the control group. As for *Matricaria chamomilla* extract, there was a direct correlation between dosage and immobilization time, and regarding reserpine, there was a significant correlation between the antagonizing effects of reserpine and *Melissa officinalis* and *Matricaria chamomilla* extracts ($P < 0.001$). Also, following the intraperitoneal injection of imipramine, there was a significant attenuation in immobilization time among the mice in the forced swim test, which was eventually antagonized by reserpine.

Table 1. Treatments for Forced Swim Test in Mice

Group (N = 8)	Treatment (I.P)
FST1 (negative control)	Normal saline 10 mL kg^{-1}
FST2	*Matricaria chamomilla* extract 50 mg kg^{-1}
FST3	*Matricaria chamomilla* extract 25 mg kg^{-1}
FST4	*Melissa officinalis* extract 25 mg kg^{-1}
FST5	*Melissa officinalis* extract 50 mg kg^{-1}
FST6 (positive control)	Imipramine hydrochloride 15 mg kg^{-1}
FST7 (negative control)	Reserpine 5 mg kg^{-1}, normal saline 10 mL kg^{-1}
FST8	Reserpine 5 mg kg^{-1}, *Melissa officinalis* extract 25 mg kg^{-1}
FST9	Reserpine 5 mg kg^{-1}, *Matricaria chamomilla* extract 50 mg kg^{-1}
FST10 (positive control)	Reserpine 5 mg kg^{-1}, imipramine hydrochloride 15 mg kg^{-1}

Table 2. Effects of the Hydroalcoholic Extracts of *Melissa officinalis* and *Matricaria chamomilla* and Imipramine on Immobility Duration in Adult Non-Reserpinized Mice[a]

Treatments	Immobility Times (s)	P Value
Normal saline 10 mL kg^{-1}	184.83 ± 5.65	-
***Matricaria chamomilla* extract 25 mg kg^{-1}**	175 ± 7.30	0.98
***Matricaria chamomilla* extract 50 mg kg^{-1}**	62.17 ± 7.91	0.00
***Melissa officinalis* extract 25 mg kg^{-1}**	128 ± 8.67	0.00
***Melissa officinalis* extract 50 mg kg^{-1}**	150.83 ± 4.84	0.055
Imipramine hydrochloride 15 mg kg^{-1}	68 ± 7.78	0.00

[a] Values are expressed as mean ± SEM. One-way ANOVA, post hoc Tukey test, (N = 8).

Table 3. Effects of the Hydroalcoholic Extracts of *Matricaria chamomilla* and *Melissa officinalis* on Immobility Duration in Adult Reserpinized Mice Compared to Imipramine[a]

Treatments	Immobility Times (s)	P Value
Reserpine 5 mg kg^{-1}, normal saline 10 mL kg^{-1}	208.60 ± 6.313	0.368
Reserpine 5 mg kg^{-1}, *Melissa officinalis* extract 25 mg kg^{-1}	211 ± 1.71	0.190
Reserpine 5 mg kg^{-1}, *Matricaria chamomilla* extract 50 mg kg^{-1}	142 ± 14.795	0.003
Reserpine 5 mg kg^{-1}, imipramine hydrochloride 15 mg kg^{-1}	182.67 ± 3.211	1.00

[a] Values are expressed as mean ± SEM. One-way ANOVA, post hoc Tukey test, (N = 8).

In a study by Moallem et al. in 2007, it was concluded that antidepressants could selectively prevent the reabsorption of serotonin and norepinephrine, which in turn, can lead to a decrease in immobilization duration and a surge in ascending activities (40). The mechanism of antidepressant effects of the *Matricaria chamomilla* and *Melissa officinalis* ethanolic extracts is unknown, but it seems that flavonoids such as apigenin, naringenin, quercetin, chrysin, catechinic acid, epicatechin, kaempferol, and fisetin can have a preventive effect against monoamine oxidases. The strongest inhibitors of the combination of ligand and benzodiazepine receptors can be used as antidepressants (41, 42). Furthermore, flavones can bind to GABA-A, which can have soothing and relaxing effects (43). Yi et al. pointed out that dietary flavonoids have multiple neuroprotective actions in central nervous pathophysiological conditions, including depression, and it was reported that naringenin can cause a potent antidepressant-like impact via central serotonergic and noradrenergic systems (44).

This study also showed that an increase in the dosage of *Matricaria chamomilla* extract from 25 to 50 mg/kg decreased the immobilization duration in the forced swim test. In addition, the flavones available in leaves and flowers can have a suppressing effect on monoamine oxidase, which in turn, can cause antidepressant effects (40, 43). It is commonly believed that improving brain monoaminergic functions is beneficial in treating depression, and

the serotonergic, noradrenergic, or dopaminergic systems have become targets for the development of antidepressants (45).

Reserpine-treated rats exhibited decreased levels of superoxide dismutase, catalase, and glutathione peroxidase in the cranial lobe of the brain (46), which could subsequently trigger oxidative stress and pseudo-depression syndrome in animals (12). Thus, blocked monoamine oxidase can prevent catecholamines from getting metabolized, which can minimize the effects of depression (47). The majority of available medications such as amitriptyline, imipramine, and clomipramine can help reduce depression and cause selective inhibition and reabsorption of both norepinephrine and serotonin (48). Previous studies reported that the intraperitoneal administration of imipramine (10, 20, and 30 mg/kg) reduced lipid and protein peroxidation and increased superoxide and catalase activities, compared to control groups, in the hippocampus and prefrontal cortex (49). Some herbal medications such *Melissa officinalis* and *Matricaria chamomilla* contain flavonoids such as quercetin, apigenin, and luteolin, which can potentially have antidepressant effects (31) and can probably inhibit monoamine oxidase. However, further experiments are needed to confirm the active anti-depressant effects of *Melissa officinalis* and *Matricaria chamomilla* and their corresponding mechanisms of action.

5.1. Conclusions

This study confirmed that the hydroalcoholic extract of *Matricaria chamomilla* at the dose of 50 mg/kg was far more effective than imipramine and the hydroalcoholic extract of *Melissa officinalis* in reducing immobilization time. Thus, the administration of *Matricaria chamomilla* may be beneficial in the management of depression.

Footnotes

Authors' Contribution: All the authors were equally involved in the study and manuscript preparation.

Conflict of Interests: The authors declare no conflict of interest related to the present work.

Ethical Considerations: All the animal procedures were performed with regard to the guidelines of the Iranian Animal Ethics Society and local university rules.

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