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Effect of a hydro-alcoholic extract of *Melissa officinalis* on passive avoidance learning and memory

Zahra Dehbani[1,2], Alireza Komaki[1*], Farshid Etaee[1,3], Siamak Shahidi[1], Masoumeh Taheri[1], Somayeh Komaki[1], Nafiseh Faraji[1]

[1]Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Biology, Islamic Azad University of Hamadan, Hamadan, Iran
[3]Rahe Sabz Addiction Rehabilitation Clinic, Hamadan University of Medical Sciences, Hamadan, Iran

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ABSTRACT

Introduction: Melissa officinalis (MO) or lemon balm is traditionally used as a sedative and anti-spasm herbal medicine. There is also evidence that this plant has effects on learning and memory. This study examined the effect of a hydro-alcoholic extract of MO on passive avoidance learning (PAL) and memory in male rats.

Methods: A total of 40 adult male Wistar rats were randomly distributed into four groups (200 to 220 g; n = 10 per group); three dose groups (50, 100, and 200 mg/kg of the hydro-alcoholic extract of MO) and vehicle control (saline) group. Saline or doses of extract were administered daily for 14 days by oral gavage. The rats were trained to enter the shuttle box to record their behavior in the PAL task. A retrieval test was performed 24 hours following training.

Results: A significant difference was seen in performance among MO groups and the control. MO administered animals had a decreased number of acquisition trials ($P<0.05$). In the retention task, MO administered animals had an increased step-through latency (SLT) ($P<0.01$), and a decreased latency in the dark compartment ($P<0.001$) compared to the control group.

Conclusion: The results of the study show that MO can improve learning and memory in the PAL task. Further investigation is needed to enhance our understanding of the neurobiological mechanisms of the MO extract and its effects on learning and memory.

Implication for health policy/practice/research/medical education:
The results of this study revealed that *Melissa officinalis* can enhance learning and memory in the passive avoidance test. The high levels of phenols and flavonoids in *M. officinalis* extracts may be responsible for its beneficial effects.

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Introduction

Melissa officinalis (MO) or lemon balm is one of the most popular therapeutic plants (1). The origin of this plant is Europe, however, it is grown throughout the world (2). It belongs to the Lamiaceae family, which is known for many aromatic and medicinal plants, and commonly is used in European traditional medicine (1). Additionally, MO is a cultured lemon-scented herb, and it is used in common Chinese medicine as a tea for its sedation and anti-spasm impacts (3, 4). Due to its unique flavor, the food manufacturing also uses this plant to taste various foods (5). Also, MO is known for its anti-viral, antioxidant, and anti-inflammatory therapeutic properties (6-8). Contemporary reports emphasize the sedative, antibacterial, and spasmolytic effects of MO, which indicate that it may improve certain disorders by reducing excitability, anxiety, and stress and improving sleep disturbance (3). Moreover, several studies suggest that MO is useful for treating a wide variety of other issues and diseases, such as HIV-1, some cancers, Alzheimer's disease (AD), hyperactivity disorder, indigestion, and hyperthyroidism (9-15). In addition to having soothing and carminative effects, MO acts as an anxiolytic and hypnotic (16). Immunostimulating effects of MO extract have also been demonstrated (17,18). The aqueous extract of MO is enriched in phenolic compounds: rosmarinic,

***Corresponding author**: Alireza Komaki (Ph.D), Tel: (+98) 81-38380267, Fax: (+98) 81-38380131, E-mail: komaki@umsha.ac.ir

protocatechuic, caftaric, caffeic, ferulic, cichoric acids and flavonoid luteolin (19).

Traditional herbal medicine suggests the use of MO may improve memory and concentration (20). A recent study demonstrated that following a single-dose administration, this herb could modulate cognitive performance and mood in healthy young subjects (3). Moreover, MO enhances the quality of life in subjects who are struggling with severe dementia, and improves agitation (21). It is reportedly valuable in controlling AD and produces a particular impact on agitation in this patient population (2).

Therefore, MO may influence cognitive function. However, the effects of MO on passive avoidance learning (PAL) have not yet been described. The primary aim of our investigation was to test whether MO could improve rodent learning and memory in the PAL.

Materials and Methods

Animals

Forty male Wistar rats (200 to 220 g) were obtained from the Razi Institute, Tehran, Iran. The rats were maintained in a room with a 12-hour dark/light cycle at 22–25°C, with 4 to 5 rats per cage. The rats had unrestricted access to food and water. Rodents were randomly distributed into four groups (10 per group); three dose groups (50,100 and 200 mg/kg of the hydro-alcoholic extract of MO) and vehicle control (saline) group. Saline or various doses of MO extract were administered daily for 14 days by oral gavage. All tests were performed in a soundproof room under the controlled light situation during the same time of day (within 11:00 AM to 3:00 PM) to diminish any confounding issues. All procedures were confirmed by the Veterinary Ethics Committee of the Hamadan University of Medical Science and were carried out based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). The investigation timeline is displayed in Figure 1.

Preparation of the extract

The leaves of MO were gathered in spring and distinguished by the Botanic Institute of Hamadan University of Medical Sciences. Then, they were dried with airflow (40°C), grounded, and then extracted with 70% ethanol. The extract was then dried in 40°C under vacuum condition, and subsequently freeze-dried (22, 23). Extract administrations were performed using aqueous suspensions of the dried extract.

Passive avoidance learning test

PAL apparatus

The PAL apparatus (shuttle box) included a light and a dark chamber with the same size (30 x 20 x 20 cm each), with transparent and dark opaque plastic walls, respectively. The dark chamber had a grid floor, which elicited an electric current (Burj Sanat Co. Iran). An opaque door separated a rectangular opening between the two compartments.

Passive avoidance training

A subject rodent was located in the light chamber facing away from the guillotine door. After five seconds, the door was opened, and following the entrance of rat to the dark chamber, the door was closed, and an electrical shock (50-Hz square wave, one mA for 1.5 seconds) was induced (24-26). Afterward, 30 seconds later the animal was delivered to the cage. After two minutes, the animal was retested and if it did not enter the dark chamber in 120 seconds, successful acquisition of a passive avoidance behavior was reported (27,28). The number of trials to the acquisition was reported as a measurement of learning (29,30).

Retention test

The retention test was conducted 24 hours following PAL acquisition (27, 31-33). The animals were located in the light chamber, similar to PAL acquisition, and after five seconds, the door was lifted, and the latency to enter the dark chamber (step-through latency; STL) and time spent in the dark chamber (TDC) were reported for up to 300 seconds. If the animal did not enter the dark side in 300 seconds, the retention test was ended, and the maximum score of 300 seconds was reported.

Statistical analysis

All results are expressed as a mean ± standard error of the mean (SEM) and processed with SPSS version 22. Data were analyzed using a one-way analysis of variance (ANOVA), followed by a Tukey post hoc analysis. Significance was set to $P<0.05$.

Results

The effects of *Melissa officinalis* on PAL acquisition

Significant differences were seen between the vehicle and drug groups in the number of acquisition trials needed to have the criterion of 120 seconds in the light chamber (Figure 2). All groups administered MO (50, 100, and

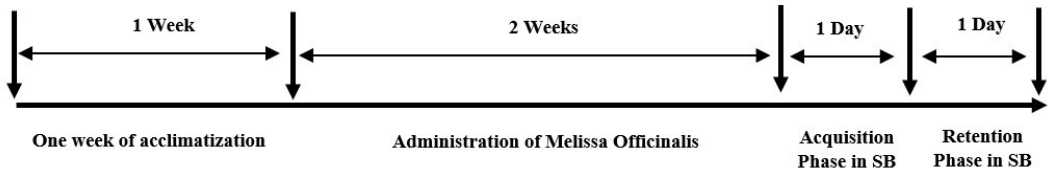

Figure 1. Timeline of experiments.

200 mg/kg) required significantly fewer trials to reach criterion than the vehicle group ($P<0.05$).

The effects of *Melissa officinalis* on the step-through latency

There were significant differences in the STL, 24 hours after the training, between the vehicle and MO groups (Figure 3). The STLs for the 50 mg/kg ($P<0.01$) and 200 mg/kg ($P<0.05$) groups were significantly greater in comparison with the vehicle. In particular, the STL for the 50 mg/kg group was significantly greater than the rest of the test groups ($P<0.05$). Significant differences were not detected between the STL for the 100 mg/kg group in comparison with the vehicle group ($P>0.05$).

The effects of *Melissa officinalis* on time spent in the dark compartment (TDC)

TDCs for all the MO groups (50, 100, and 200 mg/kg) were significantly smaller than that of the vehicle group ($P<0.001$, $P<0.01$, $P<0.05$, sequentially). The TDC for the 50 mg/kg rats was significantly smaller than the 200 mg/kg rats ($P<0.05$) (Figure 4).

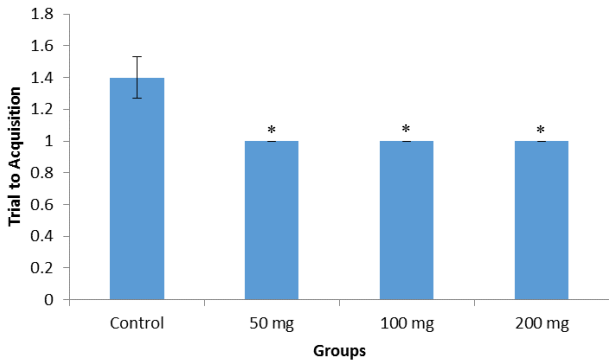

Figure 2. The impact of sub chronic oral gavage of *Melissa officinalis* extract (50, 100, and 200 mg/kg) on the number of acquisition trials. *$P<0.05$, significant differences between drug groups and vehicle (n = 10 per groups).

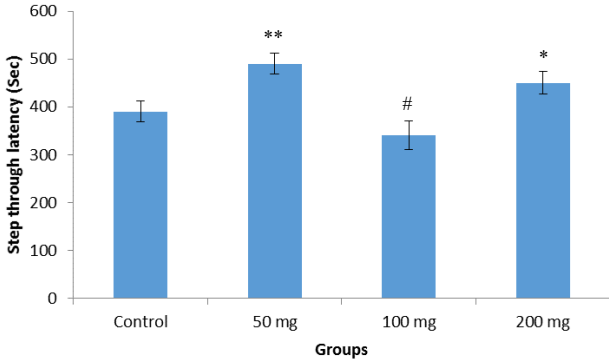

Figure 3. The effect of subchronic administration of *Melissa officinalis* extract (50, 100, and 200 mg/kg) on the step-through latency (STL) in the 24-h retention test. *$P<0.05$ and **$P<0.01$ show significant differences between drug groups and vehicle. # $P<0.05$ indicates significant differences between 50 and 200 mg/kg groups.

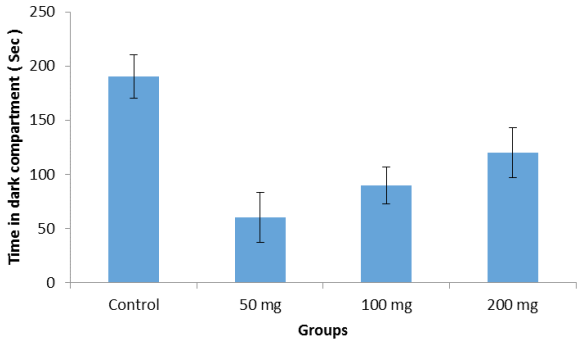

Figure 4. Effect of subchronic administration of various doses of *Melissa officinalis* extract (50, 100, and 200 mg/kg) on the time spent in the dark chamber (TDC) in the 24-h retention test. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ indicate significant differences between drug group and vehicle. # $P<0.05$ shows significant differences in comparison with the 200 mg/kg group.

Discussion

This study revealed that subchronic oral administration of the hydro-alcoholic extract of MO improved PAL learning and memory. There was a significant impact of MO on the number of trials required for PAL acquisition. In addition, there was a significant improvement in the STL and a significant reduction in the TDC during the 24-hour retention testing.

These data suggest that MO can improve learning in normal healthy rats. However, this effect is not dose-dependent. It previously reported that animals receiving 400 mg/kg of extract did not show improvement in maze performance (19). Several clinical studies using lemon balm have supported the use of MO for improvement in cognitive function (3,20). These clinical studies showed a dose-dependent, time-dependent, and response-dependent relationship in regards to its effects on memory and mood (20).

There is increasing evidence for the possible efficacy of MO in the management of AD (34,35). In one clinical study, patients with mild to moderate AD who were administrated MO extract experienced significant cognitive benefits after 16 weeks of treatment (2).

The main components of MO are triterpene acids (e.g., ursolic acid), flavonoids (e.g., luteolin), and hydroxycinnamic acid derivatives, (e.g., rosmarinic acids) (36,37). Additional notable compounds, recognized in this extract, were p-hydroxybenzoic acid and protocatechuic acid. MO extracts also have a considerable amount of caffeic acid derivatives, such as salvianolic acid (a caffeic acid trimer) (38). Finally, others have shown that linalool, citronellol, citronellal, and geranial are major chemical components in essential MO oil (39).

It has been reported that antioxidant activity can improve learning and memory (40,41). Previous data suggest that MO contains high condensation of antioxidants (42-45). The antioxidant activity of plants could be due to their phenolic compounds (27,46,47). MO has been shown

to have a high phenolic portion as well as antioxidant properties (48). MO extract contains a number of monoterpenoid aldehydes, monoterpene glycosides, polyphenolic compounds (most notably, rosmarinic acid), and flavonoids (49). Caffeic acid, and rosmarinic acid have been reported to have antioxidant properties in vitro (45, 50-52). Additionally, the antioxidant activity of MO may provide some protection against the destructive effects of free radicals in dementia (3, 50). Ursolic acid can enhance age-related cognitive deficits through activation of antioxidant enzymes and reduction of lipid peroxidation (53). Moreover, MO demonstrated antioxidant activity via prevention of linoleic acid autoxidation (54). Finally, others have demonstrated that the cytoprotective effect of MO extracts in rats was, in particular, due to its free-radical scavenging properties (18,44).

Furthermore, it has been shown that MO is active at the acetylcholine receptors in the central nervous system, modulating both ionotropic and metabotropic receptors, and following acute administration modulates mood and cognitive performance (3,55). This issue raises the possibility of benefits of MO in attenuation of aging-related cholinergic decrements, as well as in improving states of delirium, such as those associated with anticholinergic drugs (56). The anticholinesterase activity of MO extract and its primary constituent, rosmarinic acid, has been reported previously (57). In addition, chlorogenic acid inhibits cholinesterase activity and ameliorates scopolamine-induced amnesia (58). The results of previous studies suggest that MO has cholinergic receptor-binding properties, and consumption of a single dose of MO can regulate both the mood and cognitive performance of the healthy young applicants (20).

Conclusion

MO can improve learning and memory in the PAL task. The high levels of phenols and flavonoids in MO extract may be responsible for its favorable impacts of MO on learning and memory. Nevertheless, the precise mechanism and active compounds implicated in cognitive improvement have not been fully characterized. Therefore, further biochemical and pharmacological studies are warranted.

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Authors' contributions

AK designed, analyzed the data and revised the manuscript. ZD was responsible for performing the experimental work. MT provided assistance for study design. SK and NF participated in plant extraction and all animal intervention. SS and FE participated in the draft preparation. The paper has been read and approved by all authors for publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

All procedures were approved by the ethics committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1394.576). Ethical issues have been observed by the authors.

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