

CONTENTS

Title	Page No.
Certificate	iii
Declaration by the Candidate	iv
Copyright Transfer Certificate	v
Acknowledgements	vi-vii
Contents	viii-xiii
List of Figures	xiv-xv
List of Tables	xvi
List of Abbreviations	xvii-xviii
Preface	xix-xxi
CHAPTER 1: Introduction and Literature Review	1-40
1.1 Introduction	1
1.2 Prevalence and Impact of Chronic Pain	2
1.3 Etiology and Pathophysiology of Neuropathic Pain	3
1.3.1 Diabetes-induced Neuropathy	3
1.3.2 Cancer -induced Neuropathy	4
1.3.3 COVID a cause for Neuropathy	5
1.4 Pain Process	6
1.4.1 Anatomy of Pain	6
1.4.2 Pathophysiology of Pain	8
1.4.2.1 Chronic pain, central sensitization, centralized pain	8
1.4.2.2 Peripheral sensitization	9
1.4.2.3 Central sensitization	11
1.5 Pain Pathway	13
1.5.1 Ascending pain pathway	13
1.5.2 Brain regions involved in the experience of pain	15
1.5.3 Descending pain pathway	17
1.5.4 N-methyl-D-aspartate (NMDA) Receptors	17
1.6 Kinesin Proteins: Their Structure, and Function, and Role in Pain Modulation	19
1.6.1 KIF17: A Key Mediator in NR2B Subunit Trafficking and pain modulation	22

1.7 Pharmacotherapeutics for Chronic Pain: Efficacy and Constraints	23
1.7.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)	24
1.7.2 Antiepileptic	25
1.7.3 Antidepressants	26
1.7.4 Opioids	27
1.7.5 NMDA antagonists	28
1.7.6 Topical Medication	29
1.7.7 Emerging Therapies	29
1.7.8 Alternative Pharmacological Interventions for chronic pain management	29
1.7.9 Global Prevalence of Herbal Medicine used for Chronic Pain	30
1.7.10 Plants Used for Chronic Pain Management: List of plants used for pain management	32
1.8 <i>Sida cordifolia</i> Linn. for neuropathic pain management	33
1.9 <i>Sida cordifolia</i> Linn.: A traditional herb for management of neuropathic pain	34
1.9.1 Scientific Classification of <i>Sida cordifolia</i>	34
1.9.2 Traditional medicinal uses of <i>Sida cordifolia</i>	35
1.9.3 Phytochemistry of <i>Sida cordifolia</i>	36
1.9.4 Marketed formulations of <i>Sida cordifolia</i>	37
1.9.5 Preclinical studies on <i>Sida cordifolia</i>	38
CHAPTER 2: Rationale, Objectives and Plan of Work	41-46
2.1 Rationale	41
2.2 Objectives	42
2.3 Plan of work	44
2.3.1 Study I	44
2.3.2 Study II	45
2.2.3 Study III	45
CHAPTER 3: Materials & Methods	47-72
3.1 Drugs, chemicals and antibodies	47
3.2 Equipment and software	49
3.3 Plant material and preparation of extract of roots of <i>Sida cordifolia</i>	51
3.3.1 Collection, identification and authentication of <i>Sida cordifolia</i> L. Roots	51

3.3.2 Preparation of Extract Collected roots	51
3.4 Bioactivity guided fractionation	52
3.5 Phytochemical analysis	52
3.5.1. Flavonoid Content Estimation	52
3.5.2 Phenolic Content Estimation	53
3.5.3 Assessment of Antioxidant activity:	53
3.5.4 Characterization of crude extract of <i>Sida cordifolia</i> (SCE) and its aqueous fraction (SAF) by LC-MS/TOF and HRAMS respectively	54
3.6 Assessment of in-vitro anti-inflammatory activity by egg albumin protein denaturation assay	54
3.7 High performance thin layer chromatography	55
3.8 In-silico studies	56
3.8.1 Homology Modeling NCBI Database and Sequence Retrieval	56
3.8.2 Structure Prediction and Homology Modeling of KIF-17	57
3.8.3 Structure-based virtual screening	58
3.8.4 Molecular dynamics simulation	58
3.9 In-vivo studies	59
3.9.1 Experimental animals	59
3.9.2 Ethical committee approval	60
3.9.3 Animal model of neuropathic pain and experimental design	60
3.9.4 Evoked Pain Behavior Assay	62
3.9.4.1 von-Frey hair test: Static allodynia	62
3.9.4.2 Cotton swab test: Dynamic mechanical test	63
3.9.4.3 Assessment of Thermal hyperalgesia	63
3.9.4.4 Cold plate test	64
3.9.4.5 Acetone evaporation test	64
3.9.4.6 Acute nociceptive stimuli: Pinprick test	65
3.9.5 Conditioned place preference test	65
3.9.6 Behavioral neurotoxicity assays	65
3.9.6.1 Rota-rod test	66
3.9.6.2 Open Field Test	66
3.9.7 Tissue harvesting and storage	66
3.9.8 Biochemical assays	66

3.9.8.1	Estimations of lipid peroxidation (LPO)	67
3.9.8.2	Determination of reduced glutathione (GSH)	67
3.9.8.3	Quantification of superoxide dismutase (SOD)	68
3.9.8.4	Quantification of Nitrite	68
3.9.9	Molecular biology studies	69
3.9.9.1	Reverse transcription polymerase chain reaction (RT-PCR)	69
3.9.9.2	Western blotting	69
3.10	High performance thin layer chromatography HPTLC Profiling	71
3.11	Statistical analysis	72
	CHAPTER 4: Phytochemical & Pharmacological Investigations of <i>Sida cordifolia</i> Root Extract (SCE) on Nerve Injury-induced Chronic Pain	73-88
4.1	Introduction	73
4.2	Experimental procedure	75
4.3	Results and discussion	77
4.3.1	Phytochemical analysis	77
4.3.1.1	Total Phenolic and flavonoid content of SCE	77
4.3.1.2	Antioxidant assay of SCE	77
4.3.1.3	LC-MS/TOF analysis of SCE	78
4.3.2	In-vivo evaluation of <i>Sida cordifolia</i> root crude extract (SCE)	79
4.3.2.1	Anti-nociceptive activity of <i>Sida cordifolia</i> crude extract (SCE) in CCI-induced neuropathic pain model	79
4.3.3	SCE restored oxido-nitrosative stress markers in sciatic nerve of nerve injured rats	82
4.3.4	Effect of SCE on mRNA expression of pro inflammatory cytokines and neuropeptides in DRG and spinal cord of nerve injured rats	83
4.3.5	<i>Sida cordifolia</i> root extract (SCE) Downregulates NR2B mRNA Expressions in DRG & Spinal Tissues of Neuropathic Rats	86
4.4	Outcomes	87
	CHAPTER 5: Bioactivity Guided Fractionation of <i>Sida cordifolia</i> Root Extract: In-vitro and in-vivo Investigations	89-106
5.1	Introduction	89
5.2	Experimental design	89

5.3 Results and discussion	91
5.3.1 In- vitro anti- inflammatory activity of successive fractions of crude extract	91
5.3.2 In- vivo activity of SAF in CCI induced neuropathic pain model in rats	92
5.3.2.1 SAF attenuates thermal hyperalgesia in nerve injured rats	92
5.3.2.2 SAF inhibits mechanical allodynia in nerve-injured rats	94
5.3.2.3 <i>Sida cordifolia</i> aqueous fraction (SAF) restored biochemical alterations in sciatic nerve of injured rats	95
5.3.2.4 SAF suppressed mRNA protein expressions of pro inflammatory cytokines and neuropeptides in DRG of neuropathic rats	98
5.3.2.5 SAF suppressed nerve injury induced glia cell activation and neuro-inflammation spinal cord of CCI rats	100
5.3.2.6 <i>Sida cordifolia</i> Aqueous Fraction (SAF) Downregulates NR2B mRNA & Protein Expressions in DRG and Spinal Cord of Neuropathic Rats	102
5.3.3 HR-MS analysis of SAF	103
5.3.4 HPTLC Quantification of Betaine in <i>Sida cordifolia</i> aqueous fraction (SAF)	104
5.4 Outcomes	105
CHAPTER 6: Modulation of KIF-17/NR2B Crosstalk by betaine in Inflammatory Pain Rat Model	107-129
6.1 Introduction	107
6.2 Experimental design	107
6.3 Results and discussion	108
6.3.1 In-silico studies	108
6.3.1.1 Homology modeling	108
6.3.1.2 Molecular Docking	110
6.3.1.3 Molecular dynamics simulation	111
6.3.2 In-vivo studies,	114
6.3.2.1 Betaine Attenuates CCI-induced Thermal Hyperalgesia and Mechanical Allodynia After Nerve Injury	114

6.3.2.2	Betaine Attenuates CCI-induced Cold Hypersensitivity in Nerve Injured Rats	116
6.3.2.3	Betaine Attenuates CCI-induced Mechanical Hyperalgesia in Nerve Injured Rats (Pin prick test)	117
6.3.2.4	Effect of Betaine on Dynamic Allodynia in Nerve Injured	118
6.3.2.5	Betaine suppressed spontaneous ongoing pain in nerve injured rats without addiction	119
6.3.2.6	Betaine does not produce CNS side effects in nerve injured rats	122
6.3.2.7	Nerve injury induced oxidative stress in sciatic nerve is restored by betaine treatment	123
6.3.2.8	Betaine Attenuates Pro-Inflammatory Cytokines in DRG & Spinal Cord of Nerve Injured Rats	124
6.3.2.9	Betaine treatment alleviates nerve injury-induced KIF17 and NR2B expression in DRG and spinal cord of rats	127
6.4	Outcomes	129
	CHAPTER 7: Summary & Conclusions	130-133
7.1	Summary	130
7.2	Conclusion	133
	References	134-156
	List of Publications	157