# CHAPTER 6:

## Activation of peripheral cannabinoid receptors by CB-13 leads to modality specific attenuation of Chemotherapy-induced Neuropathic

*Chapter-6*

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## 6.1 Introduction

Chemotherapy, a mainstay of cancer treatment, plays a vital role in improving patient survival rates. However, its success is often overshadowed by a multitude of debilitating side effects, one of the most prominent being CINP [63]. This pain can be severe and lead to significant sensory loss, ultimately impacting quality of life [5,100,271]. Current treatment options for CINP are often unsatisfactory, with limitations including limited efficacy and potential for CNS side effects such as sedation, cognitive dysfunction, and addiction [7]. Researchers are actively investigating novel therapeutic approaches to alleviate paclitaxel-induced neuropathic pain, aiming to improve the quality of life for cancer patients undergoing chemotherapy.

Recently, there has been a notable emphasis on targeting the PNS as a strategic approach in the quest for safer analgesic treatments [181,272]. Within the PNS, the cannabinoid receptor (CBR) system has emerged as a fascinating target for pain management. Peripherally restricted cannabinoid agonists, unlike their psychoactive counterparts, lack the ability to readily cross the blood-brain barrier, thereby minimizing CNS exposure [273]. Numerous pre-clinical studies indicate that focusing on the peripheral CBR holds promise as an effective approach to alleviate symptoms of

both evoked and ongoing pain, all while avoiding adverse effects in the CNS [235,238]. Peripherally restricted cannabinoid agonists have shown significant efficacy in rodent models of cancer induced bone pain, neuropathic pain, and inflammatory pain [182,235,274].

This study delves deeper into the potential of a specific peripherally restricted cannabinoid agonist, CB-13, for managing paclitaxel-induced CINP in a rat model. CB-13 is a dual agonist, meaning it activates both CB1 and CB2 receptors [235,239]. Firstly, we investigated the efficacy of CB-13 in alleviating paclitaxel-induced neuropathic pain in rats. We will assess the impact of CB-13 on different pain modalities associated with CINP, such as mechanical hypersensitivity, cold hypersensitivity and spontaneous ongoing pain. Secondly, we explored the underlying neurobiological mechanisms by which CB-13 exerts its analgesic effects. By elucidating these mechanisms, we could gain valuable insights into the potential therapeutic application of CB-13 for managing CINP.

## 6.2 Experimental design

The rats were divided into six experimental groups: Naïve; Paclitaxel + Vehicle (0.9% sterile saline); Paclitaxel + CB13 (10, 20, 40μM/paw intraplantar (i.pl.)); and Paclitaxel + Gabapentin (60 mg/kg, s.c). Paclitaxel was diluted with 0.9% normal saline to achieve a working concentration of 2 mg/ml, derived from its commercially available concentration of 6 mg/ml. CB13 and gabapentin were prepared by dissolving in 0.9% normal saline. Gabapentin is currently among the first line drugs for the treatment of chemotherapy-induced neuropathic pain and the same has been used in all the evoked pain behavioral assays as standard treatment. The rats were allowed to acclimatize to

the test room environment and with different equipment for approximately 2-3 days before recording the pre-CINP baseline. Subsequently, pre-paclitaxel (pre-PTX) baseline testing was conducted for all the pain behavioral parameters. After the completion of baseline testing, the animals were administered with repeated paclitaxel injections to induced CINP. On day 28 following the first paclitaxel injection, the animals were treated with different doses of CB13 (10, 20, 40μM/paw i.pl.) in one paw (considered as injected paw) and gabapentin (60 mg/kg s.c) respectively. The time of drug administration was considered as zero minutes, and behavioral testing was performed before the drug treatment (pre-drug baseline) and at subsequent time points (30, 60, 120, and 240 minutes) post-drug treatment. Further, the effect of CB13 on spontaneous ongoing pain was evaluated by using conditioned place preference assay. This was followed by CNS toxicity testing using open field and rota-rod tests. After the completion of behavioral assays, on day 45 post first PTX injection, the animals were sacrificed and their sciatic nerve, L4-L5 dorsal root ganglions (DRGs), and spinal cord were harvested and kept at -80ºC for further molecular investigations.

## 6.3 Results and discussion

### 6.3.1 Peripheral CBR activation attenuates allodynia like behaviour in paclitaxel-induced neuropathic rats

We evaluated the efficacy of peripheral CB1/CB2R dual agonist, CB13, in the treatment of CINP by performing a battery of pain behavioural tests to assess both mechanical and cold allodynia. The CINP rats showed a significant decrease in the paw withdrawal threshold (PWT) to non-noxious mechanical stimuli in hind paw as compared to their respective pre-injury baselines and naïve rats ($p < 0.001$). Intraplantar

administration of CB13 (10, 20, 40μM/paw*)* significantly improved the PWT with effect starting from 30 mins, post drug administration in the injected paw. At 60 mins we observed the peak therapeutic effect (10 μM/paw, $p<0.05$; 20 μM/paw, $p<0.01$; 40μM/paw, $p<0.001$) as compared to their pre- drug baselines and vehicle treated CINP group. The effect lasted upto 120mins post drug administration. There was no improvement of threshold in the un-injected paw (Figure. 6.1A-B). The standard drug gabapentin (60mg/kg) showed significant effect which lasted upto 240mins post-administration ($p < 0.001$) as compared to vehicle treated neuropathic rats.

Paclitaxel significantly increased the response score to non-noxious cold stimuli in hind paws of rats as compared to the respective pre-injury baselines and naïve rats ($p <0.001$). Treatment with different doses of CB13 (20, 40μM/paw i.pl.) significantly decreased the response score in injected paws with effect starting from 30 mins, post drug administration. At 60 mins we observed the peak therapeutic effect (20 μM/paw, $p<0.001$; 40μM/paw, $p<0.001$) as compared to their pre- drug baselines and vehicle treated neuropathic rats. There was no decrease in the response score in the un-injected paw (Figure. 6.1C-D). The standard drug gabapentin (60mg/kg) also attenuated cold allodynia as compared to the vehicle treated neuropathic rats.

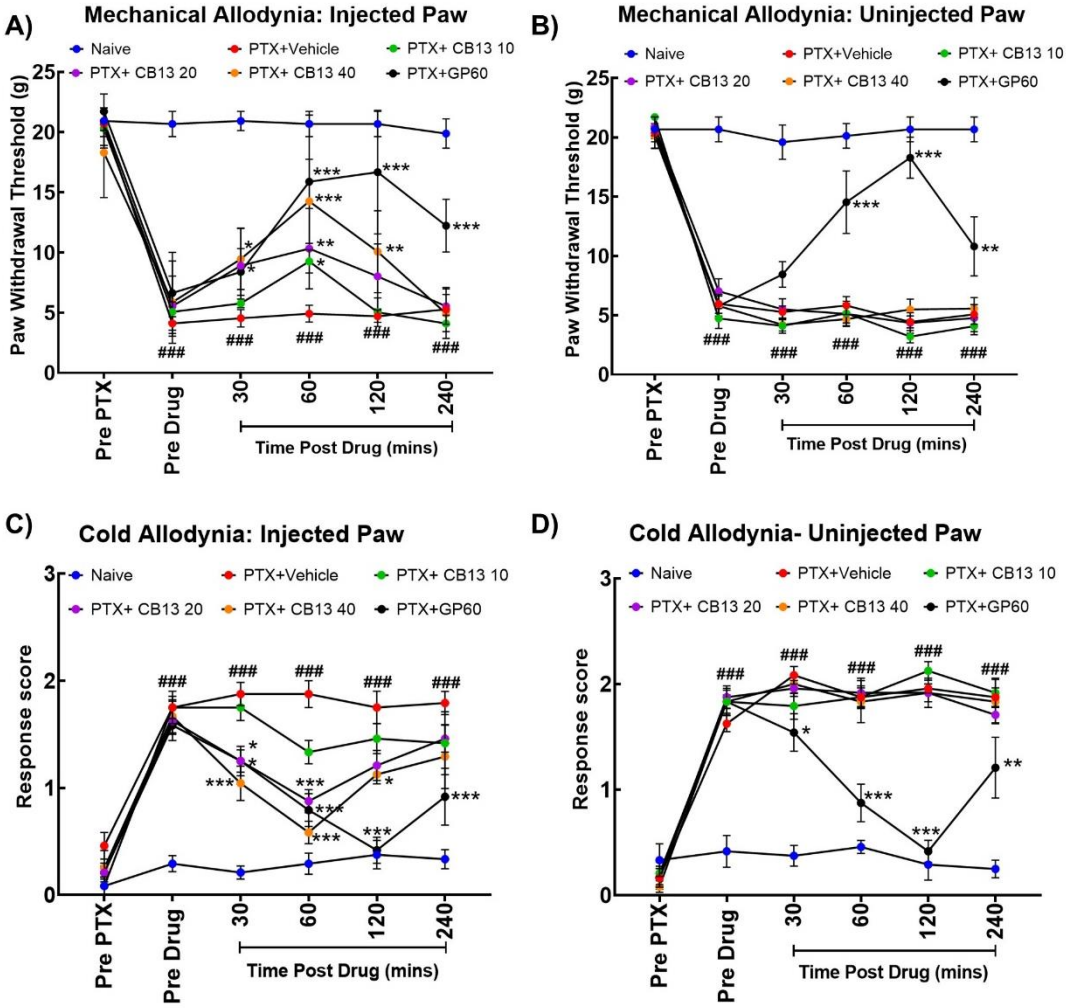



**Figure 6.1**: **Effect of CB13 on mechanical and cold allodynia induced by PTX administration. (A) von Frey hair test:** Effect of CB13 on mechanical allodynia in drug injected paw. CB13 (20, 40µM/paw i.pl.) and gabapentin (60 mg/kg s.c.) treatment significantly inhibits paclitaxel-induced hypersensitivity to non-noxious mechanical stimuli. **(B)** No effect of CB13 on mechanical allodynia in uninjected paw**. (C) Acetone spray test:** Effect of CB13 on cold allodynia in drug injected paw. CB13 (20, 40µM/paw i.pl.) and gabapentin (60 mg/kg s.c.) treatment significantly inhibits paclitaxel-induced hypersensitivity to non-noxious cold stimuli. **(D)** No effect of CB13 on mechanical hyperalgesia in uninjected paw. Data were expressed as mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=8). ### ($p<0.001$) represents significance compared to Naïve group. *($p<0.05$), **($p<0.01$), ***($p<0.001$) represents significance compared to PTX+ Vehicle group. CB13 doses: C10: 10µM/paw, C20: 20µM/paw, C40: 40µM/paw. Gabapentin: 60mg/kg.

## 6.3.2 Peripheral CBR activation does not attenuate hyperalgesia like behaviour in paclitaxel-induced neuropathic rats

CINP rats demonstrated significant mechanical and cold hyperalgesia as compared to naïve rats. In pinprick test there was a significant increase in the paw withdrawal frequency to noxious mechanical stimuli in hind paws as compared to the respective pre-injury baselines and naïve rats ($p < 0.001$). Different doses of intraplantar CB13 (20, 40μM/paw) did not decrease the paw withdrawal frequency in either paw (Figure. 6.2 A-B)**.** The standard drug gabapentin (60mg/kg) showed significant effect from 60mins which lasted upto 240mins as compared to the vehicle treated neuropathic rats.

Further we assessed the effect of CB13 on hyperresponsiveness to noxious cold stimuli, using the ice floor test. Paclitaxel administration significantly increased the number of paw lifts in response to noxious cold stimuli as compared to the respective pre-injury baselines and naïve rats ($p < 0.001$). Different doses of intraplantar CB13 (20, 40μM/paw) did not decrease the number of paw lifts in either paw (Figure. 6.2 C-D). The standard drug gabapentin showed significant effect starting at 30mins, and the effect lasted upto 240 mins post-drug treatment ($p < 0.01$) as compared to the vehicle treated neuropathic rats. These findings indicated that activation of peripheral cannabinoid receptor showed modality specific inhibition of chemotherapy-induced neuropathic pain in rats.

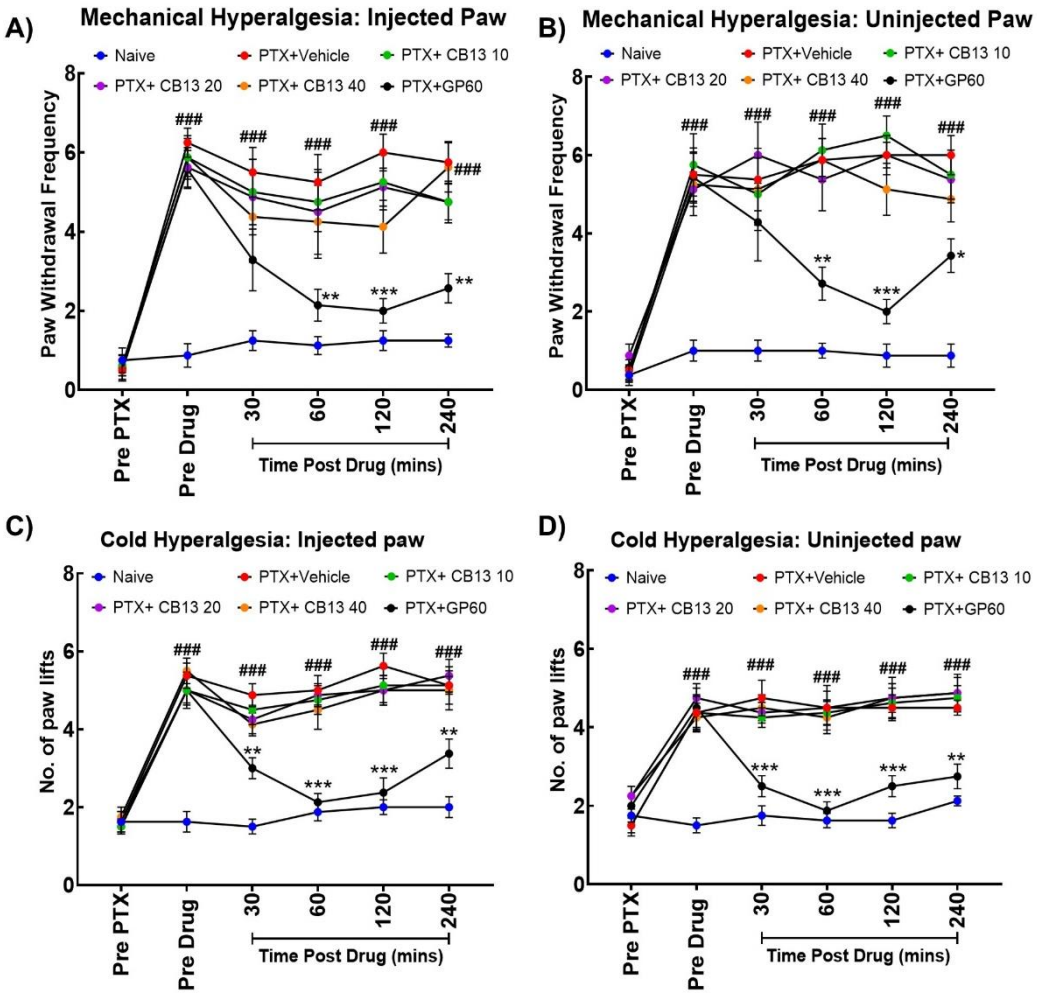



**Figure 6.2**: **Effect of CB13 on mechanical and cold hyperalgesia induced by PTX administration. (A) Pin prick test:** Effect of CB13 on mechanical hyperalgesia in drug injected paw. CB13 (20, 40µM/paw i.pl.) treatment did not inhibit paclitaxel-induced hypersensitivity to noxious mechanical stimuli. **(B)** No effect of CB13 on mechanical hyperalgesia in uninjected paw**. (C) Ice floor test:** Effect of CB13 on cold hyperalgesia in drug injected paw. CB13 (20, 40µM/paw i.pl.) treatment did not inhibit paclitaxel-induced hypersensitivity to noxious cold stimuli **(D)** No effect of CB13 on cold hyperalgesia in uninjected paw. However, gabapentin (60 mg/kg s.c.) treatment significantly inhibits paclitaxel-induced hypersensitivity to non-noxious mechanical and cold stimuli. Data were expressed as mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=8). ### ($p<0.001$) represents significance compared to Naïve group. *($p<0.05$), **($p<0.01$), ***($p<0.001$) represents

significance compared to PTX+ Vehicle group. CB13 doses: C10: 10µM/paw, C20: 20µM/paw, C40: 40µM/paw. Gabapentin: 60mg/kg.

### 6.3.3 CB13 does not attenuate spontaneous pain in paclitaxel-induced neuropathic rats

We used conditioned place preference (CPP) paradigm to assess the effect of CB13 on spontaneous pain-like behaviours in rats, a prominent symptom of CINP. CB13 (40µM/paw) treatment does not attenuate spontaneous pain in neuropathic rats as evident from no preference for the CB13-paired chamber as compared to the saline-paired chamber during the post-conditioning trial. Furthermore, there was a no change in the difference score in the chamber associated with CB13 treatment as compared to saline-paired chamber in CINP rats (Figure 6.3 A-C).

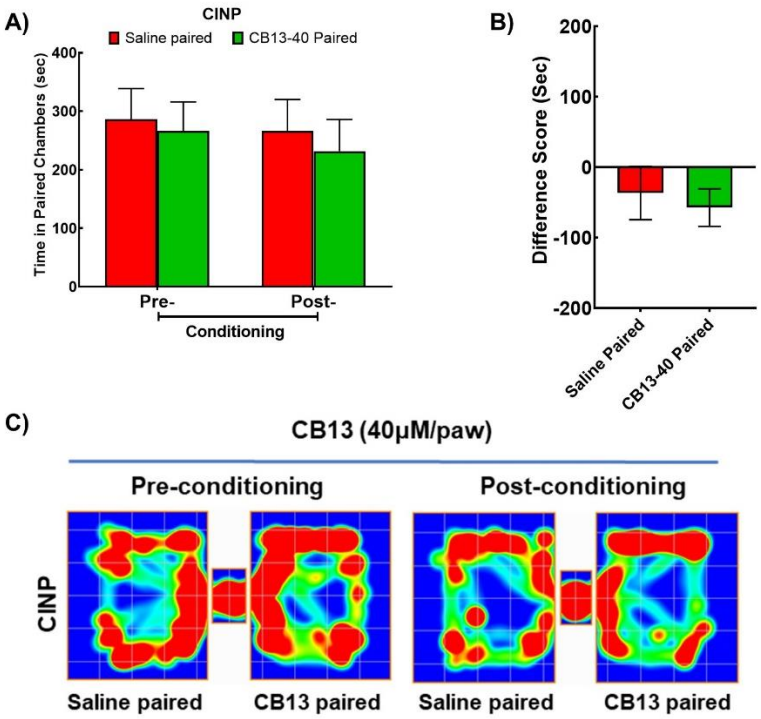



**Figure 6.3: Effect of peripheral CBR activation by CB13 on paclitaxel-induced spontaneous pain in rats. (A)** Heatmaps recorded during pre-conditioning and post-

conditioning with CB13 (40µM/paw i.pl.) v/s saline paired chambers **(B) Conditioned place preference (CPP) for CB13: CINP rats (C) Difference score.** Neuropathic (PTX) rats did not show any place preference behaviour in response to CB13 (40µM) treatment. Data were expressed as mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=6-8). CB13 doses: C40: 40µM/paw i.pl.

### 6.3.4 CB13 treatment didn't induce CNS toxicities in rats

Given the significant concerns surrounding CNS toxicities associated with available therapeutics in the market, our study aimed to assess whether CB13 (40µM/paw) leads to the emergence of any central side effects, including motor incoordination or locomotor impairment. We performed both open field test and rota-rod test to measure the locomotor activity and motor coordination of PTX-induced neuropathic rats. In open field test we have observed no difference in the average speed and total distance travelled by the rats after CB13 and gabapentin treatment. Although the morphine treated rats showed significant decrease in the total distance travelled and average speed showing its sedative property (Figure 6.4A-C).

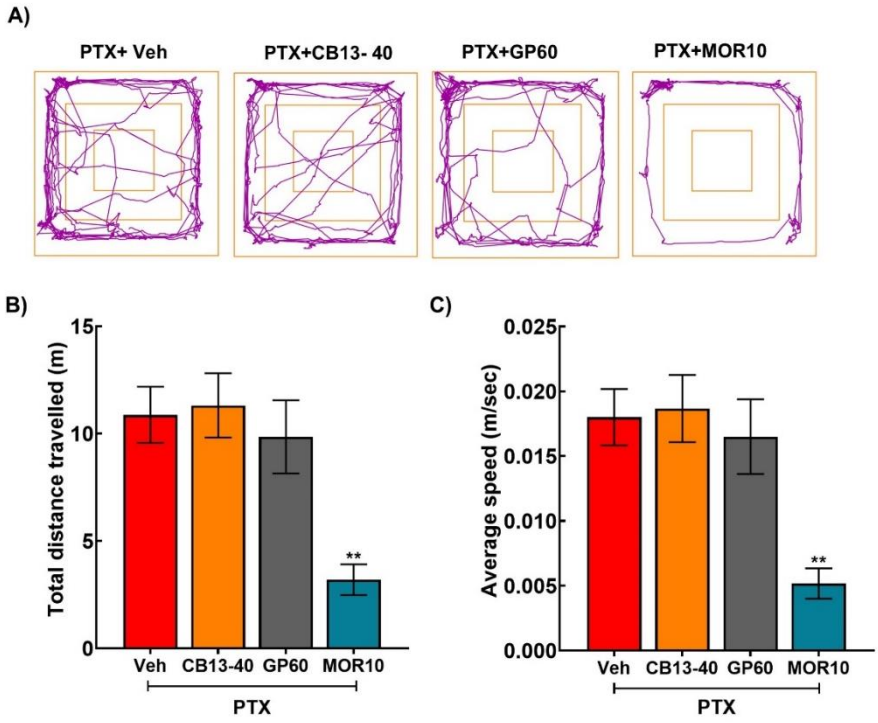


**Figure 6.4: Effect of CB13 administration on locomotor activity of rats. Open field test: (A)** Open field track plots of CINP rats treated with vehicle, CB13 (40µM/paw i.pl.), gabapentin (60mg/kg s.c.) and morphine (10mg/kg s.c.). **(B)** Total distance travelled in open field arena. **(C)** Average speed of rats treated with vehicle, CB13 (40µM/paw i.pl.), gabapentin (60mg/kg s.c.) and morphine (10mg/kg s.c.). CB13 (40µM/paw i.pl.) and gabapentin (60mg/kg s.c.) treatment did not affect the locomotor activity of CINP rats in open field arena as compared to the vehicle treated rats. However, morphine (10mg/kg s.c.) treated rats showed a significant decline in total distance travelled and average speed. Data was expressed as Mean ± SEM and analyzed by using one-way ANOVA followed by Tukey's post hoc analysis test (n=6-7). ***p<0.001 indicates statistical significance as compared to the vehicle treated rats.

In rotarod test, CB13 as well as gabapentin treatment showed no significant changes in time spent on rod as compared to their pre-drug baselines. However, it's worth noting that morphine treatment significantly decreased the fall latency of rats as compared to their pre-treatment baseline (Figure. 6.4 D) indicating its neurotoxic effects.

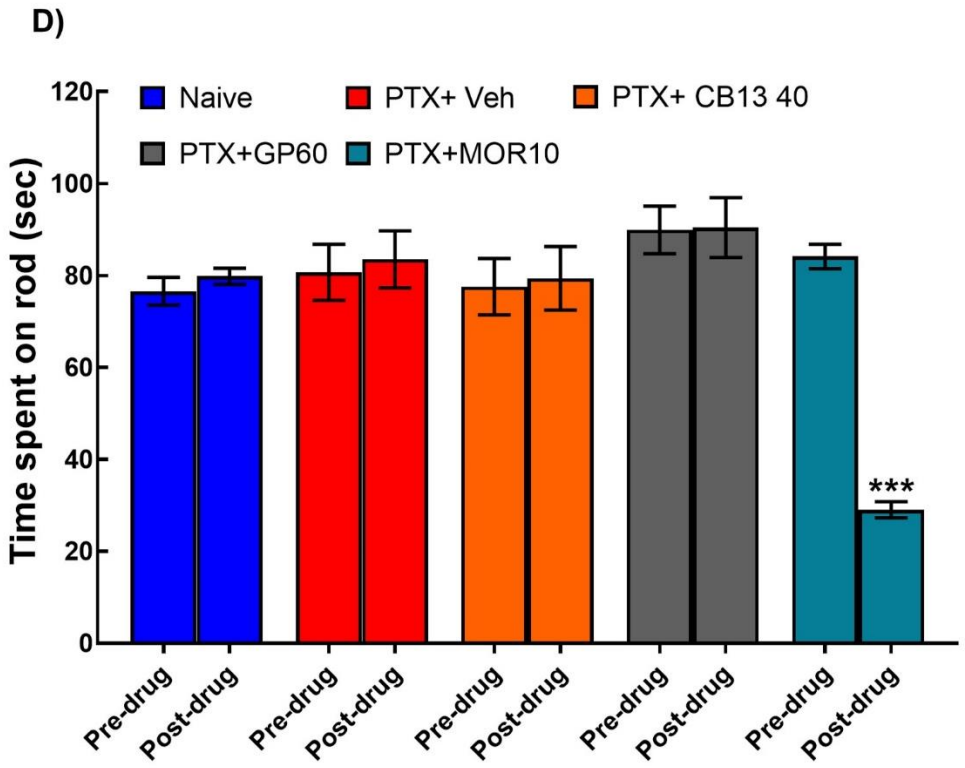



**Figure 6.4: D. Effect of CB13 administration on motor coordination of rats. Rotarod test:** CB13 (40µM/paw i.pl.) and gabapentin (60 mg/kg s.c.) treatment did not

alter the time spent by rats on rota-rod as compared to their pre-drug baseline. However, morphine (10mg/kg s.c.) treatment significantly decreased the time spent by rats on rota-rod as compared to their pre-morphine baseline. Data were presented as mean ± SEM analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=6). *$p<0.05$, and ***$p<0.001$ indicates statistical significance as compared to the PTX treated rats. $p<0.05$ was considered statistically significant. CB13 (40μM/paw i.pl.), Gabapentin (GP60): 60mg/kg s.c., Morphine MOR10: 10mg/kg s.c.

### 6.3.5 CB13 restores GSH levels but fails to suppress oxido-nitrosative parameters in the sciatic nerve of paclitaxel-induced neuropathic rats.

Generation of ROS and RNS are important features of CINP. So, we evaluated the effect of CB13 on levels of various oxidative markers and antioxidant enzymes. The level of antioxidant GSH was decreased ($p<0.001$) after PTX administration whereas nitrite ($p<0.05$) and malonaldehyde (MDA) levels ($p<0.01$) were significantly increased. Different doses of CB13 treatment restored the levels of GSH (5mg/kg, $p<0.05$ & 10mg/kg, $p<0.001$) and alleviated levels of nitrite (5 & 10mg/kg, $p<0.01$) and MDA (20 & 40μM/paw, $p<0.05$) (Figure. 6.5 A). CB13 treatment was unable to suppress the increased levels of Nitrite and MDA in the sciatic nerve of neuropathic rats (Figure. 6.5 B-C). Further, gabapentin treatment also alleviated the oxido-nitrosative stress by decreasing the levels of nitrite ($p<0.05$) and MDA ($p<0.01$) while restoring the GSH levels ($p<0.01$) in sciatic nerve of neuropathic rats.

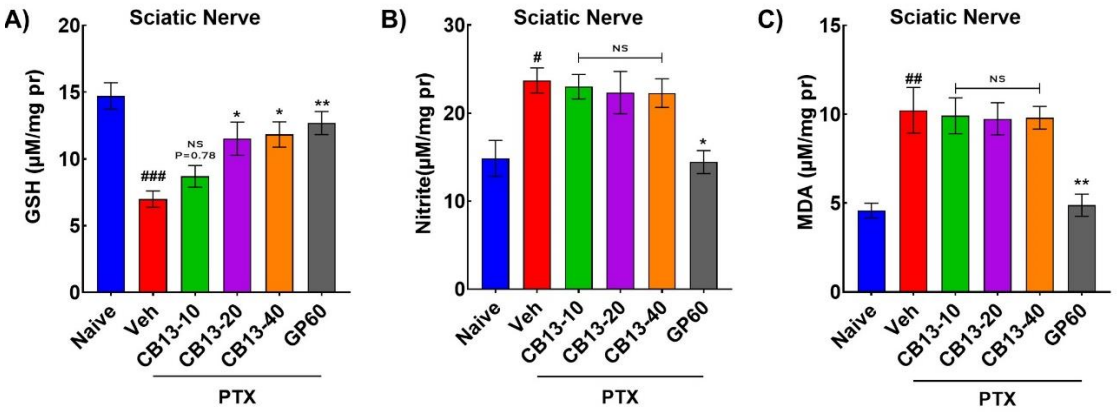



**Figure 6.5 Effect of CB13 on paclitaxel-induced oxido-nitrosative stress in sciatic nerve of rats (A) Nerve GSH:** Paclitaxel administration resulted in decreased levels of antioxidant (GSH) in sciatic nerve of rats, which was significantly restored upon treatment with CB13 (20 & 40µM/paw, i.pl.) and gabapentin (60mg/kg s.c.). **(B) Nerve nitrite and (C) Nerve malondialdehyde:** Paclitaxel administration resulted in increased nitrite and MDA levels in sciatic nerve of rats which was not decreased with CB13 treatment. However, gabapentin treatment (60mg/kg s.c.) suppressed levels of nitrite and MDA. Data were expressed as Mean ± SEM and analyzed by one-way ANOVA Post hoc analysis: Tukey's post hoc analysis test). (n=5-6). ### ($p<0.001$) represents significance compared to Naïve group. *($p<0.05$), **($p<0.01$), ***($p<0.001$) represents significance compared to PTX+ Vehicle group. CB13 doses: C10: 10µM/paw, C20: 20µM/paw, C40: 40µM/paw. Gabapentin (GP60): 60mg/kg.

## 6.3.6 CB13 alleviates CINP by downregulating TRP channels, VGCCs and NR2B expressions in DRG of neuropathic rats

The pathophysiology of paclitaxel-induced neuropathic pain involves upregulation of TRP (transient receptor potential) channels, VGSCs (voltage-gated sodium channels), and VGCCs (voltage-gated calcium channels) in DRG of rats. We observed that protein expression of TRPA1 ($P<0.001$) and mRNA expression of TRPM8 ($P<0.001$), were upregulated in the DRG of vehicle treated rats as compared to the naïve rats ($p<0.001$). Further, we also observed that protein expression of Nav1.7 were significantly upregulated in the DRG of vehicle treated rats ($P<0.001$) as compared to naïve group ($p<0.001$). Treatment with CB13 leads to significant decrease in the levels of TRPA1

(10 μM/paw, $p<0.01$; 20 & 40μM/paw, $p<0.001$) and TRPM8 (10 μM/paw, $p<0.01$; 20 & 40μM/paw, $p<0.001$). However, CB13 treatment did not downregulate the protein expression of Nav1.7. Upregulation of NR2B subunits in the DRG neurons contribute to the increased activity and sensitivity of NMDA receptors, is a key mechanism reported in CINP. Thus, we evaluated the effect of CB13 treatment on protein expression of NR2B and observed that the upregulated NR2B in DRG of PTX treated rats was significantly decreased on treatment with different doses of CB13 (20 μM/paw, $p<0.05$ & 40μM/paw, $p<0.01$) (Figure. 6.6 A-D). Gabapentin treatment also reduced the mRNA expression of TRPM8 ($p<0.001$) and protein expressions of TRPA1 ($p<0.001$) and NR2B in DRG of neuropathic rats ($p<0.01$).

Further we observed an increased mRNA expression of VGCCs (voltage-gated calcium channels)- Cav2.2 (marker for N-type calcium channel) and Cav3.2 (marker for T-type calcium channel). Treatment with different doses of CB13 resulted in a significant downregulation in mRNA expression of Cav2.2 (10, 20, 40 μM/paw, $p<0.05$) and Cav3.2 (20 μM/paw, $p<0.05$ & 40 μM/paw, $p<0.01$). Notably, gabapentin suppressed the expression of Cav2.2 but not Cav3.2 (Figure 6.6 E-F).

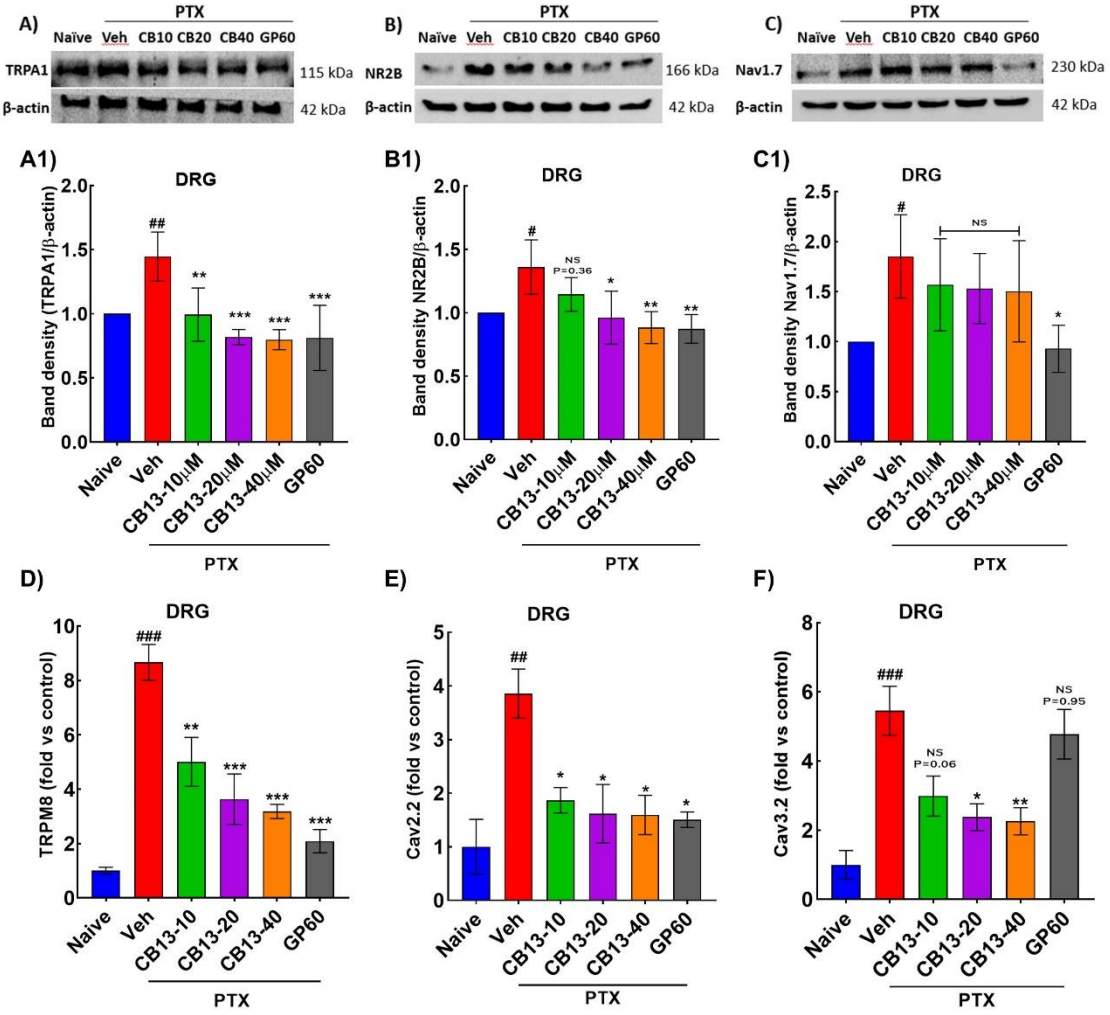



**Figure 6.6. Effect of CB13 on paclitaxel-induced increase in TRP channels, voltage gated ion channels & NR2B, in lumbar DRG of rats. (A) TRPA1 blot:** Representative blot of TRPA1 protein expression in DRG tissues of neuropathic rats. **(A1) TRPA1 protein expression:** CB13 treatment (10, 20, 40 μM/paw, i.pl) treatment significantly reversed paclitaxel-induced increase in TRPA1 expression in DRG of rats. Gabapentin treatment also showed decreased protein expression of TRPA1 in DRG of neuropathic rats. **(B) NR2B blot:** Representative blot of NR2B protein expression in DRG tissues of neuropathic rats. **(B1) NR2B protein expression:** Increased protein expression of NR2B was significantly attenuated by CB13 treatment (20, 40 μM/paw, i.pl) and gabapentin (60 mg/kg s.c.) treatment in DRG of neuropathic rats. **(C) Nav1.7 blot:** Representative blot of Nav1.7 protein expression in DRG tissues of neuropathic rats. **(C1) Nav1.7 protein expression:** Paclitaxel administration increased the Nav1.7 protein expression in DRG of rats which was unchanged by the CB13 treatment (10, 20, 40 μM/paw, i.pl). **(D) TRPM8 mRNA expression:** Increased mRNA expression of TRPM8 was significantly attenuated by CB13 treatment (10, 20, 40 μM/paw, i.pl.) and gabapentin (60 mg/kg s.c.) treatment in DRG of neuropathic rats. **(E) Cav2.2 mRNA**

**expression:** Paclitaxel administration increased the Cav2.2 mRNA expression in DRG of rats which was attenuated by CB13 treatment (10, 20, 40 μM/paw, i.pl) and gabapentin (60 mg/kg s.c.) treatment in DRG of neuropathic rats. **(F) Cav3.2 mRNA expression:** Paclitaxel administration increased the Cav3.2 mRNA expression in DRG of rats which was attenuated by CB13 treatment (20, 40 μM/paw, i.pl) but not by gabapentin (60mg/kg s.c.). Data were presented as mean ± SEM. (n=4). # ($p<0.05$), ## ($p<0.01$), ### ($p<0.001$) represents significance compared to Naïve group. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ indicates statistical significance as compared to the PTX+ Veh rats. CB13 doses: C10: 10μM/paw, C20: 20μM/paw, C40: 40μM/paw. Gabapentin (GP60): 60mg/kg.

### 6.3.7 CB13 treatment attenuates microglia activation in spinal cord of neuropathic rats

Paclitaxel administration disrupts vascular permeability and causes cytokine infiltration and microglial activation that further leads to central sensitization. Therefore, we investigated the effect of CB13 on protein expressions of ICAM-1, which is known to increase in the presence of disrupted vascular permeability, and IBA1, a marker of microglia activation. Protein expressions of ICAM1 ($p<0.01$) and IBA1 ($p<0.001$) were significantly upregulated in the spinal cord of neuropathic rats as compared to the naïve group. Treatment with CB13 leads to significantly downregulation in protein expression of IBA1 (20 μM/paw, $p<0.05$ & 40 μM/paw, $p<0.01$). However, we did not find any significant changes in ICAM1 protein expression of neuropathic rats after CB13 treatment. Furthermore, treatment with gabapentin significantly downregulated the expressions of both ICAM1 ($p<0.01$) and IBA1 ($p<0.01$) in spinal cord of neuropathic rats (Figure. 6.7A).

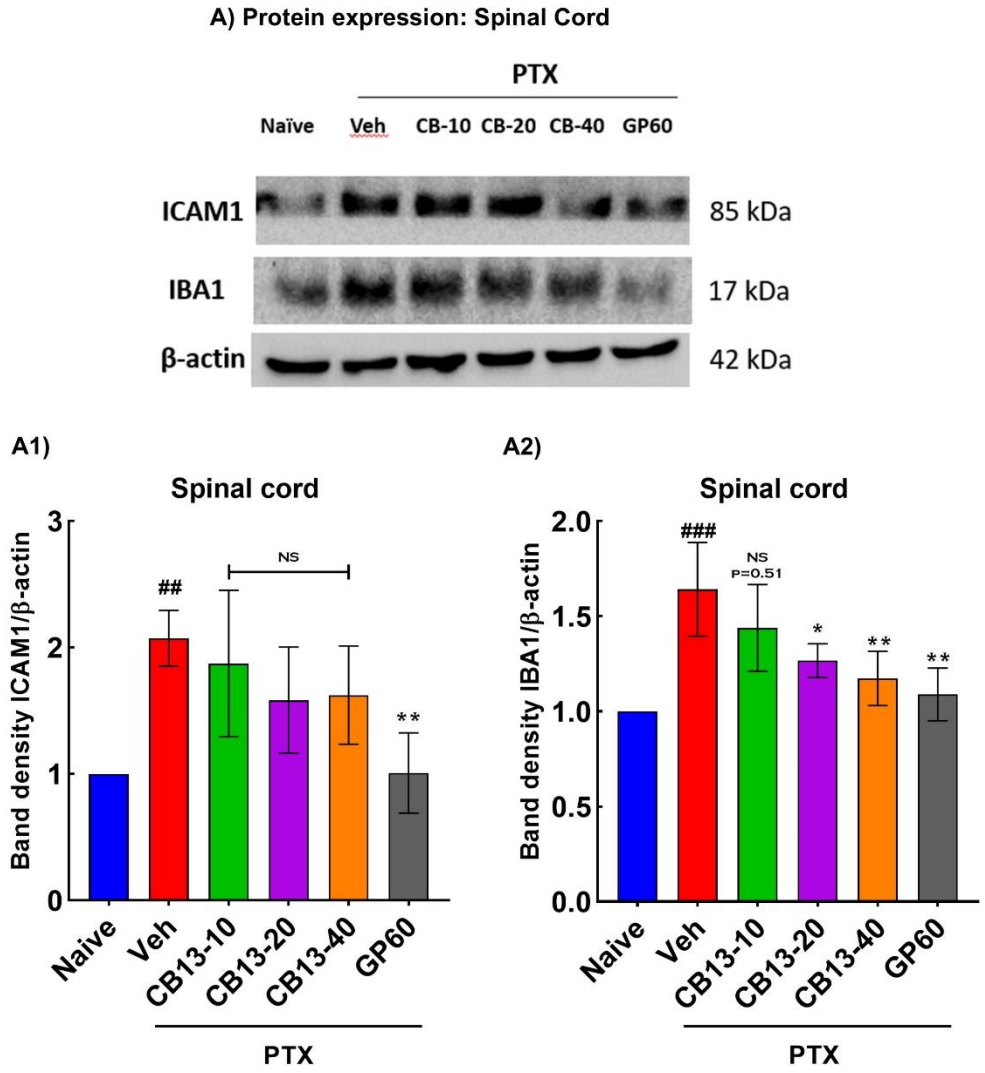
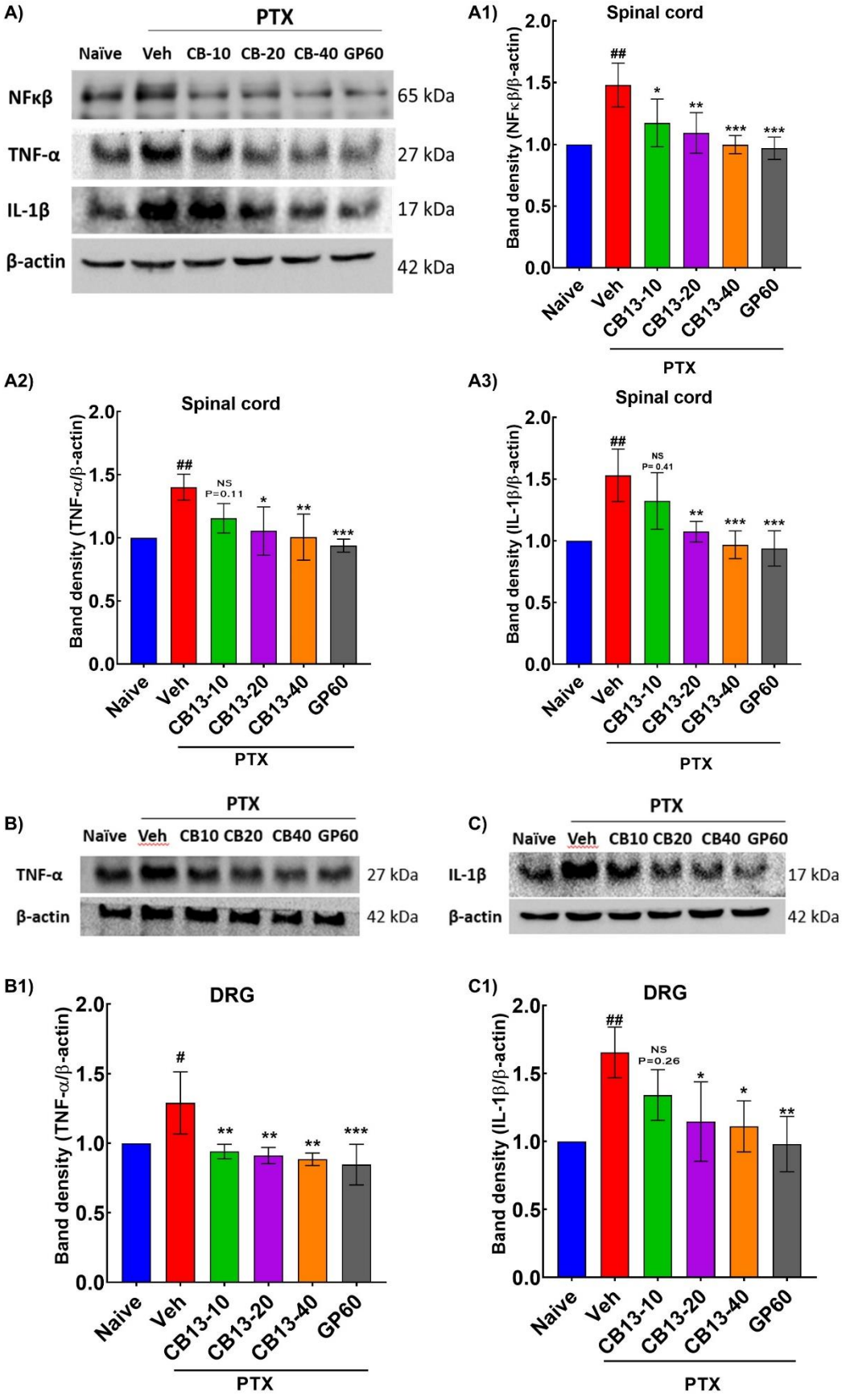



**Figure 6.7. Effect of CB13 on paclitaxel-induced increase in protein expressions of ICAM1 and IBA1 in lumbar spinal cord of rats. (A) ICAM1 and IBA1 blots:** Representative blots of ICAM1 and IBA1 protein expressions in spinal cord tissues **(A1)** Paclitaxel administration increased the ICAM1 protein expression in spinal cord of rats but CB13 was unable to alter its expression unlike gabapentin, that significantly reduced the expression of ICAM1 **(A2)** Paclitaxel administration increased the IBA1 protein expression in spinal cord of rats which was attenuated by the treatment with CB13 (20 μM/paw, $p<0.05$ & 40 μM/paw, $p<0.01$) and gabapentin (60mg/kg s.c.). Data were presented as mean ± SEM. (n=4), ##($p<0.01$), ###$p<0.001$ represents significance compared to Naïve group. *$p<0.05$, **$p < 0.01$ indicates statistical significance as compared to the PTX+ Veh rats. CB13 doses: C10: 10μM/paw, C20: 20μM/paw, C40: 40μM/paw. Gabapentin (GP60): 60mg/kg.

### 6.3.8 CB13 attenuates neuroinflammation by inhibiting release of pro-inflammatory cytokines and neuropeptides

Neuro-inflammatory mediators and neuropeptides like CGRP & Substance P plays a significant role in central sensitization during paclitaxel-induced neuropathic pain. We observed a significant increase in the protein expressions of NF-κβ, TNF-α, IL-1β and mRNA expression of CGRP and Substance P in spinal cord and DRG of neuropathic rats. CB13 treatment leads to significant decrease in protein expressions of NFκβ (10 μM/paw, $p<0.05$; 20 μM/paw, $p<0.01$ & 40 μM/paw, $p<0.001$), TNF-α (20 μM/paw, $p<0.05$ & 40 μM/paw, $p<0.01$), and IL-1β (20 μM/paw, $p<0.01$ & 40 μM/paw, $p<0.001$) in spinal cord of neuropathic rats. Simultaneously, there was a significant increase in the protein expressions of TNF-α and IL-1β in DRG of CINP rats. We observed that CB13 reduced the protein expression of both TNF-α (10, 20 & 40 μM/paw, $p<0.01$) and IL-1β (20 & 40 μM/paw, $p<0.05$), in DRG tissues of CINP rats (Figure. 6.8A-C). Further it also decreased the mRNA expressions of CGRP (10 μM/paw, $p<0.01$; 20 & 40 μM/paw, $p<0.001$) and Substance P (20 & 40 μM/paw, $p<0.01$) in lumbar spinal cord tissues of neuropathic rats (Figure. 6.9 A-B). Treatment with standard drug gabapentin leads to significant downregulation in expressions of NFκβ ($p<0.001$), TNF-α ($p<0.01$), IL-1β ($p<0.001$) and neuropeptides CGRP ($p<0.01$), Substance-P ($p<0.001$) in the lumbar spinal cord of neuropathic rats. It also decreased protein expressions of TNF-α ($p<0.05$) and IL-1β ($p<0.01$) in DRGs of neuropathic rats.


A)
PTX
Naïve Veh CB-10 CB-20 CB-40 GP60
NFκβ
TNF-α
IL-1β
β-actin
65 kDa
27 kDa
17 kDa
42 kDa
A1)
Spinal cord
Band density (NFκβ/β-actin)
A2)
Spinal cord
Band density (TNF-α/β-actin)
A3)
Spinal cord
Band density (IL-1β/β-actin)
Naive Veh CB13-10 CB13-20 CB13-40 GP60
PTX
B)
Naïve Veh CB10 CB20 CB40 GP60
TNF-α
β-actin
27 kDa
42 kDa
C)
Naïve Veh CB10 CB20 CB40 GP60
IL-1β
β-actin
17 kDa
42 kDa
B1)
DRG
Band density (TNF-α/β-actin)
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DRG
Band density (IL-1β/β-actin)

**Figure 6.8: Effect of CB13 on paclitaxel-induced neuro-inflammation and neuropeptide expression in spinal cord and DRG of rats. (A) NF-κβ, TNF-α, IL-1β blots (spinal cord):** Representative blots of NFκβ, TNF-α, IL-1β protein expressions in spinal cord of rats. **(A1) NF-κβ protein expression (spinal cord), (A2) TNF-α protein expression (spinal cord), (A3) IL-1β protein expression (spinal cord):** Paclitaxel administration significantly increased the protein expressions of NFκβ, TNF-α and IL-1β in spinal cord of rats which was attenuated by the treatment with CB13 (10, 20, 40 μM/paw, i.pl.) and gabapentin (60mg/kg s.c.). **(B) TNF-α blot (DRG):** Representative blot of TNF-α protein expression in DRG tissues. **(B1)** Paclitaxel administration increased the TNF-α protein expression in DRG of rats attenuated by the treatment with CB13 (10, 20, 40 μM/paw, i.pl.) and gabapentin (60mg/kg s.c.) **(C) IL-1β blot (DRG):** Representative blot of IL-1β protein expression in DRG tissues. **(C1)** Paclitaxel administration increased the IL-1β protein expression in DRG of rats which was attenuated by the CB13 (10, 20, 40 μM/paw, i.pl.) and gabapentin (60mg/kg s.c.) treatment. Data were presented as mean ± SEM. (n=4), #(p<0.05), ##p<0.01, ###p<0.001 represents significance compared to Naïve group. *p < 0.05, **p < 0.01, ***p<0.001 indicates statistical significance as compared to the PTX+ Veh rats. CB13 doses: C10: 10μM/paw, C20: 20μM/paw, C40: 40μM/paw. Gabapentin (GP60): 60mg/kg.

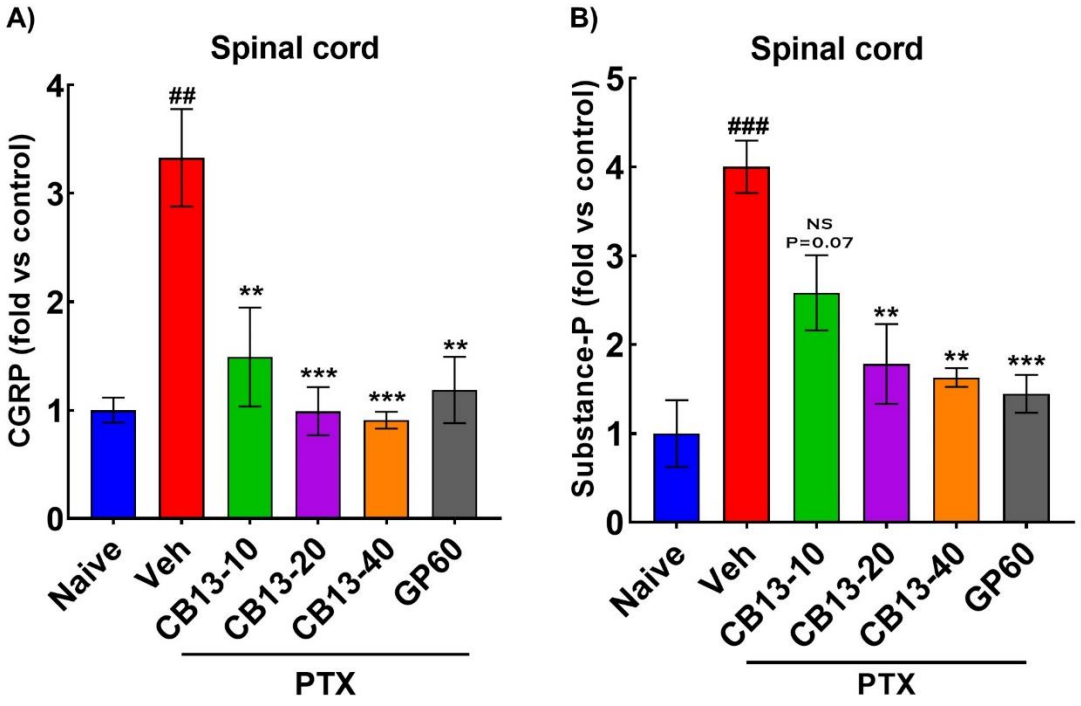


**Figure 6.9: Effect of CB13 on paclitaxel-induced increase in expression of neuropeptides in the spinal cord of rats. (A) mRNA expression of CGRP.** Paclitaxel administration significantly increased the mRNA expressions of CGRP in spinal cord of rats which was attenuated by the treatment with CB13 (10, 20, 40 μM/paw, i.pl.) and

gabapentin (60mg/kg s.c.). **(B) mRNA expression of Substance-P.** Paclitaxel administration significantly increased the mRNA expressions of Substance-P in spinal cord of rats which was attenuated by the treatment with CB13 (10, 20, 40 μM/paw, i.pl.) and gabapentin (60mg/kg s.c.). Data were presented as mean ± SEM. (n=4), ##p<0.01, ###p<0.001 represents significance compared to Naïve group. *p < 0.05, **p < 0.01, ***p<0.001 indicates statistical significance as compared to the PTX+ Veh rats. CB13 doses: C10: 10μM/paw, C20: 20μM/paw, C40: 40μM/paw. Gabapentin (GP60): 60mg/kg.

## 6.4 Outcomes

Our findings suggests that the activation of peripheral CB1/CB2 receptors effectively mitigates allodynia-like behavior in paclitaxel-induced neuropathic rats without producing CNS toxicities. This therapeutic effect is attributed to the downregulation of transient receptor potential (TRP) channels, NR2B, and voltage-gated calcium channels (VGCCs). Moreover, the observed suppression of neuroinflammatory and oxidative cascades further highlights the multifaceted impact of CB13.

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