Chapter-2

Rationale, Objectives and Plan of Work

2.1. Rationale

Pain is a subjective experience and is associated with complex somatic mechanisms and psychological influences. On the basis of mechanism pain can be grouped as nociceptive, inflammatory, or neuropathic (NP). The International Association for the Study of Pain (IASP) defines NP as "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" (Raja et al. 2020). Any lesion or disease of somatosensory system, that involves lesions in peripheral fibers and central neurons may result in wide variety of complex chronic pain syndromes. Epidemiological studies have shown that out of 20 to 25% of individuals with chronic pain may present with underlying neuropathic pain conditions accounting for 7 to 10% of neuropathic pain prevalence in general population (Bouhassira 2019; Xiong et al. 2021) Prevalence of neuropathic pain and associated comorbidities is supposed to raise higher with, increased incidences of diabetes, cancer survival and other viral and chronic diseases and ageing (Calvo et al. 2019). Severity of neuropathic pain on individual can be understated as it has been reported as "worse than death" and poor outcome and side effects of treatment available hits harder (Rojas Cabrera et al. 2020). Looking at the current scenario it is the pressing priority to find an alternate therapy that along with treating the neuropathic pain conditions improves the overall wellbeing of individuals with lesser side effects. Plant-derived medicines, as a paradigm of proactive medicine, exert fewer side effects, and a few patients have

used or expressed interest in their usage to prevent diseases and/or to improve the quality of life; additionally, such medicines have attracted the attention of scientists. *Sida cordifolia* is considered a valuable herb found in the Ayurveda system of Indian traditional medicine for its anti-inflammatory, anti-rheumatic and antipyretic potential (Patel et al. 2022). There are several mentions where roots were traditionally used for treating neuropathic pain conditions as neuralgia, and sciatica. However, so far, the possible effect of *Sida cordifolia* roots on neuropathic pain has not been investigated. The aim of this study was to evaluate the anti-allodynic potential of root extract of *Sida cordifolia* in chronic constriction induced neuropathy in rat model. Furthermore, the bioactive fraction from the entire root extract was intended to be singled out, along with the likely identification of phytoconstituents responsible for the therapeutic potential, and an investigation into the feasible mechanism of action involved will also be conducted. Involvement of proteins in alleviating the neuropathic pain, consequently responsible for its mechanism of action were investigated in DRG and spinal cord, with the help of RT-PCR and western blotting.

2.2 Objective

The aim of this study was to evaluate the anti-allodynic potential of root extract of *Sida cordifolia* in chronic constriction induced neuropathy in rat model. Involvement of proteins in alleviating the neuropathic pain, consequently responsible for its mechanism of action were investigated in DRG and spinal cord, with the help of RT-PCR and western blotting

Aim 1. Phytochemical & Pharmacological Investigations of *Sida cordifolia* Root Extract on Nerve Injury-induced Chronic Pain

Aim 1A. Collection, identification, authentication and extraction of *Sida cordifolia* roots.

Aim 1B. To perform phytochemical investigations on *Sida cordifolia* root extract.

Aim 1C. To evaluate the anti-nociceptive effect of *Sida cordifolia* root extract and molecular investigations in nerve injured rats.

Aim 2. Bioactivity Guided Fractionation of *Sida cordifolia* Root Extract: In-vitro and in-vivo Investigations further dissecting the role of KIF17/ NMDA crosstalk

Aim 2A. To perform bioactivity guided fractionation of *Sida cordifolia* roots extract (SCE)

Aim 2B To investigate the anti-nociceptive effect and mechanism of action of chosen fraction in chronic constriction injury (CCI) model of neuropathic pain in rats

Aim 3. To Investigate the Efficacy and Mechanism of Action of Betaine in Animal Model of Neuropathic Pain

Aim 3A. To study the effect of Betaine in battery of behavioral hyper-responsiveness assays in CCI model of neuropathic pain

Aim 3B. To study the effect of nerve injury on KIF17-NR2B crosstalk in DRG & spinal cord of nerve injured rats and its modulation by Betaine

2.3 Plan of work

2.3.1 Study I

Root of *Sida cordifolia* were investigated for its pain-relieving potential. Phytochemical investigation on DCM: Methanol extract (SCE) was performed along with LC-MS analysis pinpointing the bioactive players responsible for its therapeutic efficacy. Next, employing nerve-injured rat model, mimicking the chronic pain battlefield, *Sida cordifolia* whole extract, in varying concentrations, was evaluated. Its ability to attenuate the evoked (mechanical and thermal) and spontaneous pain was assessed. To delve deeper, seeking molecular insights. The efficacy of the extract to hamper the inflammatory and oxido-nitrosative stress within the sciatic nerve, the sensory relay station (dorsal root ganglion), and the pain command center (lumbar spinal cord) was analysed. Besides, key pain messengers: Substance P, CGRP, and NR2B mRNA expression within the DRG and spinal cord, uncovering its potential to modulate pain transmission and central sensitization was also studied. By integrating phytochemical analysis, behavioral assays, and molecular interrogation, this study depict the pain-relieving capabilities of *Sida cordifolia*. The animal grouping was designed as below:

Table 2.1 Animal grouping to investigate the effect DCM:Me extract of *Sida cordifolia* (SCE) on evoked and ongoing pain behavior in nerve-injured rats.

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	6
2.	Nerve injury + Vehicle	6
3.	Nerve injury + SCE 200 mg/kg p.o.	6
4.	Nerve injury + SCE 400 mg/kg p.o	6
5.	Nerve injury + SCE 800 mg/kg p.o	6
6.	Nerve injury + Gabapentin 30mg/kg i.p.	6

2.3.2 Study II

In this study. Bioactivity guided fractionation of the whole extract of *Sida cordifolia* root extract was performed. Different fraction achieved were investigated using vitro anti-inflammatory tests, unveiling the most potent fraction. Most potent fraction was than analyzed for its, pain-relieving potential in CCI (Chronic constriction injury) rat model. Subsequently, the receptor interactions, as well as the pathway modulations by most potent fraction of *Sida cordifolia* was also investigated.Further major phytoconstituents present in the aqueous fraction was identified using HRMS profiling and the same was quantified in the extract using HPTLC.

The grouping for the study was as follows:

Table 2.2 Animal grouping to investigate the effect of aqueous fraction of *Sida cordifolia* (SAF) on acute inflammatory model of pain in rats

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	6
2.	Nerve injury + Vehicle	6
3.	Nerve injury + SAF 200 mg/kg p.o.	6
4.	Nerve injury + SAF 400 mg/kg p.o	6
5.	Nerve injury + SAF 800 mg/kg p.o	6
6.	Nerve injury + Gabapentin 30mg/kg i.p.	6

2.2.3 Study III

Further, the architectural interplay between betaine a major phytoconstituents in aqueous fraction and KIF-17 using molecular dynamics simulation was investigated. Next, the effect of Betaine on evoked (mechanical and thermal) and spontaneous

ongoing pain behavior in nerve-injured rats was observed. Besides, the role of Betaine in modulating KIF17-NR2B and inflammatory axis in nerve injury-induced neuropathic pain explored. CNS associated side effects are the major limitation of currently available analgesics in clinic therefore the effect of betaine on motor coordination and locomotion activity was also observed. The animal grouping was designed as below:

Table 2.3 Animal grouping to investigate the effect Betaine on evoked and ongoing pain behavior in nerve-injured rats.

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	6
2.	Nerve injury + Vehicle	6
3.	Nerve injury + Betaine 25 mg/kg i.p.	6
4.	Nerve injury + Betaine 50 mg/kg i.p.	6
5.	Nerve injury + Betaine 100 mg/kg i.p.	6
6.	Nerve injury + Gabapentin 30mg/kg i.p.	6

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