THE INTERACTION OF LEVETIRACETAM'S ANALGESIA WITH MORPHINE INDUCED TOLERANCE IN TAIL-FLICK MICE MODEL

Preprint · August 2018

DOI: 10.13140/RG.2.2.19387.98083

CITATIONS
0

READS
10

4 authors, including:

Mehdi Saberi

40 PUBLICATIONS **159** CITATIONS

SEE PROFILE

August 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.2 • 274-283

THE INTERACTION OF LEVETIRACETAM'S ANALGESIA WITH MORPHINE INDUCED TOLERANCE IN TAIL-FLICK MICE MODEL

Vesali Mahmood, R.[1]; Ghorbani, M.[2]; Pournaghash Tehrani, S.[1]; Saberi, F.[3]; Saberi, M.[2]*

[1]Department of psychology, Faculty of Psychology and Education, University of Tehran, Tehran, Iran
[2] Department of Pharmacology and Toxicology, Applied Neuroscience Research Center, Faculty of pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran
[3]Faculty of Medicine, Iran University of Medical sciences

m_s_saber@yahoo.com

Abstract

Tolerance development to the antinociceptive effect of morphine is a major concern of its long-term administration. **Objectives:** Based on previous studies on the effect of some anticonvulsant drugs on morphine tolerance, the present study investigated the effects of Levetiracetam (LEV) on the development and expression of morphine tolerance in mice.

To evaluate the LEV effects on the development or expression of morphine tolerance, animals received LEV (60, 300 or 900 mg/kg), either 30 min before the injection of morphine (50 mg/kg) during induction period once daily for 3 consecutive days; or 30 min before injection of challenging dose of morphine (5 mg/kg), before and after morphine-induced tolerance, respectively. The analgesic effect of LEV was evaluated by tail-flick analgesiometer 30 min after injection.

LEV at the doses of 300 and 900 mg/kg significantly attenuated the development of morphine tolerance, but had no effect on expression of morphine tolerance. High dose of LEV (900 mg/kg) alone had antinociceptive effect and significantly increased the tail-flick latency time which was attenuated by naloxone.

LEV showed antinociceptive effect, and could prevent the development of tolerance presumably via opioid's receptors. Co-administration of LEV and morphine may be of great clinical implication.

Keywords: *Levetiracetam (LEV), Morphine, Tolerance, Antinociception, Tail-flick test, Mice*

Introduction

Tolerance to the effects of drugs simply means that the drug loses its effectiveness over the time and an incremental dose is required to produce the same physiological response. On the other hand, increment of the dose may induce more adverse effects and drug toxicities (1). At first, the analgesic effect of Carbamazepine in trigeminal neuralgia (2) stimulated the use of antiepileptic drugs for treatment of neuropathic pain syndromes. Then, several studies have confirmed the analgesic effects of anticonvulsant drugs in different models and species (3, 4). For instance, Carbamazepine and Gabapentine have shown to possess analgesic effects in animal models of continues pain (5). Likewise, it has also been shown that Lamotrigine is effective against trigeminal neuralgia, HIV neuropathy, central post stroke pain (6, 7) as well as tail flick test (8).

Recent studies suggest that anticonvulsants might decrease opioid consumption either by enhancing opioid analgesia or by suppressing mechanisms of opioid tolerance (4). For example, Carbamazepine (9), Phenytoin (10), Gbapentine (11), Topiramate (12) and Vigabatrin (13) have been shown to increase opioids antinociception. Other studies have also revealed that anticonvulsant drugs suppress opioid tolerance (1, 14-17). In addition it has been shown that some antiepileptic drugs such as Vigabatrine and Lamotrigine can attenuate the development and expression of tolerance to morphine-induced antinociception in mice, without having analgesic effect, alone (1, 8).

Regarding the results of these studies, it was suggested that such a suppressive effect of anticonvulsants on morphine tolerance may be a general characteristic of all anticonvulsants, but selection of more efficacious and less toxic drug may conduct the study to the clinical applications.

Levetiracetam (LEV) is a new safer and, at the same time, effective anti-epileptic drug with a unique pharmacological profile distinct from the traditional antiepileptic drugs (18). This dug was approved by the FDA in 1999 as an adjunctive therapy for the treatment of refractory partial epilepsy in adults (18). It has been shown that LEV induces an antihyperalgesic effect in two models of human neuropathic pain, suggesting a therapeutic potential in neuropathic pain patients (19, 20). In another investigation LEV has not altered nocipetive reflex threshold in no sedated animals (21). Moreover, LEV had no antihyperalgesic effect in the tibilal neuroma transposition model of neuroma pain and preoperatively LEV treated groups have shown a significant increase in pain withdrawal latency (21). Silva and coworkers have shown that post-incisional administration of LEV has no antihyperalgesic effect at any does in planter test model in rats (22). Also, Levetiracetam had no significant effect on the nociceptive threshold in normal mice, but there were significant decrease in pain threshold latency in diabetic mice, in hot plate test (23). Jungehulsing and coworkers have shown that the LEV mode of action does not exert analgesic effect in chronic CPSP (24). It has shown that LEV is able to increase the none steroidal anti-inflammatory drugs and also Caffeine analgesia effects synergistically (25).

Hence, the present study designed to investigate the effect of LEV on morphine's analgesia and its effect on the development and expression of tolerance to morphine-induced antinociception in the setting of acute nociception of tail-flick test in mice.

Methods

1.1. Animals

Ninety six adult male albino NMRI mice (Pasteur Institute, Iran) weighing average of 25 g were used in these experiments. They were kept 6 per cage (25×30×15 cm) at a room controlled temperature (23±1°C) and maintained on a 12-h light/dark cycle (light on 07:00 h) with free access to the standard rodent breeding diet and tap water. Each animal was used only once and killed immediately after the experiment. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Tehran University.

1.2. Drug preparation

Morphine sulphate (Temad Co, Iran) dissolved in saline and Levetiracetam (Bakhtar Biochem. Co,

Iran) were suspended in 0.9 % solution of NaCl. The LEV and morphine were prepared immediately before use and injected intraperitoneally (i.p.) and subcutaneously (s.c.) in a volume of 10 ml/kg, respectively.

1.3. Assessment of morphine antinociception

Nociception was assessed with the tail-flick apparatus (D'amour and Smith, 1941). Before the test, animals were allowed to adapt to the conditions of the laboratory room for at least 1 h. Moreover, animals were alternatively allocated in their restrainers for 1 min each 15 min before the test in order to attenuate the stress that has been shown to produce antinociception. Restrained animals were placed on the tail-flick apparatus (type 812, Hugo Sachs Elektronik, Germany), a noxious beam of light was focused on the tail about 4 cm from the tip, and tail-flick latency was recorded automatically. Tail-flick latency (TFL) is a spinal response and, therefore, TFL is a measurement of pain threshold at the spinal level. In order to minimize injury in the animals, a cut-off time of 8 sec was used. Latency was assessed and recorded as an average of TFL time (TFL sec).

1.4. Experimental protocols

1.4.1. Development of tolerance to morphine-induced antinociception

Tolerance induction was begun on day 1, four hours after challenge dose of morphine (4 mg/kg; s.c.) by administration of morphine (50 mg/kg; s.c.) or saline (10 ml/kg; as control) once daily for 3 days as previously has been described (8). The antinociceptive response to a challenge dose of morphine (4 mg/kg; s.c.) was determined by tail-flick test 30 min after injection on day 1 and day 4 for tolerance evaluation (n= 6 in each group). The challenge dose of morphine was selected as already described by Saberi and Chavooshi (2009)(8).

1.4.2. Effects of LEV on the development of morphine-induced tolerance

In these experiments, to evaluate the effects of LEV on the induction of morphine's tolerance, animals (n= 6) received various doses of LEV (60, 300 or 900 mg/kg; i.p.), 30 min before morphine (50 mg/kg; s.c.) or saline (10 ml/kg), once daily for 3 consecutive days. The effect of the challenge dose of morphine (4 mg/kg; s.c.) was tested on day 1 prior to and on day 4 following the induction period of morphine tolerance. The doses of LEV were selected based on the study by Ozcan et al. (2008) that evaluated the antinociceptive efficacy of LEV in a mice model for painful diabetic neuropathy (23).

1.4.3. The effect of LEV on the expression of morphine-induced tolerance

In this set of experiments, to assess the effects of LEV on the expression of morphine-induced tolerance, animals (n= 6) received different doses of LEV (60, 300 or 900 mg/kg) or saline (10 ml/kg) 30 min before challenge dose of morphine (4 mg/kg) following morphine-induced tolerance. The antinociceptive responses to the challenge dose of morphine for each animal was determined by tail-flick test on day 4 (post- tolerance) as described in experiment (2) section.

1.4.4. The antinociceptive effects of different doses of LEV in comparison to morphine and saline

In this experiment, to evaluate the antinociceptive effect of either various single doses of LEV (60, 300 or 900 mg/kg; i.p.), saline (10 ml/kg), or morphine, animals in each group (n=6) tested by tail-flick test 30 min after injection.

1.4.5. The effect of Naloxone on the antinociceptive effect of LEV

To assess the effect of naloxone on the antinociceptive effect of LEV, animals in separated groups (n= 6) received naloxone (10 mg/kg) 30 min after the injection of LEV (60, 300 or 900 mg/kg; i.p.). Then animals in each group were tested by tail-flick.

1.5. Statistical analysis

The results obtained are expressed as mean ± SEM (standard error of mean). The mean in all groups were subjected to ANOVA followed by Bonfferoni post test for multiple comparisons between groups, as needed. Data were processed by SPSS, P values less than 0.05 were considered to be statistically significant.

Results

1.1. Development of tolerance to morphine-induced antinociception

The antinociceptive response to a challenge dose of morphine (4 mg/kg; s.c.) for saline and pre-tolerant groups on day 1 and day 4 was almost similar (Figure 1). To assess the morphine tolerance in this study, animals received morphine (50 mg/kg) or saline (10 ml/kg) once daily for 3 days. Figure 1 shows the development of tolerance to morphine-induced antinociception tested by challenge dose of morphine (4 mg/kg; s.c.) on day 4. ANOVA indicated that antinociceptive response to the challenge dose of morphine on day 4 decreased significantly in morphine-treated animals that received morphine during the induction period in comparison with the saline-treated group [$F(1,9)= 228.250$, $P<0.05$; Figure 1].

1.2. Effects of LEV on the development of morphine-induced tolerance

In this set of experiment, the animals were injected with different doses of LEV (60, 300 or 900 mg/kg; i.p.), 30 min before morphine (50 mg/kg; s.c.) administration once daily for 3 days during the induction period. The lowest dose of LEV (60 mg/kg) had no significant effect on the development of morphine tolerance. ANOVA followed by bonferroni post hoc, indicated that in morphine-treated animals that received higher doses of LEV (300, 900 mg/kg) prior to morphine during the induction period, the antinociceptive responses to the challenge dose of morphine (4 mg/kg; s.c.) on day 4 significantly increased in comparison to vehicle group that received Saline as solvent of LEV [$F(3,19)=46.96$, $P<0.05$; Figure 2]. Our data revealed that pretreatment of animals with LEV significantly reduced the development of tolerance to morphine antinociceptive effect in a dose-dependent manner.

1.3. The effect of LEV on the expression of morphine-induced tolerance

We examined whether LEV blockade of morphine tolerance, required daily administration of LEV or if a single dose prior to challenge dose of morphine (tolerance expression) was sufficient. Thus, the animals received LEV (60, 300 or 900 mg/kg; i.p.) or saline (10 ml/kg), 30 min before challenge dose of morphine (4 mg/kg) prior to and following morphine-induced tolerance. ANOVA indicated that administration of LEV at any of doses could not attenuate the expression of morphine tolerance [$F(3,19)=0.403$, $P<0.05$; Figure 3].

1.4. The antinociceptive effects different doses of LEV in comparison to the morphine

To determine the antinociceptive effect of LEV, tail-flick tests were done for each group of animal, 30 min after single injection of LEV (60, 300 or 900 mg/kg; i.p.). Data analysis indicated that LEV (900 mg/kg) could significantly increase the latency when compared to saline [$F(3,19)=46.32$, $P<0.05$,; Figure 4(a)].

In addition, we also compared the high dose of LEV (900 mg/kg) with morphine group (4 mg/kg) 30 min after injection. ANOVA indicated that high dose of LEV (900 mg/kg) alone had antinociceptive effect [$F(1,9= 4.01$, $P<0.05$); Figure 4(b)].

1.5. The effect of Naloxone on the antinociceptive effect of LEV

To evaluate the effect of naloxone on the antinociceptive effect of LEV, animals received high dose of LEV (900 mg/kg) 30 min before injection of naloxone (10 mg/kg) in one group and saline (10 mg/kg) in the control group. ANOVA showed that naloxone could inhibit the antinocceptive effect of LEV [$F(1,9)=43.23$, $P<0.05$,; Figure 5].

Discussion

In this study we observed that (a) LEV decreased the development of morphine tolerance, (b) LEV had no effect on expression of tolerance to the antinociceptive effect of morphine, (c) The LEV antinociceptive effect was attenuated by naloxone a full opioid's receptors antagonist, in the tail-flick test as an acute pain model. Morphine-induced tolerance which is described as a shift to the right in the dose-response curve occurs after repeated morphine administration. In order to maintain the same level of analgesia, higher dose is necessary over time (26). This common medical problem which is usually associated with hyperalgesia, limits opioid-induced analgesia efficacy for long-term therapy. One clinical approach used to prevent opioid tolerance (and consequent hyperalgesia) is their co-administration with other drugs such as anticonvulsants and

antidepressants. In this way optimal pain relief will be obtained while providing minimize opioid doses (27). There are some studies show that NMDA receptor antagonist such as MK-80 or cycloxygenase (COX) inhibitors are able to attenuate the development of morphine tolerance (28). It is suggested that anticonvulsant drugs might be able to decrease opioid consumption by either suppressing opioid tolerance development or enhancing opioid analgesia (Gilron, 2006). These agents affect voltage- and ligand-gated channels in central pain pathways and are commonly used to treat neuropathic pain conditions. Consistent with this, our data showed that LEV pretreatment could inhibit the development of morphine tolerance dose-dependently, while had analgesic effect when administered alone. In agreement with our recent study there are other investigations revealed that some anticonvulsants can suppress opioid tolerance such as lamotrigine (8), vigabatrin (1), gabapentin (17), topiramate (15), carbamazepine (16), valproic acid (29) and felbamate (14). Ardid and co-workers have shown that LEV is able to induce antihyperalgesic effect in two models of human neuropathic pain (20). One known mechanism of LEV effects is direct reduction in high-voltage, N-type calcium channel currents in hippocampal neurons. It also facilitates GABA transmission indirectly (Ulloa et al., 2009, Poulain and Margineanu, 2002). According to previous studies, GABAergic system has a role in the progression of morphine tolerance (30). So, it may be concluded that the effect of LEV on GABA transmission could be one of the possible mechanisms explaining its inhibitory role on the development of morphine tolerance. On the other hand, morphine is a μ-opioid receptor agonist which inhibits N-type voltage-sensitive calcium channel through a G-protein coupling mechanism (31). Some investigations reveal that using N-type voltage-sensitive calcium channel blocker increases morphine analgesia whilst preventing morphine-induced tolerance and physical dependence (32). The inhibitory effect of LEV on N-type calcium channels which is the main mechanism of LEV action and also involve in opioid tolerance development, can be another reason describing the ability of LEV to attenuate morphine tolerance. Lee et al. have shown that levetiracetam modulates the presynaptic P/Q-type voltage dependent calcium channel and thus reduces glutamate release (33). There is a direct correlation between spinal cord glutamate and aspartate release and the development of morphine tolerance (34). Reducing glutamate release by LEV helps to attenuate morphine tolerance development.

In this study, naloxone (a non-selective opioid receptors antagonist) decreased the antinociceptive effect of LEV. The finding that LEV might interact with opioid receptors, can explain its inhibitory effect on morphine-induced tolerance. This result is in agreement with another study in which Micov et al. showed antihyperalgesic effect of LEV might incorporate opioid-induced antinociception (35). In a rat model of inflammatory pain, results showed that LEV produced antihyperalgesia which was, at least in part, mediated by GABAA, 5-HT and α_2-adrenergic receptors (35). As spinal cord 5-HT takes part in opioid-dependent neural events, co-administration of serotonergic drugs (such as antidepressants or antiemetics) with opioids could attenuate the opioid tolerance (36). Thus, probable serotonergic effect of LEV could be another explanation for its beneficial use in attenuating morphine induced tolerance.

It is suggested that, α_2-adrenoceptor agonists may have the potential for increasing opioid analgesia while inhibiting the development of opioid tolerance (37). Inhibition of morphine tolerance could be mediated via probable interaction of LEV with α_2-adrenergic receptors. Taken together, investigated mechanisms of LEV action which links to mechanisms producing morphine induced tolerance, could more explain its ability to attenuate opioid tolerance well. Our results suggest that LEV produced a dose-related antinociceptive effect, and its coadministration with morphine can produce therapeutic analgesia with lower dose of morphine.

In conclusion, based on the observed analgesic effects of LEV alone as well as its ability to attenuate the development of morphine tolerance, co-administration of LEV and morphine may be of great clinical implication.

Acknowledgments

This work was supported by Neuroscience Research Center, Shahid Beheshti University, M.C.,

Tehran, Iran. We thank Dr. Abolhassan Ahmadiani for his excellent technical assistance.

References

1. Chavooshi B, Saberi M, Pournaghash Tehrani S, et al. Vigabatrin attenuates the development and expression of tolerance to morphine-induced antinociception in mice. Pharmacol Biochem Behav. 2009;93(2):155-9.

2. Blom S. Trigeminal neuralgia: its treatment with a new anticonvulsant drug (G-32883). Lancet. 1962;1(7234):839-40.

3. Shannon HE, Love PL. Effects of antiepileptic drugs on attention as assessed by a five-choice serial reaction time task in rats. Epilepsy Behav. 2005;7(4):620-8.

4. Gilron I. Review article: the role of anticonvulsant drugs in postoperative pain management: a bench-to-bedside perspective. Can J Anaesth. 2006;53(6):562-71.

5. Kayser V, Christensen D. Antinociceptive effect of systemic gabapentin in mononeuropathic rats, depends on stimulus characteristics and level of test integration. Pain. 2000;88(1):53-60.

6. Backonja MM. Use of anticonvulsants for treatment of neuropathic pain. Neurology. 2002;59(5 Suppl 2):S14-7.

7. Silver M, Blum D, Grainger J, Hammer AE, Quessy S. Double-blind, placebo-controlled trial of lamotrigine in combination with other medications for neuropathic pain. J Pain Symptom Manage. 2007;34(4):446-54.

8. Saberi M, Chavooshi B. Suppressive effects of lamotrigine on the development and expression of tolerance to morphine-induced antinociception in the male mouse. Brain Res. 2009;1291:32-9.

9. Sahebgharani M, Hossein-Abad AA, Zarrindast MR. On the mechanism of carbamazepine-induced antinociception in the formalin test. Int J Neurosci. 2006;116(9):1097-113.

10. Webb J, Kamali F. Analgesic effects of lamotrigine and phenytoin on cold-induced pain: a crossover placebo-controlled study in healthy volunteers. Pain. 1998;76(3):357-63.

11. Wiffen PJ, McQuay HJ, Edwards JE, Moore RA. Gabapentin for acute and chronic pain. Cochrane Database Syst Rev. 2005(3):CD005452.

12. Lopes LS, Pereira SS, Silva LL, et al. Antinociceptive effect of topiramate in models of acute pain and diabetic neuropathy in rodents. Life Sci. 2009;84(3-4):105-10.

13. Alves ND, de Castro-Costa CM, de Carvalho AM, Santos FJ, Silveira DG. Possible analgesic effect of vigabatrin in animal experimental chronic neuropathic pain. Arq Neuropsiquiatr. 1999;57(4):916-20.

14. Kosten TA, DeCaprio JL, Rosen MI. The severity of naloxone-precipitated opiate withdrawal is attenuated by felbamate, a possible glycine antagonist. Neuropsychopharmacology. 1995;13(4):323-33.

15. Zullino DF, Cottier AC, Besson J. Topiramate in opiate withdrawal. Prog Neuropsychopharmacol Biol Psychiatry. 2002;26(6):1221-3.

16. Zullino DF, Krenz S, Favrat B, et al. The efficiency of a carbamazepine-mianserin combination scheme in opiate detoxification. Hum Psychopharmacol. 2004;19(6):425-30.

17. Gilron I, Bailey JM, Tu D, et al. Morphine, gabapentin, or their combination for neuropathic pain. N Engl J Med. 2005;352(13):1324-34.

18. Lynch BA, Lambeng N, Nocka K, et al. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Natl Acad Sci U S A. 2004;101(26):9861-6.

19. Price MJ. Levetiracetam in the treatment of neuropathic pain: three case studies. Clin J Pain. 2004;20(1):33-6.

20. Ardid D, Lamberty Y, Alloui A, et al. Antihyperalgesic effect of levetiracetam in neuropathic pain models in rats. Eur J Pharmacol. 2003;473(1):27-33.

21. Archer DP, Lamberty Y, Wang B, et al. Levetiracetam reduces anesthetic-induced hyperalgesia in rats. Anesth Analg. 2007;104(1):180-5.

22. Sliva J, Dolezal T, Prochazkova M, Votava M, Krsiak M. Preemptive levetiracetam decreases

postoperative pain in rats. Neuro Endocrinol Lett. 2008;29(6):953-7.

23. Ozcan M, Ayar A, Canpolat S, Kutlu S. Antinociceptive efficacy of levetiracetam in a mice model for painful diabetic neuropathy. Acta Anaesthesiol Scand. 2008;52(7):926-30.

24. Jungehulsing GJ, Israel H, Safar N, et al. Levetiracetam in patients with central neuropathic post-stroke pain--a randomized, double-blind, placebo-controlled trial. Eur J Neurol. 2013;20(2):331-7.

25. Tomic MA, Micov AM, Stepanovic-Petrovic RM. Levetiracetam interacts synergistically with nonsteroidal analgesics and caffeine to produce antihyperalgesia in rats. J Pain. 2013;14(11):1371-82.

26. Collin E, Poulain P, Gauvain-Piquard A, Petit G, Pichard-Leandri E. Is disease progression the major factor in morphine 'tolerance' in cancer pain treatment? Pain. 1993;55(3):319-26.

27. DuPen A, Shen D, Ersek M. Mechanisms of opioid-induced tolerance and hyperalgesia. Pain Manag Nurs. 2007;8(3):113-21.

28. Wong CS, Hsu MM, Chou R, Chou YY, Tung CS. Intrathecal cyclooxygenase inhibitor administration attenuates morphine antinociceptive tolerance in rats. Br J Anaesth. 2000;85(5):747-51.

29. Tamayo L, Contreras E. A dual action of valproic acid upon morphine analgesia and morphine withdrawal. Pharmacology. 1983;26(6):297-302.

30. Rahman AF, Takahashi M, Kaneto H. Role of GABAergic systems in the development of morphine tolerance in formalin-treated mice. Jpn J Pharmacol. 1995;68(2):207-11.

31. Wang YX, Gao D, Pettus M, Phillips C, Bowersox SS. Interactions of intrathecally administered ziconotide, a selective blocker of neuronal N-type voltage-sensitive calcium channels, with morphine on nociception in rats. Pain. 2000;84(2-3):271-81.

32. Meng G, Wu N, Zhang C, et al. Analgesic activity of ZC88, a novel N-type voltage-dependent calcium channel blocker, and its modulation of morphine analgesia, tolerance and dependence. Eur J Pharmacol. 2008;586(1-3):130-8.

33. Lee CY, Chen CC, Liou HH. Levetiracetam inhibits glutamate transmission through presynaptic P/Q-type calcium channels on the granule cells of the dentate gyrus. Br J Pharmacol. 2009;158(7):1753-62.

34. Zhao YL, Chen SR, Chen H, Pan HL. Chronic opioid potentiates presynaptic but impairs postsynaptic N-methyl-D-aspartic acid receptor activity in spinal cords: implications for opioid hyperalgesia and tolerance. J Biol Chem. 2012;287(30):25073-85.

35. Micov A, Tomic M, Popovic B, Stepanovic-Petrovic R. The antihyperalgesic effect of levetiracetam in an inflammatory model of pain in rats: mechanism of action. Br J Pharmacol. 2010;161(2):384-92.

36. Ozdemir E, Gursoy S, Bagcivan I, Durmus N, Altun A. Zimelidine attenuates the development of tolerance to morphine-induced antinociception. Indian J Pharmacol. 2012;44(2):215-8.

37. Gursoy S, Ozdemir E, Bagcivan I, Altun A, Durmus N. Effects of alpha 2-adrenoceptor agonists dexmedetomidine and guanfacine on morphine analgesia and tolerance in rats. Ups J Med Sci. 2011;116(4):238-46.

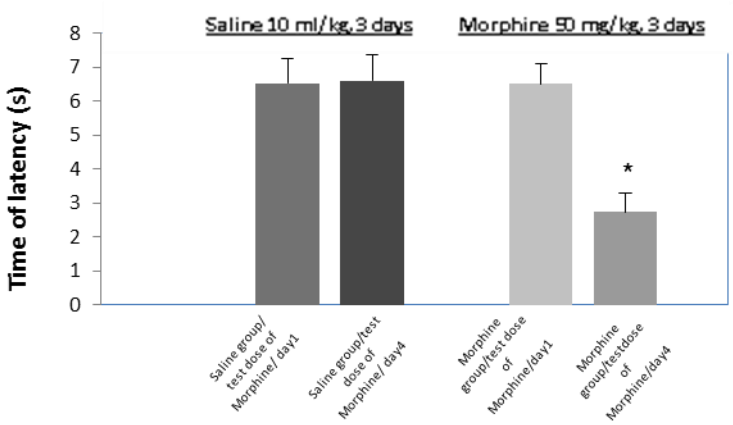

Figure 1. The effect of challenge dose of morphine (4 mg/kg; s.c.), on day 1 and following 3 consecutive days administration of saline 10 mg/kg (saline-treated group) or morphine 50 mg/kg (as an induction period) on day 4. Each column represents the Mean±SEM of latency in 6 mice. *P<0.05 in comparison to the morphine test dose group on day 1 or day 4 of saline group.

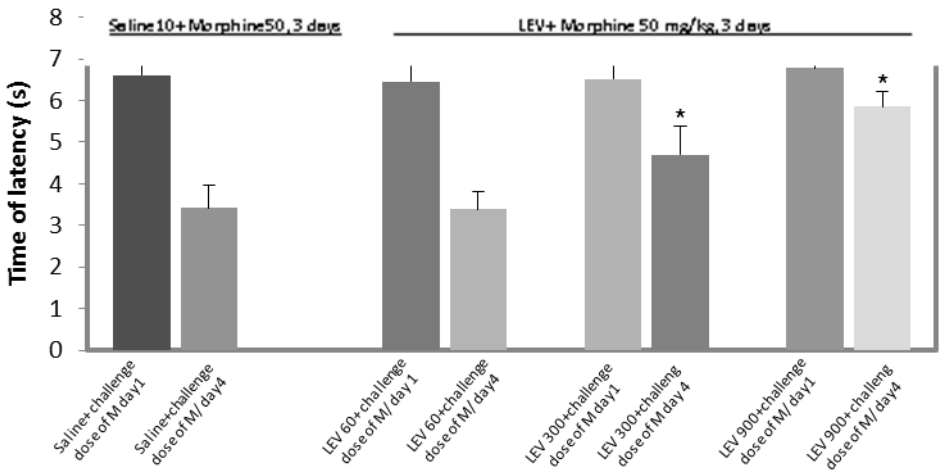

Figure 2. Effects of different doses of levetiracetam (LEV) on development of morphine tolerance. Animals received LEV (60, 300, 900 mg/kg) or vehicle (Saline, 10 ml/kg), 30 min before morphine (50 mg/kg) once daily for 3 days during the induction period. On day 1 and day 4, the tail-flick latencies were determined after injection of challenge dose of morphine (4 mg/kg). Each column represents the Mean±S.E.M. for 6 mice. *P<0.05 in comparison to the morphine tolerant group on day 4.

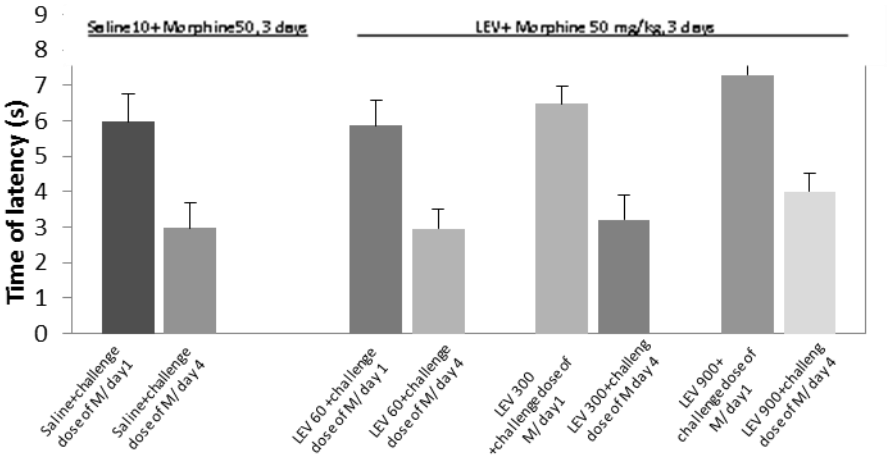

Figure 3. The effect of different doses of levetiracetam (LEV) on the expression of morphine induced tolerance following 3 days of morphine tolerance induction (50 mg/kg; once daily). Challenge dose of morphine (4 mg/kg; was injected on day 1 and day 4). Various doses of LEV (60, 300, 900 mg/kg) or saline (10 ml/kg) were administered 30 min prior to injection of challenge dose of morphine (4 mg/kg; on day 4). Data expressed as Mean±S.E.M. for 6 mice.

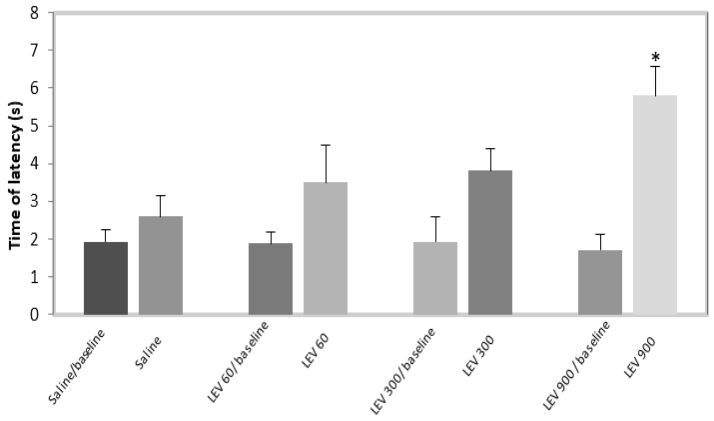

Figure 4. (a): The antinociceptive effects of the different doses of levetiracetam (LEV) alone in comparison to the saline group. (b) The antinociceptie effect of the high dose of LEV (900 mg/kg) alone in comparison to the morphine test dose (4 mg/kg). Data expressed as Mean±S.E.M. for 6 mice. *P<0.05 in comparison to the baseline of the same group.

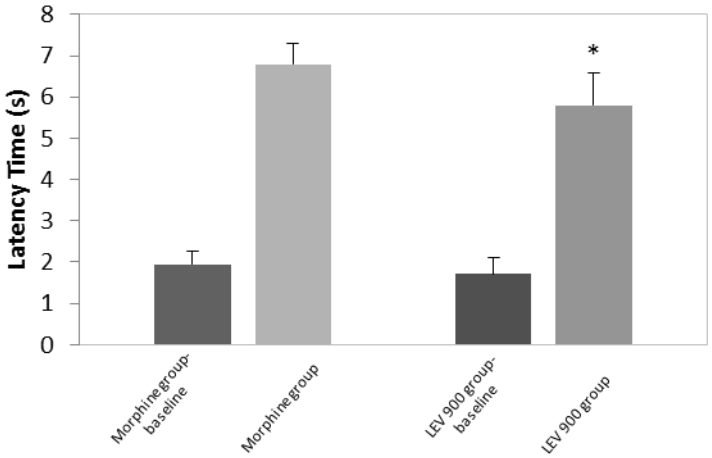

Figure 5. The effect of naloxone (10 ml/kg) on the high dose of levetiracetam (LEV, 900 mg/kg). Naloxone (10 ml/kg) was administered 30 min after injection of the high dose of LEV (900 mg/kg) in one group, and saline (10 ml/kg) in the control group. Tail-flick test was done once before injection of saline or LEV, and then 30 min after injection of naloxone. Each column is the Mean±SEM for 6 mice. *P<0.05, indicates significant when compared to the LEV alone group.