



(RESEARCH ARTICLE)



## Pharmacological approach to mechanism of action of tramadol in murine nociception and inflammation assays

Miranda Hugo F <sup>1,\*</sup>, Noriega Viviana <sup>2</sup>, Sierralta Fernando <sup>3</sup>, Sotomayor-Zárate Ramón <sup>4</sup> and Prieto Juan Carlos <sup>2,3</sup>

<sup>1</sup> Neuroscience Department, Faculty of Medicine, Universidad de Chile, Santiago, Chile.

<sup>2</sup> Cardiovascular Department; Clinical Hospital, Universidad de Chile, Santiago, Chile.

<sup>3</sup> Pharmacology Program, ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile.

<sup>4</sup> Laboratorio de Neuroquímica y Neurofarmacología, Centro de Neurobiología y Fisiopatología Integrativa, Instituto de Fisiología, Facultad de Ciencias, Universidad de Valparaíso, Chile.

Publication history: Received on 21 April 2020; revised on 05 May 2020; accepted on 07 May 2020

Article DOI: <https://doi.org/10.30574/wjarr.2020.6.2.0114>

### Abstract

The analgesic activity of tramadol has been recognized both in man and in several animal models of pain. However an extensive characterization of the opioid mechanism of action of tramadol of pain has not been reported. The objective of the present study was to evaluate the antinociceptive and anti-inflammatory activity of tramadol in different animal pain models and to determine the effect of the selective opioid antagonist: naltrexone, naltrindole and nor-binaltorphimine. The i.p. administration of tramadol induced a dose-dependent with the following order of potency: formalin hind paw, phase II > formalin hind paw, phase I > acetic acid writhing > tail flick > hot plate. Pretreatment of the mice with naltrexone (1 mg/kg i.p.) antagonized tramadol activity in the acetic acid writhing test, in the hot plate and the tail flick assays, however lack of effect in the formalin hind paw assays. Naltrindole (1 mg/kg i.p.) did not induce a significant change in all the murine assays. However, the mice pretreated with nor-binaltorphimine (1 mg/kg, i.p.) did not modified the tramadol antinociception in the acetic acid writhing and in the hot plate assays. Besides, nor-binaltorphimine pretreatment reversed significantly the tramadol effect in the tail flick and in the formalin hind paw assays. This findings suggests that tramadol effect is mediated by MOR and KOR rather DOR receptors.

**Keywords:** Nociception; Inflammation; Tramadol; Naltrexone; Naltrindole; Norbinaltorphimine; Murine assays

### 1. Introduction

The IASP (International Association for the Study of Pain) approved the following definition of pain as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” [1]. The IASP definition of pain recognizes its different components. In sensory aspect, it activates the nociceptors and the different routes and mechanisms of transmission of the painful stimulus. . On the other hand, cognitive and behavioral perception and experience represent individual and psychological personal experience [2]. The classification of pain can be done according to different variables, including duration (acute or chronic), pathogenesis (nociceptive or neuropathic), location (somatic or visceral), and others. To control pain, it is necessary to have drugs that modify its origin, can alter its perception at the central level and are capable of blocking its transmission to the central nervous system. There are no drugs that meet all the previously stated objectives, however the most frequently used in the treatment of pain are NSAIDs and opioids.

Opioids produced antinociception by activation of definite receptors located in central and peripheral nervous system. At present, five types of opioid receptors have been defined: mu receptor (MOR), kappa receptor (KOR), delta receptor

\* Corresponding author

E-mail address: [hmiranda@med.uchile.cl](mailto:hmiranda@med.uchile.cl)

(DOR), nociception receptor (NOR) and zeta receptor (ZOR) with the following subtypes: mu1, mu2, mu3, kappa1, kappa2, kappa3, delta1 and delta2 [3,4].

Opioids constitute a group of natural and synthetic medications, highlighting morphine, codeine, heroin, methadone, fentanyl, tramadol and others. Opioids are highly powerful and effective pain relievers, but it produces a high potential for side effects, as a consequence of the repeated use that causes neuron adaptation, such as tolerance, dependence and addiction [5].

Tramadol is a synthetic chiral MOR opioid drug formed for two enantiomers, (+) tramadol is a selective agonist of  $\mu$  receptors and preferentially inhibits serotonin reuptake, whereas (-) tramadol mainly inhibits noradrenaline reuptake. The synergistic actions of the two enantiomers increase the analgesic efficacy and tolerability profile of the racemic tramadol [6,7]

The antinociceptive activity of tramadol has been demonstrated in both man and animal assays of pain; however an exhaustive characterization of the opioid mechanism of action of tramadol, in animal models of pain, has not been reported. The objective of the present study was to evaluate the antinociceptive and anti-inflammatory activity of tramadol in different animal pain models and to determine the effect of the selective opioid antagonist: naltrexone, naltrindole and nor-binaltorphimine.

## 2. Material and methods

### 2.1. Animals

Male CF-1 mice (25–28 g), housed on a 12 h light-dark cycle at 22±1 °C with ccess to food and water ad libitum, were used. All animal procedures were approved by the Animal Care and Use Committee at the Faculty of Medicine, University of Chile (Protocol CBA 0852/FMUCH/2018). Animals were acclimatized to the laboratory for at least 1 h before testing, used only once during the protocol, and euthanized immediately after the algesiometric test by one intraperitoneal (i.p.) injection of 60 mg/kg of pentobarbital. The number of animals was kept at a minimum, compatible with consistent effects of the drug treatment.

### 2.2. Measurement of antinociceptive activity

Antinociception was assessed by the following murine tests:

(A) Acetic acid abdominal contraction (writhing test), as previously described [8]. Antinociception, expressed in % of maximum possible effect (% MPE), was calculated as percent inhibition of the saline control writhes (20.78±0.74, n=12).

(B) tail-flick as described previously [8]. Tail flick latencies control were 2.49±0.07 (n=12) and converted to %MPE as follows:

$$\%MPE = (\text{latency postdrug} - \text{latency control}) / (\text{cut-off time} - \text{predrug latency}) \times 100$$

(C) The formalin hind paw test described previously was used [9]. The test show 2 clear cut-periods: phase I corresponding to the 5 min immediately after formalin injection and phase II, chronicled by 10 min, a period starting 20 min after formalin injection. The control licking or biting, in sec, of the injected paw were, phase I: 133.05 ± 7.04 (n =12) and phase II: 157.83 ± 9.10 (n=12). Licking time was converted to % MPE as follow:

$$\% MPE = 100 - [(100 \times \text{post drug licking time}) / \text{control licking time}]$$

(D) The hot plate test as previously described [10]. The control licking of the forelegs were 18.96 ± 0.57 (n=12). Hot plate latencies, with a cutoff time of 30 sec to elude skin damage, were converted to % MPE as follow:

$$\% MPE = [(\text{post drug latency} - \text{control latency}) / \text{cutoff} - \text{control latency}] \times 100$$

For each NSAIDs the DE<sub>50</sub>, dose that induce 50% of MPE was calculated from lineal regression of dose-response curves.

### 2.3. Experimental design

In order to determine the antinociceptive potency of i.p. tramadol a dose-response curves produced from 0.3 to 100 mg/kg was obtained in the writhing, tail flick, formalin hind paw and hot plate tests using at least 6 animals for each at least 4 doses. To identify the participation of the opioid antagonist receptors, in the tramadol antinociception, mice were pretreated with either 1 mg/kg i.p. of naltrexone or naltrindole or nor-binaltorphimine, which are doses uses in the literature.

## 2.4. Drugs

Drugs were freshly dissolved in sterile physiological salt solution of 10 mL/Kg, for intraperitoneal administration. Tramadol hydrochloride, naltrexone hydrochloride, naltrindole hydrochloride and Nor-binaltorphimine dihydrochloride were purchased from Sigma-Aldrich Chemical Co, St.Louis, Mo, USA.

## 2.5. Statistical analysis

Results are presented as means  $\pm$  SEM. The statistical difference between the results were assessed by one-way ANOVA, followed by Tukey's post test for and p values less than 0.05 ( $p < 0.05$ ) were considered statistically significant. Statistical analyses were carried out using the program Pharm Tools Pro, version 1.27, Mc Cary Group Inc., PA, USA.

## 3. Results and discussion

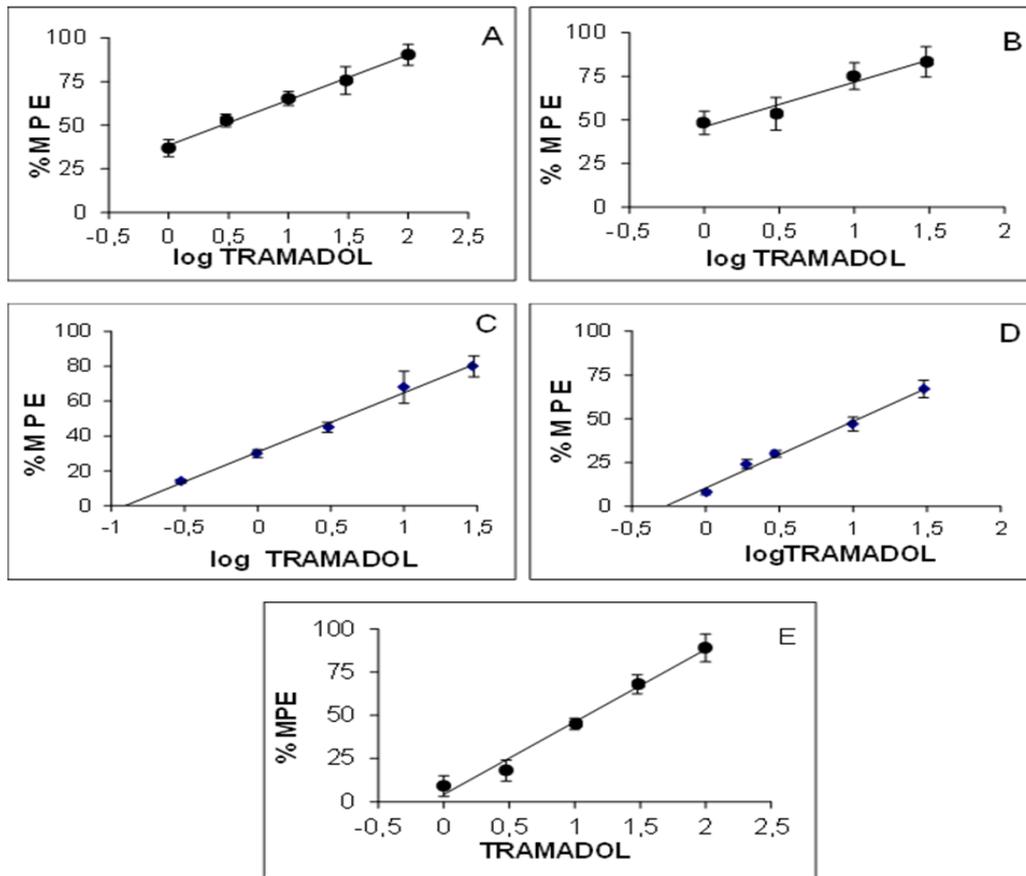
### 3.1. Antinociception induced by tramadol in the murine tests

The i.p. administration of tramadol induced a dose-dependent antinociceptive action in the acetic acid writhing, tail flick, formalin hind paw and hot plate tests. The corresponding dose-response of each assay is shown in Figure 1. The ED<sub>50</sub> antinociceptive values with their respective SEM resulting from each test is presented in table 1. The order of tramadol potency range in each assay was: formalin hind paw, phase II > formalin hind paw, phase I > acetic acid writhing > tail flick > hot plate.

**Table 1** ED<sub>50</sub> values with SEM in mg/kg for the analgesia activity of i.p.tramadol in the writhing, tail flick, hot plate and formalin hind paw of mice.

Test	ED <sub>50</sub> $\pm$ SEM (mg/kg)	Analgesic ratio <sup>a</sup>
Formalin hind paw Phase II	1.41 $\pm$ 0.31	8.73
Formalin hind paw Phase I	2.78 $\pm$ 0.22	4.43
Writhing	3.66 $\pm$ 0.26	3.36
Tail flick	3.84 $\pm$ 0.91	3.20
Hot plate	12.32 $\pm$ 1.20	1.00

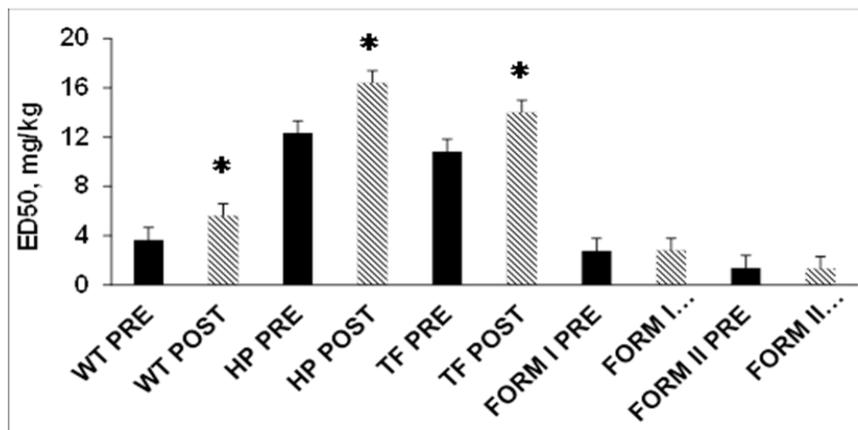
<sup>a</sup> Compared with hot plate



**Figure 1** Tramadol i.p. antinociceptive dose-response curves in (A) phase I of formalin hind paw, (B) phase of formalin hind paw, (C) acetic acid writhing, (D) tail flick and (E) hot plate assays in mice. Responses are expressed as % MPE (percentage of maximum possible effect).

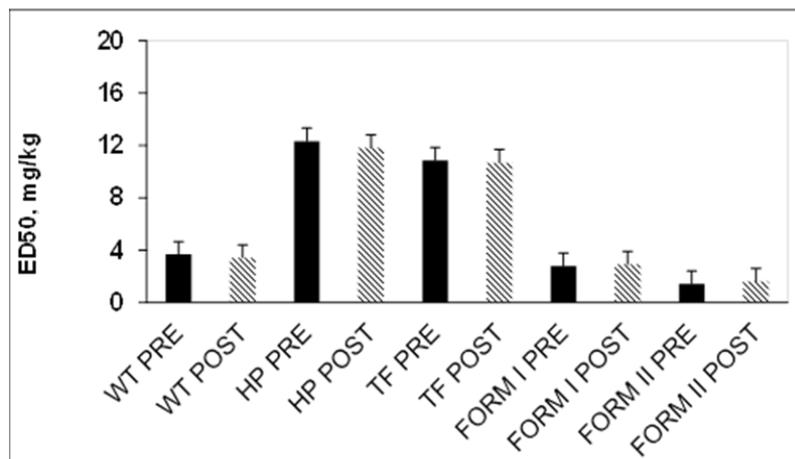
### 3.2. Effect of opioids antagonists in the tramadol antinociception

The pretreatment of the mice with naltrexone (1 mg/kg i.p.) induced a significant increment of the tramadol activity in the control writhes of the acetic acid writhing test, in the control licking of the forelegs of the hot plate and in the control latency of the tail flick assays, these changes means an antagonism in the tramadol antinociception (see figure 2). Otherwise, after pretreatment with naltrexone did not change the control licking in the formalin hind paw assays induced by tramadol. All these data are shown in figure 2.



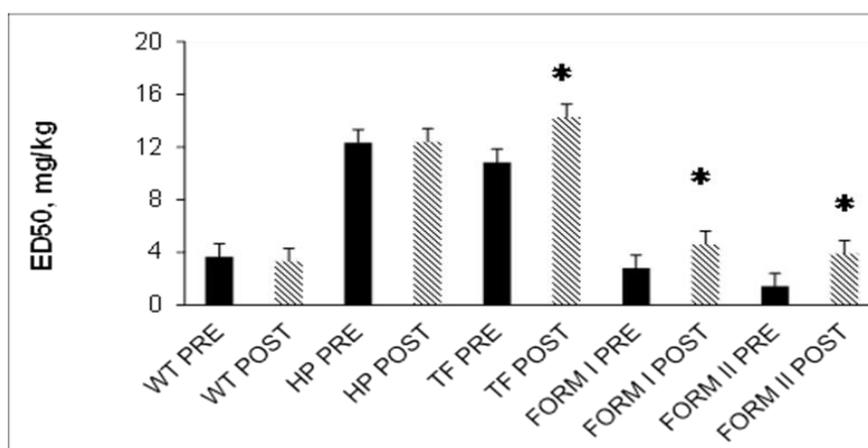
**Figure 2** Histogram of the treatment of naltrexone on the ED<sub>50</sub> of tramadol. Dark columns (PRE) show tramadol effect before and clear columns (POST) show action after administration of naltrexone in writhing test (WT), hot plate (HP), tail flick (TF) and formalin hind paw (FORM). \* P>0.05 versus pretreatment.

The pretreatment of the mice with naltrindole (1 mg/kg i.p.) did not induced a significant change of the antinociceptive activity of tramadol in the acetic acid writhing, in the hot plate assay, in tail flick and in the formalin hind paw tests. All these data are shown in figure 3.



**Figure 3** Histogram of the treatment of naltrindole on the ED<sub>50</sub> of tramadol. Dark columns (PRE) show tramadol effect before and clear columns (POST) show action after administration of naltrindole in writhing test (WT), hot plate (HP), tail flick (TF) and formalin hind paw (FORM).

In the case of the mice pretreated with nor-binaltorphimine (1 mg/kg, i.p.) did not produced a significant increase of the tramadol antinociception in the number of writhes in the acetic acid writhing and in the licking of the foreleg in the hot plate assays. Besides, nor-binaltorphimine pretreatment reversed significantly the tramadol effect in the latency of the tail flick and in the licking in the formalin hind paw assays (see figure 4).



**Figure 4** Histogram of the treatment of nor-binaltorphimine on the ED<sub>50</sub> of tramadol. Dark columns (PRE) show tramadol effect before and clear columns (POST) show action after administration of naltrindole in writhing test (WT), hot plate (HP), tail flick (TF) and formalin hind paw (FORM). \* P>0.05 versus pretreatment.

#### 4. Discussion

Animal models are primarily used to understand the mechanism of action of pain relievers and anti-inflammatory drugs. In the current study, tramadol was found to be able to induce a significant antinociceptive effect, demonstrated by the corresponding behavior of the pain test used. Thus, the administration of tramadol produced antinociception in the writhing, tail flick and hot plate. Furthermore, induce anti-inflammatory activity in the formalin hind paw. These findings are in agreement with previous reports of a similar effect of tramadol restricted to animal models of nociception such as those of hot plate [11] tail movement [12] and acetic acid writhing tests [13], nevertheless the effect of tramadol in animal inflammatory models such as formalin tests had not been reported.

To establish the role of different opioid receptors in the tramadol-induced nociceptive effect, opioid antagonists were used, which demonstrated that naltrexone reversed tramadol activity in the nociceptive pain (writhing, hot plate, and tail flick). Furthermore, naltrexone had no effect in the tonic inflammatory pain assay (formalin hind paw). The use of naltrindole did not significantly change the tramadol control values in the different tests. However, the administration of nor-binaltorphimine did not modify the effect of tramadol in the writhing and hot plate tests, on the other hand, abolished the effect of the opioid on the tail flick and the hind paw formalin.

Tramadol is a prodrug that exists as 2 enantiomers with antinociceptive activity but with different mechanisms of action. Thus, (+)-Tramadol and its metabolite O-desmethyltramadol (M1) act as selective MOR receptor agonist and inhibits serotonin reuptake. The other enantiomer, (-) inhibits noradrenaline reuptake, both synaptic reuptake increase the inhibition of descending pathways connected with pain transmission. [14-15].

The results obtained in the present study are consistent with those previously reported, although in models other than pain, in which naltrexone significantly decreases the response of discriminatory behavior to tramadol [16], the inhibition in memory recovery [17]. However, It should be noted that the effect of opioid antagonists, in the antinociception of tramadol, has only been reported in a model of cerebral ischemia pain, in which the effect of the opioid was significantly cancelled by  $\beta$ -funaltrexamine (MOR selective opioid receptor antagonist), but not naltrindole or nor-binaltorphimine [18].

The present study showed that antinociception and anti-inflammatory activity of tramadol were mediated by the MOR and KOR receptor and not by the DOR receptor. This was validated by the change in tramadol activity by naltrexone and nor-binaltorphimine and the lack of antagonism developed by naltrindole in the tests used. The explanation for these tramadol findings could be justified with the argument that opioid analgesia being a complex process in which MOR receptors can be heteromerized with  $\delta$  or  $\kappa$  opioid receptors. Based on the results of the present study, it is possible to speculate that MOR and KOR may play an important role in naltrexone antinociception. This finding is not consistent with the report by Choi et al. [19] suggesting that in the hind paw formalin trial, MOR, KOR and DOR are involved in the antinociception of naltrexone.

The mechanism of action proposed in this study for the analgesic action of tramadol is consistent with previous works in which it is proposed that O-desmethyltramadol (M1) has almost the same potency, as tapentadol and oxymorphone in MOR, KOR and DOR [20]. Nevertheless, it must be added that tramadol, described as an atypical opioid, with a special profile of opioidergic, noradrenergic and serotonergic actions, has other modulatory actions on pain, including mechanisms involved with substance P, in sodium ion channels, in adenosine, glutamate receptors, alpha-2 adrenoceptors, proinflammatory cytokines, and other modulatory effects that would regulate both central and peripheral neuronal excitability [21].

---

## 5. Conclusion

The findings of the present study demonstrated that the antinociceptive and anti-inflammatory activity of tramadol was blocked by naltrexone and nor-binaltorphimine but not naltrindole. This suggests that tramadol effect is mediated by MOR and KOR rather DOR receptors.

---

## Compliance with ethical standards

### *Acknowledgments*

*None*

### *Disclosure of conflict of interest*

The authors declare no conflict of interest.

### *Statement of ethical approval*

Experiments were performed in accordance with current Guidelines for The Care of Laboratory Animals and Ethical Guidelines for Investigation Experimental Pain approved by the Animal Care and Use Committee of the Faculty of CBA N° 852/2018.

---

## References

- [1] IASP (1979). Pain terms: a list with definitions and notes on usage: recommended by the IASP Subcommittee on Taxonomy. *PAIN* 6, 249.
- [2] Puig M. (2008). *Farmacología del dolor: analgésicos*, Fundación Grünenthal, 19-26.
- [3] Cox BM, Christie MJ, Devi L, Toll L and Traynor JR. (2015). Challenges for opioid receptor nomenclature, *IUPHAR Review* 9. *British Journal of Pharmacology* 172, 317-323.
- [4] Granier S. (2012). Structure of mu and delta opioid receptors. *Medicine Science (Paris)* 28, 870-875.
- [5] Listos J, Łupina M, Talarek S, Mazur A, Orzelska-Górka J and Kotlińska J. (2019). The Mechanisms Involved in Morphine Addiction: An Overview. *Int International Journal of Molecular Sciences*, 20, 4302-4325.
- [6] Grond S and Sablotzki A. (2004). Clinical pharmacology of tramadol. *Clinical Pharmacokinetic*, 43, 879-923.
- [7] Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD and Rawson R. (2017). Trends in Tramadol: Pharmacology, Metabolism, and Misuse. *Anesthesia & Analgesia*, 124, 44-51.
- [8] Miranda HF, Noriega V, Sierralta F, Poblete P, Aranda N and Prieto JC. (2019). Non-steroidal anti-inflammatory drugs in tonic, phasic and inflammatory mouse model. *Drug Research*, 69, 572-578.
- [9] Noriega V, Miranda HF, Prieto JC, Sotomayor-Zárate R and Sierralta F. (2020). Involvement of NO in antinociception of NSAIDs in murine hind paw. *Drug Research*, 70, 145-150.
- [10] Miranda HF, Poblete P, Sierralta F, Noriega V, Prieto JC and Zepeda RI. (2019). Interleukin-1 beta in synergism gabapentin with tramadol in murine model of diabetic neuropathy. *Inflammopharmacology*, 27, 151-155.
- [11] Meymandi MS and Keyhanfar F. (2012). Pregabalin antinociception and its interaction with tramadol in acute model of pain. *Pharmacological Reports*, 64, 576-85.
- [12] Modi H, Mazumdar B and Bhatt J. (2013). Study of interaction of tramadol with amlodipine in mice. *Indian Journal of Pharmacology*, 45, 76-79.
- [13] Zeraati F, Araghchian M and Farjoo MH. (2014). Ascorbic Acid interaction with analgesic effect of morphine and tramadol in mice. *Anesthesiology and Pain Medicine*, 4, 19529 – 19233.
- [14] Pathan H and Williams J. (2012). Basic opioid pharmacology: an update. *British Journal of Pain*, 6, 11– 16.
- [15] Beakley BD, Kaye AM and Kaye AD. (2015). Tramadol, Pharmacology, side effects, and serotonin syndrome: A Review. *Pain Physician*, 18, 395-400.
- [16] Ren YH and Zheng JW. (2000). Influence of tramadol on morphine discriminative behavior in rats. *Acta Pharmacological Sinica*, 21, 924-926.
- [17] Niknamfar S, Nouri Zadeh-Tehrani S, Sadat-Shirazi MS, Akbarabadi A, Rahimi-Movaghar A and Zarrindast MR. (2019).  $\mu$ -Opioid receptor in the CA1 involves in tramadol and morphine cross state-dependent memory. *Neuroscience Letters*, 705, 177-182.
- [18] Matsuura W, Kageyama E, Harada S and Tokuyama S. (2016). Unique action mechanisms of tramadol in global cerebral ischemia-induced mechanical allodynia. *Neuroreport*, 27, 699-704.
- [19] Choi SS, Sang KJ, Han KL, Eun-Jung H and Suh HW. (2003). Possible antinociceptive mechanisms of opioid receptor antagonists in the mouse formalin test. *Pharmacology Biochemistry and Behavior*, 75, 447-457.
- [20] Olson KM, Duron DI, Womer, Fell R and Streicher JM. (2019). Comprehensive molecular pharmacology screening reveals potential new receptor interactions for clinically relevant opioids. *Plos One*, 14, 217371-217389.
- [21] Barakat A. (2019). Revisiting Tramadol: A multi-modal agent for pain management. *CNS Drugs*, 33, 481-501.

---

## How to cite this article

Miranda HF, Noriega V, Sierralta F, Sotomayor-Zárate R and Prieto JC. (2020). Pharmacological approach to mechanism of action of tramadol in murine nociception and inflammation assays. *World Journal of Advanced Research and Reviews*, 6(2), 30-36.

---