

A Study of Acute and Chronic Anti-Nociceptive and Anti-Inflammatory Effects of Thiamine in Mice

Seyed Adel Moallem^{*1, 2 & 3}, Hossein Hosseinzadeh^{1 & 2} and Sepideh Farahi¹

¹Dept. of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad;

²Pharmaceutical Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad;

³Medical Toxicology Center, Mashhad University of Medical Sciences, Mashhad, Iran

Received 15 April 2007; revised 22 December 2007; accepted 16 January 2008

ABSTRACT

Background: Thiamine (VitB₁) is a vitamin with various important physiological functions and postulated therapeutic effects. Its use as an analgesic in neuropathic pain has been undergoing in clinical settings. However, there has been little experimental investigation on this effect. In this study, anti-nociceptive and anti-inflammatory effects of thiamine were investigated in mice. **Methods:** Three doses of thiamine (50, 100 and 125 mg/kg) were used by intraperitoneal injection in this study. Acute and chronic anti-nociceptive effects were examined using hot plate test alone and after sciatic nerve ligation, respectively. Imipramine (40 mg/kg) was used as positive control. Anti-inflammatory effects of thiamine on acute and chronic inflammation were assessed using xylene-induced edema in ears and granuloma caused by compressed cotton implantation, respectively. Sodium diclofenac (15 mg/kg) was used as positive control. Open field test was performed to differentiate the mice responses in the acute anti-nociceptive tests. **Results:** All three doses of thiamine showed significant analgesic effects in non-ligated mice and also in neuropathic pain in ligated animals. Increasing the dose of thiamine correlated with a more pronounced and sustained effect. Acute anti-inflammatory investigation showed that thiamine injected 30 or 60 minutes before xylene application reduced the weight of edematous ears. However, the effect of thiamine was less pronounced than diclofenac. Furthermore, when injected once daily for 7 days, all doses of thiamine significantly reduced the weight of the cotton disks, showing suppression of granuloma formation. **Conclusion:** Taken together, it has been shown that thiamine possesses remarkable analgesic activities and also has significant anti-inflammatory effects, confirming its clinical use in controlling pain and less in inflammation. *Iran. Biomed. J. 12 (3): 173-178, 2008*

Keywords: Thiamine, Anti-nociceptive, Anti-inflammation

INTRODUCTION

Vitamins or the heterogenic nutritional supplements are required for appropriate body function. They are classified into fat soluble (A, D, E and K) and water soluble (C, B₁, B₂, B₃, B₆, B₁₂, biotin, folic acid and pantothenic acid) vitamins [1]. Thiamine itself is not biologically active as it requires alteration to a pyrophosphate form by the action of pyrophosphokinase and Mg²⁺ to act as a cofactor in many enzymatic reactions including oxidative decarboxylation. Thiamine is actively and inactively absorbed in the ileum and jejunum [1]. Primary deficiency of thiamine could lead to the decrease of enzymes at the nerve

terminals probably due to the decrease of acetylcholine. Deficiency symptoms mostly characterized are insomnia, fatigue, heartaches, constipation, gastrointestinal distress, muscular atrophy and cramps. Furthermore, severe deficiency of thiamine results in Beriberi (Wernicke-Korsakoff syndrome) with central, peripheral nervous and cardiovascular symptoms leading to death [2]. Previous reports have related the occurrence of neuropathy with the deficiency of thiamine and thus some anti-nociceptive properties were suggested for thiamine [3]. It has been shown that prescribing high doses of vitamins B₁, B₆ and B₁₂ potentiated the anti-nociceptive effect of diclofenac, non-steroidal anti-inflammatory drugs (NSAID) and methimazole [3,

*Corresponding Author; Tel. (+98-511) 882 3255; Mobile: (+98-915) 509 0106; Fax: (+98-511) 882 3251; E-mail: moallem@mums.ac.ir

4]. A similar work reported a decrease in the spinal cord P substance in rats receiving low amounts of thiamine in their daily food supply [5]. Neuropathy could be caused by an increase in microglial cells activity [6], nitric oxide mediator production preceding inflammation [7] or protein kinase C activation [8]. Experimental induction of neuropathy could be accomplished by Bennet chronic constriction injury, Seltzer partial sciatic nerve injury or Chung spinal nerve injury [9, 10].

In most above mentioned studies, the potentiation of anti-nociceptive and anti-inflammatory effects of thiamine with NSAID has been reported. To elucidate the effect of thiamine alone in an experimental model, we investigated the anti-nociceptive and anti-inflammatory activities of thiamine in mice. Anti-nociceptive effects were examined by hot plate test without and with sciatic ligation. Xylene-induced edema in ears and granuloma caused by cotton disk implantation were used to assess acute and chronic inflammation, respectively. Since some of the evaluated effects involve behavioral responses to stimuli, open field test was performed to rule out any effect of thiamine as nervous system depressant in the acute anti-nociceptive tests.

MATERIALS AND METHODS

Animals. Male mice (BALB/c) weighing 25-30 g were obtained from the Animal Room of the School of Pharmacy, Mashhad University of Medical Sciences (Mashhad, Iran) and housed in groups of four in standard laboratory conditions in the same center. They were kept at constant room temperature ($21 \pm 2^\circ\text{C}$) under a 12/12 h light/dark cycle at least 10 days prior to testing. Commercial food pellets and tap water were freely available. They were transferred to the laboratory at least 1 hour before the start of experiments. The experiments were performed during the light portion between 08:00-12:00 a.m. to avoid circadian influences. The protocols of all animal experiments have been approved by the University Animal Care Committee.

Preparation of solutions. Thiamine is water soluble, thus appropriate solutions were prepared by dissolving desired amounts of thiamine (Hakim Co, Tehran, Iran) in normal saline. Thiamine doses were selected according to previous reports by using 300 mg/kg as a starting dose [3, 4, 11]. However, injecting various doses to groups of 5 mice revealed

that 125 mg/kg was the maximum tolerated dose. Therefore, 50, 100, and 125 mg/kg were selected. Imipramine hydrochloride 40 mg/kg (Sobhan, Tehran, Iran) and diclofenac sodium 15 mg/kg (Daru Pakhsh, Tehran, Iran) were used as positive controls for anti-nociceptive and anti-inflammatory experiments, respectively. Normal saline was used as negative control (5 ml/kg). All compounds were administered by intraperitoneal (i.p.) injection.

Acute anti-nociception by hot plate test. Anti-nociceptive experimentation was carried out using the hot plate test. Hot plate temperature was set on 55°C and cut off time was 40 s. Jumping, withdrawal of paws and/or licking of paws were counted as positive response to stimuli [12]. Groups of 10 male mice were used for each test compound. Mice responses were recorded at 0, 30, 60, 90 and 120 min after test compound administration (three doses of thiamine, imipramine or normal saline).

Chronic anti-nociception by sciatic nerve ligation. Male mice were anesthetized by ketamine (Rotexmedica Pharmaceuticals, France) 100mg/kg and xylazine (Chanelle Veterinary, Ireland) 10 mg/kg. Sciatic nerve was exposed by cutting the right leg femoral area. A metal wire was tied around the nerve. Cut skin was stitched and mice were kept in boxes [9, 12, 13]. A sham group was also added to the study. This group has received all the treatment, but the sciatic nerve was only exposed, not ligated. After two weeks, hot plate studies were carried out as explained in the previous section. Ten male mice were used in each test group (three doses of thiamine, imipramine or normal saline).

Anti-inflammatory studies. Anti-inflammatory studies consisted of acute and chronic experiments. Acute study was accomplished by evaluating the anti-inflammatory effects of thiamine by xylene-induced edema in male mice ears [12]. Thirty or sixty minutes after the i.p. injection (three doses of thiamine, diclofenac or normal saline) a drop of xylene (Merck Chemicals, Germany) was applied on each of the frontal and dorsal sides of the left ear. Two hours later, a circle of 4.5 mm in each ear was punched out and weight difference between right and left ear was recorded. Seven mice were used for each test compound. Chronic studies were pursued on male mice by employing the cotton disk-inducing granuloma test. Anesthesia was induced as mentioned earlier. Compressed cotton disks were sterilized by autoclaving, implanted under neck and

skin opening was stitched up. Test compound (three doses of thiamine, diclofenac or normal saline) was administered daily by i.p. injection. On the eighth day, the compressed cotton disk along with accompanying granuloma was removed and weight differences of cotton disks before implantation and after retrieval were recorded [12]. Seven mice were used for each group.

Open field test. The open field test is designed to measure behavioral responses such as locomotor activity and hyperactivity. Open field apparatus is made as reported [14]. Each mouse was placed in the centre of the open field, and its behavior was observed for 5 min. The parameters evaluated were the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three measures were referred to as total, peripheral, and central locomotion, respectively [14]. At the end of each test, the whole area was cleaned with a wet sponge and a dry paper towel.

Statistical analysis. Data analysis was accomplished by ANOVA and Tukey-Kramer tests (InStat software Ver. 3.0).

RESULTS

Anti-nociceptive effect of thiamine was evaluated using healthy and neuropathic mice by hot plate test which is a measure of acute and chronic pain, respectively. Thiamine at doses of 50, 100 and 125 mg/kg were selected and 40 mg/kg imipramine was used as positive control. Figure 1 demonstrates the effect of thiamine in different groups from 0 to 120 minutes after injection in healthy mice. The 50 mg/kg dose showed a significant difference with negative control ($P<0.01$) at 30 and 60 minutes, but was not significant at 90 and 120 minutes ($P>0.05$). The 100 mg/kg and 125 mg/kg doses caused significant increases in reaction times at 30, 60 and 90 minutes. At 90 minutes, the effect of 125 mg/kg dose was more significant ($P<0.001$) than the 100 mg/kg dose ($P<0.05$). But none of the groups showed a significant difference with the negative control at 120 minutes, which suggests that the effect of thiamine diminishes after 90 minutes.

Imipramine as a positive control demonstrated significant anti-nociceptive effects which was the highest at 30 minutes and lasted up to 120 minute ($P<0.001$). Maximum effect of thiamine was at 30

minutes for all groups; this lasted for 90 minutes in the 100 and 125 mg/kg groups and for 60 minutes for the 50 mg/kg group.

Chronic neuropathy was studied in mice after 14 days of inflicting the nerve injury (Fig. 2). Thiamine at 50 mg/kg caused a significant difference with the negative control ($P<0.01$) in 30 and 60 minutes, but was not significant at 90 and 120 minutes ($P>0.05$).

The dose of 100 mg/kg caused significant differences in response times at 30 and 60 minutes ($P<0.001$). The group receiving 125 mg/kg dose also showed a considerable difference with the negative control at 30 and 60 minutes ($P<0.001$) and 90 minutes ($P<0.01$). The hot plate test was also carried out for sham group after 14 days. The results were compared to the healthy group and no significant difference was detected ($P>0.05$). Comparing data from Figures 1 and 2 and also considering data from recording reaction times to pain stimuli after 7 days of sciatic nerve ligation revealed that pain threshold has diminished after nerve ligation (Fig. 3).

Acute anti-inflammatory effects of thiamine were studied by measuring edema caused by xylene in the animal's ears. Five groups of 7 mice received 5 ml/kg normal saline, 15 mg/kg diclofenac, 50, 100, or 125 mg/kg thiamine. Table 1 shows the results in mice 30 minutes after receiving the above treatments. The highest anti-inflammatory effect was produced by 125 mg/kg thiamine with inhibition percentage of 45.62%. All thiamine doses caused considerable reductions in the edema compared to

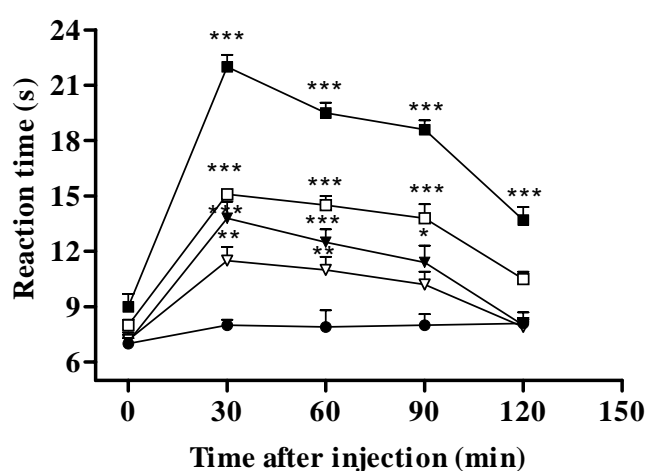


Fig. 1. Antinociception effects of three doses of thiamine, normal saline or imipramine by the hot plate test. Each point represents mean \pm standard error. *, ** and ***, $P<0.05$, $P<0.01$ and $P<0.001$, respectively. ●, normal saline; ▽, Vit B₁ 50 mg/kg; ▼, Vit B₁ 100 mg/kg; □, Vit B₁ 125 mg/kg; ■, imipramine, 40 mg/kg.

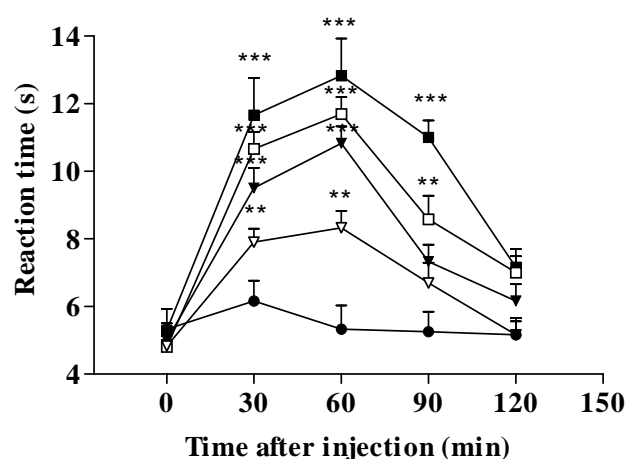


Fig. 2. Antinociception effects of three doses of thiamine, normal saline or imipramine in neuropathic mice by the hot plate test 14 days after sciatic nerve ligation. Each point represents mean \pm standard error. ** and *** depict $P < 0.01$ and $P < 0.001$, respectively. ●, normal saline; ▽, Vit B₁ 50 mg/kg; ▼, Vit B₁ 100 mg/kg; □, Vit B₁ 125 mg/kg; ■, imipramine, 40 mg/kg.

the negative control ($P < 0.01$ and $P < 0.001$). The effect of thiamine was less remarkable if xylene was applied one hour after receiving injection compared to the 30 minutes groups (Table 1). The highest anti-inflammatory effect (67.81 % inhibition) was produced by diclofenac ($P < 0.001$). Edema formation was suppressed significantly ($P < 0.01$) in mice received 100 or 125 mg/kg thiamine. However, 50 mg/kg did not manage to affect edema ($P > 0.05$).

Measuring granuloma formation was used to assess chronic anti-inflammation effect of thiamine. Five groups of seven mice received 5 ml/kg normal saline, 15 mg/kg diclofenac and 50, 100 or 125 mg/kg thiamine. Table 2 shows the corresponding results. All groups were significantly different in comparison with the negative control. There was a clear dose-response correlation between thiamine doses and reductions in granuloma formations.

Table 1. Acute anti-inflammatory effects by xylene-induced edema 30 and 60 minutes after administration of three doses of thiamine, normal saline or diclofenac as shown by mean ears weight difference \pm standard error mean and inhibition percentage.

Substance	Dose (ml/kg)	Mean ears weight difference \pm SEM (mg)		Inhibition percentage	
		30 (min)	60 (min)	30 (min)	60 (min)
Normal saline	5	10.5 \pm 0.8	-	8.2 \pm 0.7	-
Diclofenac	15	6.2 \pm 0.4***	40.57	2.6 \pm 0.3***	67.81
Thiamine	50	7.2 \pm 0.6**	31.71	6.5 \pm 0.3	20.40
Thiamine	100	6.2 \pm 0.8***	41.33	5.3 \pm 0.6**	35.13
Thiamine	125	5.7 \pm 0.4***	45.62	5.0 \pm 0.6**	38.68

Open field data (Fig. 4) revealed that compared to normal saline none of the three doses of thiamine has any depressive effect on central, peripheral or total locomotions. Therefore, none of the responses of the mice in the acute anti-nociception tests was due to a depressed nervous system and a less active animal.

DISCUSSION

Recent works have suggested vitamin B₁ in complex or separate forms potentiates anti-nociceptive and anti-inflammatory effects of NSAID or glucocorticoids [3, 4, 11]. In this study, we showed the anti-nociceptive and anti-inflammatory effects of thiamine on its own. We found the neuropathic anti-nociceptive effects of thiamine are remarkable and comparable to the positive control. This optimum effect of thiamine regarding its anti-nociceptive and anti-inflammatory effects was demonstrated using a dose of 125 mg/kg.

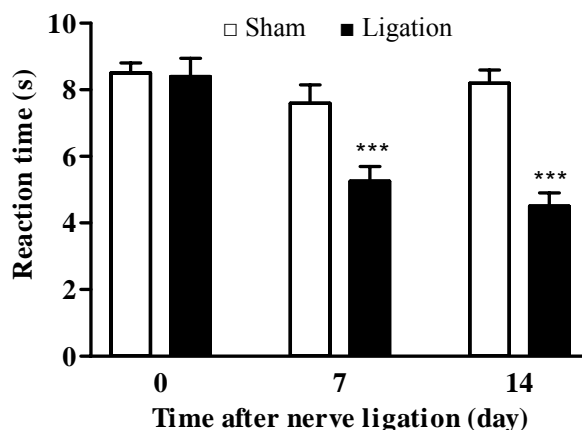


Fig. 3. Effect of sciatic nerve ligation and sham operation on reaction time to pain stimuli in the hot plate test. *** depicts $P < 0.001$.

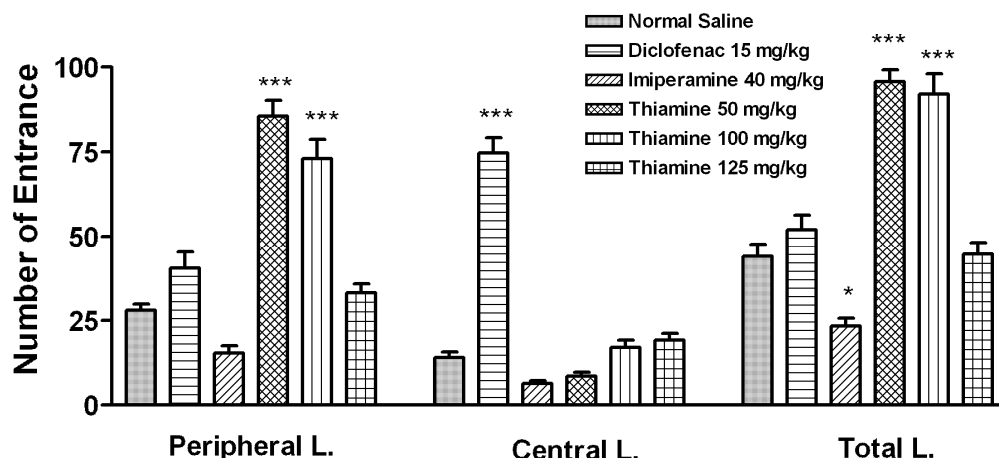


Fig. 4. Effects of drug administration on peripheral, central and total locomotion in the open field test. * and *** depict $P < 0.05$ and $P < 0.001$, respectively.

In one report, vitamins B complex including thiamine (50-100 mg/kg) induced an anti-nociceptive effect, not changed by naloxone, in the acetic acid writhing model [11]. Treatment for 7 days with above complex or thiamine alone inhibited the nociceptive response induced by formaldehyde. Similar to our fair effect of thiamine in controlling inflammation, thiamine/pyridoxine/cyanocobalamin complex partially reduced formaldehyde-induced hind paw edema.

Damage to the sciatic nerve and inflammation is accompanied by release of mediators such as calcitonin gene-related peptide, nitric oxide, and bradykinin. Also, potassium ion level and P substance in spinal C fiber increased during chronic pain. It seems that bradykinin-induced pain is dependent on local prostaglandins release that stimulates phospholipase A_2 in postsynaptic sympathetic neurons [15, 16]. Consequently, stimulation threshold of physical and thermal receptors is reduced. In the neuropathic model, the tolerance of the animals significantly decreased in

comparison to the non-ligated group. It is conceivable that the anti-nociceptive effect of thiamine, at least partially, could be explained by a reported effect of thiamine to inhibit prostaglandin E_2 , thromboxane B_2 and leukotriene E_4 [17].

Xylene's toxicity is similar to benzene which is characterized by an increase in vascular permeability and a release of inflammation mediators leading to edema. The acute anti-inflammatory effect of thiamine was more evident at 125 mg/kg dose with an inhibition of 45.62 %. It is probable that histamine, prostaglandin and/or leukotriene inhibition by thiamine could speak for the observed data. In the chronic inflammation model, the dose of 125 mg/kg showed most efficiency. Following the implantation of the cotton disk, three stages of secretory, granulation and stabilizing take place [12]. The anti-inflammatory effect of thiamine could be related to the decrease and inhibition of inflammation mediators.

Therefore, our work presents some experimental evidence supporting the administration of thiamine in controlling acute and chronic neuropathic pain, which have been controversial in clinical settings. However, more studies are needed to clarify the full extent and the mechanism of action of our findings.

ACKNOWLEDGMENTS

This study was supported by a grant from the Vice-Presidency for Research of Mashhad University of Medical Sciences, Mashhad, Iran.

Table 2. Chronic anti-inflammatory effects by cotton disk-induced granuloma method of daily administration of three doses of thiamine, normal saline or diclofenac as shown by mean cotton disks weight difference \pm standard error mean and inhibition percentage.

Substance	Dose (ml/kg)	Mean cotton disks weight difference \pm SEM (mg)	Inhibition percentage
Normal saline	5	88.0 \pm 2.6	-
Diclofenac	15	23.4 \pm 1.2***	72.37
Thiamine	50	62.3 \pm 1.8***	29.22
Thiamine	100	52.6 \pm 2.7***	40.26
Thiamine	125	39.3 \pm 1.7***	55.35

*** depicts $P < 0.001$.

REFERENCES

- Basu, T. K. and Dickerson, J. W. T. (1996) Vitamins in human health and disease. CABI Publishing, Cambridge, USA.
- Beers M. H., Porter, R. S. and Jones, T. V. (2006) The Merck manual of diagnosis and therapy, 18th edition, Merck, New Jersey, USA.
- Reyes-Garcia, G., Medina-Santillan, R., Teran-Rosales, F., Mateos-Garcia, E. and Castillo-Henkel, C. (1999) Characterization of the potentiation of the antinociceptive effect of diclofenac by vitamin B complex in the rat. *J. Pharmacol. Toxicol. Methods* 42: 73-77.
- Jurna, I. (1998) Analgesic and analgesia-potentiating action of B vitamins. *Schmerz*. 12: 136-141.
- Tadano, T., Asao, T., Aizawa, T., Sakurada, S., Abe, Y., Yonezawa, A., Ando, R., Arai, Y., Kinemuchi, H. and Kisara, K. (1995) Immunohistochemical determination of rat spinal cord substance P, and antinociceptive effect during development of thiamine deficiency. *Brain Res.* 696: 21-29.
- Stuesse, S.L., Cruce, W.L., Lovell, J.A., McBurney, D. L. and Crisp, T. (2000) Microglial proliferation in the spinal cord of aged rats with a sciatic nerve injury. *Neurosci. Lett.* 287: 121-124.
- Liu, T., Knight, K.R. and Tracey, D.G. (2000) Hyperalgesia due to nerve injury-role of peroxynitrite. *Neuroscience* 97: 125-131.
- Ohsawa, M., Narita, M., Mizoguchi, H., Suzuki, T. and Tseng, L.F. (2000) Involvement of spinal protein kinase C in thermal hyperalgesia evoked by partial sciatic nerve ligation, but not by inflammation in the mouse. *Eur. J. Pharmacol.* 403: 81-85.
- Yamamoto, T., Ohtori, S. and Chiba, T. (2000) Effects of pre-emptively administered nociceptin on the development of thermal hyperalgesia induced by two models of experimental mononeuropathy in the rat. *Brain Res.* 871:192-200.
- Decosterd, I. and Woolf, C.J. (2000) Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 87: 149-158.
- Franca, D.S., Souza, A. L., Almeida, K. R., Dolabella, S. S., Martinelli, C. and Coelho, M. M. (2001) B vitamins induce an antinociceptive effect in the acetic acid and formaldehyde models of nociception in mice. *Eur. J. Pharmacol.* 421: 157-164.
- Vogel, H. G. (2005) Drug discovery and evaluation, pharmacological assays. Springer, Berlin, Germany.
- Kontinen, V. K. and Dickenson, A. H. (2000) Effects of midazolam in the spinal nerve ligation model of neuropathic pain in rats. *Pain* 85: 425-431.
- Walsh, R.N. and Cummins, R.A. (1976) The open-field test: a critical review. *Psychol. Bull.* 83: 482-504.
- Guyton, A. C. and Hall, J. E. (2005) Textbook of medical physiology. Saunders, Philadelphia, USA.
- Levy, D. and Zochodne, D.W. (2000) Increased mRNA expression of the B1 and B2 bradykinin receptors and antinociceptive effects of their antagonists in an animal model of neuropathic pain. *Pain* 86: 265-271.
- Saareks, V., Mucha, I., Sievi, E. and Riutta, A. (1998) Nicotinic acid, vitamin B₆ and eicosanoid production. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 358 (1-Suppl.2): R726.