Medical Journal of Ahl al-Bayt University, Vol.2, No.1, Augest.2023

Selegiline Potentiates the Analgesic Effects of Diclofenac in Male Mice

Ahmed Ibraheem Rashid[1], Selman Mohammed Selman[2], Rusul Ali Kadhem[3], Hadi Fadhil Alyasari[4]

[1, 2, 3] College of Medicine/University of Babylon/ Dep. of Pharmacology, Iraq

[4] College of Medicine/University of Babylon/ Dep. of Microbiology, Iraq

Corresponding Author E-mail:

ahmed.ibraheem@uobabylon.edu.iq (AI) Med.salman.salman@uobabylon.edu.iq (S.M.), dr.rusul_alkafaji@uobabylon.edu.iq (RA), hadialyasari@gmail.com (H.A.)

Abstract

Introduction: One of the most important annoying sensory and affection is the pain which associated present or forthcoming tissue damage or referenced to such damage. Many drugs can be used for treating pain and nonsterodial anti-inflammatory drugs are the commonest agents uses for this purpose is diclofenac (Voltaren). The main actions involved for the analgesic, antipyretic, and anti-inflammatory, effect is blocking COX-1 enzyme then inhibition of prostaglandin synthesis. Selegiline is a monoamine oxidase type B that is used in advance for the management of wearing-off symptoms. One of the most important medications that is engaged in the antinociceptive mechanism which has a real function on certain CNS structure involved in the modulation of the pain like striatum, cerebral cortex, and spinal cord, is Monoamines**.** it was known recently a link between the intensity of the pain after operation and polymorphism of MAO-B in man, referring to possibility of potential action of monoamine oxidase-B in the perception of pain.

Materials and methods: Forty adult male mice were used in the research. The range of their weights were 25-30 g. Estimation of the time to twisting of the tail or flicking of the tail i.e., the action referred to the tail-flick latency. The animals were grouped into 4 groups randomly. Each group had eight animals: Group one (it is a control group): The mice in this group received N.S. similar volume of the diclofenac. Group two: each mouse received diclofenac, 3 mg/kg, IP. Group three: each mouse received 5mg/kg of selegiline, P.O. Group four: each

mouse received 5 mg/kg of selegiline, P.O. 30 minutes prior to the giving of diclofenac, 3 mg/kg, I.P.

Results: In group 2 which received diclofenac 3mg/kg, tail-flick latency significantly rised ($P < 0.05$). Effects of selegiline significantly ($P < 0.05$) prolonged after 60 minutes as compared to the baseline values of the same group and the corresponding time of control group. In the two times intervals, the tail-flick latency significantly decreased ($P<0.05$) as compared to similar time of diclofenac. Combination of selegiline with dilclofenac caused significantly ($P < 0.05$) increased.

of the time latency after thirty minutes and sixty minutes as compared to the baseline values of the same group as well as to the similar times of group 1 (control group) and selegiline group.

Keywords: Selegiline, Diclofenac, Pain, Nonsterodial anti-inflammatory drugs, Analgesic, Antipyretic, Monoamine oxidase type B, Monoamines, Male Mice.

Introduction:

One of the most important annoying sensory and affection is the pain Which associated present or forthcoming the damage of the tissues or referenced to such damage. Pain has many forms. It warns that there is tissue damage, which is cardinal for keeping away the tissue from the damage and necessarily for survival [1]. Pain may be caused by irritation of the receptors, which are called nociceptors. These receptors are free nerve endings which react to the stimulus of pain. The irritation of pain receptors can lead to feelings of pain, and these receptors may be present in internal organs, joints, and skin. The injury to the nervous system can also be in the brain and spinal cord, and peripheral nerves can induce pain [2]. There are many tests for detection and estimation of severity of pain one of them is tail flick test. In this test, the radiant heat was applied on the tail of the animal, when the mouse or rat sense a discomfort, the animal behave by a sudden movement of the tail (tail flick) and then the reaction time was measured (It is the time from the starting of the stimulation until the observation of the response of the animal)[3]. Tail flick test was used particularly to study the analgesic effect of the pharmacological agents. This test may also be employed to estimate the sensitivity of the basal thermal pain or to demonstrate the consideration of the gene differences between the control groups of the animal. Many drugs can be used for treating pain and nonsterodial anti-inflammatory drugs are the commonest agents uses for this purpose such as diclofenac. The mechanism of action of these agents are analgesic, antipyretic,

and anti-inflammatory effects. These agents inhibit the synthesis of prostaglandins by inhibition of cyclo-oxygenase-2 (COX-2) which is also called prostaglandin-endoperoxide synthase-2 (PGES-2). The drug also may have bacteriostatic effect by blocking bacterial DNA synthesis [4]. Selegiline inhibits monoamine oxidase type B (MAO-B inhibitor). It may be used in the more advance Parkinson's disease for t. management of wearing-off signs, and there much research has reported modest decreases in signs, duration of wearing-off, on-off episodes, and other symptoms. [5]. The monoamine oxidase type B (MAO-B inhibitor) its sound to be that patients with neurodegenerative disease Parkinson's disease (PD) can be clinically response to the treatment with selegiline and it is shown to be effective [6]. One of the most important medications that engaged in the antinociceptive mechanism which has a real function on certain CNS structure involved the modulation of the pain like striatum, cerebral cortex, and spinal cord, is monoamines [7]. The serious action which played the two subtypes of monoamine oxidase in the metabolism of monoamine, monoamine oxidase inhibitors denote an effective way for the management of many neurological diseases such as Parkinson's disease, anxiety, and depression [6]. Pain, interestingly, is the most symptom which is presented in some patients with pathologies of Parkinson's disease or depressio [8]. Also, it was known now that a relationship between the functional polymorphism of monoamine oxidase- type B and the intensity of the pain after surgery in the patients, referring to a potential action of mono amine oxidase-B in the pain perception [9].

Materials and methods

Animals:

Adult forty male mice (the range of the weight are 25-30 g) were used in the research. The research was done in the Animal House / University of Babylon l College of Medicine, twelve hours darck-light cycles with. Standard *diet adlibitum* and tap water were given at 25 °C. The animals, after 14 days of adaptation, were randomly divided into four groups. The number of ground meat in each group was eight mice.

Group one (control group): Each mouse received NS equal to the volume of selegilin, P.O.
Group Two: 3 mg per kg of diclofenac was administered I.P. for each mouse.
Group Three: 5mg per kg of selegilin was administered P.O. for each mouse.
Group Four: 5mg per kg of selegilin was administered P.O. 30 minutes before the administration of diclofenac, I.P.

Estimation of antinociceptive effect of the agents

Estimation of anti-nociceptive effects of agents were done by the tail flick test.[10] The animal was placed in an appropriate fixed container on the water bath allowing the last two cm of the tail of the animal deep in the water with temperature 50 ± 0.2 °C. The time was measured until the tail flicking or twisting, this time called the tail-flick latency. Each animal is verified at many time intervals; 0-minute, 30 minute, and 60 minutes after receiving the agents. In addition to that, the animals were verified for many trials, which means each animal was evaluated 3 trials in each interval divided by three to five minutes. For each animal, the time of tail-flick latency is the mean of three values of the measurements. The cut-off time, in each trial, was 3 minutes to prevent the damage of the tissue.

Drugs

Selegiline HCl tablet, 5 mg (APOTEX CORP.) was dissolved in ten ml of NS and the final concentration was 0.5 mg per ml. Ampule of diclofenac sodium 75 per 2ml (Olfen-75mg, Acino co, Switzerland) was applied in this research.

Statistical analysis

The outcomes of this research were stated as mean ± standard error mean (SEM). SPSS (version 21) did statistical analysis. For comparison between means, one-way ANOVA was used. The Values were considered as significant statistically when the P value< 0.05.

Results

1. Control group.

In the control group (group 1) which received normal saline, the time of tail-flick latency after thirty minutes show no significant changes ($P>0.05$) as compared with the time 0 as well as after sixty minutes (Figure 1 & Table 1)

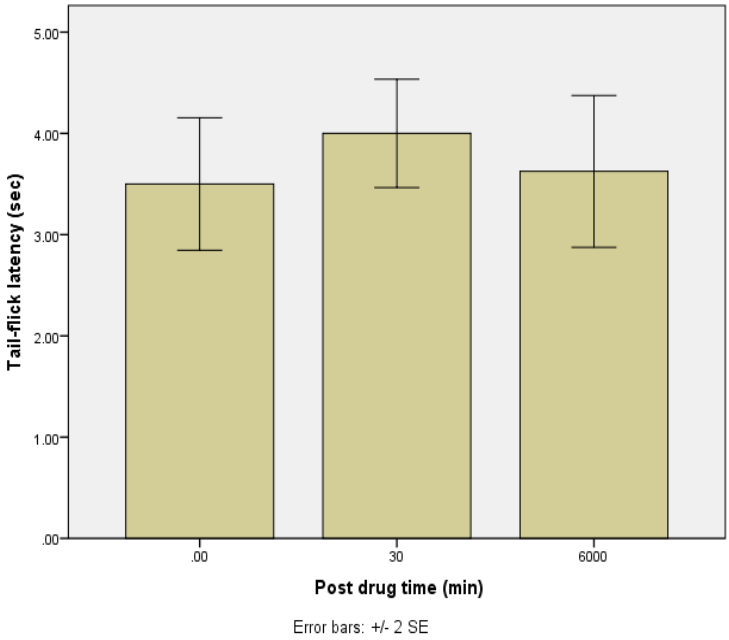

Figure No. 1: Tail-flick latency in sec (mean ± SEM) of control group. (n = 8 mice)

2. Effects of diclofenac

In group 2 which received diclofenac 3mg/kg, tail-flick latency significantly. amplified ($P < 0.05$) in the two-time intervals as compared with baseline values within the same group and the values of control group.

There were no significant changes after sixty minutes as compared with the tail-flick latency after thirty minutes ($P > 0.05$). The tail-flick latency significantly increased in the 2 times intervals as compared to analogous time of selegiline ($P > 0.05$).

(Figures 2,3, and 4 and Table 1)

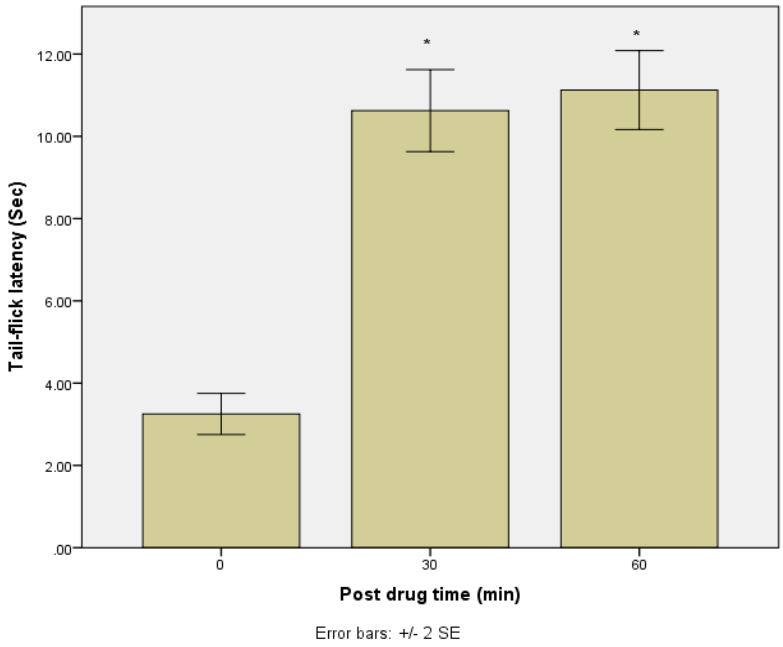

Figure No. 2: Tail-flick latency in sec (mean ± SEM) of group 2 which received diclofenac (3mg/kg). (n = 8 mice)

*= Significantly (P<0.05) increased as compared to time 0.

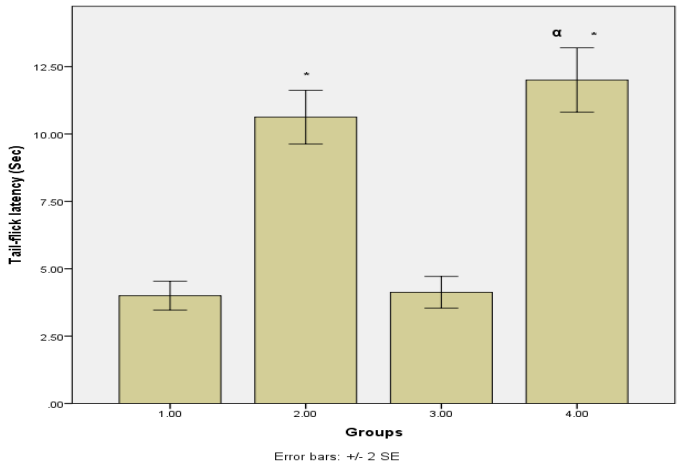

Figure No. 3: Tail-flick latency in sec (mean ± SEM) after 30 min. interval of all groups. G 1: control group. G 2(diclofenac, 3mg/kg). G 3 (selegiline, 5mg/kg). G 4 (selegiline, 5mg/kg and diclofenac (3mg/kg). (No. = 8)

*= Significantly (P<0.05) increased as compared to control group (groups 1) and group 3.
α= Significantly (P<0.05) increased as compared to group 2.

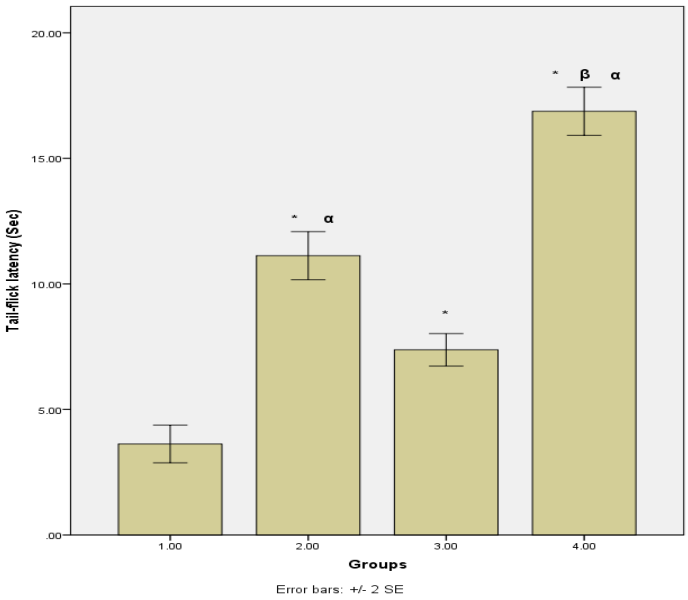

Figure No. 4: Tail-flick latency in sec (mean ± SEM) after 60 min. interval of all groups. G 1: control group. G 2(diclofenac, 3mg/kg). G 3 (selegiline, 5mg/kg). G 4 (selegiline, 5mg/kg and diclofenac (3mg/kg). (No. = 8)
*= Significantly (P<0.05) increased as compared to group 1.
α= Significantly (P<0.05) increased as compared to groups 1 and 3.
B= Significantly (P<0.05) increased as compared to group 2.

3. Effects of selegiline

There were no significant variations (P > 0.05) in the latency of the time of tail-flick in the group 3 (selegiline, 5 mg/kg) afterward thirty minutes as compared to the baseline values of the same group as well as to consistent time of group 1 (control group), while it significantly (P < 0.05) prolonged after sixty minutes as compared with the baseline values of the same group and the corresponding time of control group. The tail-flick latency significantly (P < 0.05) decreased in the 2 times intervals as compared with the corresponding time of diclofenac (Figures 3, 4, & 5 and Table 1)

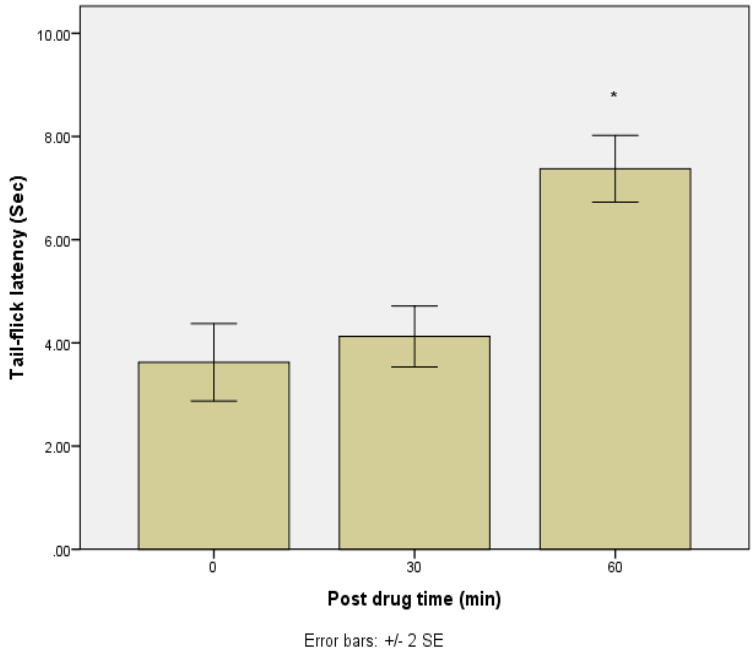

Figure No. 5: Tail-flick latency in sec (mean ± SEM) of group 3 which received selegiline (5mg/kg). (n = 8 mice)
*= Significantly(P<0.05) increased as compared to times 0 and 30.

4. Effects of combination of selegiline and dilclofenac

The latency of the time of the tail-flick test, increased significantly ($P < 0.05$) after thirty minutes and sixty minutes in the group 4 (diclofenac, 3mg per kg and selegiline, 5 mg per kg), as compared with group 1 (control group) and selegiline group. The data revealed no significant ($P > 0.05$) variations after 30 minutes as compared with an analogous time of group 2 (diclofenac group). The data, also, show that the time of tail-flick latency increased significantly ($P < 0.05$) in the combination of the selegiline and diclofenac (Figures 3, 4, and 6 and Table 1)

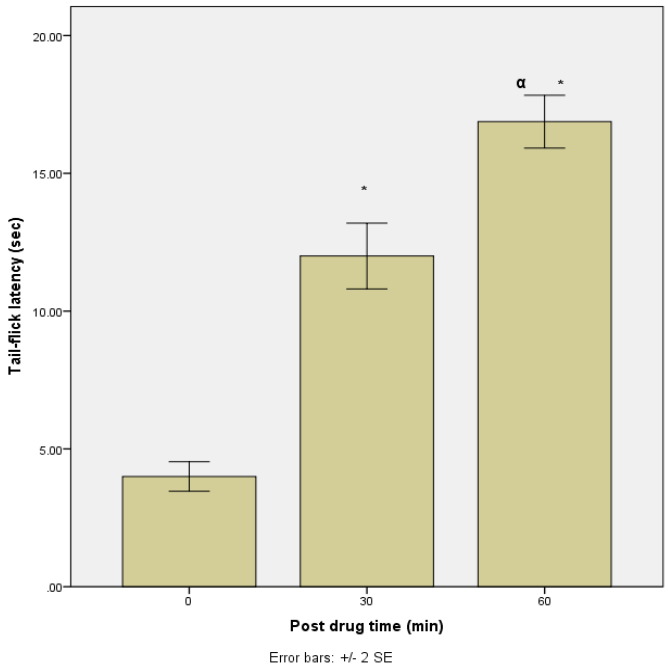

Figure No. 6: Tail-flick latency in sec (mean ± SEM) of group 4 which received combination of diclofenac (3mg/kg) and selegiline (5mg/kg). (n = 8 mice)

*= Significantly (P<0.05) increased as compared to time 0.
α= Significantly (P<0.05) increased as compared to time 30.

Table No. 1: Tail-flick latency in sec (mean ± SEM) of diclofenac (3mg/kg), selegiline (5mg/kg), and combination of selegiline (5mg/kg) and diclofenac (3mg/kg). (No. =8))

Drug	Post drug time (Min)		
	0	30	60
Saline	3.5000 ± 0.32733	4.0000 ± 0.26726	3.6250 ± 0.37500
Diclofenac	3.2500 ± 0.25000	10.6250 ± 0.49776*	11.1250 ± 0.47949*
Selegiline	3.6250 ± 0.37500	3.6250 ± 2.7383	7.3750 ± 0.32390*
Diclofenac and selegiline	4.0000 ± 0.26726	12.0000 ± 0.47949*	16.8750 ± 0.47949*

* The mean difference is significant (P< 0.05).

Discussion

This current study revealed the analgesic properties of diclofenac as an anti-inflammatory drug and the effect of selegilin that was found to have the potentiated analgesic effect of diclofenac. These results were assessed by using a tail flick test.

Adela Hilda, 2015 found that serotonin and norepinephrine reuptake inhibitors effects on the CNS, related to the management of acute pain and their efficacious effect in decreasing postoperative chronic pain. The efficacy of these agents results from its ability to inhibit the nociceptive input by modulation the descending inhibitory pathway. There are many researchers who reported the analgesic effect of duloxetine, which is like selegilin, and these results agreed with this study regarding analgesic action of selegilin [11]. Gomes Villarinho Sara (2012) found that in a study for assessment the effect of monoamine oxidase B (MAO-B), which is the main enzyme involved in the metabolism of monoamine, on the neuropathic (partial sciatic nerve ligation) pain models and on postoperative (plantar incision) in the mice. Selegiline, which is irreversible and selective monoamine oxidase inhibitor, at a dose 10 mg per kg, which is enough dose to selectively inhibit the activity of MAO-B enzyme, showed an anti-allodynic properties from 0.5-6 hours after an incisional model of pain. As well as partial ligation of the sciatic nerve in the mice also caused mechanical allodynia, which are inhibited by selegiline, 10 mg per kg, from 2-6 hours after treatment. That means, MAO-B enzyme may play a role in the development of neuropathic and posto. ative pains [12]. However, these results are in harmony with current study regarding pain control. P. K. Gillman (2005) study the interaction between opioids and selegilin like drugs and revealed that excessive intra-synaptic serotonin causes toxicity referred serotonin syndrome. However, opioids such as propoxyphene, dextromethorphan, methadone, tramadol, and pethidine (meperidine) have a weak ability to the serotonin re-uptake. So, all these drugs have been involved in the reactions of the serotonin toxicity with monoamine oxidase inhibitors which may cause some fatialities. Buprenorphine, oxycodone, codeine, and orphine are known not to be serotonin re-uptake inhibitors, and do not cause serotonin toxicity with MAOIs [13]. Grasing K (2005) study the effects of selegiline in high dose on the precipitating withdrawal symptoms of morphine in the model of addiction in rats. He reported that seligiline in high doses can reduce the morphine-reinforced behavior and these properties may occur due to the psychostimulant metabolites of seligiline [14].

The data of this study show that seligiline potentiate the analgesic effect of diclofenac in male mice. According to these results, the dose of diclofenac can be decreased if it is combined with seligiline for reducing the adverse effects of diclofenac.

Conclusion

The data of this study revealed that seligiline has antinociceptive effect in mice, as well as seligiline potentiates the analgesic effect of diclofenac.

References

1- Konttinen YT, Kemppinen P, Segerberg M, Hukkanen M, Rees R, Santavirta S, Sorsa T, Pertovaara A, Polak JM. Peripheral, and spinal neural mechanisms in arthritis, with reference to treatment of inflammation and pain. Arthritis Rheum; 1994; 37: 965-982

2- Gebhart GF, McCormack KJ. Neuronal plasticity. Implication for pain therapy. Drugs.1994; 47 (suppl 5): 1-47

3- Fender C, Fujinaga M, Maze M. Strain differences in the antinociceptive effect of nitrous oxide on the tail flick test in rats. *Anesth. Analg.* 2000; **90** (1): 195–9.

4- Lewis AJ, Furst DW. Nonsteroidal Anti-Inflammatory Druds: Mechanism and Clinicle Use. Marcel Dekker, New York, 1987

5- Riederer P, Lachenmayer L, Laux G. "Clinical applications of MAO-inhibitors". *Current Medicinal Chemistry*. 2004; **11** (15): 2033–2043.

6- Riederer P, Lachenmayer L. "Selegiline'sneuroprotective capacity revisited". *Journal of Neural Transmission*. 3003; **110** (11): 1273–1278.

7- Magyar K. "The pharmacology of selegiline". In Youdim M, Riederer P (eds.). *Monoamine Oxidases and Their Inhibitors*. 2011; International Review of Neurobiology. Vol. 100. Academic Press.

8- Knaster, P., Karlsson, H., Estlander, E. M., &Kalso, E. Psychiatric disorders as assessed with SCID in chronic pain patients: The anxiety disorders precede the onset of pain. *General Hospital Psychiatry, 2012; 34*, 46-52.

9- Yong-Xin Sun, Xiao-Hong Wang, Ai-HuaXu, Jiu-Han Zhao, Functional polymorphisms of the MAO gene with Parkinson disease susceptibility: A meta-analysis, Journal of the Neurological Sciences, 2014; 345, 1-2, (97-105).

10- Animal Models of Nociception. *Journal of Pharmacology and Experimental Therapeutics* (2001) **53** (4): 597–652.

11- Adela Hilda Onuţu Rom. Duloxetine, an antidepressant with analgesic properties – a preliminary analysis. J Anaesth Intensive Care, 2015; 22(2): 123–128.

12- Jardel Gomes Villarinho, Sara Marchesan Oliveira, Cássia Regina Silva, ThaíssaNunesCabreira, Juliano Ferreira. Involvement of monoamine oxidase B on models of postoperative and neuropathic pain in mice, 2012; 5;690(1-3):107-14

13- P. K. Gillman. Monoamine oxidase inhibitors, opioid analgesics, and serotonin toxicity British Journal of 2005, 95, 4, 434–441

14- Grasing K. and He S. Effects of high dose selegiline on morphine reinforcement and precipitated withdrawal in dependent rats. Behav. Pharmacol. 2005;16(1):1-13.