CHAPTER 5:

Peripheral mu-opioid receptor activation by dermorphin [D-Arg2, Lys4] (1–4) amide alleviates behavioral and neurobiological aberrations in rat model of chemotherapy - induced neuropathic pain

Chapter-5

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5.1 Introduction

Paclitaxel, a widely used chemotherapeutic agent, has demonstrated remarkable efficacy against various malignancies. Despite its success in cancer treatment, a distressing side effect associated with paclitaxel administration is the development of neuropathic pain, which significantly compromises patients' quality of life [266]. CINP is characterized by sensory abnormalities, such as spontaneous pain, allodynia, and hyperalgesia, burning, tingling, which can persist long after the completion of treatment [267-269]. Overall, estimates of CINP prevalence can range from 30% to 70% or more among cancer patients receiving chemotherapy. The current therapeutic options for CINP include gabapentinoids, TCAs, SNRIs like duloxetine and weak or strong opioids [7,260]. These medications predominantly work by targeting the pain centers in the CNS resulting in multiple side effects like dizziness, sedation, addiction and abuse potential [7,270]. Additionally, a significant proportion of patients with CINP do not respond favorably to current therapeutic interventions, and many eventually develop tolerance over time, rendering treatment less effective. Therefore, it becomes

imperative to prioritize the development of therapeutic interventions that can produce profound analgesia without causing CNS toxicities.

In recent years, the pursuit of targeting the PNS has gained prominence as a strategic approach in the development of safer and innovative analgesic treatments. This approach focuses on delivering medications that do not cross the blood brain barrier while directly addressing the root cause of pain at the peripheral pain site [207]. A multitude of pre-clinical reports suggest that targeting the peripheral MOR serves as a promising approach to alleviate symptoms of both evoked and ongoing pain without producing CNS adverse effects [215,221,263]. Peripherally restricted mu-opioid agonists have shown significant efficacy in rodent models of spinal cord injury, neuropathic pain, bone cancer pain, and inflammatory pain [216,217,221]. Dermorphin [D-Arg2, Lys4] (1-4) amide (DALDA), a MOR agonist, exhibits substantial hydrophilicity attributed to its three net positive charges at physiological pH, resulting in improved metabolic stability and restricted central nervous system penetration when administered systemically [220]. Notably, DALDA has demonstrated substantial promise in preclinical models of neuropathic pain pointing towards its analgesic efficacy [218,219]. Therefore, present study was designed with an aim to investigate the effect of DALDA on rat model of paclitaxel-induced neuropathic pain, along with an in-depth exploration of associated neurobiological mechanisms.

5.2 Experimental design

The rats were divided into six experimental groups: Naïve; Paclitaxel + Vehicle (0.9% sterile saline); Paclitaxel + DALDA (2.5, 5, and 10 mg/kg, *s.c*); 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. DALDA and gabapentin were prepared by dissolving in 0.9% normal saline. Gabapentin is currently among the first line drugs for the treatment of CINP and the same has been used in all the evoked pain behavioural assays as standard intervention to compare the potency of DALDA.The rats were allowed to acclimatize to the test room environment and with different equipments 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 DALDA (2.5, 5 & 10 mg/kg s.c) 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 DALDA 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 -80^{o} C for further molecular investigations.

5.3 Results and discussion

5.3.1 Peripheral MOR activation attenuates allodynia and hyperalgesia in paclitaxel-induced neuropathic rats

We evaluated the efficacy of peripheral MOR agonist, DALDA, in the treatment of CINP by performing a battery of pain behavioural tests to assess both mechanical and cold allodynia and hyperalgesia. 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 ($p < 0.001$). DALDA (2.5, 5, 10 mg/kg *s.c.)* significantly improved the PWT with effect starting from 30 mins, post drug administration. At 60 mins we observed the peak therapeutic effect (2.5mg/kg, $p<0.05$; 5 and 10 mg/kg, $p < 0.001$) as compared to their pre- drug baselines and vehicle treated CINP group. The effect lasted upto 120mins post drug administration. (Figure. 5.1A). 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 DALDA (2.5, 5, 10 mg/kg *s.c.*) significantly decreased the response score in hind paws with effect starting from 30 mins, post drug administration. At 60 mins we observed the peak therapeutic effect (2.5mg/kg; $p<0.05$, 5 and 10 mg/kg; $p < 0.001$) as compared to their pre- drug baselines and vehicle treated neuropathic rats. (Figure. 5.1B). The standard drug gabapentin (60mg/kg) also attenuated cold allodynia as compared to the vehicle treated neuropathic rats.

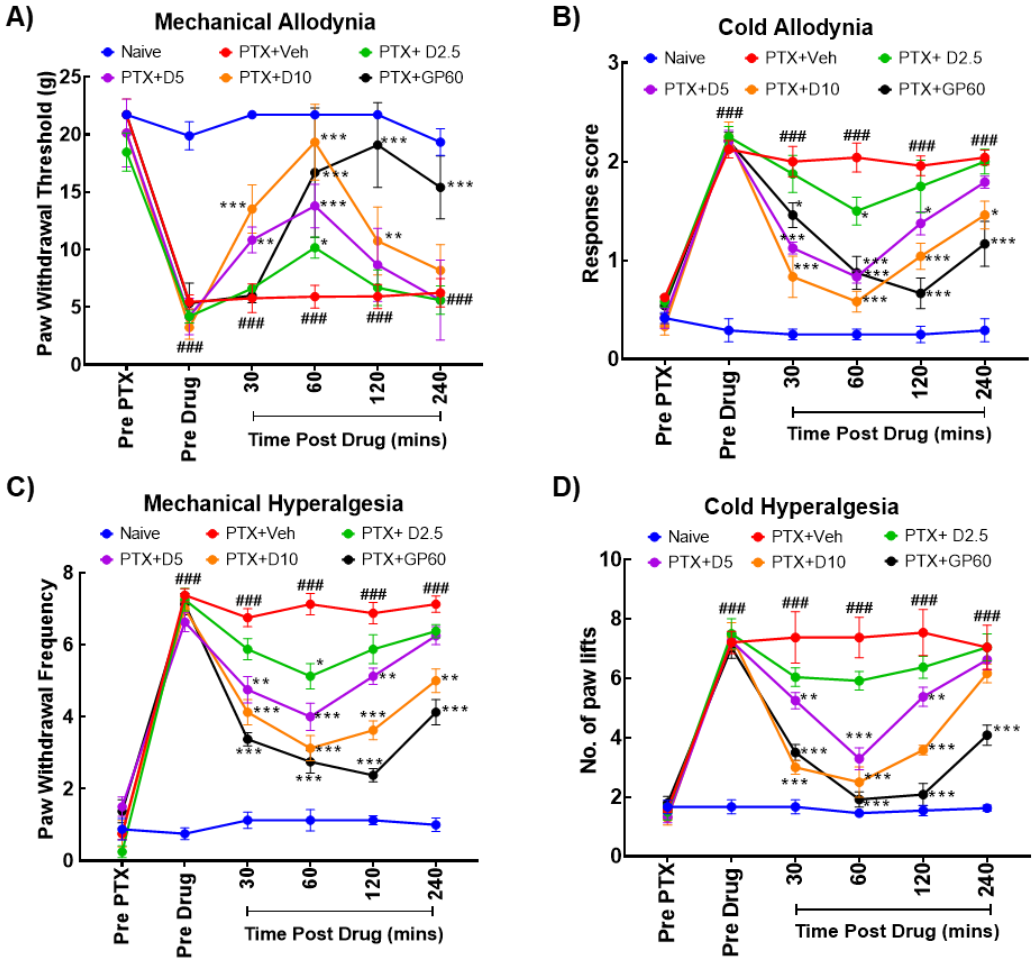

Figure 5.1: **Effect of peripheral MOR activation by DALDA on paclitaxel-induced evoked pain behaviour in rats. (A) von Frey hair test:** DALDA (2.5, 5, 10mg/kg s.c.) and gabapentin (60 mg/kg s.c.) treatment significantly inhibits paclitaxel-induced hypersensitivity to non-noxious mechanical stimuli. **(B) Acetone spray test:** Paclitaxel induces significant increase in paw withdrawal score of rats in response to non-noxious cold stimuli, which was alleviated upon treatment with DALDA (2.5, 5, 10mg/kg s.c.) and gabapentin (60mg/kg s.c.). **(C) Pin prick test:** Paclitaxel administration significantly increases the paw withdrawal frequency as compared to the pre-injury baseline in response to noxious mechanical stimuli. DALDA (2.5, 5, 10mg/kg s.c.) and gabapentin (60mg/kg s.c.) treatment significantly reduced the paw withdrawal frequency of PTX administered rats as compared to their pre-drug baseline. **(D) Ice floor test:** Paclitaxel administration led to the development of cold hypersensitivity in rats, evident from the higher count of paw lifts compared to their pre-injury baseline. Administration of DALDA (2.5, 5, 10mg/kg s.c.) and gabapentin (60 mg/kg s.c.) significantly decreased the number of paw lifts in response to noxious 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. DALDA doses: D2.5: 2.5mg/kg, D5: 5mg/kg, D10: 10mg/kg. Gabapentin (GP60): 60mg/kg.

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 ($p < 0.001$). DALDA (2.5, 5, 10 mg/kg *s.c.*) significantly decreased the PWF in hind paws with effect starting from 30 mins, post drug administration. At 60 mins we observed the peak therapeutic effect (2.5 mg/kg; $p<0.05$ and 5 and 10 mg/kg; $p < 0.001$) as compared to their pre-treatment baselines and vehicle treated neuropathic rats (Figure. 5.1C)**.** The standard drug gabapentin (60mg/kg) showed significant effect from 30mins ($p<0.001$), which lasted upto 240mins as compared to the vehicle treated neuropathic rats.

Further we assessed the effect of DALDA 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 DALDA (2.5, 5, 10 mg/kg *s.c.*) significantly decreased the number of paw lifts with effects starting from 30mins post drug administration. At 60mins we observed a peak therapeutic effect (5 and 10 mg/kg; $p<0.001$) that lasted upto 120mins post drug treatment as compared to their pre- drug baselines and vehicle treated neuropathic rats (Figure. 5.1D). The standard drug gabapentin showed significant effect starting at 30mins ($p<0.001$), and the effect lasted upto 240 mins post-drug treatment ($p < 0.001$) as compared to the vehicle treated neuropathic rats. These findings indicated that activation of peripheral MOR is can attenuate cold pain behaviour in the chemotherapy-induced neuropathic rats.

5.3.2 DALDA attenuates spontaneous pain in paclitaxel-induced neuropathic rats

We used conditioned place preference (CPP) paradigm to assess the effect of DALDA on spontaneous pain-like behaviours in rats, a prominent symptom of CINP. DALDA (10 mg/kg s.c) treatment significantly attenuates spontaneous pain in neuropathic rats as evident from substantial increase ($p<0.05$) in their preference for the DALDA-paired chamber as compared to the saline-paired chamber during the post-conditioning trial. Furthermore, there was a significant increase ($p<0.001$) in the difference score in the chamber associated with DALDA treatment as compared to saline-paired chamber in CINP rats.

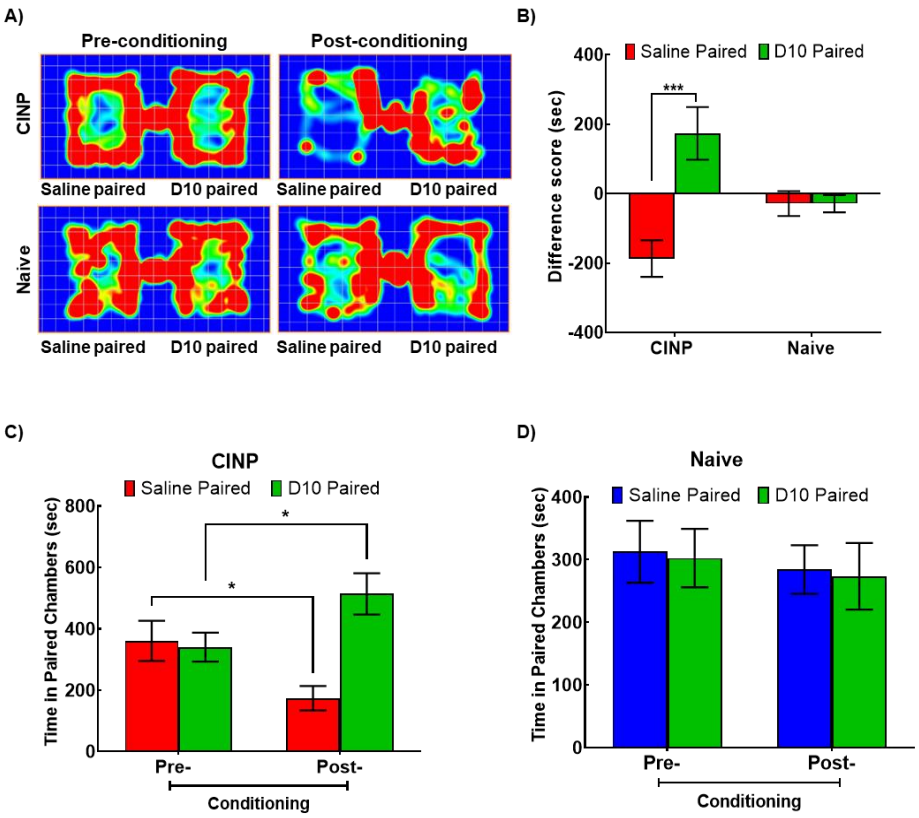

Figure 5.2: Effect of peripheral MOR activation by DALDA on paclitaxel-induced spontaneous pain in rats. (A) Heatmaps recorded during pre-conditioning and post-

conditioning with DALDA (10mg/kg s.c.) v/s saline paired chambers **(B, C, D) Conditioned place preference (CPP) for DALDA: Difference score, CINP rats, Naïve rats.** Neuropathic (PTX) rats showed significant place preference behaviour in response to DALDA (10mg/kg s.c.) treatment as indicated by increased time spent in DALDA paired chamber during post-conditioning. Notably, DALDA (10mg/kg s.c.) did not produce CPP in healthy naïve rats. Data were expressed as mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=6-8). *($p<0.05$), ***($p<0.001$) represents significant difference. DALDA dose: D10: 10mg/kg s.c.

More importantly, when we administered DALDA (10 mg/kg s.c.) in healthy naïve rats, we didn't observe any place preference behaviour (Figure. 5.2A-D). This indicates that the development of DALDA-induced CPP is dependent on pain state of the animals and is not associated with potential of drug abuse.

5.3.3 DALDA 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 DALDA 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 DALDA and gabapentin treatment. Although the morphine treated rats showed significant decrease in the total distance travelled and average speed showing its sedative property (Figure 5.3A-C). In rotarod test, DALDA 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. 5.3D) indicating its neurotoxic effects.

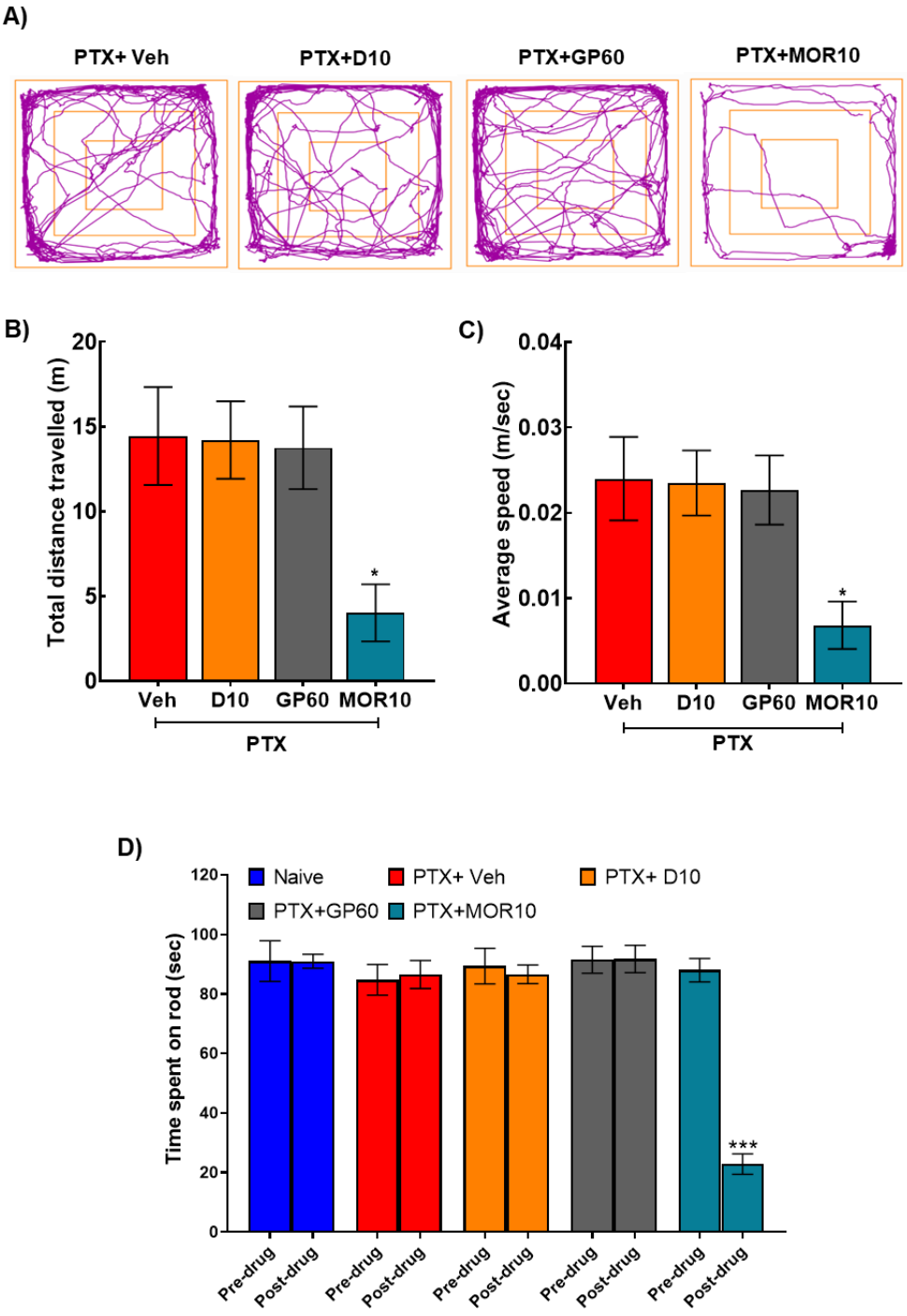

Figure 5.3: Effect of DALDA administration on locomotor activity and motor coordination of rats. Open field test: (A) Open field track plots of CINP rats treated with vehicle, DALDA (10mg/kg s.c.), gabapentin (60mg/kg s.c.) and morphine (10mg/kg s.c.). **(B)** Total distance travelled in open field arena. **(C)** Average speed of rats treated with vehicle, DALDA (10mg/kg s.c.), gabapentin (60mg/kg s.c.) and morphine (10mg/kg s.c.). DALDA (10mg/kg s.c.) 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. **(D) Rotarod test:** DALDA (10mg/kg s.c.) 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. DALDA D10: 10mg/kg s.c., Gabapentin (GP60): 60mg/kg s.c., Morphine MOR10: 10mg/kg s.c.

5.3.4 DALDA attenuates oxido-nitrosative stress in sciatic nerve of paclitaxel-induced neuropathic rats

Generation of reactive oxygen and nitrogen species (ROS and RNS) are important features of CINP. So, we evaluated the effect of DALDA on levels of various oxidative markers and antioxidant enzymes. The level of antioxidant GSH was decreased ($p<0.001$) after PTX administration whereas nitrite and malonaldehyde (MDA) levels were significantly increased ($p<0.001$). Different doses of DALDA 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 (2.5 & 5mg/kg, $p<0.01$; 10mg/kg, $p<0.001$) (Figure. 5.4 A-C). Further, gabapentin treatment also alleviated the oxido-nitrosative stress by decreasing the levels of nitrite ($p<0.01$) and MDA ($p<0.001$) while restoring the GSH levels ($p<0.001$) in sciatic nerve of neuropathic rats.

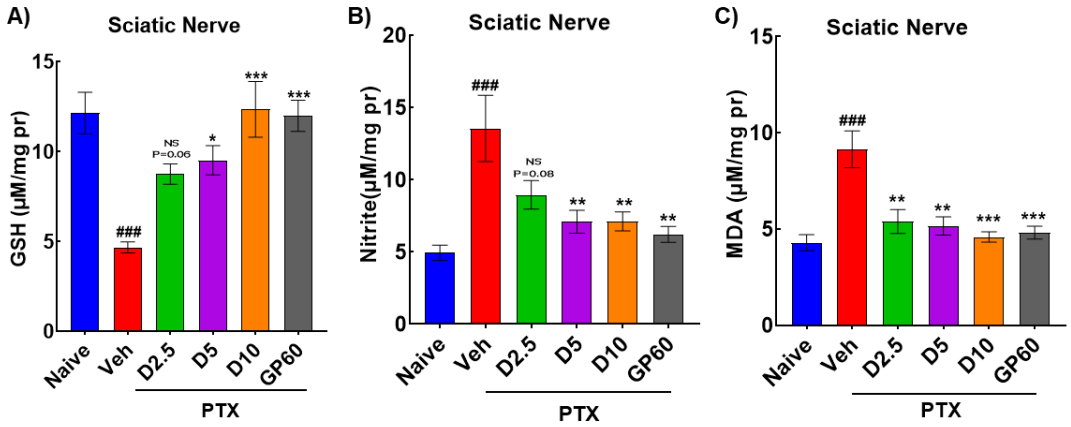

Figure 5.4 Effect of DALDA on paclitaxel-induced oxido-nitrosative stress in sciatic nerve of rats (A) Nerve GSH: Paclitaxel administration resulted in decreased levels of antioxidant (GSH) in sciatic nerve of rats, which was significantly restored upon treatment with DALDA (5 & 10 mg/kg s.c.) and gabapentin (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 significantly decreased with DALDA (2.5,5 & 10 mg/kg s.c.) and gabapentin treatment (60mg/kg s.c.). 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. DALDA doses: D2.5: 2.5mg/kg, D5: 5mg/kg, D10: 10mg/kg. Gabapentin (GP60): 60mg/kg.

5.3.5 DALDA alleviates CINP by downregulating ion channels and NR2B expressions in DRG of neuropathic rats

The pathophysiology of paclitaxel-induced neuropathic pain involves upregulation of VGSCs and TRP channels in DRG of rats. We observed that protein expression of Nav1.7 and mRNA expression of Nav1.8, were significantly upregulated in the DRG of vehicle treated rats ($F(5, 18) = 8.47$, $P<0.001$; $F(5, 18) = 7.33$, $P<0.001$) as compared to naïve group ($p<0.001$). Further we also observed that protein expression of TRPA1 and mRNA expression of TRPM8, were upregulated in the DRG of vehicle treated rats ($F(5, 18) = 7.82$, $P<0.001$; $F(5, 18) = 8.01$, $P<0.001$) as compared to the naïve rats

($p<0.001$). Treatment with DALDA leads to significant decrease in the levels of Nav1.7 (5mg/kg, $p<0.05$ and 10mg/kg $p<0.001$), Nav1.8 (5 and 10mg/kg $p<0.05$), TRPA1 (2.5 mg/kg, $p<0.05$; 5 and 10mg/kg $p<0.001$) and TRPM8 (5 and 10mg/kg, $p<0.01$) (Figure. 5.5A-C). 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 DALDA treatment on mRNA expression of NR2B and observed that the upregulated NR2B in DRG of PTX treated rats was significantly decreased on treatment with different doses of DALDA (5mg/kg, $p<0.05$; 10mg/kg, $p<0.001$). Gabapentin treatment also reduced the mRNA expressions of NR2B in DRG of neuropathic rats ($p<0.001$) (Figure. 5.5D).

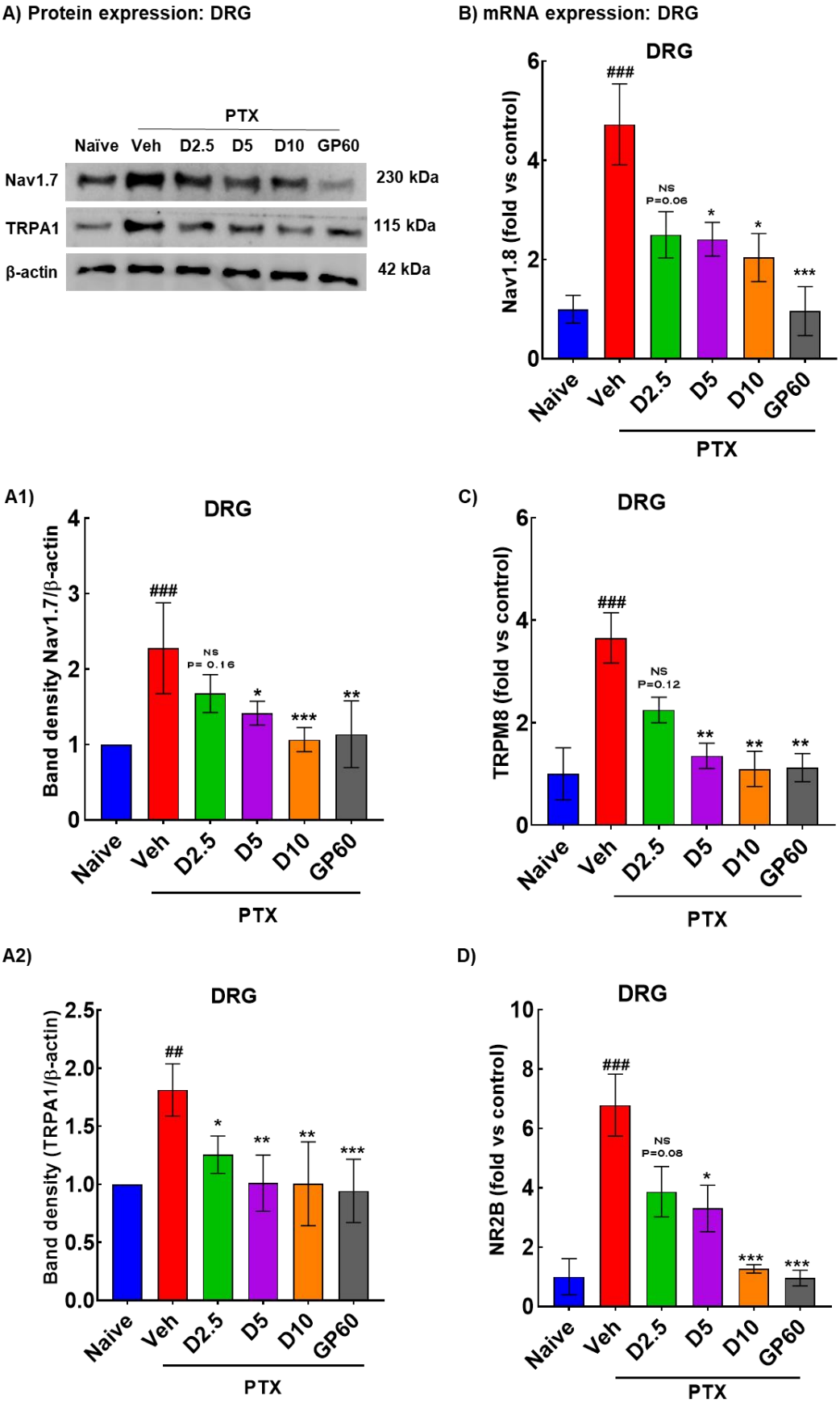
A) Protein expression: DRG
PTX
Naïve Veh D2.5 D5 D10 GP60
Nav1.7
TRPA1
β-actin
230 kDa
115 kDa
42 kDa
B) mRNA expression: DRG
DRG
Nav1.8 (fold vs control)
###
NS P=0.06
*
*
***
Naive Veh D2.5 D5 D10 GP60
PTX
A1)
DRG
Band density Nav1.7/β-actin
###
NS P= 0.16
*
***
**
Naive Veh D2.5 D5 D10 GP60
PTX
C)
DRG
TRPM8 (fold vs control)
###
NS P=0.12
**
**
**
Naive Veh D2.5 D5 D10 GP60
PTX
A2)
DRG
Band density (TRPA1/β-actin)
##
*
**
**
***
Naive Veh D2.5 D5 D10 GP60
PTX
D)
DRG
NR2B (fold vs control)
###
NS P=0.08
*
***
***
Naive Veh D2.5 D5 D10 GP60
PTX

Figure 5.5. Effect of DALDA on paclitaxel-induced increase in voltage gated sodium channels and TRP-ion channels in lumbar DRG of rats. (A) Nav1.7 and TRPA1 blot: Representative blots of Nav1.7 and TRPA1 protein expression in DRG tissues of neuropathic rats. **(A1) Nav1.7 protein expression:** Paclitaxel administration increased the Nav1.7 protein expression in DRG of rats which was attenuated by the DALDA treatment (5 & 10 mg/kg s.c.). **(A2) TRPA1 protein expression:** DALDA treatment (2.5, 5 & 10 mg/kg s.c.) treatment significantly reversed paclitaxel-induced increase in TRPA1 expression in DRG of rats. Gabapentin treatment also showed decreased protein expression of both Nav1.7 and TRPA1 in DRG of neuropathic rats. **(B) Nav1.8 mRNA expression:** Paclitaxel administration increased the Nav1.8 mRNA expression in DRG of rats which was attenuated by DALDA treatment. **(C) TRPM8 mRNA expression:** Increased mRNA expression of TRPM8 was significantly attenuated by DALDA treatment (5 & 10 mg/kg s.c.) and gabapentin (60 mg/kg s.c.) treatment in DRG of neuropathic rats. **(D) NR2B mRNA expression:** Increased mRNA expression of NR2B was significantly attenuated by DALDA treatment (2.5, 5 & 10 mg/kg s.c.) and gabapentin (60 mg/kg s.c.) treatment in DRG of neuropathic rats. 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. DALDA doses: D2.5: 2.5mg/kg, D5: 5mg/kg, D10: 10mg/kg. Gabapentin (GP60): 60mg/kg.

5.3.6 DALDA 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 DALDA 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 ($p<0.05$) and IBA1 ($p<0.01$) were significantly upregulated in the spinal cord of neuropathic rats as compared to the naïve

group. Treatment with DALDA leads to significantly downregulation in protein expression of IBA1 (5 and 10mg/kg, $p<0.01$). However, we did not find any significant changes in ICAM1 protein expression of neuropathic rats after DALDA treatment. Furthermore, treatment with gabapentin significantly downregulated the expressions of both ICAM1 ($p<0.01$) and IBA1 ($p<0.001$) in spinal cord of neuropathic rats (Figure. 5.6A).

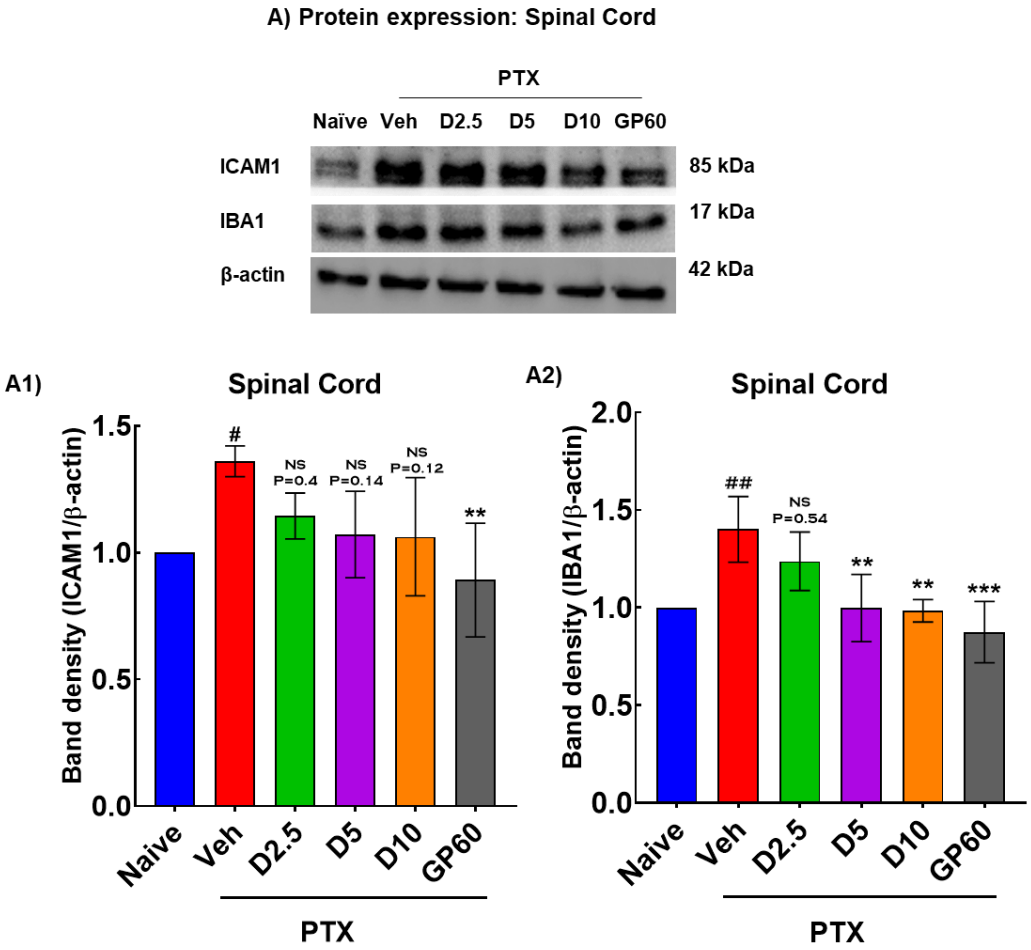

Figure 5.6. Effect of DALDA on paclitaxel-induced increase in protein expressions of ICAM1 and IBA1 in lumbar spinal cord of rats. (A) ICAM1 and IBA1 blots: Representative blots of ICAM1 and IBA1 protein expressions in spinal cord tissues **(A1)** Paclitaxel administration increased the ICAM1 protein expression in spinal cord of rats but DALDA 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 DALDA (2.5, 5 & 10 mg/kg s.c.) and gabapentin (60mg/kg s.c.). Data were presented as mean ± SEM. (n=4), #($p<0.05$), ##$p<0.01$ represents significance compared to Naïve group. **$p < 0.01$, ***$p<0.001$ indicates statistical significance as compared to the PTX+ Veh rats. DALDA doses: D2.5: 2.5mg/kg, D5: 5mg/kg, D10: 10mg/kg. Gabapentin (GP60): 60mg/kg.

5.3.7 DALDA attenuates neuroinflammation by inhibiting release of pro-inflammatory cytokines and neuropeptides

Neuroinflammatory mediators and calcitonin gene-related peptide (CGRP) play 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 IL-6 and CGRP in spinal cord and DRG of neuropathic rats. DALDA treatment leads to significant decrease in protein expressions of NFκβ (5mg/kg, $p<0.05$ and 10mg/kg, $p<0.001$) and TNF-α (5 and 10mg/kg, $p<0.05$), and mRNA expressions of IL-6 (2.5, 5 and 10mg/kg, $p<0.001$) and CGRP (2.5mg/kg, $p<0.01$; 5 and 10mg/kg, $p<0.001$) in spinal cord tissues of neuropathic rats (Figure. 5.7 A, C, D). Simultaneously, there was a significant increase in the protein expressions of TNF-α and IL-1β in DRG of CINP rats. Interestingly, we observed that DALDA selectively reduced the protein expression of IL-1β, without affecting the expression of TNF-α in DRG tissues of CINP rats. The protein expression of pro-inflammatory marker IL-1β was significantly reduced upon DALDA (5mg/kg, $p<0.05$ and 10mg/kg, $p<0.01$) treatment (Figure. 5.7B). Treatment with standard drug gabapentin leads to significant downregulation in expressions of NFκβ ($p<0.001$), TNF-α ($p<0.01$), IL-6 ($p<0.001$) and CGRP ($p<0.001$) in the spinal cord and TNF-α ($p<0.05$) and IL-1β ($p<0.01$) in DRGs of neuropathic rats.

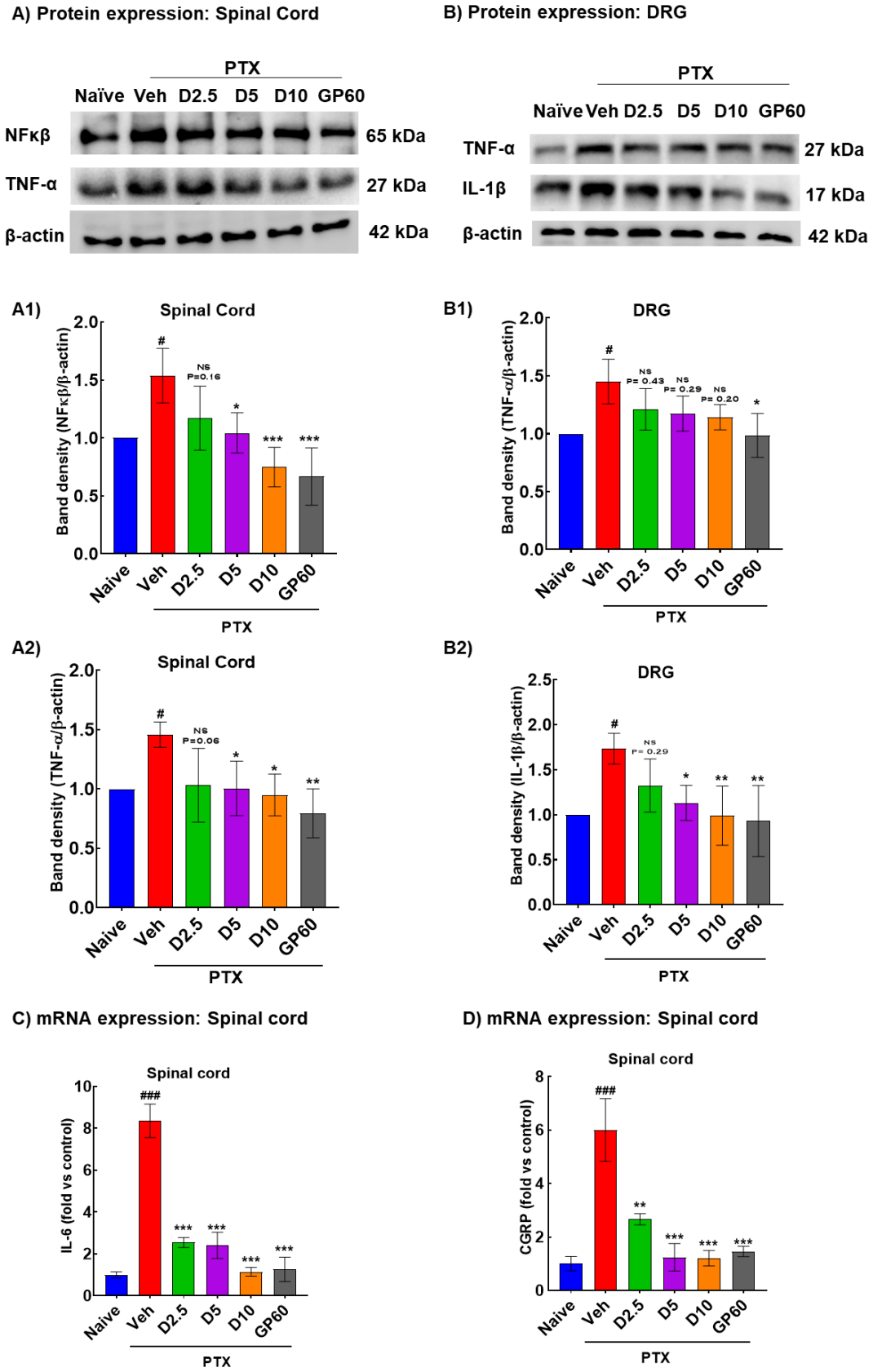
A) Protein expression: Spinal Cord
PTX
Naïve Veh D2.5 D5 D10 GP60
NFκβ
65 kDa
TNF-α
27 kDa
β-actin
42 kDa
B) Protein expression: DRG
PTX
Naïve Veh D2.5 D5 D10 GP60
TNF-α
27 kDa
IL-1β
17 kDa
β-actin
42 kDa
A1)
Spinal Cord
Band density (NFκβ/β-actin)
NS P=0.16
B1)
DRG
Band density (TNF-α/β-actin)
NS P= 0.43
NS P= 0.29
NS P= 0.20
A2)
Spinal Cord
Band density (TNF-α/β-actin)
NS P=0.06
B2)
DRG
Band density (IL-1β/β-actin)
NS P= 0.29
Naive Veh D2.5 D5 D10 GP60
PTX
C) mRNA expression: Spinal cord
Spinal cord
IL-6 (fold vs control)
D) mRNA expression: Spinal cord
Spinal cord
CGRP (fold vs control)

Figure 5.7: Effect of DALDA on paclitaxel-induced neuro-inflammation and CGRP expression in DRG and spinal cord of rats. (A) NF-κβ & TNF-α blots (spinal cord): Representative blots of NFκβ, TNF-α protein expressions in spinal cord of rats. **(A1) NF-κβ protein expression (spinal cord), (A2) TNF-α protein expression (spinal cord):** Paclitaxel administration significantly increased the protein expressions of NFκβ, TNF-α in spinal cord of rats which was attenuated by the treatment with DALDA (2.5, 5, 10mg/kg s.c) and gabapentin (60mg/kg s.c.). **(B) TNF-α & IL-1β blots (DRG)** Representative blot of TNF-α, IL-1β protein expression in DRG tissues. **(B1)** Paclitaxel administration increased the TNF-α protein expression in DRG of rats which was unaltered by the DALDA treatment unlike gabapentin, that significantly reduced the expression of TNF-α. **(B2)** Paclitaxel administration increased the IL-1β protein expression in DRG of rats which was attenuated by the DALDA (5, 10mg/kg s.c.) and gabapentin (60mg/kg s.c.) treatment. **(C) IL-6 mRNA expression (spinal cord),** and **(D) CGRP mRNA expression (spinal cord):** Paclitaxel administration also upregulates the mRNA expressions of IL-6 and CGRP in spinal cord of rats which was attenuated by the DALDA 2.5, 5, 10mg/kg s.c.) and gabapentin (60mg/kg s.c.) treatment. Data were presented as mean ± SEM. (n=4), #($p<0.05$), ###$p<0.001$ represents significance compared to Naïve group. *$p < 0.05$, **$p < 0.01$ indicates statistical significance as compared to the PTX+ Veh rats. DALDA doses: D2.5: 2.5mg/kg, D5: 5mg/kg, D10: 10mg/kg. Gabapentin (GP60): 60mg/kg.

5.4 Outcomes

In conclusion, our study unequivocally demonstrates that DALDA is highly effective in alleviating both paclitaxel-induced evoked and spontaneous ongoing pain through a multitude of mechanisms. These mechanisms include downregulation of TRP channels, VGSCs, and NR2B, as well as the inhibition of microglial activation, attenuation of neuroinflammatory responses, and modulation of oxido-nitrosative pathways. These findings offer invaluable insights into the development of innovative therapeutic strategies aimed at targeting peripheral MOR for the management of CINP.