Received: 28 July 2024 | Revised: 25 January 2025 | Accepted: 24 February 2025

DOI: 10.1002/jsf2.70004

RESEARCH ARTICLE

JSFA Reports

Lack of tolerance to the separate or combined hypnotic actions of different partitions of *Melissa officinalis* (total, ethyl acetate, and aqueous) and methanolic partition of *Albizia julibrissin*

Mahshid Rashidi[1] | Masoud Sadeghi-Dinani[2] | Mohammad Rabbani[1]

[1]Department of Pharmacology and Toxicology and Isfahan Pharmaceutical Research Centre, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

[2]Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence
Mohammad Rabbani, Department of Pharmacology & Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: rabbani@pharm.mui.ac.ir

Abstract

Objective: The present study investigates the hypnotic action of the methanolic partition of *Albizia julibrissin* (MPAJ) and various partitions (total, ethyl acetate, and aqueous) of *Melissa officinalis* (MO) in mice.

Methods: Pentobarbital-induced sleep (PIS) time prolongation was used to assess the hypnotic action of test compounds. For the tolerance study, MO and MPAJ were given alone or in combination for 7 days, and then the locomotor activity of the mice was measured as an index of tolerance. Mortality of the herbal extracts was studied after intraperitoneal (i.p.) and oral administration in mice.

Results: Separate administration of total extracts of *Melissa officinalis* and methanolic extract of *Albizia julibrissin* at 12.5 mg/kg significantly increased the PIS time. In the combination of two plants, there was no additional or synergistic increase in sleepiness when compared to the separate administration of each plant ($p < 0.05$). In contrast to diazepam, the locomotor activity of the animals was not significantly altered after 7 days of injection with MPAJ or total extract of MO (TEMO), or their combinations.

Conclusion: Separate administration of TEMO and MPAJ provides significant hypnotic effects. The combination of two extracts, however, does not result in synergistic or additive hypnotic action. The lack of additive effects could be due to possible interaction by components of the two plants. The increase in mortality rate and undesirable reactions observed after i.p. administration of MPAJ is probably due to the absorption of certain toxic compounds that are not normally absorbed after oral administration.

KEYWORDS
diazepam, hypnotic, mice, pentobarbital, plant extracts, tolerance

INTRODUCTION

Sleep is a fundamental physiological process aimed at restoring and rejuvenating the body, which may be susceptible to disruption due to several circumstances like disease, stress, and environmental noise. The presence of a chronic sleep problem has been associated with several health consequences, including diminished reaction times, impaired memory function, difficulties in emotional regulation, and alterations in immune system functioning.[1,2]

Several synthetic drugs such as benzodiazepines,[3] non-benzodiazepines,[4] antidepressants,[5] and antihistamines[6] have been used to treat people with sleep disorders. Despite their drawbacks such as tolerance and withdrawal, drugs such as benzodiazepines are still the most commonly prescribed drugs.[7] The use of herbal extracts

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2025 The Author(s). *JSFA Reports* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

is picking up ground among clinicians as well as patients as an alternative therapy, particularly for those people with mild to moderate symptoms of anxiety and sleep disorders.[8]

Lemon balm with the scientific name of *Melissa officinalis* (MO) is owned by the Lamiaceae family, indigenous to the Mediterranean, southern Europe, northern Africa, and east to the Caucasus and northern Iran.[9,10] Lemon balm has been used since ancient times as a mild sedative, anti-anxiety, and hypnotic.[11] The leaves of this plant contain flavonoids, phenolic compounds, and monoterpenoid aldehyde.[12] These components are thought to be responsible for most of the lemon balm properties, especially its antioxidant and sleep-inducing effects.[11]

Albizia julibrissin (AJ), commonly known as Persian silk tree, has dried flowers or bark that are commonly processed for various medicinal purposes. A wide range of pharmacological effects has been reported for AJ, ranging from antidepressant and anxiolytic,[13] anti-inflammation,[14] antioxidation,[15] and antitumor.[16] While the pharmacological effects of Albizia alone have been studied well, its combination with other herbs has not been investigated. In a recent study investigating the effects of different partitions of AJ for insomniac action, we showed that the methanolic partition of the plant produces stronger sleep induction than other tested partitions (Rabbani M, 2022, unpublished data). To further this work, we aimed to evaluate the combinational effects of the methanolic partition of AJ (MPAJ) with various partitions of MO on pentobarbital-induced sleep (PIS) time in mice. Herb-herb combinations could result in therapeutic benefits and occasionally with a greater efficacy.

MATERIALS AND METHODS

Reagents and chemicals

The study utilized sodium pentobarbital powder from Sigma (Germany, Lot 521 k1738) as the standard drug. Other chemicals, including Tween 80 (from Merck, Germany), ethyl acetate, methanol (from Pars Chemi, Iran), ethanol, hexane (from Ashtalk and Mojalli, Iran), diazepam ampoule (Chemidarou, Iran), and normal saline (Darou Pakhsh, Iran), were sourced from local suppliers.

Plant material

Flowers of AJ were collected from Isfahan province, Iran, in May 2021. The aerial part of MO was purchased from Pakan Bazr Company (Isfahan, Iran). The identity of plant specimens was confirmed by the Department of Pharmacognosy, and a voucher specimen of AJ (SAM-2494) and MO (SAM-3295) was registered and deposited in the Samsam Shariat Herbarium, Faculty of Pharmacy, Isfahan University of Medical Sciences, Iran.

Extract preparation

The plant samples were shade-dried and subsequently ground by an electric mill. The powdered samples of AJ (1500 g) and MO (1000 g) were separately extracted by the maceration method using 5 L of methanol: water (70:30) and 5 L of ethanol: water (70:30), respectively, with occasional stirring at room temperature. The extraction procedure was repeated three times at 3-day intervals. The resulting extracts (total extract) were concentrated to 1000 mL using a rotary vacuum evaporator (Heidolph VV 2000, Germany) under reduced pressure at 40–45°C.

A total of 500 mL of the concentrated AJ total extract was separated into aqueous and hexane phases using a decanter. The procedure was repeated three times and finally the resultant hexane partitions were pooled. The remaining aqueous layer was then separated between water and ethyl acetate using the same procedure. The aforementioned procedure was repeated for MO's total extract, yielding the ethyl acetate and aqueous partitions. The final partitions of both plant samples were concentrated by rotary evaporator and completely dried using freeze-drier equipment. The dried partitions were stored in the refrigerator until use.

Animals

Male Swiss mice (25–30 g, 6 weeks old) were collected from the animal house of the School of Pharmacy (Isfahan, Iran) and were kept in groups of six mice per cage under standard environmental conditions of humidity, temperature, and light/dark cycle. Adequate supplies of sustenance and hydration were readily accessible without restriction. All methods performed in animal studies were conducted in accordance with the criteria for the care and use of laboratory animals as qualified by The National Ethical Committee in Iran (Ethics code: IR.MUI.RESEARCH.REC.1400.479). The study aimed to minimize animal suffering and reduce the number of animals used in the tests. Experiments were conducted during the light period, and behavioral observations took place in a soundproof room to mitigate diurnal variations. Animals were randomly selected and acclimatized to laboratory conditions by being kept in their cages for 5 days prior to dosing.

Treatment

Mice received a single intraperitoneal (i.p.) injection of drugs (diazepam, 2 mg/kg; sodium pentobarbital, 50 mg/kg), plant extracts (at various doses), or vehicle (normal saline), and were applied for only one test. The injections were randomly administered between 8:00 and 13:00 to account for fluctuations in daily activity levels. For each treatment group, a minimum of six mice was used.

Determination of total phenolic content

The total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu method, following the procedure outlined by Everette.[17] Total phenolic content was determined using a standard curve based on different concentrations of gallic acid (50–500 μg/mL in methanol). The UV absorbance of the samples was read

at 765 nm, and the results were expressed as gallic acid equivalents (milligram of gallic acid equivalents per gram) of dried extract.

Pentobarbital-induced sleep (PIS) test

The sleep induction test was based on extending sleep duration through the use of pentobarbital. A single i.p. injection of vehicle, diazepam (2 mg/kg) or partitions of extracts was given 30 min prior to pentobarbital (50 mg/kg). The time interval between pentobarbital injection and the onset of sleep (loss of righting reflex) and the total duration of sleep (the time interval between the lack of the righting reflex and the acquisition of the righting reflex) was recorded.[18]

Locomotor activity test (LAT)

To assess spontaneous locomotor activity, a rectangular dark cage measuring 35 × 25 × 29 cm, equipped with an infrared tracking device, was used. This equipment records two crucial parameters (the number of movements and the time traveled) during three 5-min observation sessions, totaling 15 min, as explained by explained by Rabbani et al. (1995). The units of the activity counts were arbitrary and based on the beam breaks by movement of mice. To calculate the animal's mobility, the number of movements was multiplied by the duration of movements. Mice were injected with different substances: plant extract, diazepam, or vehicle (normal saline). After a 30-min interval, the mice were placed in the activity cage for observation.[19]

Lethal dose at 50% (LD_{50})

The LD_{50} of the extracts was determined by the methods described by Akhila et al.,[20] and Guideline 423[21] of the Organization for Economic Co-operation and Development (OECD). The total extract of MO (TEMO) and MPAJ were separately administered via p.o. and i.p. routes. Based on our previous studies, MO and AJ were first administered at 2000 and 300 mg/kg, respectively. After oral administration of MO and AJ, the animals were observed for 30 min, 4 h, 24 h, and 14 days, and their body weights were monitored. After i.p. administration of extracts, the animals were observed for 30 min, 4 h, and 24 h for signs and symptoms of toxicity. The same procedure was used for the determination of LD_{50} in combination studies.

Tolerance test

In order to assess the development of tolerance to plant extracts, locomotor activity was used. The MO and AJ extracts alone or in combination were administered (i.p.) for 7 days. Locomotor activity was measured using the above-explained method 30 min after the first dose and on the eighth day.[22]

Statistical analysis

The results are presented as mean ± SEM. To analyze the data, the researchers employed a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. *P* values less than 0.05 were considered significant. The software programs that were used for data analysis were Graph Pad Prism 8.

RESULTS

Total phenolic content

The total phenolic content of AJ and MO extracts was measured using the gallic acid standard curve. The total phenolic content of the MPAJ was found to be 36.3 mg GAE/g and for the total, the ethyl acetate and aqueous partitions of MO were 244.7, 419.3, and 234 mg GAE/g, respectively.

The effects of plant extracts on pentobarbital-induced sleept time

PIS test in mice is a classic pharmacological method for screening sedative and hypnotic properties of drugs. Figure 1 shows the effects of diazepam, AJ, and MO extracts at varying doses on PIS time. Diazepam administered at a dose of 2 mg/kg significantly extended sleep duration by approximately 2.5 times compared to the control group ($p < 0.001$, Figure 1).

The MPAJ at 12.5, 25, 50, and 100 mg/kg doses significantly increased the PIS time by 73.95, 132.27, 135.46, and 208.25% compared to the control group, respectively ($p < 0.001$, Figure 1). As shown in Figure 1, the minimum effective dose of AJ was 12.5 mg/kg, a dose that was selected for further combination studies.

In order to find the minimum effective concentration of MO's extracts, different partitions, namely, ethyl acetate, aqueous, and total extracts were examined. As shown in Figure 1, the ethyl acetate and aqueous partitions of MO up to a dose of 200 mg/kg did not significantly change the PIS time and were therefore omitted from the combination studies. The total extract, on the other hand, at the dose of 12.5 mg/kg significantly ($p < 0.001$, Figure 1) increased the duration of sleep by 190.4% and was therefore selected for further examination.

MPAJ at two doses of 12.5 and 25 mg/kg were combined with a selected dose of ethyl acetate, aqueous, or total extracts of MO. The combination of MPAJ at any of these two doses with ethyl acetate or aqueous partitions of MO did not significantly change the PIS time. When the MPAJ (at either 12.5 or 50 mg/kg) was mixed with TEMO (12.5 or 50 mg/kg), the duration of sleep was significantly increased by more than twofold ($p < 0.001$, Figure 1) compared to the control group.

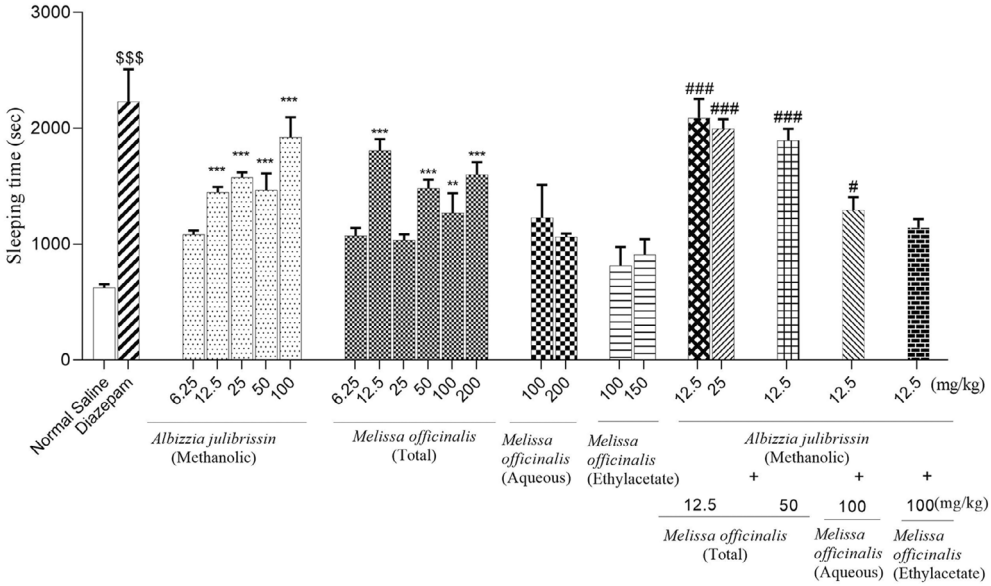


FIGURE 1 Sleep-inducing effects of *Melissa officinalis* partitions and *Albizia julibrissin* methanolic partition separately and their combinations on sleep duration in pentobarbital-induced sleep test. Normal saline (control), diazepam (2 mg/kg), methanolic extract of *Albizia julibrissin*, and various partitions (total, aqueous, and ethyl acetate) of *Melissa officinalis* were administered via the intraperitoneal route. Two doses (12.5 and 25 mg/kg) of *Albizia julibrissin* were used for combinational studies with selected doses of total (12.5 and 50 mg/kg), aqueous (100 mg/kg), and ethyl acetate (100 mg/kg) partitions of *Melissa officinalis*. All extracts were injected i.p., 30 min prior to pentobarbital administration (50 mg/kg, $n = 6$). The results are shown as Mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to the normal saline group. $^{\$\$\$}p < 0.001$ compared to the normal saline group. $^{\#\#\#}p < 0.001$ compared to the normal saline group.

The effects of plant extracts on spontaneous locomotor activity

The cumulative locomotor activity counts that measure the total activity during 15 min recording was performed to check the movement of animals after the injection of selected doses of plant extracts. Diazepam at 2 mg/kg significantly reduced mobility compared to the control group by 84.5% ($p < 0.001$, Figure 2). The selected doses of MPAJ at 12.5 mg/kg and the TEMO significantly reduced the mobility of the animals by 73.71% and 31.66% compared to the control group, respectively. The combination of MPAJ (12.5 mg/kg) and TEMO (12.5 mg/kg) further decreased the mobility of animals by 85.92% compared to the control group ($p < 0.001$, Figure 2). Figure 3 illustrates the locomotor activity counts recorded at three time intervals: 5, 10, and 15 minutes. The data indicate that diazepam significantly reduces locomotor activity across all time points. Furthermore, the compounds MPAJ and TEMO also led to a decrease in locomotor activity at these intervals, albeit to a lesser extent compared to diazepam.

Acute toxicity (LD50) of the extracts

The OECD test guideline recommendation doses were used for assessing the oral and i.p. toxicity of MPAJ and TEMO separately and in combination. Based on the above-mentioned guideline, TEMO and MPAJ were administered i.p. at 2000 mg/kg, respectively, and the animals were monitored for the first 30 min and then at 4 and 24 h. The dose of 2000 mg/kg of TEMO was found to be quite safe without causing any changes in the behavior of the animals. Based on the above protocols, the LD_{50} of MPAJ was determined to be 25 mg/kg after 24 h of i.p. injection. The i.p. administration of MOTE at 2000 mg/kg with 5 mg/kg of AJME was safe without any lethality (Figure 4).

For oral toxicity studies, TEMO and MPAJ were administered at 2000 mg/kg separately, and the animals were monitored for the first 30 min, 4 h, 24 h, and 14 days later. Both plant extracts, up to a dose of 2000 mg/kg, did not cause any mortality indicating the safety of the oral route. Similar results were found when the two plant extracts were administered orally at 2000 mg/kg.

Tolerance studies

To examine the development of tolerance, each plant extract or their combination was given via the i.p. route for seven consecutive days, and then locomotor activity was measured 30 min afterward. As shown in Figure 5, administration of diazepam at 2 mg/kg for 7 days resulted in the development of tolerance to the locomotor activity of animals. In contrast to diazepam, administration of MPAJ (12.5 mg/kg) or TEMO (12.5 mg/kg) or their combinations at these doses did not significantly change the locomotor activity of the animals after 7 days (Figure 5). As illustrated in Figure 6, the seven-day treatment with either saline or diazepam did not lead to any significant changes in the animals' body weights. However, the administration of MPAJ alone led to a reduction in body weight. In contrast, when MPAJ extract

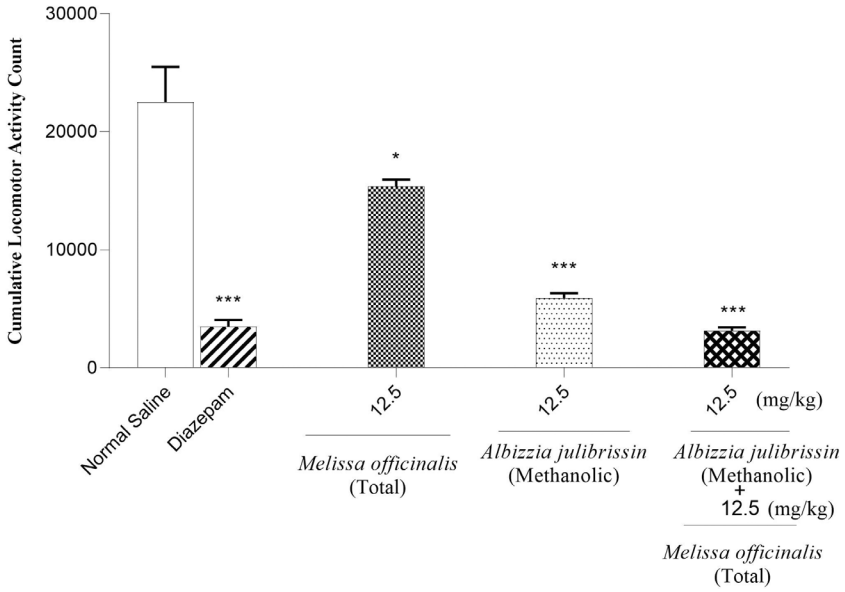


FIGURE 2 Sedative effects of the minimum effective dose of the selected partition of *Melissa officinalis* and the methanolic partition of *Albizia julibrissin* in the locomotor activity test (cumulative). Normal saline (control), diazepam at a dose of 2 mg/kg, the methanolic extract of *Albizia julibrissin*, and the total extract of *Melissa officinalis* were administered both separately and in combination as i.p. injection.Thirty minutes after each injection, the mice were placed in a locomotor activity test for a duration of 15 minutes ($n = 6$). The results are mentioned as Mean ± SEM. $^{*}p < 0.05$, $^{***}p < 0.001$ compared to the normal saline group.

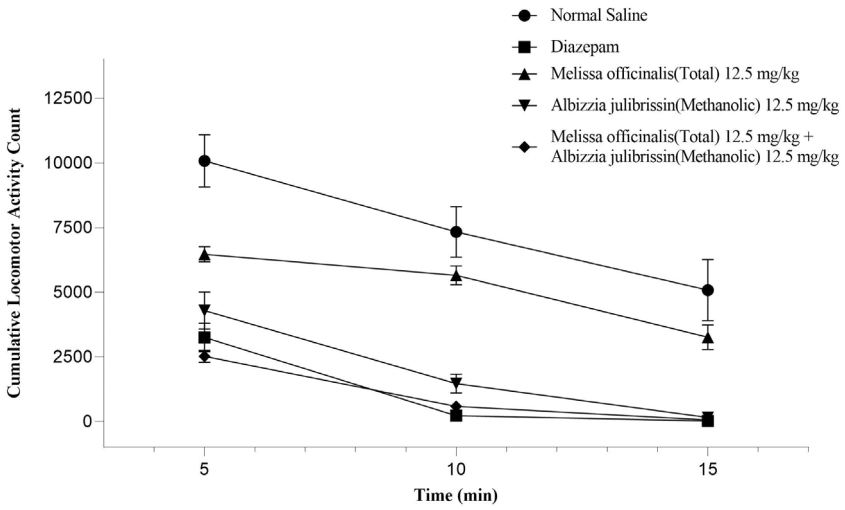


FIGURE 3 Sedative effects of the minimum effective dose of the selected partition of *Melissa officinalis* and the methanolic partition of *Albizia julibrissin* in the locomotor activity test. The results are mentioned as Mean ± SEM.

was combined with TEMO, no significant weight loss was observed (Figure 6).

DISCUSSION

The overall goal of these experiments was to determine whether the combined hypnotic effects of MPAJ and TEMO have additive or synergistic hypnotic effects. To assess the hypnotic effects of these extracts, a PIS time method was used. This is a well-known experimental approach in which the onset and duration of sleep by 50 mg/kg of pentobarbital are measured. Consistent with prior research and as anticipated, diazepam notably extended the PIS time confirming the validity of the method.[23]

To our knowledge, this is the first study in which the acute effects of extracts of AJ and MO have been considered after i.p. administration on PIS. MPAJ at a minimum dose of 12.5 mg/kg caused a twofold increase in the duration of sleep induced by

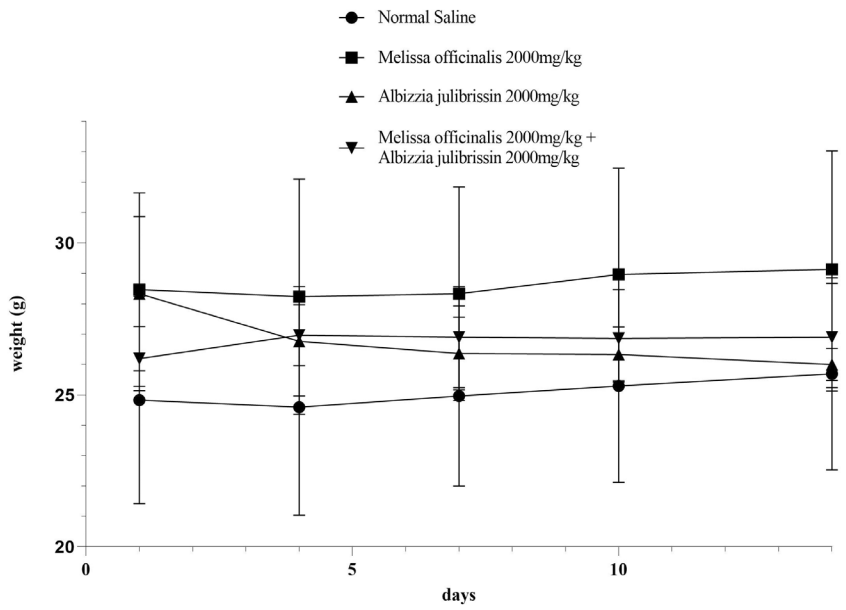


FIGURE 4 Changes in weight following oral administration of plant extracts at a dose of 2000 mg/kg, separately and in combination. Normal saline (control), methanolic partition of *Albizia julibrissin*, and total extract of *Melissa officinalis* were administered orally. The animals were monitored for weight during 14 days. Each group consisted of at least three female mice. The results are mentioned as Mean ± SEM.

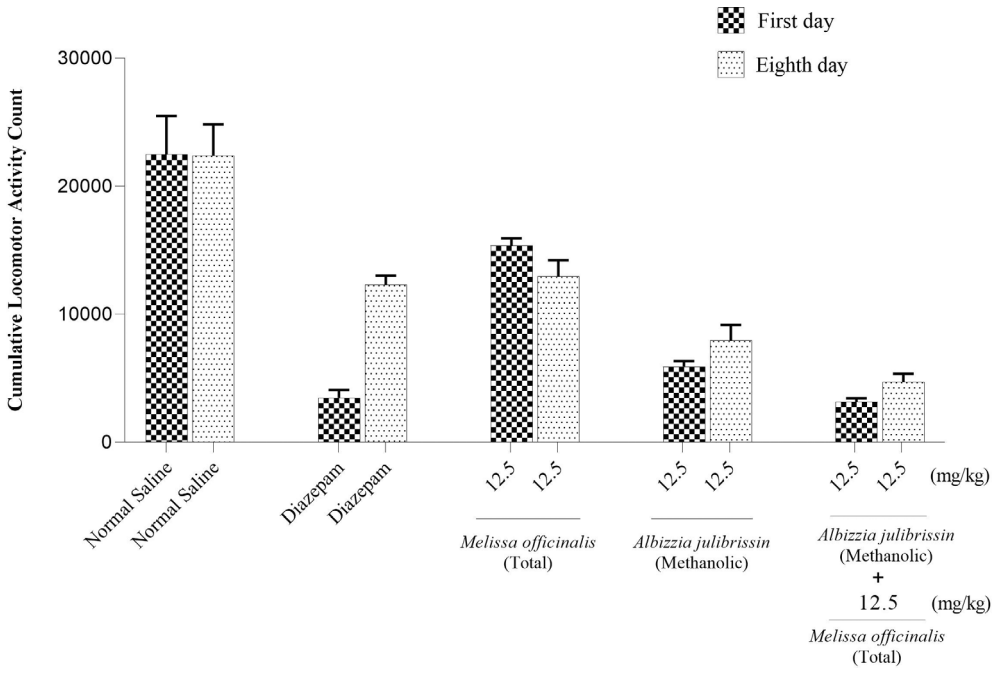


FIGURE 5 Sedative effects of the minimum effective dose of the selected partition of *Melissa officinalis* and the methanolic partition of *Albizia julibrissin* after seven consecutive days of injection compared to the first-day injection in the locomotor activity test. Normal saline (control), diazepam at a dose of 2 mg/kg, methanolic partition of *Albizia julibrissin*, and total extract of *Melissa officinalis* separately and combined as i.p. The injections were continued for seven consecutive days, and on the eighth day, 30 min after the injection, the mice were again placed in the locomotor activity test for 15 min ($n = 6$). The results are mentioned as Mean ± SEM.

pentobarbital without evidently shortening sleep latency. Two flavonoids, namely, quercitrin and isoquercitrin isolated from AJ have been identified as the main factors for its sedative effects.[24,25] These components are thought to exert their effects by increasing the level of 5-HT.[25] Another constituent of AJ is jolibroside C1, with anti-anxiety and sleep-inducing effects acting through 5-HT_{1A} and $GABA_A$ benzodiazepine receptors.[26]

Among the three partitions of MO that were tested here, the total extract provided the highest sleep-induction properties and was therefore chosen for further combination studies. According to the

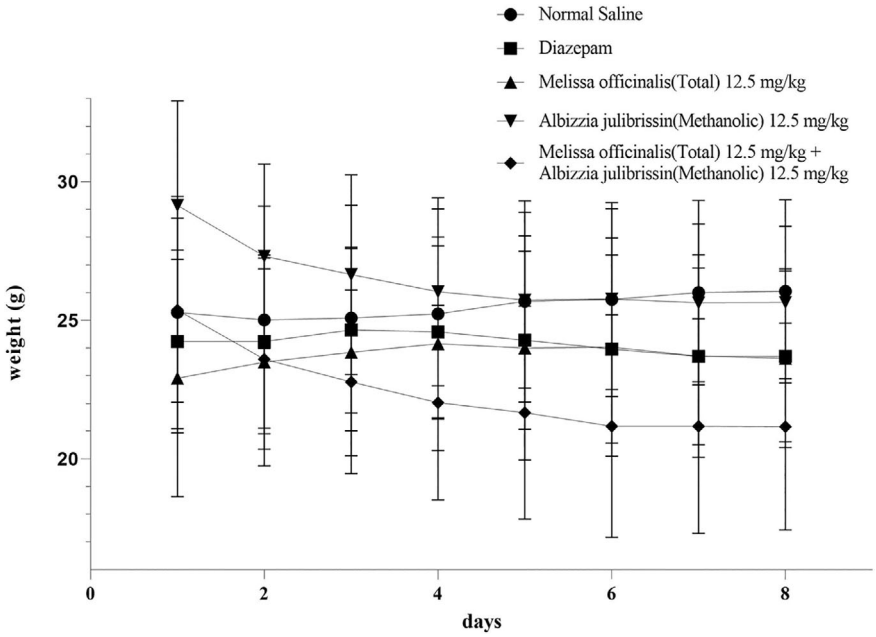


FIGURE 6 Weight changes of animals after injection of extracts and drugs for seven consecutive days. Normal saline (control), diazepam at a dose of 2 mg/kg, methanolic partition of *Albizia julibrissin*, and total extract of *Melissa officinalis* separately and combined were injected for seven consecutive days, and during this period the weight of the animals was controlled and recorded.

data obtained from the pentobarbital test, the sedative effects of the total extract were more pronounced than those of the aqueous and ethyl acetate extracts, highlighting the significance of the whole plant constituents in achieving the full effect.[27] The minimum dose of the TEMO after i.p. administration in our hands was 12.5 mg/kg. A similar study conducted by Soulimani et al. also showed the effectiveness of MO at low doses after i.p. administration in mice.[28]

The combination studies showed the lack of additive or synergistic effects by different doses of the MPAJ and TEMO. The hypnotic effect by combination of two extracts was not greater than the mathematical sum of the individual herbs. The lack of an extra hypnotic effect from combining two extracts may be due to factors such as lack of synergy, receptor saturation, diminished returns, and individual variability in responses. If they target the same receptors, saturation can occur, limiting further responses. Diminished returns may also play a role, as increased dosages yield smaller additional benefits. Additionally, pharmacokinetic differences might reduce their combined efficacy, and insufficient dosages could hinder any potential enhancement in effect.

Although the TEMO was quite safe at higher doses, the MPAJ i.p. doses appeared to be toxic at doses above 25 mg/kg. The LD_{50} of MPAJ was found to be 25 mg/kg and this limitation made it impossible to increase the dose in this study. As part of the study, we also investigated the toxicity of plants after oral administration and found no lethality in animals up to very high doses of MPAJ and TEMO. The apparent difference in the toxicity profile of the MPAJ after i.p. and oral administration could be due to the saponin compounds found in the AJ, specifically julibroside J29-31, which exhibit cytotoxic effects. When administered i.p., they are absorbed and have an effect at the injection site. However, oral administration does not result in significant absorption or specific effects. Studies on these compounds in *Albizia* species have also highlighted their cytotoxic and hemolytic properties after i.p. administration.[29] This phenomenon has also been reported with other plants. For example, the LD_{50} for Albizia glaberrima[30] and berberine[31] are reported to vary after i.p. and oral administration of the extracts.

The TEMO at the selected dose of 12.5 mg/kg did not significantly change the locomotor activity of the animal. This is in agreement with other findings in which the oral administration of MO was shown to have no profound effect on locomotor activity.[32,33] The MPAJ, on the other hand, did reduce the locomotor activity after i.p. injection of the extract at 12.5 mg/kg. No one before has studied the acute effect of MPAJ after i.p. administration on locomotor activity. In another species of *Albizia*, i.p. injection of its extract has been shown to reduce the mobility of the animals.[34] It is interesting to note that, unlike diazepam, no tolerance developed to the locomotor effects of MPAJ or TEMO after 1 week of extracts injection. Similar findings have been reported by Taiwo AE et al., after oral administration of MO in rats treated for 10 days.[32] The lowered locomotor activity is not solely due to the sedative effect of the compound; other factors such as muscle relaxation or change in body temperature may affect the movement of the animals.

The results indicated that, unlike TEMO, the groups receiving MPAJ alone or in combination with MO exhibited signs of reduction in locomotor activity. Regarding body weight, animals orally administered AJ extract experienced weight loss, whereas those receiving a combination of both plants did not, which contradicted the results of i.p. administration. This discrepancy might be due to differences in

absorption capacity between AJ and MO, as well as variations in animal genetics and administration methods. Similar differences in weight changes after oral and i.p. administration have been observed in studies.[35] A study on tamoxifen also mentioned that oral administration of drugs resulted in less stress and fewer side effects in animals compared to i.p. administration,[36] which may explain our observations in this study as well.

CONCLUSIONS

We found that the acute injections of MPAJ or TEMO at a low dose of 12.5 mg/kg prolonged the pentobarbital-induced sleep time. The combination of the two extracts, however, did not provide an additive or synergistic sedative action. The potency of herbal extract combinations can vary greatly depending on the specific combination of the herbs used. While herbal extracts are generally considered safe, combining multiple herbs can increase the risk of side effects and, in some cases, may interact with each other in unexpected ways, leading to nonadditive effects.

ACKNOWLEDGMENTS

The information in this paper originates from a Pharm.D. thesis, which received financial support from the Research Department of Isfahan University of Medical Sciences in Isfahan, Iran. The authors of this work have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

ORCID

Mahshid Rashidi https://orcid.org/0009-0001-4242-2418

REFERENCES

1. Askari VR, Rahimi VB, Ghorbani A, Rakhshandeh H. Hypnotic effect of *ocimum Basilicum* on pentobarbital-induced sleep in mice. Iran Red Crescent Med J. 2016;18:1–6.
2. Shimizu E, Sato D, Hirano Y, Ebisu H, Kagayama Y, Hanaoka H. Digital cognitive-behavioural therapy application compared with zolpidem for the treatment of insomnia: protocol for an exploratory randomised controlled trial. BMJ Open. 2024;14:1–10.
3. Chapoutot M, Peter-Derex L, Bastuji H, Leslie W, Schoendorff B, Heinzer R, et al. Cognitive behavioral therapy and acceptance and commitment therapy for the discontinuation of long-term benzodiazepine use in insomnia and anxiety disorders. Int J Environ Res Public Health. 2021;18:1–19.
4. Wang W, Fu W, Zhu H, Ma J, Zhang J, Qi J. Progress in GABAA receptor agonists for insomnia disorder. Front Pharmacol. 2024;15:1–9.
5. Wichniak A, Wierzbicka A, Jernajczyk W. Sleep and antidepressant treatment. Curr Pharm Des. 2012;18:5802–17.
6. Ozdemir PG, Karadag AS, Selvi Y, Boysan M, Bilgili SG, Aydin A, et al. Assessment of the effects of antihistamine drugs on mood, sleep quality, sleepiness, and dream anxiety. Int J Psychiatry Clin Pract. 2014;18:161–8.
7. Passarella S, Duong MT. Diagnosis and treatment of insomnia. Am J Health Syst Pharm. 2008;65:927–34.
8. Ji X, Ivers H, Beaulieu-Bonneau S, Morin CM. Complementary and alternative treatments for insomnia/insomnia-depressionanxiety symptom cluster: meta-analysis of English and Chinese literature. Sleep Med Rev. 2021;58:101445.
9. Castro-Muñoz R, Boczkaj G, Cabezas R. A perspective on missing aspects in ongoing purification research towards Melissa officinalis. Food Secur. 2023;12:1–15.
10. Awad R, Muhammad A, Durst T, Trudeau VL, Arnason JT. Bioassay-guided fractionation of lemon balm (*Melissa officinalis* L.) using an in vitro measure of GABA transaminase activity. Phytother Res. 2009;23:1075–81.
11. Kennedy DO, Little W, Haskell CF, Scholey AB. Anxiolytic effects of a combination of *Melissa officinalis* and *Valeriana officinalis* during laboratory induced stress. Phytother Res. 2006;20:96–102.
12. Miraj S, Rafieian-Kopaei K, Kiani S. *Melissa officinalis* L: a review study with an antioxidant prospective. J Evid Based Complement Altern Med. 2017;22:385–94.
13. Wang BM, Qiao P, Wang W, Song W, Liu C, Wang XY, et al. Effect of *Albiziae Flos* and Polygalae radix alone and their combination on depression-like behavior and CREB and NOX2 expression in hippocampus of chronic unpredictable stress-induced rats. Chin J Exp Tradit Med Formulae. 2021;27:32–9.
14. Sangeetha M, Chamundeeswari D, Saravana Babu C, Rose C, Gopal V. Attenuation of oxidative stress in arthritic rats by ethanolic extract of *Albizia procera* benth bark through modulation of the expression of inflammatory cytokines. J Ethnopharmacol. 2020;250:112435.
15. Sobeh M, Hassan SA, El Raey MA, Khalil WA, Hassan MAE, Wink M. Polyphenolics from *Albizia harveyi* exhibit antioxidant activities and counteract oxidative damage and ultra-structural changes of cryopreserved bull semen. Molecules. 2017;22:1993.
16. Qian Y, Han Q-H, Liu D, Tu P-F, Zeng K-W, Liang H. Anti-tumor target identification and molecular mechanism study of total saponins from *Albizia julibrissin*. Chin J Chin Mater Med. 2017;42:3661–5.
17. Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB. Thorough study of reactivity of various compound classes toward the folin-Ciocalteu reagent. J Agric Food Chem. 2010;58:8139–44.
18. Kim HD, Hong K-B, Noh DO, Suh HJ. Sleep-inducing effect of lettuce (*Lactuca sativa*) varieties on pentobarbital-induced sleep. Food Sci Biotechnol. 2017;26:807–14.
19. Rabbani M, Wright EJ, Little HJ. Tolerance to competitive NMDA antagonists, but no crosstolerance with barbiturates. Pharmacol Biochem Behav. 1995;50:9–15.
20. Akhila JS, Shyamjith D, Alwar MC. Acute toxicity studies and determination of median lethal dose. Curr Sci. 2007;93:917–20.
21. OECD. The Organization of Economic Co-operation and Development. Guidelines Test No. 423: Acute Oral toxicity-Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4. 1996 https://doi.org/10.1787/9789264071001-en
22. Marshall FH, Stratton SC, Mullings J, Ford E, Worton SP, Oakley NR, et al. Development of tolerance in mice to the sedative effects of the neuroactive steroid minaxolone following chronic exposure. Pharmacol Biochem Behav. 1997;58:1–8.
23. Chu QP, Wang LE, Cui XY, Fu HZ, Lin ZB, Lin SQ, et al. Extract of *Ganoderma lucidum* potentiates pentobarbital-induced sleep via a GABAergic mechanism. Pharmacol Biochem Behav. 2007;86:693–8.
24. Kang TH, Jeong SJ, Kim NY, Higuchi R, Kim YC. Sedative activity of two flavonol glycosides isolated from the flowers of *albizzia julibrissin* Durazz. J Ethnopharmacol. 2000;71:321–3.
25. Huang B, Wu Y, Li C, Tang Q, Zhang Y. Molecular basis and mechanism of action of Albizia julibrissin in depression treatment and clinical application of its formulae. Chin Herb Med. 2023;15:201–13.
26. Jung YH, Ha RR, Kwon SH, Hong SI, Lee KH, Kim SY, et al. Anxiolytic effects of Julibroside C1 isolated from *albizzia julibrissin* in mice. Prog Neuro-Psychopharmacol Biol Psychiatry. 2013;44:184–92.

27. Scholey A, Stough C, Benton D, editors. Lifetime nutritional influences on cognition, behaviour and psychiatric illness. Neurocognitive effects of herbal extracts. 1st ed. United Kingdom: Woodhead Publishing; 2011.
28. Soulimani R, Fleurentin J, Mortier F, Misslin R, Derrieu G, Pelt JM. Neurotropic action of the hydroalcoholic extract of *Melissa officinalis* in the mouse. Planta Med. 1991;57:105–9.
29. Kokila K, Priyadharshini SD, Sujatha V. Phytopharmacological properties of Albizia species: a review. Int J Pharm Sci. 2013;5: 70–3.
30. Adebesin IF, Akindele AJ, Adeyemi OO. Evaluation of neuropharmacological effects of aqueous leaf extract of *Albizia glaberrima* (Leguminosae) in mice. J Ethnopharmacol. 2015;160: 101–8.
31. Kheir MM, Wang Y, Hua L, Hu J, Li L, Lei F, et al. Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol. 2010;48:1105–10.
32. Taiwo AE, Leite FB, Lucena GM, Barros M, Silveira D, Silva MV, et al. Anxiolytic and antidepressant-like effects of *Melissa officinalis* (lemon balm) extract in rats: influence of administration and gender. Indian J Pharm. 2012;44:189–92.
33. João EF, Dâmaris S, Aluízio CS, Fabiana PC, Melissa S d A, Franco BL, et al. Effects of lemon balm (*Melissa officinalis*) on behavioral deficits and memory impairment of rats surviving sepsis. J Med Plant Res. 2017;11(8):153–60. https://doi.org/10.5897/JMPR2016.6266
34. He Y, Wang Q, Ye Y, Liu Z, Sun H. The ethnopharmacology, phytochemistry, pharmacology and toxicology of genus Albizia: a review. J Ethnopharmacol. 2020;257:112677.
35. Engels K, Rakov H, Zwanziger D, Hönes GS, Rehders M, Brix K, et al. Efficacy of protocols for induction of chronic hyperthyroidism in male and female mice. Endocrine. 2016;54:47–54.
36. Kiermayer C, Conrad M, Schneider M, Schmidt J, Brielmeier M. Optimization of spatiotemporal gene inactivation in mouse heart by oral application of tamoxifen citrate. Genesis. 2007;45:11–6.

How to cite this article: Rashidi M, Sadeghi-Dinani M, Rabbani M. Lack of tolerance to the separate or combined hypnotic actions of different partitions of *Melissa officinalis* (total, ethyl acetate, and aqueous) and methanolic partition of *Albizia julibrissin*. JSFA Reports. 2025;5(5):222–30. https://doi.org/10.1002/jsf2.70004