



## TO STUDY THE ANTINOCICEPTIVE EFFECT OF CINNARIZINE ALONE AND IN COMBINATION WITH TRAMADOL IN ALBINO RATS BY TAIL FLICK METHOD

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### ABSTRACT:

**Background:** Pain is the most common reason for physician consultation in most countries. It is a major symptom in many medical conditions, and can interfere with a person's quality of life and general functioning. Acute pain is usually managed with medications such as analgesics and anesthetics. Cinnarizine is a piperazine derivative and Tramadol is an opioid analgesic. Cinnarizine is also a calcium channel blocker. So, in the present study an attempt has been made to access the antinociceptive activity of Cinnarizine alone & in combination with standard drug Tramadol with the hope of pharmacological synergism and better relief from pain.

**Material & Methods:** 4 rats in 6 groups were weighed & colour coded. Basal reaction time to radiant heat was taken. Tail withdrawal time was recorded as the end point. Reaction time was taken at 0, 30 & 60 minutes on day 0, 7, 14, 21 & 28.

**Result:** All observation were done by the ANOVA followed by post hoc Tukey's. It is seen that Cinnarizine alone (in both doses) has antinociceptive activity but that is not statistically significant. But when it is given along with Tramadol it potentiates antinociceptive activity of Tramadol which is statistically significant.

**Conclusion:** The result of this study conclude that Cinnarizine alone has antinociceptive activity in both doses (i.e 2.5mg/kg & 5mg/kg) but it is statistically not significant. Tramadol shows higher antinociception with Cinnarizine in dose of 5mg/kg than with 2.5mg/kg.

**Keywords:** Antinociception, Cinnarizine, Tramadol, Tail flick test, Albino rats

### INTRODUCTION

Pain is the most common reason for physician consultation in most countries.<sup>1,2</sup> It is a major symptom in many medical conditions, and can interfere with a person's quality of life and general functioning.<sup>3</sup> Psychological factors such as social support, hypnotic suggestion, excitement, or distraction can significantly affect pain's intensity or unpleasantness.<sup>4,5</sup> Pain is an enormous global health problem. WHO has estimated that 1 in 10 adults are diagnosed with chronic pain each year. Those who experience pain can experience acute, chronic, or intermittent pain, or a combination of the all. 10% of the world's populations endure chronic pain.<sup>6</sup> Physiologists distinguish between pain and nociception. Nociception is the sensory nervous system's response to certain harmful or potentially harmful stimuli. Pain is the unpleasant emotional experience that usually accompanies nociception.

Acute pain is usually managed with medications such as analgesics and anesthetics. Management of chronic pain, however, is much more difficult and may require the coordinated efforts of a pain management team,

which typically includes medical practitioners, physician assistants and nurses.<sup>7</sup>

Several studies on the antinociceptive effect of calcium channel blockers (CCBs) + opioids like morphine have reported significantly higher antinociceptive effect than that produced by either of the drugs administered alone. In many cases, CCBs did not have any antinociceptive effect by itself, suggesting a synergistic interaction. These experimental works depended upon exposing the animals to brief thermal stimuli as in the tail-flick and the hot plate tests.<sup>8</sup>

Cinnarizine is a piperazine derivative and H<sup>1</sup> histamine antagonist. It is also a calcium channel blocker.<sup>9</sup> It is mainly used and a well established drug for the control of vomiting due to motion sickness and can inhibit smooth muscle contraction by blocking calcium channels. At the level of presynaptic terminal it also blocks voltage gated calcium channels. So when action potential reaches the axonal terminal the already blocked calcium channels do not allow Ca<sup>2+</sup> to enter the axonal terminal. It will reduce the movement of neurotransmitter toward synaptic cleft. Thus the action

potential transmission to post synaptic terminal will be attenuated.

If this study proves that Cinnarizine has antinociceptive effect then it can also be used to treat pain. So, in the present study an attempt has been made to access the antinociceptive activity of Cinnarizine alone & in combination with standard drug Tramadol with the hope of pharmacological synergism and better relief from pain.

#### Aims and Objectives of the study

- To evaluate antinociceptive effect of Cinnarizine in two different doses
- To evaluate the antinociceptive effect of Tramadol in combination with Cinnarizine in two different doses

#### Material & Methods

Healthy male wistar Albino rats were procured from authorized supplier from Kolkata and rats weighing between 150-250 gms were taken for the present study. The animals were kept in clean and dry cages with 12 h:12 h light-dark cycle at normal room temperature and humidity. They were acclimatized to the available housing condition in the cages for the period of 2 weeks before using for the experiment and were fed with standard laboratory diet consisting of soaked black gram (Kala Chana), maize and water was given *ad libitum*. Arrangements were made to ensure regular cleaning of cages and disposal of excreta and urine. The experimental protocol was approved by the Institutional Animal Ethics Committee and whole experiment was conducted according to the guidelines and ethical norms approved by Institutional Animal Ethics Committee (IAEC) Guidelines.

#### Place of study

The study was carried out in the Postgraduate research laboratory, Department of Pharmacology, Rajendra Institute of Medical Sciences, Ranchi after approval from Institutional Animal Ethics Committee (IAEC).

#### Test Drug

Cinnarizine (25mg tablet)

#### Standard Drug

Tramadol Hydrochloride (50mg tablet)

#### Inclusion criteria

- Male wistar albino rats
- Weight between 150-250 gms
- Healthy and active in their cage

#### Exclusion criteria

- Female rats (because of cyclic hormonal effect on drugs can modify results)

- Weight <150 gms & >250 gms
- Diseased and Inactive in their cage

The total duration of experiment was 28 days and the experiments were done at 1 week intervals i.e on day 0, 7, 14, 21 & 28. Rats were given respective treatments in the morning hour at 09:30-10:30 am. Experiments were conducted between 10AM to 4PM to minimize the diurnal variation.

#### Study Groups

For this study, 24 Albino rats were taken and grouped randomly into 6 groups, 4 in each group.<sup>10</sup> All the groups received the respective drugs by oral route through gavage tube.

**Group I:** Control – Received 1ml of distilled water p.o

**Group II:** Standard – Received Tramadol 10mg/kg of body weight p.o

**Group III:** Received Cinnarizine 2.5 mg/kg body weight p.o

**Group IV:** Received Cinnarizine 5 mg/kg body weight p.o

**Group V:** Received Tramadol 10mg/kg & Cinnarizine 2.5 mg/kg body weight p.o

**Group VI:** Received Tramadol 10 mg/kg & Cinnarizine 5 mg/kg body weight p.o

#### Dose calculation

Dose of the drugs were calculated from the standard clinical human dose on the basis of surface area. Surface area ratio of 200 gm rat to 70 kg man is 0.018.<sup>11,12</sup> Thus, human dose of any drug (for a 70 kg person) multiplied by 0.018 gives the value of that drug for 200gm of rat. Multiplying that product with 5 will give per kg value for rats.

**Cinnarizine:** The daily dose of Cinnarizine for motion sickness or prophylaxis of migraine is 25 & 50 mg/day for average 70 kg adult.<sup>13</sup> So, per kg value for rats came approximately 2.5 & 5mg/kg. For every group, different strengths of Cinnarizine were prepared. Now during oral administration the volume of Cinnarizine solution was adjusted according to the body weight of the rat.

**Tramadol:** The capsule was powdered and a uniform solution was made by using distilled water. The daily dose of Tramadol for acute pain is 100 mg/day for average 70 kg adult.<sup>14</sup> So, per kg value for rats came approximately 10mg/kg. Now during oral administration the volume of Tramadol solution was adjusted according to the body weight of the rat.

#### Methodology:

Analgesia was measured using modified method of D'Amour and Smith called as tail flick method using an

analgesiometer. Tail flick latencies (reaction time) of the animal were assessed. The strength of the current passing through the naked nichrome wire was kept constant at 5 amps. The distance between the heat source & tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The time taken by the animal to withdraw (flick) its tail from the hot wire was noted and taken as the 'reaction time. This tail flicking is considered as the end point of this test and time is measured. The cut-off time of 15 sec is planned to avoid any tissue damage. Normal reaction time was noted in each animal before starting the experiment. These readings were taken as reaction time at 0 minutes. Following initial reading all animals received the respective drugs orally and the effects were measured after 30 & 60 minutes in the tests. All observations were made between 10 am to 4 pm in a noiseless diffusely illuminated room.

**Principle:** The application of thermal radiation to the tail of an animal provokes the withdrawal of the tail by a brief vigorous movement. It is the reaction time of this movement that was recorded.

#### Procedure of Tail Flick test

- 4 rats in 6 groups were weighed & colour coded.
- At a time 1 animal was held in a restrainer in such a way that the tail lies over the nichrome wire of the Analgesiometer. Strength of current used is 5 ampere.
- Basal reaction time to radiant heat was taken by placing the tail on the radiant heat source.
- Tail withdrawal time was recorded as the end point
- Cut off time of 15 seconds is considered as maximum analgesia & tail was removed from source of heat to avoid any tissue damage.
- Reaction time was taken at 0, 30 & 60 minutes on day 0, 7, 14, 21 & 28.

#### Technique of oral drug administration to rats<sup>15</sup>

Oral administration of substances is done by Gavage tube. In laboratory animals, dosing by gavage tube involves removing the animal from its cage, manually restraining it, inserting a small-diameter tube (18 Gauge) into the esophagus, and delivering the drug directly into the stomach by means of a syringe. Although highly effective, care must be taken to ensure that the tube or needle does not enter the trachea or damage the esophagus or stomach.

#### Statistical analysis

All the data were recorded in microsoft excel sheets for further evaluation. Evaluation was done as mean  $\pm$  standard deviation (SD). Statistical analysis of data was carried out by employing analysis of variance (ANOVA) followed by *Post hoc Tukey's*.

#### Results:

The following results were observed.

**Table 1:** Mean Reaction time (in seconds) of Group I throughout the study period with Mean  $\pm$  S.D by Tail Flick test

Day↓	TIME (in Minute)→		
	0	30	60
0	6.21	6.37	6.37
7	5.83	5.89	6.14
14	5.73	6.56	6.39
21	6.20	6.26	6.28
28	6.23	5.74	6.08
Mean $\pm$ SD	6.04 $\pm$ 0.59	6.16 $\pm$ 0.56	6.25 $\pm$ 0.43

**Group I:** Control – received 1ml of distilled water p.o

**Table 2:** Mean Reaction time (in seconds) of Group II throughout the study period with Mean  $\pm$  S.D by Tail Flick test

Day↓	TIME (in Minute)→		
	0	30	60
0	5.86	6.75	7.31
7	5.93	7.06	8.34
14	7.08	8.57	9.25
21	6.58	8.14	9.62
28	6.58	7.69	8.89
Mean $\pm$ SD	6.41 $\pm$ 0.91	7.64 $\pm$ 0.97	8.68 $\pm$ 1.22

**Group II:** Standard – received Tramadol 10mg/kg of body weight p.o

**Table 3:** Mean Reaction time (in seconds) of Group III throughout the study period with Mean  $\pm$  S.D by Tail Flick test

Day↓	TIME (in Minute)→		
	0	30	60
0	6.52	6.64	6.97
7	5.94	5.91	6.38
14	6.46	7.19	7.79
21	6.70	7.60	7.74
28	6.32	7.11	7.64
Mean $\pm$ SD	6.39 $\pm$ 0.70	6.89 $\pm$ 0.85	7.30 $\pm$ 0.78

**Group III:** received Cinnarizine 2.5 mg/kg body weight p.o

**Table 4:** Mean Reaction time (in seconds) of Group IV throughout the study period with Mean  $\pm$  S.D by Tail Flick test

Day↓	TIME (in Minute)→		
	0	30	60
0	7.20	7.51	7.84
7	6.49	6.91	7.32
14	6.71	7.41	8.32
21	7.49	8.27	8.83
28	5.98	6.92	7.16
Mean $\pm$ SD	6.77 $\pm$ 0.86	7.40 $\pm$ 0.78	7.89 $\pm$ 0.86

**Group IV:** received Cinnarizine 5 mg/kg body weight p.o

**Table 5:** Mean Reaction time (in seconds) of Group V throughout the study period with Mean  $\pm$  S.D by Tail Flick test

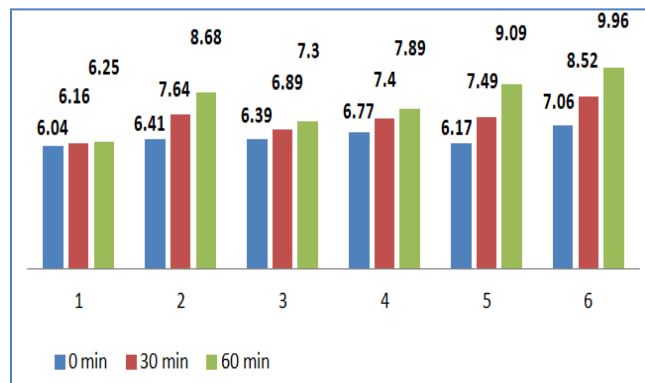
Day↓	TIME (in Minute)→		
	0	30	60
0	6.38	7.21	8.55
7	6.08	7.26	8.42
14	7.00	8.69	9.90
21	5.56	7.44	9.52
28	5.87	6.85	9.04
Mean $\pm$ SD	6.17 $\pm$ 0.94	7.49 $\pm$ 1.05	9.09 $\pm$ 1.08

**Group V:** received Tramadol 10mg/kg & Cinnarizine 2.5 mg/kg body weight p.o

**Table 6:** Mean Reaction time (in seconds) of Group VI throughout the study period with Mean  $\pm$  S.D by Tail Flick test

Day↓	TIME (in Minute)→		
	0	30	60
0	6.76	7.45	8.82
7	6.73	8.28	9.79
14	5.97	8.16	10.04
21	6.30	8.73	10.70
28	7.83	9.05	10.28
Mean $\pm$ SD	7.06 $\pm$ 1.08	8.52 $\pm$ 1.22	9.96 $\pm$ 0.96

**Group VI:** received Tramadol 10 mg/kg & Cinnarizine 5 mg/kg body weight p.o



**Figure 1:** Bar diagram showing mean reaction time of all groups (in sec) by Tail Flick Test

On observation by the ANOVA followed by post hoc Tukey's, it is seen that –

- Mean reaction time is increased in Group II, III, IV, V & VI. But significant increase ( $p$ -value $<$ 0.05) is seen in Group II, V & VI with respect to Group I, III & IV.
- Group III & IV shows increase in Mean reaction time but it is not significant ( $p$ -value $>$ 0.05) with Group I.
- Group V & VI shows significant increase in Mean reaction time in comparison to Group I ( $p$ -value $<$ 0.05) but not significant ( $p$ -value $>$ 0.05) in comparison to group II.

➤ Group VI (5mg/kg of Cinnarizine+Tramadol) potentiates antinociceptive effect of tramadol more than Group V (2.5mg/kg Cinnarizine+Tramadol) in case of acute pain.

So, it is seen that Cinnarizine alone (in both doses) has antinociceptive activity but that is not statistically significant. But when it is given along with Tramadol it potentiates antinociceptive activity of Tramadol which is statistically significant.

## Discussion

In this study we wanted to explore the antinociceptive activity of Cinnarizine alone & in combination with standard drug Tramadol. We found that cinnarizine induced an antinociceptive effect alone & also in combination with Tramadol. This finding revealed that antinociceptive effect of Cinnarizine was not comparable to Tramadol, but the combination potentiates antinociceptive activity of tramadol.

Probable mechanism for this action of Cinnarizine might be in the Calcium channel blocking activity in the neuronal synapse which attenuates pain transmission. Cinnarizine being a T-type as well as L-type  $Ca^{2+}$  channel antagonists directly act on the cytoplasmic membrane of neurons.<sup>16</sup> As the  $Ca^{2+}$  channels are blocked, on stimulation by noxious stimuli they resist the release of calcium into the synapse of 1<sup>st</sup> order neuron in the periphery. The calcium is needed by the vesicle to pour neurotransmitters into the synaptic cleft. Then only these neurotransmitters transmit the action potential to 2<sup>nd</sup> order neurons. Since the Calcium channels are blocked by Cinnarizine, there is less availability of Calcium for the vesicles containing neurotransmitter. Therefore the transmission of action potential is attenuated. This is probably the mechanism of antinociception by Cinnarizine which helps Tramadol in potentiating the effect.

**Modi et al in 2013** found that amlodipine, a calcium channel blocker, when combined with the opioid agonist tramadol, enhances antinociceptive effects of tramadol as well as prolongs the duration of its antinociceptive effect.<sup>17</sup> Blocking of calcium channel results in attenuation of synaptic transmission of nociceptive neurons. In their study they have demonstrated the role of calcium channels in antinociceptive action of calcium channel blockers. Since amlodipine also blocks calcium channels, it is likely that antinociceptive action could be due to blockade of these channels.

**Arash Bostani et al in 2013** found that calcium channel blocking efficacy of cinnarizine is analogous to flunarizine.<sup>18</sup> They showed that Cinnarizine may reduce frequency, severity and duration of migraine attacks.

This effect was said to be due to Calcium channel blocking activity.

**Sekiguchi and Kawabatain in 2013** found that the T-type CaV3.2 channel, which is involved in both central and peripheral termini of primary afferent neurons, is now thought to play an important role in the processing of pain.<sup>19</sup>

**Jarvis et al. in 2014** found that when the Calcium channels are inhibited at the molecular level, there is a significant reduction in pain.<sup>20</sup> They mainly examine the role of CaV3.2 in neuropathic pain; however, both Cav3.1 and Cav3.3 have been implicated in this process.

**Priyambada S et al in 2015** found that Cinnarizine (3.5mg/kg) has analgesic property, and it is less when compared to Flunarizine (2.6mg/kg).<sup>21</sup> Cinnarizine is a long-acting, potent inhibitor of potassium chloride-depolarization-induced peripheral vasoconstriction, acting via selective inhibition of calcium influx into depolarized cells, thereby reducing the availability of free calcium ions for induction and maintenance of contraction in smooth muscle. Directly antagonizes the stimulated influx of extracellular calcium, modifying intracellular calcium- adenosinetriphosphate balance in erythrocytes, thus increasing their flexibility and decreasing whole blood viscosity.

**Chen X et al in 2018** found that the neuronal Ca<sub>v</sub>2.2 voltage-gated Ca<sup>2+</sup> channel is implicated in mediating neurotransmitter release in nociceptive neurons. Voltage-gated calcium channels (Ca<sub>v</sub>2.2), largely localized to primary afferent terminals in laminae 1 and 2 of the dorsal horn, play an important role in pain signaling by contributing to the release of neurotransmitters such as glutamate, substance P, and calcitonin gene-related peptide (CGRP).<sup>22</sup>

**Jaksimovic SL et al in Jan'2019** demonstrated that, by targeting the Ca<sub>v</sub>3.2 isoform of T-type calcium channels and N-type High Voltage Activated (HVA) calcium channels in nociceptors, we can effectively reduce postsurgical hyperalgesia in rats. In addition, they found that epipregnanolone, a CCB exerts antinociceptive effects in healthy rats, in part by reversibly blocking T-channels in peripheral DRG neurons.<sup>23</sup>

#### Limitation of this study:

- Both the methods used were crude methods, some specific method could yield better result.
- The dose selection in this study was done according to the human absolute dose of drugs. Ideally the doses should be determined by the therapeutic plasma concentration & toxicological study with selected doses. But that would be very difficult to estimate in our experimental study set-up. This may require large number of animals & may take longer duration for

study. The ethical concern is also a limiting factor for toxicity study.

- Last but not the least, animal studies may not give convincing result in human beings & the drugs that appear to be promising in animal studies can fail in clinical trials.

#### Conclusion

The result of this study in albino rats conclude that-

- a) Cinnarizine alone has antinociceptive activity in both doses ( i.e 2.5mg/kg & 5mg/kg) but it is statistically not significant.
- b) Cinnarizine potentiates the antinociceptive activity of Tramadol in both doses.
- c) Tramadol shows higher antinociception with Cinnarizine in dose of 5mg/kg than with 2.5mg/kg.

However our study is very primitive in the method and parameters used to evaluate analgesia. Further studies need to be done in various other acute pain models using different species & in different doses to establish efficacy of Cinnarizine alone & in combination with Tramadol as an analgesic.

More elaborative studies are required to elicit the molecular mechanism of pain suppression of the studied drugs and for implementing the results of this study in actual clinical setting. So, further clinical trials using this combination should be carried out for evaluating the effect in different pain conditions.

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#### References:

1. Debono, DJ; Hoeksema, LJ; Hobbs, RD (August 2013). "Caring for Patients with Chronic Pain: Pearls and Pitfalls". *Journal of the American Osteopathic Association*. 113(8): 620–627
2. What should be the core outcomes in chronic pain clinical trials?. *Arthritis Research & Therapy*. 2004;6(4):151–4
3. Assessment of pain. *Br J Anaesth*. 2008;101(1):17–24
4. The Social Outcast: Ostracism, Social Exclusion, Rejection, & Bullying (Sydney Symposium of Social Psychology). East Sussex: Psychology Press; 2005.
5. The skin senses: Proceedings of the first International Symposium on the Skin Senses, held at the Florida State University in Tallahassee, Florida. Sensory, motivational and central control determinants of chronic pain: A new conceptual model. p. 432

6. International Association for the Study of Pain: Unrelieved pain is a major global healthcare problem
7. Terland, O.; Flatmark, T. (1999). "Drug-induced parkinsonism: Cinnarizine and flunarizine are potent uncouplers of the vacuolar H<sup>+</sup>-ATPase in catecholamine storage vesicles". *Neuropharmacology*. 38 (6): 879–882.
8. Ray SB, Mehra RD. Potentiation of opioid-induced analgesia by L-type calcium channel blockers: need for clinical trial in cancer pain. *Indian J Anaesth*. 2008;52:367–72.
9. Murphy DB, McCartney CJ, Chan VW. Novel analgesic adjuncts for brachial plexus block: A systematic review. *Anesth Analg* 2000;90:1122-8
10. Charan J, Kantharia N.D, How to calculate sample size in animal studies? *Journal of Pharmacology and Pharmacotherapeutics* | October-December 2013 | Vol 4 | Issue 4 page 303-306
11. Ghosh MN. Toxicity studies In. Ghosh MN, editor, *Fundamental of Experimental Pharmacology* 5th ed, Kolkata; Hilton and company 2011. P167-209. "Parenteral opioids for labor pain relief: A systematic review - *American Journal of Obstetrics & Gynecology*". [www.ajog.org](http://www.ajog.org). Retrieved 2015-07-11.
12. Mann, M.D, Crouse, D.A and Prentice, E.D (1991). Appropriate Anomal numbers in Biomedical Research in Light of Animal Welfare Considerations, *Laboratory Animal Science* 1991, 41 (1); 6-14
13. Dr Supriya Priyambada, Dr Rajasree N, Dr Jagadeesh P, Hanumanth N, A comparative study of analgesic effect of Flunarizine and Cinnarizine in albino rats, using digital analgesiometer, *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 14, Issue 10 Ver. V (Oct. 2015), PP 07-11*
14. Ruchi Gupta, Simmerpreet Kaur, Saru Singh, and K.S. Aujla, A comparison of Epidural Butorphanol and Tramadol for Postoperative Analgesia Using CSEA Technique, *J Anaesthesiol Clin Pharmacol*. 2011 Jan-Mar; 27(1): 35–38.
15. Zeenat Atcha et al Alternative Method of Oral Dosing for Rats, *J Am Assoc Lab Anim Sci*. 2010 May; 49(3): 335–343.
16. Tabata Y et al , T-type Calcium Channels Determine the Vulnerability of Dopaminergic Neurons to Mitochondrial Stress in Familial Parkinson Disease, *Stem Cell Reports jour* Vol. 11 p 1171–1184
17. Modi H, Mazumdar B, Bhatt J. Study of interaction of tramadol with amlodipine in mice. *Indian J Pharmacol* 2013;45:76-9.
18. Arash Bostani et al, The effects of cinnarizine versus sodium valproate in migraine prophylaxis, *International Journal of Neuroscience*, 2013; 123(7): 487–493
19. Sekiguchi F, Kawabata A (2013) T-type calcium channels: functional regulation and implication in pain signaling. *J Pharmacol Sci* 122:244–250
20. Jarvis MF, Scott VE, McGaraughty S, Chu KL, Xu J, Niforatos W, Milicic I, Joshi S, Zhang Q, Xia Z (2014) A peripherally acting, selective T-type calcium channel blocker, ABT-639, effectively reduces nociceptive and neuropathic pain in rats. *Biochem Pharmacol* 89:536–544
21. Supriya Priyambada, Rajasree N, Jagadeesh P, Hanumanth N, A comparative study of analgesic effect of Flunarizine and Cinnarizine in albino rats, using digital analgesiometer, *IOSR Journal of Dental and Medical Sciences. Volume 14, Issue 10 Ver. V (Oct. 2015), PP 07-11* DOI: 10.9790/0853-141050711
22. Chen X, Liu D, Zhou D, et al. Small-molecule Ca<sub>v</sub>α<sub>1</sub>-Ca<sub>v</sub>β antagonist suppresses neuronal voltage-gated calcium-channel trafficking. *Proc Natl Acad Sci USA*. 2018;115(45): E10566–E10575. doi:10.1073/pnas.1813157115
23. Joksimovic SL, Donald RR, Park JY, Todorovic SM. Inhibition of multiple voltage-gated calcium channels may contribute to spinally mediated analgesia by epipregnanolone in a rat model of surgical paw incision. *Channels (Austin)*. 2019;13 (1): 48–61. doi:10.1080/19336950.2018.1564420