Vitamin B_{12} and ketorolac on pain in Long Evans rats

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Abstract

Background: Effects of vitamin B_{12} on pain have been demonstrated in different animal and human studies. But comparison of these effects with similar effects of ketorolac tromethamine (KT) and their combination have not been established. **Objective:** To assess the effects of vitamin B_{12} on pain and also to compare them with those of the combinations of vitamin B_{12} with KT in rat models. **Methods:** This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2015 to February 2016. For this, 20 (twenty) Long Evans rats (215±35 gm) of both sexes were divided into control (A, with 5 ml/kg normal saline) and experimental (B1, with 15 mg/kg B_{12}; B2, with 10 mg/kg KT; B3, with B_{12}+KT) groups with 5 rats in each group. All the drugs and vitamin were administered intraperitoneally in a single dose just one hour before formalin test. To evaluate the treatments' effect on nociceptive pain, early phase (1^{st}-5^{th} minutes); on central analgesic system, interphase (6^{th}-15^{th} minutes); and on inflammatory pain, late phase (16^{th}-60^{th} minutes) of the formalin test, were observed. In all phases, total frequency of jerking and total duration of flexing and licking of the right hind paw were counted after administration of subcutaneous formalin (50 µl, 2.5%) injection. Statistical analysis was done by ANOVA, followed by Bonferroni post hoc test. In the interpretation of results, $p \leq 0.05$ was considered as significant. **Results:** B_{12} lowered only the jerking frequency and KT lowered both jerking frequency and flexing-licking duration significantly ($p \leq 0.001$) in the late phase of formalin test. On the other hand, combination of B_{12} and KT significantly ($p \leq 0.001$) lowered both the study variables in all 3 phases of formalin test. **Conclusion:** From this study it may be concluded that, vitamin B_{12} possess analgesic effects and combination of B_{12} with KT is more effective than those of their individual administration.

Keywords: Pain, Analgesic, Vitamin B_{12}, Ketorolac, Formalin Test.

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Introduction

Pain is the most unpleasant sensation among all the sensory perceptions[1]. Pain involves not only the mere recognition of the sensation of tissue damage, it is also affected by emotional and cognitive condition of an individual[2,3]. It is protective in nature, but to do so it also causes discomfort. For this, pain has been proposed as a complex, multi-dimensional appreciation[4]. Pain is not homogenous and associated with a diversity of behaviors. It may vary in quality, intensity and duration. Etiologically it has been classified into nociceptive, inflammatory, neuropathic and functional type[3,5-7].

To find out the intensity of disease, pain measurement is necessary. It is also very important for selection of a proper analgesic. For the measurement of nociceptive and inflammatory pain behaviors in animal model, the formalin test is another valid method and is sensitive for various classes of analgesic drugs. This test shows an early

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phase which reflects direct activation of nociceptors, an interphase showing the activation of central analgesic system and a late phase reflecting pain due to inflammation[8,9].

Ketorolac tromethamine (KT) is the most potent non-steroidal anti-inflammatory drug (NSAID) used to treat any pain associated with inflammation, especially postoperative pain, renal colic, arthritis, lumbago, headache and cancer pain. It is indicated for the short-term management of severe acute pain that requires immediate analgesia[10,11]. However long term use of any NSAID as well as KT can be associated with peptic ulcer and some other systemic manifestations, like coagulation disorder, nephrotoxicity as well as severe impairment of cardiac, cerebral or hepatic functions[11-14].

In recent years, many studies have been conducted throughout the world with an aim to find alternatives to the traditional analgesic drugs in order to replace them or at least to reduce the duration of drug therapy, so that their adverse effects can be minimized[14,15].

Vitamins are very good alternatives for their cost-effectiveness, of which vitamin B complex comprise eight different water soluble compounds. Among these eight vitamins, B_{12} supplementation in combination with B_1 and B_6 showed decrement of the nociceptive and inflammatory pain in a number of animal studies [16-23].

However, it has been reported that single dose of 10 mg/kg KT might be the sub effective dose against nociceptive as well as inflammatory pain [24,25]. It has also been proposed that, combined administration of B vitamins (B_1, B_6, B_{12}) with KT may have potentiating effects in nociceptive and inflammatory pain[21].

But still the information's regarding this matter is not sufficient enough to reach any final conclusion. Furthermore, no reported data was available to compare the combined analgesic effects of vitamin B_{12} with KT to that of their individual administration on nociceptive and inflammatory pain.

On the basis of this background, the present study has been designed to evaluate the analgesic effects of single administration of Ketorolac as well as its combination with vitamin B_{12} to compare their combined analgesic effects with their individual administration in Long Evans rats.

Methods

This experimental study was conducted in the Pain Laboratory, Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2015 to February 2016. All experiments and animal care were performed according to the guidelines set in the 'Manual for Care and Use of Laboratory Animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b 2002)[26].

Procurement and maintenance of animals

Twenty (20) healthy adult Long Evans rats weighing 180 to 250 gm[14] of both sexes[24,27,28] were obtained from animal house of Bangladesh University of Health Sciences (BUHS), Dhaka. All the rats were kept in the Pain Laboratory of the Department of Physiology, BSMMU, where they were housed in specially built plastic cages with 6 rats per cage under a 12/12 hour light/dark cycle[29-31]. The ambient room temperature was maintained at around 27 to 28°C, corresponding to the thermo-neutral zone for rodents[17,32]. All the rats had free access to standard laboratory food and cooled boiled water[14]. They were kept there for a period of seven consecutive days for environmental acclimatization, prior to the experiment. To avoid circadian influences all the experiments were performed at day time between 08:00 and 16:00 hours[33].

Dose schedule

The vitamin B_{12} (Biopharma, Bangladesh) and Ketorolac tromethamine (Novartis, Bangladesh) were obtained in granular form and were dissolved in normal saline (5 ml/kg body weight). On the basis of drugs and vitamin administration, all the rats were divided into 4 groups (5 rats/group). Of them control group (A) received only normal saline (5 ml/kg body weight)[33], Vitamin treated group (B1) received vitamin B_{12} (15 mg/kg body weight)[15], ketorolac treated group (B2)

received ketorolac tromethamine (KT) (10 mg/kg body weight)[24], combination treated group (B3) received vitamin B_{12} (15 mg/kg body weight)[15] and KT (10 mg/kg body weight)[15,24] in equal volume to that of normal saline, respectively. Just one hour after intraperitoneal[14,15,34,35] administration of drug and vitamin, all the rats underwent formalin test.

Formalin test

In order to make the rats accustomed to the test environment, all the rats were placed in the observation chamber (34x34x34 cm^3) of the plexiglass formalin test box in pairs for fifteen (15) minutes daily for four (4) consecutive days, and singly for three (3) days prior to the test[8,36].

On the day of experiment, the rat was intraperitoneally injected with normal saline or vitamin B_{12} or KT or combinations thereof in accordance with the experimental paradigm being followed. Just one (1) hour later, the rat was restrained manually by a thick towel and fifty (50) µl of dilute (2.5%) formalin was injected subcutaneously[36,37] into the plantar aspect of the right hind paw with an insulin syringe. Immediately thereafter, the animal was placed in the observation chamber of the plexiglass formalin test box, and pain behaviors were observed for consecutive sixty (60) minutes. A mirror fixed at an angle of 45^0 beneath the transparent floor of the chamber was used to facilitate unhindered observation. The first five (5) minutes (1^{st} to 5^{th}) was considered as the early phase, the next ten (10) minutes (6^{th} to 15^{th}) as the interphase, and the last 45 minutes (16^{th} to 60^{th}) as the late phase. During observation, the total number of jerking and the total duration of flexing plus licking of the injected paw (in seconds) per 5-minutes time block was also recorded. A stopwatch was used to count the time[8,30,36].

Results were expressed as mean ± SEM and the data were statistically analyzed by ANOVA, followed by Bonferroni's post hoc test. In the interpretation of results, p d"0.05 was accepted as the level of significance.

Results

Antinociceptive effect

The effects of single dose (intraperitoneal) of only KT and combined administration of vitamin B_{12} and KT in early and late phase of formalin test were observed. In all phases the pain behaviors were analyzed as total frequency jerking as well as total duration of flexing and licking.

In this study, in the early phase of formalin test, the mean values of both variables were lowered in all the study groups than those of the control group. However this decrement was statistically significant (p ≤0.001) only in the rats with combined treatment of KT and vitamin B_{12} both in frequency of jerking as well as in duration of flexing and licking (Figure 1).

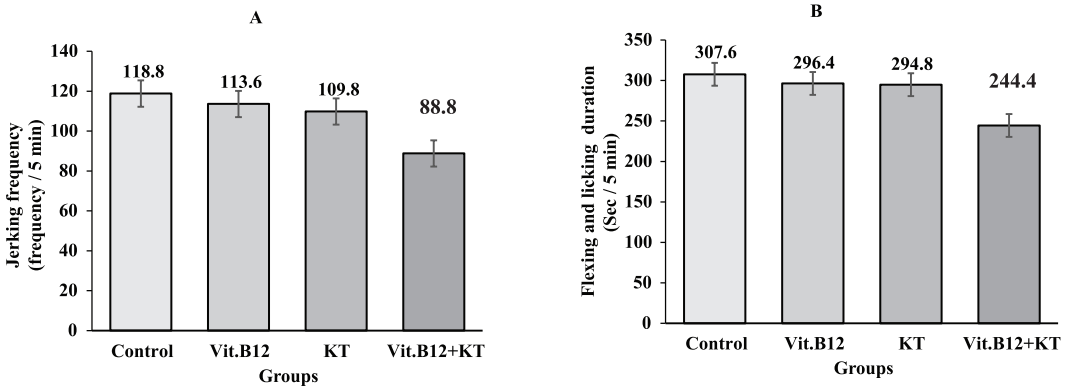

Figure 1: Frequency of jerking (A) and duration of flexing and licking (B) in early phase of formalin test in different groups of rats. Each bar symbolizes for mean ± SE for 5 rats. ***=p ≤0.001, compared to those of control. KT= Ketorolac tromethamine.

Similarly in the interphase of formalin test, combined administration of KT and vitamin B_{12} showed significant lowering of the jerking frequency ($p \leq 0.001$) (figure 2A) as well as of the duration of flexing and licking ($p \leq 0.05$) (figure 2B) when compared to control (Figure 2).

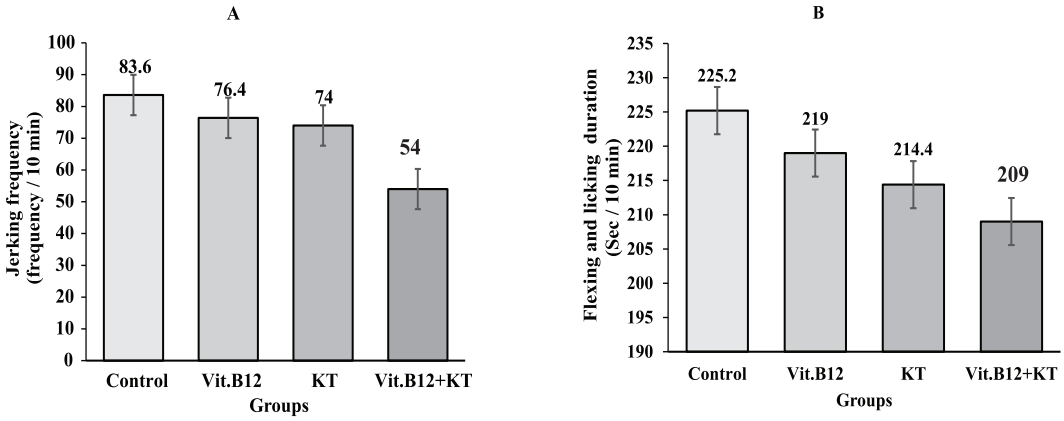

Figure 2: Frequency of jerking (A) and Duration of flexing and licking (B) in the interphase of formalin test in different groups of rats. Each bar symbolizes for mean ± SE for 5 rats. ***=p ≤ 0.001 and *=≤ p d"0.05 compared to those of control. KT= Ketorolac tromethamine.

However, in late phase of formalin test, both the study groups showed significantly reduction (p>0.001) in both the pain variables compared to that of the control group (Figure 3). In addition, rats treated with combined administration showed statistically significant (p>0.001) reduction in the duration of flexing and licking than that of individual administration of KT in this phase (Figure 3).

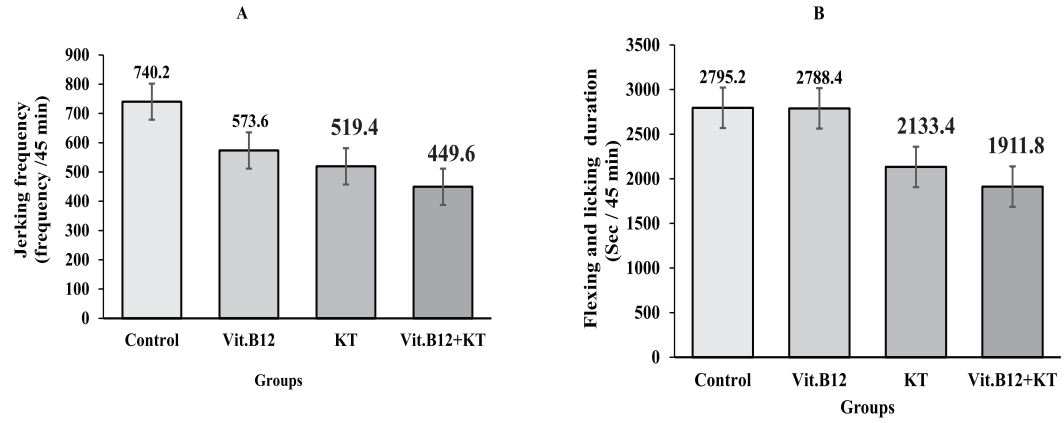

Figure 3: Frequency of jerking (A) and duration of flexing and licking (B) in late phase of formalin test in different groups of rats. Each bar symbolizes for mean ± SE for 5 rats. ***=p >0.001, compared to control and ###=p ≤ 0.001, comparison between only KT vs KT + Vit.$B_{12.}$ KT= Ketorolac tromethamine

Discussion

In this study, individual administration of B_{12} lowered only the jerking frequency in the late phase of formalin test. Similar trend of findings has been observed by Imtiaz (2011)[15], though they investigated the effect of administration of combination of B_{12} and folic acid. Furthermore, decrement of pain response by vitamin B_{12} was also reported by other researchers after single administration of vitamin B_{12} at different doses in different pain models[17,21,37].

In addition, KT lowered both jerking frequency and flexing-licking duration significantly in the late phase of formalin test of our study. Similar trend of findings has been also observed by Medina-Santillan et al. (2004)[21], though they investigated the effect of administration of individual after oral ketorolac (0.32-10 mg/kg). Furthermore, decrement of pain response was also reported by other researchers after single administration of ketorolac at different doses in different pain models[17,21,37].

On the other hand, in our study, combination of B_{12} and KT significantly lowered both the study variables in all 3 phases of formalin test in experimental rats. Similar trend of findings has been observed by Medina-Santillan et al. (2004)[21], though they investigated the effect of oral administration of individual ketorolac (0.32-10 mg/kg) as well as combinedly (100:100:1 mg/kg).

Though the exact mechanisms of these effects could not be revealed directly from the present study, several investigators of different countries proposed various suggestions on these aspects, which might be cause of our present findings. It has been suggested that, antinociceptive effect of vitamin B_{12} may be due to increase inhibitory control of afferent nociceptive neurons at the spinal cord along with decrease response of thalamic neurons to nociceptive stimulation[38,39]. Moreover, vitamin B_{12} might stimulate release of endogenous opioids or activate of opioid receptors as well as GABA metabolism, which in turn may decrease Ca^{2+} mediated release of neurotransmitters[18]. In addition, GABA might also increase conductance through K^+ channels and cause hyperpolarization of the postsynaptic membrane of the dorsal horn neurons, finally leading to decreased pain conduction[40-42].

Again, it has also been suggested that antinociceptive effect of KT include activation of the NO–cyclic GMP (cyclic guanosine monophosphate) pathway, followed by opening of ATP-sensitive K^+ channels at the peripheral level. This channel opening in turn allows K^+ to diffuse out from the post synaptic neurons to hyperpolarize it and thus decrease pain conduction[42,43].

Along with these, in this study, combined administration of vitamin B_{12} and KT have shown more effectiveness in lowering pain than the KT alone, as evidenced by more decrement in all the study variables in the combined group. It is assumed that any the above mentioned mechanisms might be activated together and synergistically acted to cause more effectiveness in lowering different pain variables after combined administration of vitamin B_{12} and KT.

Conclusion

From this study, it can be concluded that combined administration of vitamin B_{12} and KT may be more effective in lowering pain than the individual administration of KT. This data may appraise the clinicians and general populations to use vitamin B_{12} along with KT for better management of pain. Although, further experimental study is needed to elucidate the exact component and mechanism responsible for these effects.

Conflict of interest none

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References

1. Melzack R. The tragedy of needless pain. Sci Am. 1990; 262(2): 27-33.
2. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001; 413:203-210.
3. Woolf CJ. Pain: moving from symptom control toward mechanism-specific pharmacologic management. Ann Intern Med. 2 004; 140:441-451.
4. Oxenham D. Pain management & palliative care. In: Colledge NR, Walker BR, Ralston SH editors. Davidson's principles & practice of Medicine. Edinburgh; Churchill Livingstone. 2002; 279-288.
5. Riedel W, Neeck G. Nociception, pain and antinociception: current concepts. Z Rheumatol. 2001; 60(6): 404-415.
6. Medzhitov R. Inflammation: new adventures of an old flame. Cell. 2010; 140(6):771-776.
7. Nathan C. Points of control in inflammation. Nature. 2002; 420(6917): 846-52.
8. Henry JL, Yashpal K, Pitcher GM, Coderre TJ (1999). Physiological evidence that the 'interphase' in the formalin test is due to active inhibition. Pain. 82:57-63.
9. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain. 1977; 4(2):161-174.
10. Gillis JC, Brogden RN. Ketorolac. Drugs. 1997; 53(1):139-188.
11. Sam T, Mathew S, Gayathri Devi, Sandhya KV. Formulation and evaluation of ketorolac tromethamine-loaded albumin microspheres for potential intramuscular administration. AAPS PharmSciTech. 2007; 8(1):100-108.
12. Fini A, Garuti M, Fazio G, Alvarez FJ, Holgado MA: Diclofenac salts. I. fractal and thermal analysis of sodium and potassium diclofenac salts. J Pharm Sci. 2001; 90: 2049–2057.
13. Rainsford KD, Kean WF, Ehrlich GE. Review of the pharmaceutical properties and clinical effects of the topical NSAID formulation, diclofenacepolamine. Curr Med Res Opin. 2008; 24(10).
14. Juaira T. Effect of ±-tocopherol and its combination with diclofenac on pain and inflammation in rats. [Thesis] [Dhaka (Bangladesh)]: BSMMU. 2014.
15. Imtiaz M. Effect of vitamin B_{12} and folic acid and their combination on pain and inflammation in rats.[Thesis] [Dhaka (Bangladesh)]: BSMMU. 2011.
16. Bartoszyk GD, Wild A. Antinociceptive effects of pyridoxine, thiamine and cyanocobalamin in rats. ANN NY ACAD SCI. 1990; 473-476.
17. França et al. B vitamins induce an antinociceptive effect in the acetic acid and formaldehyde models of nociception in mice. Eur J Pharmacol. 2001; 421:157–164.
18. Reyes-Garcíaet al. Analgesic effect of B vitamins in formalin-induced inflammatory pain. Proc West Pharmacol Soc. 2001; 44:139-140.
19. Reyes-García G, Castillo-Henkel C, Medina-Santillán R, Terán-Rosales F, Grandos-Soto V. Mechanisms of analgesic action of B vitamins in the formalin-induced inflammatory pain. Proc West Pharmacol Soc. 2002; 45:144-146.
20. Rocha-González HI, Terán-Rosales F, Reyes-García G, Medina-Santillán R, Granados-Soto V. B vitamins increase the analgesic effect of diclofenac in the rat. Proc West Pharmacol Soc. 2004; 47:84-87.
21. Medina-Santillán R, Reyes-García G, Rocha-González HI, Granados-Soto V. B vitamins increase the analgesic effect of ketorolac in the formalin test in the rat. Proc West Pharmacol Soc. 2004; 47: 95-99.
22. Terán-Rosales F, Medina-Santillán R, Reyes-García G, Granados-Soto V. Synergistic antinociceptive interaction between acetaminophen or metamizol and

B vitamins in the formalin test. Drug Dev. Res. 2006; 66:286-294.

23. Rodríguez-Silverio J, Aguilar-Carrasco JC, Reyes-García G, MedinaSantillán R, Flores-Murrieta FJ. Antinociceptive and anti-inflammatory effects of ketoprofen are potentiated by a vitamin B mixture in the rat. 2005; 64:66-70.

24. Banode SV, Borkar AS, Badwaik RT. Effect of ketorolac on opioid induced antinociception in rats. IJMPS. 2012; 3(3):7-13.

25. Padi SSV, Naidu PS, Kulkarni SK. Involvement of peripheral prostaglandins in formalin-induced nociceptive behaviors in the orofacial area of rats. Inflammopharmacology. 2006; 14:57-61.

26. Islam KMN, Rahman ASMH, Al-Mahmud KA. Manual for care and use of laboratory animals. Animal resources branch. International Centre for Diarrhoeal Diseases Research, Bangladesh; 2001.

27. Das et al. Antinociceptive and anti-inflammatory activity of the bark extract of plumeriarubra on laboratory animals. European J Med Plants. 2013; 3(1):114-126.

28. Jami MSI, Sultana Z, Ali ME, Begum MM, Haque MM. Evaluation of analgesic and anti-inflammatory activities on ethanolic extract of terminaliachebula fruits in experimental animal models. AJPS.2014; 5:63-69.

29. Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain. 1995; 60(1):91-102.

30. Tajik H, Tamaddonfard E, Hamzeh-Gooshchi N. The effect of curcumin (active substance of turmeric) on the acetic acid-induced visceral nociception in rats. Pak J Biol Sci. 2008; 11(2):312-314.

31. Ali T, Javan M, Sonboli A, Semnanian S. Evaluation of the antinociceptive and anti-inflammatory effects of essential oil of nepeta pogonosperma jamzad et assadi in rats. DARU. 2012; 20:1-8.

32. Refinetti R, Horvath SM. Thermopreferendum of the rat: inter- and intra-subject variabilities. Behav Neural Biol. 1989; 52(1):87-94.

33. Moallem SA, Hosseinzadeh H, Farahi S. A study of acute and chronic anti-nociceptive and anti-inflammatory effects of thiamine in mice. Iran Biomed J. 2008; 12 (3):173-178.

34. Kim MJ, Hong BH, Zhang EJ, Ko YK, Lee WH. Antinociceptive effects of intraperitoneal and intrathecal vitamin E in rat formalin test. Korean Journal of Pain. 2012; 25(4):238-244.

35. Tamaddonfard E, Samadi F, Egdami K. The effects of vitamin B_{12} and diclofenac and their combination on cold and mechanical allodynia in a neuropathic pain model in rats. Veterinary Research Forum. 2013; 4(1):19-24.

36. McNamara CR, Brehm et al. (2007). TRPA1 mediates formalin-induced pain. Proceedings of the National Academy of Sciences.104 (33): 13525–13530.

37. Reyes-García et al. Analgesic effect of B vitamins in formalin-induced inflammatory pain. Proc West Pharmacol Soc. 2001; 44:139-140.

38. Fu Q-G, Carstens E, Stelzer B, Zimmermann M. B vitamins suppress spinal dorsal horn nociceptive neurons in the cat. NeurosciLett.1988; 95(1-3): 192-197.

39. Jurna I, Carlsson KH, Kömen W, Bonke D. Acute effects of vitamin B_6 and fixed combinations of vitamin B_1, B_6 and B_{12} on nociceptive activity evoked in the rat thalamus: dose-response relationship and combinations with morphine and paracetamol. KlinWochenschr. 1990; 68(2):129-35.

40. Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. BehavPharmacol. 2011; 22(56):390-404.

41. Nadine et al. ±-tocopheryl phosphate interact with the cannabinoid system in the rodent hippocampus. Free RadicBiolMedicine. 2011; 51: 1643-55.

42. Barrett KE, Barman SM, Boitano S, Brooks HL. Ganong's review of medical physiology. 23rd. New Delhi: Tata McGraw-Hill; 2010.

43. Lázaro-Ibáñez GG, Torres-López JE, Granados-Soto V. Participation of nitric oxide-cyclic GMP-ATP-sensitive K channel pathway in the antinociceptive action of ketorolac. European Journal of pharmacology. 2001; 426 (1-2):39-44.