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The effect of *Melissa officinalis* L. extract on learning and memory: Involvement of hippocampal expression of nitric oxide synthase and brain-derived neurotrophic factor in diabetic rats

Mohsen Naseri [a], Reza Arabi Mianroodi [b], Zeynab Pakzad [c], Peyman Falahati [c], Masomeh Borbor [c], Hossein Azizi [d], Sima Nasri [e,*]

[a] Traditional Medicine Clinical Trial Research Center, Shahed University, Tehran, Iran
[b] R&D Department, Research and Production Complex, Pasteur Institute of Iran, Karaj, Iran
[c] Department of Biology, Payame Noor University, Tehran, Iran
[d] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[e] Department of Biology, Payame Noor University, PO BOX19395-3697, Tehran, Iran

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ABSTRACT

Ethnopharmacological relevance: Diabetes is a systemic disease, which can cause synaptic defects in the hippocampus. Hippocampus plays a crucial role in learning and memory. *Melissa officinalis* L. has been used as for memory enhancement in Persian Medicine.

Aim of the study: The aim of this study was to evaluate the impact of the hydroalcoholic extract of *Melissa officinalis* L. on learning and memory, considering its impact on nitric oxide synthase and brain-derived neurotrophic factor expression in the hippocampus of diabetic rats.

Materials and methods: Melissa officinalis L. extract was obtained by maceration method. To evaluate phenolic and flavonoid compounds of the extract, the samples were analyzed by HPLC. The animals were randomly divided into 6 groups: vehicle-treated control, *Melissa officinalis*-treated control (50 mg/kg), vehicle-treated diabetic, and *M. officinalis*-treated diabetic (25, 50, or 100 mg/kg). Diabetes was induced by streptozotocin And *Melissa officinalis* L. was administered for 2 weeks once diabetes was induced. Passive avoidance and Y-maze tasks were performed for learning and memory assessment. At the end of learning and memory tasks, rats were sacrificed and their hippocampus removed, lysed, and homogenized. The RNA contents were purified and then used as the template for cDNA synthesis. Real-time PCR was used to evaluate nitric oxide synthase and brain-derived neurotrophic factor genes expression.

Results: Rutin was main flavonoid compound and rosmarinic acid was the main phenolic compound of the *Melissa officinalis* extract. Streptozotocin induced diabetes and impaired learning and memory in diabetic rats. *Melissa officinalis* treated-control group showed a higher alternation score in the Y-maze task and step-through latency in the passive avoidance task compared to the vehicle treated diabetic group. *Melissa officinalis*-treated rats showed a higher alternation score in the Y-maze task in all doses compared to the vehicle treated diabetic group ($P < 0.05$). In addition, in the passive avoidance task *Melissa officinalis* increased step-through latency ($P < 0.05$) but not initial latency, in all doses. Furthermore, in diabetic rats, the expression of brain-derived neurotrophic factor and nitric oxide synthase genes decreased. However, hippocampal brain-derived neurotrophic factor and nitric oxide synthase gene expression was increased in *Melissa officinalis*-treated rats compared to diabetic rats ($P < 0.05$).

Conclusions: Melissa officinalis improved learning and memory in diabetic rats, which may have occurred by increasing brain-derived neurotrophic factor and nitric oxide synthase gene expression.

* Corresponding author.
E-mail addresses: naseri@shahed.ac.ir (M. Naseri), arabi552@yahoo.com (R. Arabi Mianroodi), p_zeynab@yahoo.com (Z. Pakzad), peyman_falahati71@yahoo.com (P. Falahati), m66b88@yahoo.com (M. Borbor), azizih@modares.ac.ir (H. Azizi), s_nasri1@pnu.ac.ir (S. Nasri).

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1. Introduction

Diabetes is a systemic disease that can cause brain damages such as synaptic defects in the hippocampus (Han et al., 2019). Type 1 diabetes alters the hippocampal structure, which plays a crucial role in human learning and memory. Hyperglycemia causes a deficit in memory (Murray et al., 2014). Brain-derived neurotrophic factor (BDNF) modulates neuroplasticity. In a study by Han et al., BDNF was shown to block hyperglycemia-induced microglial activation and decrease the level of inflammatory factors such as TNF-α and IL-6 in the hippocampus of type 1 diabetic mice. BDNF reversed phosphorylated NF-κβ in streptozotocin-induced diabetes in mice (Han et al., 2019).

Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthase (NOS) (Paul and Ekambaram, 2011) and has three isoforms: neuronal NOS (NOS1), inducible NOS (NOS2), and endothelial NOS (NOS3); NOS1 and NOS3 being dominant in the central nervous system (Saeedi Saravi, 2017). Insulin is an important stimulus for NOS3 activation (Tabit, 2010).

NO synthase plays a crucial role in synaptic plasticity and learning and memory (Saeedi Saravi, 2017). Pathological conditions including epilepsy, stress, and diabetes can lead to a defective NO activity in the brain, resulting in learning and memory impairment. Furthermore, it has been shown that administration of L-arginine and NO to Alzheimer's patients prevents dementia (Paul and Ekambaram, 2011).

Melissa officinalis (M. officinalis L.*)* (lemon balm) is a member of the Lamiaceae family. *M. officinalis* L. is native to Mediterranean regions and Europe (WHO, 2005). In European traditional medicine, *Melissa officinalis* is used as folium for relieving mental stress, mild gastrointestinal symptoms and for aiding in sleep, prescribed in the form of herbal tea, powdered herbal substance, or aqueous/ethanolic extracts (European Medicines Agency, 2013). In Russian traditional medicine, it is used for the treatment of bronchial asthma (Mamedov and Craker, 2001) and also considered a tonic and adaptogen in the State Pharmacopoeia of Russia (Shikov et al., 2021).

Melissa officinalis is cultivated in Europe and America (WHO, 2005). It possesses antioxidant (Miraj et al., 2017), anti-inflammatory, antinociceptive, antispasmodic, antimutagenic, and anticancer activity, in addition to improving behavioral symptoms in anxiety disorders, cognitive impairment, insomnia, and stress (Zarei et al., 2015).

Persian medicine (PM) is a traditional healthcare system with deep historical roots, practiced since approximately ten thousand years ago (Ghaffari et al., 2015, 2017). PM having produced several thousand of manuscripts, famous scientists, and verbal sources in different languages — is among the ancient and comprehensive traditional medical systems. This system provides several therapeutic strategies for various diseases (Qaraaty et al., 2014).

In PM, *M. officinalis* L. has been prescribed for neurological disorders such as depression (Araj-Khodaei et al., 2020) and anxiety (Soltanpour et al., 2019), and is also used for sleep quality (Alijaniha et al., 2014). It has been shown that *M. officinalis* extract can improve memory in healthy and scopolamine-induced Alzheimer's rats (Soodi et al., 2014). Moreover, *M. officinalis* L. has protective effects against beta-amyloid induced toxicity in PC12 cells (Sepand et al., 2012).

From a biochemical point of view, *M. officinalis* L. extract contains flavonoids (quercitrin, rhamnocitrin, and luteolin), polyphenolic compounds (such as rosmarinic acid, caffeic acid, and protocatechuic acid), monoterpenoid aldehyde, monoterpene glycosides, triterpenes (including ursolic and oleanolic acids), sesquiterpenes, tannins, and essential oils (citral) (Miraj et al., 2017). In a previous study, *M. officinalis* extract improved learning impairment in streptozotocin-induced Alzheimer model (Sabbaghziarani et al., 2014). When used in combination with *Boswellia serrata* extract, *M. officinalis* increased memory in scopolamine treated rats (Mahboubi et al., 2016). A study concluded a single dose of *M. officinalis* L. could ameliorate cognition and mood in healthy youth (Kennedy et al., 2002). Another study suggested oral administration of the *M. officinalis* L. extract can improve learning and memory in the passive avoidance learning test, a result of its high level of phenols and flavonoids (Dehbani et al., 2019).

Regarding the effect of *M. officinalis* L. on diabetes and learning and memory, the aim of this study was to investigate the effect of the hydroalchoholic extract of *M. officinalis* L. on learning and memory, in addition to BDNF and NO synthase gene expression in the hippocampus of diabetic rats.

2. Materials and methods

2.1. Plant material

M. officinalis or lemon balm (locally called Badranjboye) was purchased in spring (2019) from Firouzeh Botanical Garden, located in Tehran province, Iran. The plant was identified and authenticated by Dr. Mohammad Kamalinejad at the Herbarium of the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran (voucher number 3380).

The plant was washed to remove any possible contamination. Aerial parts were dried at laboratory temperature, which was 30–35 °C. The amount of 250 g of the powdered dried plants was macerated in triplicate with 1000 ml hydroalcoholic solvent (%40 ethanol, 60% distilled water) for 24 h for each extraction. After extraction was done, the total mixture was filtered and the solvent was removed by rotary evaporator (Leporini et al., 2020). The extract rate was 16% of the dry weight of the plant.

2.1.1. Total phenolic assay

The total phenolic compounds (TPC) of the *M. officinalis* L. extract was determined as described earlier by Karimi et al. (2018). The amount of 1 ml of extract solved in methanol at the concentration of 0.5 mg/ml was mixed with 2.5 ml folin-ciocalteu reagent and 2 ml of 7.5% (w/v) Na_2CO_3. The mixture was vortexed for 15 s and incubated in the dark at room temperature for 90 min. The absorbance of the mixtures was measured using a visible spectrophotometer (Novaspec II Visible spectra) at 765 nm. The results were expressed as mg gallic acid equivalents/g dry weight (DW).

2.1.2. Total flavonoid assay

The total flavonoid compounds (TFC) of the extract were determined according to the procedure used by Karimi et al. (2018). An aliquot (0.1 ml) of extract in methanol at a concentration of 1 mg/ml was added to 0.3 ml 5% (w/v) $NaNO_2$ and incubated for 5 min in the dark at room temperature. The mixture was supplemented with 0.3 ml 10% (w/v) $AlCl_3$ and 2 ml 1 N NaOH, making a total volume of 5 ml after distilled water was added too. Absorbance was measured at 510 nm using a visible spectrophotometer (Novaspec II Visible spectra). The results have been expressed as mg catechin equivalents/g dry weight (DW).

2.1.3. Determination of phenolic and flavonoid compounds by HPLC

To determine the quantity and types of the phenolic and flavonoid compounds of the extract, the samples were analyzed by a high-performance liquid chromatography device (Knauer, Germany) equipped with UV–Vis Photo Diode Array (PDA) detector (Knauer- UV K2501) and Knauer K1001 pump and using an analytical column (Inertsil ODS-3,5 μm 4.6 × 150 mm, Gl Science Inc), as described by Karimi et al. (2010, 2018), but with slight modifications. The mobile phase consisted of deionized water (solvent A) and acetonitrile (solvent B). The pH of the deionized water was adjusted to 2.5 with trifluoroacetic acid. The column was equilibrated by 85% solvent A and 15% solvent B. The elution was conducted by increasing the ratio of solvent B from 15% to 85% in 50 min. Solvent B was then decreased to 15% within 5 min. This ratio was maintained for an extra 10 min for equilibration. The flow rate was 0.6 ml min^{-1} and the phenolic and flavonoid compounds of the mixture were detected at 280 nm and 350 nm, respectively. In order to quantify the bioactive compounds of the extract, a calibration curve was prepared

by injecting different standard compounds of rutin (Sigma-Aldrich, ≥94%), apigenin (Sigma-Aldrich, ≥97%), quercetin (Sigma-Aldrich, ≥95%), luteolin (Sigma-Aldrich, ≥98%), the flavonoids, and the phenolic acid of rosmarinic acid (HWI analytik GMBH, 89.3%). In order to produce calibration curves, standard solutions were prepared for each compound at different concentrations: 4.3, 8.6, 12.9, 17.2, 21.5 μg/mL for apigenin; 9.37, 18.75, 37.5, 75, 150 μg/mL for rutin and quercetin; 1.07, 5.33, 26.68 μg/mL for lutolin; and 16, 80, 120, 160, 220 μg/mL for rosmarinic acid levels. These solutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area. Each determination was conducted in triplicate and the calibration curve was fitted by linear regression.

2.2. Animals

Male Wistar rats weighing 130–150 g (purchased from Pasteur institute of Iran) were housed in an animal room with 12 h light/12 h dark cycle (23 ± 2 °C and 30–40% humidity). The animals were fed with a standard pelletized diet and had ad libitum access to tap water. Animals were handled in accordance with NIH guidelines and the guidelines of the ethical committee of Payame Noor University, with the ethical code IR.PNU.REC.1398.008.

The animals were randomly divided into 6 groups: vehicle-treated control, *M. officinalis*-treated control (50 mg/kg), vehicle-treated diabetic, and three diabetic groups treated with *M. officinalis* (25, 50, and 100 mg/kg) (Soodi et al., 2014). *M. officinalis* was solved in normal saline. Diabetes was induced with a single i.p. dose of 60 mg/kg streptozotocin (Sigma-Aldrich, Germany). 0, 1, and 2 weeks after streptozotocin injection, blood samples were collected from the subjects to measure serum glucose level using the glucose oxidase method. Animals with a serum glucose concentration higher than 250 mg/dl were considered diabetic (Mirshekar et al., 2011). Once diabetes was confirmed in the treated rats, *M. officinalis* extract was administered i.p. for 2 weeks. The timeline of the experiment is shown in Fig. 1.

2.2.1. Y-maze task

The Y-maze was made of 3 black plexiglas arms. Each rat was randomly placed at the end of one arm and allowed to move freely through the maze for 8 min. The number of arm entrances was recorded. Entry was defined as when the base of the animal's tail was entirely placed inside the arm. One correct alternation was defined as any three successive arm entries with no repeated arm entry in them (Mirshekar et al., 2011).

2.2.2. Passive avoidance learning

On days 1 and 2, each rat was located in the passive avoidance apparatus and left for 5 min to habituate. The acquisition trial occurred on day 3, where each rat was placed in the light chamber and a door was opened 5 min later. Once the rat entered the dark chamber, the door was closed and an electrical shock (50 Hz square wave, 1 mA for 1 s) was delivered to the floor of the chamber. The latency to enter the dark chamber was recorded in this phase as initial latency (IL). Rats with an IL over 60 s were excluded from the study. Subjects were individually returned to the light chamber. Latency to enter the dark chamber was calculated as step-through latency (STL) of a passive avoidance behavior on the 4th day. Cut-off time was considered as an STL of 480 s (Mirshekar et al., 2011).

2.3. Hippocampus dissection

The rats were sacrificed and their brain was removed under anesthesia. The brains were placed on dry ice and cut into right and left hemispheres. The olfactory bulb and frontal part of brain were removed from the left hemisphere. The ventral side of the brain was pulled up to remove the midbrain and reveal and separate the hippocampus. The specimen was kept frozen in −80 °C until the molecular experiment assay (Chiu et al., 2007).

2.4. RNA extraction and real-time PCR

The hippocampus was lysed and homogenized and RNA content was purified using an RNA purification kit (RNeasy Mini Kit, QIAGEN, Germany) according to the manufacturer's instructions.

The purified RNA content was first measured in a Nanodrop spectrophotometry device (Nanodrop 1000; Thermo, USA) at 260 nm and then used as the template for cDNA synthesis by reverse transcriptase enzyme using a cDNA synthesis kit (Qiagen, Netherland). The newly synthesized cDNA was used for quantifying NO synthase and BDNF mRNA using real-time PCR.

The real-time PCR mix was prepared as follows: 1 μl of the purified cDNA sample was mixed with 0.5 ml (10 pM) of forward and reverse primers (Table 1), 10 μl CYBER Green Master Mix (ABI, USA), and 8 μl DPECT water (making a total volume of 10 μl). RT-PCR cycles were executed in a real-time thermo cycler (Applied Biosystems,ABI Step One, USA) with the following instructions: denaturation at 94 °C, 20 s; annealing at 58 °C, 30 s; and extension at 72 °C, 30 s. Primer quality and PCR cycle accuracy was evaluated after 40 cycles using the melting curve for each gene. Cycle threshold (CT) values were determined using automated threshold analysis. GAPDH gene, a housekeeping gene, was used for normalizing the PCR results in order to consider loading differences (Login et al., 2009). Each test was repeated three times.

2.5. Statistical analysis

Data was analyzed using the one way ANOVA test and Tukey post-test. The results were expressed as mean ± SEM. A difference of $P < 0.05$ was considered significant.

3. Results

3.1. Chemical analysis

3.1.1. Total phenolic and flavonoid content

The total phenolic content of the plant extract was measured as

Table 1
Sequence of primers for target genes (prepared by primer-BLAST Software).

Primer	Sequence
BDNF F	GTCCCTTCTACACTTTACCTCT
BDNF R	TCTTTCACCCTTTCCACTCC
NOS3 R	AAGATTGCCTCGGTTTGTTG
NOS3 F	TATTTGATGCTCGGGACTGC
GAPDH R	CAT ACT CAG CAC CAG CAT CAC C
GAPDH F	AAG TTC AAC GGC ACA GTC AAG G

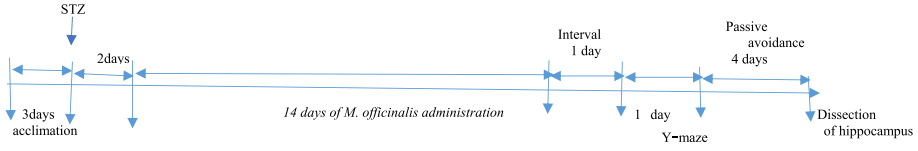


Fig. 1. Timeline of the experiment. Behavioral and molecular tests were performed after 14 days of *M. officinalis* extract administration.

191.75 ± 29.76 mg gallic acid equivalent/g DW. The evaluated amount of total flavonoid content was 215.12 ± 11.18 mg catechin equivalent/g DW.

3.1.2. HPLC results

Reversed-phase (RP) liquid chromatography was used to determine the phenolic and flavonoid compounds present in the *M. officinalis* hydroalcoholic extract. Rosmarinic acid, rutin, and apigenin were detected based on their retention times and quantified according to their respective standard calibration curves. Rutin and apigenin were two main flavonoid compounds, constituting 9.36% and 0.56% of the extract, respectively. Rosmarinic acid was the main phenolic compound of the extract, constituting 4.97% of the extract.

3.2. Body weight and glucose level

The weight of the diabetic rats significantly decreased in comparison to the control group ($P < 0.05$). However, *M. officinalis* significantly increased the weight of *M. officinalis*-treated diabetic rats compared to control diabetic ones in all doses. Moreover, while the serum glucose level elevated in diabetic rats, *M. officinalis* caused a significant drop in the serum glucose level of treated rodents compared to diabetic rats ($P < 0.05$) (Fig. 2).

3.3. Y-maze task

The alternation score of the diabetic animals decreased in comparison to control rats ($P < 0.05$). Meanwhile, *M. officinalis*-treated diabetic rats showed a higher alternation score compared to the diabetic group ($P < 0.05$). Incidentally, the most effective dose was 50 mg/kg ($P < 0.05$). In addition, there was a significant difference in the alternation score of diabetic rats, control subjects, and *M. officinalis*-treated control rats ($P < 0.05$) (Fig. 3).

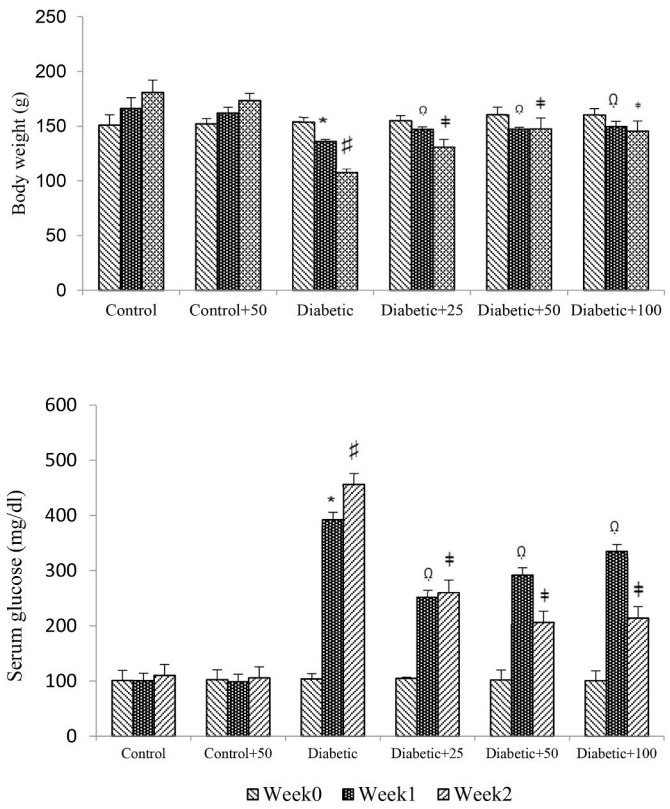


Fig. 2. Body weight and serum glucose level, 1 and 2 weeks after treatment with *M. officinalis*. *P < 0.05 difference between diabetic and control rats after the 1st week. Ω P < 0.05 difference between *M. officinalis*-treated diabetic groups and the diabetic group after the 1st week. #P < 0.05 difference between diabetic and control rats after the 2nd week. ǂ P < 0.05 difference between *M. officinalis*-treated diabetic groups and the diabetic group after the 2nd week.

3.4. Passive avoidance test

There was no significant difference among the groups in IL. Furthermore, diabetic rats showed a significant impairment in retention and recall in the passive avoidance task, demonstrated by a lower STL. *M. officinalis*-treated diabetic rats showed a greater change in this regard. In addition, the retention and recall of *M. officinalis*-treated rats were higher than other groups. The most effective dose was 50 mg/kg ($P < 0.05$) (Fig. 4).

3.5. BDNF gene expression

The results of this study revealed that diabetic rats significantly lower BDNF gene expression compared to control rats. Treating normal and diabetic rats with *M. officinalis* extract increased BDNF gene expression in the hippocampus in comparison to both the control and diabetic rats ($P < 0.05$) (Fig. 5).

3.6. NOS3 synthase gene expression

Similar to BDNF, there was a significant difference between the control and diabetic group in NOS3 synthase expression. Furthermore, a significant rise in hippocampal NOS3 expression was observed in diabetic rats that were treated with *M. officinalis* extract ($P < 0.05$).

In other words, diabetic rats receiving 50 mg/kg doses of *M. officinalis* extract expressed the NO synthase 3 gene significantly higher than diabetic rats that had not received the *M. officinalis* extract ($P < 0.05$). Moreover, nondiabetic rats treated with 50 mg/kg doses of *M. officinalis* extract had a higher NOS3 gene expression than control rats that had not received the extract, although the difference was not significant (Fig. 6).

4. Discussion

Cognitive impairment in diabetes may involve inflammation, oxidative stress, cholinergic neurotransmission, and microvascular dysfunction. In addition, it has been proven that neuronal mRNA and protein levels of NOS are decreased in the hippocampus of diabetic rats (Yazir et al., 2019).

In this research, we found that streptozotocin decreased weight and increased glucose levels in diabetic rats, while the hydroalcoholic extract of *M. officinalis* reversed this effect (Fig. 2). This finding is in line with a previous study (Chung et al., 2010).

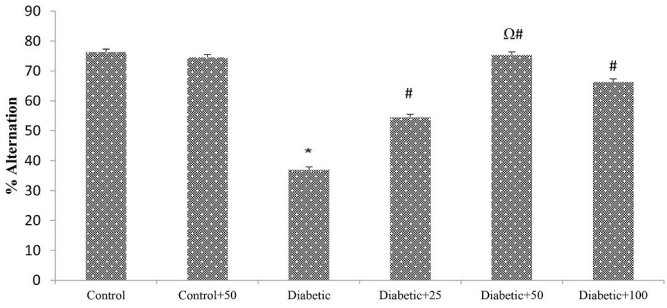
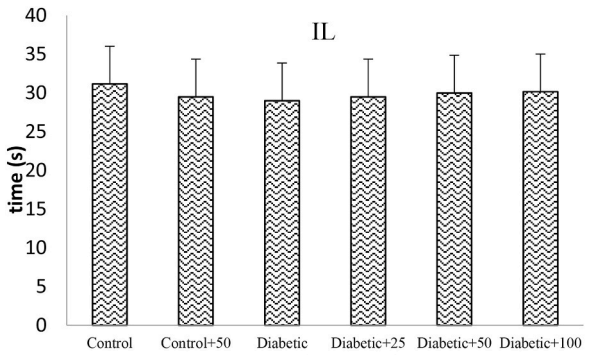


Fig. 3. Alternation score of control, treated control, diabetic, and *M. officinalis*-treated diabetic rats in the Y-maze task. *P < 0.05 difference between diabetic and control, control rats treated. #P < 0.05 difference between *M. officinalis*-treated diabetic groups and the diabetic group. Ω P < 0.05 difference between diabetic rats treated with 100, 50, and 25 mg/kg doses of *M. officinalis*.



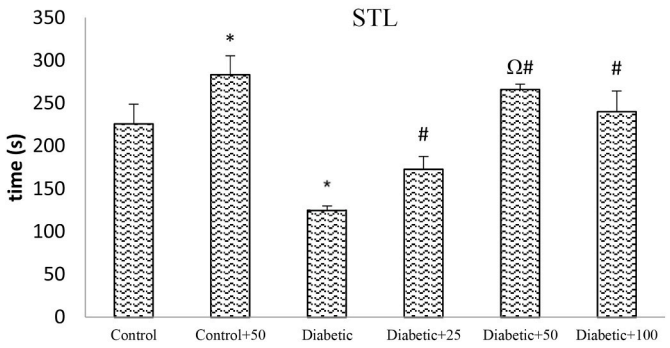


Fig. 4. Initial (IL) and step through (STL) latencies of control, treated-control, diabetic, and diabetic-treated rats. *P < 0.05 difference between diabetic control rats, treated, and control group. #P < 0.05 difference between *M. officinalis*-treated diabetic groups and diabetic group. Ω P < 0.05 difference between diabetic rats treated with 50 mg/kg and 25 mg/kg doses of *M. officinalis*.

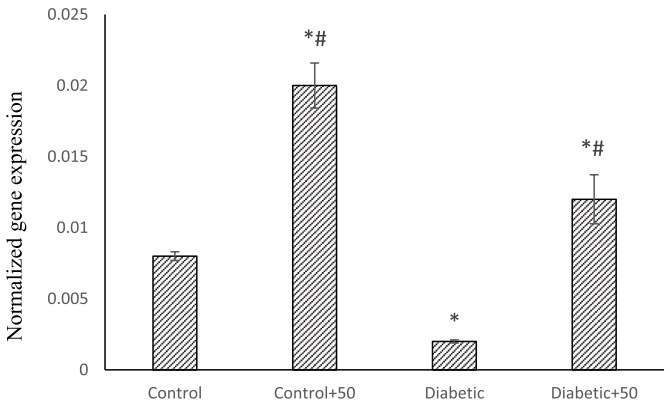


Fig. 5. BDNF gene expression in control, treated-control, diabetic, and diabetic-treated rats. *P < 0.05 difference between control and other groups, #P < 0.05 difference between the diabetic group and other groups.

M. officinalis extract is rich in flavonoids and polyphenols (Miraj et al., 2017), as confirmed by our chemical analysis findings. Flavonoids have antidiabetic effects, they modulate sugar transport by increasing insulin secretion, decreasing apoptosis, inducing pancreatic β-cells proliferation, reducing inflammation and oxidative stress, and stimulating GLUT4 translocation (Vinayagam et al., 2015).

Our results showed that the extract of the aerial part of *M. officinalis* improved learning and memory in diabetic rats (Figs. 3 and 4), which

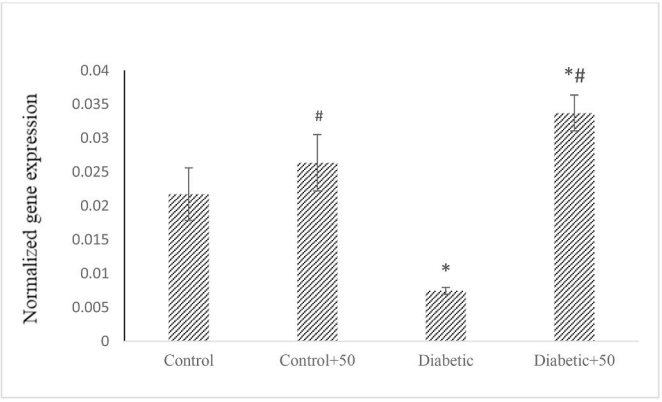


Fig. 6. Comparison of NOS3 gene expression in *M. officinalis*-treated rats with diabetic rats. *P < 0.05 difference between control and other groups, #P < 0.05 difference between the diabetic group and other groups.

confirms previous studies (Sabbaghziarani et al., 2014; Mahboubi et al., 2016; Kennedy et al., 2002; Dehbani et al., 2019). In a study by Sabbaghziaarani et al. (2014), gavage of *M. officinalis* extract ameliorated memory in Alzheimer rats, as assessed by the Morris water maze task. A research by Mahboubi et al. (2016) showed that the combination of *M. officinalis* and *Boswellia serrata* extracts improved Morris Water Maze performance in scopolamine treated rats. Dehbani et al. (2019) examined how gavaging the hydroalcoholic extract of *M. officinalis* leaves (with 70% ethanol) significantly affects passive avoidance learning in rats. Kennedy et al. (2002) investigated the positive effects of *M. officinalis* extract on cognition and mood in healthy humans.

M. officinalis has long been used for treating nervous system diseases (Mahboubi, 2019). *M. officinalis* has protective effects on Amyloid β-induced toxicity and oxidative stress in PC12 cell culture. It has been suggested that this protective effect occurs through antioxidant activity or nicotinic receptor activation (Sepand et al., 2013).

In our study, we found *M. officinalis* increased BDNF and NOS3 gene expression in diabetic-treated rats, as compared to untreated diabetic rats.

A low expression of BDNF is associated with cognitive deficits in diabetes (Zhen et al., 2013). BDNF expression drops in the hippocampus of type 1 diabetic rats (Xia et al., 2014).

A previous study suggested that mutation in the BDNF gene or BDNF knockout led to defective learning and memory (Zheng et al., 2012).

The flavonoids of *M. officinalis* are quercetin, rutin, catechin, and epicatechin (Pereira et al., 2014). However, in our study, rutin, and apigenin were detected while quercetin and luteolin were not. Apigenin improves the cognitive deficit observed in kindled mice, which is attributed to the BDNF upregulation that occurs in the hippocampus as a result of this compound (Sharma, 2020). Rutin increased memory retrieval by increasing extracellular signal-regulated kinase 1 (ERK1), strengthening the CREB[1] signaling pathway, and increasing BDNF gene expression in the hippocampus of rats with Alzheimer's (Moghbelinejad et al., 2014). Catchin prevented learning and memory impairment in senescence-accelerated mouse prone-8, which is associated with the protein kinase A/CREB pathway and BDNF (Li et al., 2009).

Pure flavonoids improve spatial memory and increase BDNF protein and mRNA levels of BDNF in the DG, CA1, and CA3 hippocampal regions. These data suggest that pure flavonoids are able to regulate BDNF metabolism/stabilization (Rendeiro et al., 2013). BDNF plays an important role in synaptic plasticity and promotes neuronal spine density (Alonso et al., 2004).

It has been showed that hippocampal NO has an important role in learning and memory. NMDA receptor contributes to memory consolidation and leads to the production of NO from L-arginine (Paul and Ekambaram, 2011).

Cerebral blood flow couples with neuronal activity, which is important for brain function and cognition. NO plays a crucial role in vasodilation, which is generated by both endothelial and neuronal NOS. Catchin activates endothelial NOS (Pervin, 2019). Rutin promotes NO production in cultured human umbilical vein endothelial cells by inducing NOS3 gene expression, NOS3 protein synthesis, and NOS3 activity (Ugusan et al., 2014).

Reduced endothelial NOS is the leading endothelial dysfunction in diabetes. Enhancing NOS3 activity in diabetes patients is a therapeutic target (Chandra et al., 2016). NOS3 is concentrated in hippocampal pyramidal cells more than any other brain area. The NOS3 of the hippocampus may affect the neural plasticity underlying learning and memory in adults (Gökcek-Sarac et al., 2012).

M. officinalis is a good source of volatile oil, flavonoid glycosides, and derivatives of caffeic acid (rosmarinic acid), which has antioxidant capabilities (Petkova et al., 2017). *M. officinalis* generates antioxidant properties and neuroprotection, possibly due to its inhibitory effects on monoamine oxidase (Hassanzadeh et al., 2011). In addition, *Melissa officinalis* extract is rich in rosmarinic acid, which prevents Alzheimer's disease from progressing (Noguchi-Shinohara et al., 2020). Rosmarinic acid reverses anxiety by increasing hippocampus BDNF (Makhathini et al., 2018).

5. Conclusion

According to the results of this study, *M. officinalis* can improve learning and memory in diabetic rats. The mechanism by which the hydroalcoholic extract of *M. officinalis* improved learning and memory in diabetic rats can be related to its impact on BDNF and NOS3 gene expression. The polyphenols of the hydroalcoholic extract of *M. officinalis* can increase the expression of these genes, as demonstrated by our real-time assays. Complementary data on how and which component of the extract affects learning and memory should be studied and discussed in future studies that purify and administer the components of the extract.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.114210.

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