Chapter-7

Summary & Conclusions

7.1 Summary

Neuropathic pain is characterized by spontaneous pain, abnormal hypersensitivity to stimuli (hyperalgesia) and nociceptive responses to non-noxious stimuli (allodynia). Pathophysiology of neuropathic pain is complex and heterogeneous factors as viral infections, autoimmune diseases metabolic disorders regulate the central or peripheral nervous system afflicting the same (Sommer et al. 2018). Origin of neuropathic pain may be either central or peripheral. Central neuropathic pain is a result of lesion or disease in brain or spinal cord, while peripheral neuropathic pain involves pathophysiological alterations in sensory fibers (C, Aβ, and Aδ) (Colloca et al. 2017b; Chen et al. 2018). Any kind of lesion or inflammation as a result of injury or infection, leads to sensitization of peripheral nociceptors which is accompanied by spontaneous discharge resulting in ongoing pain. The treatment available for the neuropathic pain is inadequate and produces several side effects. Thus, there is an unmet pharmacological need to address the management of neuropathic pain. Betaine (25, 50, and 100 mg/kg) significantly inhibits the evoked pain (mechanical and thermal) in nerve-injured rats. The analgesic effect of betaine was found to be sustained from 1 hr to 2hr and a fall in the effect was observed at 4 hr post-administration. Pain is aversive in nature and studying the ongoing component of chronic pain is of most importance in terms of developing the effective management of the disorder. CPP was performed to assess the spontaneous ongoing pain and to test different compounds against the same. Betaine and standard drug gabapentin inhibits ongoing pain without producing addiction like

morphine. Currently available pharmacotherapeutics for the management of chronic pain is coupled with the CNS toxicity which creates a substantial barrier to their clinical utility. Motor impairments are most common associated side effects with the use of analgesics thus betaine was also examined also for CNS toxicity in open field and rota trod test. The present study suggests that betaine does not exhibit the CNS toxicities associated with locomotor function and motor-incoordination of rats. *Sida cordifolia* root extract SCE, its aqueous fraction SAF and Betaine major bioactive component demonstrate explicit anti-inflammatory potential and manifest significant reduction in production of inflammatory cytokines (TNF-α, IL-1β, and IL-6) in our study. Similar kind of results are reported for betaine as potent anti-inflammatory in both animals and humans(Zhao et al. 2018), TLR-4, IL-23, ICAM-1, COX-2 (Xia et al. 2018) and antioxidant (Hassanpour et al. 2020), capabilities which are regulated by different signalling pathways at molecular and cellular level. Similar results were obtained in *chdh* (a gene for betaine synthesis) knock out mice where betaine treatment in methamphetamine induced behavioural sensitization, successfully retrieve proinflammatory and oxidative stress conditions(Okuma et al. 2004; Alvarez et al. 2011). Our results are in line with these earlier published reports and the present work demonstrate anti-inflammatory activity of betaine in DRG and spinal cord of nerve injured rats. Oxidative stress cascades get aggravated in chronic pain condition and promotes the pathophysiology of disease. Here, in present study it was observed that nerve injury induced oxidative stress was mitigated with betaine treatment. This indicated the strong multifarious effect of betaine treatment over the nerve injury pain model and its potential to be further explored as an analgesic. The TRPV1 channel mediated sensitization and upregulation of protein expression during the development

of neuropathic pain (Christoph et al. 2008; Li et al. 2017; Cernit et al. 2020). Our results are line with the previous reports and the present finding revealed that the upregulation of TRPV1 expression was found to be significantly increased in nerve injured rats which was attenuated by betaine at dose dependent manner. Kinesins are the motor protein involved in transport of various mRNA, protein and organelles in anterograde direction across the neuron. KIF17 is a member of kinesin superfamily that transport the NR2B subunit from the cytoplasm to dendritic endings, making the NMDARs functional (Lewis et al. 2018; Uniyal et al. 2021a). Recent studies have demonstrated that targeting KIF17 and NR2B cargo expression and transport could provide an alternate approach to target NMDA receptors activity. Our study has suggested that betaine treatment downregulate the KIF17 and NR2B protein expression in DRG and spinal cord tissues. This could be one of the key mechanisms by which betaine exerts its analgesics and anti-inflammatory effect. An extended firing of c-fiber nociceptors cause release in excitatory neurotransmitter glutamate, resulting in the activation of NMDA receptors in spinal cord ultimately leading to central sensitization (Raja et al. 2019; Zhang et al. 2020b; Bravo et al. 2022). Activation of NMDA receptors at peripheral level are reported to display increased sensitivity to painful stimulus and exert a significant effect on neuropathic pain behavior at molecular level. Studies suggest NR2B subunit execute a major contribution towards initiation and maintenance of neuropathic pain during the early phases of injury followed by establishing a long lasting enhanced spinal excitability (Liu et al. 2008; Zhuo 2009; Uniyal et al. 2021b). But before NR2B subunit to exert its effect, on DRG or central neurons, it is required for freshly processed subunit to be trafficked into the extra synaptic dendrites and get localized in to synapse to activate NMDAR and this transport of cargo is carried by

various kinesin proteins(Yoshihara et al. 2021; Uniyal et al. 2022b). Hence, betaine induce indirect inhibition of NMDAR regulated by KIF-17 mediated suppression of NR2B in CCI induced neuropathic pain in rat model.

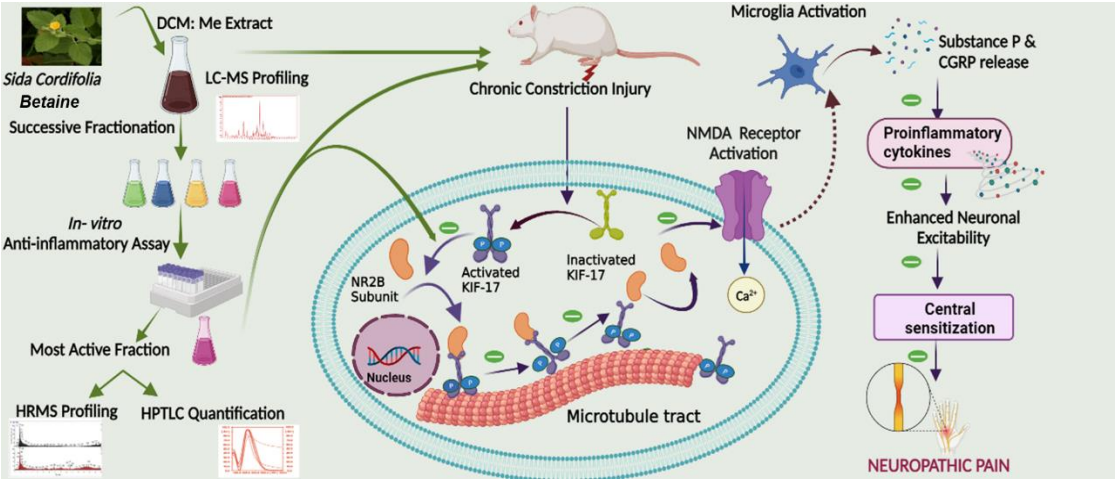

Figure 7.1 Overall Summary

7.2 Conclusion

Betaine treatment significantly attenuates evoked & spontaneous ongoing pain in nerve injured rats by inhibiting KIF-17 mediated NR2B trafficking & oxido-inflammatory signaling in DRG & spinal cord of neuropathic rats. Moreover, betaine treatment did not cause locomotor impairment & drug addiction in rats, unlike morphine. Betaine may serve as an alternative/adjuvant therapy in patients suffering from neuropathic pain.