DEVELOPMENT OF NOVEL THERAPEUTICS TARGETING TRANSIENT RECEPTOR POTENTIAL CHANNEL MEDIATED NOCICEPTION FOR THE TREATMENT OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

Thesis submitted in partial fulfilment for the

Award of Degree

DOCTOR OF PHILOSOPHY

By

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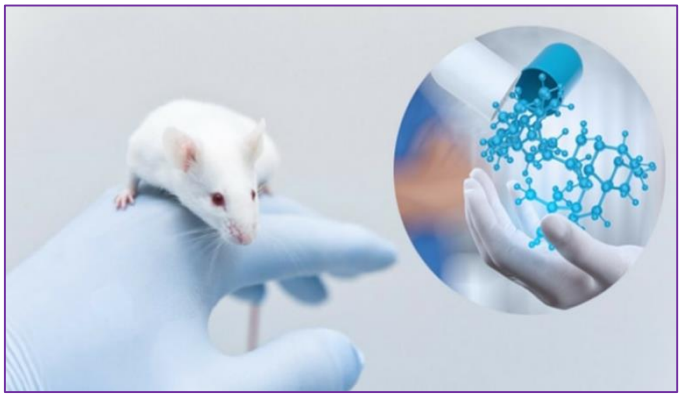
Dedicated to

"To the future generations of scientists who will continue to build upon our work, pushing the boundaries of what is known and striving for new breakthroughs in pain management"



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Date: 13.02.2024 **(Akhilesh)**

Place: Varanasi

TABLE OF CONTENTS

LIST OF FIGURE

LIST OF TABLE

LIST OF SYMBOLS AND ABBREVIATIONS

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ATP	Adenosine tri-phosphate
BDNF	Brain-derived neurotrophic factor
Ber	Bergenin
BLAST	Basic Local Alignment Search Tool
BSA	Bovine serum albumin
CaMKII	Calcium/calmodulin-dependent protein kinase II
Camp	Cyclic adenosine monophosphate
CC	Combination chemotherapy
CDK	Cyclin-dependent kinase
CGRP	Calcitonin gene-related peptide
CINP	Chemotherapy-induced neuropathic pain
CNS	Central nervous system
COX	Cyclooxygenase
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CPP	Conditioned place preference
CREB	Camp response element-binding protein
DMSO	Dimethyl sulfoxide
DRG	Dorsal root ganglion
DRG	Dorsal root ganglion
DTNB	5,5’-dithiobis (2-nitrobenzoic acid)
DTNB	5,5’-dithiobis (2-nitrobenzoic acid)
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid
ERK	Extracellular signal-related kinase
GABA	Gamma-aminobutyric acid
GP	Gabapentin
GPCR	G protein coupled receptors
GSH	Reduced glutathione
IASP	International Association for the Study of Pain
IBA1	Ionized calcium binding adaptor molecule 1
ICD	International Classification of Disease

IL-1β	Interleukin-1 Beta
IL-1β	Interleukin-1 Beta
LPO	Lipid peroxidation
LTP	Long-term potentiation
MAPK	Mitogen activated protein kinase
MD	Molecular dynamic
MDA	Malondialdehyde
NCBI	National Centre for Biotechnology Information
NF-κβ	Nuclear factor kappa β
NMDA	N-methyl-D-aspartate
NPT	Normal pressure and temperature
NR2B	N-methyl D-aspartate receptor subtype 2B
NS	Non-significant
OSM-3	Osmotic avoidance abnormal protein 3
OXL	Oxaliplatin
PDB	Protein data bank
PME	Particle Mesh Ewald
PTX	Paclitaxel
RC	Ramachandran
REPSA	Reversible reference system propagator algorithms
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TNF-α	Tumour necrosis factor-alpha
TRPA1	Transient receptor potential ankyrin 1
TRPM8	Transient receptor potential melastatin 8
TRPV1	Transient receptor potential vanilloid 1
VCAM	Vascular cell adhesion molecule
VCR	Vincristine

PREFACE

Cancer is the second leading cause of death in the world and one of the biggest threats to global public health, with reportedly 23.6 million incident cancer cases and 10.5 million cancer deaths across 204 countries and territories in 2021 (Brianna and Lee, 2023). Chemotherapy, a cornerstone in cancer treatment, has shown efficacy, but its use is accompanied by the with severe side effect of chemotherapy-induced neuropathic pain (CINP). CINP persist almost 30–90% of patients undergoing neurotoxic chemotherapy, manifesting as sensory, motor, and autonomic disorders, with significant impact on patients' quality of life. Despite several efforts made by pain scientists in preclinical studies, nearly 98% of drugs fail in clinical trials due to various factors. One major contributor to these failures is the lack of an optimal clinically representative animal model that accurately recapitulates the actual conditions associated with CINP. Therefore, there is an urgent need for novel and further development of effective therapeutic strategies for establishment of a robust animal model that accurately recapitulates the clinical condition of CINP. Based on the drug prescription record for cancer survival as well as recent literature on clinical studies, it is suggested that chemotherapeutic agents are generally prescribed in a combination of two or three drugs. Numerous studies documented that the combined chemotherapy regimen of vincristine, cisplatin and paclitaxel is the most prescribed treatment for cancers like ovarian, breast and hematological malignancies. In this context, we propose a three-step strategy to develop and validate a combination-based rat model of CINP, incorporating face validity, predictive validity, and constructive validity. Our study delves into the cellular and molecular pathways underlying CINP, with a particular focus on transient receptor potential (TRP) channels, such as TRPA1, TRPV1, and TRPM8. These channels play pivotal roles in thermal, mechanical, and cold hypersensitivity during CINP. Treatment with paclitaxel, bortezomib and cisplatin led to an increase in expression of both TRPA1/TRPV1 mediated mitogen-activated protein kinases (MAPK) pathways followed by the release of pro-inflammatory cytokines and chemokines resulting in neurogenic inflammation. Additionally, we explored the interaction between N-methyl-D-aspartate receptors (NMDARs) and TRP channels, presenting a novel approach to target NMDAR function indirectly.

Recognizing the limitations of current CINP treatments, including adverse effects and drug interactions, we shift our focus to natural phytochemicals. Bergenin, a promising compound, was investigated for its therapeutic potential against CINP. We have dissected the detailed mechanism of action of Bergenin involving modulation of TRP channels-mediated NR2B signalling in DRG and spinal cord of neuropathic rats. Virtual screening and dynamics studies were conducted to evaluate Bergenin's binding interactions and thermodynamic stability with TRPA1, TRPV1, and TRPM8.

The therapeutic potential of targeting TRPA1 in managing chemotherapy-induced peripheral neuropathy (CIPN) is widely acknowledged. However, numerous reports highlight the significant drawbacks of TRPA1 antagonists, including severe side effects such as respiratory depression, cardiovascular abnormalities, and hypersensitivity reactions. Given the debilitating nature of CIPN, it becomes imperative to explore alternative and more specific treatment approaches tailored to address these challenges. Our study introduces a ground-breaking approach to target TRPA1 nociceptors using small interfering RNA (siRNA) delivered intrathecally. Overcoming challenges in siRNA delivery into the systemic circulation, including rapid degradation, excretion, and blood-brain barrier permeability, we present a lipid-based nano-carrier delivery system. This innovative system, developed with state-of-the-art facilities, demonstrates biocompatibility, biodegradability, low toxicity, and efficient entrapment efficacy, paving the way for a potential breakthrough in CINP management through intravenous administration. In summary, our comprehensive study aims to address the scientific and clinical gaps in CINP research, offering a multifaceted approach from animal model development to novel therapeutic interventions. The potential implications of our findings extend beyond CINP, influencing the broader landscape of cancer treatment and chronic pain management. The present thesis is divided into seven chapters and a brief description is given below:

Chapter 1 is the opening chapter extensively reviews existing literature on chemotherapy-induced neuropathic pain (CINP), portraying it as a profound disorder. It delves into the motivation behind the research, providing insights into the study's background. The section elucidates definitions, terminologies, mechanisms, and constraints associated with current therapeutic approaches for CINP. A thorough

exploration of the intricate interplay of TRP channels in diverse signaling pathways integral to the neurobiology of CINP is presented. The chapter emphasizes the need for innovative animal models to better simulate the disorder's complexity, aiming to improve translational relevance and enhance the efficacy of therapeutic interventions.

Chapter 2 outlines the rationale and objectives of the research, introducing the study's hypothesis and detailing the experimental design. It articulates multiple objectives framed through a multidisciplinary, state-of-the-art approach, incorporating in-silico and in-vivo tools. The chapter serves as a concise yet thorough roadmap, highlighting the thoughtful planning and integration of diverse methodologies.

Chapter 3 providing a comprehensive account of the material and methods employed, this section offers a detailed overview of various experimental techniques, elucidating their working principles and any modifications made. The discussion encompasses *in-silico* techniques, sample size determination, CINP models, surgical procedures, tissue harvesting methods, sample processing procedures, reagent preparation and composition, as well as biochemical assays and molecular biology techniques.

Chapter 4 presents the experimental work and findings of the first study conducted to evaluate various aspects of the hypothesis. It details the development and validation of a clinically relevant, combination-based chemotherapeutic model of peripheral neuropathy using behavioral, pharmacological, and molecular tools. The three-step strategy employed demonstrates face, predictive, and constructive validities.

Chapter 5 another part of the experimental work focuses on studying the effect of Bergenin as a potential phytochemical in an animal model of chemotherapy-induced neuropathic pain. The detailed mechanism of action of Bergenin, involving modulation of TRP channels-mediated NR2B activation in DRG and spinal cord of neuropathic rats, is dissected. *In-silico* studies were performed to validate Bergenin's binding affinity with different TRP channels.

Chapter 6 summaries the development of TRPA1 siRNA-based nano-formulation for the treatment of CINP. The study evaluates the efficacy of TPRA1-siRNA liposomal formulation in animal model of CINP via intrathecal/intravenous

administration. Addressing challenges associated with siRNA delivery, a lipid-based nano-carrier delivery system is successfully engineered, demonstrating significant attributes including biocompatibility, biodegradability, low toxicity, and high entrapment efficacy.

Chapter 7 summaries and the key findings of the experimental work of the thesis and includes the discussion on the results observed in the present work and describes the advantages of new animal model over conventional monotherapy based model of CINP. It delves into detailed insights gained from the experimental work on the novel mechanisms of Bergenin and TRPA1 siRNA in their anti-nociceptive action against CINP. The chapter concludes by summarizing the overall thesis work and providing insights into the future scope of the research. Potential avenues for further investigation and application of the novel treatment strategies are outlined, emphasizing the ongoing commitment to treat CINP.