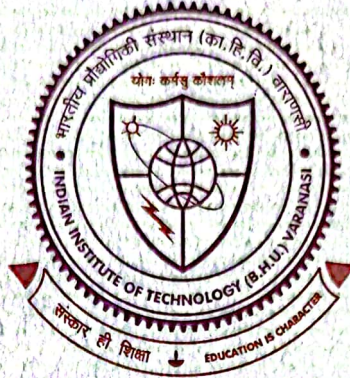


Targeted Nanomedicine and Nanotheranostics for Breast Cancer Imaging and Therapy



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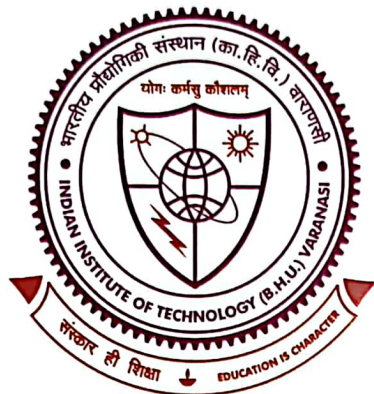
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CERTIFICATE

It is certified that the work contained in the thesis titled "**TARGETED NANOMEDICINE AND NANOTHERANOSTICS FOR BREAST CANCER IMAGING AND THERAPY**" by **ABHISHESH KUMAR MEHATA** has been carried out under my supervision and that this work has been not submitted elsewhere for a degree.

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Abbreviations and Symbols

%	Percentage
°	Degree
µg	Microgram
µl	Microliter
AFM	Atomic force microscope
ALT	Alanine aminotransferase
ANP	1-amino 4-nitro naphthalene
AST	Aspartate aminotransferase
CS	Chitosan
CM6	Coumarin-6
CI	Combination index
CLSM	Confocal laser scanning microscope
cm	Centimeter
DAPI	4',6-diamidino-2-phenylindole
DMAB	Di-dodecyldimethyl ammonium bromide
DMBA	7, 12-dimethylbenz [α] anthracene
DMEM	Dulbecco's modified Eagle's medium
DPPH	1,1-diphenyl-2-picrylhydrazyl
DRI	Dose reduction index
DSC	Differential scanning calorimetry
EDTA	Ethylenediaminetetraacetic acid
EE	Entrapment efficiency
Egen	Estrone
FA	Folic acid
FBS	Fetal bovine serum
GRAS	Generally regarded as safe
GSH	Glutathione
h	Hour
USG	Ultrasound
HPLC	High performance liquid chromatography
PA	Photoacoustic
IS	Internal standard
kD	Kilodalton
kg	Kilogram
l	Liter
LLOQ	Lower limit of quantification
LOD	Limit of detection

LOQ	Limit of quantification
UMN	Ultrasmall magnesium nanocluster
MEM	Minimum essential medium
mg	Milligram
min	Minute
ml	Milliliter
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
NPs	Nanoparticles
ng	Nanogram
nm	Nanometer
NMR	Nuclear magnetic resonance
PDI	Polydispersity index
PI	Propidium iodide
RSD	Relative standard deviation
RSM	Response surface methodology
SD	Standard deviation
sec	Second
SEM	Scanning electron microscope
Sem	Standard error of mean
PS	Particle size
PDI	Polydispersity Index
PLB	Palbociclib
TEM	Transmission electron microscope
TOS	Total solid content
w/v	Weight-by-volume
w/w	Weight-by-weight
XRD	X-ray diffraction
XPS	X-ray photoelectron spectroscopy
ZP	Zeta Potential
θ	Theta

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second, third and fifth row) or right (fourth row) side of the breast. ns ($P \geq 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and **** ($P < 0.0001$)..... 124

Abstract

Breast cancer remains the most prevalent aggressive cancer affecting women, with significant mortality rates, especially in high-income countries. Traditional cancer treatments, while effective, often cause severe non-specific cytotoxicity and lead to drug resistance with prolonged use. To address these issues, this research first focuses on developing a single-targeted nanomedicine using estrogen receptor (ER) targeted chitosan nanoparticles (NPs) loaded with Palbociclib (PLB), a chemotherapeutic agent for advanced breast cancer therapy. The ER-targeted NPs were characterized for particle size, zeta potential, polydispersity, surface morphology, drug entrapment efficiency, and *in vitro* drug release. Cytotoxicity assays, cellular uptake studies, apoptosis assays, and cell cycle analysis were conducted on ER+ breast cancer cell lines (MCF-7 and T-47D). *In vivo* studies included pharmacokinetic analysis, histopathology of vital organs, and evaluation of antitumor activity in a DMBA-induced breast cancer rat model. Results demonstrated that ER-targeted NPs showed significantly improved anticancer activity, pharmacokinetics, and tumor targeting compared to non-targeted NPs and free drugs.

Building on these findings and to further enhance therapeutic efficacy and incorporate imaging capabilities, the study further developed a dual-targeted theranostic system that targets both estrogen and folate receptors. This system used chitosan NPs loaded with both PLB and ultra-small magnesium nanoclusters (UMN), the latter serving as an imaging agent. The dual-receptor targeting employed estrone and folic acid to enhance the specificity and efficacy of the treatment. The dual-targeted NPs were characterized similarly to the single-targeted NPs, with additional evaluations of their imaging capabilities. *In vitro* and *in vivo* studies assessed the cytotoxicity, cellular uptake, pharmacokinetics, and antitumor activity of the dual-targeted NPs. Simultaneous imaging was performed using ultrasound and photoacoustic techniques to visualize tumor size,

hypoxic regions, and vascularity. Results showed that the dual-targeted NPs had significantly enhanced anticancer activity and imaging capabilities compared to non-targeted and single-targeted NPs. The dual-targeted NPs exhibited substantial accumulation in breast tumors and better visualization of tumor characteristics, indicating improved therapeutic efficacy and precise monitoring of tumor response.

Conclusively, the single-targeted ER nanomedicine and the dual-targeted theranostic system developed in this study offer promising approaches for advanced breast cancer treatment. The ER-targeted NPs demonstrated effective drug delivery and anticancer activity, while the dual-targeted system provided enhanced therapeutic efficacy combined with robust imaging capabilities, potentially leading to better management and monitoring of breast cancer.