Lamotrigine in an experimental animal model of inflammatory pain

[1]Natalia N. Rus, [2]Corina Bocşan, [2]Anca D. Buzoianu

[1] "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2] Department of Pharmacology, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Abstract. Objective: The purpose of this study is to assess the effects of lamotrigine (LTG) in experimental animal models of pain caused by inflammation. Materials and methods. The study was performed on 50 white male Wistar rats, weighing between 110 and 200 g, randomized into 5 groups: group 1 - control injected with saline solution (0.5 ml/100 g) (C), group 2 – reference pain reliever, metamizole (50 mg/kg) (I), group 3 - reference pain reliever, diclofenac (15 mg/kg) (D), group 4 - LTG (50 mg/kg) (LTG50), group 5 - LTG (20 mg/kg) (LTG20). The evolution of the inflammation was assessed by measuring the initial volume of the foot and, after induction of inflammation, by estimating the corresponding volume at different previously established times, using a plethysmometer Ugo Basile. The difference between the volume measured at different times and the baseline volume represented the volume of the induced edema and the hyperalgesic response latency produced by mechanical compression (analgesy-meter). The mechanical paw pressure test was applied basally after injection (0.5 ml/100g intraperitoneal injection). The average values, error and standard deviation were determined for each group and for each particular moment. Results: The measurement of the kaolin-injected paw volume did not show significant changes over the LTG-induced edema. Groups treated with LTG after inducing the inflammatory process reveal dose-dependent increased pain thresholds. LTG50 increased pain thresholds one hour after recording baseline values before the induction of the inflammatory process, both for the inflamed paw and for the opposite - witness paw. The antihyperalgesic effect is noticeable, compared to the control group, after one hour for LTG50 (p=0.001) and after 1 and 2 hours for LTG20 (p=0.03). Compared to reference pain relievers employed in acute and inflammatory pain, metamizole and diclofenac, LTG did not significantly increase the pain threshold. Conclusions: LTG exhibits dose-dependent antihyperalgesic effects in acute inflammatory pain. In comparison with the control group, LTG displays noticeable antihyperalgesic effect in doses of 50 mg/kg and 20 mg/kg throughout the entire testing period, with similar effect to that of diclofenac during the first hour after administration.

Key Words: antinociceptive action, pain threshold, lamotrigine, inflammatory pain.

Corresponding Author: C. Bocşan, bocsan.corina@umfcluj.ro

Introduction

Most pain-associated clinical symptoms are primarily caused by inflammation. As a cardinal sign of inflammation, pain actually has a protective role against further lesions (Kidd et al 2001). Thus, in normal conditions, inflammation isolates the damaged tissue and promotes tissue restoration, but this process may degenerate, being accused in many chronic diseases (Mungiu 2002).

Tissue damage or the presence of irritants may trigger biochemical changes in peripheral sensory nerve endings, phenomenon called inflammatory process (Winter et al 1962).

The release of proinflammatory mediators influences free sensory nerve endings and generates an enhanced action potential that is transmitted and results in the onset of pain. Persistent inflammation leads to increased excitability in sensory nerve fibers, amplifying response to nociceptive stimuli, phenomenon called hyperalgesia (Winter et al 1962; Kidd et al 2001). Pharmacological interventions in the treatment of inflammatory pain increasingly refer to the mechanisms by which proinflammatory mediators generate pain signals and initiate amplified transmission in neural circuits (Mungiu 2002).

Lamotrigine (LTG) is an antiepileptic drug of the phenyltriazine class, chemically unrelated to existing antiepileptic drugs. LTG blocks voltage-dependent sodium channels and inhibits glutamate release (Nakamura-Craig & Follenfant 1995). Inhibition of voltage-dependent sodium channels leads to the stabilization of the presynaptic membrane and the decrease in the release of excitatory neurotransmitters in spinal dorsal horn neurons. Studies show that LTG produces voltage-dependent blocking of sustained repetitive neuronal discharges in neuronal cultures (Lees & Leach 1993) and inhibits the pathological release of glutamate and glutamate-generated action potentials (Messenheimer 1995). This mechanism supports the use of LTG to control nociception and epilepsy (Messenheimer 1995). Other studies have shown the effect of LTG in the fight against hyperalgesia and allodynia due to partial peripheral nerve injury(Arguelles et al 2002). The complex mechanism of action and the use in different conditions suggests the effect of LTG on voltage-dependent N-type and P-type calcium channels, as well as the inhibition of serotonin, dopamine, acetylcholine and norepinephrine reuptake (Lees & Leach 1993; Stefani et al 1996). The effect on the GABAergic system has also been demonstrated, consisting in

the modulation of inhibitory effects prior to the effects on sodium channels (Stefani et al 1996).

Starting from our previous studies (Rus et al 2013) and on the assumption that new generation antiepileptic drugs interfere with the analgesic process of pain impulse transmission mechanisms, this study aims to test the hypothesis of LTG use in acute inflammatory pain.

Changes in pain threshold following administration of LTG were analyzed by comparison with an analgesic used in acute pain, metamizole and an anti-inflammatory drug commonly used in inflammatory conditions, diclofenac.

Materials and methods

The study was performed based on the modified method described by Winter in 1962 (Winter et al 1962).

The study was performed on 50 white male Wistar rats, weighing between 110 and 200 g, from the biobase of "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca. The animals used for experimental purposes were maintained under standard environmental and nutrition conditions, with constant temperature ($25\pm2°C$) and humidity. The natural light-dark cycle (12/12 hours) was preserved. Animals were randomized into batches of 10 rats each. Food was suspended and water was provided ad libitum for the rats 12 hours before each test. The following groups were formed:

• Group 1 - control injected with saline solution at a dosage of 0.5 ml/100g (C)
• Group 2 - reference pain reliever, metamizole at a dosage of 50 mg/kg (M)
• Group 3 - reference pain reliever, diclofenac at a dose of 15 mg/kg (D)
• Group 4 – LTG at a dosage of 50 mg/kg (LTG50)
• Group 5 – LTG at a dosage of 20 mg/kg (LTG20)

The administered drugs: LTG (purchased from CTX Life Sciences, India, batch: LMJ1100020007), metamizole (Algocalmin vials, 500 mg/ml, purchased from Zentiva Romania), diclofenac (Voltaren vials, 75 mg/3ml, purchased from Novartis Pharma AG) were dissolved or diluted in 0.9% NaCl saline solution (purchased from Infomed Fluids, Romania, batch: 013 13 02 254) and administered intraperitoneally (IP) in a single dose of 0.5 ml/100mg.

The evolution of inflammatory edema was tracked by measuring the initial volume of the foot and, after induction of inflammation, by measuring the corresponding volume at different previously established times (plethysmometer Ugo Basile). The difference between volume values measured at different times and the baseline value represented the volume of the induced edema. Induction of inflammation was performed by injection of 0.1 ml 10% Kaolin (suspended in Tween 80 soil solution) in the left paw, the right paw being a witness for the assessment of the inflammatory process and for the comparison of the analgesic effect of test substances. The evolution of the inflammatory process was monitored by measuring the volume of the paw with a plethysmometer (Ugo Basile 7140), before and after induction of inflammation at 1, 2, 4, 6 and 24 hours.

Mechanical analgesic testing was performed prior to injection (baseline) and after induction of inflammation, after 1, 2, 4, 6 and 24 hours from the injection of the substances to be investigated. Test substances were administered by IP injection 30

minutes after induction of inflammation. The effect of the systemic administration of LTG was studied by measuring the pain threshold (unit/cm^2, 1unit=10 g) corresponding to inflammatory pain using an analgesy-meter (Ugo Basile, Italy).

Statistical analysis was performed using Medcalc software version 12.5. Quantitative data were expressed as mean and standard deviation. Differences between repeated measurements regarding a continuous variable were assessed by ANOVA for repeated measures. Differences between repeated measurements regarding a continuous variable while taken into account the drug that was administered were assessed by mixed-ANOVA for repeated measures. The level of statistical significance was set at $p<0.05$.

Results

Evolution of induced edema in the experimental groups
Edema induced in the experimental groups increases gradually, reaching the maximum value 6 hours after administration of 10% kaolin. As shown in Table 1, in the groups treated with LTG, the drug did not significantly influence induced edema volume when compared to the control group or to the group treated with diclofenac as reference anti-inflammatory drug.

Changes in pain threshold after single-dose administration of investigational drugs (acute administration) in experimental models of pain caused by inflammation
The control group shows small variations in pain threshold throughout the testing period. There is an inverse correlation between pain threshold and edema at 6 hours (increased edema, decreased pain threshold). The group treated with metamizole significantly increased pain threshold values one hour after administration, metamizole being a short-acting analgesic with fast half-time.

The group treated with diclofenac at a dosage of 15 mg/kg, exhibits moderate increase in pain threshold, more obvious in the first hours following administration, corresponding to its half-life in plasma and synovial fluid concentrations.

The groups treated with LTG exhibit increased pain threshold in the first hours after administration (Table 2). One after following injection, LTG50 determines an increase in pain threshold for both the inflamed and the healthy witness paw, above the baseline value measured before induction of inflammation (Fig. 1). LTG20 also increases pain threshold values in the first 2 hours after administration, but without reaching baseline values. There were no significant correlations between edema values and pain threshold measurements for the groups treated with LTG. Groups treated with LTG exhibit significantly increased pain thresholds compared to the control group 1 hour after administration for LTG50 ($p=0.001$), and 1 hour and 2 hours ($p=0.03$) for LTG20 (Figure 2). In comparison with the groups treated with metamizole and diclofenac, LTG50 did not significantly increase pain threshold values at any of the moments chosen for testing.

Discussions and conclusions

The injection of the rat foot with 10% Kaolin, suspended in Tween 80, was used as a model of acute pain caused by inflammation, method described by Winter et al in 1962 and further amended.

Table 1. Evolution of edema after administration of kaolin

Tested group	Edema at 1 hour (ml)	Edema at 2 hours (ml)	Edema at 4 hours (ml)	Edema at 6 hours (ml)	Edema et 24 hours (ml)
C(control)	1.87±0.71	1.96±0.64	2.61±0.95	3.51±1.15	3.18±1.14
M(metamizol)	1.74±0.84	1.92±1.07	1.97±1.07	2.98±1.35	2.86±1.28
D(diclofenac)	1.83±0.84	1.83±0.51	1.76±0.48	2.1±0.96	2.44±0.7
LTG50	1.86±0.61	2.16±0.64	2.42±0.58	2.69±0.43	3.31±0.8
LTG20	2.23±0.58	2.15±0.59	2.62±0.77	2.66±0.43	3.65±0.6

Table 2. Evolution of pain threshold in the inflamed paw after acute administration of investigational drugs at the times chosen for testing (unit/cm^2)

| Tested group	Pain threshold at 1 hour (unit/cm^2)	Pain threshold at 2 hours (unit/cm^2)	Pain threshold at 4 hours (unit/cm^2)	Pain threshold at 6 hours (unit/cm^2)	Pain threshold et 24 hours (unit/cm^2)
C	4.47±1.67	4.76±1.48	4.47±1.46	3.25±1.182	4.57±1.03
M	10.72±4.14	8.10±3.50	6.11±1.64	4.97±1.19	5.70±1.77
D	7.53±1.63	7.63±2.55	5.43±0.99	5.55±1.42	5.87±1.24
LTG50	8.15±2.29	5.37±1.17	3.62±0.88	3.87±0.42	3.45±1.42
LTG20	6.05±1.46	6.3±1.49	4.55±1.21	3.9±0.81	3.57±0.81
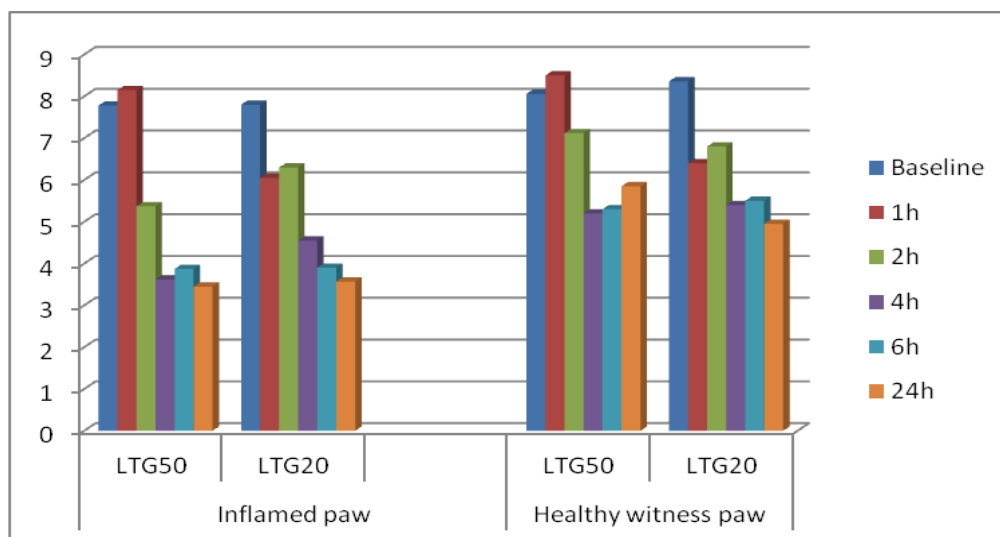
Fig. 1. Comparative evolution of pain threshold following administration of LTG, measured by means of an analgesy-meter (unit/cm^2)
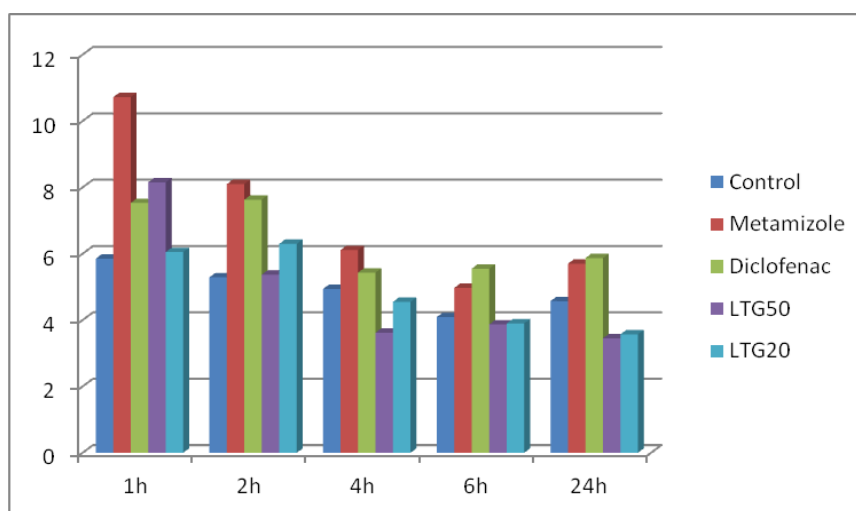

Fig. 2. Evolution of pain threshold following administration of LTG, illustrated by comparison with metamizole, diclofenac and control group (unit/cm^2)

In addition to the induction of inflammatory edema, this model is also a model of painful sensitization of primary afferent nerve fibers. Animal models of inflammation-induced pain show an increased excitability of neuronal circuits in response to the activation of the nerve endings of primary sensory neurons in the spinal cord (Fehrenbacher et al 2003). Activation of sensory nerve endings is the result of the direct action of pro-inflammatory mediators released in response to tissue aggression (Levine & Reichling1999; Mungiu 2002). Among the proinflammatory mediators that directly activate primary afferent nociceptors are bradykinin, serotonin, prostaglandins, hydrogen ions and excitatory amino acids (Levine & Reichling 1999). Considering the modulation of excitatory amino acid release in neuropathies, determined by new generation antiepileptics, the study aimed at finding a possible explanation for the action of LTG in pain caused by inflammation.

The measurement of the paw volume after the injection of kaolin did not show significant changes of the edema after administration of LTG. Studies testing the effect of LTG in inflammatory pain do not mention effects on the induced edema (Lee et al 2002). Unlike other tested AEDs, LTG is indicated in anticonvulsant therapy and psychiatric conditions, without benefiting from expanded therapeutic indications in pain, although many studies conducted in time suggest its use in neuropathic pain (Laughlin et al 2002) and even in acute pain (Nakamura-Craig & Follenfant 1995) .

After the inflammatory process is induced, the groups treated with LTG reveal dose-dependent increased pain thresholds. After one hour, LTG50 increase the pain threshold for both the inflamed and the healthy witness paw, over baseline values recorded before the induction of the inflammatory process. The antihyperalgesic effect is obvious when compared to the control group, after one hour for LTG50 and after one and two hours for LTG20. In comparison with reference pain relievers employed in acute and inflammatory pain, metamizole and diclofenac, LTG does not show a statistically significant increase in the pain threshold. Erichsen et al. obtained similar results to those in our study, suggesting that doses of 10, 30 and 60 mg/kg LTG administered intraperitoneally significantly attenuated mechanical hyperalgesia after peripheral nerve injury (Erichsen et al 2003). Employing a model of induction of inflammation similar to ours, Lee et al. found that LTG administered intrathecally has antihyperalgesic effect when administered before and after the inflammatory process was induced (Lee et al 2002). Similar to our results, they found that LTG suppresses mechanical and thermal hyperalgesia, a dose-dependent influence (Lee et al 2002), in the first 3 hours after administration. Although in vitro pharmacological studies have suggested the inhibition of voltage-dependent Na+ channel as a major mechanism of action in secondary hyperalgesia associated with joint inflammation, where LTG seems to act on the transmission of nerve impulses by means of excitatory amino acids (Lee et al 2002). Moreover, it has been shown that the development of secondary hyperalgesia associated with joint inflammation involves receptors, NMDA, nonNMDA and GABA (Rees et al 1995). The release of excitatory amino acids, nitric oxide and proinflammatory prostaglandins is involved in the development of hyperalgesia after the induction of nerve injury or of an inflammatory process, as shown by studies in animal models (Coderre & Melzack 1992; Dickenson 1997; Cairns et al 2003). Nakamura-Craig also

reported analgesic effects in models of acute pain induced by injection of PGE2 during LTG administration, before and after the induction of hyperalgesia, as well as inhibiting effects on the development of hyperalgesia induced by repeated injection of PGE2 (Nakamura-Craig & Follenfant 1995).

There is a consensus regarding the influence of NMDA receptors in the abnormal transmission of pain signals and sustained hyperalgesia, but regarding the mechanism by which LTG acts in inflammatory conditions cannot exclude the possible inhibition of excitatory amino acid release determined by Na+ channel blockade (Lee et al 2002). More recent studies show that chronically administered LTG reduces brain cyclooxygenase activity, the concentration of PGE2 and NF-κB activity (transcription factor that controls cytokine production through COX2) being important indicators of bipolar disorders (Ramadan et al 2012). The same study shows that LTG blocks the effects of excitatory amino acids mediated through NMDA receptors (Ramadan et al 2012). The most likely mechanism suggested by most experimental studies, in which LTG acts in inflammatory pain, is the modulation of excitatory amino acid release and of aberrant transmission of nerve impulses to response coordinating centers. There is need for comprehensive studies to determine whether Ca^{2+} (Stefani et al 1996) and Na^+ (Lees & Leach, 1993) channel blockade reported in some studies occurs before the modulation of excitatory amino acid release by stabilizing the presynaptic membrane.

A review of clinical trials on the use of LTG in acute and chronic pain suggests, based on efficiency coefficients (NNT number of patients needed to treated in order for 50% of them to show significant clinical improvements) and safety coefficients (NNH number of patients needed to harm in order for 50% of them to show minor or major adverse reactions), that no there is no convincing data demonstrating that LTG is effective in acute or chronic pain at doses between 200 and 400 mg/day (Wiffen et al. 2011). Nevertheless, the authors acknowledge that there are no clinical studies investigating LTG in acute pain, given that the presence of rash as adverse effect is limited in long-term therapy (Wiffen et al. 2011).

This study suggests the possibility of using LTG in acute inflammatory pain therapy provided that therapy with NSAIDs is limited. Besides the analgesic profile comparable to that of NSAIDs, LTG yields proven anti-anxiety effects (Mirza et al 2005), useful in acute pain conditions. The efficiency in acute inflammatory pain therapy may be useful in patients undergoing anti-epileptic drug treatment, where the use of conventional therapies in such situations may cause significant side effects. The treatment for acute inflammatory pain requires clinical studies to establish the effective analgesic dose of LTG and to confirm the safe use of LTG.

References

Argüelles CF, Torres-López JE, Granados-Soto V. Peripheral antinociceptive action of morphine and the synergistic interaction with lamotrigine. Anesthesiology 2002;96(4):921-5.

Cairns BE, Svensson P, Wang K, Hupfeld S, Graven-Nielsen T, Sessle BJ, et al. Activation of peripheral NMDA receptors contributes to human pain and rat afferent discharges evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2003;90(4):2098-2105.

Coderre TJ, Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 1992;12(9):3665–70.

Dickenson A. Mechanisms of central hypersensitivity: excitatory aminoacids mechanisms and their control, in A. Dickenson, JM Beson(eds). The Pharmacology of Pain. 1997;Springer Verlag: Berlin p:167-210.

Erichsen HK, Hao JX, Xu XJ, Blackburn-Munro G. A comparison of the antinociceptive effects of voltage-activated Na+ channel blockers in two rat models of neuropathic pain. Eur J Pharmacol 2003;458:275-82.

Fehrenbacher JC, Taylor CP, Vasko MR. Pregabalin and gabapentin reduce release of substance P and CGRP from rat spinal tissues only after inflammation or activation of protein kinase C. Pain 2003;105:133–44.

Kidd BL, Urban LA. Mechanisms of inflammatory pain. Br J Anaesth 2001;87(1):3-11.

Laughlin TM, Tram KV, Wilcox GL, Birnbaum AK. Comparison of antiepileptic drugs tiagabine, lamotrigine, and gabapentin in mouse models of acute, prolonged, and chronic nociception. J Pharmacol Exp Ther 2002;302(3):1168-75.

Lee TH, Wang CJ, Wu PC, Buerkle H, Lin SH, Yang LC. The thermal and mechanical anti-hyperalgesic effects of pre-versus post-intrathechal treatment with lamotrigine in a rat model of inflammatory pain. Life Sci 2002;70(25):3039-47.

Lees G, Leach MJ. Studies on the mechanism of action of the novel anticonvulsant lamotrigine (Lamictal) using primary neurological cultures from rat cortex. Brain Res 1997;612(1-2):190-9.

Levine JD, Reichling DB. "Peripheral mechanisms of inflammatory pain", in P. D. Wall, R. Melzack (eds), Textbook of Pain, 1999;Churchill Livingstone, New York, p.59-84.

Messenheimer JA. Lamotrigine. Epilepsia 1995;36(Suppl 2):S87–S94.

Mirza NR, Bright JL, Stanhope KJ, Wyatt A, Harrington NR. Lamotrigine has an anxiolytic-like profile in the rat conditioned emotional response test of anxiety: a potential role for sodium channels?. Psychopharmacology (Berl) 2005;180(1):159-68.

Mungiu OC. Tratat de algeziologie. 2002; Editura Polirom: Iasi p: 45-95.

Nakamura-Craig M, Follenfant RL. Effect of lamotrigine in the acute and chronic hyperalgesia induced by PGE2 and in the chronic hyperalgesia in rats with streptozotocin-induced diabetes. Pain 1995;63(1):33-7.

Ramadan E, Basselin M, Rao JS, Chang L, Chen M, Ma K, Rapoport SI. Lamotrigine blocks NMDA receptor initiated arachidonic acid signalling in rat brain: implications for its efficacy in bipolar disorder. Int J Neuropsychopharmacol 2012;15(7):931-43.

Rees H, Sluka KA, Westlund KN, Willis WD. The role of glutamate and GABA receptors in the generation of dorsal root reflexes by acute arthritis in the anaesthetized rat. J Physiol 1995;484(Pt. 2):437–45.

Rus NN, Bocşan C, Vesa ŞC, Coadă CA, Buzoianu AD. Topiramate in nociceptive pain - experimental analgesia study. HVM Bioflux 2013;5(2):70-76.

Stefani A, Spadoni F, Siniscalchi A, Bernardi G. Lamotrigine inhibits calcium currents in cortical neurons: functional implications. Eur J Pharmacol 1996;307(1):113-6.

Wiffen PJ, Derry S, Moore RA. Lamotrigine for acute and chronic pain. Cochrane Database Syst Rev 2011;16;(2):CD006044.

Winter CA, Risley EA, Nuss GW. Carrageen-induced oedema in hind paw of the rats – an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-547.

Authors

•Natalia N. Rus,"Iuliu Haţieganu" University of Medicine and Pharmacy, 6th Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, EU, email: nrus76@yahoo.com

•Corina Bocşan, Department of Pharmacology, "Iuliu Haţieganu" University of Medicine and Pharmacy, 6th Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, EU, email: bocsan.corina@umfcluj.ro

•Anca D. Buzoianu, Department of Pharmacology, "Iuliu Haţieganu" University of Medicine and Pharmacy, 6th Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, EU, email: abuzoianu@umfcluj.ro