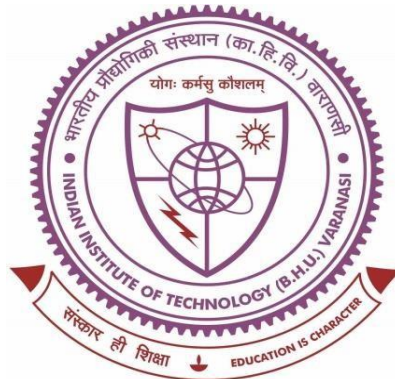


**TARGETING PERIPHERAL G-PROTEIN
COUPLED RECEPTORS FOR THE TREATMENT
OF CHEMOTHERAPY-INDUCED
NEUROPATHIC PAIN**



**Thesis submitted in partial fulfilment for the Award of
Degree**

DOCTOR OF PHILOSOPHY

By

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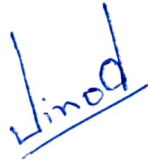
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*Dedicated to those
enduring the immense
burden of cancer and
chemotherapy-induced
neuropathic pain*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine tri-phosphate
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CBR	Cannabinoid Receptor
CCI	Chronic constriction injury
CGRP	Calcitonin gene-related peptide
CINP	Chemotherapy Induced Neuropathic Pain
CIPN	Chemotherapy-induced Peripheral Neuropathy
CNS	Central nervous system
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CPP	Conditioned place preference
DALDA	Dermorphin [d-Arg ² , Lys ⁴] (1–4) amide
DMSO	Dimethyl sulfoxide
DRG	Dorsal root ganglion
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid
GABA	Gamma-aminobutyric acid
GPCR	G protein- coupled receptors
GSH	Reduced glutathione
IASP	International Association for the Study of Pain
ICAM1	Intercellular adhesion molecule 1
LOP	Loperamide
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
MOR	Mu-opioid Receptor
NFK β	Nuclear factor kappa β
NMDA	N-methyl-D-aspartate

NS	Non-significant
PINP	Paclitaxel Induced Neuropathic Pain
PNS	Peripheral Nervous System
RIPA	Radioimmunoprecipitation Assay
SNRIs	Serotonin and Norepinephrine Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TRP	Transient Receptor Potential channels
VGCCs	Voltage Gated Calcium Channels
VGSCs	Voltage Gated Sodium Channels



PREFACE

PREFACE

Cancer is a leading cause of mortality worldwide, significantly impacting global life expectancy. In addition to the physical challenges posed by malignancy, individuals undergoing cancer treatment frequently confront the adverse impact of chemotherapy-induced neuropathic pain (CINP). This distressing consequence of cancer treatment is characterized by a myriad of symptoms, ranging from tingling and numbness to excruciating pain, significantly diminishing the quality of life for those already grappling with the immense challenges of cancer. The profound nature of CINP is highlighted by its enduring presence, persisting even after the completion of chemotherapy. The burden imposed on patients is not solely physical; psychological and emotional well-being are intricately intertwined, exacerbating the challenges faced by individuals already navigating the complexities of cancer diagnosis and treatment.

Although CINP presents significant challenges, the available therapeutic options are unfortunately quite restricted. Current treatments for neuropathic pain often come with debilitating side effects, including hepatic impairment, renal insufficiency, fatigue and central nervous system toxicities like anxiety, dizziness, sedation, respiratory depression, cognitive dysfunction, addiction, and abuse potential, compounding the challenges faced by patients. The lack of precision in targeting neuropathic pain at its source contributes to the suboptimal outcomes observed in many cancer patients.

Amidst this therapeutic void, targeting of peripheral G Protein- Coupled Receptors (GPCRs) emerges as a promising avenue for innovative interventions. Notably, the mu-opioid receptors (MORs) and cannabinoid receptors (CBRs) within

the peripheral nervous system hold significant potential for therapeutic exploitation. By focusing on these receptors at the periphery, we aim to mitigate the unwanted central side effects associated with conventional therapies. Targeting the peripheral MORs, which are key players in pain modulation, has garnered attention for providing pain relief without the unwanted cognitive and sedative side effects often observed with centrally acting opioids. Additionally, CBRs, present an intriguing prospect, as they are predominantly expressed in the peripheral nervous system and immune cells, offering a more localized and targeted approach to pain management.

Recognizing the critical need for effective therapeutics that address the specificities of CINP, this research aims to explore and elucidate the intricacies of peripheral GPCR modulation. By unraveling the complexities of MORs and CBRs within the peripheral nervous system, we aspire to develop interventions that not only alleviate the burden of pain but also do so with a precision to avoid unwanted adverse effects.

In the present work, we have performed pre-clinical investigations to evaluate the analgesic potential of peripherally restricted MOR and CBR agonists in rat model of CINP. Further, we have investigated the downstream molecular signalling resulting in mitigation of CINP. The present thesis is divided into seven chapters, the brief description of which is given below:

Chapter 1 introduces neuropathic pain as an adverse consequence experienced by cancer patients receiving chemotherapy. It discusses in detail the unique symptomology and pathophysiology of CINP. The chapter further illustrates the motivation of work and the background of the study by providing the limitations of currently available

therapeutics for the management of CINP. Additionally, a comprehensive exploration of the literature is presented, shedding light on the pivotal role played by various GPCRs in pain modulation. The mechanisms orchestrating analgesia through MORs and CBRs are intricately detailed. The literature review extends to encompass an in-depth discussion on the drugs utilized in this thesis, providing a thorough foundation for the subsequent research exploration.

Chapter 2 of this thesis is dedicated to the rationale and objectives of the work. This chapter consists of the hypothesis of the study along with experimental design. It also includes the details of different objectives that were framed using a multidisciplinary state-of-the-art approach using *in-vivo* behavioural and molecular tools.

Chapter 3 illustrates a detailed description of the material and methods used to carry out the present work. A complete overview of the different experimental techniques including the working principles and modifications performed is presented in this section. Sample size, rodent model of CINP, method of tissue harvesting, sample processing procedures, reagent preparation and composition, biochemical assays and molecular biology techniques are discussed in a detailed manner along with the source of materials and reagents used in the experimental work.

Chapter 4 presents the experimental work and findings of the first study conducted to evaluate various aspects of the hypothesis. The section starts with the development of rodent model of paclitaxel induced neuropathic pain. Here, we investigated the effect of peripheral MOR agonist on chemotherapy-induced evoked and spontaneous ongoing pain. We further evaluated if loperamide administration showed any Central Nervous System (CNS) toxicities. The section also presents the comparison of loperamide with

gabapentin and morphine activity on spontaneous ongoing pain inhibition and the addictive potential profiling of these compounds. Next, the findings from molecular studies including biochemical, RT-PCR and western blotting, are discussed in detail to elucidate the mechanism of action of loperamide.

Chapter 5 represents another part of the experimental work that has been carried out to further confirm our findings from the previous study. In this we have evaluated the effect of a preferential peripheral MOR agonist, Dermorphin [d-Arg2, Lys4] (1–4) amide (DALDA) on rat model of paclitaxel induced neuropathic pain. The study revealed the effect of DALDA on chemotherapy-induced evoked and spontaneous ongoing pain while further demonstrating its non-addictive potential. The multifaceted mechanisms underlying DALDA's efficacy in mitigating CINP are elucidated in detail.

Chapter 6 presents the experimental work and findings conducted to investigate the effect of peripheral CBR agonist, CB13 on chemotherapy-induced neuropathic pain. We further evaluated if CB13 administration showed any CNS toxicities. Next, the findings from molecular studies including biochemical, RT-PCR and western blotting, are discussed in detail to elucidate the mechanism of action of CB13.

Chapter 7 summarizes 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 peripherally restricted therapeutics targeting specific peripheral GPCRs, notably MORs and CBRs, for the management of CINP while steering clear of CNS toxicities. Finally, this chapter concludes the thesis work and illustrates the future scope of the research