Peptide biologics for effective management of breast cancer and fungal keratitis

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JULY 2025

Indian Institute of Technology Delhi (IITD), New Delhi, 2025

Peptide biologics for effective management of breast cancer and fungal keratitis

by

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Submitted

in fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2025

Guru Granth Sahib

Title:Raag Gauree, Guru Arjan Dev Ji

Page:293, Line No.:14

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salok |

ਗਿਆਨ ਅੰਜਨੁ ਗੁਰਿ ਦੀਆ ਅਗਿਆਨ ਅੰਧੇਰ ਬਿਨਾਸੁ ॥

Giaan anjan gur deea agiaan andher binaas |

ਹਰਿ ਕਿਰਪਾ ਤੇ ਸੰਤ ਭੇਟਿਆ ਨਾਨਕ ਮਨਿ ਪਰਗਾਸੁ ॥੧॥

Har kirapaa te sant bhettiaa naanak man paragaas |1|

The Guru has given the healing ointment of spiritual wisdom, and dispelled the darkness of ignorance. By the Lord's Grace, I have met the Saint; O Nanak, my mind is enlightened. ||1||

This thesis is dedicated to my beloved family, guide and friends for their unconditional support, love and motivation

CERTIFICATE

This is to certify that the thesis entitled **"Peptide biologics for effective management of breast cancer and fungal keratitis"** being submitted by **Ms. Prasanjeet Kaur** to the **Kusuma School of Biological Sciences,** Indian Institute of Technology Delhi, New Delhi for the award of the degree of **"Doctor of Philosophy"** is a record of the bonafide research work carried out by her, prepared under my supervision, in conformity with the rules and regulations of the 'Indian Institute of Technology Delhi'. The research report and the results present in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Date: 21st July 2025

Place: New Delhi

Dr. Archana Chugh

Professor

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ACKNOWLEDGEMENTS

This thesis is not just the culmination of my research, it's a journey filled with challenges, discoveries, and immense learning. Along the way, I have been fortunate to receive the support, guidance, and encouragement of many incredible individuals, without whom this work would not have been possible. I am deeply grateful to everyone who played a role in making this work a reality.

First and foremost, I feel blessed to work under the supervision of ***Prof. Archana Chugh****, whose invaluable insights, patience, and encouragement have been instrumental in shaping this research. Her mentorship has not only refined my scientific perspective but has also inspired me to push boundaries and strive for excellence. I have been fortunate to have an advisor who instilled in me the qualities of being a good and independent researcher.*

Further, I would like to extend my heartfelt gratitude to ***Dr. Shikha Yadav****,* ***Dr. Sushmita Shah and Dr. Harish Chander****, for providing valuable insights and constructive feedback in carrying out in vivo experiments. Their expertise and keen observations have helped refine my work.*

I would like to express my sincere appreciation to the ***Indian Institute of Technology, Delhi*** *and the* ***Kusuma School of Biological Sciences*** *for providing the resources and an intellectually stimulating environment that nurtured this research. A special note of thanks to the* ***Central Research Facility at IIT Delhi****, whose advanced infrastructure, state-of-the-art equipment, and technical expertise have been instrumental in carrying out crucial aspects of this research. I would also like to extend my sincere gratitude to the* ***High-Performance Computational Facility at IIT Delhi****, which provided the computational power necessary for the molecular dynamics simulations and other intensive calculations involved in this research. Heartfelt thanks to the* ***National Institute of Biologics, Noida,*** *for providing the necessary facilities and support to carry out the in vivo experiments critical to this research. I am also deeply grateful to the* ***University Grants Commission (UGC)*** *for providing financial support through the UGC Fellowship.*

I am incredibly grateful to my amazing labmates, ***Dr. Anupama, Dr. Harsha, Dr. Vivek, Dr. Pankhuri, Dr. Sujithra, Dr. Anjali, Dr. Nirupama, Dr. Manobala, Dr. Saurabh****,* ***Dr. Gagandeep, Aditi, Gagan, Malay, Mayank, Shiva, Jyoti*** *for being my support system through*

every high and low of this journey. From celebrating small victories to navigating setbacks, their encouragement, patience, and unwavering camaraderie have made this experience not just productive but truly memorable. Thank you for the insightful discussions, the laughter that kept me going, and for always having my back when experiments or life didn't go as planned. I couldn't have asked for a better team to share this journey with.

I would also like to extend my heartfelt gratitude to my incredible batchmates and friends from other labs, ***Nidhi, Pragati, Maryam, Ram, Vikas, Rituparna****,* ***Dr. Akanksha*** *who have been a constant source of motivation and support. Whether it was sharing ideas over coffee, helping troubleshoot experiments, or simply being there to listen during challenging times, their presence has made this journey so much more enriching. The friendships we have built, the late-night brainstorming sessions, and the shared struggles and successes have been invaluable. Thank you for making this experience not just about research, but also about growth, laughter, and unforgettable memories.*

I cannot express enough gratitude to my sister, ***Lajpreet Kaur****, whose unwavering support and belief in me have been my greatest source of strength. Through every challenge, she has been my confidant and my biggest cheerleader. I am also incredibly thankful to my wonderful friends,* ***Prachi, Aruna, Kirti****, who always stood by me. Our shared adventures have been a perfect balance to the demands of research. Thank you for being my support system, for celebrating every little milestone, and for making this journey unforgettable.*

Above all, I am profoundly grateful to my family for their unconditional love, patience, and unwavering support throughout this journey. Their belief in me, even during times when I doubted myself, has been my greatest source of strength. From encouraging words to endless motivation, they have been my anchor through every challenge and success. Their sacrifices, understanding, and constant reassurance have given me the confidence to push forward. This thesis is as much a result of their support as it is of my efforts, and I will always be grateful for their presence in my life.

Lastly, my deepest gratitude goes to my son, ***Hunarpratap Singh Kang****, who came into this world while I was writing this thesis. His arrival brought indescribable joy and renewed purpose to my life. Though an infant, his presence was a silent source of strength, patience, and inspiration as I worked through the final stages of my research.*

I also extend my heartfelt thanks to my husband, ***Harpal Singh Kang****, who has stood by me with unwavering love, patience, and encouragement. Thank you for your endless support, for understanding my dedication to this journey, and for always reminding me that no path is too difficult when walked with the right person.*

Finally, to everyone who, in one way or another, contributed to this journey - thank you. This thesis is as much a product of your support as it is of my efforts, and I am eternally grateful.

Prasanjeet Kaur

ABSTRACT

This thesis explores the transformative potential of peptide biologics in addressing two major medical challenges breast cancer and fungal keratitis through the innovative application of antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs). By harnessing their unique selectivity, membrane-targeting ability, and low toxicity, this work aims to push the boundaries of conventional therapeutics and offer next-generation treatment strategies.

Part I focuses on a venom-derived AMP, Ltc2a, evaluated for its anticancer efficacy. *In vitro* studies across human cancer cell lines revealed significant cytotoxicity in comparison to normal HEK293T cells. Moreover, the disruption of 3D spheroid integrity by Ltc2a was also observed. The LDH assay and PI staining revealed a membranolytic mode of action of the peptide. Interestingly, *in vivo* analysis in a murine breast cancer model demonstrated remarkable tumour suppression, reduced expression of Ki67 and CD34, and increased apoptosis confirmed through TUNEL assays. The truncated variants of Ltc2a were designed to gain insights into the region responsible for its activity. Interestingly, the truncated variants retained their helical structure but exhibited no cytotoxicity at the tested concentration, suggesting the role of specific amino acids in the activity of peptides. However, the differential uptake of variants in cancer cells vs. normal cells suggests the potential selectivity of peptides, opening avenues for targeted drug delivery. Importantly, histological evaluation of major organs and comprehensive haematological and biochemical analyses confirmed the excellent safety profile of Ltc2a, with no observable systemic toxicity. In parallel, zebrafish toxicity and bioavailability studies validated the peptide's biocompatibility and systemic distribution, supporting its translational potential.

Part II shifts focus to the CPP Tat_2, exploring its peptide membrane interactions and antifungal efficacy. This study demonstrates the successful uptake of Tat_2 across multiple mammalian cell lines, conforming its broad delivery potential. The mechanism of action of Tat_2 revealed concentration dependent uptake with lower concentration favouring endocytosis while higher concentration demonstrating direct penetration. Atomic force microscopy revealed increased membrane roughness following peptide exposure, while FESEM confirmed the absence of gross morphological changes, suggesting a selective, non-disruptive entry mechanism. Molecular dynamics simulations unravelled Tat_2's preferential interaction with anionic lipid membranes, leading to bilayer thinning and water penetration. Circular dichroism spectroscopy

showed that Tat_2 remains unstructured in lipid environments, which likely contributes to its functional flexibility.

Tat_2 also demonstrated potent antifungal activity against *Fusarium dimerum*, impairing hyphal growth via membrane disruption. It successfully penetrated the corneal epithelium in a rabbit model and resolved fungal keratitis in mice, showing anti-inflammatory properties and no detectable ocular or systemic toxicity.

In conclusion, this work presents two promising peptide candidates Ltc2a for cancer and Tat_2 for fungal infections as powerful, safe, and multifunctional biologics with the potential to revolutionize current treatment paradigms.

सार

यह शोध एंटीमाइक्रोबियल पेप्टाइड्स (एएमपी) और सेल-पेनेट्रेटिंग पेप्टाइड्स (सीपीपी) के अभिनव अनुप्रयोग के माध्यम से दो प्रमुख चिकित्सा चुनौतियों स्तन कैंसर और फंगल केराटाइटिस को संबोधित करने में पेप्टाइड बायोलॉजिक्स की परिवर्तनकारी क्षमता की खोज करता है। उनकी अनूठी चयनात्मकता, झिल्ली-लक्ष्यीकरण क्षमता और कम विषाक्तता का उपयोग करके, इस कार्य का उद्देश्य पारंपरिक चिकित्सा की सीमाओं को आगे बढ़ाना और अगली पीढ़ी की उपचार रणनीतियों की पेशकश करना है। भाग I एक विष-व्युत्पन्न एएमपी, Ltc2a पर केंद्रित है, जिसका मूल्यांकन इसकी कैंसर विरोधी प्रभावकारिता के लिए किया गया है। मानव कैंसर कोशिका रेखाओं में इन विट्रो अध्ययनों ने सामान्य HEK293T कोशिकाओं की तुलना में महत्वपूर्ण साइटोटॉक्सिसिटी का खुलासा किया। इसके अलावा, Ltc2a द्वारा 3D गोलाकार अखंडता का विघटन भी देखा गया। LDH परख और PI धुंधलापन ने पेप्टाइड की क्रिया के एक मेम्ब्रेनोलिटिक मोड का खुलासा किया। दिलचस्प बात यह है कि म्यूरिन ब्रेस्ट कैंसर मॉडल में इन विवो विश्लेषण ने उल्लेखनीय ट्यूमर दमन, Ki67 और CD34 की अभिव्यक्ति में कमी और TUNEL परख के माध्यम से पुष्टि की गई एपोप्टोसिस में वृद्धि को प्रदर्शित किया। Ltc2a के काटे गए वेरिएंट को इसकी गतिविधि के लिए जिम्मेदार क्षेत्र में अंतर्दृष्टि प्राप्त करने के लिए डिज़ाइन किया गया था। दिलचस्प बात यह है कि काटे गए वेरिएंट ने अपनी हेलिकल संरचना को बनाए रखा, लेकिन परीक्षण की गई सांद्रता पर कोई साइटोटॉक्सिसिटी नहीं दिखाई, जो पेप्टाइड्स की गतिविधि में विशिष्ट अमीनो एसिड की भूमिका का सुझाव देता है। हालांकि, कैंसर कोशिकाओं बनाम सामान्य कोशिकाओं में वेरिएंट के विभेदक अवशोषण से पेप्टाइड्स की संभावित चयनात्मकता का पता चलता है, जिससे लक्षित दवा वितरण के लिए रास्ते खुलते हैं। महत्वपूर्ण रूप से, प्रमुख अंगों के हिस्टोलॉजिकल मूल्यांकन और व्यापक हेमाटोलॉजिकल और जैव रासायनिक विश्लेषण ने Ltc2a की उत्कृष्ट सुरक्षा प्रोफ़ाइल की पुष्टि की, जिसमें कोई अवलोकन योग्य प्रणालीगत विषाक्तता नहीं थी। समानांतर में, ज़ेब्राफ़िश विषाक्तता और जैवउपलब्धता अध्ययनों ने पेप्टाइड की जैव-संगतता और प्रणालीगत वितरण को मान्य किया, जो इसकी अनुवाद क्षमता का समर्थन करता है। भाग II सीपीपी टैट2 पर ध्यान केंद्रित करता है, इसकी पेप्टाइड झिल्ली अंतःक्रियाओं और एंटीफंगल प्रभावकारिता की खोज करता है। यह अध्ययन कई स्तनधारी कोशिका रेखाओं में टैट2 के सफल अवशोषण को प्रदर्शित करता है, जो इसकी व्यापक वितरण क्षमता को दर्शाता है। टैट2 की क्रियाविधि ने सांद्रता पर निर्भर अवशोषण को प्रकट किया, जिसमें कम सांद्रता एंडोसाइटोसिस को बढ़ावा देती है जबकि उच्च सांद्रता प्रत्यक्ष प्रवेश को प्रदर्शित करती है। परमाणु बल

माइक्रोस्कोपी ने पेप्टाइड एक्सपोजर के बाद झिल्ली खुरदरापन में वृद्धि का खुलासा किया, जबकि FESEM ने सकल रूपात्मक परिवर्तनों की अनुपस्थिति की पुष्टि की, जो एक चयनात्मक, गैर-विघटनकारी प्रवेश तंत्र का सुझाव देता है। आणविक गतिशीलता सिमुलेशन ने एनायनिक लिपिड झिल्लियों के साथ टैट2 की पसंदीदा अंतःक्रिया को उजागर किया, जिससे द्विपरत पतलापन और पानी का प्रवेश हुआ। सर्कुलर डाइक्रोइज्म स्पेक्ट्रोस्कोपी ने दिखाया कि टैट2 लिपिड वातावरण में असंरचित रहता है, जो संभवतः इसके कार्यात्मक लचीलेपन में योगदान देता है। टैट2 ने फ्यूजेरियम डिमेरम के खिलाफ शक्तिशाली एंटीफंगल गतिविधि का भी प्रदर्शन किया, जो झिल्ली विघटन के माध्यम से हाइफल विकास को बाधित करता है। इसने खरगोश के मॉडल में कॉर्नियल उपकला में सफलतापूर्वक प्रवेश किया और चूहों में फंगल केराटाइटिस को ठीक किया, जिससे सूजन-रोधी गुण दिखे और कोई पता लगाने योग्य नेत्र या प्रणालीगत विषाक्तता नहीं हुई।

निष्कर्ष में, यह कार्य कैंसर के लिए Ltc2a और फंगल संक्रमण के लिए Tat2 जैसे दो आशाजनक पेप्टाइड उम्मीदवारों को शक्तिशाली, सुरक्षित और बहुक्रियाशील जैविक पदार्थों के रूप में प्रस्तुत करता है, जिनमें वर्तमान उपचार प्रतिमानों में क्रांति लाने की क्षमता है।

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ABBREVIATIONS

ACPs	Anticancer Peptides
ACE	Angiotensin Converting Enzyme
AFM	Atomic Force Microscopy
AFPs	Antifungal Peptides
AGS	Adenocarcinoma cell
ALBD	Albumin
ALPU	Alkaline phosphatase
APD3	Antimicrobial Peptide Database
AMCase	Acidic Mammalian Chitinase
AMPs	Antimicrobial Peptides
ATPase	Adenosine triphosphatase
BC	Breast Cancer
BD	*Becton Dickinson*
BIT	Bilirubin
BMI	Basal Metabolic Index
BNP	Brain Natriuretic Peptide
BRCA	Breast Cancer gene
CA	Calcium
CAGR	Compound Annual Growth Rate
CAR T-cell	Chimeric Antigen Receptor T-cell
CBA	Cytometric Bead Array

CD	Circular Dichroism
CDK4/6	Cyclin-dependent kinase 4 and 6
CFU	Colony Forming Unit
CHARMM-GUI	Chemistry at Harvard Macromolecular Mechanics-Graphical User Interface
CLRs	C-type lectin Receptors
CLSM	Confocal Laser Scanning Microscopy
CNP	C-type Natriuretic peptide
COM	Centre of mass
CPPs	Cell Penetrating Peptides
CPCSEA	Control and Supervision of Experiments on Animals Guidelines
CRENZ	Creatinine
CRF	Central Adrenocorticotropin-releasing Factor
DAB	3,3'-diaminobenzidine
DAPI	4,6-diamino-2-phenylindole
DCIS	Ductal Carcinoma *in situ*
DIC	Differential Interference Contrast
DLS	Dynamic Light Scattering
DMC1	Disruption of Meiotic Control
DMEM	Dulbecco's Modified Eagle Medium
DMPC	1,2 Dimyristoyl Glycero 3 Phosphorylcholine
DMPG	1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol, sodium salt

DMSO	Dimethyl sulfoxide
DPPC	1,2-Dipalmitoylphosphatidylcholine
DPPG	1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol
DPX	Dibutylphthalate Polystyrene Xylene
DNA	Deoxyribonucleic Acid
DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
DOPS	1,2-dioleoyl-sn-glycero-3-phospho-L-serine
Dpf	days post-fertilization
DSC	Differential Scanning Calorimeter
E.coli	*Escherechia coli*
EDTA	Ethylenediamine tetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal Growth Factor Receptor
EO	Eosinophils
ERα	Estrogen Receptor Alpha
ERBB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscopy
FITC	Fluoroscein Isothiocyanate
F.dimerum	*Fusarium dimerum*
GAGs	Glycosaminoglycans

GLP	Glucagon-like peptide
GR	Granulocytes
GROMACS	GROningen MAchine for Chemical Simulations
H&E	Hematoxylin & Eosin
Hb	Haemoglobin
HCl	Hydrochloric acid
HCT	Hematocrit
HER2	Human epidermal growth factor receptor 2
HIV	Human Immunodeficiency Viruses
Hpf	hours post-fertilization
HPLC	High Performance Liquid Chromatography
HRP	*horseradish peroxidase*
HSV	Herpes Simplex Virus
IAEC	Institutional Animal Ethics Committee
IARC	International Agency for Research on Cancer
IBC	Inflammatory Breast Cancer
IBD	Inflammatory Bowel Disease
IDC	Invasive Ductal Carcinoma
IHC	Immunohistochemistry
IL-1β	Interleukin-1β
ILC	Invasive Lobular Carcinoma
I-TASSER	Iterative Threading ASSEmbly Refinement
LCIS	Lobular Carcinoma *in situ*

LDH	Lactate dehydrogenase
LINCS	Linear Constraint Solver
LUVs	Large Unilamellar Vesicles
LY	Lymphocytes
MAPs	Membrane-active peptides
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MD	Molecular Dynamic
MFI	Mean Fluorescence Intensity
miRNA	Micro ribonucleic acid
MHC	Major Histocompatibility Complex
MIC	Minimum Inhibitory Concentration
MO	Monocytes
MPV	Mean Platelet Volume
mRNA	messenger RNA
MRE	Mean Residual Ellipticity
mTORC1	Mammalian Target of Rapamycin Complex 1
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NIB	National Institute of Biologicals
NOD	Nucleotide-binding Oligomeristion domain
NLRs	NOD-like receptors
NPs	Natriuretic Peptides

NPR	Natriuretic Peptide Receptor
NPT	Nose-Hoover Piston Thermostat
NTA	Nanoparticle Tracking Analysis
NVT	Nose-Hoover thermostat with volume and temperature
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate Buffer Saline
PCT	Procalcitonin
PD-1	Programmed Cell Death Protein 1
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PD-L1	Programmed Cell Death Ligand 1
PDW	Platelet Distribution Width
PET	Positron emission tomography
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PI3K	Phosphoinositide 3-kinase
PLT	Platelets
PMAP-23	Porcine Myeloid Antimicrobial Peptide-23
PME	Particle Mesh Ewald
PNPs	Pexiganan Nanoparticles
PR	Progesterone Receptor
PRRs	Pattern Recognition Receptors
PR	Proline-arginine

PrAMP	Proline-rich Antimicrobial Peptide
PRO	Protein
PS	Phosphatidylserine
PTU	Phenylthiourea
QCM-D	Quartz crystal microbalance with dissipation monitoring
RAAS	Renin-Angiotensin-Aldosterone System
RANKL	Receptor activator of nuclear factor-Kb, its ligand
RBC	Red Blood Cell
RDF	Radial Distribution Function
RDWCV	Red Cell Distribution Width-coefficient of Variation
RDWSD	Red Cell Distribution Width Standard Deviation
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPE	Retinal Pigment Epithelium
RPMI	Roswell Park Memorial Institute
RT	Room temperature
SBS	Short Bowel Syndrome
SDS	Sodium Dodecyl Sulphate
SGPTD	Serum Glutamic Pyruvic Transaminase
SGOTD	Serum Glutamic Oxaloacetic Transaminase
siRNA	small interfering RNA
SPECT	Single-photon emission computed tomography
T2DM	Type-2 Diabetes Mellitus

Tat	Trans-activator of transcription
TBS	Tris-buffered saline
TFE	2,2,2-trifluoroethanol
TIP3P	transferable intermolecular potential with 3 points
TME	Tumour Microenvironment
TNBC	Triple-negative Breast Cancer
TLRs	Toll-like Receptors
TST	Topical-Systemic-Targeted
TUNEL	Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labelling
UA	Uric acid
USD	United States Dollar
USFDA	U.S. Food and Drug Administration
VMD	Visual Molecular Dynamics
VPs	Venom peptides
WBC	White Blood Cell
WHO	World Health Organization