

The Effect of Palmitoylethanolamide Analogue, N-(2-Morpholinoethyl) Palmitoylethanolamide on Pain Behaviour in Mice

Gholamreza Karimi¹, Farzin Hadizadeh², Amir Mirshamsi¹, Toktam Ziaee¹

¹ Medical Toxicology Research Center, Pharmacy School, Mashhad, IRAN

² Pharmaceutical Research Center, Pharmacy School, Mashhad, IRAN

Corresponding author: Gholamreza Karimi,

Medical Toxicology Research Center, Pharmacy School, Mashhad, IRAN

E-mail: karimig@mums.ac.ir

Summary

In the present study, we investigated the antinociceptive and anti-inflammatory properties of morpholino analogue of palmitoylethanolamide (PEA) by using abdominal constriction, formalin and tail flick tests. In the writhing test, the doses of 50 and 100 mg/kg of PEA analogue showed remarkable decrease in the number of abdominal constrictions when compared to diclofenac. The doses of 50 and 100 mg/kg of PEA analogue exhibited similar effect to morphine in formalin and tail flick tests. The results demonstrated that the PEA analogue exhibit central and peripheral antinociceptive activity in a dose-dependent manner. This presents a novel analgesic compound that may target the endogenous cannabinoid system for treatment of analgesic disorders.

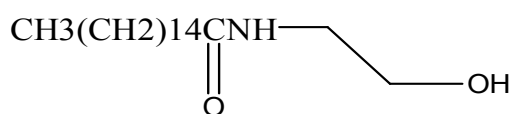
Keywords: Antinociceptive, Palmitoylethanolamide, Analogue, Cannabinoids

Introduction

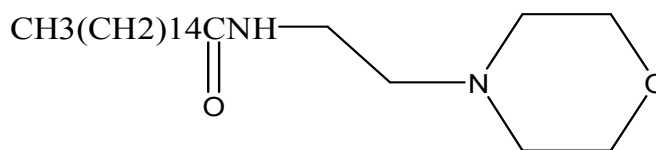
Considerable evidence demonstrating that Δ^9 -tetrahydrocannabinol (THC), the psychoactive ingredient of *Cannabis sativa*, and a number of synthetic cannabinoid (CB) receptor agonists have analgesic activity in rodent models of acute and chronic pain (1). In particular, cannabinoid agonists attenuate signs of hind paw hypersensitivity with nerve injury and demyelination associated pain (2,3). Similar findings in models of inflammatory pain are mediated via both CB1 and CB2 receptors (4,5). However, there is a need to improve the therapeutic index of cannabinoids, particularly with respect to psychotropic side effects which are mediated by CB₁ receptors expressed in brain (1). An approach which may avoid such side effects is to manipulate the endogenous cannabinoid system (6).

The endocannabinoid N- arachidonylethanolamide (anandamide), and its naturally occurring analogue, N-palmitoylethanolamide (PEA) exert potent analgesic and anti-inflammatory activities (7,8,9). Although PEA does not bind to either CB1 or CB2 receptors, its antinociception actions may be prevented by the selective CB₂ receptor antagonist SR144528 (10). Different mechanisms of PEA action have been suggested including: interaction with uncharacterized CB2 like receptor (11), interaction with the peroxisome proliferator-activated receptor - α (12) and inhibition of fatty acid amide hydrolase (FAAH), thus increasing local concentrations of anandamide and perhaps PEA (13).

Few studies with PEA analogue have been shown to produce analgesic in a number of acute and inflammatory pain (14,15). Since little is known about the modification of ethyl head chain in PEA, there is a need to further investigate the effect of substitution of ethyl head chain upon its ability to interact with cannabinoids receptors or FAAH. The present study was undertaken to characterize and functionally correlate the analgesia action of morpholino analogue of PEA in various animal models of pain.



N-palmitoylethanolamide (PEA)



PEA analogue

Materials and methods

Chemistry

Synthesis of N-palmitoylethanolamide

N-palmitoylethanolamide was prepared in one step from palmitoyl chloride and an excess of ethanolamine in dichloromethane, with a yield > 95%, ¹H-nuclear magnetic resonance (NMR) and infrared (IR) spectra were in complete accordance with its chemical structure, as previously described (16).

Synthesis of N-(2-morpholinoethyl) palmitoylamide

To 1 mmol of palmitoyl chloride in 10 mL of dichloromethane, 2 mmol of 2- (4-morpholinyl) ethanol was added in drops at room temperature. The mixture was stirred overnight. The resulting precipitate was isolated by filtration and recrystallized in ethanol; white crystals were obtained. The melting point was 100-110°C and the ¹H-NMR (CDCl₃) δ : 0.9 (3H, t, CH₃); 1.2-1.6 (26 H, m, (CH₂)₁₃); 2.2 (2H, t, CH₂CO); 2.35-2.75 (m, 6H, CH₂N); 3.30-3.48 (m, 6H, CH₂NH); 4.80 (1H, t, NHCO) was in complete accordance with its chemical structure. Its elemental analysis for C, H and N was within $\pm 0.4\%$ of the theoretic value.

Animals

All experiments were performed with male albino mice (20-25 g) obtained from our own animal facility. Animals were maintained in a room with controlled temperature $22 \pm 2^\circ\text{C}$ for 12h high/dark cycle with free access to food and water. Animal methods conducted in accordance with experimental animal guidelines of pharmacy school, Mashhad University of Medical Sciences.

Drug preparation

Palmitoylethanolamide and its analogue were prepared as a suspension in a mixture of 1: 19 (v/v) DMSO/Saline containing 0.5% (w/v) methylcellulose. The suspension was sonicated for 60 S before using. Diclofenac and morphine obtained from Daru Pakhsh Company and prepared in normal saline.

Tail flick test

Antinociceptive behavior was assessed using a modified version of that described by Connor (17). The tail was exposed to focused beam of radiant heat at a 3 cm from the tip using tail flick unit. The time necessary for the mice to withdrawal the tail was registered at 30, 60 and 90 min after intraperitoneal administration of drugs. A 10s cut-off was employed to prevent tissue damage.

Acetic acid-induced abdominal writhing

Mice received intraperitoneal injections of acetic acid (0.6% v/v), 0.15 ml per animal. Writhing episodes were monitored for a period of 20 min, starting 5 min after administration of irritant. The animals were pretreated with drugs or vehicle, 30 min before acetic acid administration (18).

Formalin test

Animals received the injection of 20 μl of formalin (2.5% v/v) into dorsal surface of the left hind paw. The time that animals spent licking the injected paw was recorded for periods of 0-15 min (early phase) and 20-50 min (late phase). The animals were pretreated with drugs or vehicle 30 min before formalin administration (19).

Statistical analysis

Results are presented as mean \pm SEM. Statistically significant differences between treatment groups were evaluated by ANOVA followed by Tukey-Kramer test. Probabilities less than 0.05 ($P < 0.05$) were taken as a representing significant differences.

Results

Tail flick test

Figure 1 and 2 show the effects of PEA and its analogue on the tail flick model. The PEA and its analogue exhibited antinociceptive activity in tail flick test. The PEA did not show dose-dependent activity and the 100 mg/kg of PEA was found to give unexpectedly remarkable decrease in antinociception at 90 min when compared to previous dose.

The PEA analogue at the doses of 10, 50 and 100 mg/kg showed similar effect to morphine. The 10, 50 and 100 mg/kg of analogue after 30 min produced an analgesic activity that was greater than PEA.

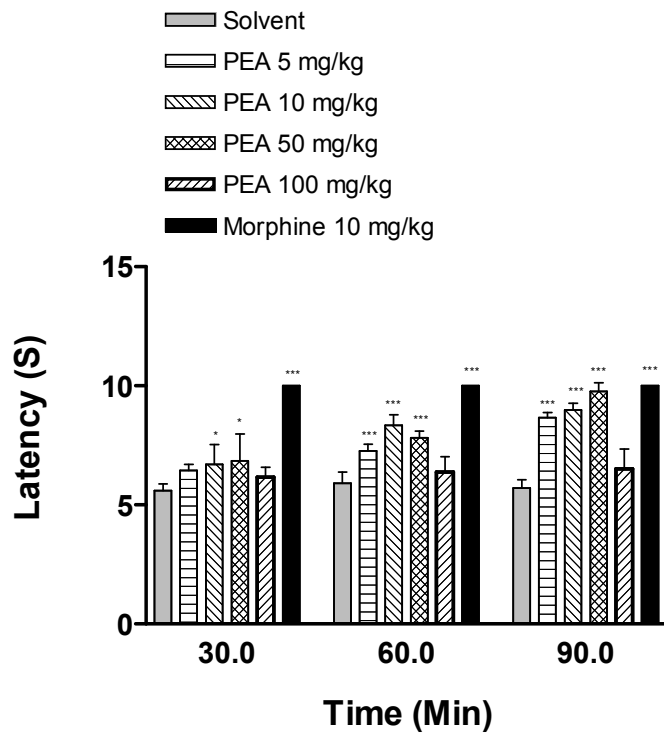


Fig 1. The effect of palmitoylethanolamide in tail flick response in mice. Data was reported as Mean \pm SEM (n=6), *P<0.05, ***P<0.001 vs solvent, Tukey-Kramer test.

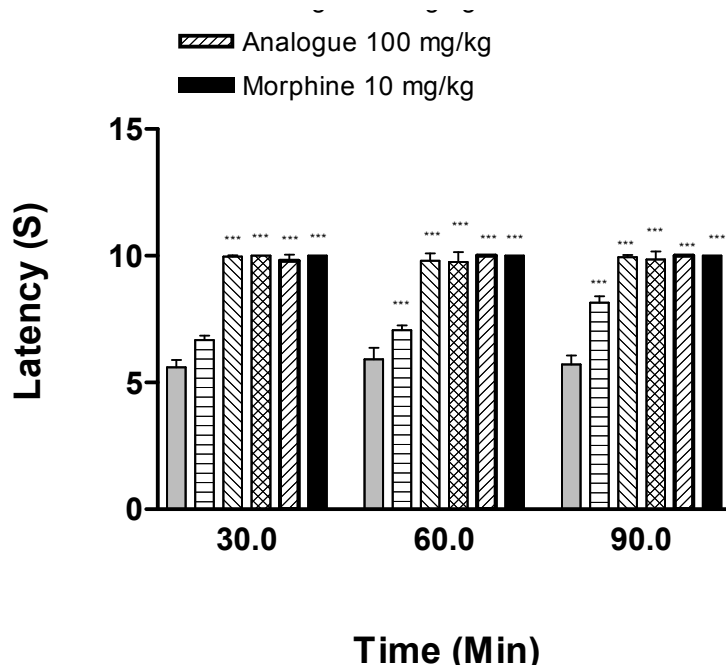


Fig 2. The effect of palmitoylethanolamide analogue in tail flick response in mice. Data was reported as Mean \pm SEM (n=6), ***P<0.001 vs solvent, Tukey-Kramer test.

Writhing test

Figure 3 and 4 show the antinociceptive profile of PEA and its analogue using the acetic acid induced abdominal constriction in mice. PEA in the doses of 50 and 100 mg/kg, significantly reduced the number of writhes. The analogue, at all doses used, exhibited a significant antinociceptive activity in a dose-dependent manner. The 100 mg/kg of PEA and the 50 and 100 mg/kg of analogue were found to give remarkable decrease in the number of abdominal constrictions when compared to diclofenac.

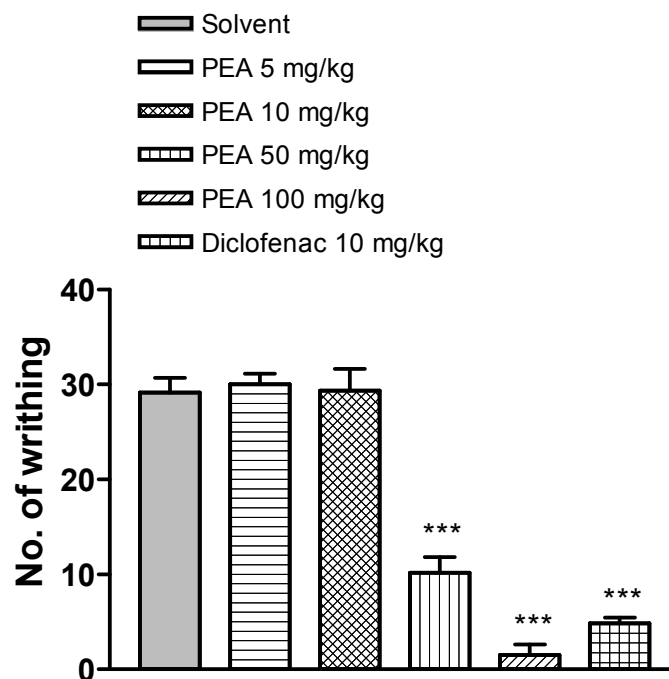


Fig 3. The effect of palmitoylethanolamide (PEA) on acetic acid-induced writhing in mice. Data was reported as Mean \pm SEM (n=6), ***P<0.001 vs solvent, Tukey-Kramer test.

Formalin test

The antinociceptive effect of PEA and its analogue using formalin test have been shown in figure 5 and 6. The PEA and its analogue exhibit significant antinociceptive activity in both phases of the formalin test, as can be seen by morphine. Interestingly, the 50 and 100 mg/kg doses of two drugs exhibited complete analgesia in the second phase of the test.

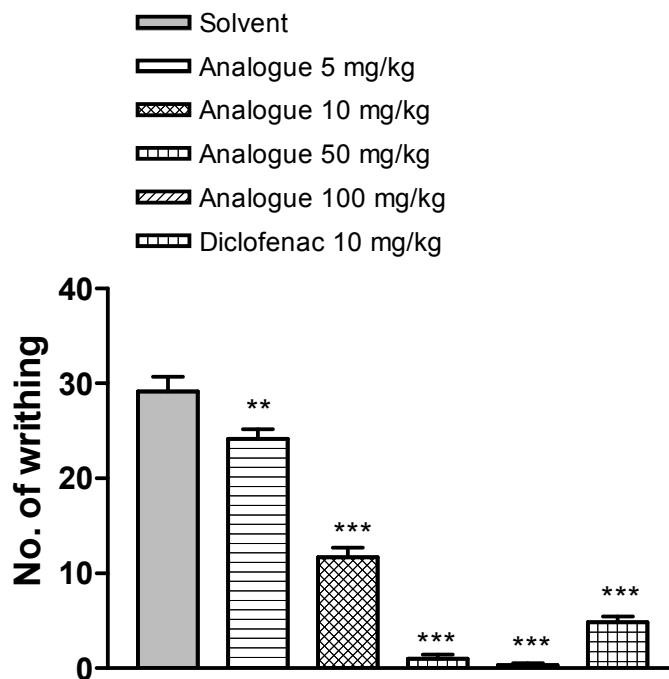


Fig 4. The effect of palmitoylethanolamide analogue on acetic acid-induced writhing in mice. Data was reported as Mean ± SEM (n=6), **P<0.01, ***P<0.001 vs solvent, Tukey-Kramer test.

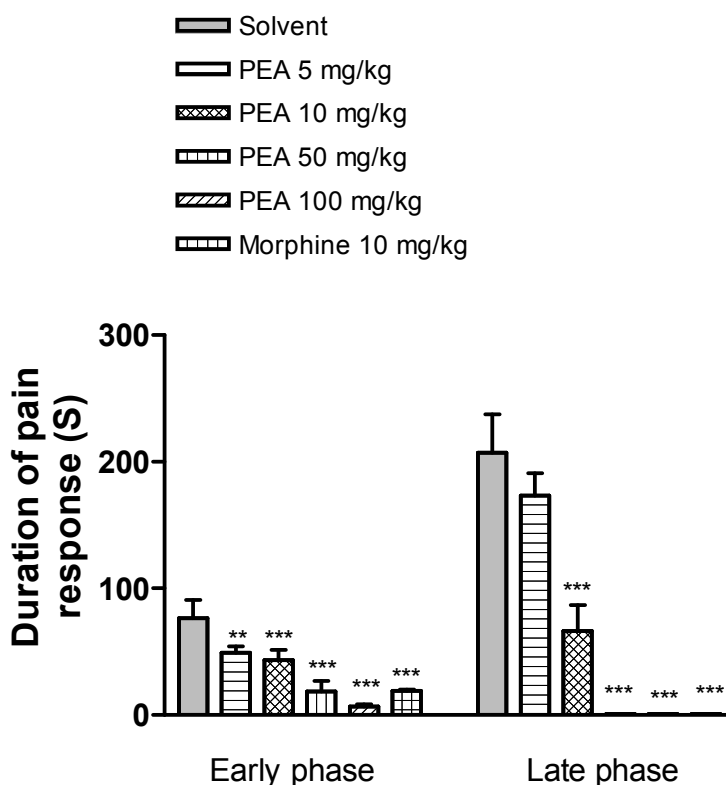


Fig 5. The effect of palmitoylethanolamide (PEA) on formalin induced nociception in mice. Data was reported as Mean ± SEM (n=6), **P<0.01, ***P<0.001 vs solvent, Tukey-Kramer test.

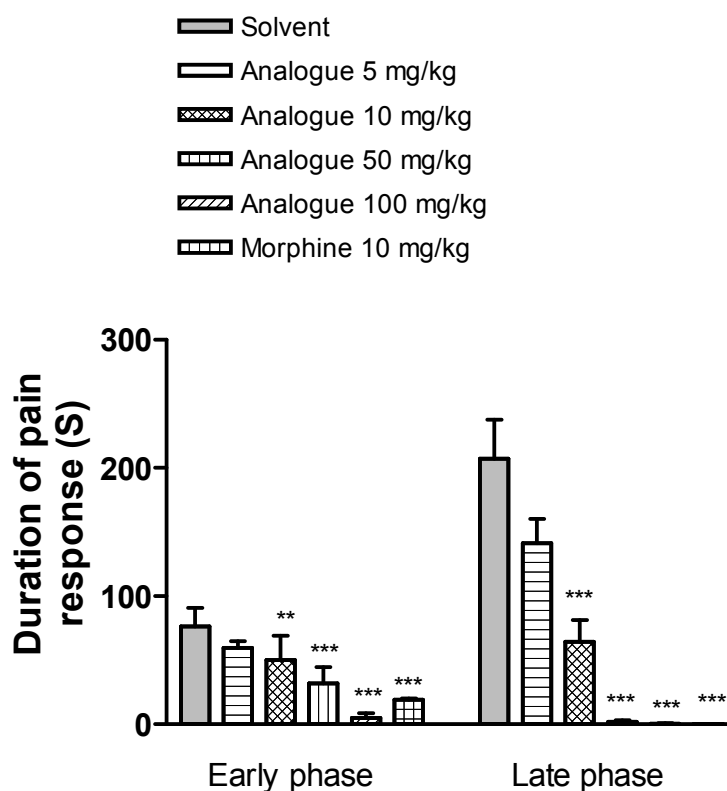


Fig 6. The effect of palmitoylethanolamide analogue on formalin induced nociception in mice. Data was reported as Mean \pm SEM (n=6), **P<0.01, ***P<0.001 vs solvent, Tukey-Kramer test.

Discussion

The present study has confirmed the possible antinociceptive and anti-inflammatory properties of morpholino analogue of PEA. The ability of analogue to reduce the number of abdominal writhings indicates the antinociceptive activity but did not signify the involvement of peripheral or central mechanisms (20). The acetic acid test was caused by release of prostaglandins in the peritoneal (17), so the observed antinociceptive activity could be due to inhibition of peripheral cyclooxygenase or direct blocking of receptors.

The formalin test produced a distinct biphasic response. The early phase is a result of direct stimulation of nociceptors by formalin and is an acute reaction observed immediately after administration of formalin. The second phase is due to inflammatory processes (21). Drugs that act centrally affect both phases while drugs that act peripherally influence the late phase (20). The ability of analogue to inhibit both phases suggested the involvement of central mechanism and is concomitant with the activity shown by centrally acting analgesic drugs like morphine.

To test if the analogue could develop central antinociceptive effect the model of tail flick was used. The effectiveness in tail flick assay could indicate analgesic brought about by inhibition of pain on spinal regions of central nervous system (20).

It has been reported that chemically and thermally induced tests elicit the selective stimulation of C and A fibers, respectively (22). In addition, the ability of analogue to inhibit the chemically and thermally-induced antinociceptive response suggested that the analogue had a characteristic of strong analgesics like opioid agonists (23).

The fact that certain drugs show desired effects over a narrow range of doses could be used to explain our findings on PEA dose-independent activity in tail flick test. These results might be related to therapeutic window phenomena in which certain drugs produce suboptimal or even decline activities when the dose was below or above the therapeutic range (24).

In conclusion, the study demonstrates the centrally and peripherally activities of PEA analogue. Further studies are, however, required to clarify its exact mechanism of action.

Acknowledgment

The authors are thankful to Vice Chancellor of Research, Mashhad University of Medical Sciences, for financial support.

References

1. Rice AS (2005) Textbook of pain: cannabinoids, First edition, New York, P. 521-540.
2. Scott DA, Wright CE, Anagus JA (2004) Evidence that CB1 and CB2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. *Pain* 109: 124-137
3. Wallace VC, Cottrel DF, Brophy PJ, Fleetwood SM (2003) Focal lysolecithin-induced demyelination of peripheral afferents results in neuropathic pain that is attenuated by cannabinoids. *J Neurosci* 23: 3227-3233
4. Bridges D, Ahmad K, Rice AS (2001) The synthetic cannabinoid WIN 55, attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. *Br J Pharmacol* 133: 586-594.
5. Fox A, Kesingland A, Gentry C, Urban L (2001) The role of central and peripheral cannabinoid receptors in the antihyperalgesic activity of cannabinoid in a model of neuropathic pain. *Pain* 92: 91-100
6. Hohmann A, Suplita R (2006) Endocannabinoid mechanisms of pain modulation. *AAPS J* 8: E693-E708
7. Jaggar SI, Hasnic FS, Sellaturay S Rice AS (1998) The antihyperalgesic actions of cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* 76: 189-199
8. Calignano A, La Rana G, Giuffrida A, Piomelli D (1998) Control of pain initiation by endogenous cannabinoids. *Nature* 394: 277-281.
9. Rice AS (2001) Cannabinoids and pain. *Curr Opin Investig Drugs* 2: 399-414
10. Calignano A, La Rana G, Piomelli D (2001) Antinociceptive activity of endogenous fatty acid amide, palmitoylethanolamide. *Eur J Pharmacol* 419: 191-198
11. Re G, Barbero R, Miolo A, Di Marzo V (2007) Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain. *Vet J* 173: 21-30
12. Lovermine J, Russo R, La Rana G, Calignano A, Piomelli D (2005) The search for palmitoylethanolamide receptor. *Life Sci* 77: 1685-1698
13. Lambert DM, Vandevoorde S, Jonsson KO, Fowler CJ (2002) The palmitoylethanolamide family. *Curr Med Chem* 9: 663-674
14. De Fillipis D, D'Amico A, Cinelli MP, Di Marzo V, Iuvone T, (2008) Adelmidrol, a palmitoylethanolamide analogue reduces chronic inflammation in a carrageenin-granuloma models in rats. *J Cell Mol Med* (In Press).

15. Wallace VC, Segerdahl AR, Lambert DM, Vandevoorde S, Blackbeard J, Hasnic F, Rice ASC (2007) The effect of palmitoylethanolamide analogue (L-29) on pain behavior in rodent models of neuropathy. *Br J Pharmacol* 151: 1117-1128
16. Lambert DM, Vandevoorde S, Diependaele G, Robert (2001) Anticonvulsant activity of palmitoylethanolamide in mice. *Epilepsia* 43: 321-327
17. Connor J, Makonnen E, Rostom A (2000) Comparison of analgesic effect of Khat extract, amphetamine and ibuprofen in mice. *J Pharm Pharmacol* 52: 107-110
18. Matheus ME, Nerrondo LF, Vieitas EC, Manezes FS, Fernandes PD (2005) Evaluation of the antinociceptive properties from *Brillantaisia palisotii* stems extracts. *J Ethnopharmacol* 102: 377-381
19. Nikfar Sh, Abdollahi M, Sharifzadeh H, Eftekhar N (1998) Interaction between lead acetate and morphine on antinociception in mice. *Gen Pharmacol* 30: 489-493
20. Gomes NM, Rezende MC, Fontes SP, Matheus ME, Fernandes PD (2007). Antinociceptive activity of *Amazonian copaiba* oils. *J. Ethnopharmacol* 109: 486-492
21. Hunskaar S, Hole K (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 30: 103-108
22. Lu Y, Pirec V, Yeomans DC (1997) Differential antinociceptive effects of spinal opioids on foot withdrawal responses evoked by C fiber or A delta nociceptor activation. *Br J Pharmacol* 121: 1210-1216
23. Yeomans DC, Pirec V, Proudfit HK (1996) Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptor in rat. *Pain* 68: 133-140
24. Katzung BG (2007). *Basic and clinical pharmacology*, Ten edition, New York, p. 34-50