

Could Melissa Officinalis Extract Restore Streptozotocin-Induced Spatial Memory Impairment in Rats?

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ABSTRACT

Introduction: Alzheimer disease is a progressive and irreversible neuropsychiatric disorder. Melissa Officinalis improves anxiety and clinical dementia symptoms caused by AD; therefore, the purpose of the present study was to evaluate the effect of Mellisa extract on spatial memory deficit induced by STZ in MWM examination of male wistar rats.

Methods: In the present study, 112 male wistar rats (220-270g) were used. Spatial learning deficit was induced by bilateral ICV injection of STZ (3mg/kg) via cannula; then, the Melissa extract in different doses administrated by gavage; and Morris Water Maze for measurement of spatial learning parameters was performed.

Results: The results of this study demonstrated that gavage of the 200mg/kg of Melissa officinalis in combination with STZ, followed by significant reduction in two parameters: Time and Distance.

Conclusion: Totally, the data indicate possible therapeutic value of M. officinalis extract on spatial learning improvement.

1. Introduction

The number of people suffering from neurological disorders has been increased lately worldwide. Alzheimer disease (AD) so called as late senile dementia, is a progressive and irreversible neurodegenerative and psychiatric disorder (which are the most common in developed countries) characterized by the fallowings: neuronal degeneration, behavioral disturbance, and me-

morial loss [1]. One of the determinant factors leading to this disorder is oxidative stress [2, 3]. Melissa officinalis L. (Lamiaceae), also known as lemon balm, is a perennial herb which grows in the south-central Europe, Asia, and northern of Iran. In Iran, this plant is called locally as Badranjbooye, Varangboo and Faranjmoshk [4]. Lemon balm extract has been found to have some compounds such as flavonoid and phenolic acids as possible free radical scavengers [4-7] and antiapoptotic factors [6, 7]. According to research done by others, some advantages

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are related to *Melissa officinalis* (*M. officinalis*): nerve-calming, memory enhancing, dementia improving, neurological disorder manipulating, cholinergic, and neurotropic acting [5, 6, and 8]. Some type of neurotoxicity models such as methamphetamine [9], ecstasy [7], STZ [2], etc. have been done up to now; neuroprotective function of *Melissa* in ecstasy model has been proven by our previous work [7]. There are well defined deteriorations related to STZ which eventually lead to AD: learning, memory, and cerebral metabolism (glucose and energy) [2]. Based on the findings in this regard, the aim of our study was to evaluate the effects of *M. officinalis* extract on spatial memory deficit induced by streptozotocin in male wistar rat in morris Water Maze (MWM).

2. Material and Methods

2.1. Animals

Wistar albino rats (220-270 g) were obtained from Laboratory Animal Center, Faculty of Pharmacy at Tehran University of Medical Sciences. In this study, animal care conditions were 12-hour periods of light and darkness, $23 \pm 2^\circ\text{C}$ temperatures and enough food and water. Some animals considered out of experiment due to conditions: movement and vision impairment, weight loss, and death.

2.2. Melissa Extraction

The extract was solved in percolation device with 70% ethanol; then, it condensed in the vacuum distillation rotary; and the waxy essence converted into powder in the freeze dryer for 48 hours.

2.3. Surgical Procedures

Rats were anesthetized with a solution of ketamine (80 mg/kg) and xylazine (15mg/kg) (intraperitoneal, Razi

Co., Iran), then placed in Stereotaxic apparatus (Steering, USA); SCALP layers cut between Bregma and Lambda with a scalpel. Lateral ventricular peculiarities regarding to Bregma characteristics (AP, ML, and DV) and according to Paxinos Watson atlas, were AP (-0.8mm from Bregma), ML ($\pm 1.5\text{mm}$ from midline), and DV (-3mm from Dura mater surface); finally, after determining the injection site, 2 guide cannula were implanted in 2 holes (created by dental drill), and fixed by dental cement. Mice were allowed 3 days rest for obtaining recovery.

2.4. Rat Training in MWM

Spatial learning of all animals was examined on 17th day of treatment under exposure to MWM (Morris, 1984), which consists of circular water pool (160 cm diameter, 60 cm height) filled with water ($25 \pm 2^\circ\text{C}$) at a depth of 25 cm. Pool start point was determined based on the geographical directions, which divided it into 4 quadrant. An escape platform (10 cm in diameter) was fixed 1 cm below water surface in the middle of one of the randomly chosen direction (north-west) of the pool. At first, animals were allowed to swim without platform for 90 sec; then, in acquisition phase, each rat permitted to swim for 4 days from 4 trial points with a maximum and interval time of 60 and 30 sec, respectively; for ones who could not find the platform, were manually guided and stabilized in the equal time; eventually, animal movements were recorded by camera aided computer, then processed and evaluated by Ethovision 3.1 software.

2.5. Experimental Groups

112 animals were classified into 3 groups (control, sham, and treatment), which in total divided into 14 sub-groups (8 animals per each one). At third day after surgery, daily administration of *Melissa* extract and distilled water was allowed by gavage; on days 5 and 7, normal

Table 1. Shows the brief description of the present procedure.

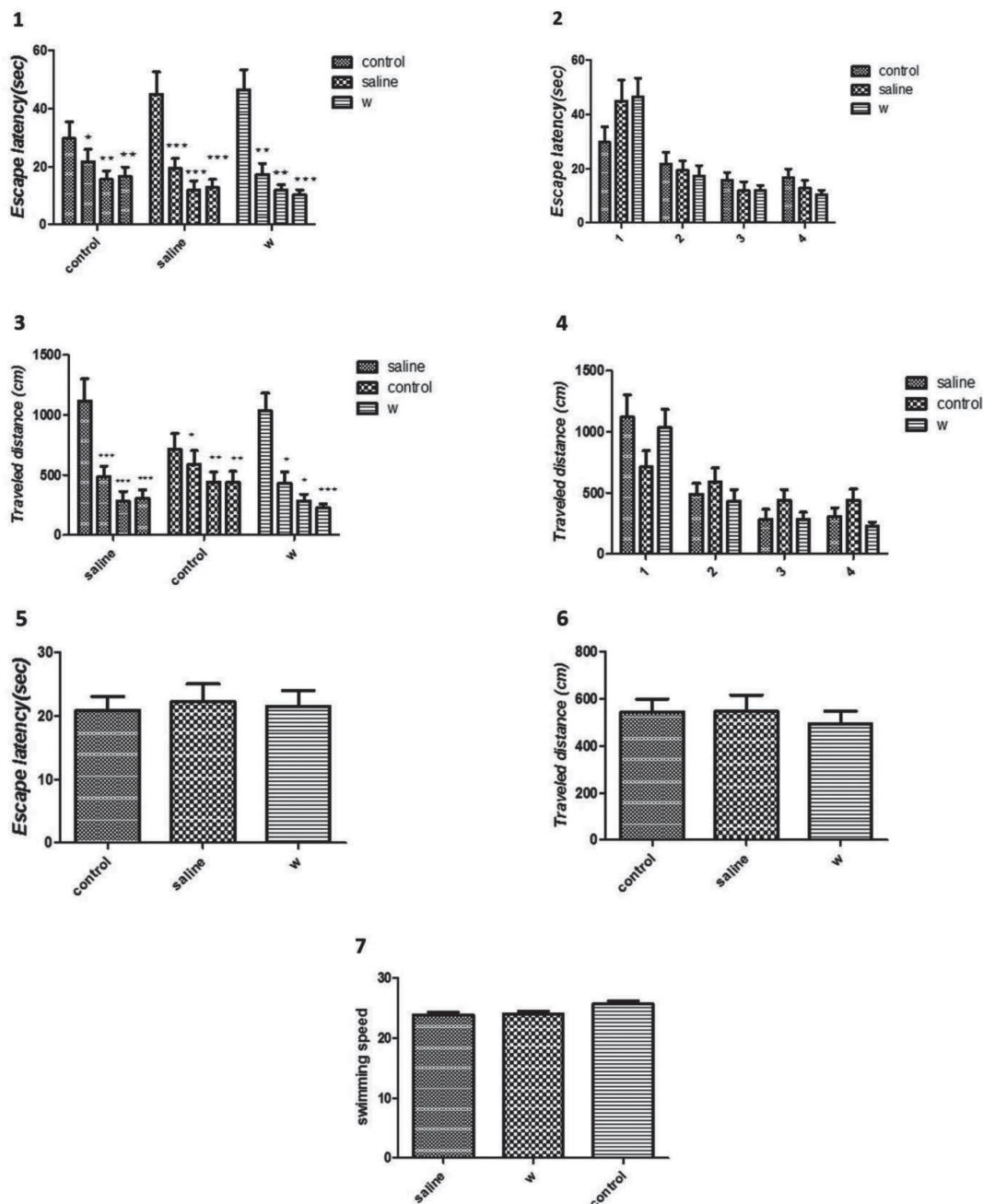
Group	Material Type		Administration Method	Dosage	Administration Time	Animal Number	
Control	-		-	-	-	8	
Sham	Normal saline		ICV	5μl (per each Ventricle)	Days 5 and 7 after surgery		
	Distilled water						
Treatment	Melissa		Gavage	2.5cc	Days 3 after surgery, Daily until 17		
	Melissa & STZ			Melissa			50mg/kg
							150 mg/kg
							200 mg/kg
							250 mg/kg
							300 mg/kg
	STZ		ICV	5μl (per each Ventricle)	Days 5 and 7 after surgery		
	STZ						

saline and STZ (3mg/kg, sigma. USA) were injected via intracerebroventricular (ICV) with a 10 μ l (1 μ l/min) Hamilton syringe; Time period for examination was considered 17 days (Table1).

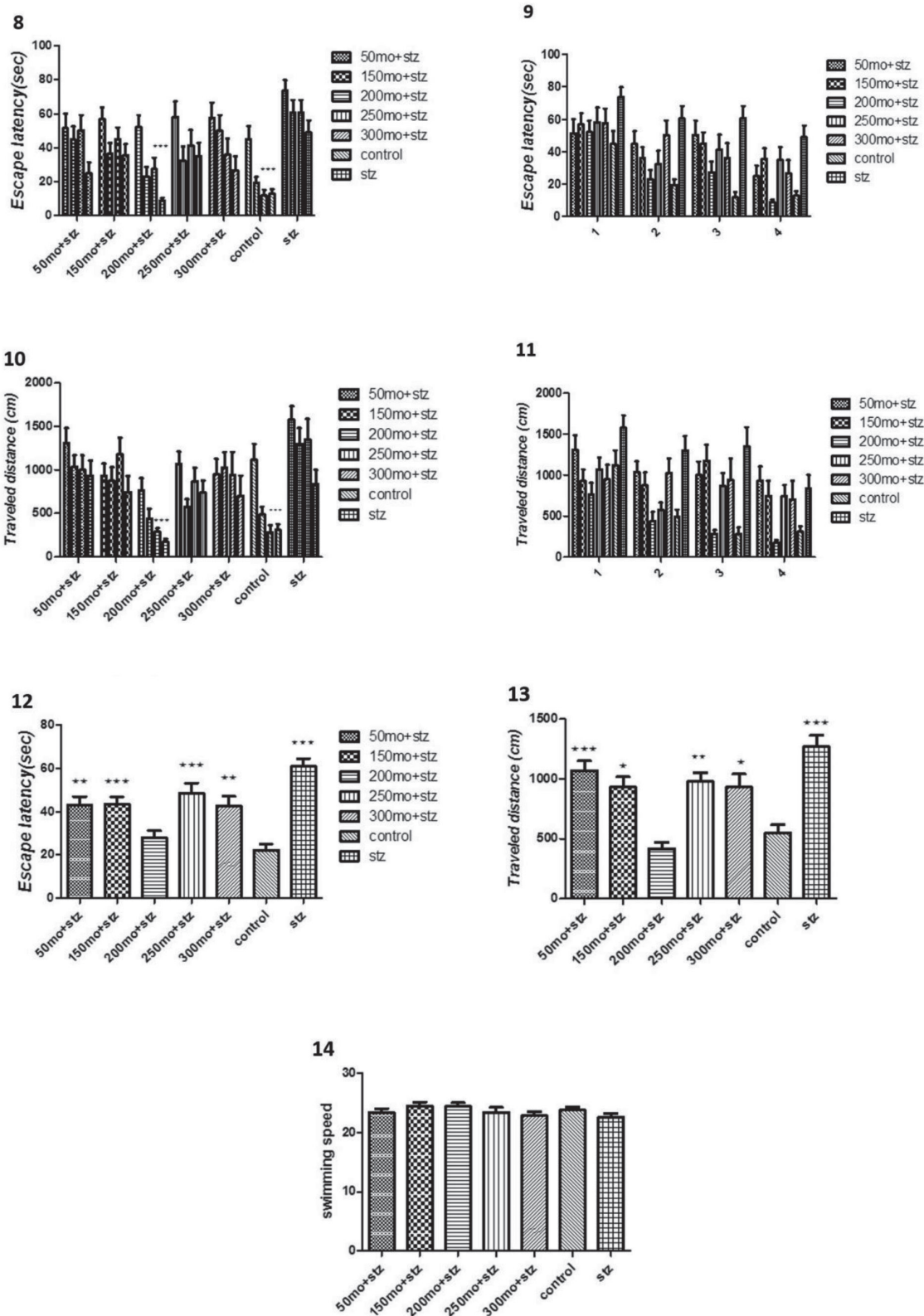
2.6. Histological Evaluations

Animals were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (15mg/kg); then their brains

were removed after perfusion, fixed in 10% formalin, and sectioned (40 μ m) by microtome (Vibroslice, Compel, England); Specimens with precise location of cannula in lateral ventricles were chosen for evaluation. After preparation of 7 μ m tissue sections, Congo Red staining was accomplished for detection of amyloid deposits, which along with nuclei must stain red, and blue, respectively.

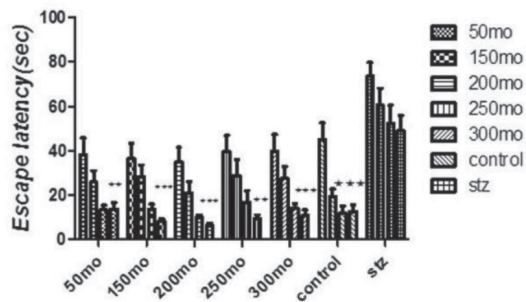


Graphs 1-7. Comparing control with sham. 1-4: Time and distance parameters over time and for each day individually; 5-7: Time, distance, and speed parameters in total 4 days. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$.

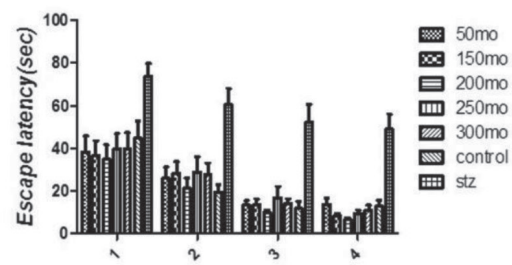


Graphs 8-14. Comparing the Melissa, different doses, - STZ combination (MO+STZ) with STZ group solely and control. 8-11: Time and distance parameters over time and for each day individually; 12-14: Time, distance, and speed parameters in total-4days. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$.

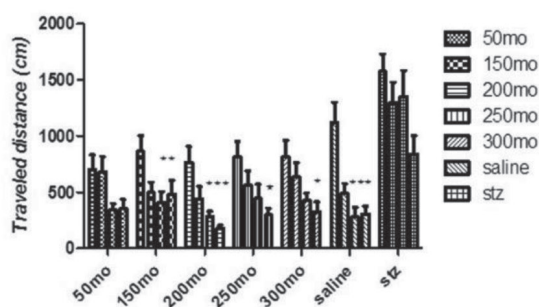
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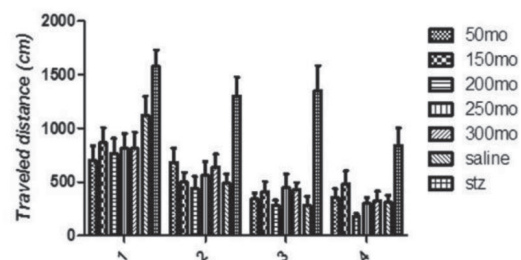
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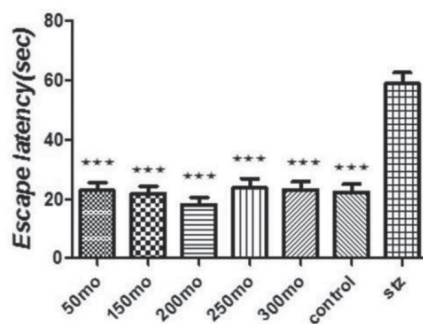
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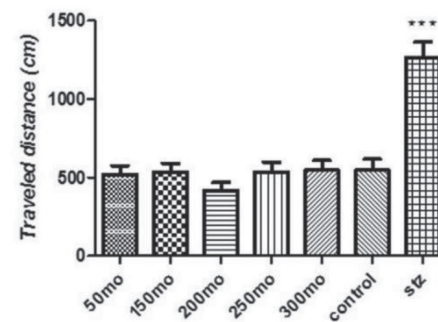
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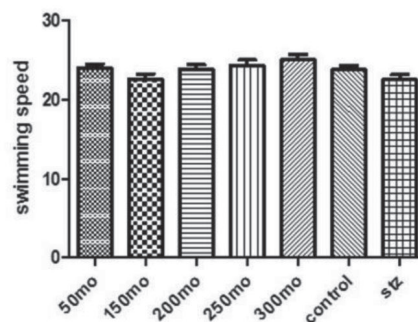
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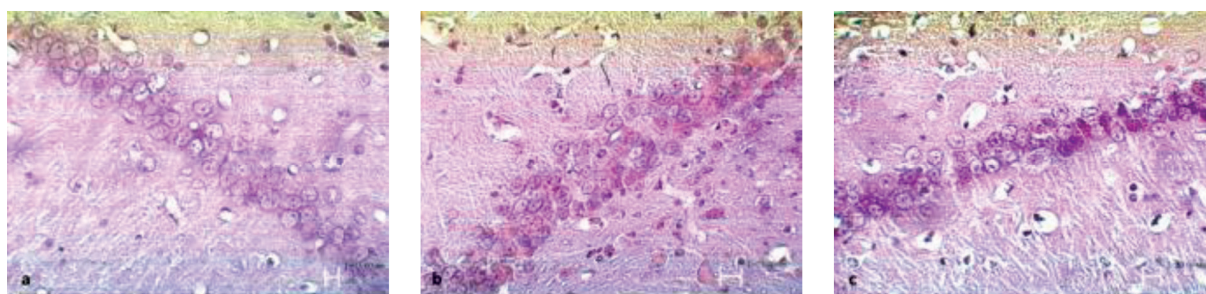
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Graphs 15-21. Comparing the Melissa, different doses (MO) with STZ group solely and control. 15-18: Time and distance parameters over time and for each day individually; 19-21: Time, distance, and speed parameters in total 4 days. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$.



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Figure 1. Amyloid Plaque production in the CA1 Hippocampus in 3 different groups. a: Control, b: STZ and c: 200 mg/kg of Mo+STZ (Congo Red Staining, X400).

2.7. Statistical Analysis

Data analysis carried out through one-way NOVA followed by Tukey's post hoc test ($P < 0.05$ considered as statistically significant) by graph pad prism 5.0 software.

3. Results

The outcome of the present study for evaluation of the effects of education on three parameters (time, distance, and speed) in order to find MWM hidden platform, by comparing each group with control group, was as follows:

The trends for the last parameter in all groups show no conspicuous alteration. Graphs 1-7 provide information about the control and sham groups. It is clear that there was a dramatic decline for the first two factors over time, but for each day individually and whole four days, the data were not meaningful.

Charts 8-14 compare the *Melissa officinalis*, different doses, - STZ combination (Mo+STZ) with STZ solely. We can see that all groups had almost fall trends for the first two factors over time. Spatial learning fell dramatically in group receiving STZ exclusively, as the most striking feature. On the other hand, the trend for group receiving 200mg/kg of Mo+STZ was upward significantly in total 4 days; otherwise, in groups receiving 50, 150, 250 and 300 mg/kg of Mo+STZ there was a slight drop for the first two parameters in 4 days in total.

Graphs 15-21 indicate *Melissa*, different doses, and STZ separately. It is apparent that there was a relatively narrow range of fluctuations in the first two items in diverse doses of *Melissa*, which was significant as compared with STZ for both each day and 4 day-period in total.

Histology Features

Amyloid plaques in the CA1 hippocampus for control (a); STZ (b); and 200mg/kg of Mo+STZ, (c) groups—respectively has been shown by Congo Red Staining (Fig. 1). As it is understood, the existence of plaques for the first (a), second (b), and last (c) groups were negative, positive, and positive, sequentially. The number of the third one was substantially less than the second.

4. Discussion

Several studies have employed some features related to *M. officinalis* as regarded i.e. nerve-calming, memory enhancing, etc., [5, 6, and 8]. In the present study, there were no obvious differences between control and sham groups, as mentioned before. This implies the reality that normal saline and distilled water could be used as suitable solvents due to not interfering with spatial learning.

The results of the present study showed that there was a significant diminishes in spatial learning in STZ group on the contrary with control that proofs well-established model of spatial learning deficit (Alzheimer). Likewise, other research indicated some characteristics of Alzheimer like alterations in behavior and brain glucose in respond to STZ injection [10, 11].

In spite of the fact that there was no conspicuous outcome in spatial memory relating to *Melissa*, 50, 150, 250, and 300mg/kg doses, - STZ combination rather than STZ group, which affirms no beneficial aspects of using these doses, providing data form *Melissa*, 200 mg/kg, - STZ incorporation imply the noticeable improvement in maze performance. Recent search in this regard confirm that administration of *M. officinalis* (200 mg/kg) can attenuate learning impairment [5]. The results of our study represent the effectiveness of *Melissa* dose-

dependency in therapy protocols. Studies assert that Melissa and especially its ethanol extract have the binding capability to acetylcholine (ACh) receptors (Nicotinic and Muscarinic) [12-14]. Ferreira et al confirm the fact of inhibitory effects of this extract on acetyl cholinesterase enzyme (AChE) [15] which itself inhibit ACh, a molecule with fundamental role in learning and memory also related to disorders improvement [16]; Ryan and Byrne propose the inhibitory capability (on AChE) for "Monoterpenes" which exist in the Melissa extract [17]; To sum up, the resulting recovery with consumption of 200mg/kg dose of Melissa extract and STZ simultaneously attribute to its inhibitory effects on AChE or binding ability to discussed receptors.

Objective data of the present study declare another promising privilege for concurrent use of Melissa, 200 mg/kg, - STZ in reduction of stress and agitation, in despite of STZ and other Melissa- STZ combined groups. Some other pros assertion by other scholars are as follows: improve cognitive deficit and sedative effects [18-20], reduce Alzheimer caused stress [21], recover mood related problems [22-24], and diminish mild to moderate Alzheimer caused agitation; probably, these functions are due to the activation of Ach receptors in CNS [12-14].

It was also noted that there is no significant alteration with groups receiving different doses of Melissa extract as opposed to control; it means that no pros and cons are in association with its usage on educational process. Nevertheless, the upshot was considerably higher as compared with STZ. Our result was equivalent with other research in regard with memory strengthening [25, 26].

Another point to notice was the reduction of amyloid plaques by using 200 mg/kg Melissa keep up with STZ in comparison with STZ group; free radical production [27], and further neuron apoptosis [16] is the consequence of beta-amyloid; breaking down this process by Melissa shows its antioxidant and neuroprotective effects due to probable inhibitory effects on monoamine oxidase (MAO) [13, 7].

Generally, owe to beneficial aspects of Melissa in special learning, it could be considered as a suitable choice of treatment in patients suffering from neurodegenerative disorders.

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