Advances in Bioresearch
Adv. Biores., Vol 7 (3) May 2016: 73-77
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

Effects of Hydro-Alcoholic Extract of *Melissa Officinalis* (Lemon Balm) on Morphine State – Dependent Learning in Nicotine-Treated Mice

[1*]**Ramesh Ahmadi,** [1]**Azadeh Karimi Anjiraki;** [1]**Saeede Pishghadam**
[1] Department of Biology, Islamic Azad University, Qom Branch, Qom, Iran
Corresponding author: Ramesh Ahmadi, 15thKhordad St, Islamic Azad University, Qom Branch, Qom, Iran.
E-mail: ramahmd@yahoo.com

ABSTRACT
State -dependent learning is the phenomenon through which memory retrieval is most efficient when an individual is in the same state of consciousness as they were when the memory was formed. M. officinalis has also terpenoides that is useful in memory and learning. Moreover nicotine can affect on memory and learning by nicotinic receptors of hippocampus cholinergic system. The present study was conducted on the effects of Melissa Officinalis (MO) extract on SDL in mice.p 66 male mice were selected and allocated to 8 groups (n=6).To study memory in mice using passive avoidance method to measure step-down latency. The obtained results showed that injection of pre- training of nicotine (0.1mg/kg) and pre – testing of Melisa officinalis extract25 mg/kg intraperitoneally can improve memory and also and pre – testing of nicotine (0.1mg/kg) and pre- training of extract (25 mg/kg(intraperitoneally can improve memory . Interaction effect of nicotine and Varangboo improves memory.
Keywords; *Melisa Officinalis Extract (Varangboo) , Steady State- Dependent Learning , Nicotine, Mice*

Received 20/01/2016 Accepted 24/04/2016

How to cite this article:
Ramesh A, Azadeh K, 1Saeede P. Effects of Hydro-Alcoholic Extract of *Melissa Officinalis* (Lemon Balm) on Morphine State – Dependent Learning in Nicotine- Treated Mice. Adv. Biores. Vol 7 [3] May 2016: 73-77.DOI: 10.15515/abr.0976-4585.7.3.7377

INTRODUCTION

M. officinalis enhances memory and relieves stress [1]. One of the main properties of *M. officinalis* is its antioxidant property which is due to the presence of special compounds in it. *M. officinalis* extract has polyphenolic compounds, such as quercetin, gallic acid, as well as flavonoids, aldehyde, and tannin compounds. Due to its medicinal properties, *M. officinalis* has been used in other studies for examining its effectson Alzheimer, memory, learning, and depression. [2] .Melissa Officinalis L. (Labiatae) has been frequently used in Iranian traditional medicine to treat neurological disorders such as depression and anxiety, and it is also mentioned as a memory enhancing herb [3].The extract of *M. officinalis* has a cholinergic property [4].

As well as neurotropic action [5] Nicotine enhances attention and cognitive performance [6].nAChR agonists, including nicotine, enhance cognition, memory and promote learning by actions in a number of different brain circuits; however, the underlying mechanisms are yet not completely under- stood [7-9].Neuronal nicotinic acetylcholine receptors (nAChRs) are widely expressed throughout the brain [10].

The aim of present article is to give a brief overview of effects of hydro-alcoholic extract of Melissa officinalis (Lemon Balm) on morphine state – dependent learning in mice.

MATERIALS AND METHODS

Animals

Mice weighed 25±30 g were used at the beginning of the study. The animals were purchased from the Pasteur institute of Iran. 6 animals were housed per cage in clear polycarbonate cages, maintained under a 12:12-h light/ dark cycle, and given access to food and water *ad libitum* .Behavioral testing was done

over a 3-day period. Animals were kept under standard conditions (temperature 20–21^{0}C, 60–65% relative humidity) and returned to 12–12 h light/dark cycle and handled once a day for approximately 10min for 6–7days.

Plant extraction procedure

Dried leaves were ground to a fine powder.

The powdered leaves (50g) were macerated in distilled water (500ml) at room temperature for 24h. Subsequently, the mixture was filtered using Whatman filter paper. The filtrate was concentrated over the vapor of the water bath and dried under vacuum. The yield of extract was 31.6% (w/w)

RESULTS

pre- training and pre- testing effect of morphine on step down

Latency (SDL)

The effect of pre-training and pre-testing administration of morphine on stat-dependent learning was examined in three groups. As figure 1 shows, in group which received morphine (1,3 mg/kg) before training, significant decrease was observed (.**P<0.01) compared to control group on the step down latencies (SDL). Compared to control group, significant increase was observed (*p<0.05) in the step-down latencies (STD) in group which received morphine (1,3mg/kg) before testing. Moreover, morphine (3 mg/kg) could enhance memory as much as control. Also, significant main effect (Significant difference) was not observed in groups which received morphine (1,3mg/kg) before testing and morphine (3mg/kg) before training, comparing with control group.

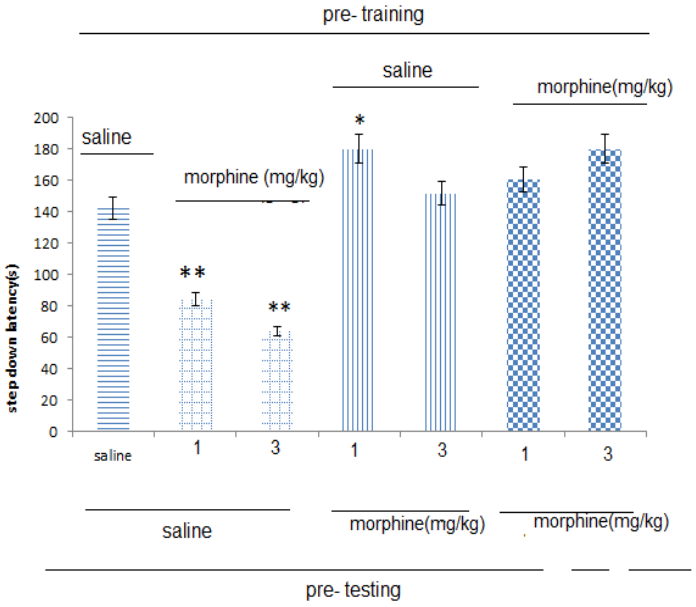

Figure 1: pre- training and pre- testing effect of morphine on state-dependent learning
Significant difference between saline + saline (**P<0.01)
Significant difference between saline +saline (*P<0.05)
Data are the means ± SEM

Pre- training and pre- testing effect of nicotine on step down

Latency (SDL)

Figure 2 shows significant increase and decrease was observed respectively (*P<0.05) in animals that received nicotine (0.01 and 0.1 mg/kg) before training, compared with control group (saline) (***P<0.001) in the step-down latencies (STD). Compared with control group (saline), significant decrease was observed (**P<0.01) in effect of pre-testing administration of nicotine in animals that received nicotine (0.1 mg/kg). Furthermore, significant increase was observed in the step down latencies (SDL) in lower dose of ethanol (0.25g/kg) (*P<0.05) in the animals that received ethanol (1,0.25 g/kg) before training and nicotine (0.01 mg/kg) on the test day, compared with control group (saline).

Effect of pre- testing and pre – training of *M. officinalis* on step down

Latency (SDL)

As shown in Figure 3, comparative effect of Administered varangboo (25 mg/kg) before testing significant difference was not observed. significant increase was observed (##p<0.01) in effect of morphine

(3mg/kg) before training and saline before testing compared with morphine (3mg/kg) before training and varangboo before testing , on step down latencies (SDL). A significant increase was observed in animals that received nicotine (0.1mg/kg) before training and saline before testing compared with a group which received nicotine (0.1mg/kg) before training and varangboo (25mg/kg) before testing on SDL (●●p<0.01). A significant increase was observed in a group that received morphine (3mg/kg) and nicotine (0.1mg/kg) before training and varangboo before testing compared with a group which received morphine (3 mg/kg) before training and varangboo before testing (▪▪p<0.01). A significant increase was observed (ΔP<0.05) on SDL in other comparison between morphine + nicotine+ varangboo with saline + varangboo .

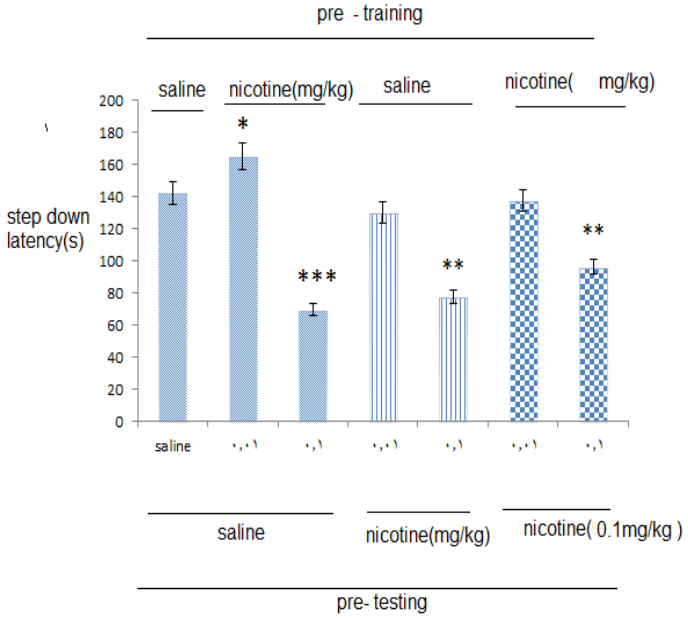

Figure2. The effects of nicotine on inhibitory avoidance memory
Seven groups of animals were used. Five groups of animals received pre-training and pre testing , saline , or different doses of nicotine (0.1, 0.01 mg/kg) .Two groups of animals received pre-training injections of ethanol(1, 0.25g/kg) and nicotine (0.01mg/kg) on the test day . Data are the means ± SEM * significant difference with saline +saline group

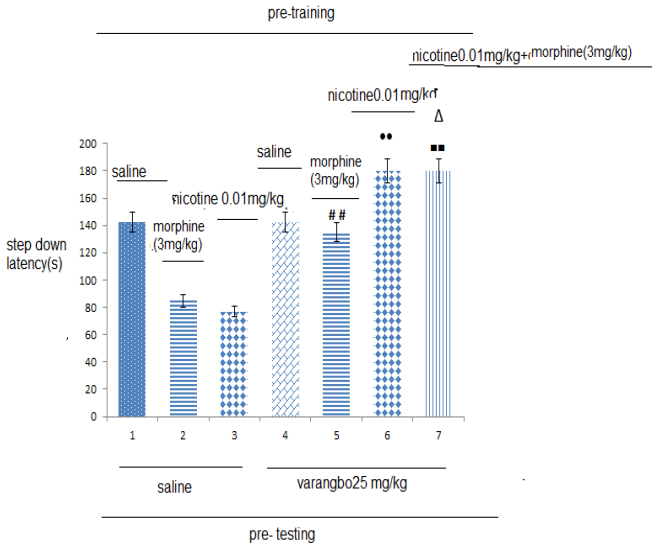

Figure 3:pre- training effect of varangboo (25mg/kg) on control (saline) group in step down latency(STD) Significant difference between morphine + saline (##P<0.01). Significant difference between nicotine +saline (••P<0.01). Significant difference between varangboo+ morphine (P<0.01) . Significant difference between varangboo+saline (p<0.05); Data are the means ± SEM

DISCUSSION

This experiment demonstrated that morphine caused state- dependent learning in mice treated by *M. officinalis* .There are several conflicting evidences showing the effect of morphine on learning and memory [11, 12].

It is well known that pre- or post-training administration of morphine and ethanol have impairing effects on passive avoidance tasks and interestingly, pre-test administration of the same doses of these drugs were able to recur memory impairment [13-16]. It has been suggested that the activation of the NMDA receptor is required for long-term potentiation (LTP) in the hippocampus, amygdala, and medial septum (17) .This mechanism has been implicated in memory formation; the involvement of the glutamate-receptor system and LTP is strongly linked to new learning and memory in animal models (18). As seen in figure 2, pre- training administration of nicotine (0.1 mg/kg) might not recur memory impairment. Memory is defined as a system for the storage and retrieval of learned information with physiological properties of its own(19). Several studies have shown that chronic exposure to morphine or heroin leads to the impairment of hippocampal LTP [20, 21; 22] and induces cognitive deficiencies, as shown by poor performances on memory task of heroin abusers (23) or chronic opiate-treated rodents [24; 25; 20; 26]. Injection of morphine to male rats impairs specifically spatial learning in MWM [27,28,29]. However, the mechanisms underlying these effects of opiates are poorly understood.The pre-training administration of morphine impaired the memory. When 24h later the same dose of morphine was administered before testing, the memory was retained [30, 31]. This is known as morphine state-dependency (morphine St-D) [30,31]. Also, it has been reported that opioids have positive effects on synaptic plasticity in hippocampus [32].

Several investigations have shown that there is a close relationship among the cholinergic system and opioids in memory performance [33, 34].

Reversal of morphine-induced memory impairment in mice by withdrawal in Morris water maze: possible involvement of cholinergic system [35].

M. officinalis also enhances memory and relieves stress (1) Ferreira et al confirm the fact of inhibitory effects of this extract on acetyl cholin¬esterase enzyme (AChE) [36.], which inhibits ACh itself, a molecule with fundamental role in learning and memory is also related to disorders improvement [37].

CONCLUSION

Results implicate that *Melissa Officinalis* has shown increasing effects on step down latency (SDL) in mice. These results propose the potential use of *Melissa Officinalis* has neuroprotective agent in learning and memory.

REFERENCES

1. Akhondzadeh, S. , Noroozian , M. , Mohammadi, M. , Ohadinia , S. , Jamshidi , . AH, .et al. (2003). Melissa officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized, placebo controlled trial. J. Neurol. Neurosurg. Psychiatr.74: 863 – 6.
2. Pereira , RP. , Fachinetto , R. , de Souza Prestes, A. , Puntel , RL. , Santos da Silva, GN. , et al.(2009). Antioxidant effects of different extracts from *Melissa officinalis, Matricari arecutita* and Cymbopogoncitratus. Neurochem . Res, 34: 973-983.
3. Naghibi, F. ,Mosaddegh, M . , Mohammadi Motamed ,S. , Ghorbani, . (2005).Labiatae Family in folk medicine in Iran: from ethnobotany to pharmacology. Iran J Pharm Res. 2:63-79.
4. Wake G. , Court J. , Pickering , A. , Lewis R. , Wilkins ,R. , Perry ,E. (2000) .CNS acetylcholine receptor activity ineuropean medicinal plants traditionally used to improve failing memory. J Ethnopharmacol. 69:105-114.
5. Soulimani, R. , Fleurentin , J. , Mortier F, Misslin , R, Derrieu , G. , Pelt , J. M., .(1991).Neurotropic action of the hydroalcoholic extract of *Melissa officinalis* in the mouse. Planta Med. 57:105-109.
6. Newhouse, PA, Potter, A, Singh, A. (2004) .Effects of nicotinic stimulation on cognitive performance. CurrOpinPharmacol4:36–46.
7. Levin,E.D.,Briggs,S.J.,Christopher,N.C.,andAuman,J.T.(1994).Working memory performance and cholinergic effects in the ventral tegmental area and substantianigra. Brain Res. 657, 165–170.
8. Levin,E.D. ,and Simon,B.B.(1998). Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology(Berl) . 138, 217–230.
9. Socci,D. J. ,Sanberg, P.R.,and Arendash,G.W.(1995).Nicotine enhances Morris water maze performance of young and aged rats. Neurobiol.Aging . 16, 857–860.
10. Gotti,C.,Moretti,M.,Gaimarri,A.,Zanardi,A.,Clementi,F.,andZoli,M.(2007).Heterogeneity and complexity of native brain nicotinic receptors. Biochem. Pharmacol . 74, 1102–1111.
11. Means, L .W. ,Holsten, R . D. ,(1996) . Long M and High K M. Scopolamine- and morphine- induced deficits in water maze alternation: failure to attenuate with glucose. Neurobiol. Learn. Mem .66: 167-75 (5).

12. Sala, M. ,Braida , D. , Leone, MP. , Calcaterra , P. , Frattola ,D and Gori E. . (1994) . Chronic morphine affects working memory during treatment and withdrawl in rats: possible residual long-term impairment. Behav.Pharmacol. 5: 570-80.
13. Nishimura, M. ,Shigi, Y. , Kaneto, H. , (1990). State dependent and/or direct memory retrieval by morphine in mice.Psychopharmacology . 100, 27–30.
14. Nakagawa, Y. , Iwasaki, I. , (1995) . Involvement of benzodiazepine/GABA-Areceptor complex in ethanol-induced state-dependent learning in rats. Brain Res. 686, 70–76.
15. Nakagawa, Y. , Iwasaki, I. , (1996) . Ethanol-induced state-dependent learning is mediated by .5-hydroxytryptamine3 receptors but not by N-methyl-Daspartate receptor complex. Brain Res. 706, 227–232.
16. Khavandgar, S. ,Homayoun, H. , Torkaman-Boutorabi, A. , Zarrindast, M.R. ,(2002). The effects of adenosine receptor agonists and antagonists on morphinestate-dependent memory of passive avoidance. Neurobiol.Learn.Mem . 78, 390–405.
17. Rockstroh, S. ,Emre, M. , Tarral A. , Pokorny, R . (1996) .Effects of the novel NMDA-receptor antagonist SDZ EAA 494 on memory and attention in humans. Psychopharmacology.124:261–266.
18. Lozano, VC . , Armengaud , C . , Gauthier. , M . (2001) . Memory impairment induced by cholinergic antagonists injected into the mushroom bodies of the honeybee. J Comp Physiol. 187:249–254.
19. Izquierdo, I. (1979). Effect of naloxone and morphine on various forms of memory in the rat: Possible role of endogenous opiate mechanisms in memory consolidation. Psychopharmacology, 69, 199-203.
20. Pu, L. , Bao, GB. , Xu, NJ. , Ma, L. , and Pei G. (2002) .Hippocampal long-term potentation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates. J Neurosci . 22: 1914-1921.
21. Salmanzadeh, F. , Fathollahi , Y. , Semnanian , S. , Shafizadeh, M .(2003) . Dependenceon morphine impairs the induction of long-term potentiation inthe CA1 region of rat hippocampal slices. Brain Res 965:108 –113.
22. Bao, G. , Kang, L. , Li ,H. , Li, Y. , Pu , L. , Xia , P. , Ma ,L. , Pei, G. (2007). Morphine and heroin differentially modulate in vivo hippocampal LTP in opiate dependent rat. Neuropsychopharmacology .32:1738 –1749.
23. Guerra, D. , Sole´, A. , Camí, J. , Toben, ˜a A . (1987). Neuropsychological performance in opiate addicts after rapid detoxification. Drug Alcohol Depend . 20:261–270.
24. Spain, JW. , and Newsom , GC. (1991) . Chronic opioids impair acquisition of both radial maze and Y-mazechoice escape. Psychopharmacology (Berl) . 105: 101-106.
25. Li, Z. , Wu, CF. , Pei, G. , and Xu, NJ. (2001) . Reversal of morphine induced memory impairment in mice by withdrawal in Morris water maze: possible involvement of cholinergic system. Pharmacol Biochem Behav . 68: 507-513.
26. Miladi Gorji ,H. , Rashidy-Pour, A. , Fathollahi, Y . (2008). Effects of morphine dependence on the performance of rats in reference of the water maze. PhysiolBehav 93:622– 627.
27. McNamara, RK. , and Skelton, RW. (1991) .Retraining morphine impairs acquisition and performance in theMorris water maze: motivation reduction rather than amnesia. Psychobiology . 19: 313-322.
28. Classen, W. , and Mondadori , C. (1984) . Facilitation or inhibition of memory by morphine: a question of experimental parameters. Experientia . 40: 506-509.
29. McNamara, RK. , and Skelton, RW. (1992) .Pharmacological dissociation between the spatial learning deficitsproduced by morphine and diazepam. Psychopharmacology;108: 147-152.
30. Izquierdo, I. , and Dias, R. D. (1983) . Effect of ACTH, epinephrine, betaendorphine, naloxone, and of the combination of naloxone or betaendorphine with ACTH or epinephrine on memory consolidation. Psychoneuroendocrinology . 8, 81–87.
31. Bruins Slot, L. A. , and Colpaert, F. C. (1999) Opiate state of memory: receptor mechanisms. Journal of Neuroscience . 19, 10520–10529
32. Xie, CW. , and Lewis, DV. (1995) . Endogenous opioids regulate long-term potentiating of synaptic inhibition in the dentate gyrus of rat hippocampus. J Neurosci; 15: 3788-3795.
33. Ukai, M. , Lin, H.P. (2002) . Involvement of mu(1)-opioid receptors and cholinergic neurotransmission in the endomorphins-induced impairment of passive avoidance learning in mice. Behav. Brain Res. 129, 197–201.
34. Zarrindast, M.R. , Nouraei, N. ,Khalilzadeh, A . , Askari, E. (2006a) . Influence of acute and sub-chronic nicotine pretreatment on morphine state-dependent learning. Behav. Brain Res. 173, 268–273-
35. Li ,Z. , Wu ,CF. , Pei ,G. , Xu , NJ. (2001) . Reversal of morphine-induced memory impairment in mice by withdrawal in Morris water maze: possible involvement of cholinergic system Pharmacol Biochem Behav. Mar . 68(3):507-13.)
36. Ferreira ,A. , Proneca ,C. , Serralheiro, MLM. , Araujo , MEM . (2006) .The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. Journal of Ethnopharmacology.108: 31-37
37. Behl ,C. (1999). Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. Progress in Neurobiology. 57: 301-323