Chapter-4

Deciphering the Role of KIF17-NR2B Signaling in Nerve Injury Mediated Evoked and Ongoing Pain and its Modulation by Pan-Aurora Kinase Inhibition

4.1 Introduction

In highly polarized neurons the delivery and sorting of organelle is also carried out with the help of the kinesin superfamily (KIFs). Any impairment in the expression and functioning of KIFs results in maladaptive neuronal circuits that results in improper propagation of neuronal signals [163,164]. Kinesin family member 17 (KIF17) is a homodimeric kinesin motor protein that belongs to the osmotic avoidance abnormal protein 3 (OSM3)/KIF17 family. This adenosine triphosphate (ATP)-dependent motors transport the synthesized cargos in a retrograde direction to the +end of the synaptic membrane. KIF17 consists of the tail domain that binds to the postsynaptic density-95/Discs large/Zona occludens-1 (PDZ) domain of mammalian lin ten protein (mLin-10). Further, the mLin 10 binds to the large scaffolding cargo complex that includes the NR2B subunit of NMDAR [53]. The dynamic living mammalian neuron studies have confirmed the trafficking of NR2B subunit by KIF17 kinesin nanomotors [104]. Kinesins are localized into the cytoplasm in an auto-inhibited state. After their activation by various kinase enzymes (like Cyclin-dependent kinase 5 (CDK-5) and Ca 2+ /calmodulin kinase 2), kinesins attach to their respective cargos (vesicles containing receptors) [88]. The binding is facilitated by scaffolding adapter proteins and finally, the whole complex binds to the microtubule and movement occurs across the axon. On

reaching the synaptic membrane the whole complex gets disassembled and the receptor is delivered to the dendritic surface and made functional. Researchers are targeting kinesins and enzymes responsible for the surface delivery of receptors for pain relief [102,131,165].

There are many regulatory proteins (like kinases) involved in the activation and regulation of kinesin for the delivery of receptors to form motor-protein complexes. Aurora kinase is a serine-threonine kinase involved in cell division. Recently it has been shown that aurora kinase B plays a critical role in spinal microgliosis during the neuropathic pain condition in nerve-injured rats [166]. Tozasertib is known for being one of the strongest inhibitors of aurora kinase, with an inhibitory effect at nanomolar concentrations [167]. Until now, interest in the study of tozasertib as an inhibitor of aurora kinases has been determined in terms of its use in the treatment of cancer, including myelogenous leukemia [168].

Regardless of demonstrated preclinical effectiveness, the NMDA receptor antagonist failed to treat neuropathic pain in clinical trials [98,169]. The major reason behind this is NMDA receptor interaction with other nociceptive units and secondary messenger systems. Another issue with the use of NMDA antagonists is the occurrence of severe adverse effects which further suppress the clinical utility of this class of pharmacological agents [98]. However, because of the significant involvement of the NMDA receptor system during chronic neuropathic pain, its therapeutic targeting cannot be ignored. Therefore, an indirect approach of targeting the NMDA receptor function by interfering with receptor maturation, synthesis, and transport to the synaptic membrane could be an effective strategy for the treatment of neuropathic pain. KIF17 has been recently known to be involved in the pathophysiology of chronic pain

[125,128,145]. Thus, exploring the exogenous ligands that interfere with the KIF17-NR2B cargo system can provide a novel line of therapeutics for the management of neuropathic pain. Aurora kinase are the enzymes that regulate the molecular and functional diversity of kinesin proteins [140,170,171]. Hence, in the present study, we have examined the effect of Tozasertib, a pan-Aurora kinase inhibitor, on the KIF17-NR2B crosstalk in dorsal root ganglion and spinal cord of nerve-injured rats and its effect on nerve injury induced chronic pain.

4.2 Experimental procedure

First, we investigated the architectural interplay between aurora kinase and tozasertib using molecular dynamics simulation. Next, we investigated the effect of tozasertib on chronic pain rats were divided into six experimental groups with a minimum of eight rats/group. The first group consists of naïve healthy rats, while the second group was disease control group where nerve-injured rats were administered with the vehicle of tozasertib. Rats in the third, fourth and fifth groups belonged to different test groups where nerve-injured rats were treated with intraperitoneal tozasertib at 10 mg/kg, 20 mg/kg, and 40 mg/kg respectively. The sixth group consists of the standard control group in which nerve-injured rats were treated with gabapentin (30 mg/kg *i.p*). For open field, rota-rod, and place preference studies we used another four groups of nerve-injured rats which were treated with vehicle, tozasertib (40 mg/kg *i.p*), gabapentin (30 mg/kg *i.p*) and morphine (10 mg/kg *i.p*) respectively. On 14^{th}-day post nerve injury pain behaviors including heat hyperalgesia (Hargreaves test), cold hyperalgesia (ice floor test), thermal allodynia (acetone drop test), dynamic mechanical allodynia (cotton swab test), static mechanical allodynia (von-Frey test) and mechanical

hyperalgesia (pin-prick test) were assessed at 0, 0.5, 1, 2, 4 hr post administration of drugs. Next the animals were sacrifices and DRG, spinal cord and sciatic nerve were harvested for molecular analysis. rtPCR and western blotting was performed to assess the expression of NR2B, KIF17 and inflammatory cytokines in respective tissues.

To study the effect of tozasertib on normal nociceptive threshold we have divided rats (n=8/groups) in naïve or healthy, naïve+ treatment with different dosage of tozasertib (10 mg/kg, 20 mg/kg and 40 mg/kg), naïve + gabapentin (30 mg/kg *i.p*) and naïve + morphine (10 mg/kg *i.p*) groups.

4.3 Results and discussion

1.3.1 In-silico studies

Tozasertib is an investigational drug and undergone clinical trials for lymphoblastic leukemia, myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndromes, and colorectal cancer [172]. The in-vitro studies have suggested that tozasertib can regulate cell death via caspase-3, Receptor-interacting serine/threonine-protein kinase 1 (RIPK-1) and poly (ADP-ribose) polymerase, mast cell responsiveness via NFkB signaling, epigenetic functioning via histone deacetylases, and other downstream cellular processes via cyclin B, ERK, and cdc25c which is an M-phase inducer phosphatase 3 enzymes [173,174]. Although the crystal structure of Aurora kinase with co-crystallized tozasertib is reported earlier but as a general limitation of crystal structures, it captures only a single pose. However, the protein-ligand interactions are always dynamic in nature. To overcome this limitation and to investigate the architectural interplay of tozasertib with aurora kinase, we performed a classical molecular dynamics simulation of the complex obtained from the

crystal structure (PDB code: 3E5A) [175]. Figure 4.1A shows the conformation of tozasertib in the aurora kinase A binding site according to the crystallographic data. Amino acids Leu139, Phe144, Lys162, Leu210, Tyr212, Thr217, Lys224, Leu263, and Asp274 are located in the immediate vicinity of the drug sorbed in the binding site. Amino acid residues Gly140, Val147, Leu194, Glu211, Pro214, Leu215 are positioned within 4 Å from the tozasertib molecule as well.

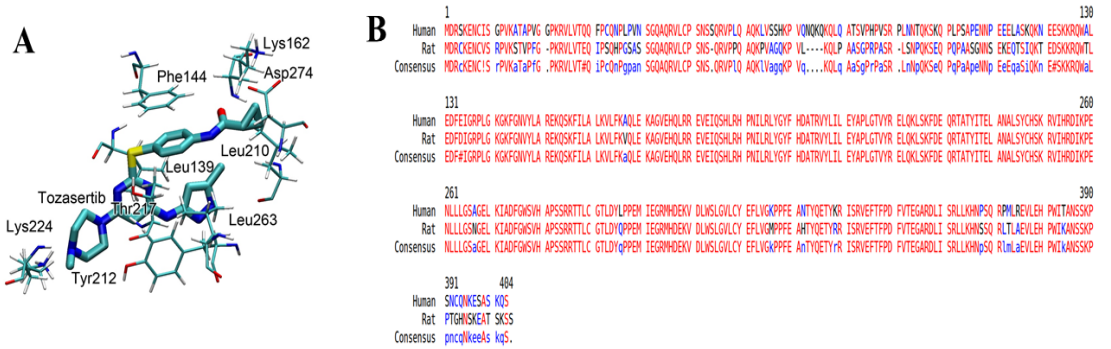


Figure 4.1. A) The molecule of tozasertib bound in the binding site of human aurora kinase A according to X-ray data (structure code 3E5A) [175]. The hydrogen atoms in the protein molecule were added using Nanoscale Molecular Dynamics (NAMD) molecular modeling software [176]. **B) Comparative analysis of primary sequences of human and rat aurora kinase A.** The symbol # is displayed in the consensus sequence when a polar amino acid is replaced with a polar one (glutamine, glutamate, asparagine, aspartate), ! – when valine is replaced with isoleucine. The bottom line is the so-called consensus sequence. It is a generalized sequence that is obtained by comparing letters in the alignment columns. When homology is high, the consensus line represents the predominant amino acid, indicated by a red capital letter. When homology is low, the consensus line represents the predominant amino acid indicated by a lowercase blue letter.

At the next stage, we performed the alignment of the primary sequences of human and rat aurora kinase A (Figure 4.1B). According to the alignment result, the primary sequence identity between human and rat aurora kinase A is 82.9%. All amino acids of the binding site for tozasertib are identical in the kinases of these organisms. For this reason, we suppose that the effectiveness of the interaction of the drug with human and

rat aurora kinase A will be similar; therefore, in vitro and ex vivo experiments are most likely to give similar results for these species. However, the pharmacokinetics of tozasertib may differ due to the fact that rats and humans have different concentrations of transport proteins in the blood and different ratios of the concentrations of enzymes involved in metabolism [177,178]. These differences might lead to failure when studying the efficacy of tozasertib in patients. Further clinical trials will help to evaluate the effectiveness of the drug for the management of neuropathic pain syndrome in humans.

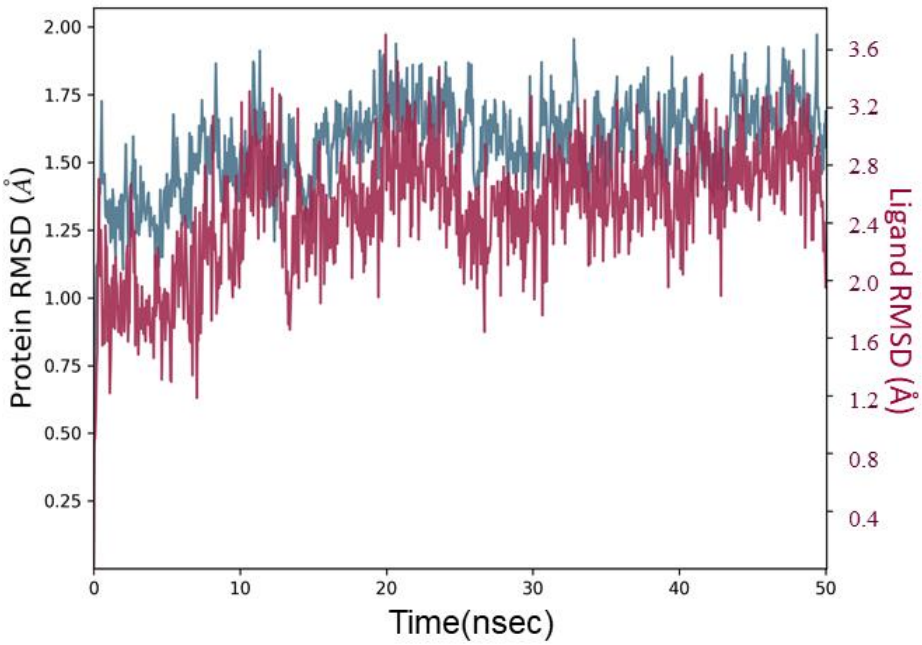


Figure 4.2. Protein-ligand RMSD for tozasertib and aurora kinase A. Left Y-axis shows RMSD evolution for the protein. The stability of ligand is represented by right Y-axis.

For the first time we have conducted studies on the architectural interaction between Tozasertib and Aurora Kinase using molecular dynamics simulation. The MD results made it possible not only to determine the location and structure of the protein pockets for binding to tozasertib molecules but also to assess their degree of

conformational fluctuations. For tozasertib Figures 2-3 carries all the analysis data of molecular dynamics (MD) trajectory. The root-mean-square deviation (RMSD) plot (Figure 4.2) shows that the protein RMSD remains between 1 to 2 Å indicating stable conformation of the protein during the simulation timeframe. Tozasertib interacts with the ATP binding site of aurora kinase A and inhibits the phosphorylation process. During the initial 15 ns the protein and ligand RMSD fluctuates a little bit which indicated equilibration.

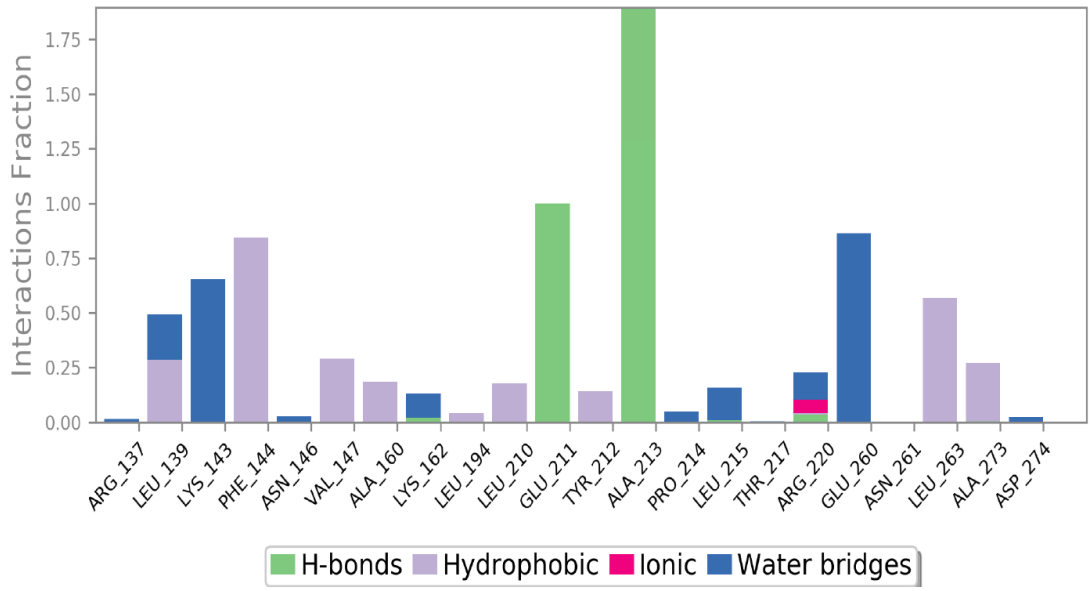


Figure 4.3 Protein-ligand contacts for tozasertib and aurora kinase. Four types of interaction were categorized and studies namely hydrogen bonding, hydrophobic interactions, ionic or polar interaction, and water bridges.

Later, from 15 to 50 ns the ligand-protein complex is converged to a stable state and the RMSD remains between 1-2 Å and 2-3.5 Å for Aurora kinase A and tozasertib, respectively. The interaction of the ligand with the individual amino acid residues in the binding pocket of aurora kinase A is quantified and depicted in Figure 4.3. It can be seen from the plot that the ligand interacted with the aurora kinase A active site with ALA_213 and GLU_211 subunits through hydrogen bonding.

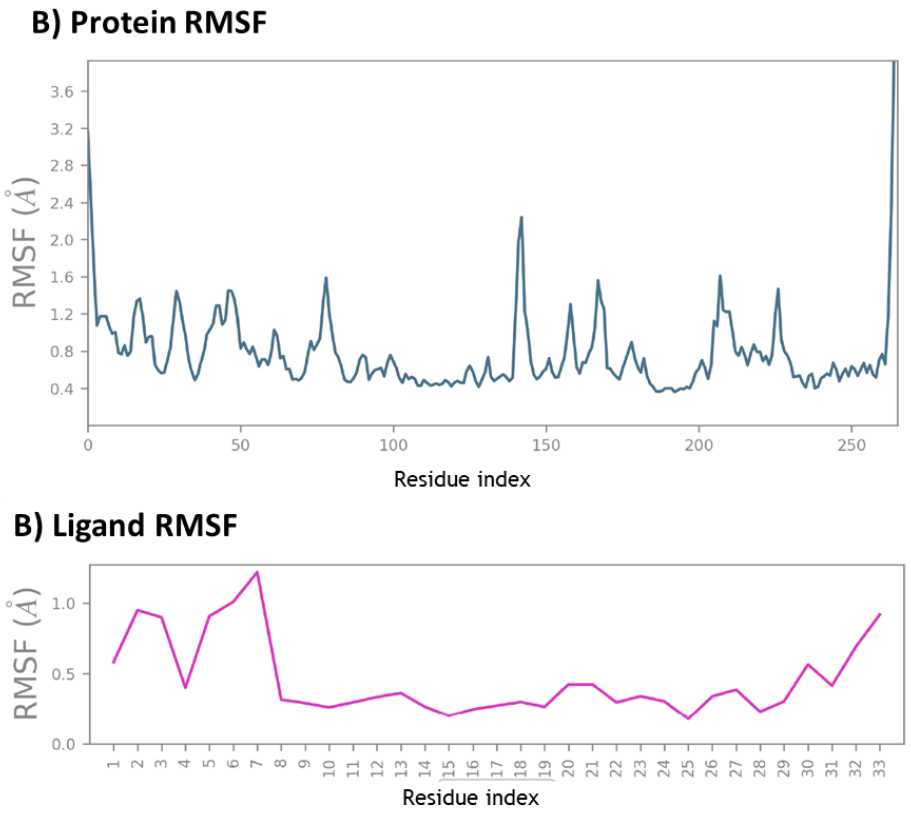


Figure 4.4. **Root mean square fluctuation for tozasertib and aurora kinase: A)** RMSF for aurora kinase, **B**) RMSF for tozasertib.

The hydrogen bonding played a major role in the interaction of this ligand with aurora kinase A active site. Further, the hydrophobic bonding with PHE_144 and water bridges with GLU_260 and LYS_143 were also observed. Finally, we found ligand-protein polar interaction or ionic interaction with the ARG_260 subunit. Furthermore, in order to examine the flexibility of the structure of the protein we have calculated the root mean square fluctuation (RMSF) (Figure 4.4A). The whole protein had an RMSF value between 0.4 to 2.2 Å except the N and C-terminal amino acids which showed moderate movement throughout the 50 ns long simulation. RMSF plot of ligand (Figure 4.4B) indicated that the ligand largely stayed bound to the active site amino-acids as the residues were observed consistently throughout the simulation.

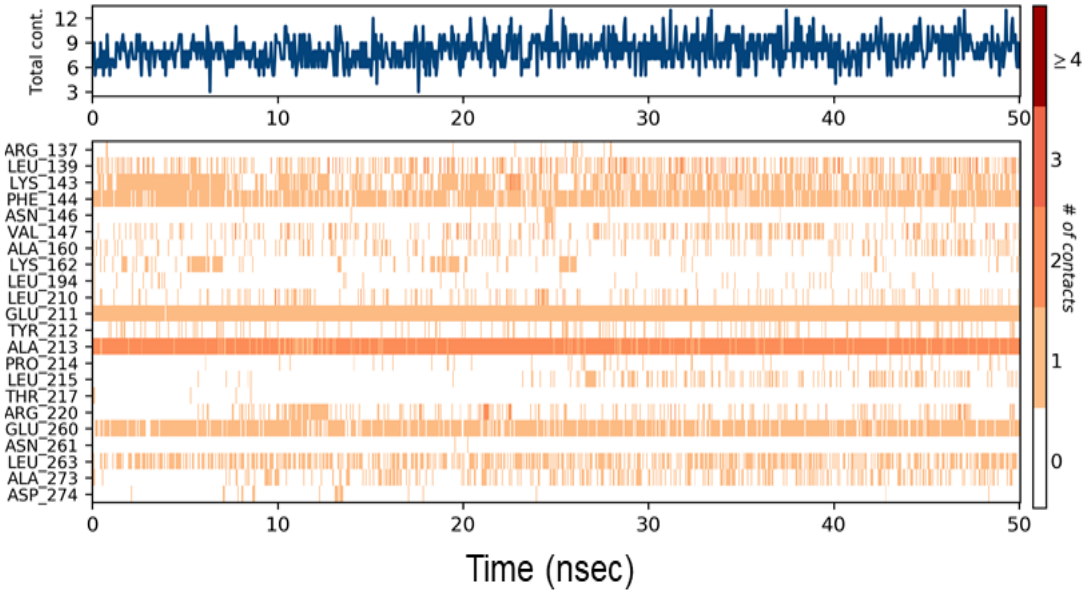


Figure 4.5 Timeline representation of the interactions and contacts for tozasertib and aurora kinase A.

Finally, a timeline of protein-ligand interactions in the form of hydrogen bonds, hydrophobic interaction, polar interaction, and water bridges was plotted as shown in Figure 4.5 throughout the simulation time of 50 ns. The top panel indicates the total number of specific protein-ligand contacts and the bottom panel indicates residue level interaction of the ligand. The results have demonstrated that there were a minimum of six contacts between ligand and protein throughout the simulation which is indicative of good ligand-protein interactions.

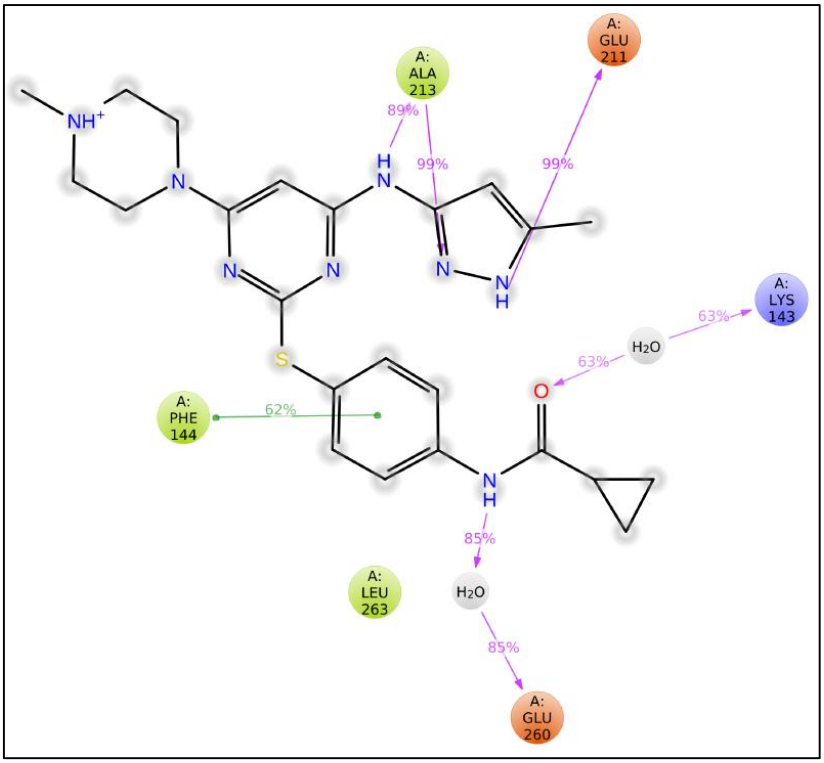


Figure 4.6 A Schematic of detailed ligand atom interactions with the protein residues

Figure 4.6 shows specific interactions of the ligand with active site amino acid residues of aurora kinase A. Thus, the information obtained by the molecular modeling methods reveals the details of the interaction between tozasertib and aurora kinase and explains why tozasertib is such a potent inhibitor of this enzyme.

1.3.2 Effect of tozasertib on nerve injury-induced pain-like behavior in rats

1.3.2.1 Tozasertib treatment attenuates thermal hyperalgesia in nerve injured rats

We have performed a battery of pain behavioral assays ranging from thermal, mechanical, cold to spontaneous ongoing pain to assess the efficacy of pan aurora kinase inhibition on neuropathic pain. Chronic constriction injury (CCI) induced nerve injury led to significant decrease in ipsilateral paw withdrawal latencies (PWL) of nerve

injured rats ($p<0.001$) as compared to their pre injury baselines and healthy naïve group (Figure 4.7A). Tozasertib treatment (10, 20, and 40 mg/kg *i.p*) significantly attenuates the thermal hyperalgesia as evident by an increase in PWL of nerve injured rats as compared to their pre-drug baselines. An increase in latency trend was observed at 30 min post-tozasertib administration which was absent in vehicle treated nerve injured rats (Figure 4.7A). The significant effect of tozasertib started at 1 hr post-administration i.e 10 mg/kg ($p=0.002$), 20 mg/kg ($p<0.001$) and 40 mg/kg ($p<0.001$). The peak therapeutic effect of tozasertib 10, 20, and 40 mg/kg ($p<0.001$) was observed at 2 hr post-drug administration which was lasted up to 4 hours followed by a decline in the effect of tozasertib. However, 10 mg/kg dose did not show significant effect at 4 hr post-drug administration in restoring nerve injury-induced decreased PWL. The maximum possible effect of tozasertib was found to be around 100% at 2 hrs with the dose of 40 mg/kg (Figure 1A). The percentage MPE in figure 4.7B has suggested the dose-dependent effect of tozasertib in attenuation of nerve injury induced-thermal hyperalgesia. A significant effect on thermal hyperalgesia was observed across the groups [$F (5, 42) = 322$; $p<0.001$] and time points [$F (5.03, 211) = 304$; $p<0.001$]. Moreover, contralateral PWL (figure 4.9A) of nerve injured rats were not altered before and after, CCI surgery and drug treatments (tozasertib and gabapentin).

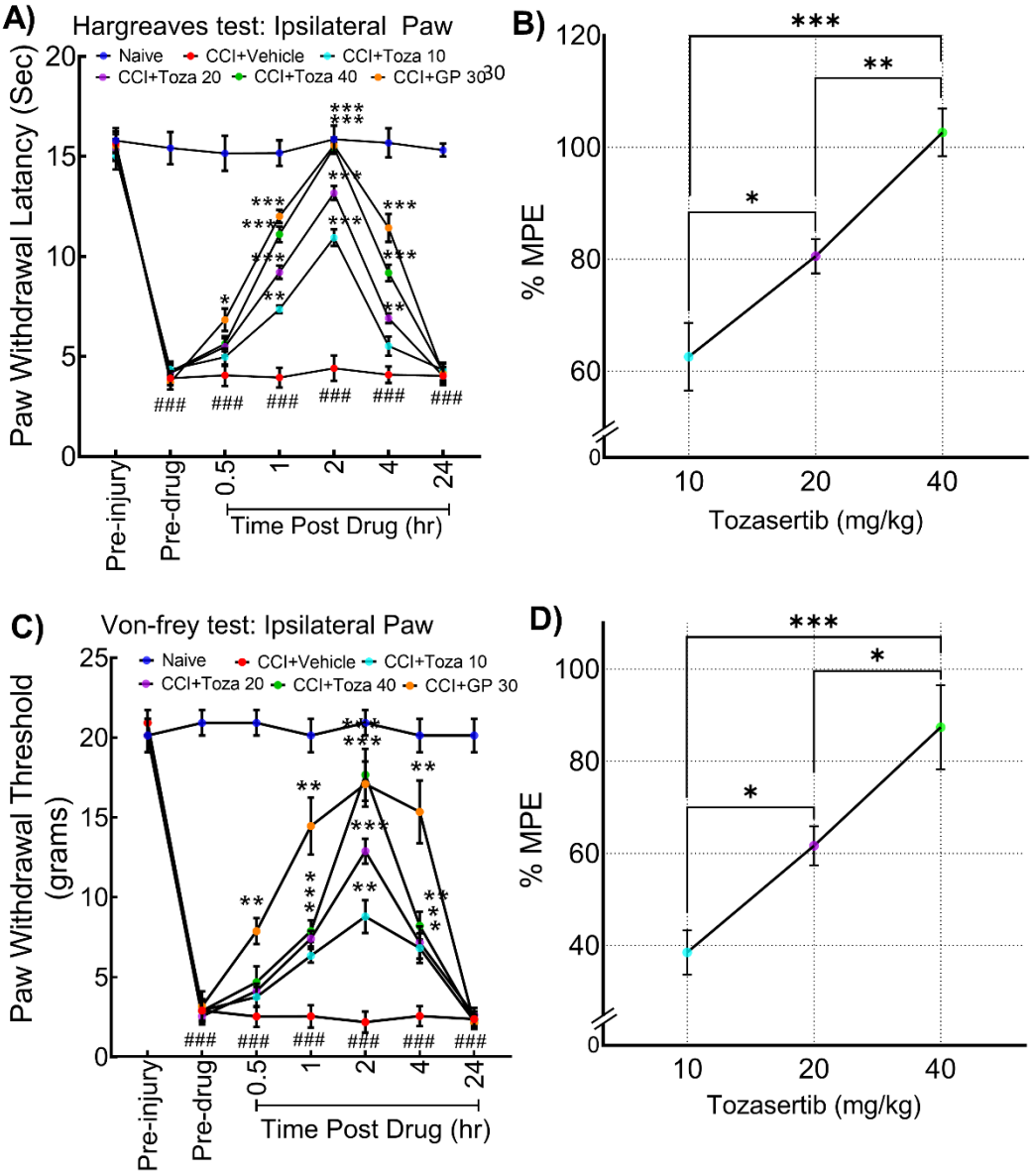


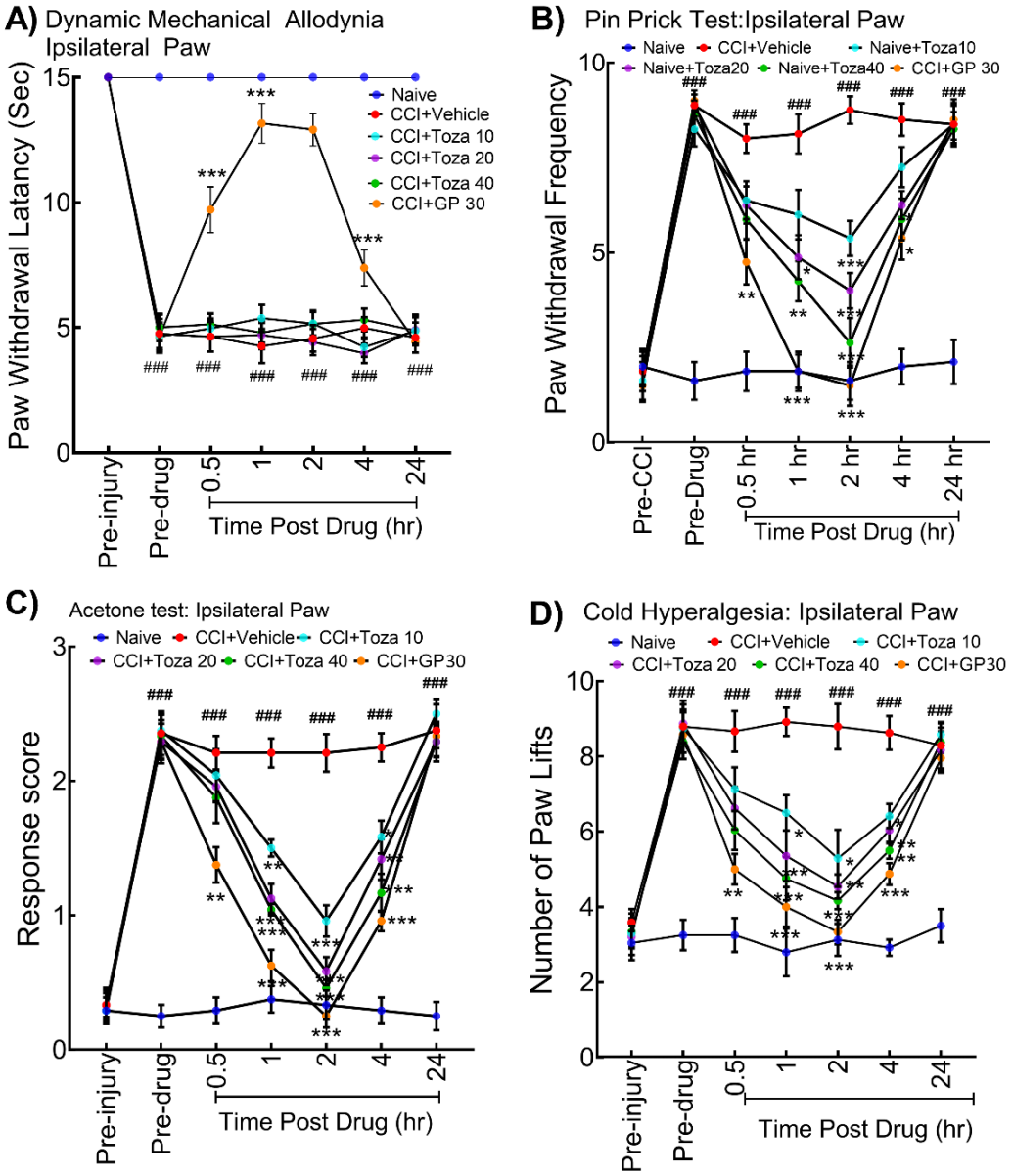
Figure 4.7 Effect of pan-aurora kinase inhibition on pain-like behavior in nerve-injured rats. (A) Hargreaves test: Nerve injury significantly decreased the paw withdrawal latency (PWL) of rats on day 14 post-CCI surgery as compared to their pre-injury baseline. Tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) administration resulted in a significant and dose-dependent increase in PWL of nerve injured rats as compared to their pre-drug baseline. **(B) von Frey hair test:** Nerve injury-induced significant decrease in paw withdrawal threshold (PWT) of rats which was significantly restored in a dose-dependent manner on treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*). Data were presented as mean ± SEM. $^{###}$P<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***p<0.001 indicates statistical significance as compared to the nerve injured rats. P<0.05 was considered statistically significant. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

The standard drug gabapentin (30mg/kg *i.p*) showed significant effect at 30 min post-administration ($p=0.04$), 1 hr ($P<0.001$), 2 hr ($p<0.001$), and the effect lasted for the 4-hr post-administration ($p<0.001$) as compared to the vehicle treated nerve injured rats.

1.3.2.2 Tozasertib treatment inhibits mechanical allodynia (static but not dynamic) and hyperalgesia in nerve-injured rats

Mechanical allodynia and mechanical hyperalgesia are one of the prominent symptoms in patients suffering from neuropathic pain. We tested the effect of tozasertib on both static and dynamic mechanical allodynia by using von-Frey hair test and cotton bud test respectively. We observed a significant effect across the groups [$F (5, 42) = 133$; $p<0.001$] and time points [$F (4.00, 168) = 230$; $p<0.001$] in paw withdrawal threshold (PWT) of nerve injured and drug treated rats in von-Frey hair test. We found a significant decrease in static allodynia as ipsilateral paw withdrawal thresholds at day 14 post-CCI surgery were significantly decreased as compared to their pre-injury baselines and naïve group rats ($p<0.001$) (Figure 4.7C). Tozasertib treatment (10, 20 and 40 mg/kg *i.p*) significantly restored the decreased paw withdrawal threshold of nerve injured rats in a dose-dependent manner. At 1 hr post-administration, tozasertib (10 mg/kg) ($p=0.002$), 20 mg/kg ($p=0.010$) and 40 mg/kg ($p=0.001$) showed a significant effect as compared to their pre-drug baselines and vehicle treated nerve injured rats. The peak anti-allodynic effect was observed at 2 hr (10 mg/kg $p=0.002$; 20 and 40 mg/kg $p<0.001$) post- tozasertib administration which lasted for 4 hrs (10 and 20mg/kg, $p=0.04$; 40 mg/kg $p=0.003$). The % MPE data suggested that tozasertib showed a dose-dependent effect at 10 mg/kg, 20 mg/kg, and 40 mg/kg doses (Figure 4.7D). Gabapentin (30 mg/kg *i.p*) treatment also attenuated the mechanical allodynia in

nerve injured rats as compared to the vehicle treated nerve injured rats. The contralateral PWT remains unaffected before and after, CCI induced nerve injury and treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30 mg/kg *i.p*) (figure S1B). Next, we examined the effect of tozasertib on the dynamic component of the mechanical allodynia using cotton swab test. Nerve injured rats developed significant [$F(5, 42) = 118$; $P<0.0001$) dynamic mechanical allodynia after CCI surgery as evident by the decrease in paw withdrawal latency post non-noxious stimuli application as compared to their pre-CCI baseline and naïve rats ($P<0.001$). Interestingly, tozasertib (10, 20, and 40 mg/kg *i.p*) treatment did not showed inhibition of dynamic mechanical allodynia in nerve injured rats (Figure 4.8A). However, we did not observe a significant effect on dynamic mechanical allodynia before and after, nerve injury and drug treatments (tozasertib and gabapentin) as compared to their pre-injury and pre-drug baselines. The Aδ and C fibers regulates the static mechanical allodynia, whereas, the Aβ fibers are involved in the mediation of dynamic mechanical allodynia [148,179]. We found that static mechanical allodynia was significantly blocked on treatment with Tozasertib without producing any inhibition of dynamic allodynia in nerve-injured rats. Compounds such as morphine that do not inhibit dynamic mechanical allodynia also known to exert their activity through Aδ and C fibers [180]. The mechanical hyperalgesia was assessed using the pinprick test.



4.8 Effect of pan-Aurora kinase inhibition on (A) Cotton swab test: CCI-induced nerve injury significantly decreased paw withdrawal latency (PWL) in rats as compared to their pre-CCI baseline. Gabapentin (30mg/kg *i.p*) but not tozasertib (10, 20 and 40 mg/kg *i.p*) treatment led to significant increase in PWL of nerve injured rats as compared to their pre-treatment baseline. **(B) Pinprick test:** CCI-induced nerve injury resulted in increased paw withdrawal frequency (PWF) to noxious mechanical stimuli in rats which was significantly attenuated on treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) as compared to their pre-drug baseline. **(C) Acetone test:** Nerve injury-induced cold hypersensitivity on day 14-post CCI surgery in rats which was significantly attenuated by tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*). **(D) Cold hyperalgesia test:** CCI surgery-induced cold hyperalgesia in rats in response to noxious cold stimuli. Treatment with tozasertib (10,

20 and 40 mg/kg *i.p*) and gabapentin (30 mg/kg *i.p*) significantly decreased number of paw lifts in nerve injured rats as compared to their pre-drug baseline.

We have observed a significant effect across the groups ($p<0.01$) in paw withdrawal frequency (PWF) of nerve injured and drug-treated rats. Nerve injured rats showed a significant ($p<0.001$) increase in ipsilateral PWF as compared to their pre-injury baselines and naïve rats (4.8B). Tozasertib treatment significantly decreased the PWF of nerve injured rats at 1 hr (20mg/kg, $p=0.01$; and 40mg/kg $p=0.002$), 2 hr ($p<0.001$) and 4 hr (20 mg/kg, $p=0.02$ and 40 mg/kg $p=0.03$) post drug-administration as compared to the vehicle treated group. However, 10 mg/kg tozasertib did not produced significant inhibition of mechanical hyperalgesia specifically at 1 hr and 4 hr post-drug administration. Gabapentin (30mg/kg *i.p*) treated nerve-injured rats showed a significant increase in PWF at different time points as compared to the vehicle treated nerve injured rats. There was no significant effect observed in contralateral PWF before and after CCI induced nerve injury and treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30 mg/kg *i.p*) (Figure 3.9D)

1.3.2.3 Tozasertib attenuates cold allodynia and cold hyperalgesia in nerve injured rats

Hypersensitivity induced by non-noxious and noxious thermal stimuli is notable trait among neuropathic pain patient. Here we investigated the effect of tozasertib on cold allodynia and cold hyperalgesia in nerve injured rats. Acetone drop test was used to evaluate the non-noxious stimuli evoked cold allodynia in CCI injured rats. Two-way ANOVA followed by Bonferroni's multiple comparison suggested a significant effect across the groups [$F(5, 42) = 173$; $P<0.001$] and time points [$F(4.75, 199) = 155$; $P<0.001$] in response score of vehicle and drug treated nerve injured rats. CCI injury has significantly increased the response score to non-noxious cold stimuli in

ipsilateral paw as compared to the respective pre-injury baselines and naïve rats ($p<0.001$) (Figure 4.8C). Tozasertib treatment significantly decrease the response score at 1 hr (10 mg/kg, $p=0.002$; 20 and 40 mg/kg, $p<0.001$), 2 hr ($p<0.001$) and 4 hr (10mg.kg, $p=0.02$; 20mg/kg, $p=0.009$ and 40 mg/kg, $p<0.001$) post administration as compared to their respective pre-treatment baselines and vehicle treated nerve injured rats (Figure 4.8C). Gabapentin (30mg/kg *i.p*) also decreased the cold allodynia evident by a significant decrease in ipsilateral paw response score at 0.5 hr ($p=0.007$), 1 hr ($p<0.001$), 2 hr ($p<0.001$) and 4 hr ($p<0.001$) post administration as compared to the vehicle treated CCI injured rats. Further we tested the effect of tozasertib on noxious cold stimuli (0°C) induced cold hyperalgesia (Figure 4.8D). A significant effect on number of ipsilateral paw lifts was observed across the groups [$F (5.28, 222) = 63.2$; $p<0.001$] and time point [$F (5, 42) = 74.3$; $p<0.001$] in vehicle and drug administered nerve injured rats. We have found that CCI induced nerve injury has increased the number of ipsilateral paw lifts significantly in presence of noxious cold stimulus as compared to their pre-injury baselines and naïve rats ($p<0.001$) (figure 4.8D). There was a significant reduction in number of paw lifts post tozasertib administration at 1 hr (10 mg/kg, $p=0.02$; 20 mg/kg, $p=0.01$ and 40 mg/kg<0.001), 2 hr (10 mg/kg, $p=0.04$; 20mg/kg, $p=0.001$ and 40 mg/kg $p<0.001$) and 4 hr (10 mg/kg, $p=0.02$; 20 mg/kg, $p=0.008$ and 40 mg/kg, $p=0.001$) as compared to the vehicle treated nerve injured rats.

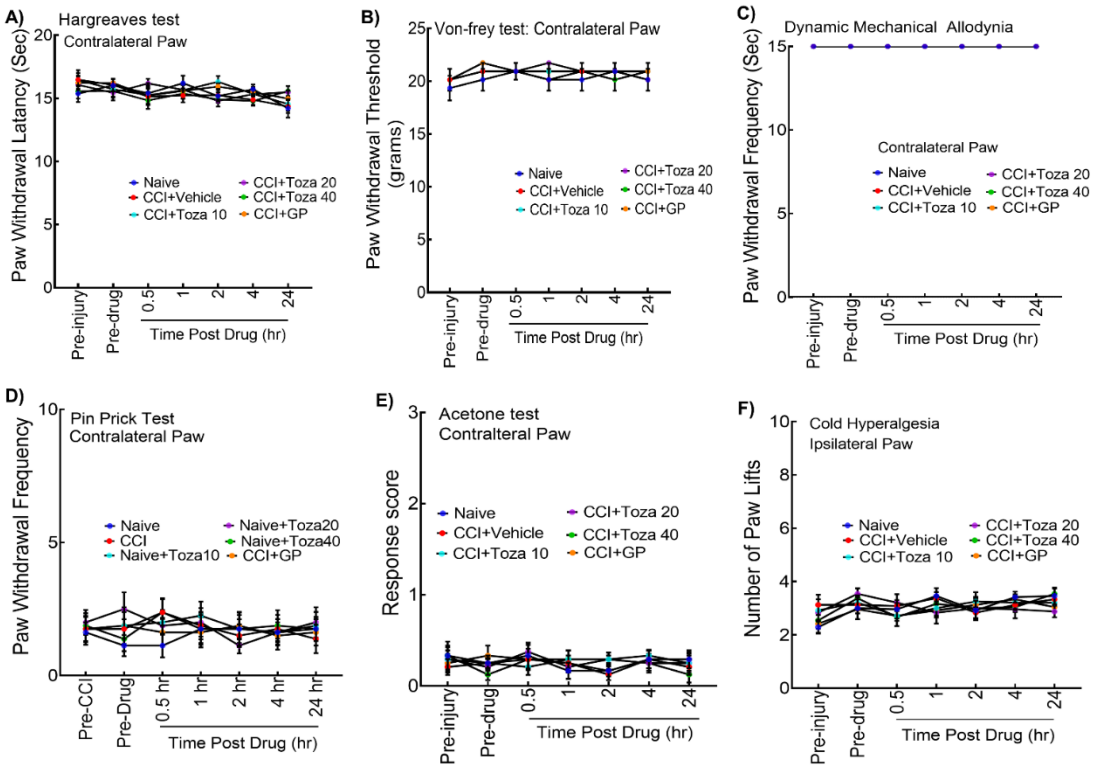


Figure 4.9 Effect of tozasertib on pain-like behavior in the contralateral paw of nerve injured rats. (A) Hargreaves test: Nerve injured rats did not showed any significant drop in their contralateral paw withdrawal latency (PWL) as compared to their pre-CCI baseline. Moreover, treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) did not showed any changes in contralateral PWL of nerve injured rats as compared to their pre-treatment baseline. **(B) von Frey hair test:** Contralateral paw withdrawal threshhold (PWT) of nerve injured rats remain unaltered as compared to their pre-injury baseline which was further remian unaffected after treatement with tozasertib (10, 20 and 40 mg/kg) and gabapentin (30mg/kg *i.p*). **(C) Cotton swab test**: Cotton swab test showed no significant changes in contralateral PWL of nerve injured rats before and after, CCI surgery and drug treatment (tozasertib and gabapentin). **(D) Pin prick test:** Contralateral paw withdrawal frequency (PWF) of rats was not altered in pin prick test before and after nerve injury. Moreover, treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) did not show any significant changes in contralateral PWF of nerve injured rats as compared to their pre-treatment baseline. **(E) Acetone test:** Contralateral paw hypersensitivity of nerve injured and drug (tozasertib and gabapentin) treated rats remain unaltered as compared to their pre-injury and pre-treatment baseline. **(F) Cold hyperalgesia test:** Contralateral paw lifts of rats in reponse to thermal noxius stimuli were not affected post-CCI and before and after treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*). Data were presented as mean ± SEM. P<0.05 was considered statistically significant. Doses: Toza10, Tozasertib 10mg/kg;

Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

Gabapentin also significantly decreased the number of ipsilateral paw lifts as compared to the CCI injured rats post 0.5 hr ($p=0.002$), 1 hr ($p<0.001$), 2 hr ($p<0.001$) and 4 hr ($p<0.05$) of administration. However, CCI induced nerve injury and drug treatments did not show a significant effect on contralateral, allodynic response score and noxious stimuli induced cold hypersensitivity in rats as compared to their respective pre-injury and pre-drug baselines (Figure 4.9 E and F).

1.3.3 Tozasertib did not altered the pain threshold of naïve uninjured rats

To evaluate the effect of tozasertib on pain threshold in naïve rats we have performed the tail-flick and tail clip test with morphine as a positive control. Both the test represents the hyperalgesic response to noxious thermal and mechanical stimulus respectively. A two-way ANOVA followed by Bonferroni's multiple comparisons suggested that in the tail-flick test there was a significant effect across the groups [$F(5, 42) = 33.5$; $p<0.001$] and time points [$F(3.45, 145) = 3.64$; $p<0.001$] in vehicle and drug treated nerve injured rats. We observed that the morphine significantly increased tail-flick latency (Figure 4.10A and B) as compared to naïve rats ($p<0.001$). The maximum possible effect post morphine administration was observed at 30 min ($p<0.001$) and the activity sustained up to 4 hours (Figure 4.10B).

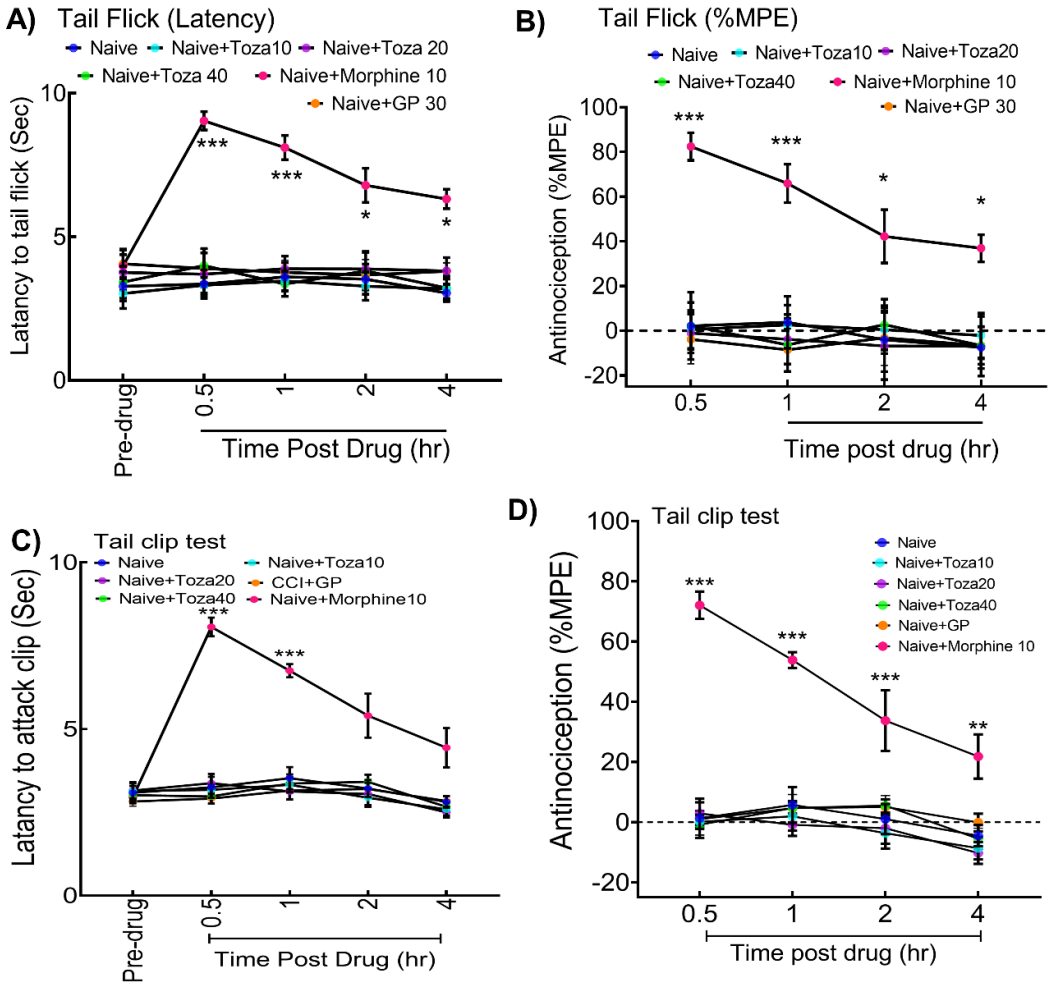


Figure 4.10 Effect of tozasertib on normal pain threshold of healthy naïve rats. (A and B) Tail flick test: Tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*). treatment showed no significant effect on normal pain threshold of naïve rats in response to noxious thermal stimuli. However, morphine (10 mg/kg *i.p*) treatment significantly increased the baseline tail-flick latency of naïve rats. **(C and D) Tail clip test:** Tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*).treatment did not alter the baseline threshold value of naïve rats in response to noxious mechanical stimuli. However, morphine (10 mg/kg *i.p*) treatment significantly increased the latency to attack in healthy naïve rats. Data were presented as mean ± SEM. $^{###}$P<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***P<0.001 indicates statistical significance as compared to the CCI rats. P<0.05 was considered statistically significant. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. Naïve, Healthy rats.

In the tail clip test, we observed a significant effect post two-way ANOVA followed by Bonferroni's multiple comparisons across the groups [F (5, 42) = 50.1; p<0.001] and time points F (2.60, 109) = 18.9; p<0.001] in vehicle and drug treated rats with CCI injury. Morphine increased the latency to noxious mechanical stimulus significantly as compared to the naïve rats (p<0.001) (Figure 4.10C). The significant effect of morphine persisted across the whole duration of the test i.e., 4 hrs (Figure 4.10 D). However, the pan-Aurora kinase inhibitor tozasertib (10, 20, and 40mg/kg *i.p*) did not altered the nociceptive latency in both tail-flick and tail clip tests. Gabapentin treatment also did not alter the pain threshold in both the tests as compared to the naïve rats. These findings suggest that tozasertib treatment does not interfere with the normal nociception in healthy naïve rats unlike morphine and suggest the potential application of this molecule during chronic pain condition.

1.3.4 Tozasertib inhibits spontaneous ongoing pain behavior in nerve-injured rats

In neuropathic pain patients, apart from the sensory hyper-sensation the emotional pain components also deteriorate the quality of life. Spontaneous ongoing pain is one of the important component of neuropathic pain and is increasingly being accepted as an parameter of overall well-being in pain patients [16]. Ongoing pain is a challenging clinical problem that -persist in patients with chronic pain condition. We have performed the Conditioned place preference (CPP) paradigm to assess the effect of tozasertib on spontaneous ongoing pain-like behavior in rats (Figure 4.11 A, B and C, and 4.12). Tozasertib (40mg/kg *i.p)* significantly (p<0.001) attenuates ongoing pain in nerve-injured rats as evident from increase in preference to the tozasertib-paired chamber as compared to the vehicle-paired chamber during post-conditioning trial (Figure 4.11 A and B). Moreover, there was a significant (p<0.001) increase in-

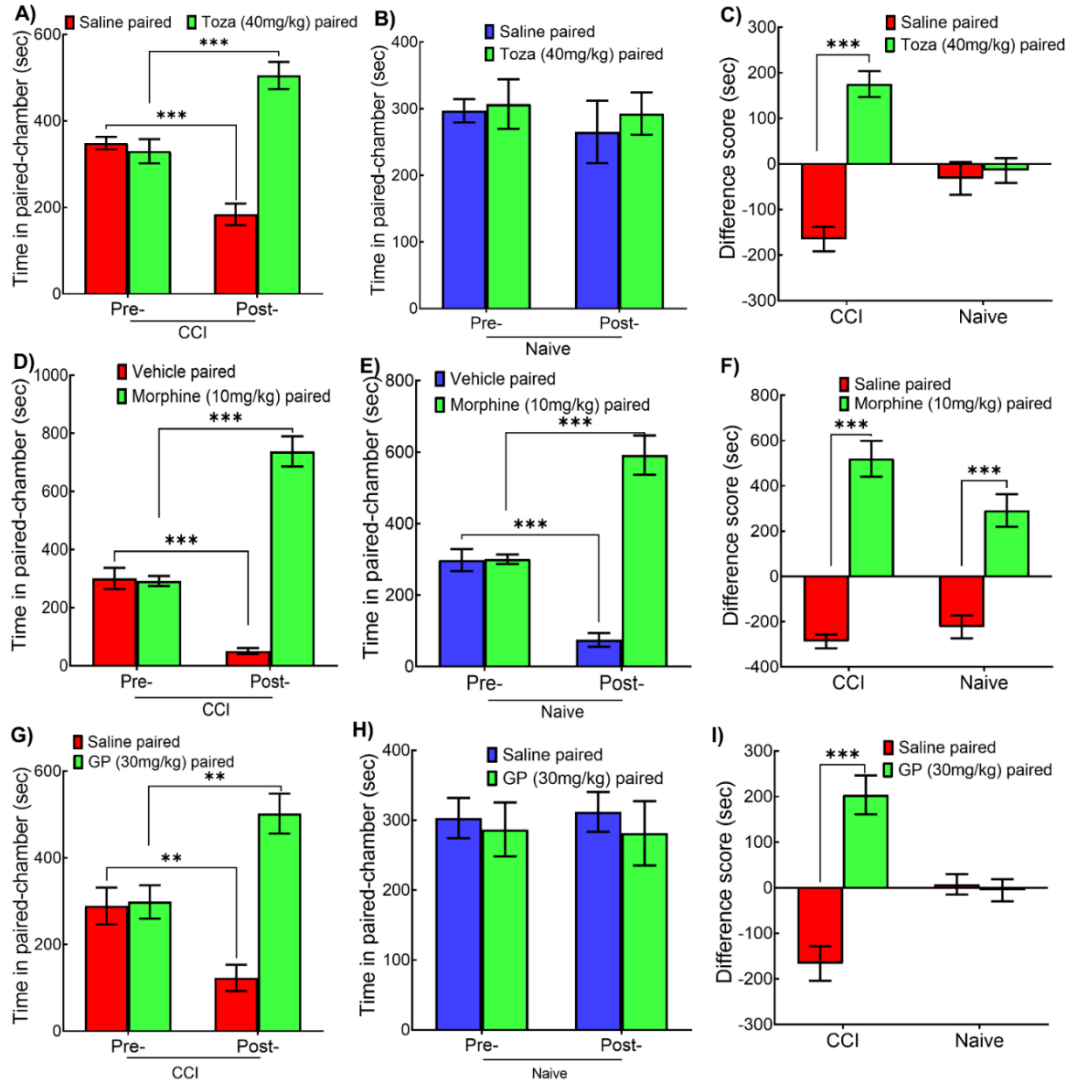


Figure 4.11 Effect of tozasertib, a pan-Aurora kinase inhibitor, on spontaneous ongoing pain in nerve injured rats. (A, B and C) Conditioned place preference (CPP) for tozasertib: Nerve-injured rats showed significant place preference behavior in tozasertib (40mg/kg *i.p.*) paired chamber as compared to the pre-conditioning baseline. Interestingly, tozasertib (40mg/kg *i.p.*) treatment did not produced CPP in healthy naïve rats. **(D, E, and F) CPP for morphine:** Morphine (10mg/kg *i.p.*) treatment significantly attenuates CCI-induced ongoing pain but also developed enhanced CPP in healthy naïve rats which indicates the analgesic and addictive potential of morphine. **(G, H and I) CPP for gabapentin:** Gabapentin (30mg/kg *i.p.*) treatment induced CPP in nerve injured rats but did not produce place preference in healthy naïve rats. Data were presented as mean ± SEM. $^{###}$P<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***p<0.001 indicates statistical significance as compared to the CCI rats. P<0.05 was considered statistically significant. Doses: Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

-difference score in tozasertib paired chamber as compared to the vehicle paired chamber in nerve injured rats (Figure 4.11 C). Interestingly, tozasertib (40mg/kg i.p) did not induce CPP in naïve rats as there was no significant change in preference to the tozasertib v/s vehicle paired chamber during post-conditioning trial (Figure 4.11 B). This suggest that tozasertib induced CPP is dependent on relief of pain-induced due to nerve injury and the molecule does not suffer from abuse liability potential. Furthermore, we have found that gabapentin treatment also induced significant ($p<0.01$) place preference behavior exclusively in nerve injured rats only (Figure 4.11 G, H and I; Figure 4.14). However, morphine treatment induced a significant place preference behavior in both nerve injured and naïve rats ($p<0001$) pointing towards its analgesic and addictive properties (Figure 4.11 D, E and F; Figure 4.13). The present findings suggest that -inhibition of aurora kinase produced inhibition of both evoked and spontaneous ongoing pain in nerve injured rats without causing drug addiction.

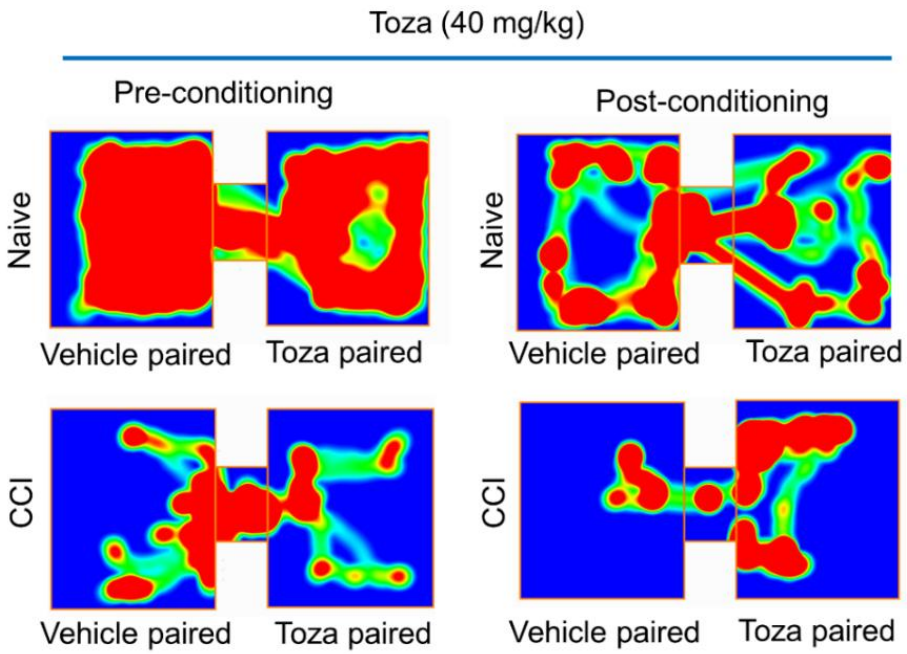


Figure 4.12 Effect of tozasertib on spontaneous ongoing pain behavior in nerve injured rats. Figure shows heat maps recorded during pre-conditioning and post-conditioning with vehicle v/s tozasertib (40mg/kg *i.p*) paired chambers.

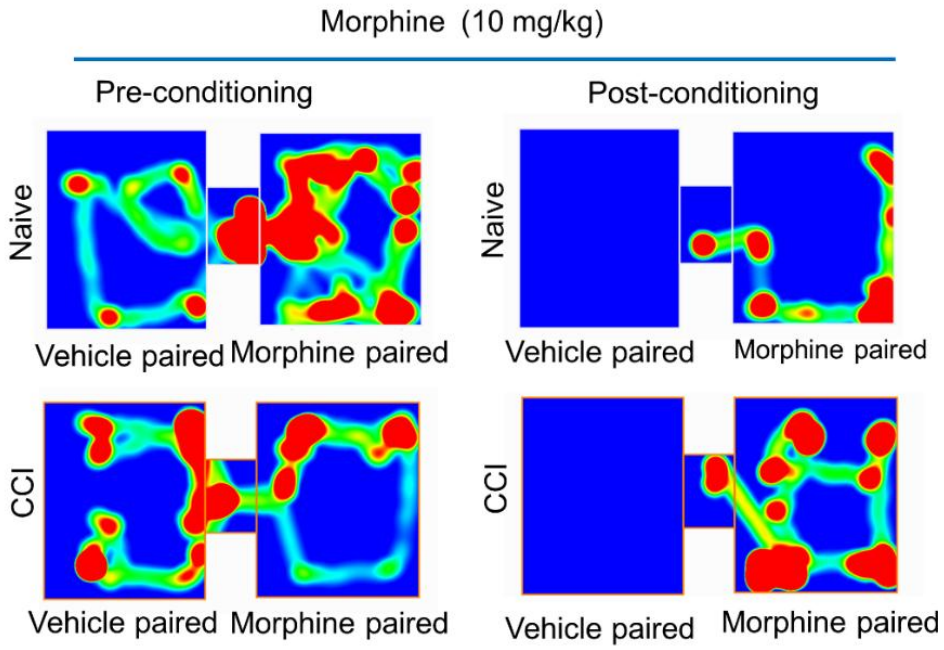


Figure 4.13 Effect of morphine on spontaneous ongoing pain behavior in nerve injured rats. Figure shows heat maps recorded during pre-conditioning and post-conditioning with vehicle v/s morphine (10mg/kg *i.p*) paired chambers. CCI, Chronic Constriction Injury.

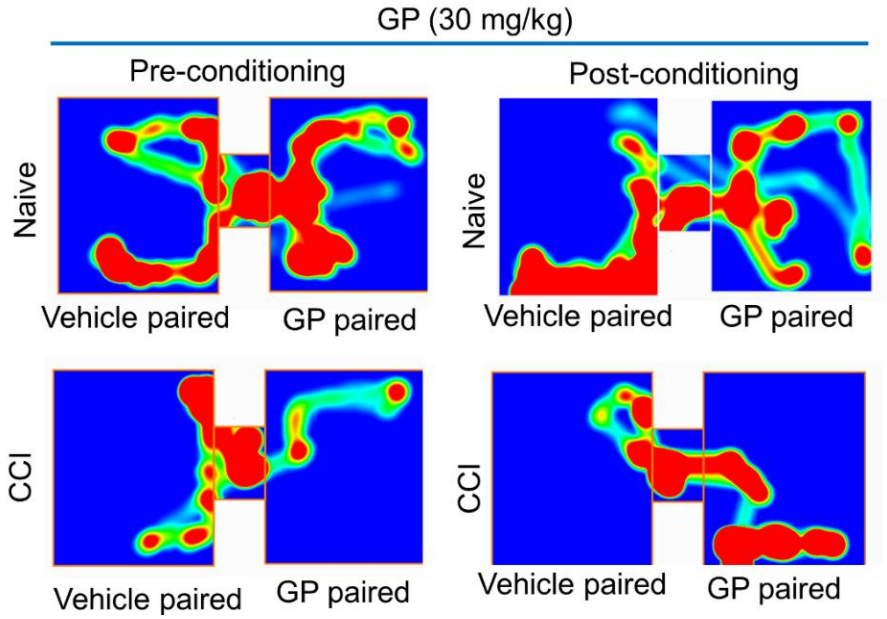


Figure 4.14 Effect of gabapentin on spontaneous ongoing pain behavior in nerve injured rats. Figure shows heat maps recorded during pre-conditioning and post-conditioning with **vehicle** v/s gabapentin (30mg/kg *i.p*). CCI, Chronic Constriction Injury.

1.3.5 Tozasertib does not affect locomotor or exploratory activity of nerve injured rats

CNS side effects are the most troublesome feature of most of the analgesics available in the clinic for the treatment of neuropathic pain [65]. Therefore, we have decided to perform the in-vivo neurotoxicity assays using open field and rota-rod test. We investigated the effects of tozasertib on motor incoordination and locomotor activity of rats. In the open field test, we observed that tozasertib (40mg/kg *i.p*) treatment did not alter the exploratory or locomotor behavior of nerve injured rats (Figure 4.15A, B, and C) as there was no significant change in the total distance travelled and average speed of tozasertib treated nerve injured rats as compared to the naïve rats. Moreover, treatment with gabapentin (30mg/kg *i.p*) also produced no observable effects on the locomotor behavior of nerve injured rats. However, morphine (10mg/kg *i.p*) administration led to significant ($p<0.05$) impairment in the locomotor activity of rats as compared to its pre-treatment baseline and vehicle treated nerve injured rats. In rotarod test also, tozasertib (40mg/kg *i.p*) as well as gabapentin (30mg/kg *i.p*) treatment showed no significant change in time spent on rod as compared to the nerve injured rats (Figure 4.15D). While morphine treatment significantly reduced ($p<0.001$) the fall latency of rats in rota-rod test as compared to its pre-treatment baseline pointing towards its sedative properties.

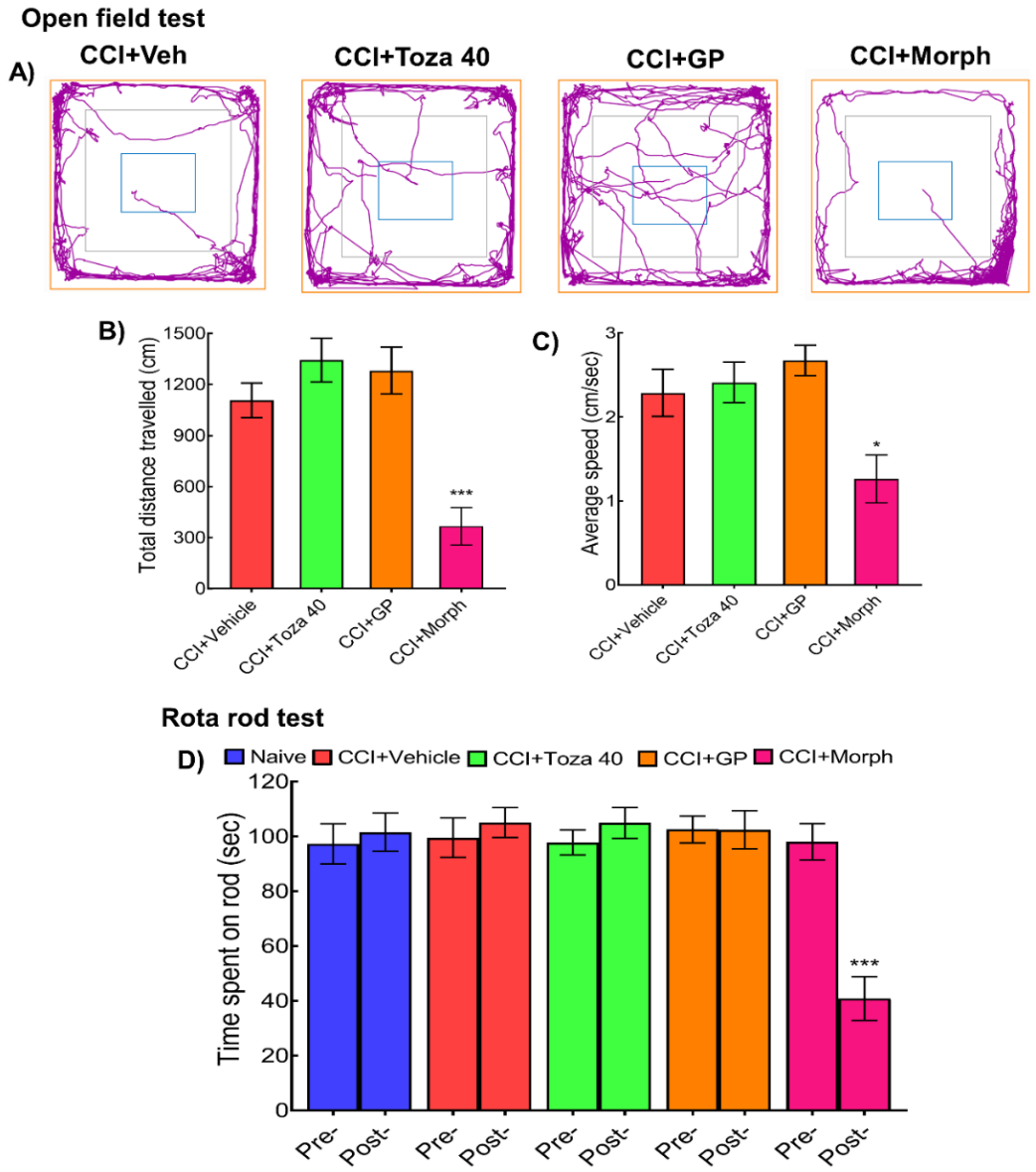


Figure 4.15 Effect of tozasertib on locomotor activity of nerve injured rats. (A, B and C) Open field test: Tozasertib (40mg/kg *i.p.*) and gabapentin (30mg/kg *i.p.*) treatment did not affect the locomotor activity of nerve injured rats in open field arena as compared to the vehicle treated rats. However, morphine (10mg/kg *i.p.*) treated rats showed a significant decline in total distance travelled and average speed. **(A)** Open field track plots of nerve injured rats treated with vehicle, tozasertib, gabapentin and morphine. **(B)** Total distance travelled by nerve injured rats after treatment with vehicle, tozasertib, gabapentin and morphine. **(C)** Average speed of nerve injured rats in open field arena after treatment with vehicle, tozasertib, gabapentin and morphine. **(D) Rotarod test:** Tozasertib (40mg/kg *i.p.*) and gabapentin (30 mg/kg *i.p*) treated rats did not show significant decrease in fall-time in rota-rod test as compared to their pre-treatment baseline. However, morphine (10mg/kg *i.p.*) treatment significantly decreased fall-time of rats as compared to the pre-morphine baseline. Data were

presented as mean ± SEM. ###p<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***P<0.001 indicates statistical significance as compared to the CCI rats. P<0.05 was considered statistically significant. Doses: Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

1.3.6 Tozasertib treatment attenuates inflammatory signaling in nerve injured rats

Inflammatory cytokines are the key modulatory mechanisms behind the development of pain pathophysiology. In chronic pain condition activation of NMDA stimulates release of cytokines that further promotes the pathophysiology of the disorder. One-way ANOVA followed by Tukey's multiple comparison test suggested a significant effect across the groups on mRNA and protein levels of ipsilateral L4-L5, DRG [F (5, 12) = 6.98; p<0.01 and F (5, 18) = 10.2; P<0.001 respectively] and spinal [F (5, 18) = 28.6; P<0.001 and F (5, 12) = 6.98; p<0.01 respectively] tissue of vehicle and drug treated nerve injured rats. CCI-induced nerve injury significantly increased the nuclear factor kappa β (NFκβ) mRNA and protein expressions in L4-L5, DRG (p<0.001 and p<0.01 respectively) and spinal cord (p<0.001 and p<0.01 respectively) tissues which was significantly attenuated on treatment with tozasertib at 10mg/kg (p<0.05 and p<0.01 respectively), 20mg/kg (p<0.001 and p<0.01 respectively), and 40 mg/kg (p<0.001) (Figure 4.16). Further, we measured the mRNA levels of inflammatory cytokines such as interleukin-6 (IL-6), interleukin 1 β (IL1β), and tumour necrosis factor-α (TNF-α) in ipsilateral L4-L5, DRG and spinal cord tissues of rats. One-way ANOVA followed by Tukey's multiple comparison demonstrated a significant effect across the groups on DRG tissues and spinal cord IL-6 [F (5, 18) = 9.66; p<0.001 and F (5, 18) = 9.64; p<0.001 respectively], IL1β [F (5, 18) = 11.1: p<0.001 and F (5, 18) = 16.4; p<0.001 respectively] and TNF-α [F (5, 18) = 20.9; p<0.001 and F (5, 18) = 15.0; p<0.001 respectively] levels in vehicle and drug treated

nerve injured rats (Figure 4.17). Nerve injury-induced significant increase in ipsilateral L4-L5, DRG and spinal expression of IL-6 ($p<0.001$), IL1β ($p<0.001$), and TNF-α ($p<0.001$) as compared to the naïve rats which was significantly attenuated on treatment with different doses of tozasertib (10mg/kg, 20mg/kg and 40 mg/kg). Gabapentin (30mg/kg) treatment also reduced the mRNA expression of IL-6 ($p<0.001$) IL1β ($p<0.001$), and TNF-α ($p<0.001$) in DRG and spinal cord tissues of nerve injured rats as compared to the vehicle treatment.

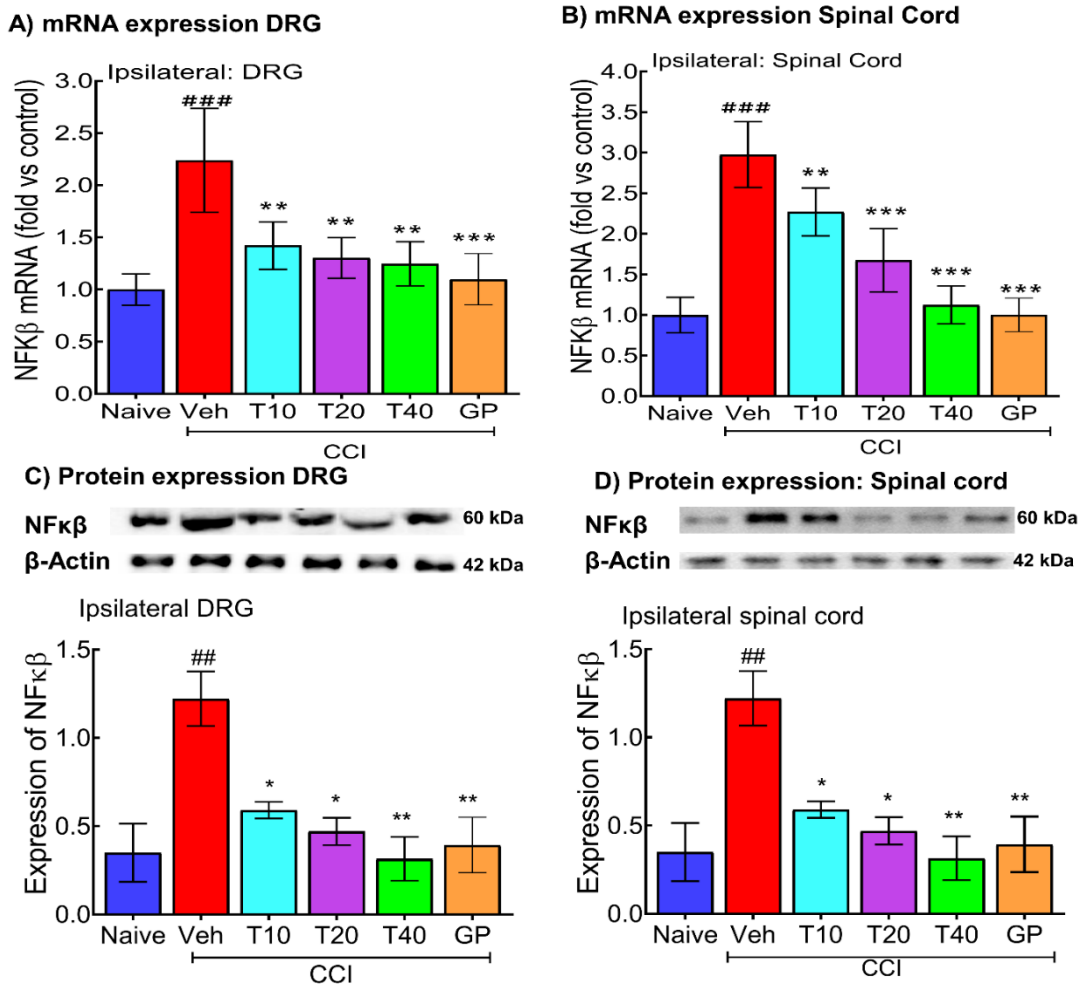


Figure 4.16 Effect of tozasertib on NFkβ mRNA and protein expressions in dorsal root ganglion and spinal cord of nerve injured rats. mRNA expressions: CCI induced-nerve injury increased mRNA expressions of NFkβ in in ipsilateral L4-L5, DRG **(A)** and spinal cord **(B)** of rats, which was significantly reversed on treatment with tozasertib (10, 20 and 40 mg/kg *i.p.*) and gabapentin (30mg/kg *i.p*). **Protein expressions:** Tozasertib (10, 20 and 40 mg/kg *i.p.*) and gabapentin (30mg/kg *i.p*) treatment significantly reduced nerve injury-induced induced protein expressions of

NFkβ in ipsilateral L4-L5, DRG **(C)** and spinal cord (**D)** of nerve injured rats. Data were presented as mean ± SEM. ###p<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***p<0.001 indicates statistical significance as compared to the CCI rats. p<0.05 was considered statistically significant. For western blot n=3 was used whereas for rtPCR n=4 biological and n=3 technical replicates were used. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

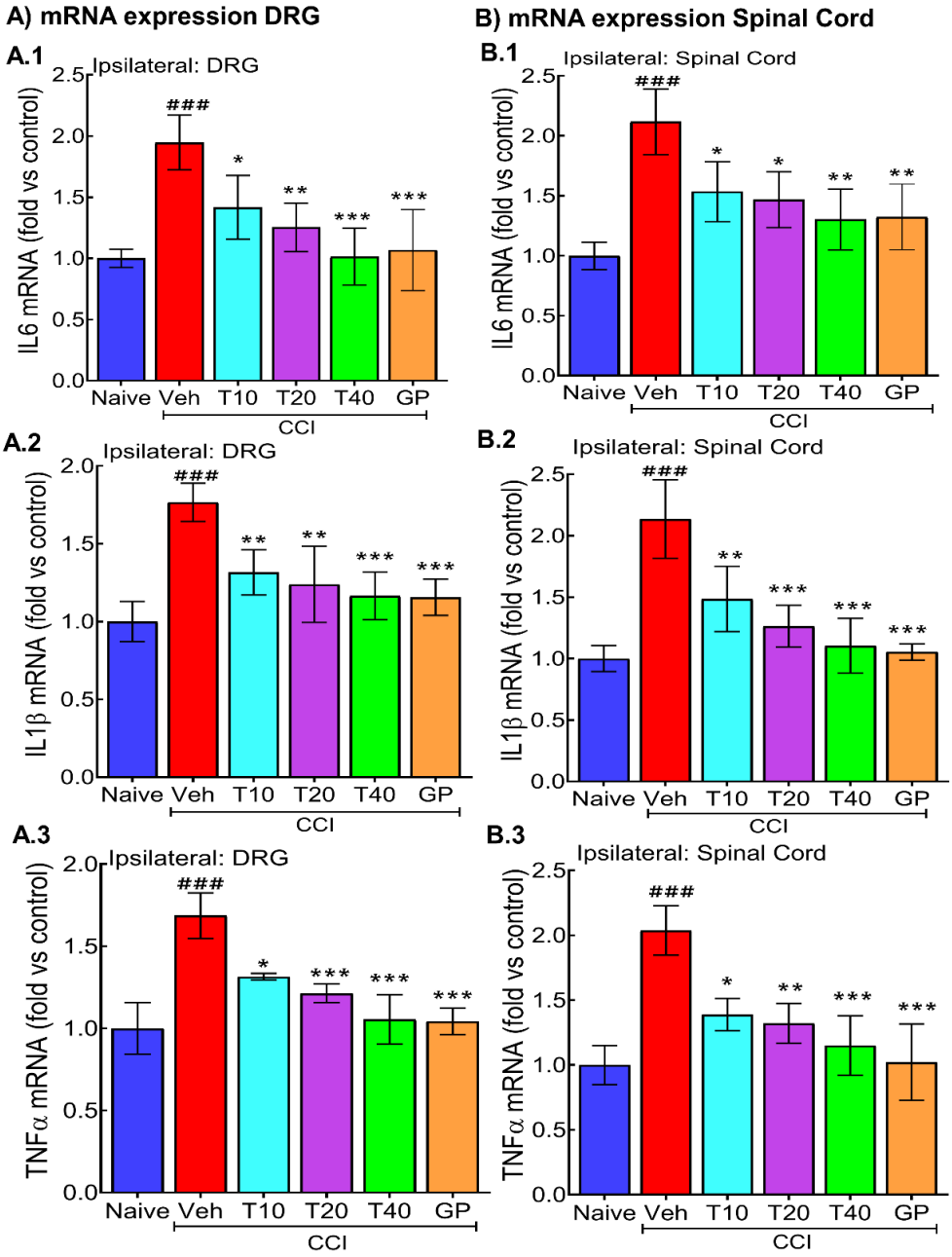


Figure 4.17 Effect of pan-Aurora kinase inhibition on nerve injury-induced inflammatory signaling in dorsal root ganglion and spinal cord of rats. (A) DRG mRNA expressions: Nerve injury-induced significant increase in IL-6 **(A1)**, IL1β **(A2)**, TNF-α **(A3)** in ipsilateral DRG of rats which was significantly attenuated by

tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) treatment. **(B) Spinal mRNA expressions:** Tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) treatment significantly attenuates CCI-induced increase in mRNA expressions of IL-6 **(B1)**, IL1β **(B2**) and TNF-α **(B3)** in ipsilateral L4-L5 spinal cord of rats. n=4 biological and n=3 technical replicates. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

1.3.7 Tozasertib interferes with KIF17 and NR2B expression in the spinal cord and dorsal root ganglion of nerve-injured rats

1.3.7.1 Tozasertib significantly reduced nerve injury induced-NR2B mRNA and protein expression in spinal and DRG tissue

NR2b subunit is involved in the development and maintenance of chronic pain [120,181]. The surface expression of NR2b is higher in chronic pain which causes activation of several downstream pathways including Ca^{++} signaling, protein kinase A (PKA), protein kinase C (PKC), extracellular signal-regulated kinase (ERK), and mitogen activated protein kinase (MAPK) pathways [53]. These multifarious pathways cause central sensitization and enhanced pain transduction and perception. rtPCR and western blot studies were conducted to investigate the effect of aurora kinase inhibition on mRNA and protein expression of NR2B in spinal cord and DRG tissues of nerve injured rats. Using one-way ANOVA followed by Tukey's multiple comparison test, we found a significant effect across the groups on both mRNA and protein expression of NR2B in ipsilateral L4-L5, DRG [$F(5, 18) = 34.7$; $p<0.001$ and $F(5, 12) = 15.3$; $p<0.001$ respectively] and spinal cord [$F(5, 18) = 30.7$; $p<0.05$ and $F(5, 12) = 14.1$; $p<0.001$ respectively] tissues of vehicle and drug treated nerve injured rats.

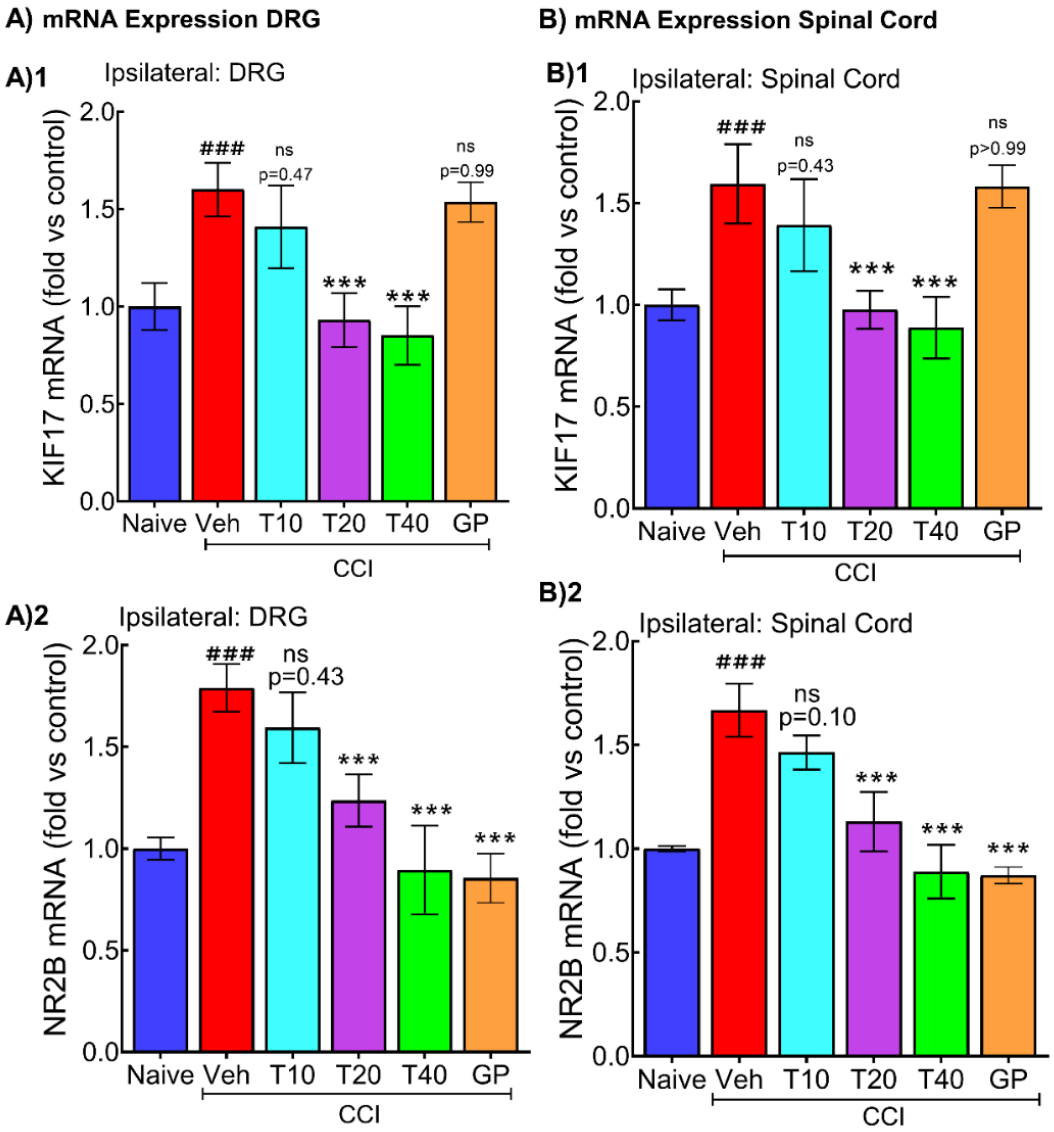


Figure 4.18 Effect of tozasertib on KIF17 and NR2B mRNA expressions in dorsal root ganglion (DRG) and spinal cord of nerve injured rats. (A) DRG mRNA expression: CCI surgery significantly increased mRNA expressions of KIF17 **(A1)** and NR2B **(A2)** in ipsilateral L4-L5 DRG of rats which was significantly attenuated by tozasertib treatment (20 and 40 mg/kg *i.p.*). Gabapentin (30mg/kg *i.p*) treatment significantly reduced the ipsilateral L4-L5 DRG mRNA expression of NR2B but does not have any effect on KIF17 mRNA expression. **(B) Spinal mRNA expression:** Nerve injury-induced significant increase in ipsilateral L4-L5 spinal expressions of KIF17 and NR2B which was significantly decreased on treatment with tozasertib (20 and 40 mg/kg *i.p.*). While, gabapentin (30mg/kg *i.p*) treatment significantly decreased NR2B expression without affecting KIF17 expression in ipsilateral L4-L5 spinal cord of nerve injured rats. Data were presented as mean ± SEM. $^{###}p<0.001$ indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***p<0.001 indicates statistical significance as compared to the CCI rats. p<0.05 was

considered statistically significant. n=4 biological and n=3 technical replicates. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

We found that nerve injury has significantly increased the mRNA and protein expression of NR2B in the DRG ($p<0.001$ and $p<0.01$ respectively) and spinal cord ($p<0.001$ and $p<0.01$ respectively) as compared to the naïve rats (Figure 4.18 and figure 4.20). Tozasertib treatment significantly decreased the ipsilateral L4-L5, spinal and DRG expression of NR2B mRNA and protein at 20mg/kg ($p<0.001$) and 40mg/kg ($p<0.001$) doses as compared to the vehicle treated nerve injured rats. Gabapentin treatment also decreased the NR2B mRNA and protein expression in both DRG tissues ($p<0.001$) and spinal cord ($p<0.001$) as compared to the vehicle treated nerve injured rats (Figure 4.18 and figure 4.19). It indicates that both tozasertib and gabapentin may involve the NR2B mediated mechanism during the attenuation of nerve injury-induced neuropathic pain. We did not observe a significant on contralateral L4-L5, DRG and spinal NR2B expression in nerve injured rats and drug treated rats (tozasertib and gabapentin) as compared to the naïve rats (Figure 4.19).

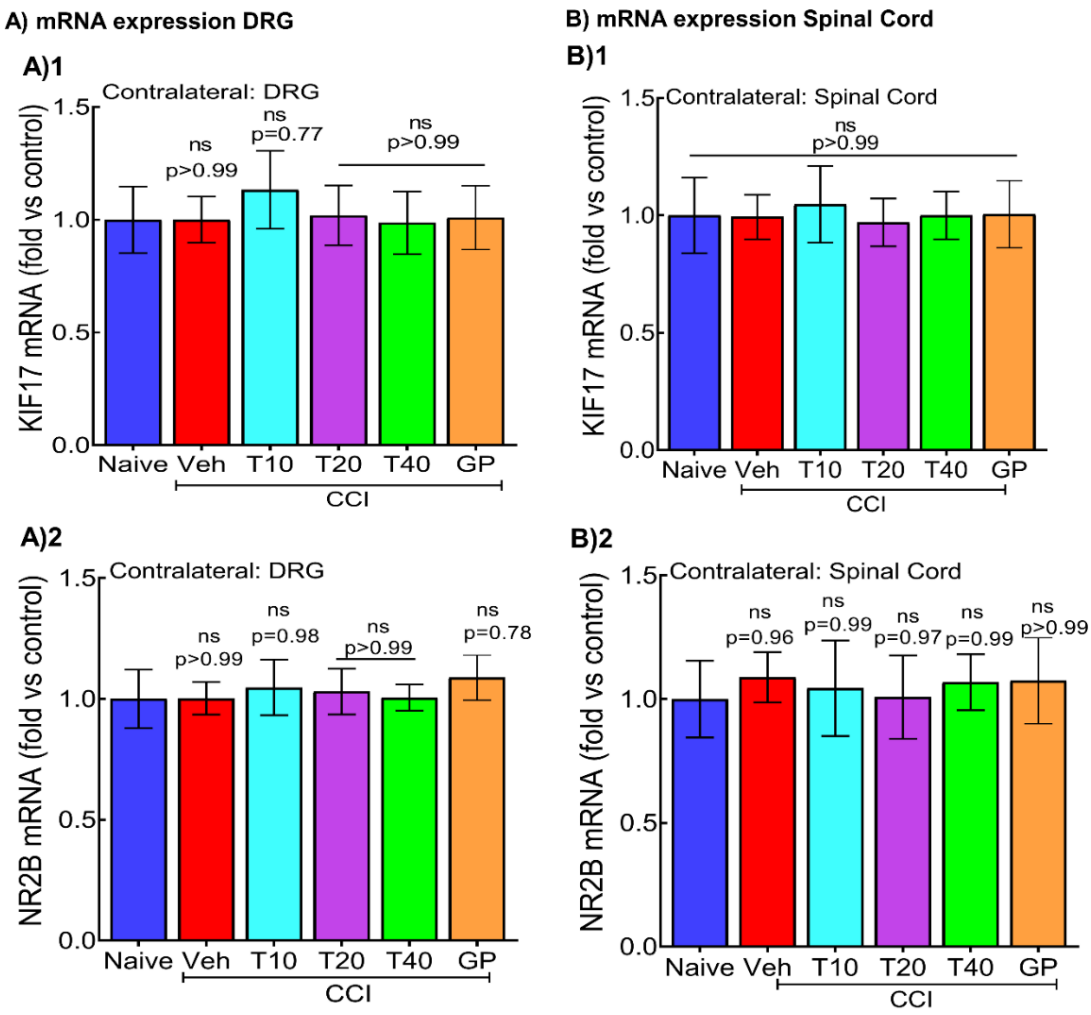


Figure 4.19 Effect of tozasertib on KIF17 and NR2B mRNA expressions in contralateral dorsal root ganglion and spinal cord of nerve injured rats. (A) DRG mRNA expression: CCI induced-nerve injury and treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) did not affect contralateral L4-L5 DRG mRNA expressions of KIF17 **(A1)** and NR2B **(A2)** as compared to the naïve rats. **(B) Spinal mRNA expressions:** CCI-induced nerve injury did not affect the spinal mRNA expressions of KIF17 and NR2B. Moreover, treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) also did not affect the contralateral L4-L5 spinal mRNA expressions of KIF17 **(B1)** and NR2B **(B2)**. Data were presented as mean ± SEM. P<0.05 was considered statistically significant. n=4 biological and n=3 technical replicates. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

1.3.7.2 Tozasertib but not gabapentin suppressed the nerve injury-induced KIF17 expression

KIF17 nanomotor is the major trafficking partner of NR2B subunit to make the NMDAR functionalized into the synaptic membrane. We observed that CCI injury increased the expression of KIF17 mRNA and protein in ipsilateral L4-L5,-

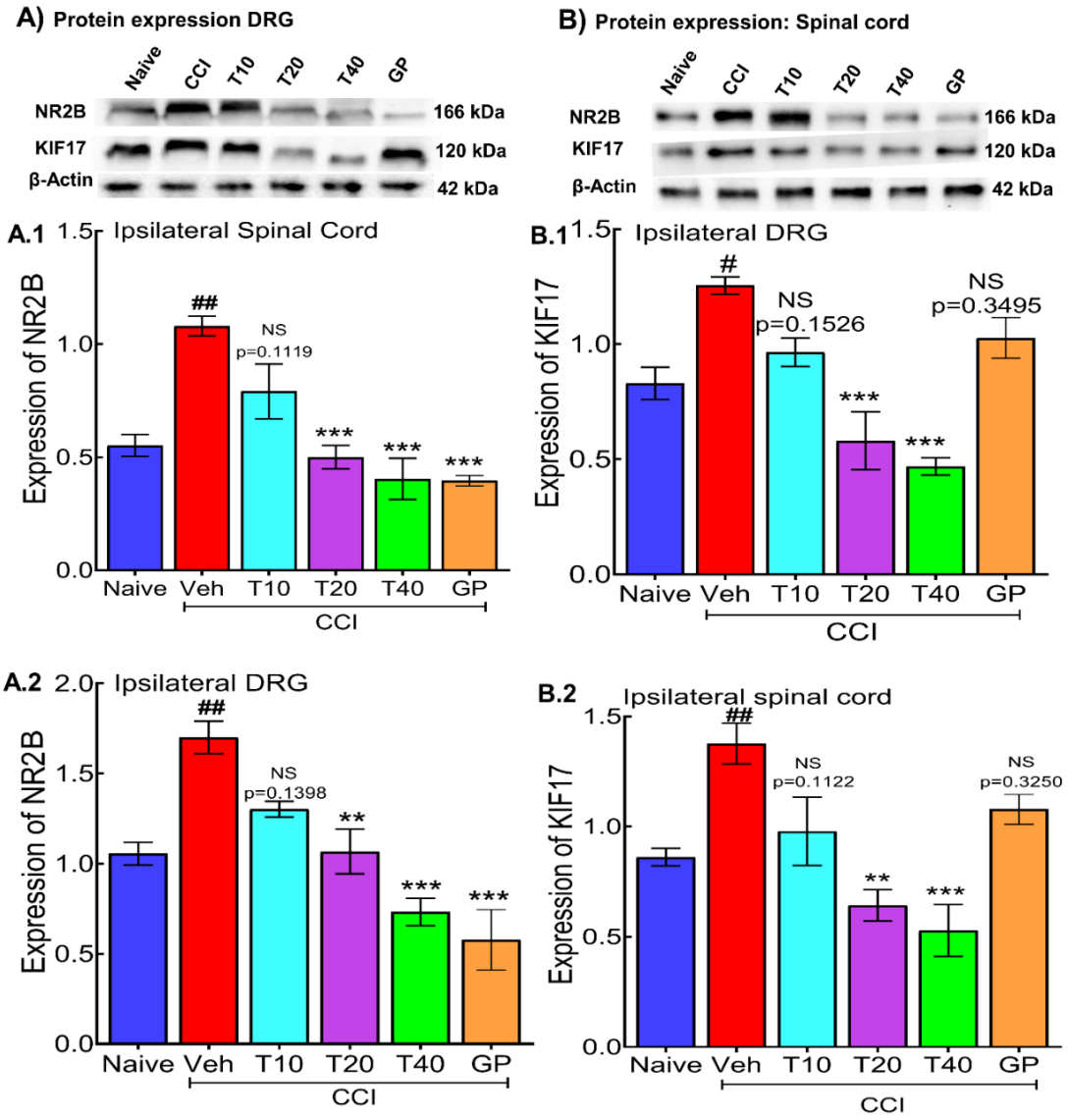


Figure 4.20 Effect of pan-aurora kinase inhibition on elevated protein expressions of KIF17 and NR2B in dorsal root ganglion and spinal cord of nerve injured rats. **(A) DRG protein expressions (A1 and A2)** Tozasertib treatment (20 and 40 mg/kg *i.p.*) attenuates CCI surgery-induced increase in protein expressions of KIF17 and NR2B in ipsilateral L4-L5 DRG of nerve injured rats. Whereas gabapentin (30mg/kg *i.p*) treatment reduced only NR2B without affecting KIF17 protein expressions in

ipsilateral L4-L5 DRG of nerve injured rats **(B) Spinal protein expressions (B1 and B2)** Nerve injury-induced significant increase in protein expressions of KIF17 and NR2B in ipsilateral L4-L5 spinal cord tissues of rats which was significantly attenuated on treatment with tozasertib (20 and 40 mg/kg *i.p.*). While, treatment with gabapentin (30mg/kg *i.p*) significantly reduced the spinal expressions NR2B without affecting KIF17 levels in nerve injured rats. Data were presented as mean ± SEM. $^{###}p<0.001$ indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***P<0.001 indicates statistical significance as compared to the CCI rats. p<0.05 was considered statistically significant. n=3. Doses: Toza10, Tozasertib 10mg/kg; Toza 20, Tozasertib 20 mg/kg; Toza40, Tozasertib 40 mg/kg; GP, Gabapentin 30mg/kg. CCI, Chronic Constriction Injury.

- DRG [F (5, 18) = 20.1; p<0.001 and F (5, 12) = 14.5; p<0.001 respectively] and spinal cord [F (5, 18) = 18.1; p<0.001 and F (5, 12) = 9.77; p<0.001 respectively] tissues of rats as compared to the naïve rats. Tozasertib treatment suppressed the ipsilateral L4-L5, DRG and spinal expression of KIF17 mRNA and protein at 20mg/kg (p<0.01 and respectively p<0.001) and 40 mg/kg (p<0.001) as compared to the vehicle treated nerve injured rats (Figure 4.18 and 4.20). Gabapentin (30mg/kg *i.p*) treatment did not produce any significant effect on the KIF17 expression in ipsilateral L4-L5, DRG and spinal cord tissues as compared to the vehicle treated nerve injured rats. From these findings, we conclude that inhibition of aurora kinase enzyme interferes with KIF17-NR2B cargo system during neuropathic pain. Whereas, gabapentin mediated pain relief does not involve the KIF17 mediated signaling in nerve injured rats. CCI induced nerve injury and, treatment with tozasertib (10, 20 and 40 mg/kg *i.p*) and gabapentin (30mg/kg *i.p*) did not show a significant effect on contralateral L4-L5, DRG and spinal expressions of KIF17 protein as compared to the naïve rats (Figure 4.19).

4.4 Outcomes

Pan-Aurora kinase inhibition produce the evoked and spontaneous ongoing pain relief by regulating KIF17-NMDA expression and neuroinflammation in the spinal cord

and dorsal root ganglion of nerve injured rats. The present study demonstrates a novel line of action towards the development of potential therapeutics by targeting aurora kinase for the treatment of neuropathic pain.