Original Article

The Role of Duloxetine in Changing the Process of Tolerance to Morphine Analgesic Effects in Male Rats

Alireza Parvizpur[1], Kiarash Fekri[1,2], Laleh Fekri[1], Parvin Ghadimi[1], Mohammad Charkhpour[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Introduction: Among various neurological systems involved in the development of morphine tolerance, serotonergic and adrenergic systems are very significant. In this study, we used duloxetine to further investigate the association between serotonergic and noradrenergic systems and the occurrence of opioid tolerance. **Materials and Methods:** Six groups of male Wistar rats were studied including saline, morphine, morphine + duloxetine (15, 30, and 60 mg.kg^{-1}.day^{-1}), and duloxetine-treated groups. Base latency time (BL) was determined using hot plate test (50 ± 0.5ºC). The latency times were reported as MPE% (maximum possible effect) and AUC (area under the curve) was calculated for each MPE%-Time curve (to evaluate global analgesic effect). **Results:** Morphine-treated group showed tolerance on the 9th day. As the same way, the groups treated with morphine and duloxetine (15, 30, 60 mg/kg) showed tolerance on the 13th, 17th, and 23rd days, respectively. Duloxetine-treated group was tolerated on the 11th day. There was a significant difference between the mean AUC in morphine + duloxetine (60 mg/kg^{-1}/day^{-1}) and morphine-treated groups. **Conclusion:** Previous studies revealed that chronic administration of morphine would reduce serotonin release in the central nervous system (CNS). This study showed the effective role of duloxetine and the serotonergic system in postponing the tolerance to analgesic effects of morphine.

Keywords: *Analgesic, duloxetine, morphine, rat, tolerance*

Introduction

There are various perspectives on the concept of pain. Generally, pain can be discussed as an unpleasant feeling resulting from physical or mental damages and it has various negative impacts on the quality of life. Therefore, finding a suitable and rational solution to control pain in patients is necessary.[1-3] Morphine, codeine, their semisynthetic derivatives like oxycodone, synthetic phenylpiperidines like meperidine and synthetic pseudopiperidines such as methadone belong to a group of medications called opioid analgesics.[4,5] The most involved classes of the opioid receptors in the mechanism of action of these analgesics are Mu, Delta, and Kappa. These receptors are found both in the central nervous system and in the peripheral regions.[6] Tolerance is one of the most challenging problems during the administration of these analgesics.[7] Tolerance is seen in almost all patients using opioid analgesics. The development of tolerance causes the patient to increase doses of the same drug or to use a more powerful opioid. This leads to more severe side effects including respiratory depression and an increased risk of drug dependence. In some cases, even the highest dose of an opioid would not produce the desired analgesic effect, if the tolerance occurs.[8,9] Morphine is one of the most effective available analgesics. However, the benefits of this drug for the treatment of chronic pain are limited by tolerance and dependence.[10] In recent years, many attempts have been made to find out the mechanisms of the tolerance to opioid analgesic effects.[11] Many attempts have also been made to find pharmacological solutions to deal with morphine tolerance.[12] So far, the roles for various neural systems in tolerating the effects of opioid analgesia have been studied, including studies of glutamatergic, dopaminergic, and serotonergic systems.[13-19] As stated, opioids apply part of their analgesic effect by stimulating the serotonergic system.[11] Duloxetine acts by inhibiting the reuptake of serotonin and norepinephrine.[20] In addition to treating depression, duloxetine is being used to treat different types of pain which can be caused by conditions like diabetic peripheral neuropathy, fibromyalgia, and stress urinary incontinence.[21-27] In this study, duloxetine was used as a reuptake inhibitor of serotonin

How to cite this article: Parvizpur A, Fekri K, Fekri L, Ghadimi P, Charkhpour M. The role of duloxetine in changing the process of tolerance to morphine analgesic effects in male rats. J Rep Pharma Sci 2020;9:215-20.

Received: 26 Aug 2019
Accepted: 08 Jul 2020
Published: 07 Oct 2020

Address for correspondence:
Dr. Mohammad Charkhpour, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. E-mail: charkhpour@tbzmed.ac.ir

Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_87_19

Quick Response Code:

and norepinephrine in order to investigate the role of these two systems in the development process of morphine tolerance.

Materials and Methods

Drugs

Morphine sulfate ampoules were purchased from Darou Pakhsh Pharmaceutical Mfg Co (Tehran, Iran). Duloxetine enteric-coated capsules were purchased from Eli Lilly and Company (Indiana) and were dissolved in normal saline (0.9% w/v, Shahid Ghazi Pharmaceutical, Tehran, Iran).

Animals

Forty-eight male Wistar rats weighing 225 g to 275 g were divided into 6 groups as following: saline-treated group (1 ml. $kg^{-1}.day^{-1}$, i.p.), morphine-treated group (10 $mg.kg^{-1}.day^{-1}$, i.p.), morphine and duloxetine-treated (15, 30, and 60 $mg.kg^{-1}.day^{-1}$, i.p.) groups and duloxetine-treated group (60 $mg.kg^{-1}.day^{-1}$, i.p.). Duloxetine doses were administered immediately following morphine injections. The animals were housed in standard cages in a room maintained at the ambient temperature (21°C–23°C) with an alternating 12-h light–dark cycle. Food and water were available ad libitum. Each animal was used only once in all experiments. All the procedures were in accordance with the international guidelines. The study protocol was designed and approved by the Ethics Committee for the Use of Animals in Research at Tabriz University of Medical Sciences.

Hot plate test

Hot plate test has been known as a suitable test for evaluating the acute pain and analgesic effects of morphine in in-vivo studies. At first, the temperature of the hot plate was adjusted to 50 ± 0.5°C. After stabilizing the temperature of the device, the rats were placed gently on it one by one and the time it took for each animal to react was recorded (The latency time). On the first day before starting the injections, the hot plate test was performed two times for each rat at an interval of one hour, the latency times were recorded and the mean was considered as base latency time (BL). After starting the injections, the latency times were recorded every other day and were considered as test latency times (TL) and then MPE% was calculated as follows:

$$MPE\% = \left[(TL - BL) \ / \ (Time_{cut-off} - BL) \right] \times 100$$

Equation 1

where $Time_{cut-off}$ is the maximum time the animal can be placed on the device. In our research, it was considered 45 s. The day in which BL and TL did not show any significant differences was considered as the tolerance day (marked by black arrows in the figures). To evaluate global analgesic effect, the AUC of the MPE%-Time curve was calculated.[28]

Statistical analysis

MPE% was expressed as mean ± standard error of mean (SEM) for each group and area under the curve was calculated for each MPE%—time chart. The data were analyzed with analysis of variance (ANOVA) followed by the multiple comparison test of Tukey and differences between means were considered statistically significant if $P < 0.05$.

Results

Effect of chronic morphine administration (10 $mg.kg^{-1}.day^{-1}$, i.p.) in the development of the tolerance to its analgesic effects

As shown in Figure 1, the analgesic effect of morphine was significant until the 9th day. Therefore the 9th day was considered as the tolerance day.

Effect of duloxetine administration in inhibiting the tolerance to analgesic effects of morphine (10 $mg.kg^{-1}.day^{-1}$, i.p.)

As shown in Figure 2, various doses of duloxetine (15, 30, and 60 $mg.kg^{-1}$) would postpone the development of the tolerance

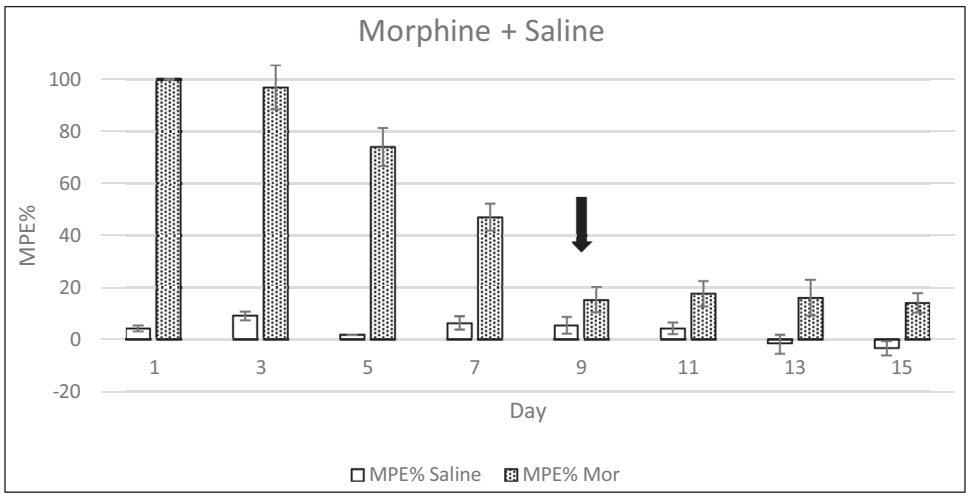

Figure 1: Effect of chronic morphine administration (10 mg.kg.day-1, i.p.) in the development of the tolerance to its analgesic effects. The day in which BL and TL did not show any significant differences was considered as the tolerance day (marked by black arrow in the figure)

to analgesic effects of morphine. In duloxetine-treated groups (15, 30, and 60 mg/kg), the tolerance to analgesic effects of morphine occurred on the 13th, 17th, and 23rd days, respectively.

Investigating the analgesic effects of duloxetine (60 mg. kg^{-1}, i.p.)

The results obtained from the duloxetine-treated group (without morphine administration), are shown in the following figure. According to Figure 3, the results obtained from this group are significantly different from the control group. The 11th day was considered as the tolerance day.

In Figure 4, AUC (area under the curve) was compared between the groups. The results indicated that there was a significant difference between the mean AUC in morphine + duloxetine (60 mg/kg) and morphine-treated groups.

Discussion

The aim of this study was to investigate the effects of duloxetine on the development of morphine tolerance. According to the results, the development of morphine tolerance in duloxetine-treated rats has been postponed in a dose-dependent manner.

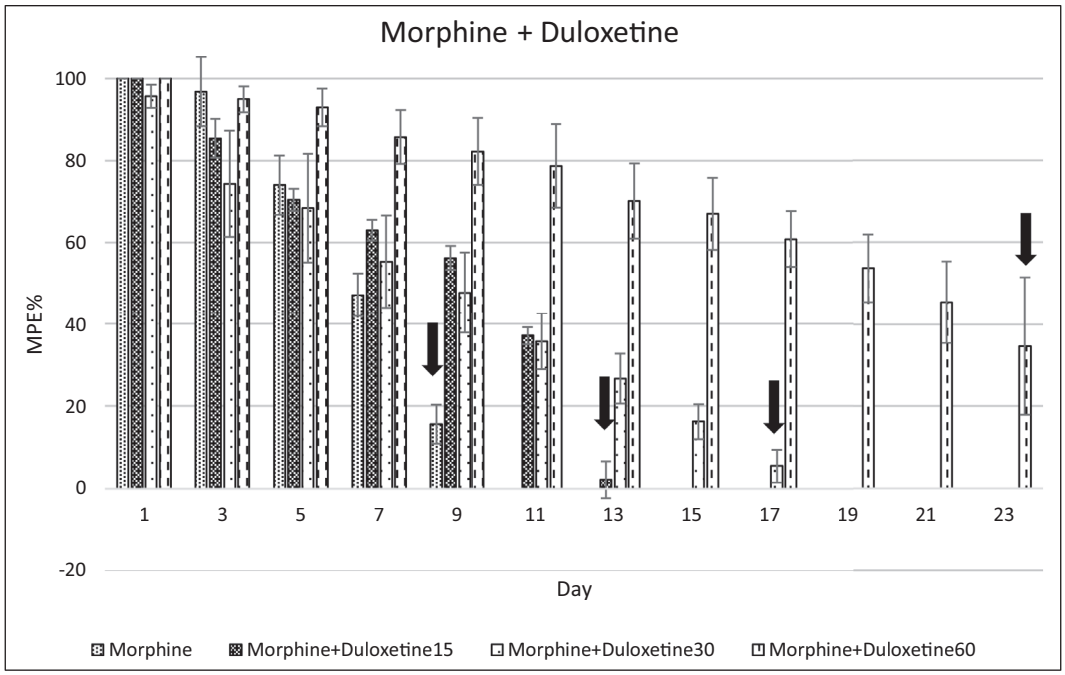

Figure 2: Effect of duloxetine administration in inhibiting the tolerance to analgesic effects of morphine (10 mg/kg, i.p.). The day in which BL and TL did not show any significant differences was considered as the tolerance day (marked by black arrows in the figure)

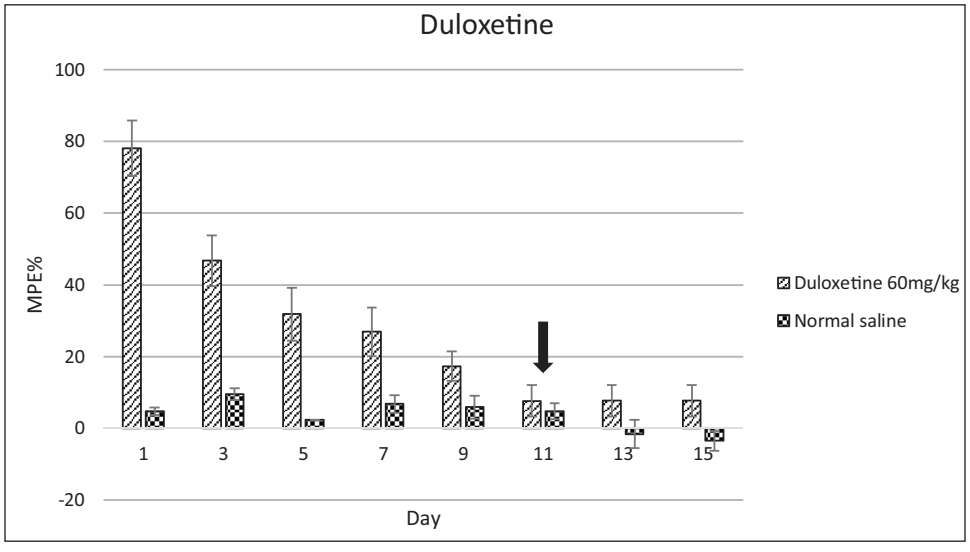

Figure 3: Analgesic effects of duloxetine (60 mg/kg, i.p.). The day in which BL and TL did not show any significant differences was considered as the tolerance day (marked by black arrow in the figure)

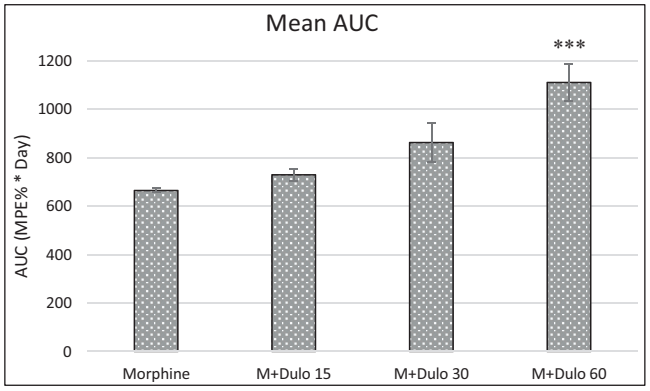

Figure 4: Comparison of the mean AUC between groups: * *P* < 0.001 compared to morphine group**

Opiates are widely used as one of the best groups of analgesics which reduce acute and chronic pains. Dependence and tolerance to the analgesic effects of morphine are two important limiting challenges during the administration of these drugs.[12] Therefore, identifying agents reducing the tolerance to opioid analgesic effects would lead to improved pain management.[29]

Numerous research activities have been conducted on the analgesic activity of selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs).[30,31]

Duloxetine is an antidepressant acting through inhibiting the reuptake of serotonin and norepinephrine. It also has a low tendency to bind to adrenergic receptors as well as serotonin, histamine, muscarinic, cholinergic, and dopamine receptors.[32] Studies investigating the effect of serotonin and norepinephrine reuptake inhibitors (especially duloxetine) in relieving chronic pains, showed that the effect of duloxetine on relieving neuropathic pain and fibromyalgia was probably due to its ability to increase the level of serotonin and norepinephrine.[33-35] These findings were in accordance with our results in the 6th group. Pathways involved in the sensation of pain include nerves entering posterior horn of the spinal cord. These descending fibers which inhibit the transfer of pain signals in the spinal neurons are likely to act through hyperpolarization of sensory neurons using endogenous opioid, serotonin, and norepinephrine as the primary mediators.[36] Previous studies showed that continuous injection of duloxetine into rats affects serotonergic and noradrenergic parameters. This drug appears to be responsible for the desensitization of 5-HT_{1A} autoreceptors and also the $alpha_2$ adrenergic heteroreceptors in the serotonergic terminals, and thus reduces the serotonin and norepinephrine function on its receptors.[32] In our study, tolerance to duloxetine (in duloxetine group) may be developed in such a way.

The results of previous studies on the synergistic effects of the medicines inhibiting the reuptake of monoamines (such as duloxetine) and morphine to produce analgesic effects indicated that inhibition of both norepinephrine and serotonin carriers was required for producing synergistic analgesic effects with opioids. Also, high levels of serotonin and its effect on serotonin receptors (5-HT_3) may reduce the potential synergistic effects with opioids (such as morphine).[37-41] Our study also focuses on synergistic effects between morphine and duloxetine and postponing the tolerance to the analgesic effects of morphine.

In an attempt to investigate the effect of serotonin and norepinephrine reuptake inhibitors (such as amitriptyline and venlafaxine) and serotonin receptor agonist (dihydroergotamine) on the analgesic effects of morphine in male rats and the tolerance to these effects, Ozmedir *et al*.[42] reported that chronic administration of morphine resulted in a decrease in serotonergic activity in the posterior raphe nuclei. As a result, it can be assumed that the serotonergic system in the posterior raphe nuclei would play an important role in developing tolerance to the analgesic effects of morphine. Also, during this study, coadministration of morphine with serotonin and norepinephrine reuptake inhibitors (amitriptyline and venlafaxine) increased the analgesic effects of morphine and decreased the development of tolerance to these effects. In our study, duloxetine probably delayed the onset of the tolerance to morphine by increasing serotonin levels and its effects on the serotonergic system in the posterior raphe nuclei.

Venlafaxine (a reuptake inhibitor of serotonin and norepinephrine) has analgesic effects. This effect is not antagonized by naloxone, which indicates that the opioid system does not play a role in SNRIs' analgesic activity and inhibiting serotonin and norepinephrine reuptake is the main mechanism for the anxiolytic activity of venlafaxine.[43] This mechanism may also play an important role in the duloxetine antinociceptive activity.

On the contrary, Arends *et al*.[44] have shown that chronic administration of morphine, decreases serotonin release from the terminals of serotonergic neurons, and at the same time, tolerance to morphine is induced in a short time. Acute administration of morphine increases serotonin synthesis and release, whereas chronic morphine administration reduces serotonin release from nerve terminals.[28,45] In our study, duloxetine is also likely to slow down the onset of morphine tolerance by increasing serotonin levels. In sum, duloxetine can postpone morphine tolerance through the mechanisms discussed.

Conclusion

According to the results, duloxetine postponed the tolerance to the analgesic effects of morphine significantly, which could be hoped to achieve a pharmacological treatment that would eliminate or delay the clinical problems associated with long-term use of opioids. These effects are often applied through nor-adrenergic and serotonergic systems but other systems such as dopaminergic, histaminergic, and cholinergic systems may also be involved. Further clinical studies are needed to find out the exact mechanisms and clinical effects.

Financial support and sponsorship

This research was financially supported by Tabriz University of Medical Sciences, Tabriz, Iran.

Conflicts of interest

There are no conflicts of interest.

References

1. Ambola RB, Ajong NV, Tufon EN. Non-pharmacological interventions for pain management used by Nurses at the Mezam Polyclinic Bamenda, Cameroon. Res J Pharmacol Pharmacodyn 2016;8:157.
2. Karabulut N, Gürçayır D, Aktaş YY. Non-pharmacological interventions for pain management used by nursing students in Turkey. Kontakt 2016;18:e22-e9.
3. Brennan F, Carr DB, Cousins M. Pain management: A fundamental human right. Anesth Analg 2007;105:205-21.
4. Chang G, Chen L, Mao J. Opioid tolerance and hyperalgesia. Med Clin North Am 2007;91:199-211.
5. Jamison RN, Mao J. Opioid analgesics. Mayo Clin Proc 2015;90:957-68.
6. Chen KY, Chen L, Mao J. Buprenorphine-naloxone therapy in pain management. Anesthesiology 2014;120:1262-74.
7. Martyn JAJ, Mao J, Bittner EA. Opioid tolerance in critical illness. N Engl J Med 2019;380:365-78.
8. Wang ZJ, Wang LX. Phosphorylation: A molecular switch in opioid tolerance. Life Sci 2006;79:1681-91.
9. Mercer SL, Coop A. Opioid analgesics and P-glycoprotein efflux transporters: A potential systems-level contribution to analgesic tolerance. Curr Top Med Chem 2011;11:1157-64.
10. Kim JA, Bartlett S, He L, Nielsen CK, Chang AM, Kharazia V, *et al*. Morphine-induced receptor endocytosis in a novel knockin mouse reduces tolerance and dependence. Curr Biol 2008;18:129-35.
11. Ozdemir E, Gursoy S, Bagcivan I, Durmus N, Altun A. Zimelidine attenuates the development of tolerance to morphine-induced antinociception. Indian J Pharmacol 2012;44:215-8.
12. Habibi Asl B, Majidi Z, Fekri K, Delazar A, Vaez H. Evaluation of the effect of aerial parts of scrophularia atropatana grossh total extracts on analgesic activity and morphine induced tolerance in mice. Pharm Sci 2018;24:112-7.
13. Aley KO, Levine JD. Different mechanisms mediate development and expression of tolerance and dependence for peripheral μ-opioid antinociception in rat. J Neurosci 1997;17:8018-23.
14. el-Kadi AO, Sharif SI. The role of 5-HT in the expression of morphine withdrawal in mice. Life Sci 1995;57:511-6.
15. Hassanzadeh K, Habibi-asl B, Roshangar L, Nemati M, Ansarin M, Farajnia S. Intracerebroventricular administration of riluzole prevents morphine-induced apoptosis in the lumbar region of the rat spinal cord. Pharmacol Rep 2010;62:664-73.
16. Jolas T, Nestler E, Aghajanian G. Chronic morphine increases GABA tone on serotonergic neurons of the dorsal raphe nucleus: Association with an up-regulation of the cyclic AMP pathway. Neuroscience 1999;95:433-43.
17. Li JY, Wong CH, Huang EY, Lin YC, Chen YL, Tan PP, *et al*. Modulations of spinal serotonin activity affect the development of morphine tolerance. Anesth Analg 2001;92:1563-8.
18. Singh VP, Jain NK, Kulkarni SK. Fluoxetine suppresses morphine tolerance and dependence: Modulation of NO-cgmp/DA/serotoninergic pathways. Methods Find Exp Clin Pharmacol 2003;25:273-80.
19. Zarrindast M-R, Dinkoub Z, Homayoun H, Bakhtiarian A, Khavandgar S. Dopamine receptor mechanism (s) and morphine tolerance in mice. J Psychopharmacol 2002;16:261-6.
20. Chalon SA, Granier LA, Vandenhende FR, Bieck PR, Bymaster FP, Joliat MJ, *et al*. Duloxetine increases serotonin and norepinephrine availability in healthy subjects: A double-blind, controlled study. Neuropsychopharmacology 2003;28:1685-93.
21. Goldstein DJ, Lu Y, Detke MJ, Hudson J, Iyengar S, Demitrack MA. Effects of duloxetine on painful physical symptoms associated with depression. Psychosomatics 2004;45:17-28.
22. Goldstein DJ, Mallinckrodt C, Lu Y, Demitrack MA. Duloxetine in the treatment of major depressive disorder: A double-blind clinical trial. J Clin Psychiatry 2002;63:225-31.
23. Perahia DG, Pritchett YL, Desaiah D, Raskin J. Efficacy of duloxetine in painful symptoms: An analgesic or antidepressant effect? Int Clin Psychopharmacol 2006;21:311-7.
24. Raskin J, Pritchett YL, Wang F, D'Souza DN, Waninger AL, Iyengar S, *et al*. A double-blind, randomized multicenter trial comparing duloxetine with placebo in the management of diabetic peripheral neuropathic pain. Pain Med 2005;6:346-56.
25. Russell IJ, Mease PJ, Smith TR, Kajdasz DK, Wohlreich MM, Detke MJ, *et al*. Efficacy and safety of duloxetine for treatment of fibromyalgia in patients with or without major depressive disorder: Results from a 6-month, randomized, double-blind, placebo-controlled, fixed-dose trial. Pain 2008;136:432-44.
26. Katofiasc MA, Nissen J, Audia JE, Thor KB. Comparison of the effects of serotonin selective, norepinephrine selective, and dual serotonin and norepinephrine reuptake inhibitors on lower urinary tract function in cats. Life Sci 2002;71:1227-36.
27. Vey EL, Kovelman I. Adverse events, toxicity and post-mortem data on duloxetine: Case reports and literature survey. J Forensic Leg Med 2010;17:175-85.
28. Mohajjel Nayebi A, Charkhpour M. Role of 5-HT1A and 5-HT2 receptors of dorsal and median raphe nucleus in tolerance to morphine analgesia in rats. Pharmacol Biochem Behav 2006;83:203-7.
29. Nayebi AM, Rezazadeh H, Parsa Y. Effect of fluoxetine on tolerance to the analgesic effect of morphine in mice with skin cancer. Pharmacol Rep 2009;61:453-8.
30. Lee YC, Chen PP. A review of ssris and snris in neuropathic pain. Expert Opin Pharmacother 2010;11:2813-25.
31. McCleane G. Antidepressants as analgesics. CNS Drugs 2008;22:139-56.
32. Gould GG, Javors MA, Frazer A. Effect of chronic administration of duloxetine on serotonin and norepinephrine transporter binding sites in rat brain. Biol Psychiatry 2007;61:210-5.
33. Goldstein DJ, Lu Y, Detke MJ, Lee TC, Iyengar S. Duloxetine vs. Placebo in patients with painful diabetic neuropathy. Pain 2005;116:109-18.
34. Iyengar S, Webster AA, Hemrick-Luecke SK, Xu JY, Simmons RM. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. J Pharmacol Exp Ther 2004;311:576-84.
35. Jones CK, Peters SC, Shannon HE. Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. J Pharmacol Exp Ther 2005;312:726-32.
36. Marks DM, Shah MJ, Patkar AA, Masand PS, Park GY, Pae CU. Serotonin-norepinephrine reuptake inhibitors for pain control: Premise and promise. Curr Neuropharmacol 2009;7:331-6.
37. Chen S, Wu N, Fraser K, Boulanger L, Zhao Y. Opioid use and healthcare costs among patients with DPNP initiating

duloxetine versus other treatments. Curr Med Res Opin 2010;26: 2507-16.
38. Ho KY, Tay W, Yeo MC, Liu H, Yeo SJ, Chia SL, *et al*. Duloxetine reduces morphine requirements after knee replacement surgery. Br J Anaesth 2010;105:371-6.
39. Pertovaara A. The noradrenergic pain regulation system: A potential target for pain therapy. Eur J Pharmacol 2013;716:2-7.
40. Obata H, Kimura M, Nakajima K, Tobe M, Nishikawa K, Saito S. Monoamine-dependent, opioid-independent antihypersensitivity effects of intrathecally administered milnacipran, a serotonin noradrenaline reuptake inhibitor, in a postoperative pain model in rats. J Pharmacol Exp Ther 2010;334: 1059-65.
41. Shen F, Tsuruda PR, Smith JA, Obedencio GP, Martin WJ. Relative contributions of norepinephrine and serotonin transporters to antinociceptive synergy between monoamine reuptake inhibitors and morphine in the rat formalin model. PLoS One 2013;8:e74891.
42. Ozdemir E, Gursoy S, Bagcivan I. The effects of serotonin/ norepinephrine reuptake inhibitors and serotonin receptor agonist on morphine analgesia and tolerance in rats. J Physiol Sci 2012;62:317-23.
43. Berrocoso E, Mico JA. In vivo effect of venlafaxine on locus coeruleus neurons: Role of opioid, α2-adrenergic, and 5-hydroxytryptamine1A receptors. J Pharmacol and Exp Ther 2007;322:101-7.
44. Arends RH, Hayashi TG, Luger TJ, Shen DD. Cotreatment with racemic fenfluramine inhibits the development of tolerance to morphine analgesia in rats. J Pharmacol Exp Ther 1998;286:585-92.
45. Harris GC, Aston-Jones G. Augmented accumbal serotonin levels decrease the preference for a morphine associated environment during withdrawal. Neuropsychopharmacology 2001;24:75-85.