Chapter-6

Modulation of KIF-17/NR2B Crosstalk by betaine in Neuropathic Pain Rat Model

6.1 Introduction

Betaine is a naturally occurring, zwitterionic amino acid derivative widely distributed in plants, animals and human beings. Betaine modulates the NMDA receptors and attenuate glutamate induced neurotoxicity in chicken embryonic brain cells, without effecting calcium ion channels. Betaine acts as partial agonist at the glycine site of NMDA receptor and regulate excitatory post-synaptic potentiation, long-term potentiation, and PSD-95 protein expression levels, along with 5-HT levels and exert its antidepressant effects (Muhammad et al. 2019). Recent studies have also revealed that betaine can modulate the kinesin activity and thereby exert the antinociceptive effect. Here, for the first time the effect of betaine on nerve injury induced evoked and ongoing pain along with dissection of the molecular mechanisms mediated through KIF17/NR2b signaling was studied.

6.2 Experimental design

In this study the effect of betaine on chronic pain rats were divided into six experimental groups with a minimum of eight rats/group was analyzed. 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. Rats in the third, fourth and fifth groups belonged to different test groups where nerve-injured rats were treated with intraperitoneal betaine at 25mg/kg, 50 mg/kg, and 100 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 sanother four groups of nerve-injured rats which were treated with vehicle, tozasertib (100 mg/kg *i.p*), gabapentin (30 mg/kg *i.p*) and morphine (10 mg/kg *i.p*) respectively were used. 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. RT-PCR and western blotting was performed to assess the expression of NR2B, KIF17 and inflammatory cytokines in respective tissues.

To study the effect of betaine on normal nociceptive threshold rats were divided inti different groups (n=8/groups) in naïve or healthy, naïve+ treatment with different dosage of betaine (25mg/kg, 50 mg/kg and 100 mg/kg), naïve + gabapentin (30 mg/kg *i.p*) and naïve + morphine (10 mg/kg *i.p*).

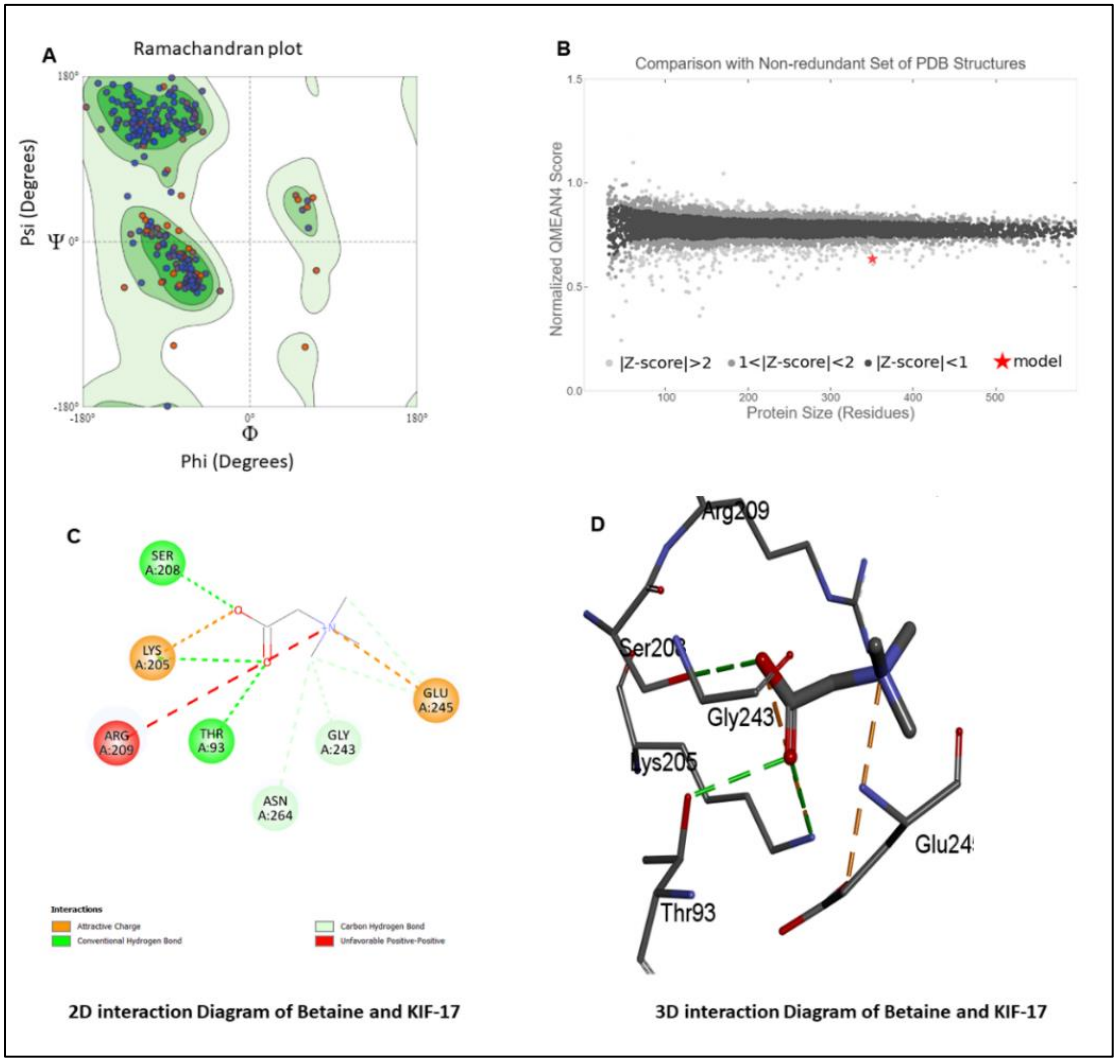
6.3 Results and discussion

6.3.1 *In-silico* studies

6.3.1.6 Homology modeling

The evaluation of the construct model was verified by the RC plot and Profile-3D analysis tools respectively. Two best models for RC analysis among the various models prepared were selected using the Swiss-model tool and the model with

significant proportion of residues lying in the most favored regions was selected for further analysis. The degree of confidence that is percentage of residues in the most favored region was used for graphical representation. The plot of the energy minimized model of KIF-17 was generated for the graphical representation, on the y-axis the Psi angles and on the x-axis the Phi angles are represented respectively. The area of disallowed region, generously allowed region, allowed region and low energy region are represented as the four quadrants of the plot In the most recommended region the KIF-17 exhibited 95.32% of the residues, 5.9% within the moreover allowed region, 0.9 % in the generously allowed region, whereas in the disallowed region 0% was found as shown in Figure 1A and 1B. Using the Verify3D and ERRAT it was found that the generated model was having 82.21% and 49.395 unfolded structures (SAVES server). Further, the PROSA Z-score and QMEANS values were observed as −6.9 and −4.26 respectively (Figure 1B). Thus, the finding from the Ramachandran plot, ERRAT, Verify-3D, and QMEANS confirm that the generated model is reliable and of good quality.



6.3.1.6 Molecular Docking

In the first molecular docking study, the 3D structure of KIF-17 and betaine was prepared and constructed. A receptor search region was defined in AutoDock Vina, encompassing the active site of KIF-17. The active sites were highlighted in red, including residues (Lys 205, Glu 245, Ser 208, Thr 93, and Arg 209), to facilitate the identification of the active site. A grid box was then designed to coordinate all of the active sites as the search region. After the docking process was completed, five docked structures were obtained, with distinguishable energy scores of −10.4, −8.1, −7.5, −7.2,

and −6.6 kcal/mol. In the lowest energy structure, betaine binds to the active site of KIF-17. This docking result demonstrates that betaine can act as an inhibitor with the potential to bind to KIF-17. The docking figures were depicted in the **Figure (6.2 C and D.)**

6.3.1.6 Molecular dynamics simulation

Molecular dynamics simulation was used to investigate the architectural interplay between KIF17 and betaine. The analysis of the root mean square fluctuation (RMSF), root mean square deviation (RMSD), number of hydrogen bonds, the radius of gyration (Rgyr), and the average distance were performed under physiological environment and the 100ns was kept as the simulation time during MD trajectory. The MD trajectory and its data analysis can be found in (Figure 6.2 A-F). The correlation of the fluctuation of the stability and the quality of the system to its initial coordinates from the structure has been established. The GROMACS rmsd utility was used to generate the RMSD plot for the KIF-17 and betaine. Initially small fluctuations were inferred at the initial step from the exhibited value of 0.3 nm, which was later stabilized resulting in less fluctuation (RMSD was observed between 0.2 to 0.7 nm for protein and ligand) throughout the simulation time interval during the simulation run in the targeted protein (Figure 6.2 A). This suggested that for the simulation process, protein and ligand get gradually stabilized as the RMSD value lies between the acceptable range that is 0.2 to 0.7 nm for the protein and 0.2 to 0.5 nm for the ligand. Furthermore, GROMACS RMSF utility was used to record the protein structure's flexibility during the interaction period of the simulation so as to predict the dynamic behaviors of the amino acid residues [24]. Internal fluctuations were not found as presented by the

finding from the graph during the simulation time of 100ns (Figure 6.2 B). Moreover, protein's RMSF value was found to lie in the acceptable range of 0.1 to 1 nm. The ligand represented RMSF value in (Figure 6.2 C) with the value between 0.02 to 0.25 nm hence confirming the its stability in the system which further suggests that in the normal way the ligand is rigidly bound to the active site of the amino acids. To determine the radius of gyration of the protein in for acquiring the level of compactness in the structure as well as an insight into the overall dimensions of the protein, gyrate utility in GROMACS was used (Figure 6.2 D). Finally, the H Bond, hydrophobic bond, ionic interaction, and water bridges which were the various interactions found between ligand and protein were present which are the essential factors responsible for the stability and formation of the protein-ligand complex. H-bond utility in GROMACS was used to plot the average number of hydrogen bonds throughout the 100 ns simulation time with a cut-off value of 0.35 nm (Figure 6.2 E). Throughout the 100ns simulation majority of the hydrogen bonds were found in the crystal structure were stored. The h bond utility was used to examine the average distance of hydrogen bonds as shown in the graph in (Figure 6.2 F). Finally, it was observed that the average distance of 0.3 nm which was within the acceptable range of hydrogen bond formation hence suggesting adequate ligand-protein interaction.

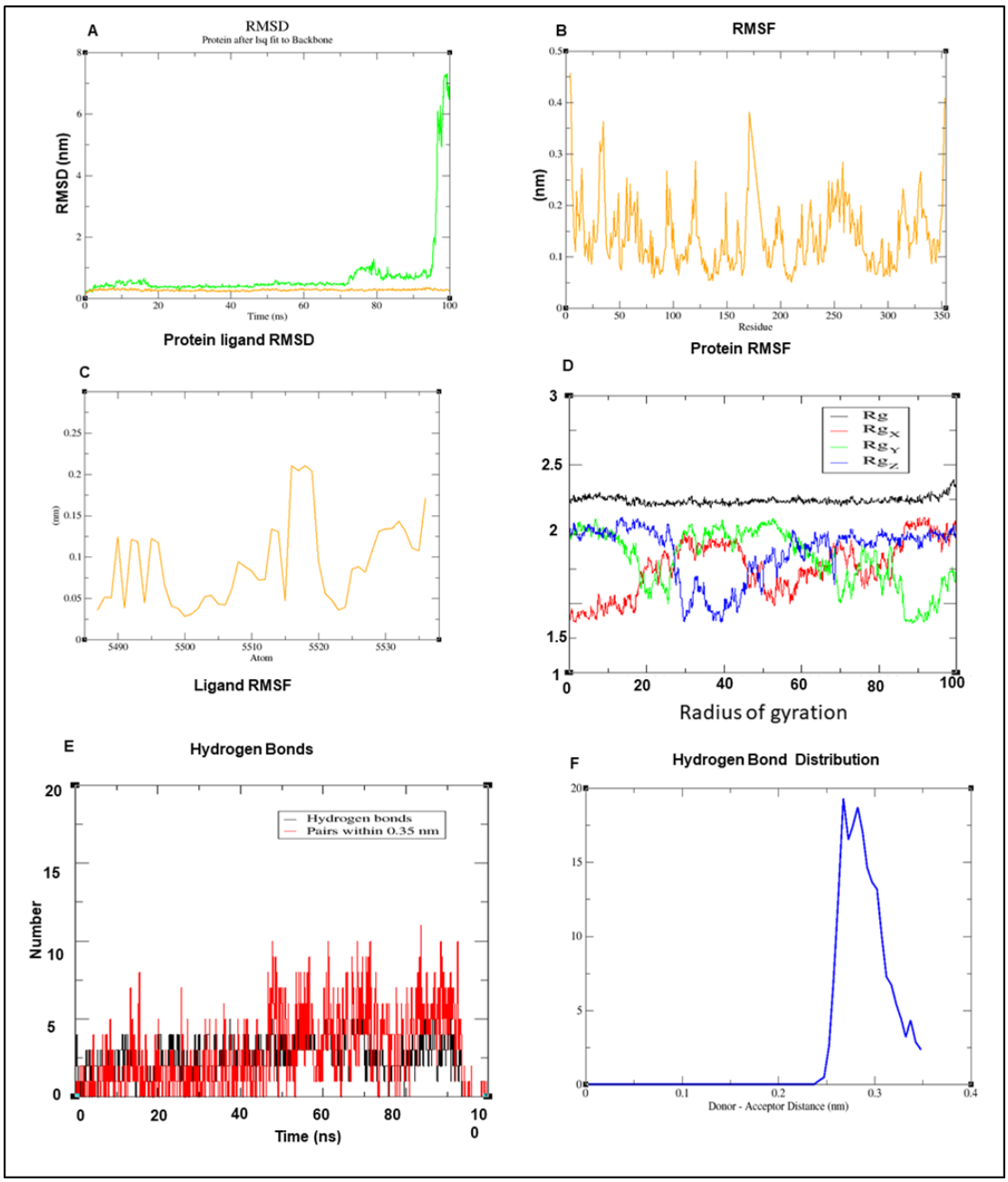


Fig 6.2. Molecular dynamics simulation trajectories of betaine and KIF-17 complex. A) Protein- RMSD **B)** RMSF of KIF-17 **C)** RMSF of betaine **D)** Radius of gyration for KIF-17 **E)** Total hydrogen bond formed during MD simulation 100 ns trajectory **F)** Average distance of hydrogen bond

6.3.2 *In-vivo* studies

6.3.2.6 Betaine Attenuates CCI-induced Mechanical Allodynia and Thermal Hyperalgesia in Nerve Injured Rats

Wide number of pain behavioral assays such as thermal, mechanical, cold and spontaneous ongoing pain to assess the efficacy of betaine on nerve injury induced-chronic pain in rats were performed. Nerve injury led to significant decrease in paw withdrawal threshold (PWT) in ipsilateral paw of CCI rats ($p<0.001$) as compared to their pre-injury baseline and naïve rats **(Figure 6.3 A).** It was observed that rats treated with betaine was significantly improved the ipsilateral paw withdrawal threshold (static allodynia) in von-Frey hair test as compared to the vehicle administrated rats. The effect of betaine was observed at 1hr and 2hr at different doses (25, 50 and 100 mg/kg *i.p.*). Further, the standard drug gabapentin also rescued the mechanical allodynia at 0.5 hr,1hr and 2hr post administration (**Figure 6.3 A)**. However, contralateral paw recording was found to be unchanged in response to the non-noxious mechanical stimulus. Heat hyperalgesia was significantly inhibited with betaine treatment (25, 50, and 100 mg/kg *i.p*) as evident by increase in PWL of neuropathic rats as compared to their pre-drug baseline. Increase in latency trends after 30-minutes post betaine administration as compared to the vehicle treated CCI rats was observed **(Figure 6.3 C).** The statistical significant effect of betaine has started at 1 hr post-administration i.e 25 50 and 100 mg/kg ($p<0.001$). The peak effect of betaine was observed at 2hr post drug administration and which was persisted up to 4hr followed by a decreases the effect of betaine. However, no significant effect on 4 hr after betaine administration at the doses of 25, 50 mg/kg, *i.p.* to restoring in nerve injury- induced decreased PWL was seen. A significant effect of heat hyperalgesia was observed across the group interaction

[F (5,48) =322; p<0.001] and time points [F (9.03, 221) = 314; p<0.001]. Additionally, contralateral PWL did not changed before and after nerve injury and different drug treatments (gabapentin and betaine).

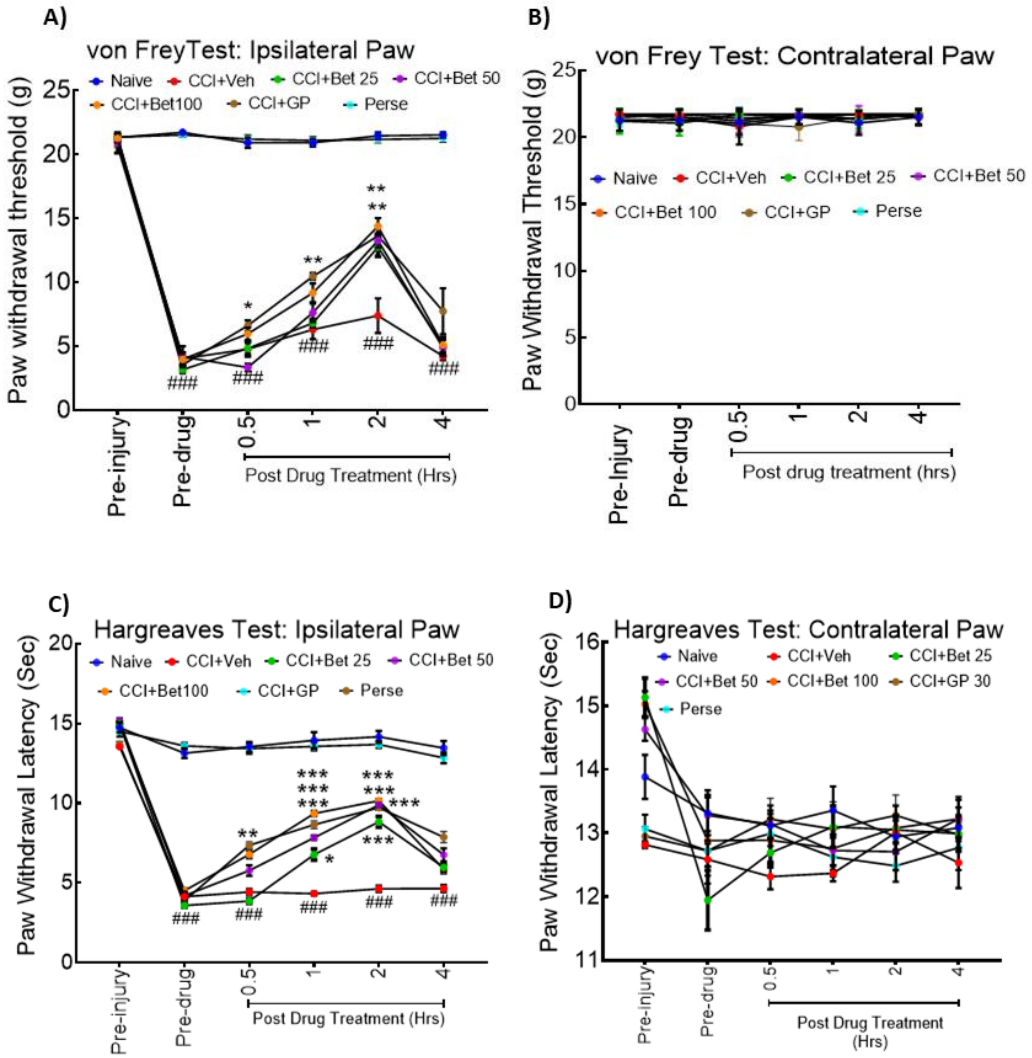


Figure 6.3. Effect of betaine on CCI-induced heat hyperalgesia in nerve injured rats. **A&B)** Ipsilateral and contralateral paw withdrawal threshold **C&D)** Ipsilateral and contralateral paw withdrawal latency. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison). ### represents significance compared to the control group (p<0.001), * (p<0.05) ** (p<0.01) and *** (p<0.001) represents significance compared to the CCI group. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

6.3.2.6 Betaine Attenuates CCI-induced Cold Hypersensitivity in Nerve Injured Rats

Next, acetone test to evaluate the effect of cold-innocuous stimulus on CCI rats was performed. There was a significant elevation of paw withdrawal score on day 14th post CCI injury as compared to pre-injury baseline **(Figure 6.4 A**). Treatment with betaine at 25, 50 and 100 mg/kg *i.p* and gabapentin 30 mg/kg, *i.p.* dose led to significantly decreases ipsilateral paw pain hypersensitivity as compared to vehicle treated rats which further suggests that anti-allodynic effect of this natural compound in nerve injured rats. Moreover, no significant pain alternation in contralateral paw response, was observed **(Figure 6.4 B).** Two-way ANOVA followed by Bonferroni's multiple comparison test was performed was applied and a significant effect was fund to across the groups interaction [$F(5, 42) = 173$; $P<0.001$] and time points [$F(4.75, 199) = 155$; $P<0.001$]. Further, the effect of betaine on cold hyperalgesia was examined. It was observed that nerve injury led to significant increases the number of ipsilateral paw lifts in the presence of noxious cold stimulus as compared to their pre- injury baseline and healthy control rats ($p<0.001$) **(Figure 6.4 A).** There was a significant reduction in number of paw lifts post betaine administration at 1 hr (25 mg/kg, $p<0.02$; 50 mg/kg, $p<0.01$ and 100 mg/kg $P<0.001$), 2 hr (25 mg/kg, $p<0.06$; 50 mg/kg, $p<0.001$ and 100 mg/kg $p<0.001$) as compared vehicle administered CCI rats. Additionally, gabapentin also significantly attenuated the number of ipsilateral paw lifts as compared to CCI rats post 1 hr ($p<0.001$), 2 hr ($p<0.001$) of administration. Moreover, no significant effect on contralateral paw was observed on allodynic response score and noxious stimuli induced cold hypersensitivity in rats as compared to their respective

pre-injury baselines and pre-drug baselines on nerve injury and drug treatment **(Fig 6.4 B)**

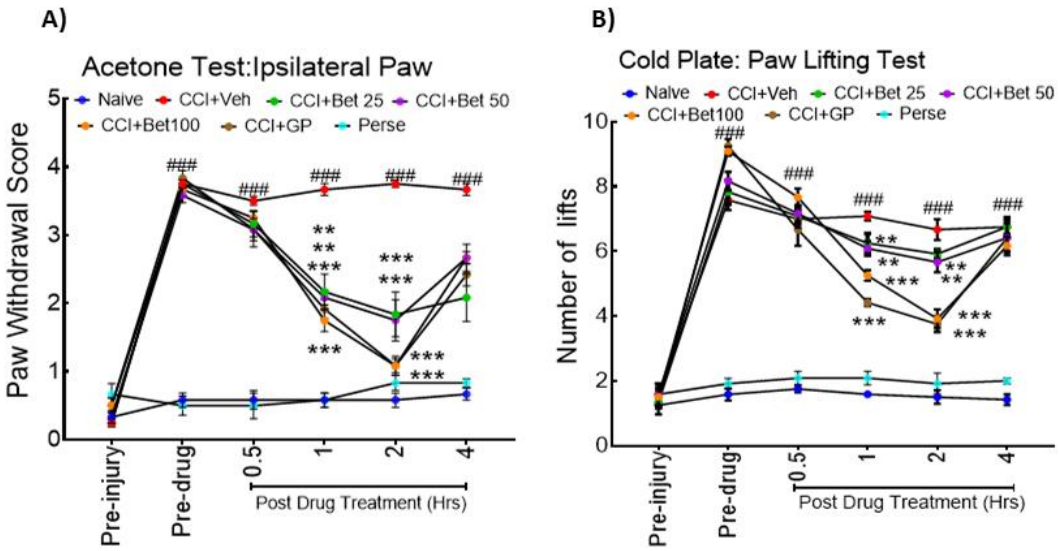


Fig 6.4. Effect of betaine on nerve injury-induced cold pain behavior in rats. **A)** Paw withdrawal response, **B)** Paw lifts. . Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison). ### represents significance compared to the control group ($p<0.001$), * ($p<0.05$) ** ($p<0.01$) and *** ($p<0.001$) represents significance compared to the CCI group. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

6.3.2.6 Betaine Attenuates CCI-induced Mechanical Hyperalgesia in Nerve Injured Rats (Pin prick test)

Next mechanical hyperalgesia in CCI rats was examined. Nerve-injury led to significant increase in the frequency of paw withdrawal when compared to the naïve rats, assessed in pinprick test. Treatment with betaine (25, 50, and 100mg/kg) produced significant attenuation in ipsilateral paw hyperalgesia at 1 and 2 hr post administration as compared to the vehicle treated rats. Gabapentin treated rats also showed significant decrease in paw withdrawal frequency at 0.5hr, 1hr, 2hr post administration **(Figure**

6.5 A). There was no observed effect of nerve injury as well as treatment on contralateral paw withdrawal frequencies in rats.

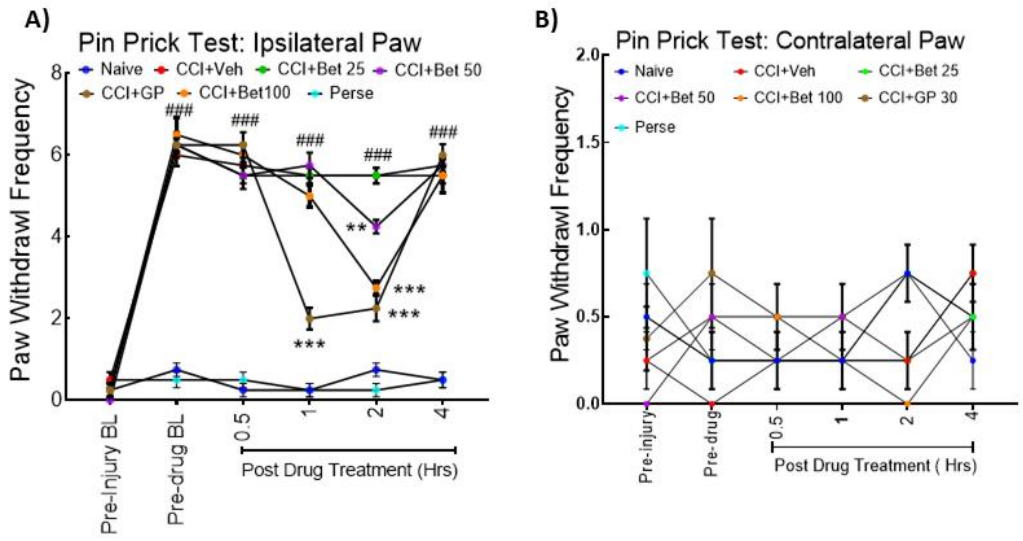


Figure 6.5. Effect of betaine in mechanical hyperplasia in nerve injured rats. **A & B)** Ipsilateral and contralateral paw withdrawal latency. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison). ### represents significance compared to the control group ($p<0.001$), * ($p<0.05$) ** ($p<0.01$) and *** ($p<0.001$) represents significance compared to the CCI group. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

6.3.2.4 Effect of Betaine on Dynamic Allodynia in Nerve Injured Rats

Furthermore, the evaluation of the dynamic component of allodynia using the cotton swab test revealed noteworthy findings. Following nerve injury, a substantial increase in the latency to paw withdrawal was observed exclusively in the ipsilateral paws of rats, with no discernible effect noted in contralateral paw responses. Notably, administration of betaine at varying doses (25, 50, and 100 mg/kg i.p.) and gabapentin (30 mg/kg i.p.) led to a significant amelioration in dynamic allodynia behavior among nerve-injured rats compared to those treated with the vehicle alone **(Figure 6.6 A-B).** Importantly, these beneficial effects were confined to the ipsilateral paw, and no observable alterations were detected in the contralateral paw of rats, emphasizing the

specificity of the treatments on the affected side. These findings underscore the potential of both betaine and gabapentin in targeting the dynamic aspects of allodynia associated with nerve injury.

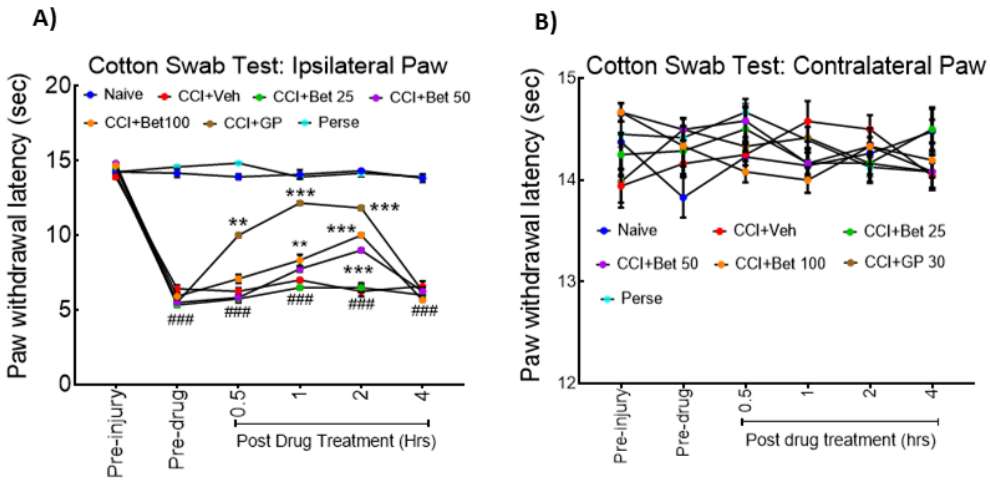


Figure 6.6. Betaine does not affect dynamic allodynia in nerve injured rats. **A & B)** Ipsilateral and contralateral paw withdrawal latency. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison). ### represents significance compared to the control group ($p<0.001$), * ($p<0.05$) ** ($p<0.01$) and *** ($p<0.001$) represents significance compared to the CCI group. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

6.3.2.5 Betaine suppressed spontaneous ongoing pain in nerve injured rats without addiction Spontaneous ongoing pain is an essential component of chronic pain, and it is widely accepted that compounds having activity against evoked and ongoing component pain are more likely to show better results in clinical trials. Spontaneous ongoing pain using conditioned place preference (CPP) was tested **(Figure 6.7).** Betaine (100 mg/kg i.p.) ($p<0.001$) administration significantly inhibits the ongoing pain in nerve injured rats as indicated by the increased CPP to drug paired chamber as compared to the saline paired **(Figure 6.7A-B).**

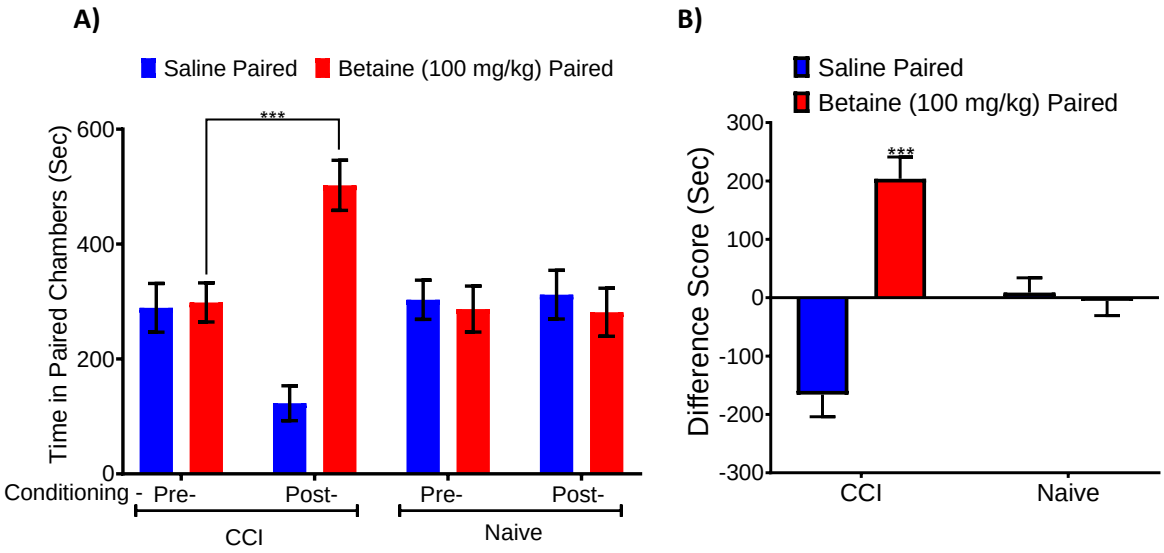


Figure 6.7. Effect of betaine on ongoing pain in nerve injured rats. A) CPP in nerve injured rats & naïve rats. **B)** Difference score. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison). ### represents significance compared to the control group ($p<0.001$), * ($p<0.05$) ** ($p<0.01$) and *** ($p<0.001$) represents significance compared to the CCI group. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg.

Interestingly, betaine does not produce CPP in naïve rats which indicate its addiction free property (Fig. 6.7B). Further, gabapentin administration also improved ongoing pain in nerve injured rats whereas there was no change in CPP to drug paired chamber in naïve rats when compared to the sa**line paired chamber** (Fig 6.8 A-B)**.** Next, CPP for morphine was performed and it was observed that nerve injured as well as naïve rats showed increase preference to the drug paired chamber as compared to the saline paired chamber. This suggests the analgesic as well as addictive potential of morphine **(Figure 6.9 A-B).**

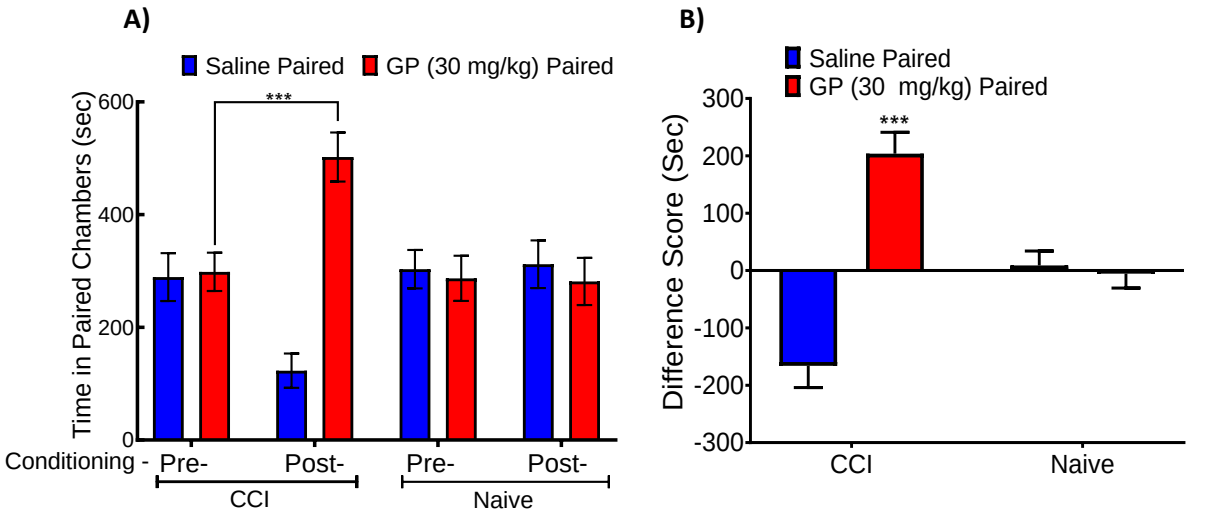


Figure 6.8. Gabapentin attenuates ongoing pain in nerve injured rats. A) CPP in nerve injured rats & naïve rats B) Difference score. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=8). ** and *** represents significance ($p<0.01$) and ($p<0.001$) respectively. Gabapentin 30mg/kg *i.p.*

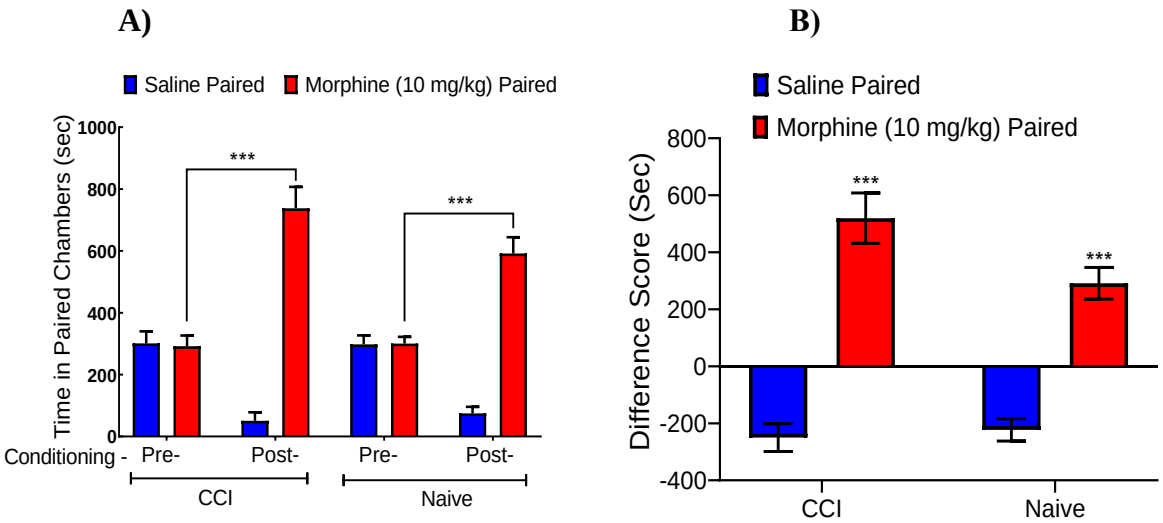


Figure 6.9. Effect of morphine on CPP in nerve injured and naïve rats. A) CPP in nerve injured rats & naïve **B)** difference score. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) ** ($p<0.01$) and *** ($p<0.001$) represents significance levels

6.3.2.6 Betaine does not produce CNS side effects in nerve injured rats

Locomotor side effects are very common with analgesics which creates a substantial barrier to the chronic pain management. Thus, the effect of betaine on locomotor activity in open field test and on motor coordination in Rota rod test was analyzed. The result of open field test showed that the betaine and gabapentin does not affect the total distance travelled and average speed of rats as compared to the vehicle treated rats. Whereas morphine treatment significant decreased the average speed and distance travelled by rats in open field test **(Figure 6.10 A).** Further, the rota rod test revealed that betaine and gabapentin treated rats did not exhibit motor incoordination as suggested by the latency to fall off the rod. However, morphine treated rats spent significantly less time on rod as compared to the vehicle treated rats **(Figure 6.10 B-C)** These results indicate that betaine does not produce CNS side effects like morphine in nerve injured rats. Thus, betaine has a broad safety profile as compared to the morphine like drugs.

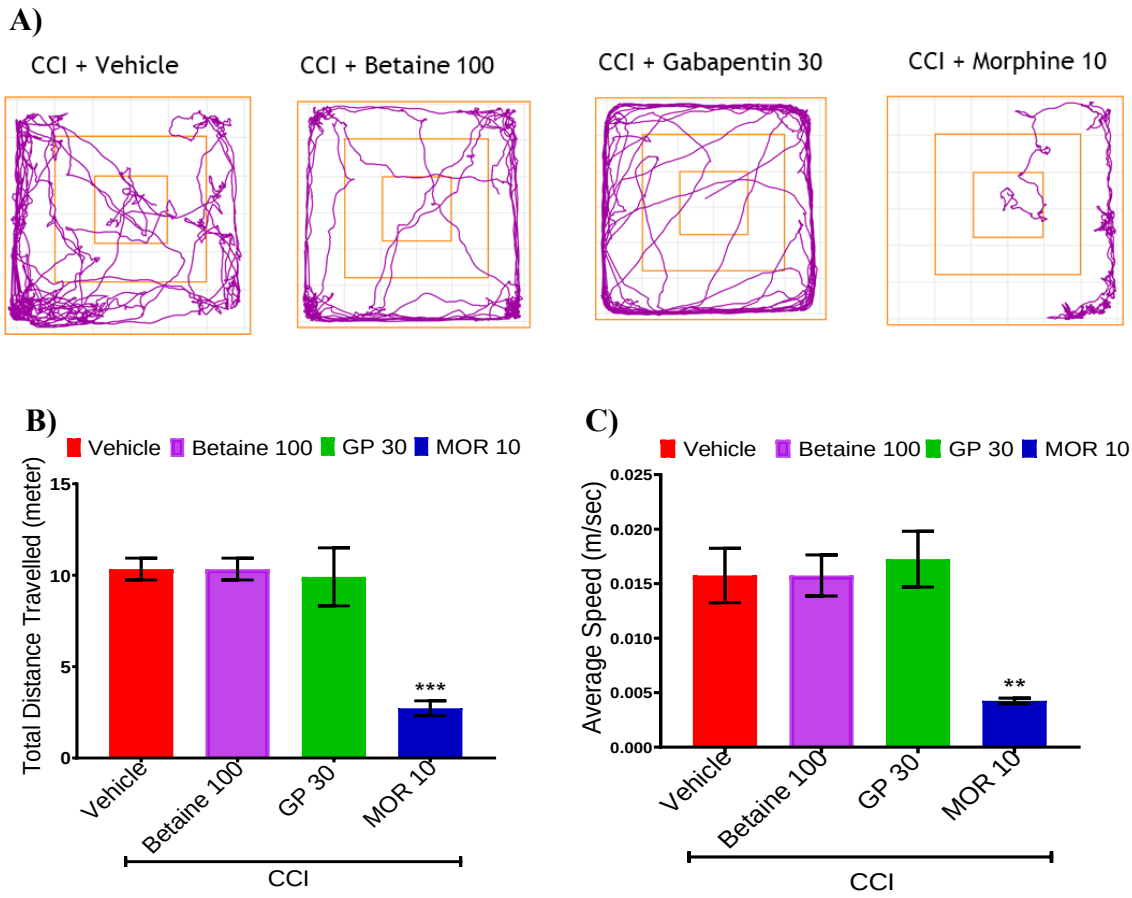


Figure 6.10. Effect of betaine on locomotor activity of nerve injured rats. **A)** Track images **B)** Total distance travelled **C)** Average speed. Data were expressed as Mean ± SEM and analyzed by two-way ANOVA (Bonferroni's multiple comparison) (n=8). ### represents significance compared to the control group * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) represents significance compared to the CCI group. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

6.3.2.7 Nerve injury induced oxidative stress in sciatic nerve is restored by betaine treatment Oxidative stress imbalance is commonly associated with any physical insult or injury. Biochemical assays to evaluate the levels the antioxidant enzyme GSH and SOD as well as the oxidative markers MDA and nitrite to assess the extent of oxidative stress were performed. An increase in the levels of MDA and nitrite was observed in the sciatic nerve of the nerve injured rats which was found to be attenuated by the treatment with betaine and the standard drug gabapentin **(Figure 6.11 A-B).** Furthermore, the levels of anti-oxidant enzymes GSH and SOD was observed to be

significantly reduced in the nerve injured rats which was found to be restored by the betaine and gabapentin treatment **(Figure 6.11 C-D).** These results indicate the antioxidant effect of betaine and gabapentin which could contribute to the analgesic effect of both compounds.

6.3.2.8 Betaine attenuates pro-inflammatory cytokines in DRG & spinal cord of nerve injured rats The inflammatory drive from the peripheral nervous system to the central, activates various transcription factors and pronociceptive genes that are essentially required for the neuropathic pain development and maintenance. One-way ANOVA followed by Tukey's multiple comparison test indicated a significant effect on IL1β and TNF-α mRNA expressions on the ipsilateral L4-L5, DRG [$F(6, 12) = 6.98$; $p<0.01$ and $F(5, 18) = 10.2$; $P<0.001$ respectively] and spinal cord [$F(5, 18) = 28.6$; $P<0.001$ and $F(5, 12) = 6.98$; $p<0.01$ respectively] of vehicle and drug-treated nerve-injured rats. Betaine at 25, 50, 100 mg/kg and standard drug gabapentin significantly inhibited overexpression of TNF-α and IL1β in the DRG as well as spinal cord of nerve injured rats **(Figure 6.11 A1- A2 & B1-B2).** A significant increase in mRNA expression of CGRP and substance P which are released as a result of tissue injury, in the DRG and spinal cord tissues as compared to the naïve rats was observed. Treatment with betaine (25, 50 and 100 mg/kg *i.p.*) significantly reduced the nerve injury induced elevated expressions of CGRP and substance P in DRG and spinal cord of rats as compared to the vehicle treatment. One-way ANOVA followed by Tukey's multiple comparison demonstrated a significant effect across the groups on DRG tissues and spinal cord CGRP [$F(6, 18) = 8.66$; $p<0.001$ and $F(8, 18) = 9.64$; $p<0.001$ respectively], Substance P [$F(5, 18) = 11.1$: $p<0.001$ and $F(5, 18) = 18.4$; $p<0.001$

respectively] levels in vehicle and drug treated nerve injured rats **(Figure 6.11 A3-A4 & B3- B4)**

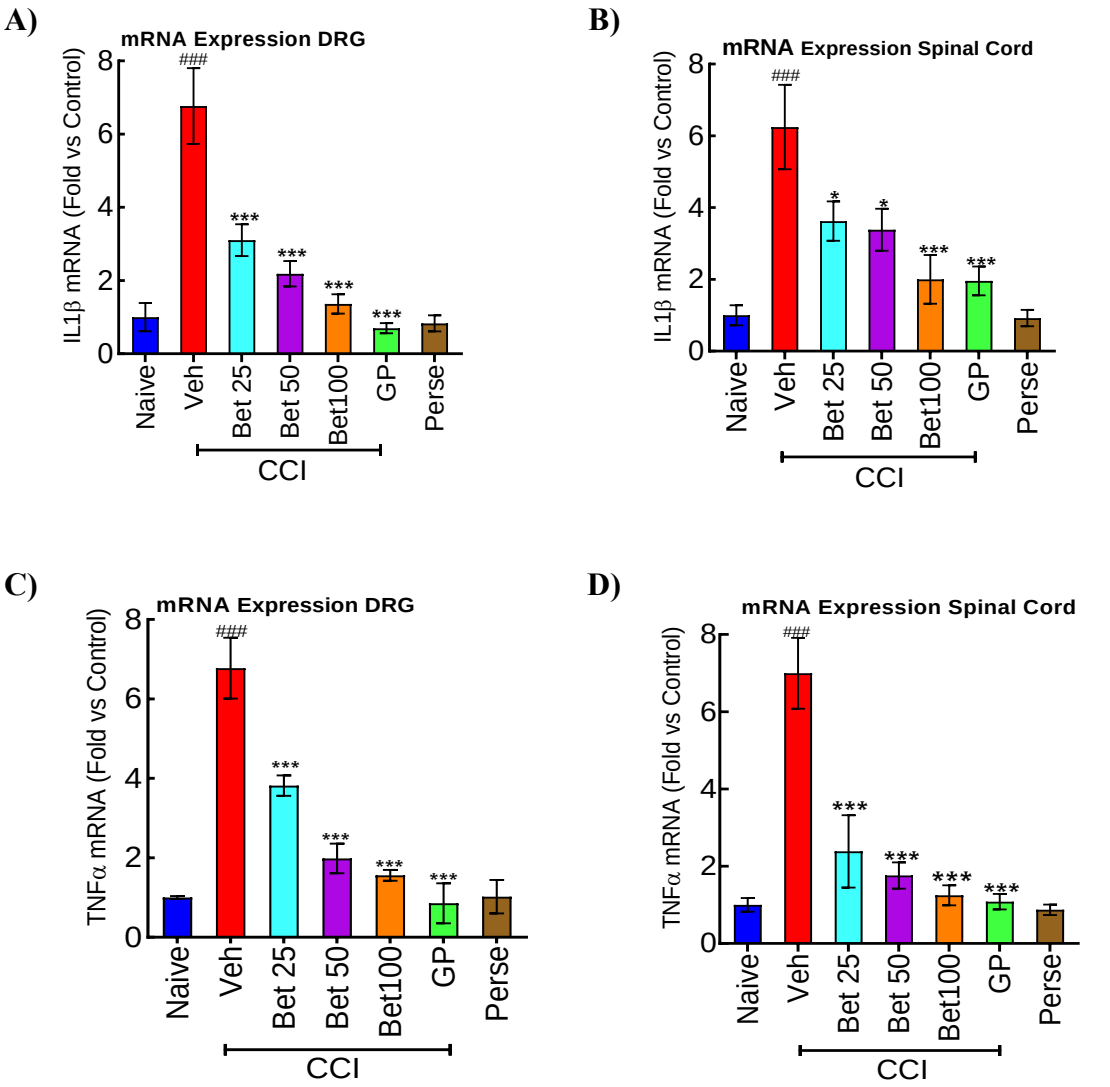


Figure 6.11. Effect of betaine on pro- inflammatory cytokines in CCI rats mRNA expression of IL1 β in DRG & spinal cord **(A and B)** and TNFα **(C and D)** in nerve injured rats. Data were presented as mean ± SEM. ###P<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***P<0.001 indicates statistical significance as compared to the CCI rats. RT-PCR n=4 biological and n=3 technical replicates were used. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

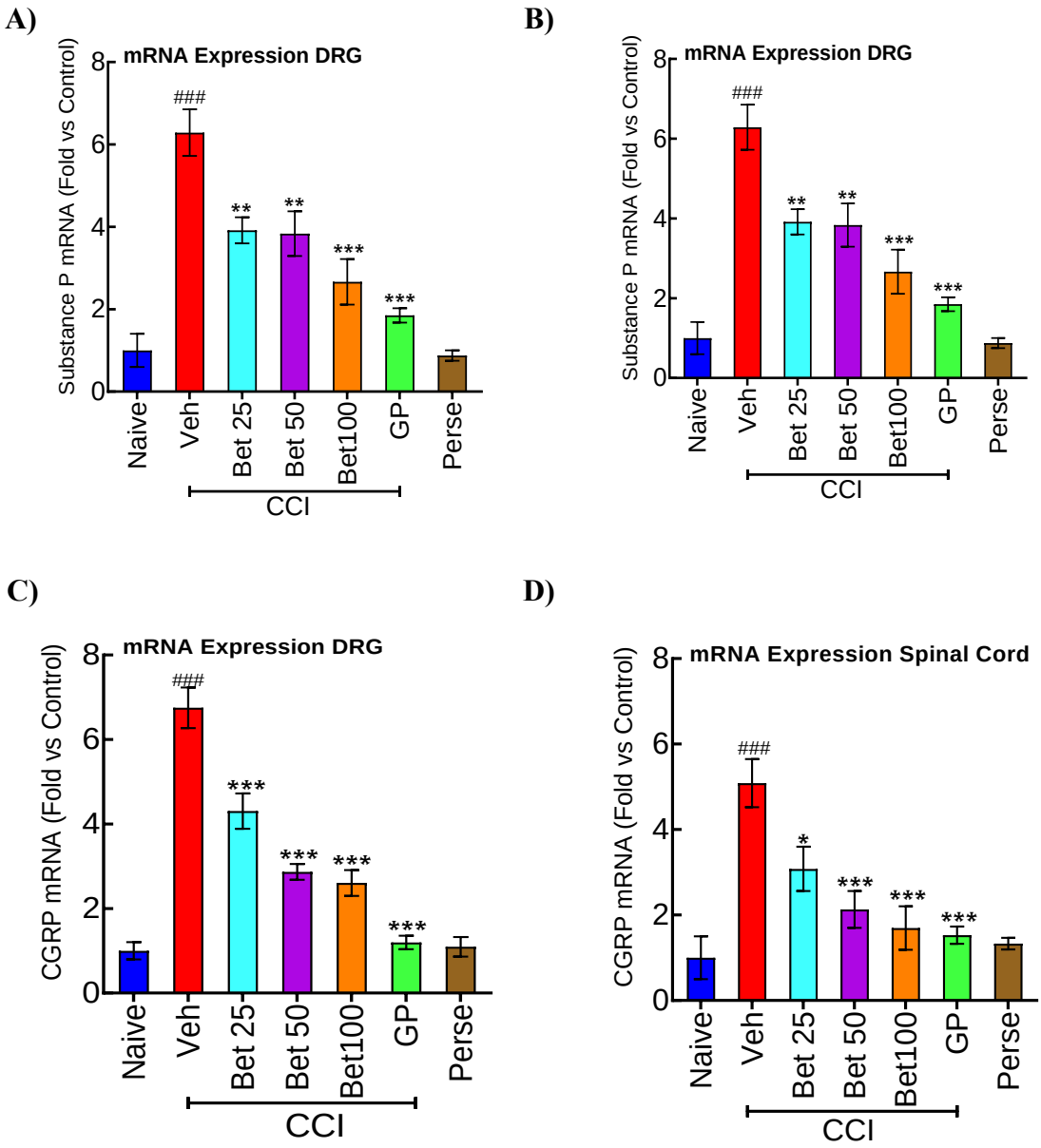


Figure 6.12. Effect of betaine on neuropeptides in CCI rats. mRNA expression of Substance P **(A and B)** and CGRP **(C and D)** in DRG & spinal cord of nerve injured rats. Data were presented as mean ± SEM. ###P<0.001 indicates statistical significance as compared to the Naïve rats. *p<0.05, **p<0.01, and ***P<0.001 indicates statistical significance as compared to the CCI rats. RT-PCR n=4 biological and n=3 technical replicates were used. Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg

6.3.2.9 Betaine treatment alleviates nerve injury-induced KIF17 and NR2B expression in DRG and spinal cord of rats

KIF17 and NR2B interplay has been recently studied in chronic pain condition and it has been demonstrated that the involvement of the same is crucial for the development and of pain progression. Moreover, a recent study has documented that betaine can interfere with kinesin mediated mechanisms under chronic pain condition. Thus, the effect of betaine on KIF-17 and NR2B interaction in CCI rats was investigated. It was observed that nerve injury led to the increases in KIF-17 and NR2B expression in DRG and spinal cord of nerve injured rats as compared to the healthy control rats. Treatment with betaine significantly decreased the protein expression of KIF17 and NR2B in both DRG and spinal cord of nerve injured rats as compared to the vehicle treated rats (Figure 6.12 A1-A2 & B1-B2). Gabapentin had no effect on the KIF17 expression whereas it significantly decreased the NR2B expression in DRG and spinal cord. These results indicate that betaine produce analgesia via different mechanism which is dependent on KIF-17 signaling.

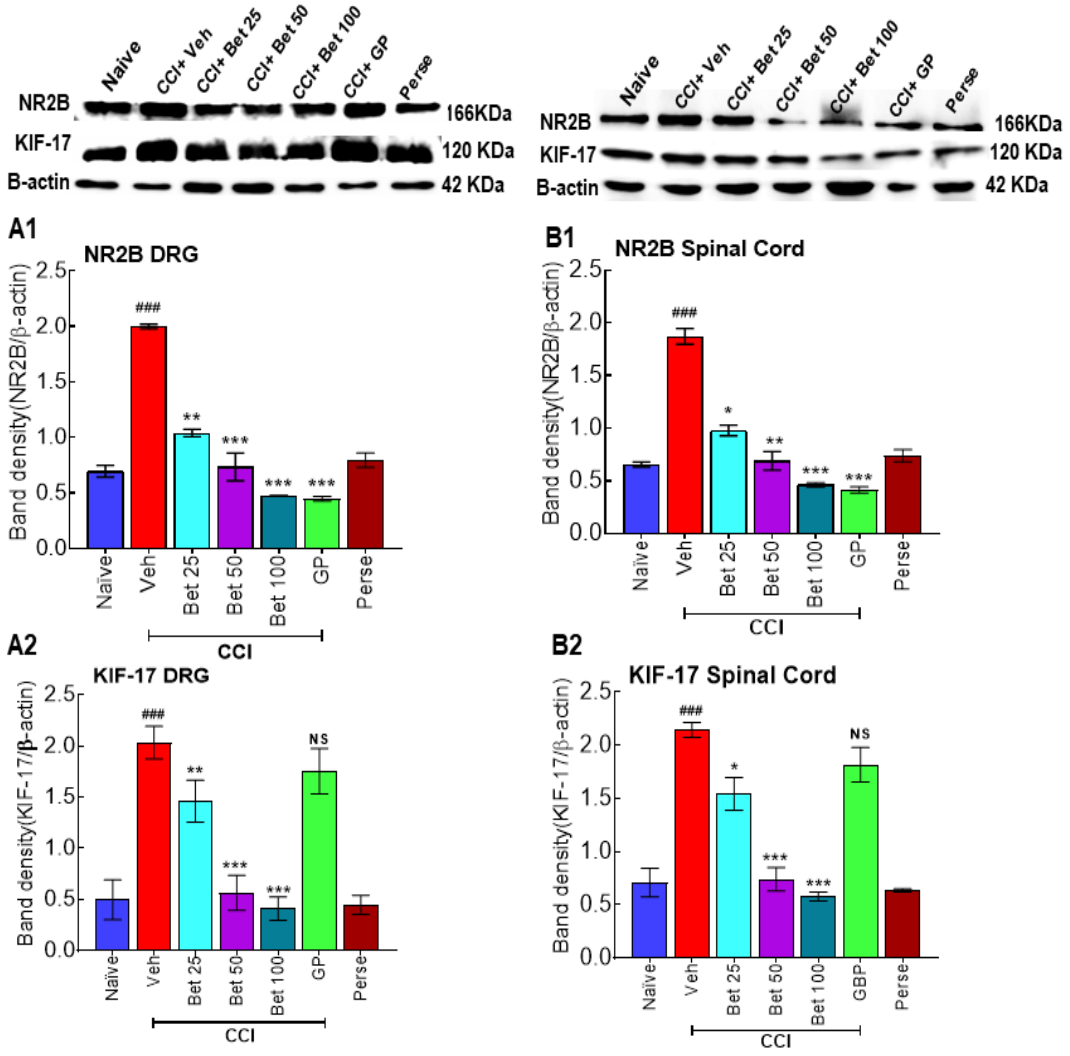


Figure 6.13. Effect of betaine on NR2B & KIF-17 expressions in nerve injured rats. Betaine significantly decreased protein and mRNA expression of NR2B and KIF-17 in the DRG (A1 & A2) and spinal cord (B1 &B2) of CCI rats. Data were presented as mean ± SEM. ##P<0.01 and ###P<0.001 indicates statistical 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. For western blot n=3 was used whereas for RT-PCR n=4 biological and n=3 technical replicates were used. Doses: Betaine dose Bet25: 25mg/kg, Bet50: 50mg/kg and Bet100: 100 mg/kg, GP30; Gabapentin 30 mg/kg.

6.4 Outcomes

Betaine inhibits evoked pain behaviour in nerve injured rats. Further, it was observed that betaine inhibits spontaneous ongoing pain in CCI rats but did not increase CPP in naïve rats unlike morphine, pointing toward its non-addictive potential. Further, the neuroinflammatory markers including TNFα, IL1β, substance P and CGRP were significantly suppressed by betaine in nerve injured rats. Betaine decreases the nerve injury induced expression of NR2B and KIF-17 in DRG and spinal cord regions of neuropathic rats. More importantly, betaine did not show any CNS-associated side effects unlike morphine as measured using open field test