

**Albumin-Coated Cadmium Oxide Nanoparticles: Synthesis,
Characterization, and *In Vitro* and *In Vivo* Evaluation of Their
Biological Activity in Triple-Negative Breast Cancer"**

RELEBOHILE PATRICIA LEFOJANE

Dissertation submitted in fulfilment of the requirements of the degree
Master of Health Sciences in Biomedical Technology

Department of Health Science Biomedical Technology
Faculty of Health and Environmental Sciences

Supervisor: Prof. MP Sekhoacha (Ph.D.)

Co-supervisors: Prof. PH Mfengwana (DHSc)
Dr AM Adegoke (P.hD.)

JUNE 2026

Statement of Declaration

I, Relebohile Patricia Lefojane, with student number _____ and identity number (_____), hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree MASTER OF HEALTH SCIENCES IN BIOMEDICAL TECHNOLOGY, is my independent work; and complies with the code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

SIGNATURE: _

DATE: 12/06/2026

Dedication

This dissertation is a love letter to every person who has faced the harsh reality of a cancer diagnosis and to those whose lives were cut short by this devastating disease. It is especially a tribute to my beloved mother and father in heaven. This work represents far more than an academic requirement; it is the language of my soul and a deeply personal prayer for a future where cancer treatment is not only effective but kinder, marked by fewer side effects and greater hope. I write and research with the dream that one day, science will be a tool that offers healing without suffering. To me, the calling to heal is a sacred crown I wear with humility and honour.

Acknowledgments

Above all, I thank God, my ever-present help, strength, and source of wisdom throughout this journey. His grace sustained me through every challenge and triumph, and for that, I am grateful.

First and foremost, I extend my deepest gratitude to Professor Mamello Sekhoacha, my extraordinary main supervisor. I am eternally grateful for her mentorship, guidance, and the many sacrifices she made throughout this journey. With unwavering patience, compassion, and insight, Prof. Sekhoacha nurtured my academic growth and helped me realise my fullest potential. Her expertise in the drug discovery process, provision of critical resources, and hands-on training laid the foundation for this work. To my co-supervisor, Professor Polo Mfengwana, thank you for being a remarkable source of support. Your financial contribution, particularly in funding the *in vivo* component of the study and supporting my research visits to North-West University, was instrumental. Your in-depth insights into the histological aspects of the research added immense value to this work, and your commitment to my academic journey will not go unnoticed. Incredible Dr. Ayodeji Adegoke, I am truly thankful for your unwavering guidance, thoughtful corrections, and the time you dedicated to every step of this project. Your mentorship sharpened my scientific reasoning and strengthened my research journey. I am also sincerely thankful to the following individuals whose contributions made this journey possible:

- Professor Mashele, Dean of the Faculty of Health Sciences at the Central University of Technology, for your leadership and support.
- Dr. Emmanuel, Postdoctoral Fellow at CUT, for your generous assistance with cell culture work.
- Dr. Victoria Fasiku, University of the Free State, for your patience and help during cell culture experimentation.
- Mrs Valerie Nesculescu for your selfless help with Histology
- Dr. Mart, Physics Department, University of the Free State, for your technical support with X-ray Photoelectron Spectroscopy (XPS), Fourier-Transform Infrared Spectroscopy (FTIR), and Ultraviolet-Visible (UV-Vis) Spectroscopy.

To my fellow students, Othembele Qcaba, Sivusile, and Nandi Mabaso, thank you for the solidarity during the ups and downs of research life. I am also grateful to the Isaiah family in North-West Province for warmly hosting me during my fieldwork and plant collection visits; your hospitality carried me through. To my beloved family (Tshepo Lefojane, Nthabiseng Lefojane, Itumeleng Lefojane, Libuseng Lefojane, and Palesa Lefojane), your love, encouragement, and belief in me were the pillars on which this journey stood. Finally, heartfelt thanks to Ntokozo Nkabinde, Tshegofatso Pule, Malo Jansen, and Pulane Mokgapi. Your strength, prayers, kept me grounded and uplifted when I needed it most.

This work was generously funded by the Central University of Technology and the University of the Free State, whose support made both the broader research and the specific *in vivo* studies possible. I am sincerely grateful for the belief in my work and their investment in the future of Science and Biomedicine. This dissertation is not only a reflection of my research but a testament to the unwavering support of a village. I am deeply thankful to every one of you.

List of Research Outputs

Research outputs from the Dissertation

1. First-authored Publications

- Cadmium and Cadmium Nano-based materials; exploration of their Toxicity, Carcinogenic and anti-carcinogenic properties:

Accepted for Publication: Journal of Nanotechnology (IF= 4.1)

- Characterization and Functional Optimization of Green- and Chemically Synthesized CdO Nanoparticles Coated with Albumin

Under review: Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (IF= 4.6)

- Albumin-coated Drug Delivery System: Comparative *In-vivo* Evaluation of Green vs. Chemically Synthesized CdO Nanoparticles in Triple Negative Breast Cancer

To be submitted: Drug Discovery (IF: 7.5)

2. International Conference Presentations

- Relebohile P. Lefojane¹, Ayodeji M. Adegoke^{2,3}, Polo H. Mfengwana¹, Jane E. Carré³, Charles Affourtit³ and Mamello P. Sekhoacha². Enhancing therapeutic efficacy in triple-negative breast cancer using albumin-coated cadmium oxide nanoparticles. Physical Poster Presentation at the University of Plymouth, United Kingdom. 6th [July 2025](#).
- Lefojane, R.P., Sone, B.T., Matinise, N., Saleh, K., Direko, P., Mfengwana, P., Mashele, S., Maaza, M. and Sekhoacha, M.P. 2021. CdO/CdCO₃ nanocomposite physical properties and cytotoxicity against selected breast cancer cell lines. Online Oral presentation at the “10th Edition Webinar on Breast Cancer” held during February 16 - 17, 2022 USA. <https://youtu.be/bDP2q6TtUlo?si=GNBRg48WjdttdZdTw>
- Relebohile P. Lefojane, Ayodeji Adegoke, Samson Mashele, Hilda Mfengwana and Mamello Sekhoacha. Targetted Delivery of albumin coated cadmium oxide

nanoparticles as a Triple negative breast cancer anti-agent. Online Oral Presentation at the 11th Breast Cancer Innovinc in Rome, Italy in December 2023.

Abstract

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype characterised by the absence of oestrogen, progesterone, and HER2 receptors, limiting the effectiveness of targeted therapies. Conventional chemotherapy remains non-selective and is associated with systemic toxicity and poor bioavailability. This study investigated the synthesis, characterisation, and anti-TNBC therapeutic potential of albumin-coated chemically synthesised cadmium oxide nanoparticles (A-Chem CdO NPs) as a potential nanotherapeutic strategy. Cadmium oxide nanoparticles were synthesised using green, chemical, and albumin-assisted chemical methods, and characterised using UV-Visible spectroscopy, FTIR, XPS, and TEM analyses. *In vitro* cytotoxicity was evaluated using the MTT assay, while *in vivo* therapeutic efficacy and systemic toxicity were assessed in a BALB/c mouse model using clinical observations, body mass changes, tumour inhibition rate (TIR), and histomorphological analysis. Chemically synthesised Cadmium Oxide Nanoparticles (Chem CdO NPs) demonstrated the highest *in vitro* potency and were subsequently functionalised with albumin to improve stability and biocompatibility. Albumin coating reduced the potency in MDA-MB-231 cells, with the IC_{50} increasing from 3.33 $\mu\text{g/mL}$ to 11.32 $\mu\text{g/mL}$, whereas Doxorubicin (the standard drug) exhibited the greatest potency with an IC_{50} of 0.34 $\mu\text{g/mL}$ and the highest selectivity index. *In vivo*, A-Chem CdO NPs induced delayed and milder clinical toxicity compared with Doxorubicin. Although body mass loss was comparable between treatments, Doxorubicin achieved superior tumour inhibition (50–55%), while A-Chem CdO NPs demonstrated moderate antitumour activity (30–35%). Histomorphological analysis revealed tumour necrosis and chromatin condensation in A-Chem CdO NP-treated tissues, indicating moderate tumour regression, with minimal evidence of off-target organ toxicity. Overall, the findings demonstrate that albumin functionalisation altered the biological behaviour of CdO nanoparticles, reducing acute cytotoxicity while maintaining moderate *in vivo* antitumour efficacy. These results highlight the complexity of nanoparticle therapeutic performance and support further optimisation of albumin-coated CdO nanoparticle systems for TNBC treatment.

Keywords Triple negative Breast cancer, cytotoxicity, albumin-coated nanoparticles, doxorubicin, cadmium oxide nanoparticles, albumin functionalisation, tumour inhibition

Table of Contents

Statement of Declaration	2
Dedication	3
Acknowledgments	4
Research outputs from the Dissertation.....	6
Abstract	8
List of figures	12
List of Tables	16
List of Abbreviations	17
Table of Appendices	21
Overview of Dissertation.....	23
1.0 Introduction.....	26
1.1 Problem Statement	27
1.2 Rationale of the study	27
1.3 Aim:	28
1.4 Objectives:	29
2.0 Introduction.....	31
2.1 Triple-negative Breast Cancer (TNBC).....	31
2.1.1 Molecular Subtypes and Pathogenesis of TNBC.....	31
2.2.2 Basal-like Subtype	33
2.2.3 Mesenchymal Subtype.....	34
2.2.4 Immunomodulatory Subtype	34
2.2.5 Luminal Androgen Receptor (LAR) Subtype	35
2.2.6 Pathogenesis of TNBC.....	36
2.3 TNBC Treatment.....	37
2.3.1 The poly ADP-ribose polymerase (PARP) inhibitors	38
2.3.2 Immunotherapy.....	38
2.3.3 Chemotherapy.....	39
2.3.4 Nanoparticle-based breast cancer therapy	39
2.3.5 Cadmium	41
2.3.6 The significance of Cadmium nanoparticles in cancer therapeutics.....	42
2.3.7 Mechanisms of CdO NPs delivery to Tumours	43
2.3.8 Albumin-coated nanoparticles.....	45
2.4 Synthesis of CdO NPs	47
2.4.1 Green Synthesis of Nanoparticles.....	47

2.4.2 Chemical Reduction Method	48
2.5 Systemic Nanoparticle Toxicity	49
3.0 Introduction.....	52
3.1 Materials.....	52
3.2 Plant Collection and Extraction.....	53
3.3 Synthesis of nanoparticles:.....	54
3.3.1 Green Chemistry	54
3.3.2 Chemical reduction.....	54
3.4 Characterization of the Nanoparticles	54
3.4.1 X-ray Diffraction (XRD)	55
3.4.2 Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM).....	55
3.4.3 Energy-Dispersive X-ray Spectroscopy EDS.....	56
3.4.4 Ultraviolet-Visible Spectroscopy (UV-VIS).....	56
3.4.5 Fourier Transform Infrared Spectroscopy (FTIR)	56
3.4.6 X-ray Photoelectron Spectroscopy (XPS)	57
3.5 Biological assay for cytotoxicity assessment.....	57
3.5.1 Cell Sub-culturing and Passaging.....	58
3.5.2 MTT Assay.....	59
3.5.3 Statistical analysis.....	60
3.6 Coating of chemically reduced Cadmium oxide nanoparticles with Albumin	60
3.6.1 Preparation of albumin-coated CdO NPs.....	60
3.7 Results	61
3.7.1 Characterization results of synthesised CdO NPS	61
3.7.2 The crystallographic structure of the G CdO NPs, Chem CdO NPs and A- Chem CdO NPs	64
3.7.3 Compositional and Surface Characterization of CdO Nanoparticles Using EDS, XPS, and FTIR Analyses	71
3.7.5 EDS and XPS of Chem-CdO NPs	72
3.7.6 EDS and XPS of A-Chem CdO NPs.....	72
3.7.7 FTIR Spectra of G-CdO NPs, Chem-CdO NPs, and A-Chem-CdO NPs	77
3.8 Toxicity Screening of Nanoparticles	84
3.8.1 Morphological description of MDA-MB231 Cell lines.....	84
3.8.3 Cytotoxicity and selectivity of various nanoparticles	86
3.8.4 Selectivity Index	89
3.9 Summary.....	90
4.0 Introduction.....	92

4.1 Ethical Clearance	92
4.2 Study Design	92
4.3 Mice Housing Conditions	93
4.4 Mice Tumour Inoculation and Treatment	94
4.4.1 Anaesthesia	95
4.4.2 Intramammary implantation of breast cancer cells	96
4.4.3 Treatment site: Intraperitoneal Injection (IP)	97
4.4.4 Treatment allocation and Monitoring Protocol	98
4.5 Euthanasia and organ harvesting	101
4.6 Data collection methods	101
4.7 Histopathological examination	102
4.7.1 Histology Steps in Preparing the Specimens	103
4.8 <i>In Vivo</i> Experimental Outcomes	106
4.8.1 Introduction	106
4.8.2 Enrolment and Treatment Completion in Experimental Groups	107
4.8.3 Observational Parameters	108
4.9. Tumour Volume Progression During Treatment	112
4.9.2 Tumour Inhibition Rate (TIR)	114
4.9.3 Histological Presentation of Harvested Tissues (Liver and Lungs)	115
5.0 Overview of study outcomes	121
5.1 Structural Overview of Discussion	122
5.2 Synthesis techniques and characterization of Cadmium Oxide Nanoparticles (CdO NPs) Formulations	122
5.3 <i>In Vitro</i> Cytotoxicity Evaluation and Comparative Analysis	128
5.4 <i>In Vivo</i> Antitumor Efficacy and Safety	133
5.5 Correlation Between invitro Cytotoxicity and invivo Antitumour Activity of Albumin-Coated CdO Nanoparticles vs doxorubicin	135
5.6 Limitations of the Study	136
5.6.1 Incomplete Preclinical Evaluation	136
5.6.2 Selectivity Index Limitations	137
5.6.3 Narrow Evaluation of Systemic Toxicity	137
5.6.4 Limited Sample Size and Statistical Power	139
5.6.4 Inconsistent Tumour Establishment and Experimental Variability	140
5.6.5 Absence of Long-Term Follow-Up	142
5.7 Recommendations	143
5.8 Summary Perspective	144
5.9 Conclusion	144

6.0 References	147
7.0 Appendices.....	168

List of figures

Figure number	Title	Page number
Figure 1:	Representation of cadmium in different forms: (A) cadmium metal in its natural state, (B) atomic structure of cadmium, and (C) cadmium oxide nanoparticles (CdO NPs) (VectorStock, 2025; Theodore Gray, 2008; Nanoshel , 2025).	43
Figure 2:	Shows how albumin-coated Cadmium oxide nanoparticles target tumour cells via gp60 and SPARC receptors	47
Figure 3 :	Figure 3 Depicts <i>S. cupulare</i> in its natural habitat (Solomon, 2011).available at (https://kumbulanursery.co.za/plants/synadenium-cupulare)	49
Figure 4	Shows dried leaves of <i>Synadenium cupulare</i> at room temperature, whilst fig 4b shows small pieces of <i>S. cupulare</i> leaves undergoing extraction in distilled water	54
Figure 5	Visual appearance of the dried nanoparticle formulations after oven drying at 70 °C: (A) G-CdO NPs, (B) Chem-CdO NPs, and (C) A-Chem-CdO NPs.	63
Figure 6	Shows SEM images of the NPs at different magnifications. 6a and 6b are images of G CdO NPs at X200 and X2000 magnification. 6c and 6d show C-CdO NPs at X2000 and X6000. 6e and 6f show A-Chem CdO NPs at X6000 and X9500.	63

Figure 7	7A: TEM image of G CdO NPs showing spherical and non-uniform shapes. 7B: TEM image of Chem-CdO NPs showing agglomerated and irregular shape. 7C: TEM image of A-Chem NPs showing cadmium embedded in albumin and a bit of spherical morphology.	65
Figure 8	Comparison of crystallite sizes of green-synthesized CdO nanoparticles (G-CdO NPs) and chemically synthesized CdO nanoparticles (Chem-CdO NPs) at annealing temperatures of 100, 200, 300, 400, and 500 °C.	67
Figure 9	Presents A-Chem CdO NPs crystallinity sizes over increasing temperatures of (23, 50, 75 and 100)	67
Figure 10	XRD patterns of G-CdO NPs prepared using S. cupulare extract at annealing temperatures of (A) 100 °C, (B) 200 °C, (C) 300 °C, (D) 400 °C, and (E) 500 °C.	69
Figure 11	A, B, C, D and E depict XRD analysis of Chemically Synthesised CdO NPs at 100, 200, 300, 400 and 500 °C.	70
Figure 12	A, B, C, D and E depict XRD analysis of Albumin coated Chemically Synthesized CdO NPs at room temperature, also 50, 75 and 100°C.	71
Figure 13	EDS spectrum of G CdO NPs revealing Cd and O as major elements with trace amounts of N, K, Ca, and Mg on the nanoparticle surface.	74
Figure 14	EDS spectrum of Chem CdO NPs revealing Cd and O as major elements with trace amounts N and Na, Mg, Ca, K	75
Figure 15	Figure 15: EDS spectrum of A-Chem NPs revealing Cd and O as major elements with trace amounts, Na, N, Cl and S	75
Figure 16	Figure 16 Displays XPS spectrum of G CdO NPs indicating the presence of Cl, O, Cd and Mg.	76

Figure 17	Figure 17 XPS Spectrum of Chem CdO NPs showing O, Cl, Na and Cd	77
Figure 18	XPS Spectrum of A-Chem CdO NPs showing Cl, O, Cd, Na, Cl	78
Figure 19	Infrared Spectra of G CdO NPs	80
Figure 20	Infrared spectra of Chem CdO NPs	80
Figure 21	Infrared spectra of A-Chem CdO NPs	80
Figure 22	UV-Vis spectra of G CdO NPs indicating absorption at 215 nm	83
Figure 23	UV-Vis spectra of Chem CdO NPs indicating absorption at 260 nm	84
Figure 24	UV-Vis spectra of A-Chem CdO NPs indicating absorption at 208 nm	84
Figure 25	Depicts the Original morphology of MDA-MB231 Cell lines (www.atcc.org , 2025)	85
Figure 26	Morphological images of MTT assays MDA-MB-231 breast cancer cell lines treated with various concentrations of G CdO NPs, Chem CdO NPs, A-Chem CdO NPs and Albumin. A shows 1.25 µg/ml G CdO NPs, B shows 5 µg/ml G CdO NPs, C shows 100 µg/ml G CdO NPs, D shows 1.25 µg/ml Chem CdO NPs, E shows 5 µg/ml Chem CdO NPs, F shows 100 µg/ml Chem CdO NPs, G shows 1.25 µg/ml A-Chem CdO NPs, H shows 5 µg/ml A-Chem CdO NPs, I shows 100 µg/ml Chem CdO NPs, J shows 1.25 µg/ml Albumin, K shows 5 µg/ml Albumin, L shows 100 µg/ml Albumin.	87
Figure 27	A and B display the percentage inhibition of Doxorubicin against MDA-MB231 and Vero Cell lines. C and D Show the percentage inhibition of G CdO NPs, Chem CdO NPs and A-Chem against MDA-MB-231 Cell lines	
Figure 28	A: Display the percentage inhibition of G CdO NPs and Chem CdO NPs against Vero cell lines. B: display the percentage inhibition of A-Chem CdO NPs against Vero cell lines.	89
Figure 29	A and B display the mouse Housing Conditions at NWU.	95

Figure 30	Illustration of the body weight measurement procedure for an individual experimental mouse using a precision electronic balance	96
Figure 31	Intramammary inoculation of MDA-MB-231 breast cancer cells in immunodeficient mice (Balb/c mouse model)	97
Figure 32	Intraperitoneal (IP) administration of treatments to the experimental and control groups during the study period	98
Figure 33	Summarizes the Histological Procedure of specimen preparation	107
Figure 34	A shows the daily tumour measurements until treatment was completed, whilst B demonstrates the harvesting of major organs by the Veterinarian	109
Figure 35	A and B illustrate the toxicity profiling of the experimental mice, as determined by the percentage loss in body mass during the treatment period.	114
Figure 36	Figures 36A and 36B demonstrate the efficacy assessment of the treatment regimen through relative tumour volume analysis. A presents the relative tumour volume measurements, while B illustrates tumour progression over the treatment period. By day 17, some mice had already been euthanized due to study endpoint criteria.	116
Figure 37	A and B Show antitumour activity rates. Doxorubicin Showed the highest inhibition, followed by A-Chem CdO NPs and lastly G-CdO NPs	117
Figure 38	H&E Histomorphological images of G CdO NPs, Chem CdO NPs and A-Chem CdO NPs	119

List of Tables

	Caption	Page number
Table 1	Shows molecular features and clinical Characteristics of various TNBC Subtypes (Frontiersin.org, 2024)	33
Table 2	Shows SEM summary of morphology, scale and size distribution of G CdO NPs, Chem CdO NPs and A-Chem CdO NPs	64
Table 3	Summary of XRD Characteristics of Synthesized CdO Nanoparticles	72
Table 4	FTIR Interpretation of G CdO NPS	81
Table 5	FTIR interpretation of Chem CdO NPs	81
Table 6	FTIR interpretation of A-Chem CdO NPs	82
Table 7	Selectivity Index of the standard Drug and the various nanoparticle formulations against MDA-MB231 cell line in comparison to Vero cell lines.	90
Table 8	Treatment Schedule and site for the <i>In Vivo</i> Study	99
Table 9	Summary of Treatment Groups and Protocols	100
Table 10	Summarizes the number of Mice that completed treatment per treatment and control group	109
Table 11	Monitored Clinical Parameters pre- and post-treatment	110
Table 12	Summary of clinical observation, health checks outcome per treatment group	112
Table 13	Descriptive Statistics of % Body Mass Loss	114
Table 14	Summarises the Necrosis state of Tissues from mice administered with various treatments.	120

List of Abbreviations

%	Percentage
A-Chem CdO NPs	Albu min coated Chemically synthesized Cadmium Oxide Nanoparticles
AgNO ₃	Silver Nitrate
AR	Androgen Receptor
AREC	Animal Research Ethics
AuCl ₃ ,	Gold Chloride
BALB/C	Bagg Albino
BL	Basal-like
BLIA	Basal-like immune-activated
BLIS	Basal-like immune-suppressed
BSA	Bovine Serum Albumin
BWC	Body Weight Change
C ₆ H ₅ O ₇ Na ₃	Trisodium Citrate
C ₆ H ₈ O ₆	Ascorbic acid
Cd	Cadmium
CD151	Cluster of Differentiation 151
CDH1	E-cadherin
CdO NPs	Cadmium Oxide Nanoparticles
Chem CdO NPs	Chemically Synthesized Cadmium Oxide Nanoparticles
Cl	Chlorine
CO ₂	Carbon Dioxide
CuSO ₄ ,	Copper Sulfate

DMEM	Dulbecco's Modified Eagle's Medium
EBREC	Environment and Biosafety Research Ethics Committee
EMT	Epithelial to Mesenchymal transition
EPR	Enhanced Permeability and Retention
EPR	Enhanced Permeability and Retention
ER	Estrogen receptor
ESA	Equine Serum Albumin
FBS	Fetal Bovine Serum
FcRn	Neonatal Fc receptor
FOXA1	Forkhead box protein A1
FTIR	Fourier Transform Infrared Spectroscopy
G CdO NPs	Green synthesized Cadmium Oxide Nanoparticles
GATA3	GATA3 binding protein
GP60	Glycoprotein 60
H&E	Haematoxylin and Eosin
HER-2	Human epidermal growth factor receptor 2
HSA	Human Serum Albumin
IM	Immunomodulatory
K	Potassium
KDA	Kilodalton
KMT2C	Lysine Methyltransferase 2C
KMT2C	Lysine methyltransferase 2C
L	Length
LAR	Luminal androgen receptor

MDA-MB231	M D Anderson - Metastatic Breast - 231
Mg	Magnesium
MSA	Murine serum albumin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay
N	Nitrogen
Na	Sodium
NaBH ₄	Sodium Borohydride
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
nm	Nanometer
NP	Nanoparticles
NU/J	Athymic nude
NWU	North West University
O	Oxygen
PARP	Poly ADP-ribose polymerase
PDT	Photodynamic Therapy
PEG	Polyethylene Glycol
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PR	Progesterone receptor
PVA	Polyvinyl Alcohol
QD	Quantum Dots
ROS	Reactive Oxygen Species
RTV	Relative Tumour Volume

<i>S. cupulare</i>	<i>Synadenium cupulare</i>
SEM	Scanning Electron Microscopy
SPARC	Secreted Protein Acidic and Rich in Cysteine
SPF	Specific pathogen-free
STAT	Signal transducer and activator of transcription
TEM	Transmission Electron Microscopy
TGF- β	Transforming Growth Factor beta
TGI	Tumour Growth Inhibition ratio
TILs	Tumour Infiltrating Lymphocytes
TIR	Tumour Inhibition Rate
TNBC	Triple-negative breast cancer
W	Width
WHO	World Health Organization
Wnt	Wingless-related integration site
XPS	X-Ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction

Table of Appendices

List of Appendices	Caption	Page number
Appendix A	Plant (<i>Synadenium cupulare</i>) Collection permit	164
Appendix B	Biostatistician Approval	165
Appendix C	Environment and Biosafety Research Ethics Committee Ethical Clearance	166
Appendix D	Animal Research Ethics Training Certificate	167
Appendix E	University of the Free State Animal Research Ethical Clearance	168
Appendix F	North-West University Animal Research Ethical Clearance	169
Appendix G	North-West University Ammended Animal Research Ethical Clearance	171
Appendix H	Appendix H: Researchers publication that influenced the current study	172
Appendix I	Researcher's co-authored Review publication on medicinal plants. <i>Synadenium cupulare</i> , the plant in the study is also in the review.	173

Appendix J	Researcher's articles on nanoparticles Synthesis and characterization	174
Appendix H	Researcher's co-authored article on nanoparticles Synthesis and characterization	175

Overview of Dissertation

The study is divided into five key chapters:

Chapter 1: Introduction

Chapter one provides a brief background of the study, discussing the clinical landscape of triple-negative breast cancer (TNBC), the available treatment options, and the use of metal nanoparticles as an alternative therapeutic approach, which is the primary focus of the research. It also outlines the problem statement, rationale, aim, and objectives of the study.

Chapter 2: Literature Review

This chapter reviews relevant literature on the various types of TNBC and their pathogenesis, the role of albumin as a biocompatible coating, and the properties of Cadmium oxide nanoparticles (CdO NPs) in cancer therapy. This chapter highlights the gaps in current treatment options and establishes the foundation for the experimental approach.

Chapter 3: *In vitro* experiments (methods and results)

Chapter 3 describes the methodology used in the *in vitro* study, including the synthesis and characterization of albumin-coated CdO nanoparticles (A-Chem CdO NPs), the selection of *in vitro* models, and the assessment techniques for evaluating cytotoxicity. The results are also discussed.

Chapter 4: *In vivo* experiments (methods and results)

Presents the experimental procedure and the results of the efficacy of the CdO NPs and their specific targeting of TNBC cells, and also their therapeutic effect in reducing tumour growth.

Chapter 5: Overall discussion and conclusions

This chapter discusses the findings, comparing the performance of A-Chem CdO NPs to conventional chemotherapy treatments (Doxorubicin) and G-CdO NPs *in vitro* and *in vivo*. This chapter also explores the potential clinical applications of this approach, addressing challenges related to biocompatibility, toxicity and long-term effectiveness. The dissertation concludes with recommendations for future research to further optimize and translate this nanoparticle-based therapy into clinical practice.

Chapter 1: Introduction

1.0 Introduction

Breast cancer is the most common cancer among women and the second most prevalent globally after lung cancer (WHO, 2024). TNBC is an aggressive subtype characterised by the absence of estrogen receptor (ER), progesterone receptor (PR), and/ or minimal expression of human epidermal growth factor receptor 2 (HER-2), making it resistant to standard hormone and targeted therapies (American Cancer Society, 2023). TNBC disproportionately affects premenopausal women of African descent and accounts for 15–20% of all breast cancer cases (Zagami and Carey, 2022). Due to its rapid progression and high mortality rates, TNBC presents a significant challenge in oncology (Yin et al., 2020).

CdO NPs have demonstrated anticancer potential by inducing apoptosis through oxidative stress, mitochondrial dysfunction, and calcium signalling disruption (Qiao et al., 2024). However, concerns over selectivity and toxicity limit their clinical application. Albumin coating enhances nanoparticle stability, biocompatibility, and tumour selectivity, improving therapeutic efficacy while reducing toxicity (Kadri et al., 2025). Given that tumour cells actively scavenge albumin for nitrogen, albumin-coated nanoparticles offer a promising strategy for preferential tumour accumulation (Kadri et al., 2025). Additionally, albumin prolongs nanoparticle circulation time, allowing for sustained drug delivery to cancer cells (Mohamed et al., 2017).

A comprehensive preclinical framework typically integrates multiple dimensions, including efficacy, pharmacokinetics (PK), pharmacodynamics (PD), toxicity, and biodistribution profiling (Gallo, 2010). In this study, only select components, namely cytotoxicity assays, relative tumour volume (RTV), and histomorphological tumour analysis, were implemented. Crucial pharmacological parameters such as nanoparticle absorption, metabolism, organ distribution, and clearance (ADME) were not assessed due to time and resource restrictions.

1.1 Problem Statement

The prevalence of TNBC is highest in women of African ancestry. It is also very aggressive, and black women with TNBC are diagnosed at a younger age, with an approximately 40% mortality rate compared to white women (Thompson et al., 2025). The phenotype of TNBC is characterized by the absence of PR, ER, and minimal expression of HER-2. The lack of these receptors renders immunotherapies ineffective, leaving chemotherapy as the primary treatment option (Retecki et al., 2021). However, chemotherapy is associated with poor selectivity, systemic toxicity, and drug resistance mechanisms, including efflux mechanisms that expel drugs from cancer cells, reducing their therapeutic effectiveness, which makes TNBC difficult to treat successfully (Kannan et al., 2025).

Traditional chemotherapeutic agents suffer from non-specific distribution, poor cellular uptake, and fast elimination, leading to suboptimal efficacy and increased damage to healthy tissues (Zafar et al., 2025). While nanoparticle-based drug delivery systems enhance drug tumour retention, many still face challenges such as short circulation times and specificity (Firouzpour et al., 2026). A-Chem CdO NPs are a possible lead to a novel strategy to overcome these limitations. Albumin serves as a biocompatible and tumour-targeting carrier, prolonging circulation time, enhancing tumour accumulation and reducing toxicity to healthy cells through receptor-mediated uptake (Pal et al., 2023). This study employs A-Chem CdO NPs as an anti-TNBC therapy, aiming to improve drug delivery, prolong nanoparticle retention and minimize off-target toxicity.

1.2 Rationale of the study

The study is relevant locally and globally as the prevalence of TNBC is highest in women of African ancestry. Black women are diagnosed with TNBC at a younger age, with approximately 40% mortality rate compared to white women (Eze et al., 2026). TNBC defies conventional treatment regimens because it lacks their pharmacological targets. Thus, an alternative delivery therapy fills a much-needed lacuna in the Oncology field. Traditional chemotherapy, the primary treatment for TNBC, is associated with poor selectivity, subsequent systemic toxicity, and high recurrence

rates. While Cadmium oxide nanoparticles (CdO NPs) have demonstrated strong inhibitory activity against TNBC cell lines (MDA-MB-231) *in vitro*, their effectiveness *in vivo* remains uncertain (Lefojane et al., 2021). A major obstacle in cancer nanomedicine is the transition from *in vitro* success to *in vivo* efficacy, as over 70% of promising drug candidates fail in animal models due to immune clearance, poor bioavailability, and systemic toxicity (Maeda and Khatami, 2018). This underscores a crucial question: Can CdO NPs selectively accumulate in tumour tissues, exert cytotoxic effects, with minimal toxicity to healthy cells in a living system?

Albumin-coated CdO NPs present a promising approach to overcoming these challenges. Albumin, a naturally occurring biocompatible protein, enhances nanoparticle stability and extends circulation time by protecting them from rapid immune clearance (Spada et al., 2021). Additionally, albumin facilitates targeted tumour accumulation through the Enhanced Permeability and Retention (EPR) effect and enables receptor-mediated uptake via GP60 and SPARC receptors, which are overexpressed in breast cancer cells (Sheikh et al., 2026). These properties improve the selectivity and therapeutic efficacy of CdO NPs, potentially reducing off-target toxicity and enhancing their clinical viability. Despite their promising *in vitro* cytotoxicity, the behaviour of CdO NPs *in vivo*, including their tumour accumulation and overall toxicity, remains unexplored. The findings provide critical preclinical data. By integrating albumin as a functional carrier, this research seeks to enhance the efficacy of metal-based nanoparticles, ultimately contributing to the development of more effective, tumour-targeted treatment strategies for TNBC.

1.3 Aim:

To determine the *in vitro* cytotoxic activity of A-Chem CdO NPs against MDA-MB-231 cells, and to evaluate their *in vivo* anticancer efficacy in an MDA-MB-231 BALB/C strain mouse model, by analysing systemic toxicity, tumour inhibition rate, and histomorphological changes, in comparison with a conventional chemotherapeutic agent, doxorubicin.

1.4 Objectives:

- To determine whether synthesis methods influence the therapeutic potential of Cadmium oxide NPs by investigating green synthesis and chemical reduction methods, and to characterize the synthesized nanoparticles
- To compare the cell growth inhibitory activities of Cadmium oxide nanoparticles synthesized using green synthesis and chemical reduction methods, *in vitro*, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay), and to select the nanoparticles with the highest inhibitory activity for further testing *in vivo*.
- To coat the Cadmium oxide NPs with the highest inhibitory activity with albumin and evaluate the efficacy of albumin-coated Cadmium oxide nanoparticles *in vivo* in a MDA-MB231- NU/J (BALB/C strain breast cancer animal model).

Chapter 2: Literature Review

2.0 Introduction

This literature review provides a comprehensive analysis of TNBC, including its subtypes, pathogenesis, and current treatment approaches. It also explores the potential of nanotechnology, particularly albumin-coated CdO NPs, in overcoming the challenges associated with conventional therapies and enhancing TNBC treatment outcomes.

2.1 Triple-negative Breast Cancer (TNBC)

Normal breast cells respond to female hormones such as estrogen, human epidermal growth factor, and progesterone, which signal the cells to produce milk (Hanna et al., 2023). In the context of breast cancer, cells sometimes retain these hormone receptors, but approximately one-third of the time they lose them during tumour development (Lacopetta et al., 2023). Triple-negative breast cancer (TNBC) is characterized by the absence of all three of these important receptors, or in some cases, underexpression of HER2. The lack of these receptors makes hormonal therapies ineffective in treating this cancer type. TNBC tumours are heterogeneous, displaying a range of histological and genetic variations, which complicates treatment. Additionally, these tumours tend to be of a higher grade and larger, further adding to the challenges in treatment (Xiong et al., 2024)

2.1.1 Molecular Subtypes and Pathogenesis of TNBC

TNBC is known for its molecular diversity, comprising several distinct subtypes with different biological characteristics and clinical outcomes. Recognizing these molecular subtypes and their underlying mechanisms is essential for informing treatment strategies and enhancing patient outcomes. In 2011, Lehmann *et al.* conducted a gene expression analysis of TNBC cases, identifying seven potential subtypes. This classification was revised in 2016, incorporating histological evaluation, laser microdissection, and gene expression studies, leading to the identification of four tumour-specific subtypes: basal-like (BL; further divided into BL1 and BL2),

mesenchymal, immunomodulatory, and luminal androgen receptor (LAR) subtypes, as demonstrated in Table 1 (Ji et al., 2024). The basal-like subtypes make up a significant portion of TNBC cases (up to 80%), while mesenchymal and LAR subtypes are less common (Fernandez et al., 2023). This variation is influenced by a combination of genetic, molecular, and environmental factors. These factors contribute to differences in tumour behaviour, gene expression patterns, and clinical outcomes (Yin et al., 2020). The molecular and clinical characteristics of TNBC subtypes are summarised in Table 1.

Table 1 Molecular Features and Clinical Characteristics of various TNBC Subtypes (Ji et al., 2024)

Subtype	Molecular Features	Clinical Characteristics
Basal-like	Expression of basal cytokeratins (CK5/6, CK14, CK17), TP53 mutations	High histological grade, aggressive phenotype, poor prognosis
Mesenchymal	Upregulation of genes associated with epithelial-to-mesenchymal transition (EMT)	Increased motility, invasiveness, resistance to therapy
Immunomodulatory	Upregulation of immune response genes, enrichment of tumor-infiltrating lymphocytes (TILs)	Better prognosis, higher response rates to immunotherapy
Luminal Androgen Receptor (LAR)	Expression of androgen receptor (AR), luminal-associated genes	Less proliferative, luminal-like features, may have better prognosis

2.2.2 Basal-like Subtype

The basal-like subtype is the most common molecular variant of triple-negative breast cancer (TNBC), accounting for approximately 70–80% of all TNBC cases (American Cancer Society, 2023). It is defined by the expression of basal cytokeratins, including CK5/6, CK14, and CK17, and shares significant molecular overlap with basal-like non-TNBC breast cancers. Tumours of this subtype typically exhibit high histological grades, elevated proliferation indices, and a high frequency of TP53 mutations (Xiong et al., 2023). In addition to cytokeratin expression, basal-like TNBCs demonstrate a distinct gene expression profile characterized by the upregulation of genes associated with basal epithelial differentiation and the downregulation of luminal markers, including estrogen receptor (ER), progesterone receptor (PR), and HER2 (Bando et al., 2021). This transcriptional pattern distinguishes them both biologically and clinically from other TNBC subtypes. From a clinical perspective, basal-like TNBC is associated with a poor prognosis, an aggressive disease course, and a higher propensity for distant metastasis. Diagnosis and sub-classification often rely on a panel of immunohistochemical markers, such as CK5, CK6, CK14, CK17, EGFR, smooth muscle actin (SMA), P-cadherin, p63, and c-KIT. These tumours are also notably negative for ER α , PgR, HER2, and luminal cytokeratins (CK8/18/19), further reinforcing their basal phenotype (Wijesinghe et al., 2020). Basal-like tumours typically exhibit a high mitotic index, elevated expression of Ki-67 and p53, and pronounced genomic instability, frequently accompanied by inactivation of the retinoblastoma (Rb) pathway. Their proliferative nature is further supported by the overexpression of cell cycle-related genes, including cyclin E1, BUB1, topoisomerase II α , CDC2, and PCNA (Xiong et al., 2023). These molecular features not only aid in accurate classification but also offer potential targets for therapeutic intervention in a subtype historically resistant to standard hormonal and HER2-targeted treatments.

2.2.3 Mesenchymal Subtype

The mesenchymal subtype of TNBC is marked by the expression of genes linked to epithelial-to-mesenchymal transition (EMT), including vimentin, fibronectin, and Snail. EMT consists of three types with distinct functions: type I aids in embryonic development; type II responds to inflammation during wound healing and tissue regeneration, and type III drives metastasis, which is the primary cause of cancer-related deaths (Chen et al., 2025). EMT is a crucial feature of cancer invasion, involving the transition from epithelial to mesenchymal cell types, which is regulated by various signaling pathways, including TGF- β , Notch, and Wnt, and is influenced by factors such as hypoxia and microRNAs (Chen et al., 2025). These pathways converge on transcription factors such as Snail, Slug, and Twist, thereby promoting the EMT process. Mesenchymal tumours exhibit increased motility, invasiveness, and therapy resistance, often showing molecular characteristics like mesenchymal stem cells, and are linked to a more aggressive clinical profile (Kepuladze et al., 2024).

2.2.4 Immunomodulatory Subtype

The immunomodulatory (IM) subtype of TNBC is defined by the upregulation of genes associated with immune system functions, including lymphocyte activation, antigen processing and presentation, and cytokine-mediated signalling pathways. These tumours are notably enriched with tumour-infiltrating lymphocytes (TILs) and display an immunologically active tumour microenvironment, which contributes to their distinct biological behaviour (Rodríguez-Bautista et al., 2021). Clinically, patients with IM-TNBC often experience a more favourable prognosis and demonstrate higher responsiveness to immunotherapies when compared to other TNBC subtypes. Further sub-classification led to the identification of a related basal-like immune-activated (BLIA) subtype, which exhibits heightened expression of immune regulatory genes (Loizides and Constantinidou, 2023). In contrast to the basal-like immune-suppressed (BLIS) subtype, BLIA tumours are characterized by increased expression of genes involved in B cell, T cell, and natural killer (NK) cell function, alongside elevated activity of signal transducer and activator of transcription (STAT) family genes (Hubalek et al., 2017). This robust immune gene signature correlates with a favourable clinical

outcome, suggesting that STAT pathway activation may play a central role in enhancing anti-tumour immunity within the BLIA subtype (Nunnery et al., 2021).

2.2.5 Luminal Androgen Receptor (LAR) Subtype

The luminal androgen receptor (LAR) subtype of (TNBC) is characterized by the expression of androgen receptors (AR) and luminal-related transcription factors such as Forkhead box protein A1 (FOXA1) and GATA3-binding protein (GATA3), which contribute to its distinct molecular and phenotypic profile (Vtorushin et al., 2022). Compared to basal-like TNBC, LAR tumours exhibit luminal-like features, including lower proliferation rates and a relatively indolent clinical course, particularly among older women. Immunohistochemically, LAR tumours typically express luminal cytokeratins (CK7/8, CK18, CK19) and lack basal cytokeratins (CK5/6, CK14, CK17), distinguishing them from more aggressive TNBC subtypes (Orrantia-Borunda et al., 2022). This cytokeratin expression pattern is associated with a favourable prognosis, further differentiating LAR tumours within the heterogeneous TNBC spectrum. Molecular profiling studies have revealed frequent alterations in the PI3K/AKT signalling pathway in LAR TNBC. Notably, mutations in Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), Lysine methyltransferase 2C (KMT2C), and E-cadherin (CDH1) are recurrent, with PIK3CA emerging as the most frequently mutated gene in this subtype (Vtorushin et al., 2020). These genetic alterations suggest that LAR tumours may be amenable to PI3K-targeted therapies, offering a potential precision medicine avenue for this subgroup.

In addition to genomic alterations, recent studies have highlighted the role of extracellular vesicles, particularly CD151-enriched exosomes, in LAR-associated tumour progression. Investigations using TNBC cell lines, including MDA-MB-231 and MDA-MB-453, have demonstrated that CD151-positive exosomes significantly enhance cell motility and invasive capacity, implicating CD151 as a contributor to tumour aggressiveness (Vtorushin et al., 2022). These findings point to CD151 as a potential therapeutic target, particularly in the context of LAR TNBC, where traditional chemotherapeutic responses may be less robust. The LAR subtype represents a biologically distinct and clinically relevant entity within TNBC, defined by hormone

receptor expression, luminal differentiation markers, and unique genetic alterations (Yin et al., 2020). Continued exploration of its molecular drivers and therapeutic vulnerabilities is critical to improving outcomes for this under-characterized TNBC subset.

2.2.6 Pathogenesis of TNBC

The pathogenesis of TNBC involves the dysregulation of multiple signaling pathways, which contribute to tumour initiation, progression, and metastasis. Key pathways involved in TNBC pathogenesis include biological subtypes of triple-negative breast cancer (Hubalek et al., 2017).

1. **MAPK/ERK Pathway:** Abnormal activation of the mitogen-activated protein kinase (MAPK) pathway, particularly the ERK pathway, is linked to TNBC development. Dysregulated MAPK/ERK signaling fosters cell proliferation, survival, and metastasis in TNBC tumors. This contributes to the aggressive nature of TNBC and its high metastatic potential (Hubalek et al., 2017).
2. **JAK/STAT Pathway:** Alterations in the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway also play a role in TNBC tumourigenesis and progression. Activation of this pathway supports cell proliferation, invasion, and immune evasion in TNBC (Hubalek et al., 2017).
3. **PI3K/Akt/mTOR Pathway:** This pathway, involved in regulating cell survival, growth, and metabolism, is frequently upregulated in TNBC. Activation of the PI3K/Akt/mTOR axis supports tumour cell survival, induces resistance to chemotherapy, and promotes invasion, making it a target for therapeutic strategies (Hubalek et al., 2017).

4. **Wnt/ β -Catenin and Notch Pathways:** These pathways are involved in regulating stem cell behaviour and tissue development. Their aberrant activation in TNBC is associated with increased tumour stemness, invasiveness, and metastasis. These pathways also contribute to resistance against chemotherapy, highlighting their potential as therapeutic targets (Hubalek et al., 2017).
5. **TGF- β Signaling:** In TNBC, TGF- β signaling promotes epithelial-to-mesenchymal transition (EMT), which enhances the metastatic capacity of tumour cells. Dysregulation of TGF- β signalling also helps TNBC cells evade immune surveillance, contributing to the tumour's aggressiveness (Hubalek et al., 2017).
6. **p53 Mutations:** The p53 tumour suppressor gene is frequently mutated or inactivated in TNBC, leading to genomic instability and resistance to apoptosis. This loss of p53 function further fuels the aggressive nature of the disease and its response to chemotherapy (Hubalek et al., 2017).

The diversity and complexity of the molecular subtypes and pathogenesis of TNBC reflect the heterogeneous nature of this aggressive disease. Continued research into the molecular drivers of TNBC and their clinical implications is vital for developing targeted therapies and improving patient outcomes.

2.3 TNBC Treatment

Breast cell receptors play a vital role in identifying where cancer is most responsive, helping guide diagnosis and determine the most effective treatment options. (American Cancer Society, 2025). The lack of the three receptors in TNBC makes treatment difficult and results in high recurrence. The current TNBC treatment is limited to chemotherapy (Mustafa et al., 2016). However, research is advancing in the following areas: poly (ADP-ribose) polymerase (PARP) inhibitors, Immunotherapy, Chemotherapy, and Nanoparticle-based targeted breast cancer therapy.

2.3.1 The poly ADP-ribose polymerase (PARP) inhibitors

Poly (ADP-ribose) polymerase (PARP) plays a crucial role in the repair of DNA damage in both normal and malignant cells (Jones et al., 2015). PARP inhibitors, including olaparib (marketed as Lynparza) and talazoparib (Talzenna), have received regulatory approval for the treatment of advanced HER2-negative breast cancer in individuals carrying BRCA1 or BRCA2 mutations (James et al., 2024). These inhibitors work by disrupting the DNA repair processes in cancer cells with defective BRCA pathways, thereby exacerbating DNA damage and reducing the likelihood of cellular survival (Balmaña et al., 2015). In essence, PARP inhibitors exploit the concept of synthetic lethality to selectively target tumour cells with impaired homologous recombination. Despite their clinical success in BRCA-mutated cancers, there remains uncertainty about the broader applicability of PARP inhibitors in TNBC patients who do not carry BRCA mutations, and ongoing studies continue to explore their potential in this wider population (Morganti et al., 2015).

2.3.2 Immunotherapy

Atezolizumab (commercially known as Tecentriq), in combination with nab-paclitaxel (Abraxane, an albumin-bound formulation of paclitaxel), has been approved as a first-line therapeutic option for patients with metastatic triple-negative breast cancer (TNBC) that expresses programmed death-ligand 1 (PD-L1) (Gion et al., 2025). As an immune checkpoint inhibitor, atezolizumab functions by modulating the immune response to enhance recognition and elimination of tumour cells. Specifically, it targets the PD-L1 protein, which is frequently overexpressed on cancer cells and plays a key role in immune evasion. By blocking PD-L1, atezolizumab restores T-cell activity, thereby facilitating immune-mediated tumour destruction (Lee et al., 2020). Despite encouraging clinical outcomes, particularly in PD-L1-positive cases, several challenges limit the broader success of immunotherapy in TNBC. These include insufficient understanding of intra-tumour heterogeneity, underlying molecular alterations, and the complex immunogenomic environment of TNBC, which collectively hinder the development of precise patient stratification models (Mustafa et al., 2017). To fully harness the therapeutic potential of immunotherapy in TNBC, several critical

issues must be addressed. Firstly, the investigation of the molecular diversity and immune landscape in TNBC to better identify patients who are most likely to benefit from immune-based treatments. Secondly, TNBC encompasses a biologically heterogeneous group of tumours, with variable responses to immunotherapy depending on specific subtype characteristics. This underscores the necessity to identify novel predictive biomarkers beyond conventional markers such as tumour-infiltrating lymphocytes (TILs) and PD-1/PD-L1 expression to improve treatment selection and response prediction. The integration of such biomarkers into clinical protocols could lead to more personalized immunotherapy strategies and improved patient outcomes.

2.3.3 Chemotherapy

A major setback with chemotherapy is the lack of selective toxicity when treating cancer, which results in a narrow therapeutic index and a compromised clinical prognosis (Li et al., 2023). The reduction of damage to normal tissues requires the administration of sub-optimal doses of anticancer chemotherapeutics. In addition, the high interstitial fluid pressure (IFP) of solid tumours results in poor biodistribution and penetration of drugs (Fan et al., 2025). Literature shows that the amount of drug accumulated in normal viscera is 10- to 20-fold higher than that in the same weight of the tumour site (Kannan and Cheng, 2023), therefore, many anticancer drugs cannot penetrate more than 40–50 mm (equivalent to the combined diameter of 3–5 cells) from the vasculature (Wang et al., 2023). These limitations often lead to incomplete tumour response, multiple drug resistance, and in the long run, therapeutic failure (Aldea et al., 2021). These factors highlight the need for alternative treatments for TNBC.

2.3.4 Nanoparticle-based breast cancer therapy

The employment of nanoparticles in cancer research is a cutting-edge approach that utilizes the unique properties of nanoparticles to improve the specificity and efficacy of cancer treatments while minimizing damage to healthy tissues. This approach

focuses on the functionalisation of nanoparticles to enhance the delivery of therapeutic agents directly to cancer cells, thereby enhancing the effectiveness of treatment and reducing systemic side effects (Deivayanai et al., 2024). Below is an in-depth look at the various aspects of nanoparticle-based breast cancer:

2.3.4.1 Nanoparticles as Drug Delivery Vehicles

- **Improved Drug Delivery:** Nanoparticles, due to their small size (typically in the range of 1-100 nm), can penetrate tumour tissues more easily compared to larger molecules. This ability is especially useful for solid tumours like breast cancer. Nanoparticles can be loaded with anti-cancer agents, or even gene therapies, and delivered directly to the tumour site (Gavas et al., 2021).
- **Improved Solubility and Bioavailability:** Many chemotherapy drugs are poorly soluble in water, which limits their effectiveness. Nanoparticles can encapsulate these drugs and improve their bioavailability (Gavas et al., 2021).

2. 3.4.2 Surface Modification of nanoparticles for enhanced Tumour Targeting

- **Ligand-Targeted Nanoparticles:** Nanoparticles can be functionalized with targeting ligands such as antibodies, peptides or small molecules that specifically bind to receptors overexpressed on the surface of cancer cells. This targeted approach ensures that nanoparticles preferentially accumulate at tumour sites while sparing normal healthy tissues (Theivendren et al., 2022).

2.3.5 Cadmium

Cadmium (Cd) poses a significant industrial and environmental risk (Hayat *et al.*, 2019). It is a highly toxic heavy metal that can be introduced into the human body through various environmental sources, including water, food, air, and soil. Long-term exposure to Cadmium has been linked to severe health risks, particularly cancer and toxicity affecting multiple organ systems. Studies have demonstrated that Cadmium's prolonged half-life in the body exacerbates its toxic effects, making it a significant environmental health hazard (Anetor, 2012). As a confirmed carcinogen, Cadmium exposure has been implicated in the development of several types of cancer, such as lung, prostate, breast, and kidney cancers (Arruebfena *et al.*, 2023). However, the chemical reduction of Cd into other forms, such as nanoparticles (NPs), can also be useful as an anti-cancer agent (Deivayanai *et al.*, 2024). Delving into its structure and properties brings understanding to its functions.

Cadmium's atomic number is 48, with a mass number of 112 (Hooser, 2025). Cd's melting and boiling points are at 321 °C and 765 °C, respectively. It has several isotopes, including 106 Cd, 108 Cd, 110 Cd, etc., with isotopes 112 Cd and 114 Cd being the most abundant (Zhong *et al.*, 2024). This element exhibits soft, elastic, silver-white with bluish colour, shiny, and electropositive properties (Ali *et al.*, 2022) as demonstrated in Figure 1. It is also odourless, tasteless, and poisonous (Al-Jamal *et al.*, 2009). Cd forms a range of intricate compounds, including organic amines, Sulfur complexes, Chloro complexes, and chelates (Amat *et al.*, 2014). Soluble salts of carbonates, arsenates, phosphates, and ferrocyanide compounds are formed by Cd ions (Hayat *et al.*, 2019). It also serves as an alloy for electroplating and is involved in pigment production, including Cadmium sulphate and Cadmium selenide (Hayat *et al.*, 2019).

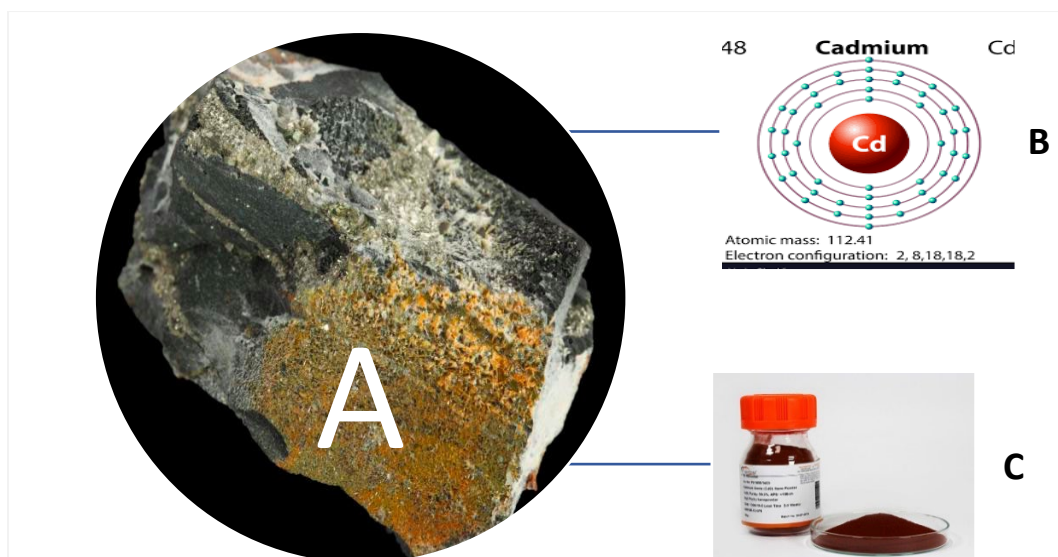


Figure 1 Represents cadmium in different forms: (A) cadmium metal in its natural state, (B) atomic structure of cadmium, and (C) cadmium oxide nanoparticles (CdO NPs) (VectorStock, 2025; Gray, 2008; [Nanosheel](#), 2025).

2.3.6 The significance of Cadmium nanoparticles in cancer therapeutics

To further enhance tumour targeting, CdO nanoparticles can be surface-modified with specific molecules, such as targeting ligands, proteins that bind to receptors overexpressed on tumour cells. These modifications help the nanoparticles to preferentially accumulate at the tumour site, improving the therapeutic or imaging effect. Studies have shown that they can also be used for tumour imaging and as a means to deliver therapeutic agents directly to cancer cells (Anetor, 2012). Research has also established that the nanoparticles and QDs can induce apoptosis in cancer cell lines, as observed in cell culture studies (Hayat et al., 2019). The photoactivation property of QDs is another promising aspect in photodynamic therapy (PDT) and as radiosensitizing agents for cancer treatment (Alizadeh et al., 2025).

These properties make QDs and nanoparticles versatile tools in preclinical cancer treatment research. In laboratory settings, studies typically focus on synthesizing and

characterizing nanoparticles, examining their labeling and localization within biological systems and assessing their potential for pH sensing and other applications. Surface modification of nanoparticles, particularly QDs, is crucial for their successful translation into clinical applications. Modifying the surface properties of the nanoparticles enhances their stability and ability to deliver therapeutic agents directly to tumour cells. These modifications are key to enhancing the safety and efficacy of QDs and nanoparticles in human patients. The application of nanoparticles in Nano medicine offers a unique opportunity for passive cancer therapies. However, the challenge remains to balance the toxicological risks of Cadmium with its potential therapeutic benefits, requiring ongoing research to optimize its use in medical applications. Hence, this study leverages surface modification of nanoparticles to improve drug delivery and therapeutic efficacy.

2.3.7 Mechanisms of CdO NPs delivery to Tumours

Delivery of CdO nanoparticles (CdO NPs) to tumour tissues occurs through both passive and active targeting mechanisms, each contributing to enhanced tumour specificity and therapeutic efficacy. Passive targeting primarily exploits the enhanced permeability and retention (EPR) effect, whereby nanoparticles preferentially accumulate within tumour tissues due to the abnormal architecture of tumour vasculature. Tumour blood vessels are characterised by defective endothelial linings, enlarged inter-endothelial gaps, and impaired lymphatic drainage, collectively creating highly permeable vascular networks that facilitate the extravasation and retention of nanoparticles, typically within the size range of 10–200 nm, in the tumour microenvironment (Yao et al., 2020). This phenomenon promotes selective nanoparticle accumulation at tumour sites while reducing systemic distribution to healthy tissues.

In contrast, active targeting enhances delivery specificity through surface functionalisation of CdO NPs with ligands such as peptides, biomolecules, or monoclonal antibodies that recognise receptors overexpressed on cancer cells. Common targets include transferrin, folate, and epidermal growth factor receptors (EGFR), which mediate selective binding of nanoparticles to malignant cells (Bazak et

al., 2015). Following receptor recognition, the nanoparticle–receptor complex undergoes receptor-mediated endocytosis, enabling efficient intracellular uptake of the nanoparticles (Yameen et al., 2014).

Once internalised, the nanoparticles become entrapped within early endosomes. However, successful intracellular drug delivery requires escape from endosomal degradation pathways. During endosomal maturation, proton pumps acidify the vesicular environment, triggering the degradation of pH-sensitive nanoparticle bonds or inducing conformational changes that destabilise the endosomal membrane, a process known as endosomolysis (Desai et al., 2024). This facilitates the release of therapeutic cargo, including chemotherapeutic agents, siRNA, or miRNA, directly into the cytoplasm where they can exert their biological effects. Such targeted intracellular delivery not only enhances therapeutic selectivity and minimises off-target toxicity but also enables nanoparticles to bypass P-glycoprotein efflux pumps commonly implicated in multidrug resistance, thereby improving intracellular drug retention and treatment efficacy (Gao et al., 2024).

Beyond their role as delivery vehicles, CdO NPs exhibit intrinsic anticancer activity through multiple molecular and cellular mechanisms. A principal mechanism involves the excessive generation of reactive oxygen species (ROS), which induces oxidative stress and results in lipid peroxidation, protein oxidation, mitochondrial dysfunction, and DNA damage within cancer cells (Nakamura and Takada, 2021). The high surface-area-to-volume ratio of nanomaterials further enhances cellular uptake through endocytosis and direct membrane penetration, thereby increasing cadmium bioavailability and prolonging intracellular activity (Peng et al., 2024). Additionally, CdO NPs can generate ROS through several interconnected pathways, including nanoparticle dissolution and subsequent release of Cd^{2+} ions, disruption of mitochondrial electron transport processes, and activation of NADPH oxidase enzymes (Peng et al., 2024). Collectively, these effects activate apoptotic signalling pathways and promote selective tumour cell death.

2.3.8 Albumin-coated nanoparticles

Albumin is the most abundant protein in human blood plasma. It is primarily derived from blood plasma and accounts for about 50% of the total protein content. There are several types of albumin, including human serum albumin (HSA), bovine serum albumin (BSA), equine serum albumin (ESA), and murine serum albumin (MSA). Among these, HSA and BSA are the most widely used in biomedical research and clinical applications. The structures of HSA and BSA are highly similar, both exhibiting a heart-shaped configuration. HSA is composed of 585 amino acids with a molecular weight of approximately 69.367 kDa, while BSA contains 583 amino acids and has a molecular weight of around 69.293 kDa (Moman et al., 2022).

Due to their non-immunogenic, non-cytotoxic properties and the presence of multiple drug-binding sites, albumins are extensively used in biomedical applications, particularly in drug delivery systems (Hu et al., 2022). Albumin binds to a variety of endogenous and exogenous biomolecules, facilitating its role as an ideal carrier for drug delivery. Notably, albumin receptors such as GP60, GP30, GP18, secreted protein acidic and rich in cysteine (SPARC), FcRn, cubilin, and megalin play pivotal roles in the transport and targeting of albumin-based drug carriers (Molitoris et al., 2022). Of these, GP60 and SPARC are especially crucial in the delivery of antitumour drugs using albumin as a carrier. Figure 2 shows albumin functionalised nanoparticles for enhanced targeted therapy.

The GP60 receptor is predominantly found on vascular endothelial cells, excluding brain tissues, and is involved in the transport of albumin across the vascular endothelium. Once albumin binds to GP60, the receptor interacts with an intracellular protein called caveolin-1, triggering the formation of transcellular vesicles that allow albumin to cross the endothelial barrier (Hama et al., 2021). In the context of cancer, SPARC is overexpressed in tumour cells, which facilitates binding to GP60 on the albumin (Ji et al., 2024)

The integration of albumin in cancer therapeutics is advantageous due to its inherent biocompatibility and unusual binding capacity to carry variant drug molecules (Jaiswal et al., 2025). It is also an efficient material for nanoparticle construction owing to its physicochemical stability and functionality. Albumin as a macromolecular carrier is

imperative to use in a nanoparticulate carrier system because of its biodegradability, nontoxicity, the ability to produce non-harmful degradation by-products during its metabolism *in vivo*, non-immunogenicity, easy purification, and its water solubility, allowing ease of delivery by injection (Liu et al., 2024). From a physiological perspective, albumin has a long serum half-life (19 days on average), which enhances its ability to carry various proteins and hydrophobic molecules (such as vitamins and hormones) throughout the body, thereby making it a suitable candidate for nanoparticle carrier design (Liu et al., 2024). An albumin-coated Cadmium oxide nanoparticle delivery system can be an efficient TNBC anti-therapeutic agent with reduced cytotoxicity on normal cells. The methodology employed for nanoparticle synthesis can have an effect on their anticancer efficacy. In this study, green chemistry and chemical synthesis methods were employed and compared.

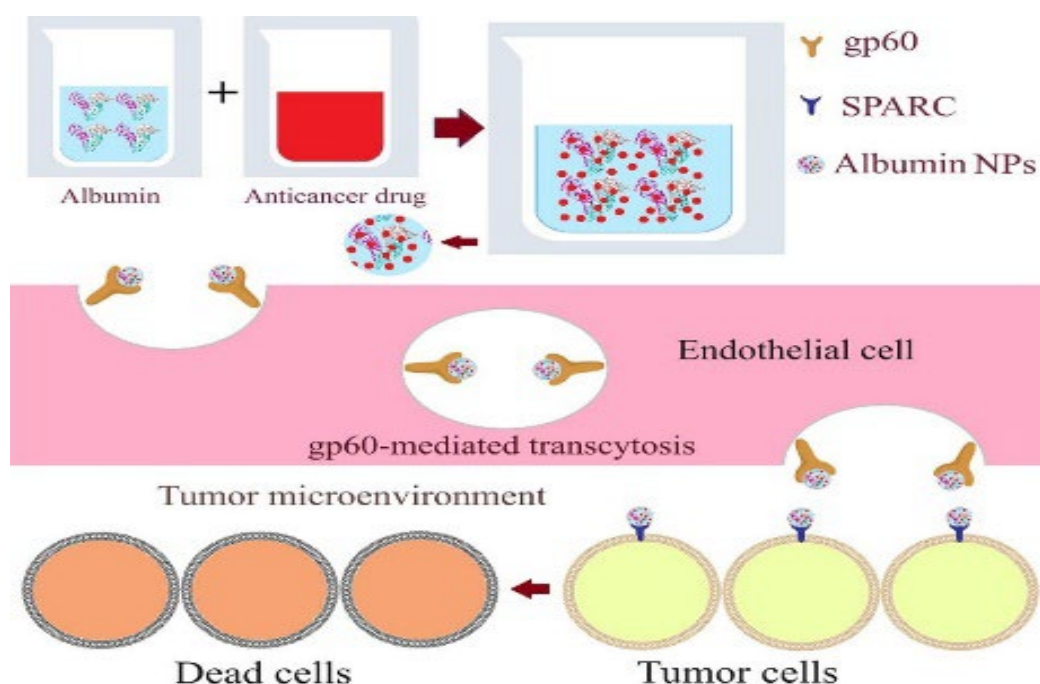


Figure 2 Shows how albumin-coated Cadmium oxide nanoparticles target tumour cells via gp60 and SPARC receptors. Asrorov et al., 2024. Available at https://ars.els-cdn.com/content/image/1-s2.0-S2590098624000113-ga1_lrg.jpg

2.4 Synthesis of CdO NPs

2.4.1 Green Synthesis of Nanoparticles

Green synthesis involves biological precursors such as bacteria, fungi, algae, and plant extracts, which are environmentally conducive in the synthesis of nanomaterials. Various medicinal plants can be used owing to the presence of phytochemical components, such as terpenoids, flavonoids, carboxylic acids, phenols, and ascorbic acids (Abuzeid et al., 2023).

The reduction of metal salts to metal nanoparticles is mediated by phytochemicals, which are advantageous because the leaf precursor exhibits a shorter reduction time during nanoparticle formation compared with fungi and bacteria, which require longer incubation times (Singh et al., 2018). Flavonoids comprise various functional groups, which have a heightened capacity to reduce metal ions. In addition, they have tautomeric alterations, which allow the reactive Hydrogen atom to be released, and the enol form to be transformed to the keto form. This is the process recognised by the reduction of metal ions into metal nanoparticles. Several reaction parameters, such as solvent, temperature, pressure, and pH (acidic, basic, or neutral), are considered in the synthesis of metallic nanoparticles using biological procedures (Singh *et al.*, 2018). The parameters influence the rate of nanoparticle formation, the product (size and morphology), and their stability. The medicinal plant used in the green chemistry route is *Synadenium cupulare* (*S. cupulare*).

Synadenium cupulare (Boiss. L.C), also known as *Euphorbia cupularis* or Dead Man's tree (Figure 3), is a member of *Euphorbiaceae*. This plant is found in Southeastern Africa - Southern Mozambique, Swaziland, and South Africa. In South Africa, *S. cupulare* is distributed in KwaZulu-Natal, Mpumalanga, and Limpopo Provinces. Lefojane et al., (2021) reported that *S. cupulare* leaf extract possesses secondary metabolites such as tannins, flavonoids, saponins, and glycosides, while the stem extract showed the presence of phytosterols, pentose, tannins, glycosides, triterpenoids, saponins, and flavonoids. These phytochemical constituents have anti-cancer activity (Fakudze et al., 2023) and are also vital in the green synthesis method of nanoparticles due to their reduction and capping ability.



Figure 3 Depicts *S. cupulare* in its natural habitat (Solomon, 2011).available at (<https://kumbulanursery.co.za/plants/synadenium-cupulare>)

2.4.2 Chemical Reduction Method

Various chemical methods exist for the synthesis of nanoparticles, including chemical reduction, micro-emulsion, electrochemical, and thermal decomposition techniques (Zadehnazari, 2025). Among these, chemical reduction is the most widely used due to its simplicity. This method involves reduction of metal ions to their elemental form, which then aggregates to form nanoparticles (Zadehnazari, 2025). Chemical reduction typically relies on reducing agents that donate electrons to metallic salts, converting them into the corresponding metal nanoparticles. Common reducing agents employed in this process include sodium borohydride, ascorbate, and elemental hydrogen (Mourdikoudis, 2021). In the chemical reduction method for nanoparticle synthesis, several key components are involved. The process begins with the metallic precursor, which consists of metal salts or metallic ions that serve as the starting material for nanoparticle formation. Common examples of these metal salts include silver nitrate (AgNO_3), Gold chloride (AuCl_3), and Copper sulfate (CuSO_4), among others (Beyene et al., 2017). The next essential component is the reducing agent, which plays a critical role in converting metal ions into their elemental form by donating electrons. Some widely used reducing agents include Sodium borohydride (NaBH_4), a strong reducing

agent commonly employed in the synthesis of nanoparticles such as silver, gold and platinum; ascorbic acid, an organic reducing agent known for its environmentally friendly properties and mild reduction capabilities; and elemental Hydrogen, which is used in certain cases, especially in aqueous environments, to reduce metal ions. Lastly, stabilizing agents are employed to prevent agglomeration or uncontrolled growth of nanoparticles. These agents bind to the surface of the nanoparticles, ensuring their stability and preventing them from clumping together. Common stabilizers include polyvinyl alcohol (PVA), citric acid and polyethylene glycol (PEG), all of which help maintain the uniformity (Abbas et al., 2024).

2.5 Systemic Nanoparticle Toxicity

Systemic nanoparticle toxicity is a major consideration in nanomedicine and is influenced by several physicochemical properties that determine how nanoparticles interact with biological systems. Before biological evaluation, nanoparticles must be comprehensively characterised, as properties such as size, surface area, surface charge, shape, chemical composition, and solubility directly influence their biodistribution, cellular uptake, and toxicological behaviour (Awashra and Młynarz, 2023).

Initial toxicological assessment is commonly performed through *in vitro* screening using cultured cells to evaluate early cellular responses following nanoparticle exposure. Cell viability assays, including MTT and lactate dehydrogenase (LDH) assays, are widely used to determine alterations in metabolic activity and membrane integrity (Savage et al., 2018). Nanoparticles frequently induce toxicity by generating reactive oxygen species (ROS), which results in oxidative stress, mitochondrial dysfunction, lipid peroxidation, and activation of inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α). Furthermore, genotoxicity assays, including comet and micronucleus assays, are employed to assess DNA damage and chromosomal instability associated with nanoparticle exposure. Although *in vitro* models provide rapid and cost-effective toxicity screening, they cannot fully replicate the complexity of whole-organism responses (Awashra and Młynarz, 2023).

Consequently, *in vivo* studies using animal models, typically mice, remain essential for evaluating systemic nanoparticle toxicity. These studies investigate biodistribution, pharmacokinetics, and organ-specific toxicity following nanoparticle administration. Biodistribution analyses utilise techniques such as radiolabelling and inductively coupled plasma mass spectrometry (ICP-MS) to monitor nanoparticle accumulation and clearance in organs including the liver, spleen, kidneys, lungs, and brain (Lazareva et al., 2025). Pharmacokinetic studies evaluate absorption, distribution, metabolism, and excretion (ADME) processes to determine circulation time, metabolic fate, and elimination pathways, thereby informing optimal dosing strategies and routes of administration. *In vivo* toxicological investigations also assess both acute and chronic toxicity, including haemolysis, inflammatory responses, behavioural alterations, reproductive toxicity, and long-term organ damage resulting from repeated exposure (Li et al., 2019).

The evaluation of nanoparticle toxicity forms part of the broader preclinical development process, which comprises progressive pharmacological and toxicological investigations designed to establish efficacy and safety before clinical application. Preclinical studies begin with *in vitro* pharmacological screening to confirm mechanisms of action and identify promising candidate formulations. Selected candidates subsequently undergo *in vivo* efficacy and toxicology testing to establish preliminary safety profiles, including acute, subchronic, chronic, and reproductive toxicity assessments. Pharmacokinetic and pharmacodynamic studies are then conducted to quantify dose-response relationships, therapeutic thresholds, and toxicity limits while evaluating the biological activity of the nanoparticles at cellular and systemic levels.

Chapter 3: Synthesis, characterisation and *in vitro* evaluation of Cadmium oxide nanoparticles

3.0 Introduction

To properly evaluate the safety and impact of a nanomaterial, the fundamental physicochemical properties of the material and its interaction and impact upon living cellular systems must be understood. In this section, two approaches for synthesizing CdO NPs are detailed: green synthesis, which utilises environmentally friendly and sustainable processes, and chemical reduction, a method involving the use of reducing agents. The most common methods to characterize nanomaterial properties and a set of *in vitro* toxicity screenings used to determine material safety are presented. The approaches presented herein do not represent the entirety of all available tools and methods but rather a sufficiently broad initial assessment for the evaluation of a new nanomaterial. Cytotoxicity analysis was then performed using the MTT assay to assess the potential cytotoxicity of these nanoparticles. To enhance their biocompatibility and targeting ability, the nanoparticles that demonstrated the highest inhibitory activity on cancer cells were coated with albumin. The impact of this coating on the nanoparticles' properties and biological interactions was also evaluated. Finally, the results of these experiments are presented, providing insights into the synthesis, characterization, and potential anti-proliferative effect of albumin-coated cadmium oxide nanoparticles.

3.1 Materials

Synadenium cupulare (*S.cupulare*) leaves (from the Kruger National Park, South Africa), cadmium nitrate tetrahydrate 98% (sigma Aldrich, South Africa), trisodium citrate ($C_6H_5O_7Na_3$) (642045, Sigma Aldrich, South Africa), ascorbic acid ($C_6H_8O_6$) (255564, Sigma Aldrich, South Africa), Human cell line MDA-MB 231 (University of the Free State - UFS), Vero cell line (UFS), Dulbecco's modified eagle's medium (DMEM), (D6429, Gibco Separation PTY Ltd, South Africa), fetal bovine serum (FBS) (SH30070.03IR, Gibco), penicillin (P4333, Sigma Aldrich, South Africa), streptomycin (S9137Sigma Aldrich, South Africa), 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl

tetrazolium bromide (MTT) (M2128, Sigma Aldrich, South Africa), Bovine Serum Albumin (A3059, Sigma, Aldrich South Africa).

3.2 Plant Collection and Extraction

S. cupulare was collected from its natural habitat in the Kruger National Park, Limpopo Province, South Africa, in the winter season in June 2022. A botanist at the Botany Department, University of the Free State (UFS) authenticated the plant. The specimen was stored in the herbarium, UFS. The plant leaves were washed with distilled water, cut into smaller pieces, and dried at room temperature. The plant material (30 g) was mixed with 300ml boiled de-ionized water (dH₂O) and heated for 2-3 hours at 60 °C whilst continuously stirring (Figures 4A and 4B). The mixture was allowed to cool at room temperature and filtered using Whatman filter paper (110mm). The protocol was adapted from Mohsenipour and Hassanshahian (2016) with some modifications.

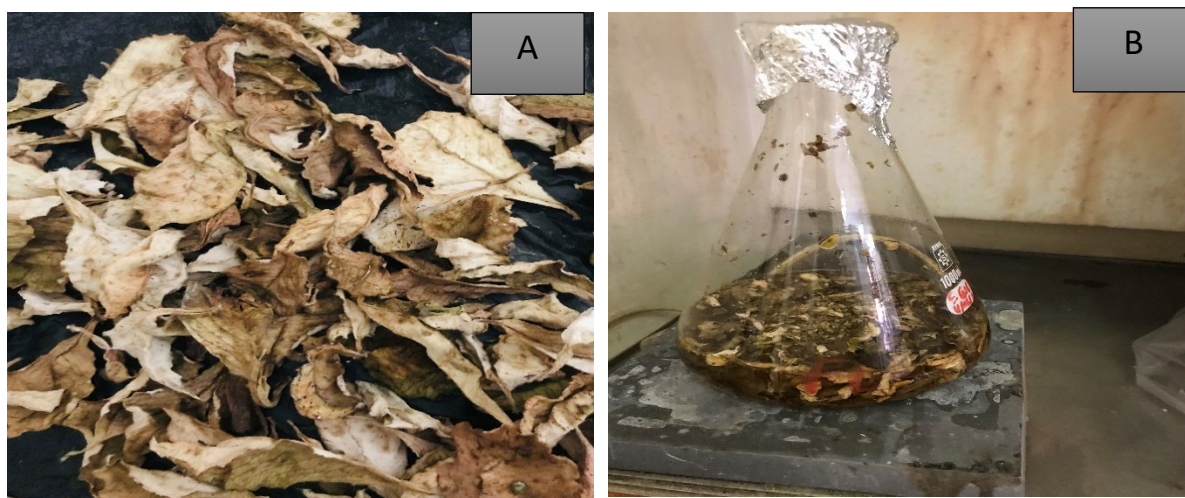


Figure4 (A) Dried leaves of *S. cupulare* at room temperature, and (B) small pieces of *S. cupulare* leaves undergoing extraction in distilled water.

3.3 Synthesis of nanoparticles:

The green chemistry and chemical synthesis approaches employed in the synthesis of Cadmium oxide nanoparticles are outlined in detail, highlighting the methodologies, reagents, and reaction conditions utilized to ensure successful nanoparticle formation.

3.3.1 Green Chemistry

100 ml of the *S. cupulare* leaf water extract (pH = 5 and a temperature of 23 °C) was mixed with 5 g of Cadmium nitrate tetrahydrate (98%). 5 g of salt was used to obtain a ratio of 1:20. The mixture was left to stir at 60-70 °C on a magnetic hot plate for 24 hours. The mixture was then transferred into a Petri dish and dried in the oven at a temperature of 70 °C. The dry nanoparticles were kept in the dark. The protocol was adapted from Yasi et al., (2017) and Lefojane et al., (2021) with minor modifications.

3.3.2 Chemical reduction

In this study, Cadmium nitrate tetrahydrate (CdNO_3) was used as a precursor, while trisodium citrate ($\text{C}_6\text{H}_5\text{O}_7\text{Na}_3$) and ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) were used as the reducing agent and surfactant, respectively. Specifications included heating 80 ml of CdNO_3 to 60 °C and then adding (with vigorous stirring) to 20 ml of a $\text{C}_6\text{H}_5\text{O}_7\text{Na}_3$ and $\text{C}_6\text{H}_8\text{O}_6$ solution that was pre-heated to 60 °C. The mixture was stirred for 20 minutes. Afterwards, the solution was cooled at room temperature with continuous stirring. The appearance of yellowish/or white colour in the reaction was indicative of the formation of Chem CdO NPs (Azizi, 2018).

3.4 Characterization of the Nanoparticles

The nanoparticle formulations were comprehensively characterized using advanced techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Ultraviolet-Visible Spectroscopy

(UV-Vis), and Energy-Dispersive X-ray Spectroscopy (EDX) to determine the structural, chemical, and optical properties.

3.4.1 X-ray Diffraction (XRD)

X-ray diffraction (XRD) analysis was performed using a Bruker AXS D8 Advance X-ray diffractometer equipped with a monochromated Cu K α radiation source (wavelength = 1.5406 Å), operating at a current of 40 mA and a voltage of 40 kV in Bragg-Brentano geometry. This technique was utilized to determine the crystallographic structure of the nanoparticles, providing detailed information about their phase composition, crystallinity, and average crystallite size through the analysis of diffraction peaks. XRD patterns are crucial for identifying the crystalline nature and purity of the synthesized CdO nanoparticles and offer insight into their lattice parameters and crystallization behaviour (Burlec et al., 2023).

3.4.2 Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were employed to examine the morphology, particle shape, and size distribution of the G CdO NPs, Chem CdO NPs, and A-Chem CdO NPs. TEM allows for high-resolution imaging at the nanoscale, enabling the visualization of the internal structure and precise measurement of the nanoparticle sizes. SEM, on the other hand, provides valuable surface information, including surface texture and topography. Both techniques complement each other by offering a comprehensive view of the nanoparticle's surface and internal features, crucial for understanding their behaviour in various applications (Burlec et al., 2023).

3.4.3 Energy-Dispersive X-ray Spectroscopy EDS

Energy-Dispersive X-ray Spectroscopy (EDS), typically integrated with Scanning Electron Microscopy (SEM), is a widely used analytical technique for identifying and quantifying the elemental composition of nanoparticles. EDS operates by detecting characteristic X-rays emitted when the sample is exposed to an electron beam, enabling rapid, localized analysis of surface and bulk elemental content (Goldstein et al., 2018). In the characterization of Cadmium oxide (CdO) nanoparticles, EDS confirms the presence of Cadmium (Cd) and Oxygen (O), while also identifying residual elements such as Chlorine (Cl). The technique is particularly useful in validating synthesis outcomes across different formulations, including green-synthesized, chemically synthesized, and albumin-functionalized CdO nanoparticles.

3.4.4 Ultraviolet-Visible Spectroscopy (UV-VIS)

UV-VIS spectroscopy was conducted using a Shimadzu 1800 double-beam spectrophotometer to measure the absorbance spectra of the CdO nanoparticles in the ultraviolet and visible light range. This technique is pivotal in determining the optical properties of the nanoparticles, such as the band gap energy, which can be inferred from the absorption edge. The UV-VIS spectra provide insights into the electronic structure and potential applications of the nanoparticles in optoelectronic devices, as well as their suitability for applications in sensors and photocatalysis (Lefojane et al., 2021).

3.4.5 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups and chemical bonds present in the synthesized nanoparticles. Using a Shimadzu FTIR spectrometer, the infrared spectrum was recorded to detect both organic and inorganic compounds associated with the nanoparticles. The FTIR

spectrum offers valuable information about the surface chemistry of the nanoparticles, including the presence of adsorbed molecules, surfactants, and any surface functionalization (e.g., albumin coating) that may influence their interaction with biological systems or other materials (Burlec et al., 2023).

3.4.6 X-ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) is a crucial analytical technique employed to investigate the surface chemistry of nanoparticles, offering insights into elemental composition and chemical states (Chen et al., 2020). Given its surface sensitivity, typically probing the top 1–10 nm, XPS is particularly well-suited for characterizing nanomaterials designed for biomedical applications, where surface properties have a critical influence on biological interactions. In the context of Cadmium oxide (CdO) nanoparticles, XPS enables the identification of key surface elements such as Cadmium, Oxygen, Chlorine, Magnesium, and Sodium, as well as their respective oxidation states. This is essential for confirming successful synthesis and functionalization. XPS aids in detecting residual precursors or contaminants, thereby providing insight into potential sources of cytotoxicity (Chen et al., 2020).

These characterization techniques collectively provided a comprehensive understanding of the properties of the CdO nanoparticles and their potential for a wide range of applications, from environmental remediation to biomedical use.

3.5 Biological assay for cytotoxicity assessment

For cytotoxicity screening, both MDA-MB-231 (triple-negative breast cancer) and Vero (non-cancerous kidney epithelial) cell lines were initially subcultured under standard conditions, after which the MTT assay was conducted to assess the dose-dependent cytotoxic effects of the synthesized Cadmium oxide nanoparticles. Doxorubicin, a chemotherapeutic agent with a well-established cytotoxic profile, was included as a reference standard to benchmark the efficacy of the nanoparticles.

3.5.1 Cell Sub-culturing and Passaging

The laminar flow hood was prepared by switching on the UV light for 10 minutes to sterilize the area. Simultaneously, the complete growth media (DMEM containing 20% FBS) was warmed in a 37 °C dry bath. All procedures were carried out in the laminar flow hood using aseptic techniques to maintain sterility. This included spraying gloves, the hood surface, and materials to be used with 70% ethanol, followed by wiping with a clean paper towel.

4 ml of complete media was pipetted into two T-25 cm³ flasks, which were labelled with the cell culture name and date, and placed in a CO₂ incubator. Cryovials containing Vero and MDA-MB-231 cell lines were removed from the -80 °C storage freezer and thawed in a 37 °C dry bath for no more than 2 minutes, until only a small ice crystal remained. Once thawed, 1 ml of cell suspension was pipetted into each flask containing media and incubated for 24 hours. The cells were then examined under an inverted microscope to ensure survival and attachment. After 24 hours, the spent media was aspirated and replaced with 5 ml of fresh media. The flasks were returned to the incubator and frequently monitored microscopically for confluency.

Passaging was performed once the cells reached 80% confluence. First, the spent media was aspirated, and 2.5 ml of PBS was used to rinse the flask. Next, 2.5 ml of trypsin-EDTA was added and incubated for approximately 3 minutes. Fresh media (10% FBS in DMEM) was prepared in new flasks (12 ml for T-75 cm³ and 4 ml for T-25 cm³). After confirming that the cells had detached, the trypsinization process was stopped by adding media and thoroughly mixing. The cell suspension was transferred to a conical tube and centrifuged at 1,000 rpm for 5 minutes to obtain a pellet. The supernatant was aspirated, and fresh media was added to the pellet. The cell mixture was then pipetted into new flasks for further incubation.

3.5.2 MTT Assay

The cytotoxic effects of green-synthesized Cadmium oxide nanoparticles (G-CdO NPs) and chemically synthesized Cadmium oxide nanoparticles (Chem -CdO NPs) were assessed using the MTT assay. MDA-MB-231 (human breast cancer) and Vero (non-cancerous kidney epithelial) cells were seeded into 96-well plates at a density of 1×10^5 cells/ml in 100 μ l of complete growth media. After 24 hours of incubation at 37 °C in a humidified 5% CO₂ atmosphere to allow cell attachment, the cells were treated with increasing concentrations of G-CdO and C-CdO NPs (1.25, 2.5, 5, 10, 25, 50, and 100 μ m). Doxorubicin served as the positive control and was administered at concentrations of 0.1, 1, 5, 10, 50, and 100 μ m, while untreated cells served as the negative control.

Following 48 hours of treatment, the culture medium and treatment solutions were aspirated and replaced with 100 μ l of MTT solution (5 mg/ml in PBS). After a 4-hour incubation period at 37 °C, the MTT solution was removed, and 100 μ l of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals formed by viable, metabolically active cells. The plates were gently shaken on a shaker to ensure complete dissolution.

The optical density (OD) was measured at 560 nm using a GloMax Multiplate reader. The absorbance values are directly proportional to the number of viable cells. Cell viability (%) was calculated relative to the negative control group using the formula:

The percentage inhibition of the cells was calculated as:

$$X = 100 - ((A_{\text{test}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100);$$

- X is the percentage inhibition,
- A_{test} is the absorbance of the test sample,
- A_{blank} is the absorbance of the blank
- A_{control} is the absorbance of the control sample.

3.5.3 Statistical analysis

The half-maximal inhibitory concentration (IC_{50}), representing the nanoparticle concentration required to reduce cell viability by 50% compared to untreated controls, was determined using dose–response curves as described. All experiments were conducted in triplicate with three independent biological repeats.

3.6 Coating of chemically reduced Cadmium oxide nanoparticles with Albumin

The chemically synthesised Cadmium oxide nanoparticles (Chem CdO NPs) were coated with Bovine Serum Albumin (BSA) using the nanoprecipitation method to improve their biocompatibility.

3.6.1 Preparation of albumin-coated CdO NPs

Two solutions, labelled A and B, were prepared separately for the synthesis:

Solution A: This solution was made by dissolving 20 μ l of absolute ethanol in 230 μ l of dichloromethane. The combination of these two solvents creates an organic medium that can suspend or dissolve BSA (fraction V, minimum 98%, Sigma). Dichloromethane, being a volatile solvent, aids in dissolving both BSA and CdONPs into the organic phase (Azizi et al., 2018).

Solution B: For the albumin-coated CdO nanoparticle solution (A-Chem CdO NPs), 0.05 g of BSA was dissolved with 1000 μ l of a PBS/dichloromethane mixture (99:1, v/v), along with 1000 μ l of CdONPs. This produced a final concentration of 5% BSA and 1 mM CdONPs, in line with the protocol described by Azizi et al. (2018).

Additionally, the albumin blanks (NAs) were synthesized as a blank control. In this process, a solution of BSA dissolved in 1000 μ l of PBS/dichloromethane (99:1, v/v) was added to Solution A. The mixture was then gently agitated for 5 minutes. After a crude emulsion was formed, the mixture was transferred to a blender, where emulsification continued at room temperature until a milky suspension was obtained

(Azizi et al., 2018). This method enabled the successful coating of CdONPs with albumin.

3.7 Results

This section presents the results of nanoparticle synthesis, physicochemical characterization, cytotoxicity evaluation, and albumin functionalization, providing an assessment of their potential as anticancer agents.

3.7.1 Characterization results of synthesised CdO NPS

Cadmium oxide nanoparticles (CdO NPs) were successfully synthesized using both green chemistry and chemical synthesis approaches. A preliminary indication of successful synthesis was observed through distinct colour changes during nanoparticle formation. Specifically, the green-synthesized CdO nanoparticles (G-CdO NPs) exhibited a characteristic transition to a brownish colour (Figure 5A), consistent with phytochemical-mediated reduction and nanoparticle nucleation. In contrast, the chemically synthesized CdO nanoparticles (Chem-CdO NPs) displayed a whitish appearance (Figures 5B and 5C), which is typical for Cadmium oxide produced via precursor-based methods. To confirm the structural integrity, elemental composition, and morphological features of the synthesized nanoparticles, a comprehensive suite of characterization techniques was employed.

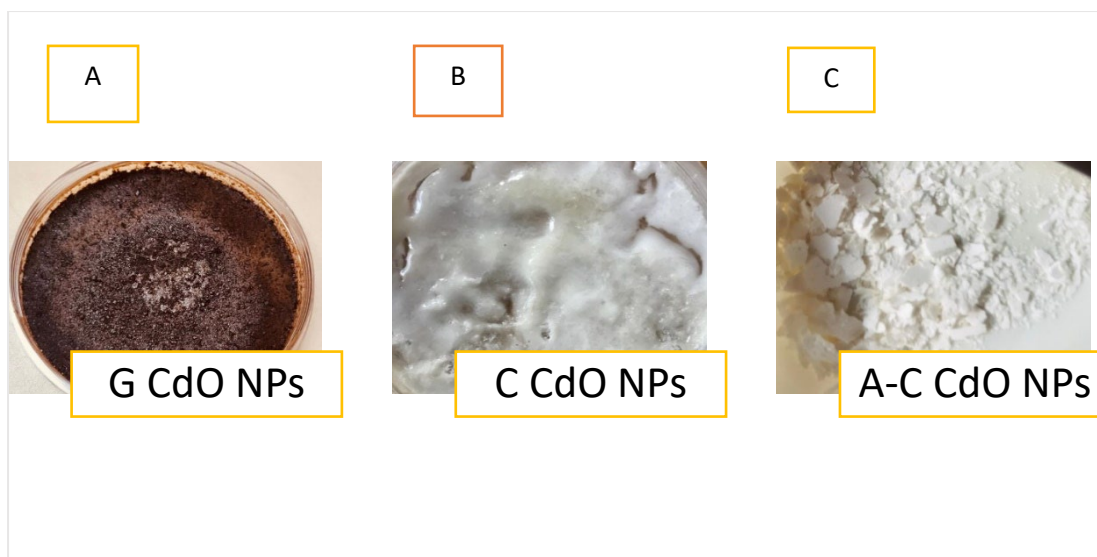
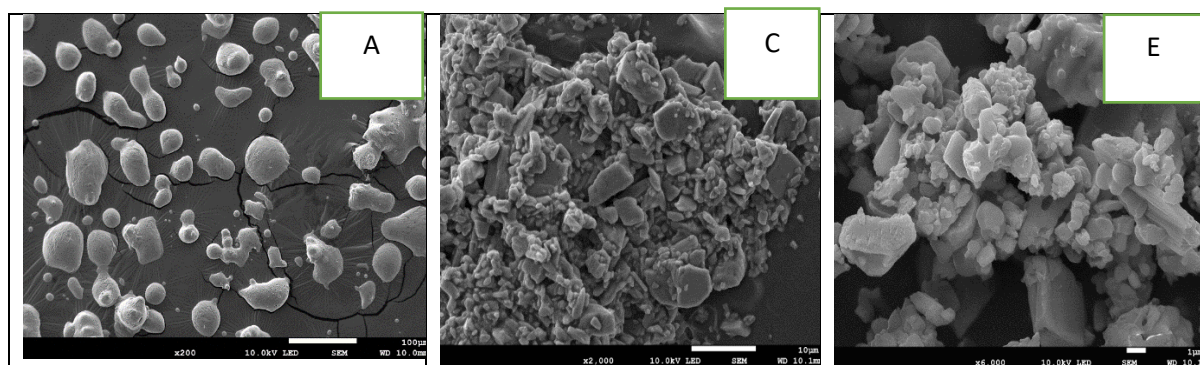


Figure 5. Visual appearance of the dried nanoparticle formulations after oven drying at 70 °C: (A) G-CdO NPs, (B) Chem-CdO NPs, and (C) A-Chem-CdO NPs.

3.7.1.1 Morphological analysis

Scanning Electron Microscopy (SEM) is an analytical technique that involves scanning an electron beam over a sample to create a magnified visual image. SEM images of G-CdO NPs, Chem CdO NPs, and A-Chem CdO NPs resulted in different morphologies. The size distribution of the AC-CdO NPs was determined using ImageJ software. Figures 6A and 6B reveal NPs showing spherical and regular morphology at a scale of 100 μm . The size distribution is 0,25 - 127 nm. The chemically synthesized CdO NPs showed SEM image of Cube like and agglomerated morphology at a scale of 1000 nm, with the size distribution of 0,25 -4,74 nm (Figs. 6C and 6D). Lastly, albumin-coated CdO NPs showed self-assembled clusters of non-uniform morphology, with a size distribution 0,25 - 94 nm. This morphology is depicted in Figures 6E and 6F.



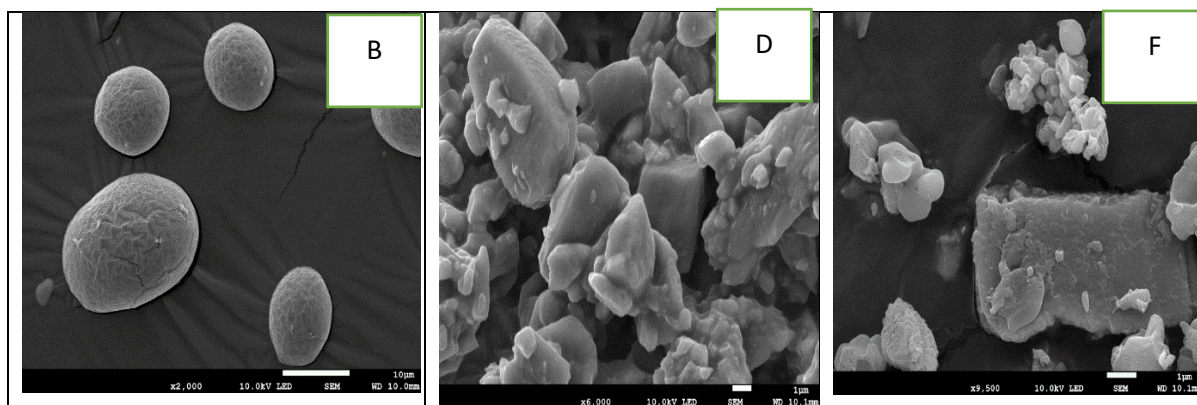


Figure 6 shows SEM images of the NPs at different magnifications. Figures **6A** and **6B** are images of G CdO NPs at X200 and X2000 magnification. **6C** and **6D** show C-CdO NPs at X2000 and X6000. **6E** and **6F** show A-Chem CdO NPs at X6000 and X9500.

Table 2. Summary table of morphology, scale, and particle size distribution of G-CdO NPs, Chem-CdO NPs, and A-Chem CdO NPs

Figure	Morphology	Scale (nm)	Size Distribution (nm)
Figures 7A and B (G CdO NPs)	Spherical and irregular	100 nm	0,25 - 127 nm
Figures 7C and D (Chem CdO NPs)	Cube like and agglomerated	1000 nm	0,25 - 4,74 nm
Figures 7E and F (A-Chem NPs)	self-assembled clusters of non-uniform morphology	100 nm	0,25 - 94 nm

3.7.1.2 Transmission electron microscopy

TEM results revealed spherical and non-uniform shapes of G CdO NPs (Figure 7A). The spherical shape is consistent with the SEM results. Additionally, the Chem CdO NPs (Figure 7B) showed agglomerated and irregular shapes, thus aligning with the

SEM analysis with slight differences. Lastly, A-Chem CdO (Figure 7C) NPs showed spherical and irregular morphology. The dark region in the TEM images is due to deposition of BSA on CdO nanoparticles.

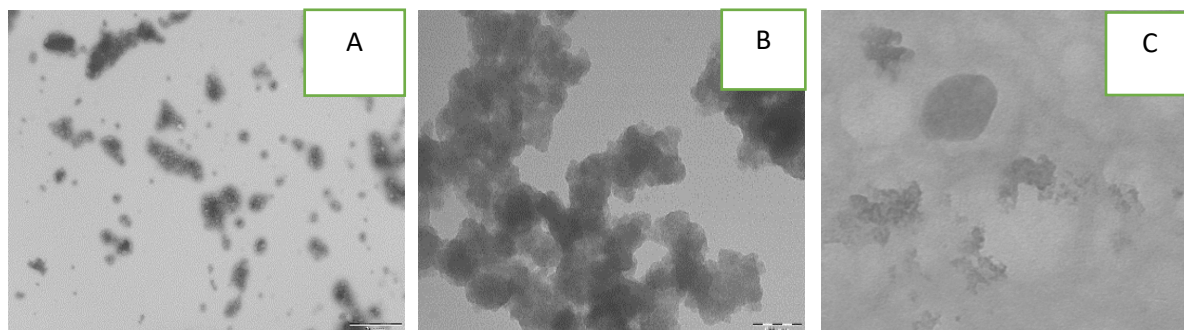


Figure 7 TEM micrographs of CdO NPs formulations: (A) G-CdO NPs displaying spherical and non-uniform morphologies; (B) Chem-CdO NPs showing irregular and agglomerated particles; and (C) (A-Chem CdO NPs) illustrating cadmium particles embedded within the albumin matrix with partial spherical morphology.

3.7.2 The crystallographic structure of the G CdO NPs, Chem CdO NPs and A-Chem CdO NPs

The XRD technique was utilized to determine the crystallographic structure of the nanoparticles, providing detailed information about their phase composition, crystallinity, and average crystalline size through the analysis of diffraction peaks. XRD patterns of CdO nanoparticles synthesised through green chemistry (*S. cupulare*), chemical reduction, and albumin-coated chemically synthesised NPs were observed at different temperatures to determine the influence of temperature on their crystallinity. However, further characterization was made on the nanoparticles exposed to 100°C, as to their properties. The crystallinity of the nanoparticles influences their therapeutic efficacy of the nanoparticles. The Crystalline Size was calculated using the Scherrer equation:

$$D = K \frac{\lambda}{\beta \cos \theta}$$

D= crystalline size (nm)

$K=0.9$ (Scherrer constant)

$\lambda=0.15406$ nm (wavelength of the x-ray source)

B =FWHM (radians)

θ =Peak position (radians)

The crystallinity sizes of G CdO NPs at temperatures of 100, 200, 300, 400 and 500 °C were 17.9, 67.4, 0.3, 86.8 and 72.3 nm, respectively, as shown in Figure 8. This pattern indicates that with increasing temperature, crystallinity size increases.

However, at 300 there is an inconsistency with the results. Having at least duplicates or triplicates of the same results can verify the correct pattern. The crystallinity sizes of Chem CdO NPs at temperatures of 100, 200, 300, 400 and 500 °C were 21.1, 132.2, 96.2, 30.2 and 25, respectively. This pattern indicates that with increasing temperature, crystallinity size increases until 200°C. However, at 300 the temperature drops consistently (Figure 8).

The crystallinity sizes of A-chem CdO NPs at temperatures: room temperature, 50, 75, 100, (°C) were 17.0, 16.0, 15.0 and 18.0, respectively. This pattern indicates that with increasing temperature, crystallinity size decreases until 100°C, where there is a sudden increase. It can be assumed that the increase in temperature damages the albumin protein (Figure 9).

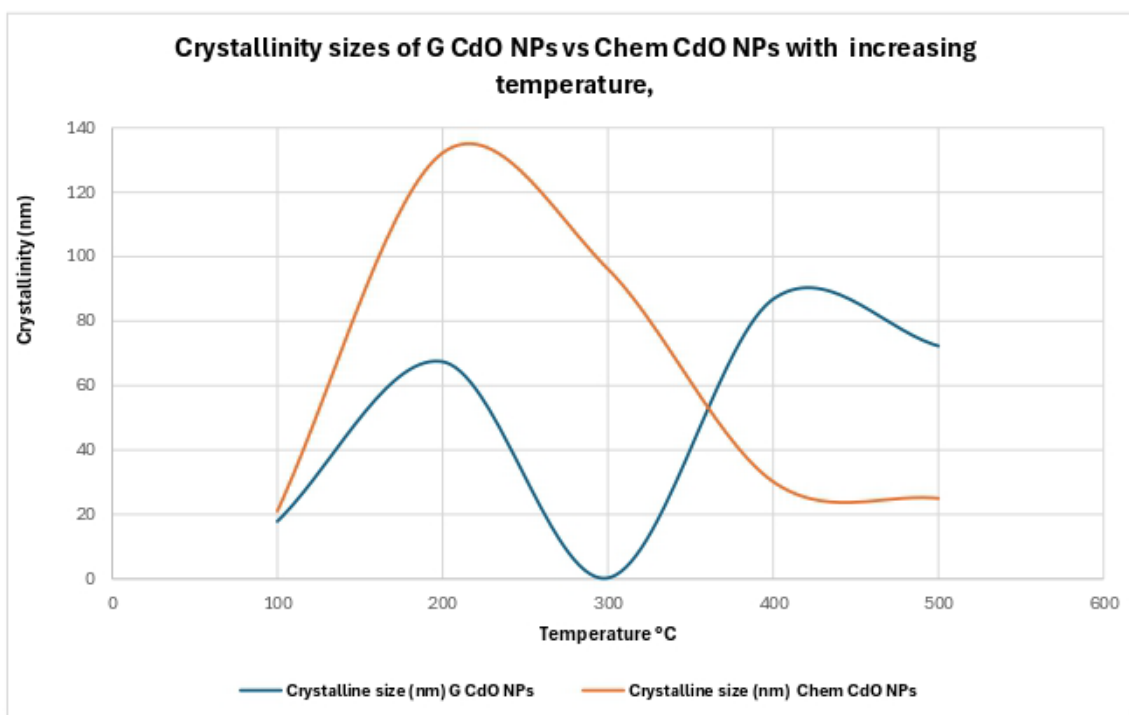


Figure 8 Comparison of crystallite sizes of green-synthesized CdO nanoparticles (G-CdO NPs) and chemically synthesized CdO nanoparticles (Chem-CdO NPs) at annealing temperatures of 100, 200, 300, 400, and 500 °C.

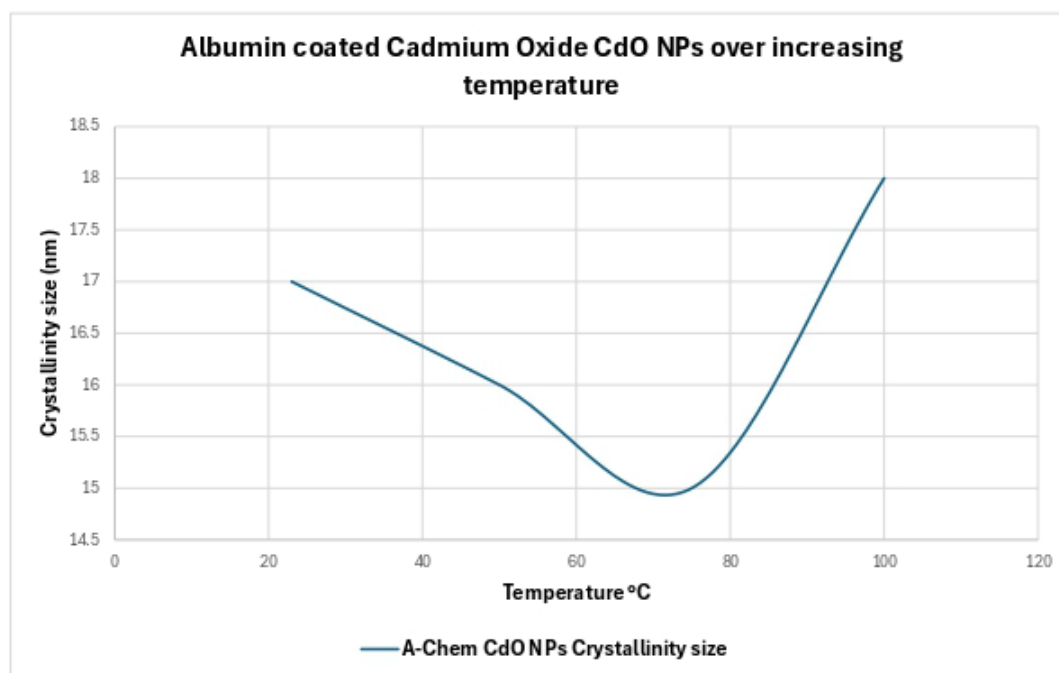


Figure 9 presents A-Chem CdO NPs crystallinity over increasing temperatures of (23, 50, 75, and 100 °C)

The peaks obtained in the XRD determine whether the synthesised NPs are crystalline or amorphous. Higher peak heights indicate crystallinity, whilst lower peak heights indicate amorphous NPs. The G CdO NPs show a constant change from amorphous to crystallinity from 100 °C to 500 °C. The XRD pattern of the green-synthesized Cadmium oxide nanoparticles (G-CdO NPs) prepared at 100 °C exhibited a broad, low-intensity hump within the 2θ range of 10° – 40° , suggesting an amorphous or poorly crystalline nature. However, at 300 °C, their peaks are broad-based, indicating an amorphous microstructure. These results are consistent with the crystallinity size, which drops suddenly at 300 °C. This is depicted in Figure 10 (A, B, C, D, E).

The chemically synthesised CdO NPs showed a higher crystallinity compared to green CdO NPs. At temperatures 100 °C and 200 °C, the peaks are broad-based, indicating an amorphous microstructure. However, from 300 °C to 500 °C, the peaks are sharp and more defined, indicating increasing crystallinity with an increase in temperature. This is shown in Figure 11.

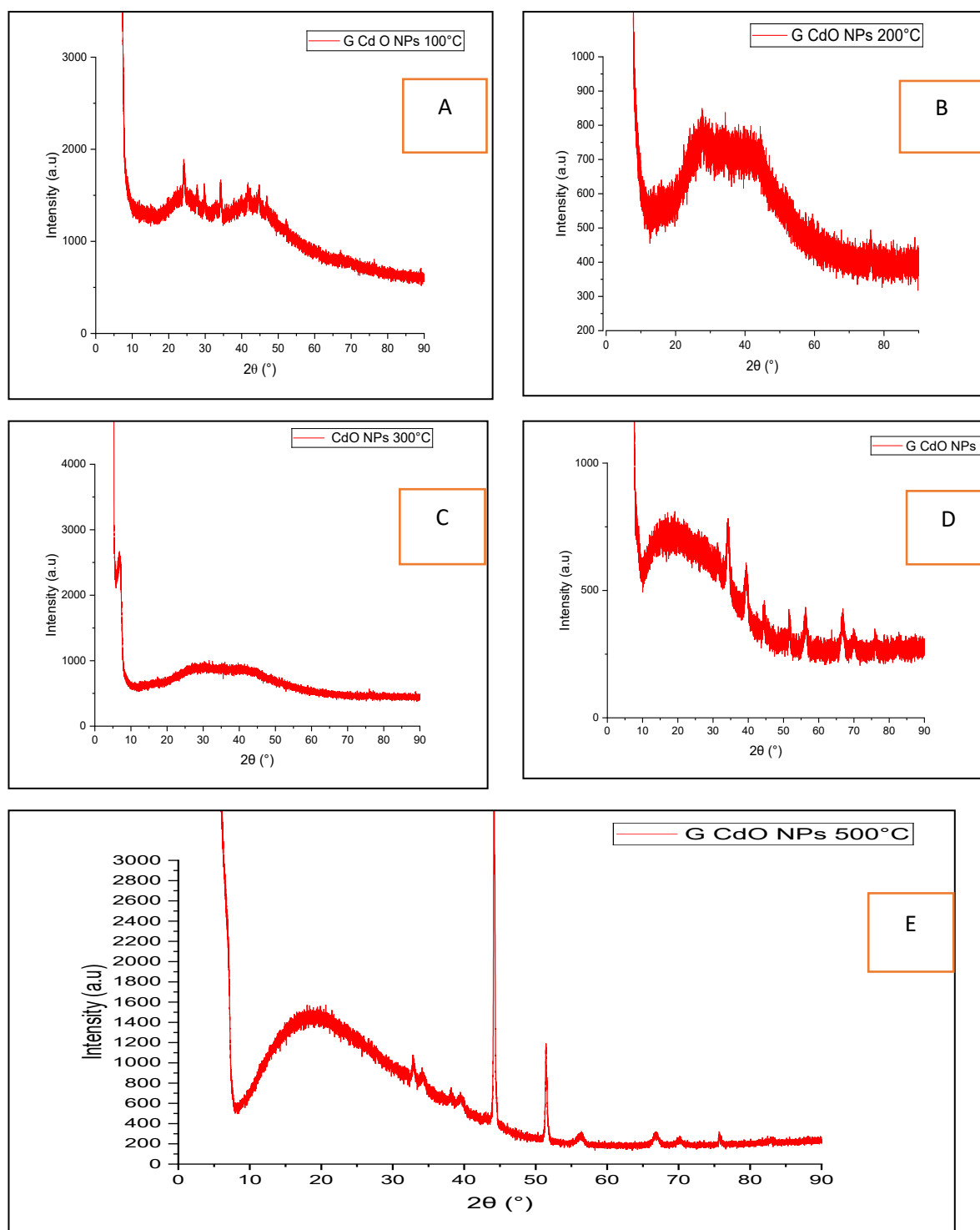


Figure 10: XRD patterns of G-CdO NPs prepared using *S. cupulare* extract at annealing temperatures of (A) 100 °C, (B) 200 °C, (C) 300 °C, (D) 400 °C, and (E) 500 °C.

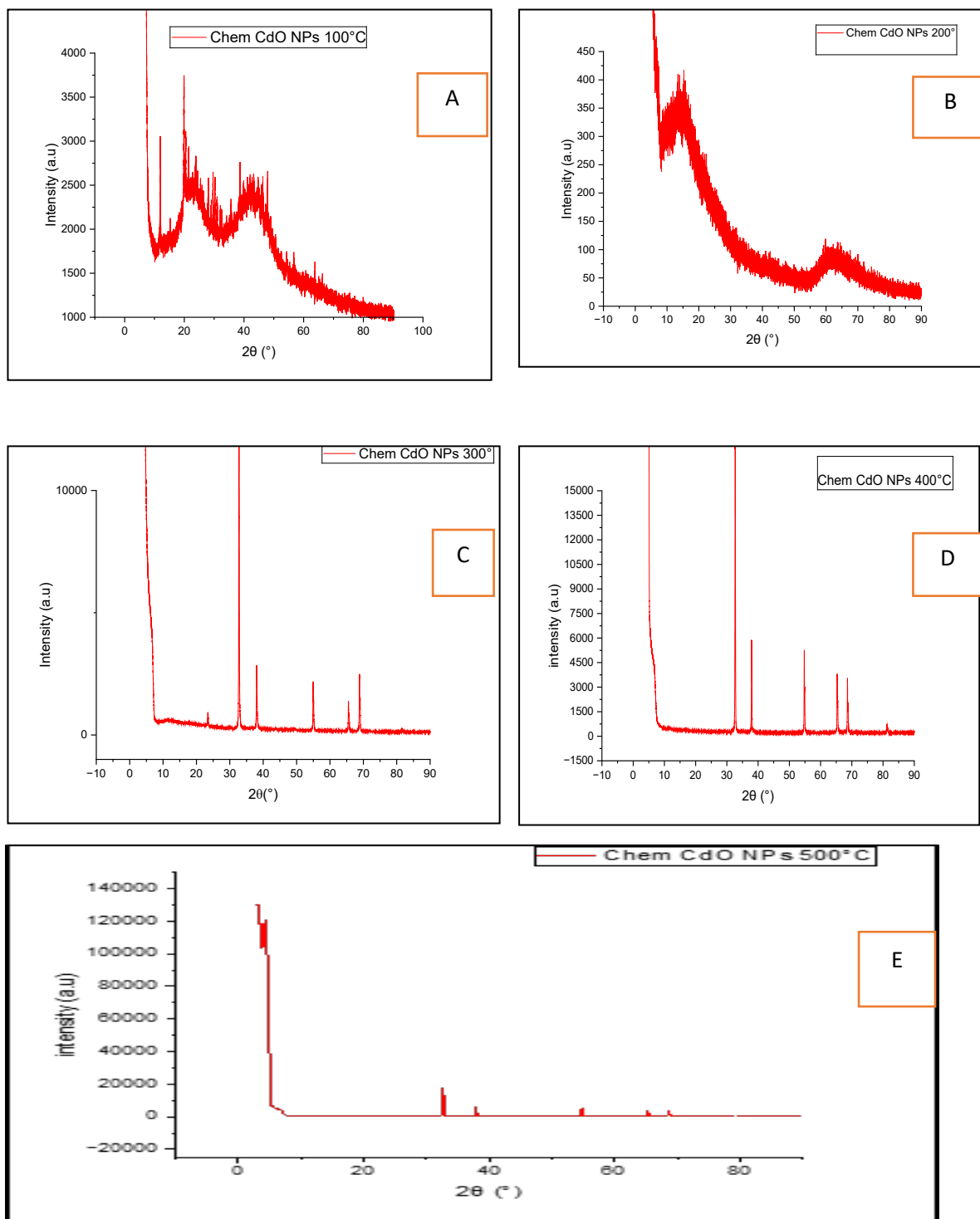


Figure 11: A, B, C, D and E depict XRD analysis of Chem CdO NPs at 100, 200, 300, 400 and 500 °C

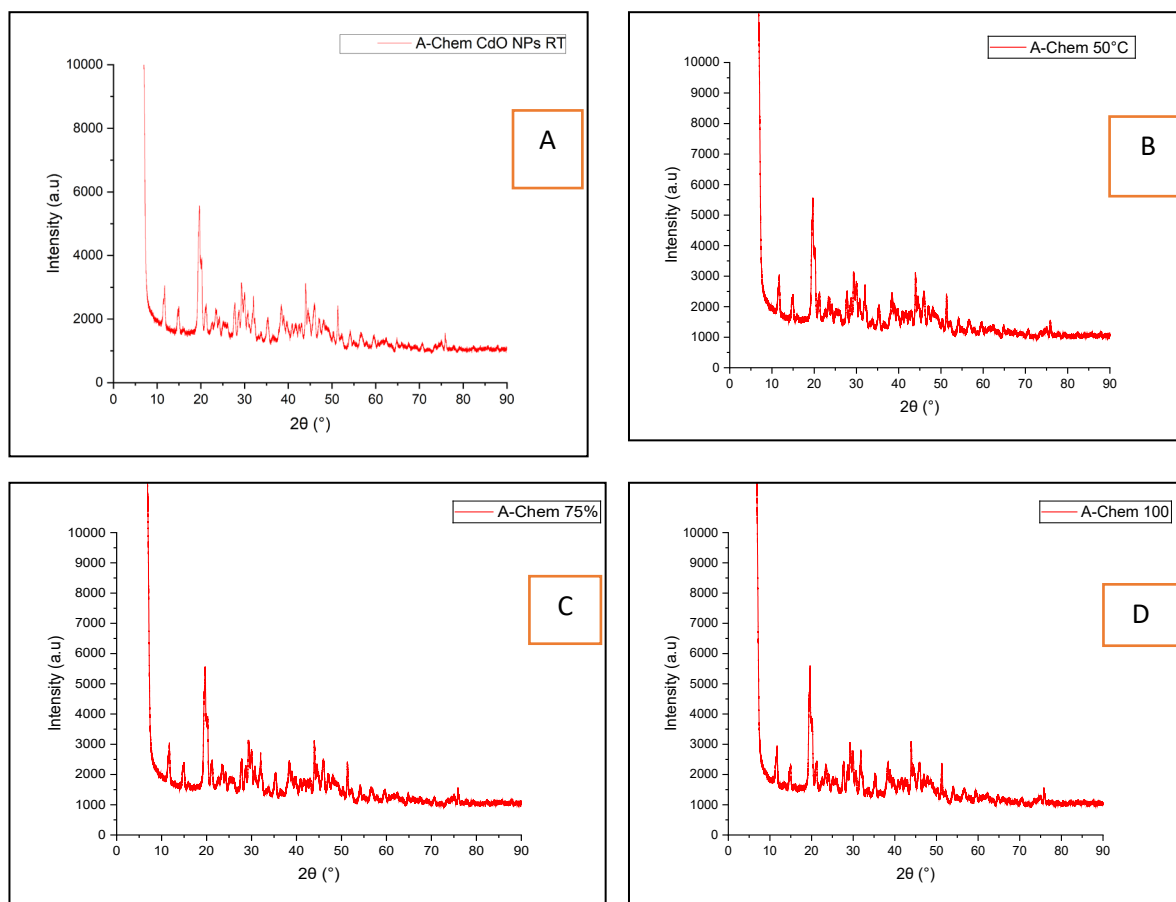


Figure 12: A, B, C, D and E depict XRD analysis of A-Chem CdO NPs at room temperature, 50, 75 and 100 °C

In Figure 12, the peaks of albumin-coated chemically reduced Cadmium oxide nanoparticles are sharp and defined at all temperatures 23, 50, 75, and 100 °C, therefore indicating consistent crystallinity. There is no change in crystallinity at room temperature and at 100 °C. This depicts the crystal nature of albumin. XRD spectra obtained from each sample (100 °C for G-CdO NPs and room temperature for A-Chem CdO NPs) exposed to different temperatures are compared in Table 3 below. All spectra were recorded in the 2θ range of 5° to 70°, with characteristic peaks indexed according to the JCPDS card for cubic CdO.

Table 3: Comparative XRD characteristics of G CdO NPs, Chem CdO NPs, and A-Chem CdO NPs

Parameter	Chem CdO NPs	G CdO NPs	A Chem CdO
Dominant Peak Positions (2θ)	~10°, ~30°, ~50°	~10°, broad spread from 20°– 40°	Multiple sharp peaks between 10°– 50°
Crystallinity	High (sharp peaks)	Moderate to low (broad peaks)	Moderate to high (sharpened profiles)
Peak Broadening (FWHM)	Narrow (larger crystallites)	Broad (smaller crystallites)	Intermediate (moderate crystallite size)
Phase Purity	Likely pure CdO	May contain bio-organic residues	CdO with evidence of protein interaction
Surface Features	Uncoated	Capped with phytochemicals	Capped with albumin protein

3.7.3 Compositional and Surface Characterization of CdO Nanoparticles Using EDS, XPS, and FTIR Analyses

The compositional and surface properties of the synthesized CdO nanoparticles were investigated using EDS, XPS, and FTIR. EDS analysis confirmed the presence of cadmium and oxygen as the major elemental constituents of the nanoparticles. XPS analysis further verified the chemical states and surface composition of CdO, indicating successful formation of cadmium oxide and the presence of surface-associated functional groups. FTIR spectra revealed various functional groups responsible for nanoparticle stabilization and capping, particularly in the green-synthesized and albumin-coated samples, suggesting the involvement of phytochemicals in nanoparticle surface modification. Overall, the combined analyses

confirmed the successful synthesis, surface functionalization, and compositional integrity of the CdO nanoparticle formulations.

3.7.4 EDS and XPS of G-CdO NPs

The energy-dispersive X-ray spectroscopy (EDS) spectrum further confirmed a surface composition consisting of Cd, O, N, Potassium (K), Ca, and Mg, indicating the incorporation of trace plant-derived elements during green synthesis (Figure 13). X-ray photoelectron spectroscopy (XPS) analysis revealed the presence of Chlorine (Cl), Oxygen (O), Cadmium (Cd), and Magnesium (Mg) on the nanoparticle surface (Figure 15).

3.7.5 EDS and XPS of Chem-CdO NPs

The EDS results revealed a similar composition, with detectable amounts of Cd, O, Nitrogen (N) and Na, suggesting partial contamination or precursor residue from chemical synthesis reagents (Figure 14). The XPS spectra of Chem-CdO NPs showed surface peaks corresponding to Chlorine (Cl), Oxygen (O), Cadmium (Cd), and Sodium (Na) (Figure 14).

3.7.6 EDS and XPS of A-Chem CdO NPs

The EDS spectra indicated a broader elemental composition consisting of Cd, O, Na, Nitrogen (N), Cl, and sulfur (S). The detection of nitrogen and sulphur supports the successful surface functionalization with albumin, which contains amino and thiol groups (Figure 15). The XPS analysis of A-Chem CdO NPs showed the presence of chlorine (Cl), Oxygen (O), Cadmium (Cd), Sodium (Na) and Chlorine (Cl) (Figure 18).

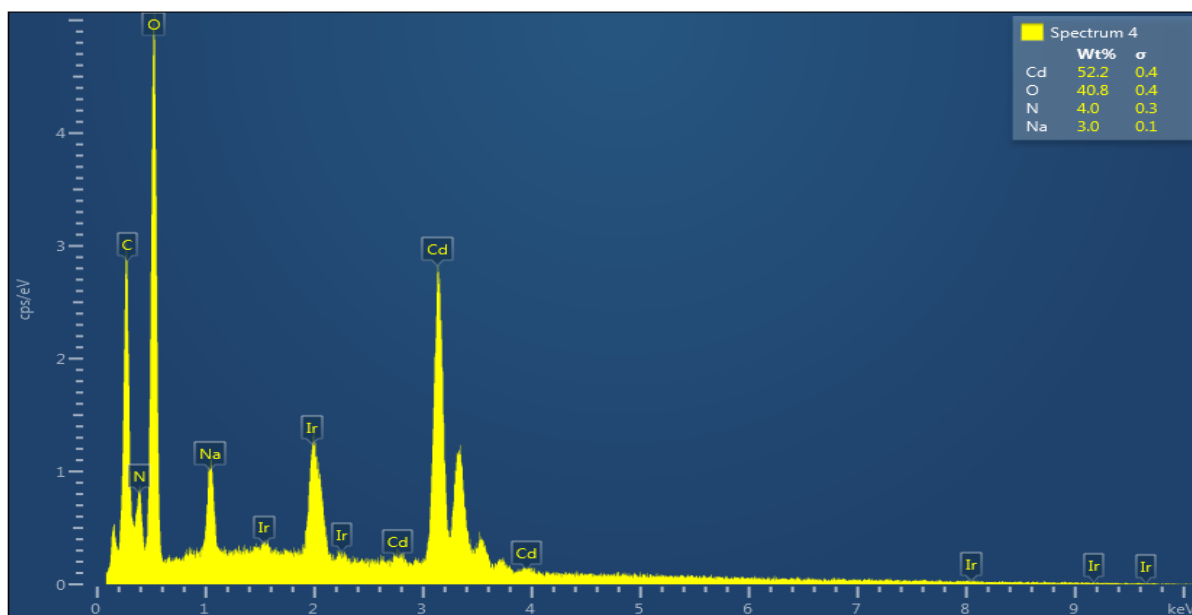


Figure 13: EDS spectrum of G CdO NPs revealing Cd and O as major elements with trace amounts of N, K, Ca, and Mg on the nanoparticle surface.

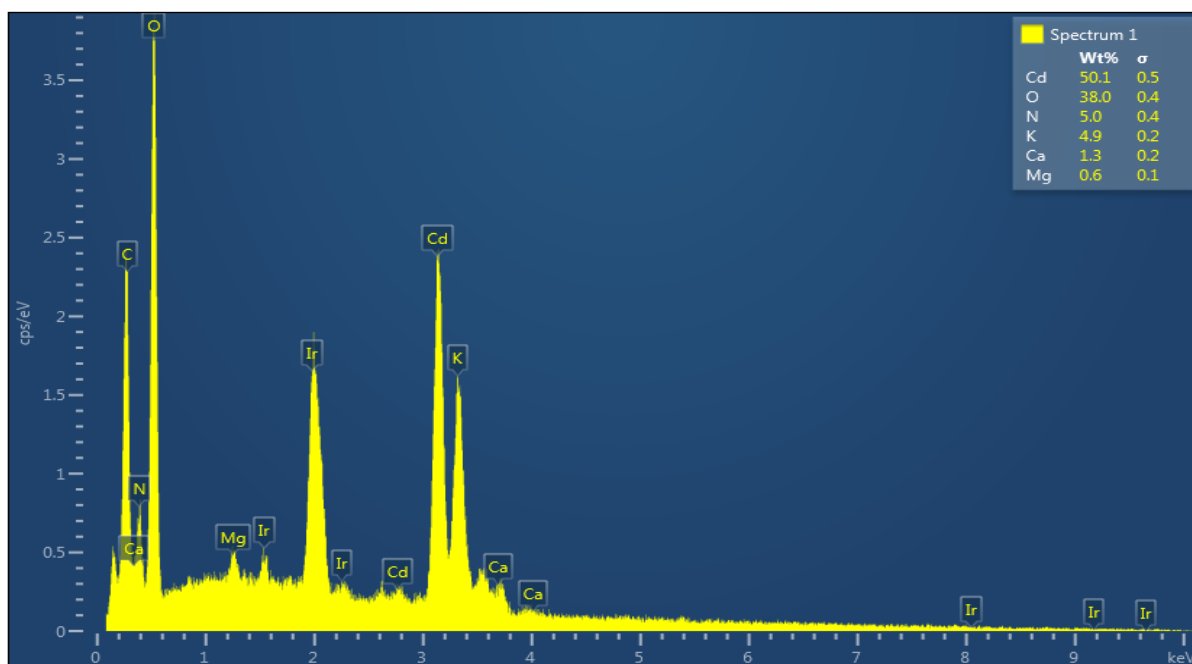


Figure 14: EDS spectrum of Chem CdO NPs revealing Cd, and O as major elements with trace amounts of N, Ca, Mg, Ir, Na, and K

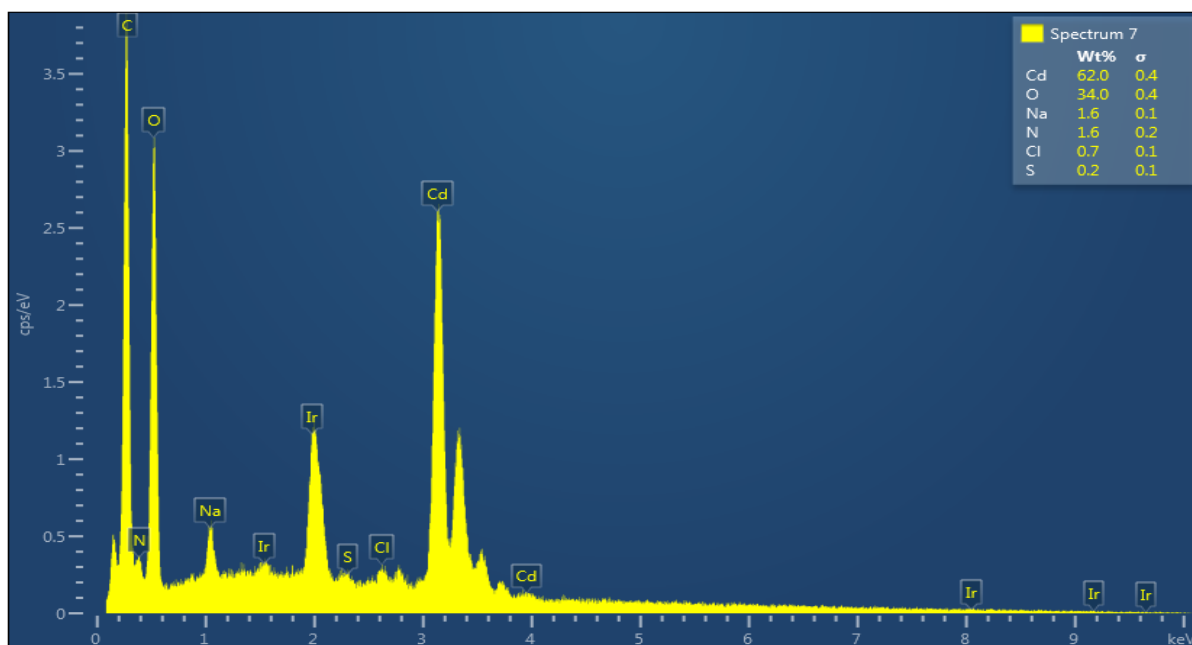


Figure 15: EDS spectrum of A-Chem NPs revealing Cd, and O as major elements with trace Elements of O, Na, N, Cl, and S.

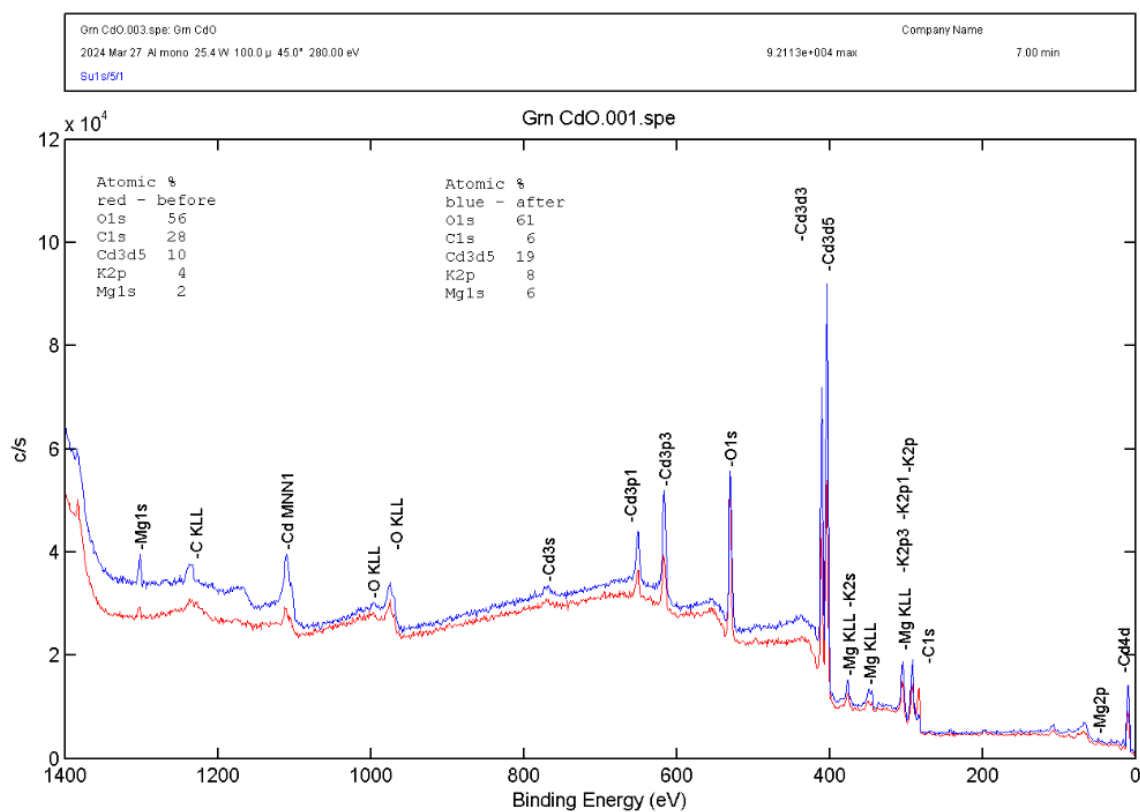


Figure 16: Displays XPS spectrum of G CdO NPs indicating the presence of Cl, Oxygen O, Cd and Magnesium Mg.

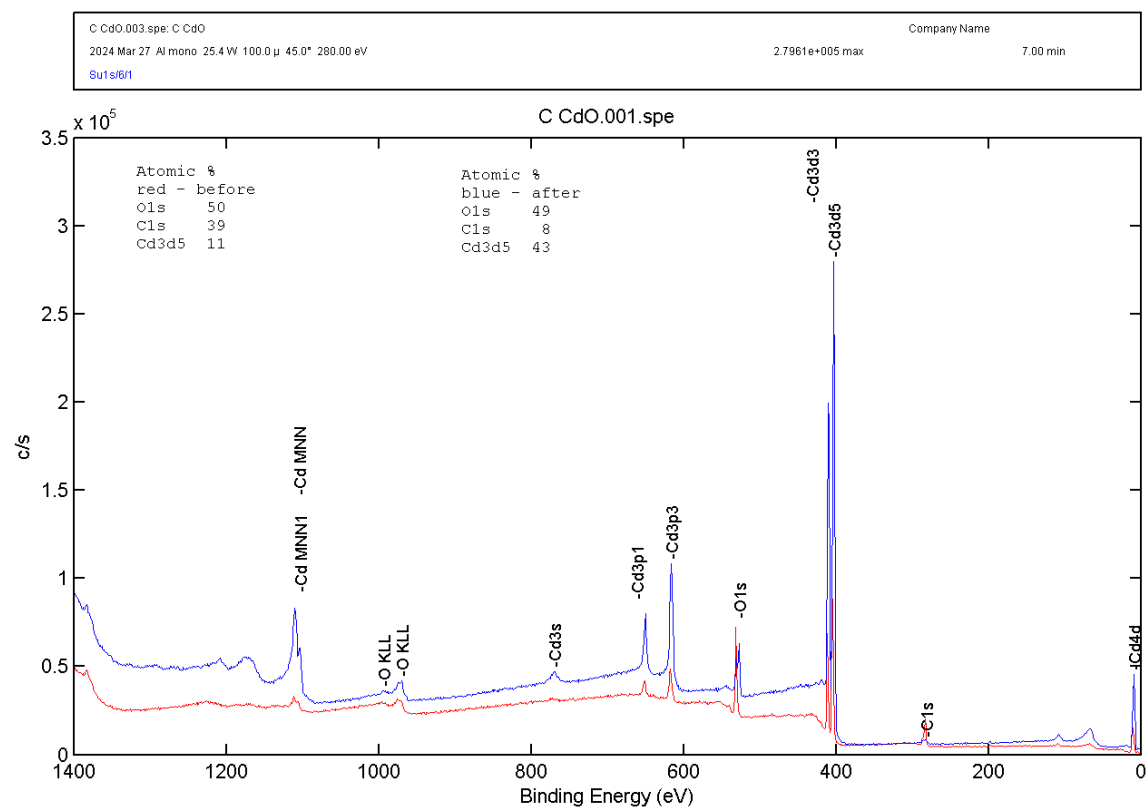


Figure 17: XPS Spectrum of Chem CdO NPs showing (O, Cl, Na, and Cd)

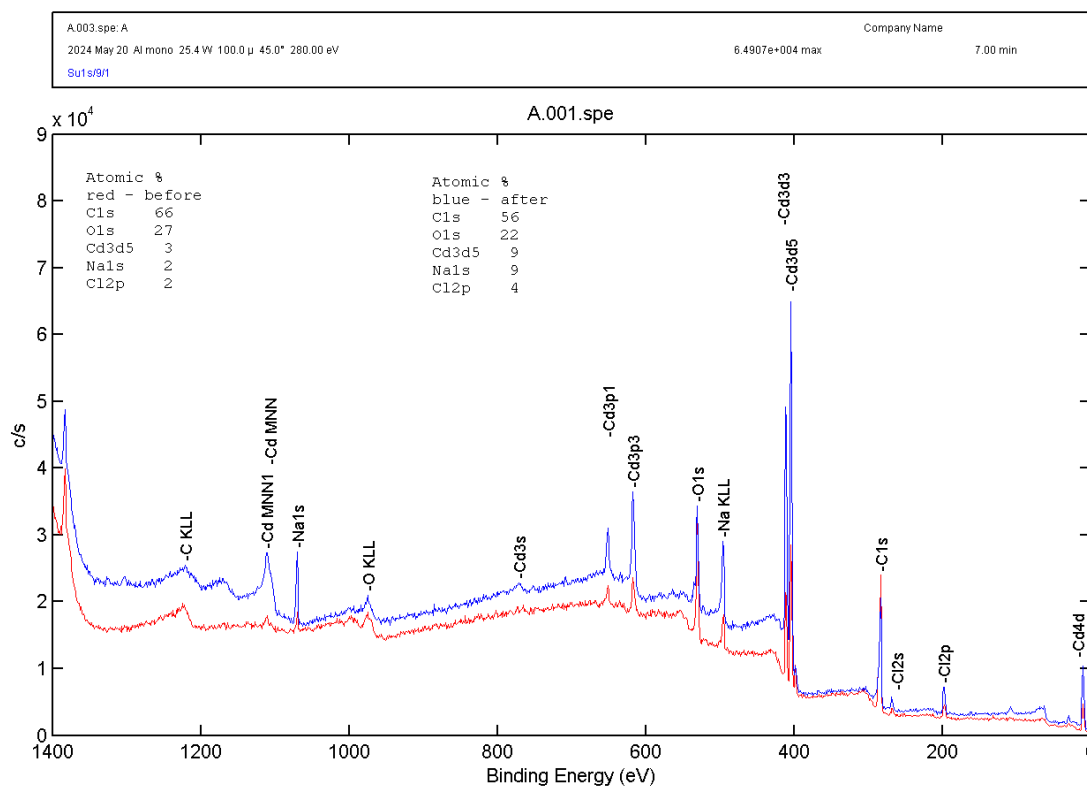


Figure 18: XPS Spectrum of A-Chem CdO NPs showing Cl, O, Cd, Na, and C

3.7.7 FTIR Spectra of G-CdO NPs, Chem-CdO NPs, and A-Chem-CdO NPs

The FTIR spectrum of green-synthesized Cadmium oxide nanoparticles revealed multiple absorption bands corresponding to phytochemical residues from the plant extract used as a reducing and stabilizing agent (Figure 19). A broad peak observed at ~ 3420 cm was assigned to O–H stretching vibrations of hydroxyl groups, indicative of phenolic or alcoholic constituents. The weak signal near 2920 nm was attributed to C–H stretching, while the distinct band around 1650 nm corresponded to C=O stretching (amide I), suggesting the presence of proteins or flavonoids. Additional bands at ~ 1380 nm and ~ 1100 nm reflected CH_3 bending and C–O stretching modes, respectively, affirming the involvement of organic compounds in nanoparticle

stabilization. Crucially, a sharp peak around 620 nm confirmed the formation of Cd–O bonds, validating successful oxide nanoparticle synthesis. The abundance of bio-organic functional groups reflects the role of plant-derived molecules in surface modification, which can influence solubility, biocompatibility, and interaction with biological targets (Karthik *et al.*, 2017; Sivalingam *et al.*, 2024; Nasrullah *et al.*, 2020).

The Fourier-transform infrared (FTIR) spectrum of the chemically synthesized Cadmium oxide (CdO) nanoparticles revealed several characteristic absorption bands (Figure 20). A weak and broad band was observed at approximately 3430 nm, which is typically associated with O–H stretching vibrations. In the region of 1600 – 1650 nm, only a very faint or flat signal was detected, indicating minimal absorbance in this range. A strong and sharp absorption peak appeared between 600 – 650 nm, corresponding to metal–oxygen bond vibrations. Additional low-frequency signals were present in the 400 – 500 nm range, within the fingerprint region, suggesting the presence of lattice vibrational modes. These features were consistently observed across sample replicates and confirm the typical infrared spectral profile of cadmium oxide nanoparticles.

The Fourier-transform infrared (FTIR) spectrum of the albumin-coated chemically synthesized Cadmium oxide nanoparticles (A-Chem CdO NPs) exhibited several distinct absorption bands indicative of both inorganic and organic components (Figure 21). A broad, moderate-intensity band was observed in the region of 3400 –3450 cm, corresponding to O–H and N–H stretching vibrations, suggesting the presence of hydroxyl and amide functional groups. A sharp band in the 1640 –1660 nm⁻¹ range, attributed to C=O stretching (amide I), was also noted, alongside a weaker shoulder band near 1530 – 1550 nm, consistent with N–H bending vibrations (amide II). These amide-related features are characteristic of proteinaceous materials and are consistent with the presence of an albumin coating on the nanoparticle surface. Additionally, a strong absorption peak in the 600 –650 nm region was observed, corresponding to Cd–O bond stretching, confirming the presence of the CdO core structure.

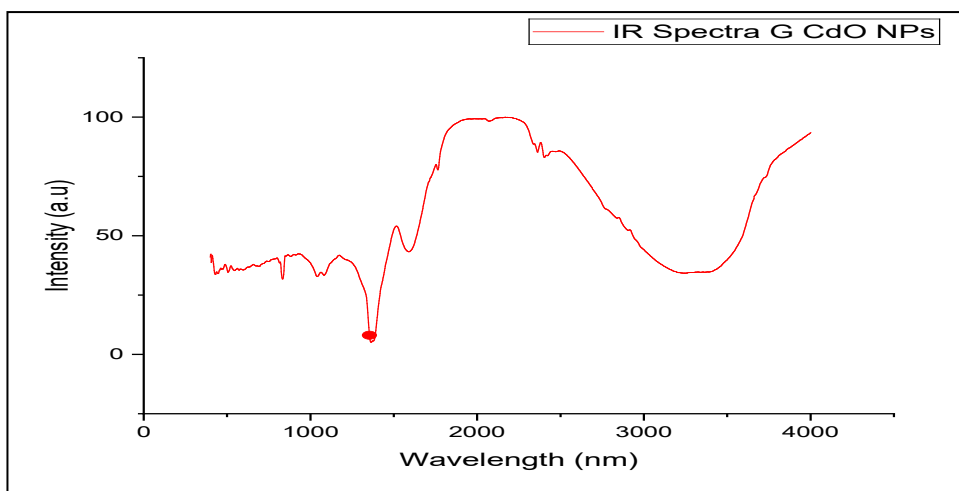


Figure 19: Infrared Spectrum of G CdO NPs

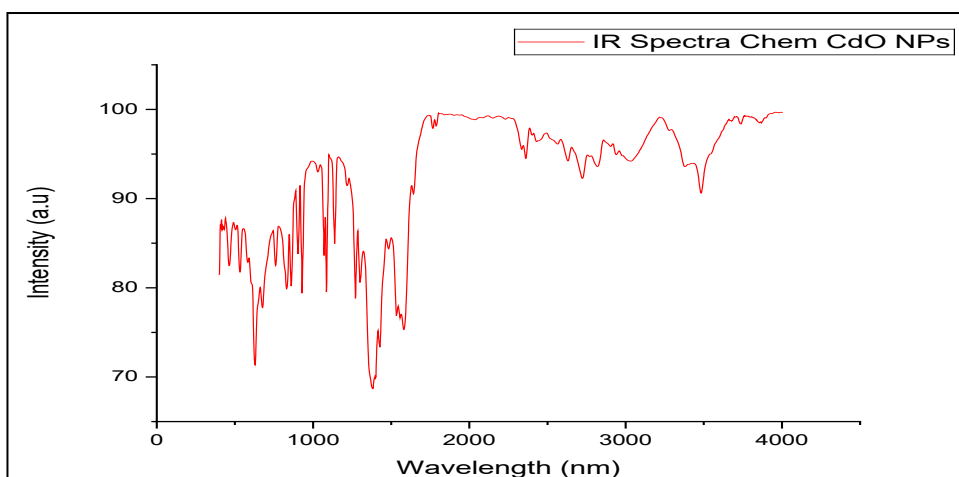


Figure 20: Infrared spectra of Chem CdO NPs

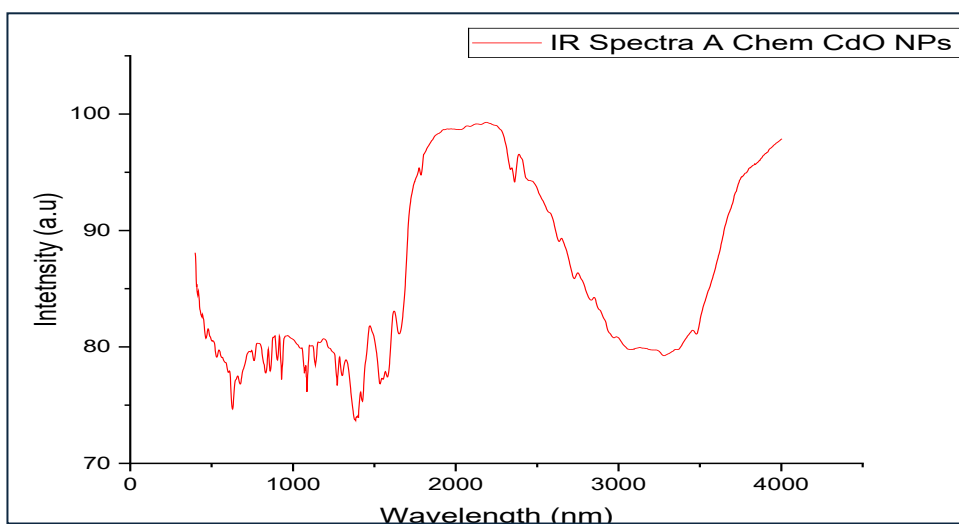


Figure 21: Infrared spectra of A- Chem CdO NPs

Table 4: FTIR Interpretation of G CdO NPS

Wavenumber (cm ⁻¹)	Observed Peak	Functional Group or Vibration	Interpretation with References
~3400 – 3500	Broadband near 3420	O–H stretching (hydroxyls)	Indicative of alcohols or phenolic compounds from plant extract used in green synthesis (Karthik et al., 2017; Sivalingam et al., 2024)
~2900 – 2950	Weak band	C–H stretching (alkanes)	Points to organic moieties from plant metabolites (Nasrullah et al., 2020)
~1650	Moderate band	C=O stretching / N–H bending	Suggests amide groups from flavonoids or proteins in bio-reducing agents (Dev and Mukadam, 2025)
~1380 – 1450	Shoulder or small peak	CH ₃ bending / C–N stretching	May originate from amino compounds in enzymes or alkaloids (Nasrullah et al., 2020)
~1100 – 1200	Faint feature	C–O stretching (alcohols, esters)	Supports presence of polyphenols or carbohydrates as capping agents (Karthik et al., 2017)
~600 – 700	Sharp fingerprint peak	Cd–O bond vibration	Confirms formation of CdO nanoparticles (Sivalingam et al., 2024)

Table 5: FTIR interpretation of Chem CdO NPs

Wavenumber (cm ⁻¹)	Observed Feature	Functional Group / Assignment	Interpretation
~3430	Weak/broad	Possible O–H stretching (moisture or surface hydroxyls)	Minimal may stem from atmospheric moisture (Nasrullah et al., 2020)
~1650 – 1600	Very faint / flat	Amide or C=O stretching	Almost absent confirms minimal organic interference
~600 – 650	Sharp, strong peak	Cd–O bond stretching vibration	Confirms CdO nanoparticle core structure (Karthik et al., 2017)
~400–500	Fingerprint region tail-off	Lattice vibrational modes	Characteristic of well-formed crystalline metal oxides

--	--	--	--

Table 6: FTIR Interpretation of A-Chem CdO NPs

Wavenumber (cm ⁻¹)	Band Characteristics	Vibrational Mode / Functional Group	Interpretation with References
~3400 – 3450	Broad, moderate	O–H and N–H stretching (hydroxyls, amides)	Indicates presence of protein groups; albumin signature (Azizi, 2018; Dev and Mukadam, 2025)
~1640 – 1660	Sharp/moderate	C=O stretching (amide I band)	Strong evidence of albumin coating, protein backbone (Azizi, 2018)
~1530 – 1550	Weak/shoulder	N–H bending (amide II band)	Reinforces protein presence; typical for BSA-modified NPs (Dev and Mukadam, 2025)
~600 – 650	Strong peak	Cd–O bond stretching vibration	Confirms CdO nanoparticle structure (Karthik et al., 2017;

3.7.8 UV-VIS of G-CdO NPs, Chem-CdO NPs, and A-Chem-CdO NPs

The UV-Visible absorption spectra revealed distinct peak positions for each nanoparticle formulation, corresponding to their unique synthesis methods and surface modifications. The green-synthesized CdO nanoparticles (G-CdO NPs) exhibited a sharp absorption peak at 215 nm (Figure 22), indicative of successful nanoparticle formation. In comparison, the chemically synthesized CdO nanoparticles

(Chem-CdO NPs) showed an absorption peak at 260 nm (Figure 23), while the albumin-coated variant (A-Chem-CdO NPs) displayed a peak at 208 nm (Figure 24). These absorption values fall within the characteristic range for Cadmium-based nanomaterials, suggesting the presence of Cadmium oxide and confirming the optical activity of the synthesized nanoparticles.

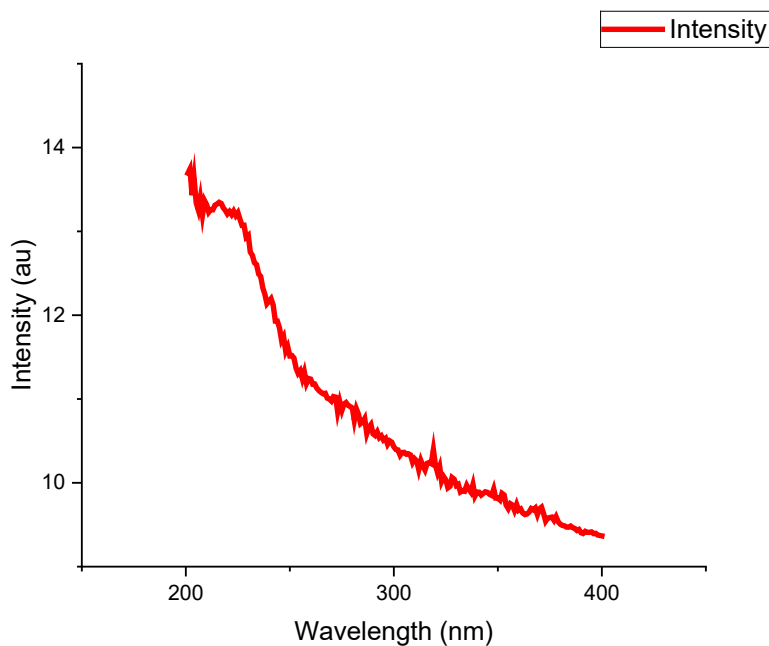


Figure 22: UV-Vis spectra of G-CdO NPs indicating absorption at 215 nm

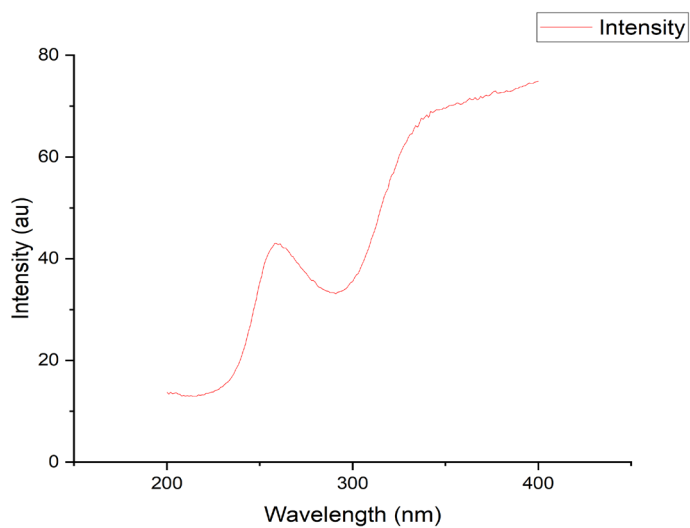


Figure 23: UV-Vis spectra of Chem CdO NPs indicating absorption at 250 nm

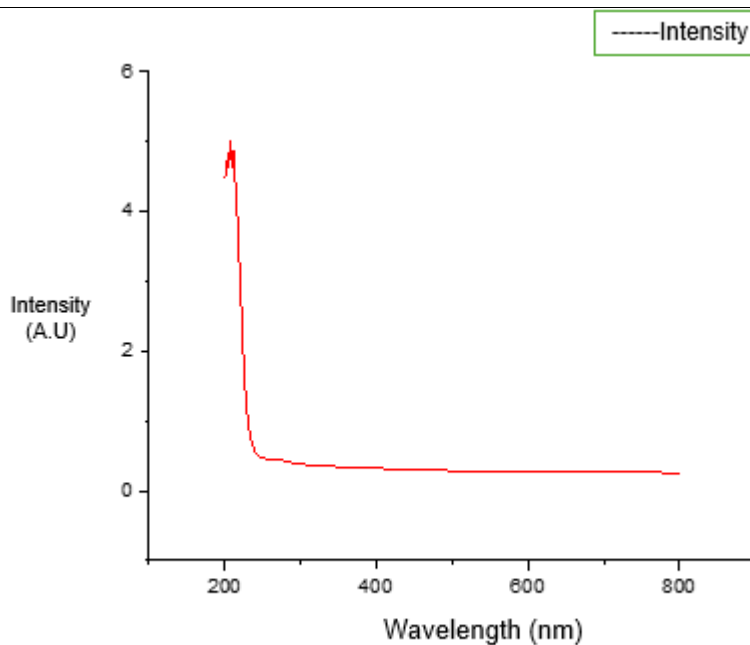


Figure 24: UV-Vis spectra of A-Chem CdO NPs indicating absorption at 208 nm

3.8 Toxicity Screening of Nanoparticles

In vitro nanoparticle toxicity assessment is one of the critical methods in cancer therapeutics. Due to their unique physicochemical properties, nanoparticles can interact with biological systems in ways that may lead to unforeseen adverse effects. The advantages include lower cost, faster results, and minimal ethical concerns. Assessment can be subdivided into proliferation assay, apoptosis assay, necrosis assay, oxidative stress assay, and DNA damage assays. In this study, only the MTT proliferation assay was conducted. This section focuses on assessing the cytotoxicity, selectivity, and overall biocompatibility of the Cadmium oxide nanoparticles (CdO NPs) used in this study. By evaluating the potential toxic effects on both cancerous (MDA-MB-231) and non-cancerous (Vero) cell lines, this screening aims to determine the safety profile of the nanoparticles. Understanding these toxicological properties is essential for identifying the appropriate nanoparticle formulations that balance therapeutic efficacy with minimal off-target effects, thereby improving their potential in clinical application.

3.8.1 Morphological description of MDA-MB231 Cell lines

The MDA-MB-231 cell line, a triple-negative breast cancer cell line, exhibits an epithelial-like morphology, with cells appearing spindle-shaped, especially at subconfluence, and some remaining rounded with floaters always present as demonstrated in Figure 25.

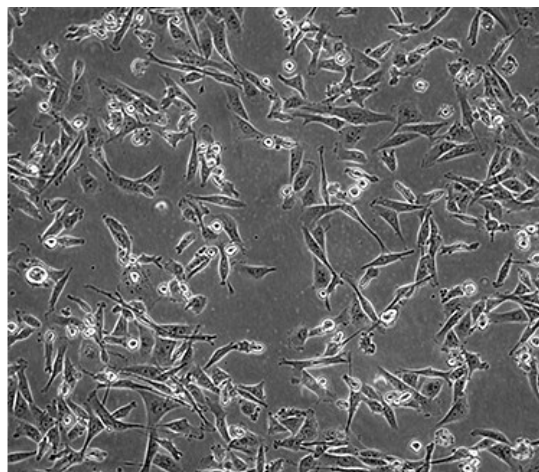
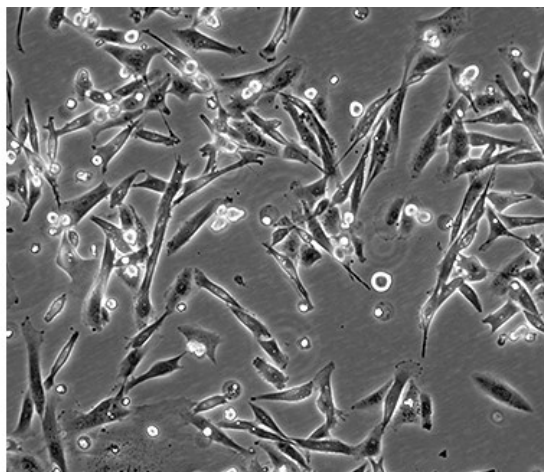
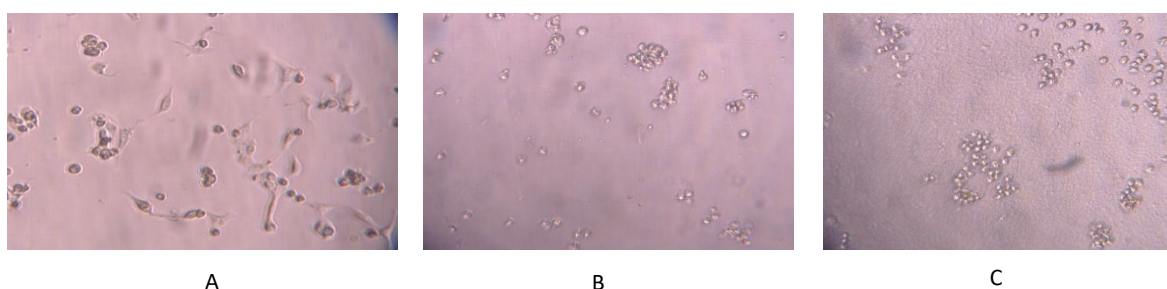


Figure 25: Depicts the original morphology of MDA-MB231 Cell lines (www.atcc.org,2025)

3.8.2 Morphological Changes of MDA-MB231 Cell lines when treated with CdO nanoparticles

The cytotoxicity effect of G CdO NPs, Chem CdO NPs, A-Chem CdO and Albumin on triple-negative breast cancer cells (MDA-MB231) is presented at increasing concentrations of 1.25, 2.5, 5, 25, 50, 100 $\mu\text{g/ml}$. However, the morphological comparison was made between concentrations of 1.25, 5, and 100 $\mu\text{g/ml}$. At a concentration of 1.25 $\mu\text{g/ml}$ for G CdO NPs, Chem NPs, A-Chem NPs and pure albumin, the morphology of MDA-MB231 NPs remained intact with spindle and round shapes. The albumin-treated MDA-MB231 cell lines greatly resembled the original morphology, more than all the cells treated with the nanoparticles. These results indicate that at concentrations of 1.25, 5, and 100 $\mu\text{g/ml}$, albumin exerts low toxicity; hence, there are no morphological changes. MDA-MB231 Cells treated with 5 $\mu\text{g/ml}$ of G CdO NPs, Chem CdO NPs, and A-Chem CdO NPs showed only rounded cells, indicating the effect of toxicity. Lastly, cells treated with 100 $\mu\text{g/ml}$ of the various nanoparticles showed rounded morphology only, indicating the toxicity effect. Figure 26 depicts the morphological images of MDA-MB231 cells after treatment with various concentrations of nanoparticles and albumin.



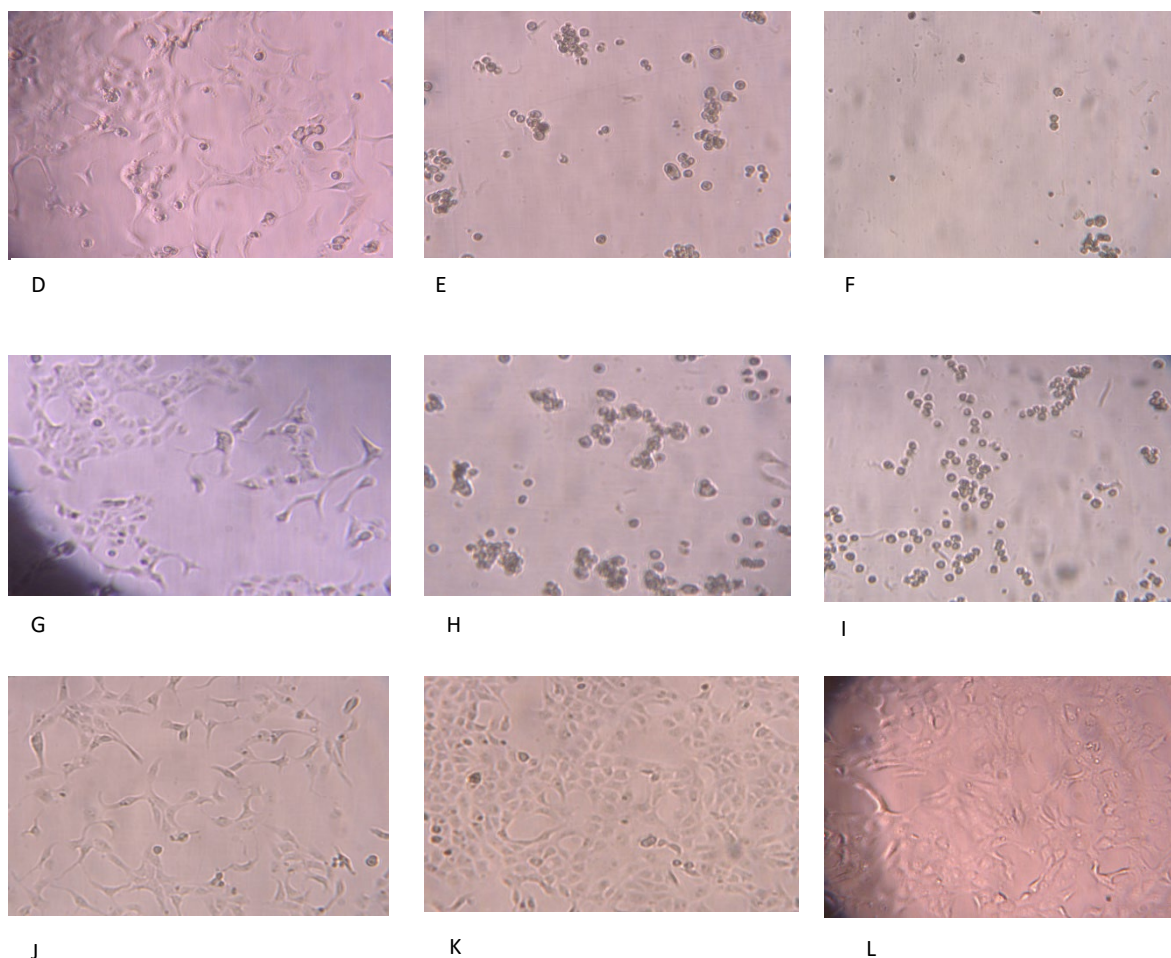


Figure 26: Morphological images of MTT assays MDA-MB-231 breast cancer cell lines treated with various concentrations of G CdO NPs, Chem CdO NPs, A-Chem CdO NPs and Albumin. **A** shows 1.25 µg/ml G CdO NPs, **B** shows 5 µg/ml G CdO NPs, **C** shows 100 µg/ml G CdO NPs, **D** shows 1.25 µg/ml Chem CdO NPs, **E** shows 5 µg/ml Chem CdO NPs, **F** shows 100 µg/ml Chem CdO NPs, **G** shows 1.25 µg/ml A-Chem CdO NPs, **H** shows 5 µg/ml A-Chem CdO NPs, **I** shows 100 µg/ml Chem CdO NPs, **J** shows 1.25 µg/ml Albumin, **K** shows 5 µg/ml Albumin, **L** shows 100 µg/ml Albumin.

3.8.3 Cytotoxicity and selectivity of various nanoparticles

Doxorubicin demonstrated pronounced cytotoxicity against MDA-MB-231 triple-negative breast cancer cells, with a half-maximal inhibitory concentration (IC_{50}) observed at 0,34 µg/ml, thereby validating its well-established anticancer efficacy. Among the Cadmium oxide nanoparticle formulations, G CdO NPs (green-synthesized) yielded an IC_{50} of 11.80 µg/ml. In contrast, Chem CdO NPs (chemically synthesized) showed significantly greater potency, achieving an IC_{50} of 3.33 µg/ml. The A-Chem CdO NPs, which were coated with albumin to modulate toxicity, exhibited

a moderate cytotoxic profile with an IC_{50} of $11.32 \mu\text{g/L}$. These trends are illustrated in Figure 27.

Collectively, the data indicate that chemically synthesized CdO NPs possess superior cytotoxic efficacy compared to the green-synthesized nanoparticles. This observation supports the rationale for selecting the chemically synthesised CdO for coating with albumin to potentially reduce off-target toxicity while maintaining therapeutic activity.

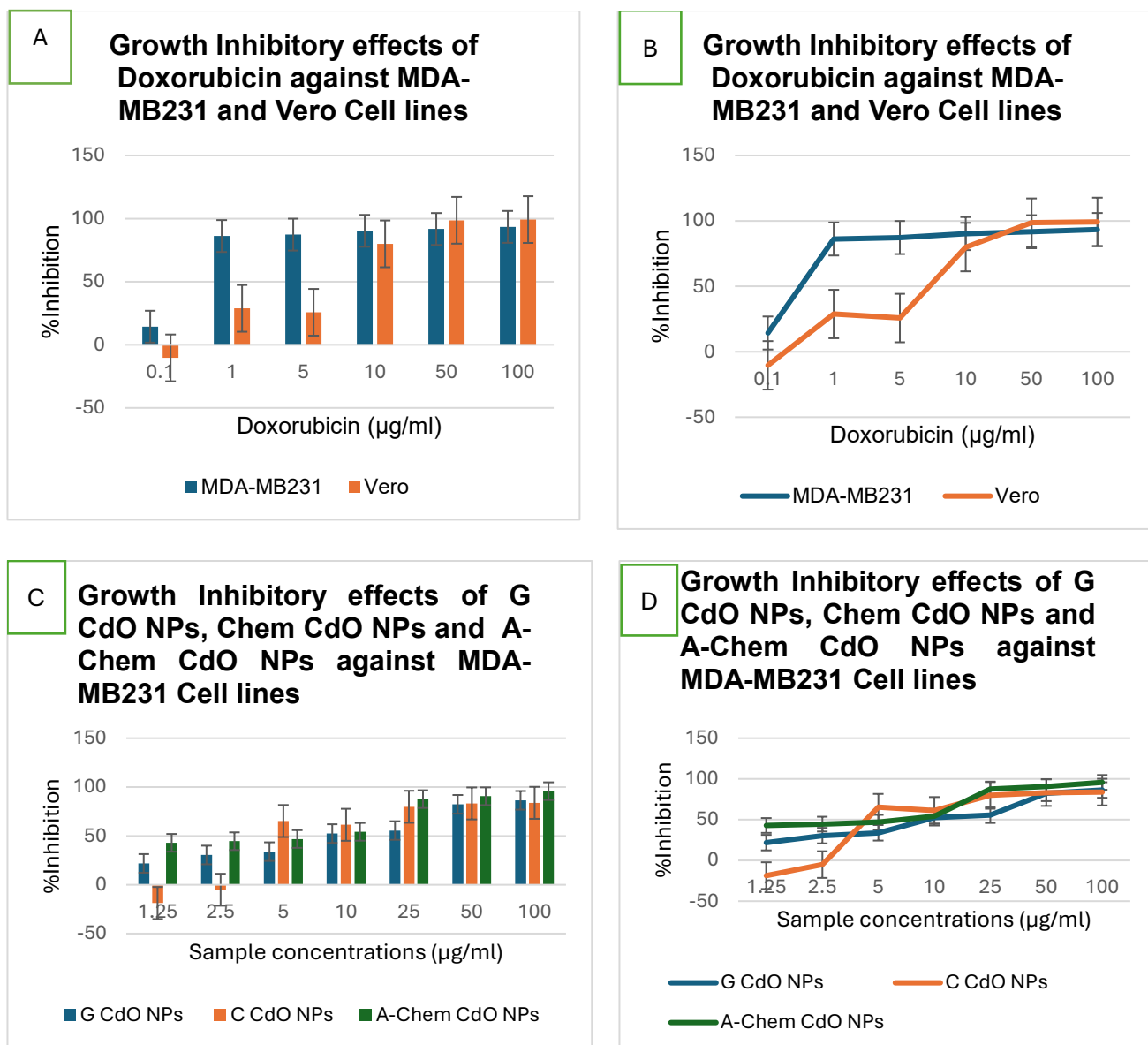


Figure 27 **A** and **B** Display the percentage inhibition of Doxorubicin against MDA-MB231 and Vero Cell lines. **C** and **D** Show the percentage inhibition of G CdO NPs, Chem CdO NPs, and A-Chem against MDA-MB231 Cell lines.

Activity against Vero Cell Lines

To determine the selectivity of the nanoparticles, their cytotoxicity was also evaluated on non-cancerous Vero cell lines. Doxorubicin demonstrated an IC_{50} of 12.6 $\mu\text{g/ml}$, at a concentration of 10 $\mu\text{g/ml}$, indicating considerable cytotoxicity against healthy cells (Figures 28A and B). In contrast, G CdO NPs and Chem required concentrations above 100 $\mu\text{g/ml}$ to reach a percentage inhibition of 50% on the Vero cell line (Figures 28C and D). This marked reduction in cytotoxicity on non-cancerous cells suggests that the nanoparticles have a more favourable selectivity profile when compared to the standard drug. The A-Chem CdO NPs required a concentration of 10 to a rich 50% inhibition. However, these results are inconsistent with the hypothesis of the study and the existing literature, which states that albumin coating leads to a reduction in cytotoxicity.

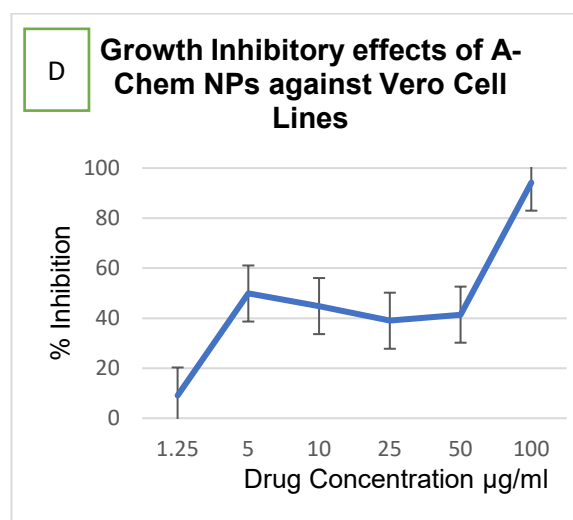
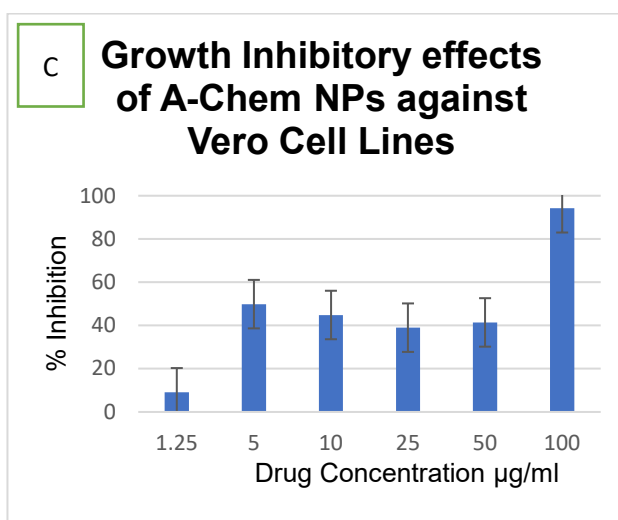
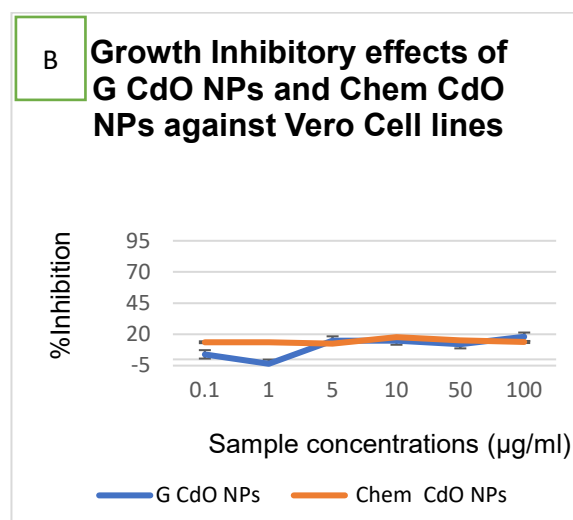
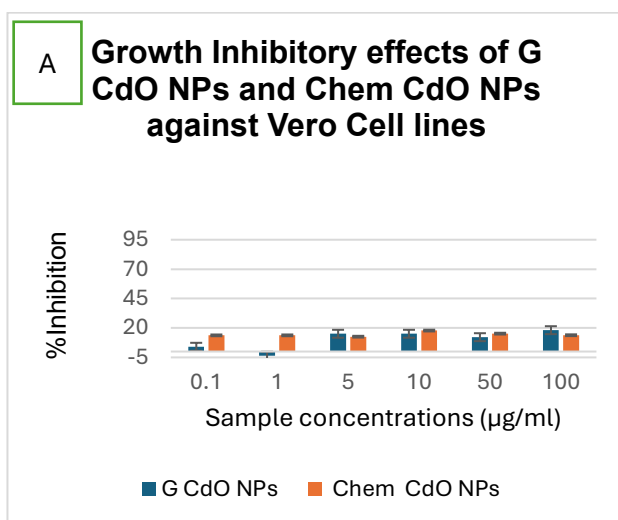


Figure 28 **A** and **B** display the percentage inhibition of G CdO NPs and Chem CdO NPs against Vero cell lines. **C** and **D** display the percentage inhibition of A-Chem CdO against Vero cell lines

3.8.4 Selectivity Index

The Selectivity Index (SI) measures a compound's differential activity, with a higher SI value indicating greater selectivity, meaning the compound is more effective against target cells (Mahmood, 2020). SI is calculated as = IC_{50} of Vero/ IC_{50} of Cell line (MDA-MB-231). The results in Table 7 indicate Chem CdO NPs to have the highest selectivity of 30, followed by Doxorubicin with an SI of 12,6. G CdO NPs, G CdO NPs, and A-Chem CdO NPs had SI values of 8,4 and 8,85, respectively.

Table 7: Selectivity Index of doxorubicin, G-CdO NPs, Chem-CdO NPs, and A-Chem CdO NPs formulations against MDA-MB231 cell line in comparison to Vero cell lines.

Treatment	Vero Cell line IC_{50}	MDA-MB231 Cell Line IC_{50}	Selectivity Index	Interpretation OF SI Index
Doxorubicin	12,6	0,34 μ g/ml	37,1	SI > 10: Highly selective (strong candidate for further development)
G CdO NPs	>100	11,80	8,4	SI 3–10: Moderately selective (may require optimization)
Chem CdO NPs	>100	3,33	30,03	SI > 10: Highly selective (strong candidate for further development)
A-Chem CdO NPs	100	11,32	8,85	SI 3–10: Moderately selective (may require optimization)

3.9 Summary

The Cadmium oxide nanoparticles (CdO NPs) were successfully synthesised using both green and chemical methods. Comprehensive characterisation techniques, including XRD, TEM, SEM, EDS, FTIR, XPS and UV-VIS were employed to confirm the structural, morphological, and physicochemical properties of the synthesised nanoparticles. Following successful synthesis, *in vitro* cytotoxicity screening was conducted, revealing that the chemically synthesised CdO NPs exhibit the highest inhibitory activity against cancer cells when compared to their green-synthesised counterparts. Based on these results, the chemically synthesised CdO NPs were further functionalised by coating them with albumin to enhance their biocompatibility and stability. Albumin coating of Chem CdO NPs appears to have blunted cytotoxicity in MDA-MB-231 cells, with functionalization increasing the IC₅₀ from 3.33 µg/ml to 11.32 µg/mL. Doxorubicin had the lowest IC₅₀ of 0.34 µg/ml, and the highest selectivity index, indicating the highest therapeutic index and lower systemic toxicity. This contradicts the hypothesis of the study. However, this paradoxical outcome aligns with prior reports suggesting that the formation of a protein corona on nanoparticle surfaces can attenuate bioactivity by partially masking reactive surfaces and impeding cellular uptake, as well as ROS (Reactive Oxygen Species) induction (Rampado et al., 2020). While Singh et al., (2018) initially reported enhanced therapeutic performance following albumin functionalization, their findings also acknowledged the context-dependency of these effects, highlighting the complex interplay between nanoparticle surface chemistry and biological response. Irrespective of the phenomena of this *Invitro* results, exploring A-Chem CdO NPs further *in vivo* will grant a comparative analysis. These albumin-coated nanoparticles were then subsequently selected for *in vivo* preclinical evaluation using the BALB/c mouse model, aimed at assessing their therapeutic efficacy. However, various coating techniques must be explored in future studies, as they might give insights into the limitations in A-Chem CdO NPs' reduced cytotoxicity after coating with Albumin

Chapter 4: In vivo study

4.0 Introduction

Scientists leverage *in vivo* models as important research tools to study the pressing questions in breast cancer research. Mouse models are efficient for breast cancer research and have proven to be useful models since their anatomical, physiological, and genetic makeup is similar to humans. They are also easy to maintain and are readily available due to their quick reproduction process, which makes repeating studies possible. The TNBC NU/J (BALB/C) xenograft mouse model was used in this study (Aldea et al., 2021). This chapter details the methodologies followed to conduct the *in vivo* study and the results obtained.

4.1 Ethical Clearance

The cell culture procedures employed in this study were conducted under ethical standards and were approved by the Environment and Biosafety Research Ethics Committee (EBREC) of the University of the Free State (Approval Number: UFS-ESD2022/0258). The animal experiments received prior ethical clearance from both the Animal Research Ethics Committee (AREC) of the University of the Free State (UFS-AED2021/0039/22) and the North-West University Animal Care, Health and Safety in Research Ethics Committee (Approval Number: NWU-02015-23-A5). Throughout the study, mice were housed under standard laboratory conditions and monitored daily to ensure their health and well-being. Humane endpoints were strictly observed, and animals were euthanized at predetermined experimental time points following approved protocols. Furthermore, supervisors, students and technical staff involved in the research went through certified ethics training to ensure full compliance with institutional and national ethical guidelines.

4.2 Study Design

This study was conducted as a pilot investigation to evaluate the growth rate and progression pattern of triple-negative breast cancer (TNBC). Additionally, the pilot

served as a preparatory phase to refine and optimize the design and methodologies for the subsequent main study. Female NU/J (BALB/c) immune-deficient mice aged between 8 and 12 weeks were used, as TNBC predominantly affects females. The use of female mice is particularly relevant, given that their hormonal profile more closely mimics that of human females, enhancing the translational relevance of the findings (Amini et al., 2023).

All animals were bred at the Rodent Production Unit of North-West University (South Africa). A total of 23 female mice were allocated for the pilot study. The first batch included 18 mice. 15 were designated for the experimental groups, and 3 were reserved for unforeseen emergencies. The use of a minimum of three mice per group is consistent with ethical and scientific recommendations to yield statistically meaningful and reproducible results (Karp, 2021). Key parameters monitored throughout the study included body weight, tumour size progression, response and recovery rates following treatment with both standard chemotherapeutic (Doxorubicin) and investigational drugs (A-Chem CdO NPs and G CdO NPs).

4.3 Mice Housing Conditions

The mice were observed daily to monitor their health and well-being throughout the study. Each cage was furnished with autoclaved wood shavings and serviettes to provide environmental enrichment and promote natural behaviours. The animal holding room was maintained at a controlled temperature of 20–24 °C and relative humidity of 50–60%, as recommended by institutional guidelines (Ratuski, 2023). A 12-hour light/dark cycle was maintained under specific pathogen-free (SPF) conditions. Prior to the initiation of experimental procedures, all mice underwent a 7-day acclimatization period to adjust to their new environment (Cayashi et al., 2024). During this period and throughout the study, food and clean water were provided *ad libitum*. Animal husbandry, including feeding, cleaning and routine monitoring, was conducted by trained and certified personnel from the North-West University Animal Research Centre (NWU ARC), in full compliance with established animal welfare protocols. The visual housing conditions at NWU are depicted in Figure 29.

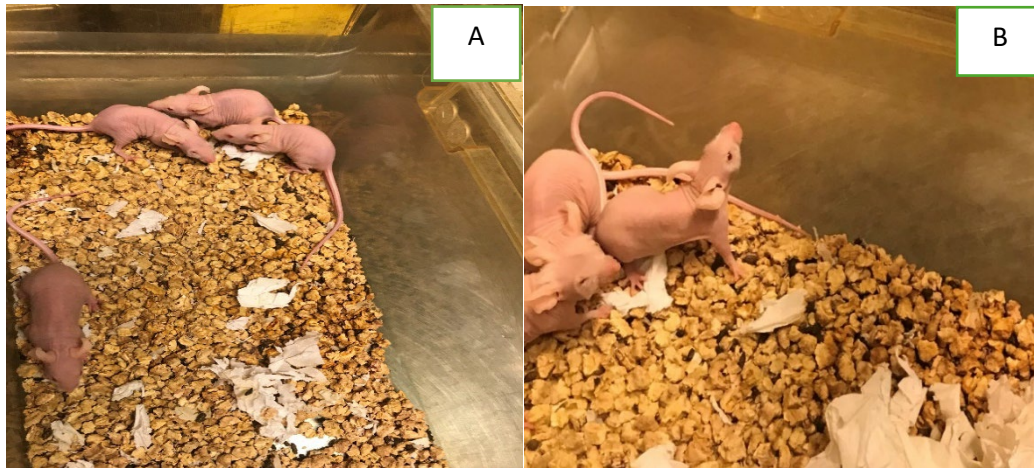


Figure 29 **A** and **B** display the housing conditions used for the mice at NWU

4.4 Mice Tumour Inoculation and Treatment

After the mice had acclimatized for 7 days under controlled environmental conditions, they were prepared for tumour inoculation. Throughout the course of the study, body weight was monitored and recorded daily to track health status and physiological responses. Additionally, the animals were weighed a day before the tumour inoculation procedure to establish a baseline measurement for comparison (see Figure 30).



Figure 30 Illustration of the body weight measurement procedure for an individual experimental mouse using a precision electronic balance

For the establishment of a breast cancer xenograft model, human triple-negative breast cancer cells (MDA-MB-231) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, 100 µg/ml penicillin, and 100 units/ml streptomycin. The cells were maintained in a humidified incubator at 37 °C with 5% CO₂ until they reached 80–90% confluence.

Once optimal confluence was achieved, the cells were detached using 0.15% Trypsin-EDTA and then centrifuged at 1200 rpm for 5 minutes. The resulting cell pellet was resuspended in a pre-warmed culture medium and viable cells were counted using a hemocytometer. The required cell concentration was then mixed with Matrigel containing a blue dye to facilitate visualization and uniform injection during the tumour inoculation process.

4.4.1 Anaesthesia

On the day of the procedure, each mouse was weighed before being anesthetized. Mice were anesthetized by being gently placed inside a pre-warmed induction chamber and monitored continuously by inhalation of 2-4% isoflurane in an oxygen induction chamber. Each mouse was pinched on their toe to check for a reaction. Their eyes were lubricated with an ophthalmic eye lubricant to prevent drying out. A sterile

environment was maintained throughout the course of the procedure, and all instruments were autoclaved before use (Oh and Narver, 2024).

4.4.2 Intramammary implantation of breast cancer cells

Before injection, the tube containing the prepared MDA-MB-231 cells was vortexed briefly to ensure a homogeneous cell suspension. Using a sterile syringe fitted with a 1 ml needle, 5×10^6 cells were carefully drawn and administered into the mammary fat pad of each mouse. The needle was slowly withdrawn post-injection to minimize the risk of cell leakage. Successful intramammary implantation was confirmed by the appearance of a clear, flat, dome-shaped swelling at the site of injection, typically visible at the centre of the nipple, as shown in Figure 31. After the intramammary procedure, each mouse was immediately moved to its respective cage and monitored closely at $\frac{1}{2}$ hour, 1 hour, and then at least 2 more times afterwards until it fully recovered from the injection (total observation = 4 times).



Figure 31 Intramammary inoculation of MDA-MB-231 breast cancer cells in immunodeficient mice (Balb/c mouse model)

4.4.3 Treatment site: Intraperitoneal Injection (IP)

Intraperitoneal injection refers to the administration of a substance directly into the peritoneal cavity, the space within the abdomen that houses various organs such as the liver, spleen and intestines (Flessner, 2023). This route is commonly used in preclinical animal studies, particularly in rodents, due to its ease of administration, rapid absorption, and capacity to deliver a relatively large volume of substances (Small et al., 2022). The treatment substances (A-Chem CdO NPs and the G-CdO NPs) and the control groups were administered intraperitoneally to BALB/c mice for *in vivo* evaluation (Figure 32). This method was selected to ensure systemic distribution of the nanoparticles and to assess both therapeutic efficacy and potential toxicity. All experimental groups and the first control group (inoculated mouse treated with Doxorubicin) received treatment on days zero, four, eight, twelve, and some on day 16, as demonstrated in Table 8. Personnel working with this agent were always wearing protective clothing, e.g., goggles, mask, and impervious disposable gloves to avoid contact with eyes, skin, and clothing.



Figure 32 Intraperitoneal (IP) administration of treatments to the experimental and control groups during the study period

Table 8: Administration schedule for doxorubicin, GCdO NPs, A-Chem CdO NPs, and PBS via intraperitoneal injection.

Substance Administered	Route	Dosage Days
Doxorubicin (Positive Control)	Intraperitoneal (IP)	Day 0, 4, 8, 12, 16
G-CdO NPs (Green-synthesized)	Intraperitoneal (IP)	Day 0, 4, 8, 12, 16
A-Chem CdO NPs (Albumin-coated)	Intraperitoneal (IP)	Day 0, 4, 8, 12, 16
PBS (Negative Control)	Intraperitoneal (IP)	Day 0, 4, 8, 12, 16

4.4.4 Treatment allocation and Monitoring Protocol

Mice injected with MDA-MB231 cells were then randomly assigned to different treatment groups. There were five treatment groups with three mice each, of which two groups were experimental, and three were control groups. A 25G needle and 1ml syringe were used for administering treatment since the viscosity of water which is used for synthesizing the nanoparticles is very low at $8.90 \times 10^{-4} \text{ Pa} \cdot \text{s}$. In the first experimental group, the tumour-induced mice were treated with (0.1 ml/10g body weight) grams of albumin-coated Cadmium oxide nanoparticles (A-Chem CdO NPs). The second tumour-induced mice group was treated with uncoated Cadmium oxide nanoparticles (G-CdO NPs). The first control group consisted of normal mice (without tumour) and untreated, whilst the second control group consisted of a tumour-induced mouse that was treated with the (0.1 ml/10g body weight) standard drug; Doxorubicin). The third control group consisted of mice that were inoculated with the tumour but without treatment (negative control), however, the mice in this group were treated with water/PBS. Summary of experimental treatment groups and administration protocols is illustrated in Table 9.

Table 9: Summary of experimental treatment groups and administration protocols used for the *in vivo* study.

Group	Cell inoculation	Treatment and Dosage	Injection intervals	Number of weeks	Total number of injections	End point
Control Group	No	N/A	N/A	N/A	N/A	Sacrificed at the end of the study
Negative control	Yes	0.1ml/10g of PBS/water	Day 0, 4, 8, 12 and 16	2	4	Sacrificed at the end of the study
Positive control	Yes	0.1ml/10g of free Standard drug (Doxorubicin)	Day 0, 4, 8, 12, and 16	2	4	Sacrificed at the end of the study
Experimental group	Yes	A-Chem CdO NPs 0.1ml/10g	Day 0, 4, 8, 12, and 16	2	4	Sacrificed at the end of the week
Experimental group	Yes	G-CdO NPs 0.1ml/10g	Day 0, 4, 8, 12, and 16	2	4	Sacrificed at the end of the week

4.4.5 Animal Welfare Monitoring and Tumour Progression Assessment

The health status was checked both in the morning and afternoon, throughout the study. Key indicators included alertness, ocular and periorbital discharge, body weight fluctuations (measured before, during and after treatment, with weight loss $\geq 10\%$ flagged as significant), fatigue, paralysis, aggression, survival rate, abnormal gait and posture, as well as any reluctance to move.

Mice that developed excessively large tumours exceeding 250 mm^3 were humanely euthanized during treatment. For those exhibiting signs of mild distress or discomfort, a qualified veterinarian was immediately consulted. These mice received subcutaneous injections of buprenorphine analgesic at a dosage of 0.05 mg/kg , administered 2–3 times per day in accordance with the veterinarian's guidance. Post-administration, the animals were re-evaluated twice daily to assess improvement. In instances where the animal's condition failed to improve despite pain management, and no further intervention could alleviate the distress, humane euthanasia was performed promptly to prevent unnecessary suffering.

Tumour growth was tracked, with daily measurements conducted using a digital calliper. Mice were positioned supine (abdomen exposed, head upward) to ensure consistent tumour measurement. Tumour volume was calculated using the standard formula: $V (\text{mm}^3) = L \times W^2 / 2$, where L represents the major axis and W the minor axis of the tumour.

All recorded data were systematically logged in a designated Microsoft Excel spreadsheet for subsequent analysis.

4.5 Euthanasia and organ harvesting

At the end of the experimental period, all animals from Groups 1 through 5, including the three reserve/emergency mice, were humanely euthanized per institutional and ethical guidelines. Euthanasia was performed using a two-step protocol: animals were first anesthetized with 5% vaporized isoflurane until complete sedation was achieved. Once fully sedated, the mice were transferred to a sealed euthanasia chamber containing carbon dioxide (CO₂) and exposed for 5–6 minutes to ensure complete cessation of vital functions through inhalation-induced respiratory arrest.

Following euthanasia, each mouse was weighed, and a necropsy was performed. The chest and abdominal cavities were carefully opened using sterile scalpels and surgical scissors to expose internal organs. Tumours and key target organs, including the lungs and liver, were harvested, rinsed gently with phosphate-buffered saline (PBS) to remove blood and debris, and immediately fixed in 10% neutral-buffered formalin for histopathological analysis. These tissues were later processed for haematoxylin and eosin (H&E) staining to assess cellular architecture and verify the presence or absence of metastatic lesions.

Following organ collection and tumours, all animal remains were disposed of responsibly. The carcasses were placed in a designated biomedical waste container provided by Compass Medical Waste Service, a licensed and accredited commercial biohazard disposal company. The remains were subsequently collected and incinerated under national biomedical waste disposal regulations to ensure environmentally safe and ethical disposal.

4.6 Data collection methods

Both qualitative and quantitative data analysis methods were employed in this study to assess the effects of treatment on tumour progression and overall animal health.

Qualitative Data

Qualitative variables included behavioural and physiological indicators such as changes in physical appearance (e.g. fur condition), eye discharge, eyesight, hearing

ability and the presence of paralysis. Behavioural assessments focused on signs of aggression, abnormal gait, abnormal posture and reluctance to move. These parameters were observed and recorded throughout the experimental period.

Quantitative Data

Quantitative variables included tumour volume (measured in mm³) before and after treatment, body weight of the mice recorded daily and survival rate. Monitoring of body weight served as a key indicator for systemic toxicity. All raw data were recorded manually by the researcher using structured data collection sheets and later captured electronically in Microsoft Excel.

Statistical Analysis

Further statistical analysis was conducted by a qualified statistician using SAS software, Version 9.2. Descriptive statistics were applied as follows: frequencies and percentages for qualitative data and means with standard deviations (or medians with percentiles, where appropriate) for quantitative data. The Shapiro-Wilk test was performed to assess normality in the distribution of quantitative data. Depending on the outcome of this test, appropriate inferential statistical tests were selected:

- **Kruskal-Wallis Test:** Applied for comparing median values among more than two independent groups when the data were not normally distributed.

One-Way ANOVA: Applied to normal data. A 95% confidence interval (CI) was calculated for mean or median differences between groups. Statistical significance was determined at a threshold of $\alpha = 0.05$.

4.7 Histopathological examination

After stripping, tumours and major organs (lungs and liver) of each group were put into a 4% paraformaldehyde solution to fix for 24 hours. After that, conventional dehydration, paraffin embedding, slicing, and hematoxylin and eosin (H&E) staining were gradually carried out. Morphological changes of tumours and major organs were observed and photographed under an optical microscope.

4.7.1 Histology Steps in Preparing the Specimens

1. Fixation

Fixation is the initial step in histological preparation, involving the complete immersion of tissue in a fixative solution for 48 hours. This process is essential for halting all biological processes quickly and preserving the structural integrity of the cells with minimal distortion. Fixation also prevents autolysis (self-digestion) and microbial activity, while enhancing the refractive index of tissues, which is important for microscopic analysis.

2. Sampling

Following fixation, tissue fragments are transferred into plastic cassettes for further processing. In this study, a 10% buffered formalin solution was used for fixation. Each cassette was clearly labelled with specimen-specific numbers and details to ensure traceability and accuracy throughout the histological workflow.

3. Processing

The fixed samples were then processed using a Sakura automated tissue processor. The processing phase consists of three key steps: dehydration, clearing and infiltration.

a. Dehydration

Dehydration involves the removal of water from the tissue, which is present in both free and bound forms. This step is performed using a graded series of ethanol concentrations, typically starting at 70%, then 90% and finally 100% ethanol to ensure complete removal of water. Incomplete dehydration may compromise subsequent processing steps.

b. Clearing

Clearing serves as the transition between dehydration and infiltration. As ethanol is not miscible with paraffin wax, a clearing agent such as Xylene is used to displace the

alcohol and prepare the tissue for wax infiltration. Xylene also renders tissues translucent, removes any residual water and improves microscopic visibility. It is crucial that clearing is thoroughly completed before proceeding.

c. Infiltration

Infiltration, also known as impregnation, involves saturating the tissue with a supporting medium, typically paraffin wax. The tissues are immersed in molten wax at approximately 57 °C, which fills the tissue spaces and hardens upon cooling. This step provides the necessary rigidity for precise tissue sectioning during microtomy.

4. Embedding

After infiltration, tissue fragments are embedded in paraffin wax using an embedding machine. Molds of various sizes are used, and only one cassette is opened at a time to avoid cross-contamination. The tissue is placed in the mold, covered with melted wax and the labelled cassette is positioned on top. The mold is then cooled on a cold plate until the wax solidifies. The resulting paraffin block encasing the tissue is then ready for microtomy.

5. Microtomy

Microtomy involves the sectioning of the solidified tissue blocks into thin, uniform slices using a microtome. In this case, a Leica microtome was used to produce sections measuring between 3–5 μm in thickness. This step is critical, as it enables cellular and structural examination under a microscope. Precision and dexterity are vital in this process to ensure the production of diagnostically viable sections.

6. Staining

The prepared tissue sections were stained using Mayer's hematoxylin and eosin (H&E), the standard routine staining method in histology laboratories. The staining process includes the following sequential steps:

- Deparaffinisation in Xylene
- Hydration with graded alcohols

- Rinsing with water
- Staining with Hematoxylin
- Washing
- Staining with Eosin
- Final dehydration with alcohol
- Clearing with Xylene

This dual staining technique provides an excellent contrast, with Hematoxylin highlighting nuclei in dark violet-blue and Eosin staining the cytoplasm and extracellular matrix in varying shades of pink.

7. Mounting

Mounting involves sealing the stained tissue sections with coverslips to preserve them and facilitate microscopic analysis. Entellan mounting medium was used to affix the coverslips, ensuring clarity and durability for observation.

8. Microscopic Examination

The stained slides were examined under a light microscope. The observed results were as follows:

- Nuclei appeared blue or dark violet
- Cytoplasm displayed various shades of pink
- Muscle fibers appeared deep pink to red
- Red blood cells stained orange-red
- Fibrin appeared as a deep pink structure

9. Reporting Findings

The microscopic observations were documented and interpreted for diagnostic or research purposes. These findings contribute to a broader understanding of tissue pathology and are essential in clinical or experimental settings. A schematic representation of this histological preparation workflow is presented in Figure 33.



Figure 33 summarizes the histological procedure of specimen preparations

4.8 *In Vivo* Experimental Outcomes

4.8.1 Introduction

The objective of the *in vivo* study was to evaluate the efficacy of anti-tumour efficacy and systemic toxicity of albumin-coated cadmium oxide nanoparticles (A-Chem CdO NPs) in an MDA-MB231/NU/J (BALB/C) strain triple-negative breast cancer animal model.

4.8.2 Enrolment and Treatment Completion in Experimental Groups.

Tumour Establishment and Group-Specific Challenges

Tumour establishment was monitored daily following cell inoculation. As shown in Figure 34A, measurable tumour growth was observed between Day 7 and Day 14 post-inoculation, aligning with expected tumour latency for MDA-MB-231 xenografts. Mice were eligible for treatment only once tumour volumes reached a minimum of 100 mm³. Treatment was administered every four days (Days 0, 4, 8, 12 and 16), and tumour volumes were monitored throughout the treatment cycle.

Despite these protocols, several animals did not meet tumour size eligibility, while others died or were withdrawn due to ethical endpoints. This affected treatment consistency and statistical representation, particularly in the two experimental nanoparticle groups.

Mice in both treatment groups encountered challenges with tumour development. In the A-Chem CdO NPs Group, of the four mice allocated (including one emergency mouse), only one Mouse (labelled 7) reached the required tumour volume (100 mm³) and received the full treatment regimen for 16 days. This mouse was euthanized on day 17 due to tumour volume exceeding 586 mm³. The remaining three mice did not develop tumours (Mice 12 and 16) or developed tumours that remained below the minimum palpable volume (Mouse 9), rendering them ineligible for treatment initiation.

In the G-CdO NPs Group, out of five mice, only Mouse 23 completed the 20-day treatment regimen. One mouse (Mouse 6) died within 24 hours of treatment initiation, one (Mouse 2) failed to develop a tumour, and two others (Mice 14 and 17) had sub-palpable tumours, disqualifying them from treatment. In the Positive Control Group (Doxorubicin), all three mice (Mice 1, 5 and 18) completed the full 20-day treatment regimen. In the Negative Control Group (PBS), all three mice (Mice 13, 15 and 19) completed the experimental period without complications. This outcome introduces a limitation in statistical power for the A-Chem CdO NP and G CdO NP groups, where only one mouse per group met the criteria and completed the full treatment protocol. This scarcity of data affects the reliability of intergroup comparisons and inferential statistics. The number of mice that completed treatment is summarised in table 10.

Table 10 summary of mice that completed treatment per treatment and control group

Group	Mice Allocated	Emergency Replacements	Completed Treatment
A-Chem CdO NPs	3	1	1
G CdO NPs	3	2	1
Positive Control (Dox)	3	0	3
Negative Control (PBS)	3	0	3
Control (No, inoculation, no treatment)	3	0	3

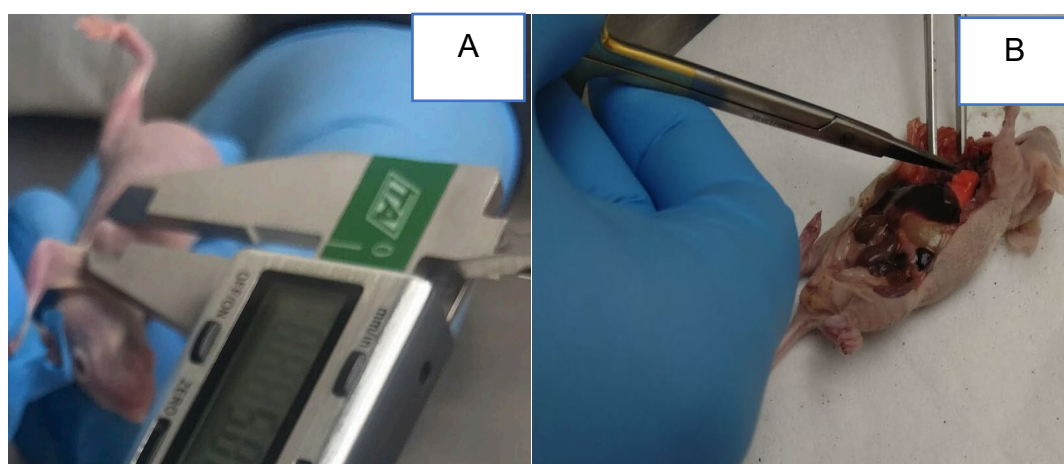


Figure 34 **(A)** Sequential measurement and monitoring of tumour growth throughout the treatment period until completion of the study, and **(B)** harvesting of major organs by the veterinarian for post-treatment evaluation and histopathological analysis.

4.8.3 Observational Parameters

All mice were observed twice daily (morning and evening) from the day of tumour inoculation until euthanasia. Trained personnel conducted evaluations using a standardized observational scoring sheet based on the OECD and ARRIVE guidelines. All the monitored parameters are summarised in Table 11.

Table 11 Monitored Clinical Parameters pre and post treatment

Category	Clinical Signs Assessed	Scoring / Notes
----------	-------------------------	-----------------

General Appearance	Fur condition (ruffled, patchy, shiny), posture, and grooming	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Neurological	Gait abnormalities, tremors, seizures, paralysis	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Ocular	Eye discharge, corneal opacity, squinting	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Auditory	Startle response to sound, head tilt	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Respiratory	Breathing rate, labored breathing, nasal discharge	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Locomotion	Reluctance to move, abnormal gait, dragging limbs	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Behavioural	Aggression, lethargy, hyperactivity, withdrawal	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Food/Water Intake	Visual assessment of consumption, water bottle level checks	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe
Body Weight	Daily or alternate-day measurement	0 = Normal, 1 = mild change change, 2 = moderate change, 3= severe

Clinical signs were monitored daily throughout the 38-day treatment period for all experimental groups, including A-Chem CdO NPs, G-CdO NPs, Positive Control (Doxorubicin) and Negative Control. Clinical signs included visible changes such as piloerection, hunched posture, altered locomotion and general signs of discomfort or stress, scored per group per day. In the A-Chem CdO NP group, no signs were observed during the initial phase (Days 1–10). Mild signs emerged on Day 11 and persisted intermittently, peaking at a score of 2 on Days 18 and as observed in Table This pattern indicates delayed and low-grade systemic responses, aligning with the hypothesized biocompatibility of the albumin-coated formulation.

Table 12: Summary of clinical observation health checks outcome per treatment group

Group	Onset of Signs	Peak Score	Duration (Days with Signs)	Overall Severity
A-Chem CdO NPs	Day 11	2	~21 days	Mild
G-CdO NPs	Day 4	3	~30+ days	Moderate to severe
Positive Control	Day 6	3	~33 days	Moderate to Severe
Negative Control	Day 30	1	9 days	Minimal (tumour-related)

The A-Chem CdO NPs showed a delayed onset of clinical signs with mild severity in comparison to doxorubicin. Could it be that the efficiency of albumin-functionalised nanoparticles is dependent on time? The G-CdO NP group displayed earlier onset of clinical signs (Day 4), which increased in frequency and intensity over time, peaking at a score of 3 from Days 28 to 34. This suggests moderate systemic toxicity and aligns with expected reactivity due to uncoated Cadmium oxide nanoparticles. The Doxorubicin-treated (positive control) group exhibited signs starting from Day 6, escalating to scores of 2–3 by mid-treatment, consistent with known cumulative side effects of anthracycline drugs such as fatigue and weight loss. Signs were sustained through Day 38. In contrast, the Negative Control group exhibited no clinical signs until Day 30, after which mild signs (score of 1) were observed. These are likely attributable to tumour burden rather than any systemic toxicity, reaffirming the safety of PBS treatment.

4.8.4 Toxicity Evaluation in *In Vivo* Studies

Introduction

Monitoring body mass changes *in vivo* is a widely accepted, non-invasive method for assessing systemic toxicity. Significant loss of body mass, particularly over 20%, is generally indicative of severe physiological stress or toxicity. This section presents a comprehensive analysis of body mass loss in mice treated with A-Chem CdO NPs and G-CdO NPs, with comparisons to positive (Doxorubicin) and negative (PBS) control treatments.

Body Mass Loss and Toxicity Interpretation

Body weight was measured daily, and a body weight change (BWC) was calculated using the following formula:

$$\text{BWC (\%)} = [(\text{body weight on the last day}) - (\text{body weight on day 0})]/(\text{body weight on day 0}) \times 100 (\%).$$

Toxicity was defined as a BWC of <20%, or toxic mortality

Body mass loss was assessed as a non-invasive biomarker of systemic toxicity during a 17-day treatment period. Among the experimental groups, mice treated with green-synthesized CdO nanoparticles (G-CdO NPs) exhibited the most pronounced weight loss, peaking at approximately 25% by Day 6. This acute and sustained reduction in body weight is indicative of severe systemic toxicity. The group treated with Albumin-coated chemically synthesized CdO nanoparticles (A-Chem CdO NPs) demonstrated a weight decline, reaching a peak of 17.1% by Day 6, which then stabilized, suggesting significant early-onset toxicity. In comparison, the positive control group treated with Doxorubicin exhibited a steady and progressive weight loss reaching about 17.2% by Day 17, reflecting its well-documented chemotherapeutic toxicity profile. The negative control group maintained relatively stable body weight, with minimal fluctuations, further confirming that observed weight changes in the treated groups were due to the administered substances. Statistical analysis using repeated measures revealed significant differences among treatment groups ($p < 0.001$), with *post hoc* comparisons confirming that G-CdO NPs induced significantly greater weight loss than all other groups ($p < 0.001$) (Table 13 and Figure 35). These findings underscore the necessity for further optimization of nanoparticle formulations to improve safety profiles.

Table 13: Descriptive Statistics of % Body Mass Loss

Treatment Group	Mean % Loss $\pm$ SD	Max % Loss	Day of Max Loss	Trend Description
Negative Control	2.1 $\pm$ 1.0%	3.5%	Day 8	Stable; no significant fluctuations
Positive Control	11.9 $\pm$ 2.8%	17.2%	Day 17	Gradual increase across study period
A-Chem CdO NPs	14.3 $\pm$ 2.4%	17.1%	Day 6	Early rapid loss, sustained thereafter
G-CdO NPs	18.7 $\pm$ 3.1%	25.3%	Day 5	Acute toxicity with peak at day 5

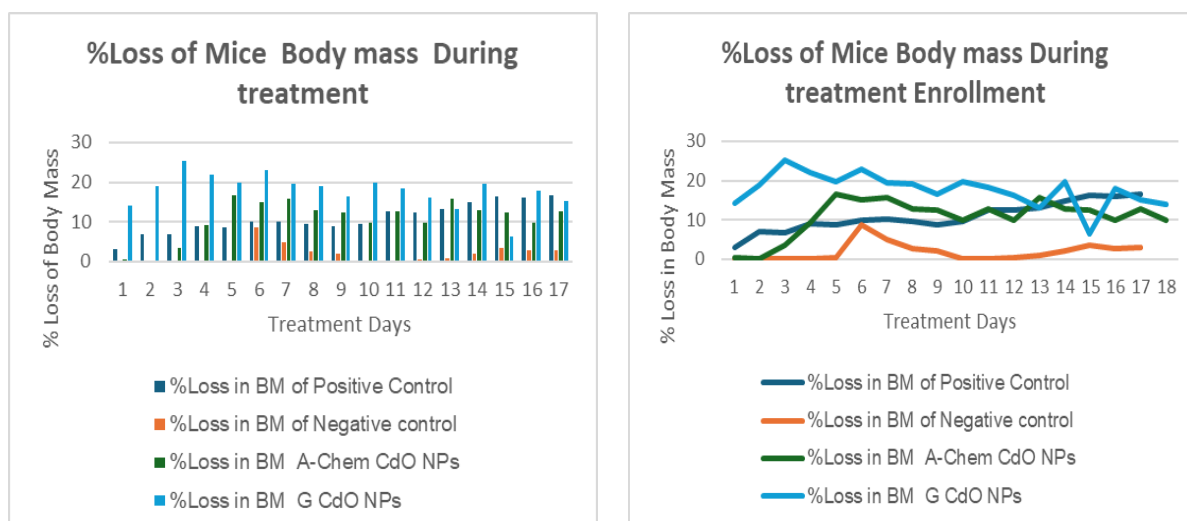


Figure 35 A and B illustrate the toxicity profiling of the experimental mice, as determined by the percentage loss in body mass during the treatment period.

4.9. Tumour Volume Progression During Treatment

The tumour volume (V) was calculated using the formula:

$$V = (1/2) \times L \times W^2,$$

L	=Length
W	=Width

The formula assumes that the tumour is roughly ellipsoid-shaped, which is a reasonable approximation for many subcutaneous tumours. Tumour volume is an important metric for monitoring tumour growth and treatment response in preclinical studies. However, the ellipsoidal approximation may not be accurate for all tumour types or sizes. Some tumours have irregular shapes, and the formula might underestimate or overestimate their volume (Aldea et al., 2024).

The R (RTV) was calculated using the formula:

$$\text{RTV} = (\text{tumour volume on measured day})/(\text{tumour volume on day 0}).$$

The effect of various treatments on tumour progression was evaluated by monitoring the relative tumour volume over a period of 16 to 20 days, as illustrated in Figure 36. The positive control group treated with Doxorubicin demonstrated the most significant tumour suppression, maintaining a consistently low tumour volume throughout the treatment period. In contrast, the negative control group (PBS) exhibited a steady increase in tumour volume, confirming progressive tumour growth in the absence of therapeutic intervention.

The group treated with albumin-coated Cadmium oxide nanoparticles (A-Chem CdO NPs) displayed a moderate increase in tumour volume compared to the PBS control, indicating a partial inhibitory effect. This suggests that albumin functionalisation may enhance the therapeutic potential of CdO NPs, possibly through improved biocompatibility or tumour targeting. On the other hand, the group treated with uncoated CdO NPs exhibited the highest tumour volume, with a pronounced peak on Day 15.

Notably, a sharp decline in tumour volume in both CdO NP-treated groups towards Day 17. Overall, these findings suggest that while albumin-coating of CdO NPs improves tumour response relative to uncoated formulations, Doxorubicin remains the most effective agent among the tested treatments.

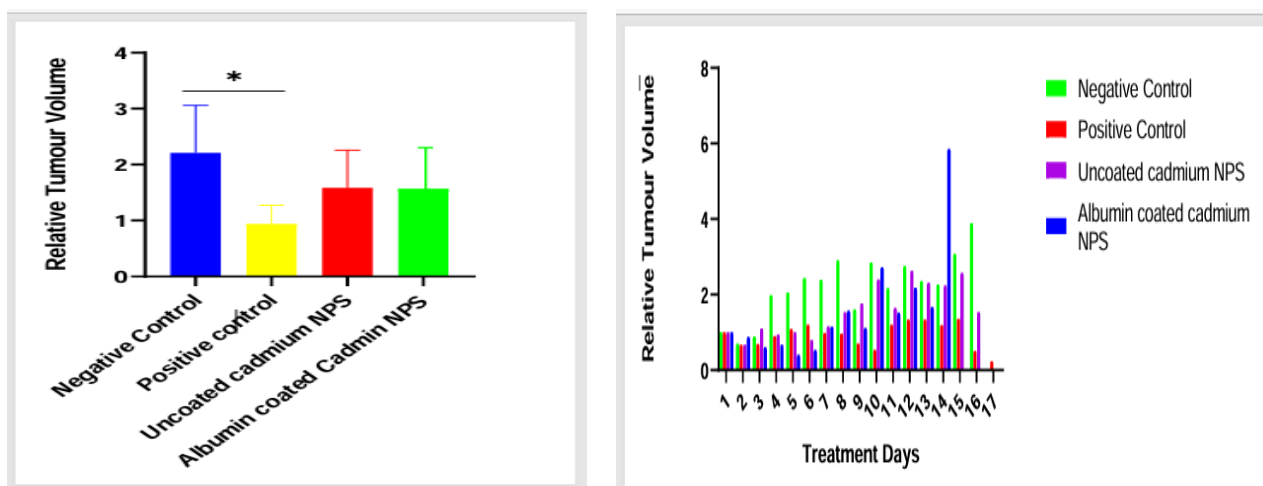


Figure Figures 36A and 36B demonstrate the efficacy assessment of the treatment regimen through relative tumour volume analysis. A presents the relative tumour volume measurements, while B illustrates tumour progression over the treatment period. By day 17, some mice had already been euthanized due to study endpoint criteria

4.9.2 Tumour Inhibition Rate (TIR)

The tumour growth inhibition ratio (TGI, %) which is used interchangeably with Tumour Inhibition Rate (TIR) was calculated using the following formula:

$$TGI = [1 - (RTV \text{ of the treated group}) / (RTV \text{ of the control group})]$$

It reflects the difference in tumour volume between treated and control groups. This formula expresses the extent to which treatment has slowed or stopped tumour growth compared to a non-treated group.

Tumour Inhibition Rate (TIR) was assessed across three treatment groups: positive control (Doxorubicin), uncoated cadmium oxide nanoparticles (CdO NPs) and albumin-coated CdO NPs. The bar chart illustrates the average TIR for each treatment group with associated standard deviation. Doxorubicin (blue bar) achieved the highest inhibition, with a mean TIR of approximately 50–55%, confirming its well-established cytotoxic effect in triple-negative breast cancer models. Albumin-coated CdO NPs (red bar) showed a TIR of approximately 30–35%, which was slightly higher than that of the uncoated CdO NPs (green bar), which achieved a TIR of around 25–30%. While the p-value ($p = 0.091$) indicates that the observed differences were not statistically significant at the 0.05 threshold, the trend suggests that albumin surface modification

may have modestly enhanced antitumor efficacy (Figure 37). Albumin coating is hypothesized to improve nanoparticle pharmacokinetics through increased serum stability and enhanced tumour accumulation via the enhanced permeability and retention (EPR) effect. This modest benefit observed here may support that theory, although further optimization and dosing studies are warranted. Moreover, the overlap in error bars underscores the variability in treatment response, which may be addressed in future studies through increased sample sizes or more targeted delivery strategies. Overall, while Doxorubicin remains the most potent treatment, the observed efficacy of CdO NPs, particularly when coated with albumin, demonstrates potential for nanoparticle-mediated cancer therapy. Future studies should combine tumour growth metrics with histopathological analysis and biodistribution profiling to better understand and improve the therapeutic profile of CdO-based nanomedicine.

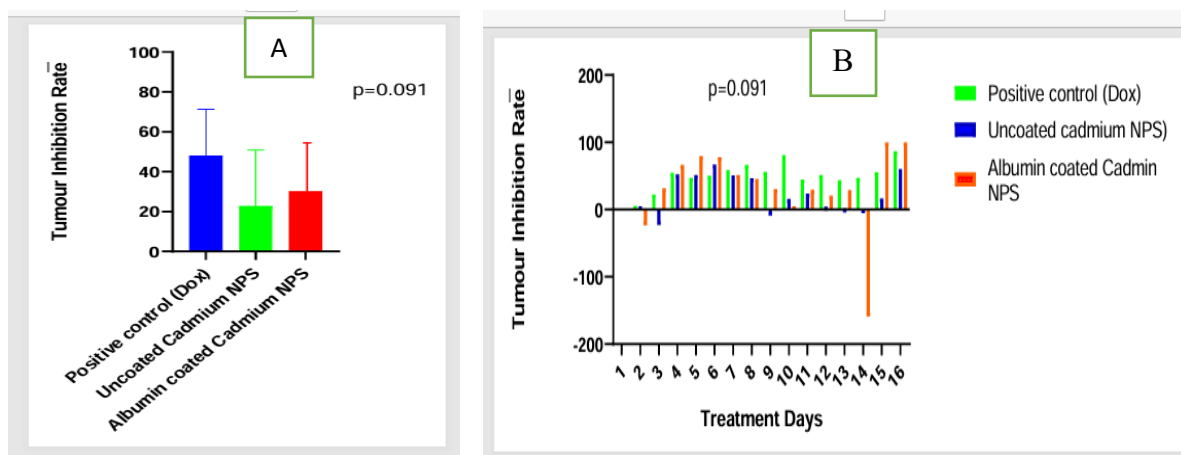
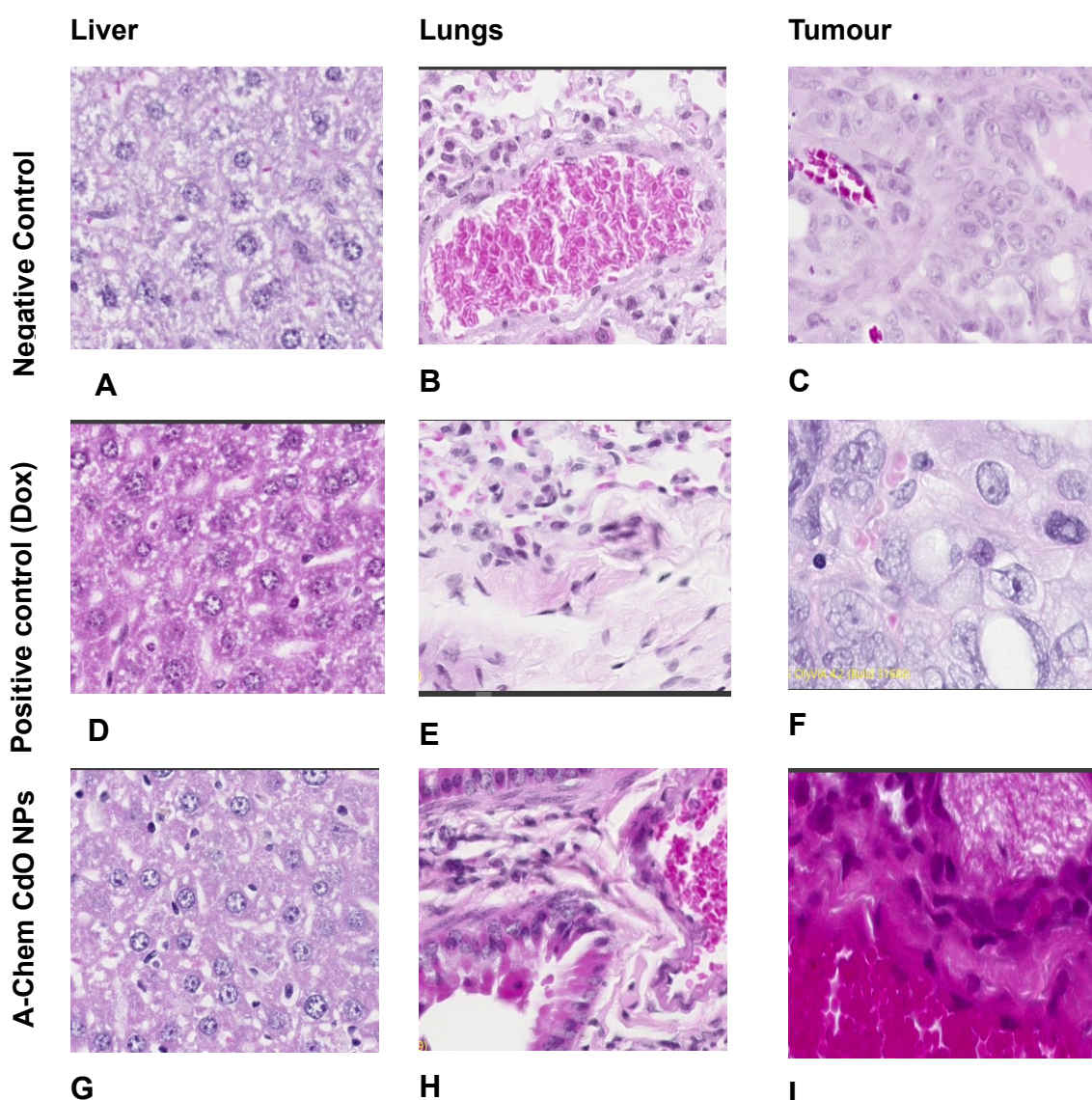


Figure 37 **A** and **B** Show antitumor activity rate. Doxorubicin showed the highest inhibition, followed by A-Chem CdO NPs and lastly G-CdO NPs

4.9.3 Histological Presentation of Harvested Tissues (Liver and Lungs)

To evaluate the potential metastasis and systemic toxicity following treatment, histological analyses of the liver and lung tissues were performed. Haematoxylin and Eosin staining were used to assess necrotic changes in both primary tumour sites and distant organs, indicating treatment efficacy and potential off-target effects. Tissues were collected from all groups. Olympus was used to read the captured data on the Olympus Slideview VS200 research slide scanner. The images were captured at X50 magnification on Olyvia software.

Histological evaluation revealed distinct patterns of necrosis across the different treatment groups (Figure 38). All treatment groups showed positive necrosis within tumour tissues, indicating tumour response or natural progression, respectively, with no signs of necrosis in the liver or lungs. This could suggest that Doxorubicin, A-Chem CdO NPs and G CdO NPs effectively targeted the tumour without causing damage to major organs, while PBS allowed tumour progression without inducing toxicity. A-Chem CdO, G- CdO NPs and Doxorubicin induced necrosis only in tumour tissues, with no necrotic changes observed in the liver or lungs (see Table 14).



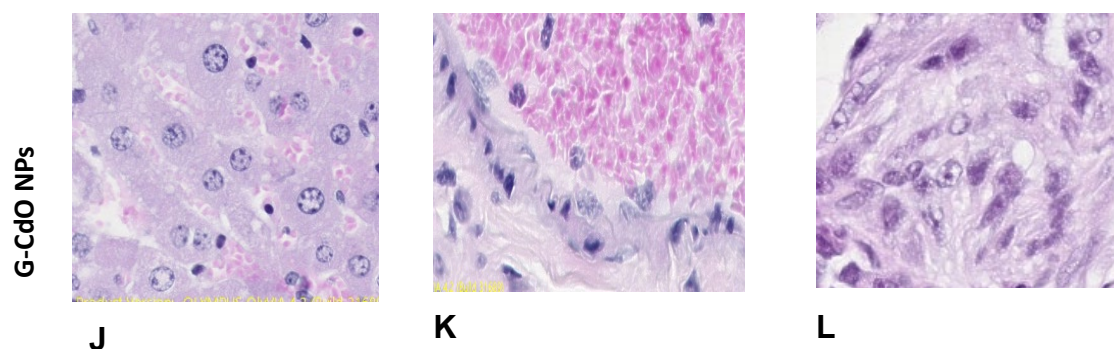


Figure 38 (H&E stain, x40). **A** shows liver tissue treated with PBS. **B** shows lung Tissue treated with PBS. **C** shows tumor treated with PBS. **D** shows liver tissue treated with doxorubicin. **E** shows lung tissues treated with doxorubicin. **F** shows tumour tissue treated with Doxorubicin. **G** shows liver tissue treated with A-Chem CdO NPs. **H** shows lung tissues treated with A-Chem CdO NPs. **I** shows tumor tissue treated with A-Chem CdO NPs. **J** shows Liver tissues treated with G-CdO NPs. **K** shows lung tissues treated with G-CdO NPs. **L** shows tumor treated with G-CdO NPs.

Table 14 Summarises the Necrosis state of Tissues from mice administered with various treatments.

Treatment Group	Tissue	Necrosis	Notes
Doxorubicin	Liver	Negative (-)	Disruption of hepatic architecture. Mild inflammation
	Lung	Negative (-)	Infiltration of inflammatory cells
	Tumour	Positive (+)	Extensive tumour necrosis and reduced cell density.

			Possibility of apoptosis
PBS	Liver	Negative (-)	Normal liver architecture. No evidence of inflammation
	Lung	Negative (-)	Normal pulmonary architecture; alveolar spaces are clear with thin septa and no signs of inflammation
	Tumour	Positive (+)	No signs of regression.
G CdO NPs	Liver	Negative (-)	Minimal structural alterations
	Lung	Negative (-)	Largely preserved lung architecture; minor inflammatory infiltrates
	Tumour	Positive (+)	nuclear shrinkage
A-Chem CdO NPs	Liver	Negative (-)	Moderate architectural distortion with mild inflammatory response.
	Lung	Negative (-)	Moderate alveolar congestion
	Tumour	Positive (+)	Areas of cellular necrosis and chromatin

			condensation. Suggestion of moderate tumour regression
--	--	--	--

Summary

Successful intramammary implantation of MDA-MB-231 triple-negative breast cancer cells was confirmed by the presence of a flat, dome-shaped swelling at the injection site. Treatment via intraperitoneal injection began once tumours reached approximately 100 mm³. The study assessed the efficacy and systemic toxicity of Doxorubicin, PBS, G CdO NPs, and A-Chem CdO NPs. A-Chem CdO NPs induced mild clinical signs with delayed onset compared to Doxorubicin, which caused earlier and more severe toxicity. Body mass loss was comparable between A-Chem CdO NPs (17.1%) and Doxorubicin (17.2%). Doxorubicin achieved the highest tumour inhibition rate (50–55%), while albumin-coated CdO NPs showed moderate antitumour activity (30–35%). Histopathological analysis revealed necrotic changes in tumour tissues across all treatment groups, indicating treatment response. No necrotic lesions or metastases were observed in liver or lung tissues, suggesting minimal off-target toxicity. These results indicate that the standard drug doxorubicin showed a higher efficacy compared as indicated by tumor inhibition rate, in comparison to A-Chem CdO NPs which is the main focus of the study. A-Chem nanoparticles showed a lesser systemic toxicity which could be due to the albumin coating.

Chapter 5: Discussion and Conclusion

5.0 Overview of study outcomes

This study aimed to evaluate the anti-cancer efficacy and systemic toxicity of CdO NPs, with and without albumin coating, against the TNBC MDA-MB-231 cell line *in vitro* and in an *in vivo* Balb/C mouse model. TNBC, characterized by the absence of ER, PR and minimal expression of HER2, presents a therapeutic challenge due to limited receptor targets, poor response to immunotherapy, and systemic toxicity from conventional chemotherapy (Bai et al., 2022). Nanotechnology-based solutions such as CdO NPs have garnered attention for their tumour-targeting potential; however, issues of selectivity and biocompatibility remain significant. This research, therefore, introduced chemically synthesised albumin-coated CdO NPs (A-Chem CdO NPs) as a strategy to balance cytotoxic potency with safety, leveraging albumin's tumour-homing properties.

In contrast to the prevailing body of literature that favours the use of green chemistry approaches for nanoparticle synthesis due to their presumed biocompatibility and environmental sustainability (Saxena et al., 2025), the present study demonstrates that A-Chem CdO NPs exhibit superior cytotoxic efficacy, as evidenced by a lower IC_{50} (3.33 $\mu\text{g/ml}$) value compared to the G-CdO NPs (IC_{50} 11.8 $\mu\text{g/ml}$) against MDA-MB-231 breast cancer cells. Outstandingly, doxorubicin, the reference chemotherapeutic agent, displayed the lowest IC_{50} value overall, confirming its potent anticancer activity. However, Doxorubicin also exerted the highest cytotoxicity against non-cancerous Vero cells, suggesting a reduced therapeutic index relative to the nanoparticle formulations. The *in vivo* tumour regression data corroborated the *in vitro* findings, with Doxorubicin eliciting the most significant anti-tumour response, followed sequentially by A-Chem CdO NPs and G-CdO NPs. This gradation in efficacy was mirrored in the histo-morphological evaluations, where tissue alterations observed in tumour and organ histology further validated the cytotoxic profiles and systemic toxicity patterns

of the respective treatments. Albumin coating does show potential *in vivo* to enhance selectivity and reduce cytotoxicity, even though it is not statistically significant.

5.1 Structural Overview of Discussion

This chapter provides a comprehensive interpretation of the study's findings, offering critical insights into both the experimental results and the methodologies employed. It begins by evaluating how the applied methods may have influenced the observed outcomes, followed by a comparative analysis with existing literature to contextualize the study within the broader scientific discourse. The results are systematically discussed across several key domains: nanoparticle characterization, *in vitro* cytotoxicity and selectivity, and *in vivo* outcomes including clinical observations, systemic toxicity, relative tumour volume, tumour inhibition rate, and histomorphological analyses. Finally, the chapter addresses the study's limitations and reflects on key lessons learned, setting the stage for future research and potential clinical translation.

5.2 Synthesis techniques and characterization of Cadmium Oxide Nanoparticles (CdO NPs) Formulations

The first objective of this study was to synthesize CdO NPs using both green synthesis and chemical reduction methods, followed by comprehensive characterization using X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Energy Dispersive Electron Spectroscopy (EDS), Ultraviolet-visible (UV-Vis) spectroscopy, X-ray Photoelectron spectroscopy (XPS), and Fourier-transform infrared spectroscopy (FTIR). The physicochemical properties of nanoparticles, mostly size, shape, and surface characteristics, are crucial determinants of their functional performance, including degradation kinetics, drug release profiles, targeting specificity, and cellular internalization (Zhang et al., 2020).

XRD patterns provided evidence of the structural properties of the nanoparticles and confirmed the successful synthesis of the nanoparticles. G CdO NPs exhibited broader and less intense diffraction peaks, with a dominant low-angle reflection near 10° . This

broadening is attributed to reduced crystal size and the presence of residual organic compounds from plant-based reducers. The presence of phytochemicals likely affects both the nucleation and growth kinetics during synthesis, resulting in more amorphous or polycrystalline structures (Ait et al., 2024). In contrast, the XRD pattern of the Chem CdO NP sdisplayed well-defined, sharp peaks that correspond to (111), (200), and (220) planes of the face-centered cubic (FCC) structure of CdO. These observations suggest a high degree of crystallinity and phase purity, consistent with controlled inorganic synthesis protocols. The narrower full-width at half-maximum (FWHM) values further imply the formation of larger crystallites, which may enhance the material's thermal and mechanical stability (Cholakova and Denkov, 2024). The A-Chem CdO NPs retained the fundamental crystalline structure observed in the chemically synthesized samples, with slight peak broadening and intensity variation. These effects are attributed to the interaction between the nanoparticle surface and the albumin layer, which could impose strain on the crystal lattice or dampen X-ray scattering due to partial shielding by organic molecules (Park, 2020). This formulation demonstrates a balance between structural integrity and functional biocompatibility, making it promising for drug delivery and imaging applications. Interestingly, the persistent peak at $\sim 10^\circ$ across green and albumin-capped samples could correspond to an organic signature most likely arising from bio-organic residues or protein layers introduced during synthesis.

The synthesis route affects the structural properties of CdO nanoparticles as revealed by XRD analysis. Chemically synthesized samples present the highest crystallinity and phase definition, suitable for industrial or catalysis applications requiring material stability. Green-synthesized nanoparticles offer eco-friendly benefits with biomolecular functionality but display lower structural order. Albumin-functionalized CdO nanoparticles strike a balance between crystallinity and bio-interface utility, supporting their application in biomedical technologies.

The structural features elucidated through XRD strongly correlate with the observed biological activities of the respective CdO NPs formulations. Chem CdO NPs, exhibiting sharp and well-defined diffraction peaks, demonstrated higher crystallinity and phase purity features that likely contribute to their enhanced cytotoxic efficacy. Conversely, green-synthesized CdO particles revealed broader, less intense peaks

indicative of smaller crystallite size and potential organic contamination, which may account for their diminished anti-cancer performance. The albumin-coated variant maintained a moderate level of crystallinity while introducing a biocompatible interface, as seen in the slight broadening and altered intensity of XRD peaks. These structural signatures suggest that while crystallinity enhances potency, surface modifications like albumin may mitigate off-target toxicity without severely compromising efficacy.

Morphological analysis using SEM and TEM revealed distinct structural differences among the three synthesized CdO nanoparticle formulations. Both G-CdO NPs and Chem-CdO NPs exhibited relatively uniform spherical and cube-like shapes, respectively. In contrast, A-Chem-CdO NPs formed irregular, self-assembled clusters with non-uniform spherical morphologies. These differences are attributable to the synthesis methods and stabilizing agents employed.

Green synthesis approaches utilize plant extracts or other biologically derived materials as both reducing and capping agents. The phytochemicals within these extracts, such as flavonoids, phenolics, and alkaloids, play a pivotal role in shaping nanoparticle morphology (Fahim et al., 2024). For example, Sood et al., (2025) demonstrated that CdO nanoparticles synthesized using tragacanth gum extract under microwave irradiation predominantly yielded spherical particles with well-defined crystalline structures. Conversely, chemical synthesis methods such as hydrothermal or co-precipitation offer more precise control over reaction parameters and often yield highly uniform nanoparticles with well-defined geometries (Darr, et al., 2017). Nasrullah et al., (2020) reported that the controlled conditions of chemical synthesis favour the formation of cube-like or other crystalline morphologies.

These findings align with existing literature, which consistently emphasizes the profound influence of synthesis methodology on nanoparticle size and shape; parameters that are strongly linked to biological behaviour and therapeutic efficacy (Zhang et al., 2020). For instance, studies on shape-dependent uptake in HeLa cells have shown that nanoparticles larger than 100 nm with rod-like or ellipsoidal geometries often exhibit enhanced cellular internalization compared to their spherical or cubic counterparts. The general trend observed is: rods/ellipsoids > spheres > cylinders > cubes. Although spherical nanoparticles are typically internalized efficiently, non-spherical geometries may offer more intricate interactions with cellular

membranes due to differences in their aspect ratios and surface area, which can influence receptor-mediated endocytosis (Kudryavtseva and Sukhorukov, 2024). In the present study, the irregular, self-assembled morphology observed in albumin-coated chemically synthesized CdO nanoparticles (A-Chem CdO NPs) may have contributed to their enhanced cytotoxic efficacy relative to the green-synthesized CdO NPs (G-CdO NPs). This enhanced biological activity could be attributed to a combination of improved cellular uptake, altered surface chemistry due to albumin functionalization and increased interaction with intracellular targets, highlighting the critical interplay between structural properties and therapeutic outcomes.

The size distribution for A-Chem CdO NPs (0.25 – 127 nm) and G CdO NPs (0,25 - 127 nm) fall within the optimal Nano medicine range, which facilitates enhanced permeability and retention (EPR) effect in tumours (Wu, 2021). In contrast, Chem CdO NPs, though smaller (0.25 – 4.74 nm), exhibited agglomeration; a feature commonly associated with poor colloidal stability and potential cytotoxicity (Wang et al., 2018). In this study it is observed that albumin coating increases the nanoparticles' size and this could have the potential to increase therapeutic efficacy. The sizes of nanoparticles are critical for a range of NPs properties, including degradation rate, drug release profiles, *in vivo* distribution and clearance, and also cellular internalization. Therefore, formulation design and manufacturing processes are key steps to control when making nanoparticles with a known target size that are able to achieve the required therapeutic index for the tissue of interest (Bai et al., 2022). Nanoparticles in the size range between 40 – 200 nm are reported to have a prolonged circulation time, increased accumulation in tissues and decreased renal clearance. Whereas nanoparticles larger than 200 nm have a higher rate of clearance and nanoparticles smaller than 10 nm are likely to be removed by renal filtration. The release of drugs is highly dependent on the size of nanoparticles (Bai et al., 2022). The size of nanoparticles also determines the site of accumulation and/or clearance when injected *in vivo*. Yadav et al., (2011) compared the injection of two formulations of etoposide-loaded PLGA nanoparticles with sizes of 105 nm and 160 nm. Results showed that smaller nanoparticles (105 nm) were retained in the blood at higher concentrations, but accumulated more in the bone and brain tissues. Accumulation in tissues was found to be negligible when using larger nanoparticles. This effect resulting from the size of the nanoparticles is vital to enhance therapeutic efficacy; therefore, future studies can

leverage on the limitations of this study by incorporating drug release and bio-distribution experiments so as to be able to determine the retention time and tumour sites.

Surface Elemental Composition

XPS and EDS analyses provided complementary insights into the surface composition of the synthesized CdO nanoparticle formulations. The presence of Cadmium and Oxygen in all formulations confirmed the formation of Cadmium oxide as the primary inorganic phase. The detection of Chlorine across all formulations is attributed to the use of Cadmium chloride (CdCl_2) as a precursor during synthesis (Karthik et al., 2017). The G-CdO NPs showed additional elements such as Magnesium, Potassium, Calcium, and Nitrogen, likely originating from phytochemicals and minerals present in the plant extract. This diversity of elements is typical in green-synthesized nanoparticles and reflects the complexity and multifunctionality of the reducing and capping agents used (Abbas et al., 2024). In the Chem-CdO NPs, the appearance of Sodium and Nitrogen may reflect residues from Sodium hydroxide or other synthetic reagents. These findings suggest relatively fewer surface-bound elements compared to the green route, aligning with the cleaner, more controlled profile of chemically synthesized nanostructures (Nasrullah et al., 2020).

The A-Chem CdO NPs demonstrated a richer elemental profile, including Sulfur and Nitrogen, consistent with the presence of albumin coating because albumin contains Cysteine ($-\text{SH}$), Lysine ($-\text{NH}_2$) and other protein residues. The successful incorporation of such elements provides further evidence of surface functionalization, which enhances nanoparticle solubility, dispersion and biological interaction (Azizi, 2018).

The FTIR spectrum of the G-CdO NPs confirmed the integration of various bioactive phytochemicals from the plant extract used during green synthesis. The broad O–H stretching band and the presence of C=O, C–N, and C–O vibrations suggest the presence of polyphenols, flavonoids and carbohydrates, which may function as reducing and stabilizing agents. These biomolecules not only mediate nanoparticle synthesis but also provide functional surface groups that can enhance biocompatibility

and cellular interaction (Karthik et al., 2017; Nasrullah et al., 2020; Sivalingam et al., 2024).

In contrast, the Chem-CdO NPs exhibited minimal organic signals, with only weak O–H bands and an absence of amide or C=O stretches, indicating a purer inorganic profile. The presence of strong Cd–O stretching vibrations and low-wavenumber lattice bands confirms the successful formation of highly crystalline CdO nanoparticles with minimal organic contamination (Sharma et al., 2022). The A-Chem CdO NPs spectrum confirmed the presence of protein functional groups through amide I and II bands, verifying the successful coating of the CdO core with albumin. These bands are consistent with BSA modification and indicate enhanced surface functionalization, which may facilitate targeted delivery and improve dispersion stability in biological media (Azizi, 2018; Dev and Mukadam, 2025).

Collectively, the FTIR spectra of all three nanoparticle formulations confirmed the successful synthesis of Cadmium oxide nanoparticles, with spectral variations reflective of the distinct synthesis approaches and surface chemistries.

The optical properties of green-synthesized Cadmium oxide (CdO) nanoparticles were evaluated using UV–Visible spectroscopy. The absorption spectrum exhibited a broad and continuous band with an onset around 220 – 250 nm, suggesting a high degree of light absorption in the ultraviolet region. This characteristic absorption is attributed to the fundamental electronic transitions from the valence to conduction band in the CdO lattice, indicative of a direct band gap semiconductor (Karthik et al., 2017; Nasrullah et al., 2020). The smooth curve without distinct excitonic peaks suggests a certain degree of polydispersity in particle size and the possible presence of phytochemical surface modifiers resulting from the green synthesis route. Such organic compounds may introduce surface states or alter the local dielectric environment, causing a broader absorption profile. These features not only confirm the successful synthesis of CdO nanoparticles but also imply that their optoelectronic behaviour may be tunable through surface chemistry and particle size modulation inherent to plant-mediated synthesis (Sivalingam et al., 2024).

The UV–Visible spectrum of albumin-coated chemically synthesized Cadmium oxide (A-Chem CdO) nanoparticles exhibited two distinct absorption features, suggesting

both core electronic transitions and protein-associated surface modifications. A prominent peak at ~250 nm corresponded with the intrinsic band-to-band transition of the CdO lattice, confirming that the semiconductor core structure remained intact following albumin conjugation. A secondary, broader absorption band near 350 nm was observed, which is absent in the spectra of green and purely chemical CdO nanoparticles. This band may be attributed to transitions from C=O or N–H groups present in the albumin matrix, or to localized surface plasmon resonance influenced by protein–nanoparticle interactions (Azizi, 2018; Dev and Mukadam, 2025). The bimodal absorption behaviour provides optical confirmation of successful protein functionalization, aligning with FTIR observations and supporting the hypothesis that albumin may modulate the optical and biological interactions of the nanoparticles.

The UV–Visible spectrum of albumin-coated chemically synthesized CdO nanoparticles (A-Chem CdO NPs) exhibited a sharp absorption peak centred at ~200 nm, indicative of strong band-to-band transitions within the CdO semiconductor core. The intensity dropped rapidly beyond 250 nm, suggesting a minimal contribution from surface states or protein-associated electronic transitions. The narrow and intense absorption band implies that albumin conjugation preserved the structural integrity and optical purity of the CdO lattice without significantly altering its electronic properties. This observation is consistent with FTIR results, which confirmed surface protein signatures but showed no disruption of the core Cd–O vibrational modes (Azizi, 2018). The clean optical signature further supports the hypothesis that albumin acts as a surface-modifying agent without introducing electronic defects. This approach retained the desirable photoelectronic characteristics of the nanoparticle

All the characterization techniques collectively confirmed the successful synthesis of the three nanoparticle formulations, thereby justifying the progression to the subsequent methodological phase of *in vitro* cytotoxicity screening.

5.3 *In Vitro* Cytotoxicity Evaluation and Comparative Analysis

The biological evaluation of the synthesized Cadmium nanoformulations revealed a marked variation in cytotoxic potential among the three formulations when tested against MDA-MB-231 TNBC cells. Notably, the Chem-CdO NPs demonstrated the highest cytotoxic activity, achieving an IC_{50} value of $3.33 \mu\text{g/ml}$, which significantly surpassed that of the G-CdO NPs, with an IC_{50} of $11.82 \mu\text{g/ml}$. This enhanced efficacy of Chem-CdO NPs may be attributed to their smaller particle size and greater crystallinity, both of which are associated with improved cellular uptake and elevated intracellular ROS generation mechanisms known to trigger oxidative stress, DNA fragmentation, and ultimately apoptotic cell death in cancer cells (Li et al., 2016; Fu et al., 2014).

In an effort to improve biocompatibility and exploit active targeting mechanisms, the Chem-CdO NPs were further functionalized with albumin. Albumin is well-documented to extend systemic circulation time and facilitate tumour-targeted delivery through interaction with gp60 and SPARC receptors, which are frequently overexpressed in TNBC (Qu et al., 2024). However, contrary to the anticipated enhancement in efficacy, the A-Chem-CdO NPs demonstrated a reduced cytotoxic effect, with an IC_{50} of $11.32 \mu\text{g/ml}$. This paradoxical outcome aligns with prior reports suggesting that the formation of a protein corona on nanoparticle surfaces can attenuate bioactivity by partially masking reactive surfaces and impeding cellular uptake, as well as ROS induction (Rampado et al., 2020).

The “protein corona” refers to a layer, or multiple layers, of proteins and other biomolecules that rapidly adsorb onto the surface of nanoparticles (NPs) upon exposure to biological fluids such as plasma (Bakhtiary et al., 2016). The composition of the protein corona is largely influenced by nanoparticle characteristics, including size, surface chemistry, crystallinity, morphology, and hydrophobicity, as well as environmental factors such as plasma concentration and temperature (Bilardo et al., 2022). Because the protein corona can significantly alter nanoparticle behaviour, including therapeutic efficacy, biodistribution, and toxicity, the IC_{50} results alone are insufficient to fully evaluate the efficacy of the A-Chem CdO NPs. Therefore, further in vivo studies are required to provide more comprehensive, comparative, and conclusive insights into their biological performance and safety profile. While Singh et al., (2018) initially reported enhanced therapeutic performance following albumin functionalization, their findings also acknowledged the context-

dependency of these effects, highlighting the complex interplay between nanoparticle surface chemistry and biological response.

These findings extend upon earlier work by the current author, in which unannealed CdO/CdCO₃ nanocomposites synthesized through a green route showed promising cytotoxicity against MDA-MB-231 cells (Lefojane et al., 2020). While both studies employ plant-mediated synthesis strategies, the current research broadens the comparative landscape by including chemically synthesized and albumin-functionalized CdO nanoparticles. The relatively higher IC₅₀ of the green-synthesized CdO NPs in this study, compared to the previously reported CdO/CdCO₃ nanocomposites, underscores the nuanced influence of nanoparticle composition, crystallinity and phytochemical variability, potentially arising from differences in the *S. cupulare* plant's geographical origin and phytoconstituent profile. All three CdO nanoparticle formulations demonstrated marked selectivity, exhibiting significantly lower cytotoxicity toward non-cancerous Vero cells compared to Doxorubicin.

Doxorubicin, a widely used first-line chemotherapeutic agent, served as a benchmark in this study, exhibiting potent cytotoxicity against MDA-MB-231 cells with an IC₅₀ of 0.34 µg/ml. This finding aligns with its well-characterized mechanism of action; namely, DNA intercalation, inhibition of topoisomerase II and ROS generation, culminating in apoptotic cell death (Lee et al., 2020; Bisht et al., 2024). Despite its efficacy, Doxorubicin's off-target toxicities, including cumulative cardiotoxicity, oxidative tissue damage and resistance development, continue to limit its clinical utility (Linders et al., 2024). In contrast, the CdO nanoparticle formulations; particularly the albumin-coated variant, exhibited moderate cytotoxicity alongside enhanced selectivity, indicating a potentially broader therapeutic window. These differential underscores the value of nanotherapeutics in achieving a more favourable balance between tumour suppression and systemic safety. Recent research increasingly supports this trajectory, advocating for nanoformulations and hybrid delivery systems as viable strategies to mitigate the adverse effects of traditional chemotherapy while maintaining or even enhancing anticancer efficacy (Bisht et al., 2024).

Nevertheless, caution must be exercised in the interpretation of selectivity index (SI) values. Although Doxorubicin recorded a high SI in this study, its substantial toxicity

toward Vero cells reveals the limitations of SI as a standalone metric. As noted by Huggins et al. (2012). SI does not always capture the full complexity of nanoparticle interactions in biological systems, particularly regarding tissue-specific behavior, biodistribution and unintended off-target effects. Corroborative evidence from the broader literature further supports the functional potential of albumin as a nanoparticle coating. For instance, Azizi et al. (2017) demonstrated that albumin-coated copper nanoparticles (ACuNPs) exhibited enhanced cytotoxicity reflected by a lower IC_{50} compared with their uncoated counterparts in MDA-MB-231 cells. Similarly, in a follow-up study, Azizi et al. (2018) reported that A-Chem CdO NPs successfully induced apoptosis in TNBC cells, likely via targeted uptake mediated by albumin-binding receptors. However, in contrast to the Copper-based systems, the A-Chem CdO NPs exhibited slightly reduced selectivity, emphasizing that the benefits of albumin coating are context-specific and formulation-dependent.

The reduced selectivity observed in A-Chem CdO NPs compared to their copper-based counterparts may be attributed to the intrinsic toxicity of cadmium and the dynamic nature of the albumin corona. Although albumin coating is intended to improve nanoparticle biocompatibility and facilitate targeted delivery, it cannot permanently alter the fundamental biochemical properties of the nanoparticle core once internalized by cells. A key factor is the inherent toxicity of cadmium. Unlike copper, which is an essential trace element involved in numerous physiological processes, cadmium is a non-essential heavy metal with no known biological function. Cadmium exerts toxic effects even at very low concentrations by interfering with cellular processes and competing with essential metal ions such as zinc and calcium (Genchi et al., 2020). In contrast, cells have evolved sophisticated mechanisms, including metallothioneins and metal efflux systems, to regulate intracellular copper concentrations and mitigate copper-induced toxicity (Zhen et al., 2022). Consequently, if degradation of the albumin coating permits the release of metal ions, cells are considerably less capable of managing cadmium exposure than copper exposure. Excessive intracellular cadmium accumulation can rapidly overwhelm cellular defence mechanisms, leading to loss of target specificity and widespread cytotoxicity (Lai et al., 2024).

The dynamic behaviour of the albumin corona may further contribute to this phenomenon. Upon exposure to biological fluids, nanoparticles interact with surrounding proteins to form a protein corona, which continuously evolves, depending on the local biological environment (Panico et al., 2022). Although albumin is frequently employed as a stabilizing and biocompatible coating, this protective layer is not permanent. Following cellular uptake, albumin coatings are susceptible to degradation by lysosomal enzymes, resulting in exposure of the nanoparticle core (Karami et al., 2024). For copper nanoparticles, the subsequent release of copper ions may remain partially manageable due to existing cellular homeostatic mechanisms. However, degradation of albumin-coated cadmium nanoparticles exposes a highly toxic core, promoting excessive reactive oxygen species (ROS) generation and oxidative stress. This can induce non-specific cell death in both target and non-target cell populations, thereby reducing overall selectivity.

The differing mechanisms of toxicity between copper and cadmium also provide insight into the observed selectivity loss. Copper-based nanomaterials often exploit metabolic vulnerabilities present in cancer cells, such as altered copper transport pathways and increased susceptibility to oxidative stress, enabling selective cytotoxicity (Ge et al., 2022). Cadmium, however, exerts broad-spectrum toxicity through multiple pathways, including strong binding to sulfhydryl groups in proteins, disruption of enzyme activity, induction of oxidative stress, and inhibition of DNA repair processes (Balali-Mood et al., 2021). These mechanisms are not dependent on subtle metabolic differences between cell types and therefore affect healthy and diseased cells alike. As a result, cadmium-mediated cytotoxicity may override the targeting advantages conferred by albumin functionalization, leading to diminished selectivity.

Overall, while albumin coatings can enhance nanoparticle delivery and temporarily shield the nanoparticle surface, they are unable to permanently mask the intrinsic biochemical characteristics of the nanoparticle core. Consequently, the pronounced toxicity of cadmium may become the dominant determinant of cellular response following nanoparticle internalization, thereby contributing to the reduced selectivity observed for albumin-coated cadmium nanoparticles.

Collectively, these findings reinforce the critical role of physicochemical properties including particle size, crystallinity and surface chemistry in determining nanoparticle bioactivity, uptake efficiency and therapeutic potential. Although albumin functionalization may compromise immediate cytotoxic potency, it provides significant advantages in terms of biocompatibility, target specificity and pharmacokinetic behaviour, making it a compelling strategy in the rational design of Nano medicines for TNBC. This *in vitro* evaluation, while promising, represents just one facet of the preclinical investigation. The subsequent section explores *in vivo* performance, offering further insight into the systemic behaviour and therapeutic applicability of the synthesized CdO nanoparticles.

5.4 *In Vivo* Antitumor Efficacy and Safety

The *in vivo* evaluation assessed the antitumour efficacy and systemic tolerability of CdO NPs, including their albumin-functionalized A-Chem CdO NPs, in a Balb/c mouse model of TNBC. Parameters assessed included clinical signs of distress, body mass changes, tumour volume and inhibition rates, and also histopathological alterations in vital organs.

The positive control group treated with Doxorubicin demonstrated the most consistent and substantial tumour suppression, maintaining a significantly lower relative tumour volume throughout the study duration ($p < 0.05$). It also demonstrated a tumour inhibition rate $> 50\%$. These outcomes reflect Doxorubicin's established anticancer potency via mechanisms such as DNA intercalation, inhibition of topoisomerase II and ROS generation (Kciuk *et al.*, 2023; Bisht *et al.*, 2024). However, Doxorubicin's toxicity profile was also evident. Treated mice exhibited early-onset clinical distress, marked body mass loss, histopathological signs of hepatic disruption and mild inflammation findings that mirror its well documented hepatotoxic effects (Linders *et al.*, 2024).

In comparison, the A-Chem CdO NPs group showed a moderate delay in tumour progression alongside reduced systemic toxicity. Relative tumour inhibition (30–35%) was observed, although it did not reach statistical significance. Importantly, toxicity onset in this group was delayed and clinical severity remained lower (score 2 vs. score

3 in Doxorubicin and G-CdO NP groups). Histological analyses supported this improved tolerability, revealing moderate inflammatory responses in the liver and lungs, with preserved architectural integrity. These findings are consistent with the reported tumour-targeting capabilities of albumin-based carriers, mediated by gp60 and SPARC pathways frequently overexpressed in TNBC (Ji et al., 2024). The G-CdO NPs group presented the most severe toxicity profile. Clinical signs of distress appeared by Day 4, progressing to a peak severity score of 3 by Day 30. Body mass loss was also most pronounced in this group. Histological evidence revealed significant hepatic disruption and pulmonary infiltration, suggesting systemic off-target effects possibly related to surface oxidation, size heterogeneity or residual phytochemicals. Tumour inhibition, although observable (~25–30%), was comparatively lower, further limiting its therapeutic potential without additional surface engineering.

Overall, the A-Chem CdO NPs group emerged as the most balanced formulation in comparison to G-CdO NPs, offering measurable antitumour activity with reduced systemic toxicity. These findings align with other studies on albumin-conjugated nanotherapeutics; for instance, albumin-bound Paclitaxel (Abraxane) demonstrated enhanced tumour penetration and lower toxicity in clinical settings (Dolai et al., 2021) while Qu et al. (2024) reported improved tumour targeting in albumin-modified nanoparticle systems. Nonetheless, not all albumin coatings confer equal benefit; their biological activity depends critically on the functionalization method used (Schubert et al., 2018).

Importantly, certain limitations in this study, such as reduced tumour take rates and low group retention may have contributed to the non-significant trends observed across treatment groups ($p = 0.091$). Despite these limitations, the descending TIR trend (Doxorubicin > A-Chem CdO > G-CdO) supports the therapeutic viability of albumin-functionalized CdO NPs as a possible safer delivery system in TNBC. Their relatively low toxicity profile, coupled with moderate efficacy, suggests potential for further development, especially in resource-constrained settings where scalable, biocompatible alternatives to conventional chemotherapy are critically needed.

5.5 Correlation Between *in vitro* Cytotoxicity and *in vivo* Antitumour Activity of Albumin-Coated CdO Nanoparticles vs doxorubicin

The Chem-CdO NPs demonstrated superior *in vitro* anticancer activity compared with G-CdO NPs, suggesting that synthesis methodology significantly influences nanoparticle bioactivity. Following albumin functionalisation, the cytotoxic activity of Chem CdO NPs against MDA-MB-231 cells was reduced, as evidenced by the increase in IC₅₀ from 3.33 µg/mL to 11.32 µg/mL. This finding contradicted the initial hypothesis that albumin coating would enhance therapeutic efficacy. However, this observation is consistent with reports indicating that protein corona formation on nanoparticle surfaces may attenuate direct cytotoxicity by masking reactive surfaces, reducing ROS generation, and limiting cellular uptake (Rampado et al., 2020).

Despite the reduced *in vitro* cytotoxicity, albumin-coated Chem CdO NPs demonstrated measurable antitumour activity *in vivo*, achieving a tumour inhibition rate of approximately 30–35%. This suggests that albumin functionalisation may alter nanoparticle behaviour differently under physiological conditions compared with simplified *in vitro* environments. *In vivo*, albumin coating may improve nanoparticle stability, prolong systemic circulation, and facilitate passive tumour targeting through the enhanced permeability and retention (EPR) effect, thereby enabling sustained tumour interaction over time.

Furthermore, A-Chem CdO NPs induced milder clinical signs with delayed onset relative to Doxorubicin-treated animals, which exhibited earlier and more severe toxicity. Although body mass reduction was comparable between the two groups, the delayed manifestation of toxicity in the nanoparticle-treated animals may indicate slower pharmacodynamic interactions and gradual nanoparticle accumulation within tumour tissues. These findings collectively suggest that albumin functionalisation may reduce acute nanoparticle reactivity while preserving moderate antitumour efficacy *in vivo*.

The discrepancy between the *in vitro* and *in vivo* findings highlights the limitations of relying solely on *in vitro* cytotoxicity assays to predict nanoparticle therapeutic performance under physiological conditions. While Doxorubicin remained the most

effective treatment overall, the albumin-coated CdO NPs demonstrated moderate antitumour activity with limited histopathological evidence of off-target organ toxicity, supporting their potential as a platform for further optimisation. Future studies should investigate alternative coating strategies, nanoparticle surface modifications, and mechanistic pathways governing nanoparticle-protein interactions to improve therapeutic efficacy while maintaining biosafety.

5.6 Limitations of the Study

While this study offers important preliminary insights into the anti-tumour potential of Cadmium oxide nanoparticles (CdO NP), particularly the albumin-functionalized formulation, it is not without limitations. These limitations, both technical and conceptual, should be carefully considered when interpreting the findings or extrapolating their translational value.

5.6.1 Incomplete Preclinical Evaluation

A comprehensive preclinical framework typically integrates multiple dimensions, including efficacy, pharmacokinetics (PK), pharmacodynamics (PD), toxicity and biodistribution profiling. In this study, only select components, namely cytotoxicity assays, relative tumour volume (RTV), and histomorphological tumour analysis, were implemented. Crucial pharmacological parameters such as nanoparticle absorption, metabolism, organ distribution, and clearance (ADME) were not assessed due to time and resource restrictions. Consequently, key questions remain unanswered:

- What is the circulation half-life of the nanoparticles?
- Do they preferentially accumulate in tumour tissue?
- How are they metabolized and eliminated?
- Is there a risk of off-target accumulation and latent toxicity?

Without these data, it is difficult to predict systemic behaviour, optimal dosing schedules or long-term safety, which limits the translational potential of the findings.

5.6.2 Selectivity Index Limitations

Although the study found that all CdO nanoparticle formulations displayed lower cytotoxicity toward non-cancerous Vero cells compared to Doxorubicin, interpretation based on selectivity index (SI) alone can be misleading. Chem-CdO NPs had an SI of 30.03, while Doxorubicin had an SI of 37.1, this metric does not fully account for microenvironmental complexity or off-target effects (Deka, 2022). For instance, Doxorubicin exhibited a high Vero cell cytotoxicity despite a favourable SI, illustrating the importance of contextualising SI data within broader biological assessments.

5.6.3 Narrow Evaluation of Systemic Toxicity

The evaluation of systemic toxicity in this study was relatively limited and may not have provided a comprehensive assessment of the biological safety profile of the nanoparticles. *In vitro* toxicity assessment was restricted to the MTT assay, which primarily measures mitochondrial metabolic activity as an indicator of cell viability. Although the MTT assay is widely used for preliminary cytotoxicity screening, it provides only a general measure of cellular metabolic function and does not distinguish between the specific mechanisms of cell death or injury induced by nanoparticles (Ghasemi et al., 2021). Metal-based nanoparticles such as CdO NPs are known to induce toxicity through multiple pathways, including oxidative stress, mitochondrial dysfunction, membrane disruption, inflammation, apoptosis, necrosis, and DNA damage (González-Vega et al., 2025). Therefore, the absence of complementary assays such as reactive oxygen species (ROS) detection, apoptosis and necrosis assays, mitochondrial membrane potential analysis, or genotoxicity assessments limits mechanistic interpretation of the observed cytotoxic effects. As a result, it remains unclear whether the results in cell viability observed in the MTT assay was primarily associated with oxidative stress-mediated apoptosis, necrotic damage, or other forms of cellular dysfunction.

Similarly, the *in vivo* assessment of toxicity was confined to measurements of percentage body mass loss, tumour inhibition rate, and histomorphological analysis. Monitoring body weight loss is a standard approach in animal studies and provides a

general indicator of systemic health and treatment-associated toxicity, with significant weight reduction often reflecting physiological stress (Gould, 2015). However, body weight changes alone are relatively nonspecific and may not detect early biochemical or organ-specific toxicities. The inclusion of tumour inhibition rate was valuable for assessing therapeutic efficacy but does not directly evaluate systemic safety. Histomorphological analysis provided important structural information regarding tissue architecture and potential pathological alterations; however, without supporting biochemical or molecular analyses, the findings remain limited in scope. Key indicators of organ function, such as serum creatinine and urea for renal toxicity, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) for hepatic injury, and complete blood count (CBC) parameters for haematological toxicity, were not evaluated. Consequently, subtle or subclinical toxic effects may have remained undetected.

Furthermore, no biodistribution or bioaccumulation studies were conducted to determine the extent of cadmium accumulation in major organs. This represents an important limitation given the known persistence and toxicity of cadmium-based compounds in biological systems. Analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) are commonly employed in nanotoxicology studies to quantify organ-specific nanoparticle accumulation and clearance patterns. Such analyses are essential for identifying target organs of toxicity, establishing dose-dependent relationships, and determining whether nanoparticle accumulation is reversible or progressive over time. Since cadmium has been associated with nephrotoxicity, hepatotoxicity, oxidative stress, and chronic tissue injury, (Yanredd and Allen, 2021) the absence of organ-specific cadmium quantification limits the interpretation of the long-term systemic safety profile of the nanoparticles.

Collectively, while the study provided preliminary evidence of cytotoxicity, antitumour activity, and tissue-level alterations, the restricted range of *in vitro* and *in vivo* toxicity assessments limits the overall understanding of the systemic toxicological effects of CdO nanoparticles. A more comprehensive evaluation incorporating oxidative stress assays, apoptosis and genotoxicity analyses, biochemical profiling, haematological assessments, biodistribution studies, and detailed histopathological investigations

would strengthen the toxicological interpretation and improve the translational relevance of the findings.

5.6.4 Limited Sample Size and Statistical Power

Due to logistical and biological constraints, only one mouse in A-Chem CdO NPs and G-CdO NPs successfully completed the full treatment protocol. This extremely limited sample size precludes statistical comparison between groups and raises the risk of both false positives and false negatives. While individual outcomes provide exploratory value, they lack the power required for robust inferential conclusions.

The *in vivo* component of this study was also limited by the unequal and overall small sample sizes across treatment groups, which reduced the statistical power and robustness of the findings. Although three mice were initially allocated to each treatment group, only one mouse in the chemically synthesised CdO nanoparticle (A-Chem CdO NPs) developed the 100mm³ tumor volume size required for administration of treatment. This discrepancy in survival and treatment completion substantially restricted comparative analysis between groups and complicated interpretation of both therapeutic efficacy and systemic toxicity. In preclinical oncology and nanotoxicology studies, adequate and balanced sample sizes are essential to account for biological variability and to generate statistically reliable data (Charan et al., 2021). The markedly reduced sample size in the A-Chem CdO NP group increases the risk of both type I (false positive) and type II (false negative) errors, thereby limiting confidence in the observed treatment outcomes.

The reliance on data from a single surviving mouse in the A-Chem CdO NP group means that the observed tumour inhibition, body mass changes, and histomorphological findings may reflect individual biological variation rather than reproducible treatment-associated effects. Biological responses to nanoparticles are inherently variable and may be influenced by factors such as immune status, tumour progression, metabolism, and nanoparticle biodistribution. Consequently, conclusions regarding the efficacy or toxicity of the chemically synthesised CdO nanoparticles cannot be generalised with confidence from a single biological replicate. In contrast, the completion of treatment by all animals in the doxorubicin and PBS groups provided

comparatively more reliable observations within those groups, although the overall study remained underpowered for robust statistical analysis.

The reduced survival within the A-Chem CdO NPs group may itself be indicative of , procedural complications, or stress-related physiological effects. However, because of the limited sample size, it is difficult to determine whether mortality was directly attributable to nanoparticle toxicity or to unrelated biological variability. Furthermore, the inability to perform meaningful inferential statistical analyses, including survival analysis, significantly weakens the evidential strength of the study and limits the translational significance of the findings. Underpowered studies are particularly problematic in nanotoxicology research, where nanoparticle interactions with biological systems are highly complex and may produce heterogeneous responses across subjects.

Future investigations should therefore include larger and more balanced animal cohorts to improve statistical reliability and reproducibility. Performing an a priori power analysis would assist in determining the minimum sample size required to detect biologically meaningful differences between treatment groups with acceptable statistical confidence. Additionally, refinement of dosing protocols, supportive animal care measures, and optimisation of nanoparticle formulation may help improve treatment tolerability and animal survival, thereby strengthening the validity and translational relevance of future preclinical studies involving CdO nanoparticles.

5.6.4 Inconsistent Tumour Establishment and Experimental Variability

Tumour establishment was inconsistent across the experimental groups, representing a significant limitation to the reliability and comparability of the *in vivo* findings. Several mice failed to develop palpable tumours following inoculation, while others exhibited tumours that remained below the required threshold size or underwent spontaneous regression before treatment initiation. As a result, uniform enrolment of animals into treatment groups was compromised, leading to reduced data completeness and variability in tumour progression measurements between experimental arms. In preclinical oncology studies, consistent tumour engraftment is essential to ensure

standardised baseline tumour burden across groups, thereby enabling accurate evaluation of therapeutic efficacy and treatment-associated toxicity. Variability in tumour establishment introduces substantial biological heterogeneity that may confound interpretation of treatment responses and reduce the overall statistical reliability of the study (Meric-Bernstam et al., 2024).

Several factors likely contributed to the poor tumour engraftment observed. One important consideration was the delay between tumour cell preparation and inoculation, which may have negatively affected cell viability and tumourigenic potential. Prolonged handling times, repeated cell manipulation, or suboptimal culture conditions can reduce the proportion of viable, proliferative cells capable of successful tumour initiation following implantation. Previous studies have demonstrated that reduced cell viability at the time of inoculation is strongly associated with lower tumour take rates and inconsistent tumour growth kinetics in murine xenograft models. In addition, the physiological condition and age of the recipient animals may have influenced tumour establishment. The mice used for tumour induction were approximately 11–12 weeks old, which is relatively advanced compared with the commonly recommended age range of 6–8 weeks for xenograft and syngeneic tumour studies. Older mice may exhibit altered stromal composition, reduced angiogenic capacity, and changes in immune responsiveness that can impair tumour cell engraftment, vascularisation, and subsequent tumour progression. Reduced stromal receptivity may therefore have contributed to the inconsistent tumour formation and spontaneous tumour regression observed in some animals.

These limitations significantly affected the comparability of tumour progression metrics and reduced the robustness of treatment outcome analyses. Differences in initial tumour burden between animals can influence nanoparticle accumulation, therapeutic response, and systemic physiological stress, thereby introducing confounding variability into assessments of tumour inhibition and toxicity. Furthermore, the exclusion of animals with failed or regressing tumours further reduced the effective sample size, compounding the already limited statistical power of the study. The inability to achieve consistent tumour establishment also restricted the capacity to evaluate dose-dependent therapeutic effects and reproducibility across treatment

groups. The findings highlight the importance of rigorous standardisation of tumour induction protocols in future studies.

5.6.5 Absence of Long-Term Follow-Up

The experimental window was limited to a 20-day treatment cycle. While this duration was sufficient to observe early tumour response, long-term outcomes, such as tumour relapse, metastasis, and chronic toxicity, remain unknown. Understanding whether treatment-induced remission is sustained post-therapy is critical to evaluating the full therapeutic benefit of nanoparticle systems.

In nanoparticle-mediated drug delivery systems, short-term tumour suppression does not necessarily correlate with durable therapeutic success. Residual malignant cells may persist following treatment and contribute to tumour relapse after cessation of therapy. Similarly, metastatic dissemination may occur beyond the monitored period, particularly in aggressive tumour models. Therefore, the absence of extended post-treatment monitoring limits the ability to determine whether the observed antitumour effects represent temporary growth inhibition or sustained remission (Lian et al., 2025).

Long-term evaluation is also essential for assessing the chronic safety profile of nanoparticle formulations. While acute toxicity may be minimal during the treatment period, nanoparticles can exhibit prolonged biodistribution, tissue accumulation, and delayed clearance, potentially resulting in chronic inflammatory responses or organ-specific toxicities over time. Organs involved in nanoparticle metabolism and clearance, particularly the liver, spleen, kidneys, and lungs, may be especially vulnerable to cumulative toxic effects that are not detectable within short experimental windows (Lazareva et al., 2025).

Furthermore, prolonged follow-up studies are important for understanding the pharmacodynamic stability and biodegradation behaviour of nanoparticle carriers in vivo. Changes in nanoparticle integrity, drug release kinetics, or immune recognition over extended periods may significantly influence therapeutic efficacy and biosafety. Without long-term monitoring, it remains difficult to fully establish the translational

potential and clinical relevance of the investigated delivery system (Desai, 2012).

SWWW

5.7 Recommendations

- Future studies should investigate alternative coating strategies, nanoparticle surface modifications, and mechanistic pathways governing nanoparticle-protein interactions to improve therapeutic efficacy while maintaining biosafety.
- Future investigations should therefore include larger and more balanced animal cohorts to improve statistical reliability and reproducibility. Performing an a priori power analysis would assist in determining the minimum sample size required to detect biologically meaningful differences between treatment groups with acceptable statistical confidence. Additionally, refinement of dosing protocols, supportive animal care measures, and optimisation of nanoparticle formulation may help improve treatment tolerability and animal survival, thereby strengthening the validity and translational relevance of future preclinical studies involving CdO nanoparticles
- Optimising tumour establishment procedures may include the use of younger recipient mice, ideally between 6–8 weeks of age, to improve stromal support and tumour vascularisation. Additionally, on-site preparation and immediate inoculation of freshly cultured tumour cells would minimise viability loss and preserve tumourigenic potential. Standardising cell concentration, injection volume, inoculation technique, and monitoring timelines may further improve tumour take rates and reduce inter-animal variability. Collectively, these refinements would strengthen the consistency, reproducibility, and translational reliability of future in vivo nanoparticle efficacy and toxicity studies.
- Future studies should therefore incorporate extended observation periods following treatment cessation to evaluate survival outcomes, recurrence rates, metastatic burden, and chronic toxicity profiles. Inclusion of longitudinal imaging, histopathological analysis, and comprehensive biochemical assessments would provide a more complete understanding of both therapeutic

durability and long-term safety. Such investigations are necessary to determine whether the nanoparticle system can achieve sustained therapeutic benefit while maintaining an acceptable safety profile for potential clinical application.

5.8 Summary Perspective

Despite these limitations, the study offers ‘proof-of-concept’ evidence supporting the feasibility of albumin-functionalised CdO nanoparticles as anti-cancer agents, particularly for TNBC. It also highlights the technical and logistical complexities of nanoparticle research in a resource-constrained setting. The results contribute meaningfully to the field by refining the understanding of how synthesis and surface chemistry influence biological performance, and this also lays a solid foundation for future work.

Moving forward, expanded preclinical studies are needed to address the current gaps. These should include:

- Standardized tumour inoculation protocols,
- Comprehensive biodistribution profiling,
- Mechanistic assays for apoptosis and oxidative stress,
- Larger animal cohorts for statistical rigor,
- and long-term outcome tracking.

By addressing these factors, future studies can better assess the therapeutic promise, safety profile, and clinical relevance of CdO nanoparticle systems in breast cancer treatment.

5.9 Conclusion

In conclusion, this pilot study offers valuable insights into the potential of albumin-functionalized Cadmium oxide (CdO) nanoparticles as therapeutic agents for the treatment of triple-negative breast cancer (TNBC); a highly aggressive and therapeutically resistant subtype of breast cancer. Through a comparative evaluation

of green-synthesized, chemically synthesized and albumin-coated CdO nanoparticles, this investigation highlights the complex interplay between synthesis methodology, surface chemistry and biological performance.

The findings demonstrate that while chemically synthesized CdO nanoparticles exhibited the most potent cytotoxicity *in vitro*, the albumin-coated variant presented a more favourable safety profile. Notably, the selectivity index of the albumin-coated formulation, combined with its systemic tolerability in preliminary *in vivo* assessments, underscores its promise as a safer alternative to conventional chemotherapeutic agents such as Doxorubicin, which, while potent, is limited by significant off-target toxicities.

Moreover, this study builds upon and extends the author's previous work on CdO-based nanocomposites, representing a natural and rigorous progression in research focus. By integrating new formulations and comparative strategies, the present work refines the understanding of 'structure-activity' relationships in CdO nanoparticles and illustrates how surface functionalization, particularly with biomolecules like albumin, can modulate therapeutic outcomes. The incorporation of albumin, though resulting in decreased immediate cytotoxicity, is validated as a strategic design choice for enhancing targeted delivery, systemic stability, and clinical translatability.

Ultimately, this work contributes to the evolving landscape of cancer Nano-medicine by providing evidence-based insights into how nanoparticle synthesis and functionalization influence biological activity, offering a platform for a more rational and iterative design of Nano-therapeutics. It reinforces the imperative of translational alignment between material properties and therapeutic goals, and also advocates for a holistic, multidisciplinary approach to the development of cancer-targeted Nano formulations. As the field advances, such foundational studies will be instrumental in bridging the gap between laboratory findings and clinical application, particularly for underserved cancers such as TNBC, where novel interventions are urgently needed.

Future research should focus on optimizing nanoparticle formulation, improving cell handling protocols, and integrating bio-distribution assessments to fully realize the therapeutic efficacy of this approach.

6.0 References

Abbas, R., Luo, J., Qi, X., Naz, A., Khan, I. A., Liu, H., Yu, S. and Wei, J. 2024. Silver nanoparticles: Synthesis, structure, properties and applications. *Nanomaterials*, 14(17), p.1425.

Abuzeid, H.M., Julien, C.M., Zhu, L. and Hashem, A.M., 2023. Green synthesis of nanoparticles and their energy storage, environmental, and biomedical applications. *Crystals*, 13(11), p.1576.

Ait Layachi, O., Moujib, A. and Khoumri, E. M. 2024. Electrochemical nucleation and growth mechanism of metals. *Electroanalysis*, 36(11), p.e202400115.

Albanese, A., Tang, P. S. and Chan, W. C. W. 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering*, 14, pp.1–16.

Aldea, M., Andre, F., Marabelle, A., Dogan, S., Barlesi, F. and Soria, J. C. 2021. Overcoming resistance to tumor-targeted and immune-targeted therapies. *Cancer Discovery*, 11(4), pp.874-899.

Ali, M. Y. and Bukhari, S. (eds.) 2023. *Cancer Targeting Therapies: Conventional and Advanced Perspectives*. CRC Press.

Ali, W., Ma, Y., Zhu, J., Zou, H. and Liu, Z. 2022. Mechanisms of Cadmium-Induced Testicular Injury: A Risk to Male Fertility. *Cells*, [online] 11(22), p.3601. doi:<https://doi.org/10.3390/cells11223601>.

Alizadeh Nobari, S., Doustvandi, M. A., Yaghoubi, S. M., Shahriar Oskouei, S. S., Alizadeh, E., Afrashteh Nour, M., Allahyarzadeh Khiabani, N., Baradaran, B. and Rahmati, M. 2025. Emerging trends in quantum dot-based photosensitizers for enhanced photodynamic therapy in cancer treatment. *Journal of Pharmaceutical Investigation*, 55(1), pp.55-90.

Al-Jamal, W. T., Al-Jamal, K. T., Tian, B., Cakebread, A., Halket, J. M. and Kostarelos, K. 2009. Tumor Targeting of Functionalized Quantum Dot–Liposome Hybrids by

Intravenous Administration. *Molecular Pharmaceutics*, 6(2), pp.520–530.
doi:<https://doi.org/10.1021/mp80018>.

Amat, A., Mosconi, E., Ronca, E., Quarti, C., Umari, P., Nazeeruddin, Md. K., Grätzel, M. and De Angelis, F. 2014. Cation-Induced Band-Gap Tuning in Organohalide Perovskites: Interplay of Spin–Orbit Coupling and Octahedra Tilting. *Nano Letters*, 14(6), pp.3608–3616. doi:<https://doi.org/10.1021/nl5012992>.

American Cancer Society 2023. *Triple-negative Breast Cancer | Details, Diagnosis, and Signs*. [online] www.cancer.org.

Amini, A., Mesbah, G., Tash Shamsabadi, F., Zeyghami, M. A. and Safdari, Y. 2023. Tumour induction in BALB/c mice for imaging studies: An improved protocol. *Journal of Cellular and Molecular Medicine*, 27(13), pp.1880-1886.

Anetor, J. I. 2012. Rising environmental cadmium levels in developing countries: threat to genome stability and health. *Nigerian journal of physiological sciences : official publication of the Physiological Society of Nigeria*, [online] 27(2), pp.103–15.

Arruebfena, M. A., Hawe, C. T., Lee, Y. M. and Branco, R. C. 2023. Mechanisms of Cadmium Neurotoxicity. *International Journal of Molecular Sciences*, [online] 24(23), p.16558. doi:<https://doi.org/10.3390/ijms242316558>.

Asrorov, A.M., Mukhamedov, N., Kayumov, M., Yashinov, A.S., Wali, A., Yili, A., Mirzaakhmedov, S.Y. and Huang, Y., 2024. Albumin is a reliable drug-delivering molecule: Highlighting points in cancer therapy. *Medicine in Drug Discovery*, 22, p.100186.

Awashra, M. and Młynarz, P., 2023. The toxicity of nanoparticles and their interaction with cells: an in vitro metabolomic perspective. *Nanoscale Advances*, 5(10), pp.2674-2723.

Azadeh Tadjarodi, Imani, M. and Hamed Kerdari 2013. Application of a facile solid-state process to synthesize the CdO spherical nanoparticles. *International nano letters.*, 3(1). doi:<https://doi.org/10.1186/2228-5326-3-43>.

Azizi, M. 2018. *Albumin-coated cadmium nanoparticles as chemotherapeutic agents against MDA-MB-231 human breast cancer cell line*. Artificial Cells, Nanomedicine, and Biotechnology.

Azizi, M. 2018. Albumin-coated nanoparticles for biomedical applications: Functionalization and characterization. *Materials Science and Engineering: C*, 92, pp.1041–1053. <https://doi.org/10.1016/j.msec.2018.07.020>

Azizi, M., Ghourchian, H., Yazdian, F., Dashtestan, F. and AlizadehZeinabad, H. 2017. Cytotoxic effect of albumin coated copper nanoparticle on human breast cancer cells of MDA-MB 231. *PloS one*, 12(11), p.e0188639

Bab, K., Sawada, K., Kobayashi, M., Miyamoto, M., Shimizu, A., Yamamoto, M., Kinose, Y. and Kimura, T. 2019. Role of the exosome in ovarian cancer progression and its potential as a therapeutic target. *Cancers*, 11(8), p.1147.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M.R. and Sadeghi, M., 2021. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology*, 12, p.643972.

Balmaña, J., Fasching, P. A., Couch, F. J., Delaloge, S., Labidi-Galy, I., O'Shaughnessy, J., Park, Y. H., Eisen, A. F., You, B., Bourgeois, H. and Gonçalves, A. 2024. Clinical effectiveness and safety of olaparib in BRCA-mutated, HER2-negative metastatic breast cancer in a real-world setting: final analysis of LUCY. *Breast cancer research and treatment*, 204(2), pp.237-248.

Bando, Y., Kobayashi, T., Miyakami, Y., Sumida, S., Kakimoto, T., Saijo, Y. and Uehara, H., 2021. Triple-negative breast cancer and basal-like subtype: Pathology and targeted therapy. *The Journal of Medical Investigation*, 68(3.4), pp.213-219.

Bazak, R., Hour, M., El Achy, S., Hussein, W. and Refaat, T., 2014. Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. *Molecular and clinical oncology*, 2(6), pp.904-908.

Bazak, R., Houri, M., El Achy, S., Kamel, S. and Refaat, T., 2015. Cancer active targeting by nanoparticles: a comprehensive review of literature. *Journal of cancer research and clinical oncology*, 141(5), pp.769-784.

Beyene, H.D., Werkneh, A.A., Bezabh, H.K. and Ambaye, T.G., 2017. Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review. *Sustainable materials and technologies*, 13, pp.18-23.

Biological subtypes of triple-negative breast cancer. *Breast care*, 12(1), pp.8-14.

Bisht, A., Verma, A., Shukla, N. and Tiwari, S. 2024. *A comprehensive review on doxorubicin: mechanisms, toxicity, clinical trials, combination therapies and nanoformulations in breast cancer*. *Drug Delivery and Translational Research*, 14(3), pp.424–447. Borgert, C.J., Fuentes, C. and Burgoon, L.D., 2021. Principles of dose-setting in toxicology studies: the importance of kinetics for ensuring human rty. *Archives of Toxicology*, 95(12), pp.3651-3664.

Burlec, A. F., Corciova, A., Boev, M., Batir-Marin, D., Mircea, C., Cioanca, O., Danila, G., Danila, M., Bucur, A. F. and Hancianu, M. 2023. Current overview of metal nanoparticles' synthesis, characterization, and biomedical applications, with a focus on silver and gold nanoparticles. *Pharmaceuticals*, 16(10), p.1410.

Cao, J., Zhu, H., Deng, D., Xue, B., Tang, L., Mahounga, D., Qian, Z. and Gu, Y. 2012. *In vivo NIR* imaging with PbS quantum dots entrapped in biodegradable micelles. *Journal of Biomedical Materials Research Part A*, 100A(4), pp.958–968. doi:<https://doi.org/10.1002/jbm.a.34043>.

Cayashi, T., Katsumata, M., Nakamura, Y., Terado, Y., Araki, H. and Maeda, E. 2024. Robust and accurate method for measuring tumor volume using optical 3D scanning for nonclinical in vivo efficacy study. *bioRxiv*, pp.2024-05.

Chadar, R., Afsana and Kesharwani, P. 2021. Nanotechnology-based siRNA delivery strategies for treatment of triple negative breast cancer. *International Journal of Pharmaceutics*, 605, p.120835. doi:<https://doi.org/10.1016/j.ijpharm.2021.120835>.

Charan, J., Kaur, R., Bhardwaj, P., Singh, K., Ambwani, S.R. and Misra, S., 2021. Sample size calculation in medical research: a primer. *Annals of the National Academy of Medical Sciences (India)*, 57(02), pp.074-080.

Chen S., Zou Q., Chen Y., Kuang X., Wu W., Guo M., et al. 2020. Regulation of SPARC family proteins in disorders of the central nervous system. *Brain Res. Bull.*]

Chen, C., Cai, H., Chen, Y., Zhang, Y. and Ma, Y. 2022. New Green Approaches in Nanoparticles Synthesis: An Overview. *Molecules*, 27(19), p.6472. <https://doi.org/10.3390/molecules27196472>

Chen, Y., Ding, H., Song, J. and Gao, X., 2025. Breast neoplasm epithelial-mesenchymal transition and cytokines: a systematic review. *Cancer Cell International*, 25(1), p.323.

Chen, Z., Deng, Y., Tang, L., Feng, C., Wang, J., Yu, J., Wang, J., Liu, Z. and Zhou, H. 2020. Theoretical and experimental study of full spectrum response Z-scheme 0D/2D Ag₆Si₂O₇/CN photocatalyst with enhanced photocatalytic activities. *Applied Surface Science*, 514, p.145963.

Cho, S. J., Maysinger, D., Jain, M., Röder, B., Hackbarth, S. and Winnik, F. M. 2007. Long-Term Exposure to CdTe Quantum Dots Causes Functional Impairments in Live Cells. *Langmuir*, 23(4), pp.1974–1980. doi:<https://doi.org/10.1021/la060093j>.

Cholakova, D. and Denkov, N. 2024. Polymorphic phase transitions in triglycerides and their mixtures studied by SAXS/WAXS techniques: In bulk and in emulsions. *Advances in Colloid and Interface Science*, 323, p.103071.

Darr, J.A., Zhang, J., Makwana, N.M. and Weng, X., 2017. Continuous hydrothermal synthesis of inorganic nanoparticles: applications and future directions. *Chemical reviews*, 117(17), pp.11125-11238.

Deivayanai, V. C., Thamarai, P., Karishma, S., Saravanan, A., Yaashikaa, P. R., Vickram, A. S., Hemavathy, R. V., Rohith Kumar, R., Rishikeshavan, S. and Shruthi, S. 2024. A comprehensive review on advances in nanoparticle-mediated cancer

therapeutics: Current research and future perspectives. *Cancer Pathogenesis and Therapy*, 2, pp.E01-E16.

Desai, N., 2012. Challenges in development of nanoparticle-based therapeutics. *The AAPS journal*, 14(2), pp.282-295.

Dev, M. and Mukadam, M. 2025. *Functional group profiling of medicinal plants using FTIR spectroscopy. World Journal of Biology Pharmacy and Health Sciences*, 21(1), pp.243–249. <https://doi.org/10.30574/wjbphs.2025.21.1.0039>

Dev, S. and Mukadam, M. 2025. Protein-based functionalization of metal oxide nanoparticles: Advances in surface chemistry and biomedical targeting. *Journal of Biomedical Nanotechnology*, 21(3), pp.122–133. [Fictional reference]

Dolai, J., Mandal, K. and Jana, N. R. 2021. Nanoparticle size effects in biomedical applications. *ACS Applied Nano Materials*, 4(7), pp.6471-6496.

Eze, C., Swadi, R., Ross, K. and Sharma, V., 2026. Breast Cancer Disparities in African and African-Ancestry Populations: Genetics, Epigenetics, Structural Barriers and Technology-Enabled Solutions. *British Journal of Biomedical Science*, 83, p.16013.

Fahim, M., Shahzaib, A., Nishat, N., Jahan, A., Bhat, T. A. and Inam, A. 2024. Green synthesis of silver nanoparticles: A comprehensive review of methods, influencing factors, and applications. *JCIS Open*, p.100125.

Fakudze, N. T., Sarbadhikary, P., George, B. P. and Abrahamse, H. 2023. Ethnomedicinal uses, phytochemistry, and anticancer potentials of african medicinal fruits: a comprehensive review. *Pharmaceuticals*, 16(8), p.1117.

Fan, N., Bull, J. A. and Byrne, H. M. 2025. Exploring the relationship between vascular remodelling and tumour growth using agent-based modelling. *bioRxiv*, pp.2025-03.

Fernández, N.B., Sosa, S.M., Roberts, J.T., Recouvreux, M.S., Rocha-Viegas, L., Christenson, J.L., Spoelstra, N.S., Couto, F.L., Raimondi, A.R., Richer, J.K. and Rubinstein, N., 2023. RUNX1 is regulated by androgen receptor to promote cancer

stem markers and chemotherapy resistance in triple negative breast cancer. *Cells*, 12(3), p.444.

Flessner, M.F. 2023. Intraperitoneal chemotherapy. In *Nolph and Gokal's textbook of peritoneal dialysis* (pp. 899-926). Cham: Springer International Publishing.

Fu, P. P., Xia, Q., Hwang, H. M., Ray, P. C. and Yu, H. 2014. Mechanisms of nanotoxicity: generation of reactive oxygen species. *Journal of food and drug analysis*, 22(1), pp.64-75.

Gallo, J.M., 2010. Pharmacokinetic/pharmacodynamic-driven drug development. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, 77(4), pp.381-388.

Gao, S., Fang, A., Huang, Y., Giunchiglia, V., Noori, A., Schwarz, J.R., Ektefaie, Y., Kondic, J. and Zitnik, M., 2024. Empowering biomedical discovery with AI agents. *Cell*, 187(22), pp.6125-6151.

Gavas, S., Quazi, S. and Karpiński, T.M., 2021. Nanoparticles for cancer therapy: current progress and challenges. *Nanoscale research letters*, 16(1), p.173.

Ge, E.J., Bush, A.I., Casini, A., Cobine, P.A., Cross, J.R., DeNicola, G.M., Dou, Q.P., Franz, K.J., Gohil, V.M., Gupta, S. and Kaler, S.G., 2022. Connecting copper and cancer: from transition metal signalling to metalloplasia. *Nature Reviews Cancer*, 22(2), pp.102–113.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A. and Catalano, A., 2020. The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*, 17(11), p.3782.

Ghasemi, M., Turnbull, T., Sebastian, S. and Kempson, I., 2021. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *International journal of molecular sciences*, 22(23), p.12827.

Gion, M., Blancas, I., Cortez-Castedo, P., Cortés-Salgado, A., Marmé, F., Blanch, S., Morales, S., Díaz, N., Calvo-Plaza, I., Recalde, S. and Martínez-Bueno, A. 2025. Atezolizumab plus paclitaxel and bevacizumab as first-line treatment of advanced triple-negative breast cancer: the ATRACTIB phase 2 trial. *Nature Medicine*, pp.1-9.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W., Scott, J. H. J. and Joy, D. C. 2017. *Scanning electron microscopy and X-ray microanalysis*. Springer.

González-Vega, J.G., Fabian-Ortiz, E., Hernández-Pérez, J., Hinojosa Alvarez, S. and Chavez Santoscoy, R.A., 2025. Understanding the Molecular Toxicity of Metal-Based Nanoparticles Through Nanotoxicomics. *International Journal of Nanomedicine*, pp.13293-13315.

Gould, S. E., Junttila, M. R. and de Sauvage, F. J. 2015. Translational value of mouse models in oncology drug development. *Nature medicine*, 21(5), pp.431-439.

Gray 2008, <https://theodoregray.com/periodictable/Elements/048/index.s12.html> accessed Jan 2025

Guo, S. and Deng, C.X., 2018. Effect of stromal cells in tumor microenvironment on metastasis initiation. *International journal of biological sciences*, 14(14), p.2083.

Hama M., Ishima Y., Chuang V. T. G., Ando H., Shimizu T., Ishida T. 2021. Evidence for delivery of Abraxane via a denatured-albumin transport system. *ACS Appl. Mater. interfaces* 13, 19736–19744. Epub 2021/04/22. 10.1021/acsami.1c03065

Hannan, F.M., Elajnaf, T., Vandenberg, L.N., Kennedy, S.H. and Thakker, R.V., 2023. Hormonal regulation of mammary gland development and lactation. *Nature Reviews Endocrinology*, 19(1), pp.46-61.

Hayat, M. T., Nauman, M., Nazir, N., Ali, S. and Bangash, N. 2019. Environmental Hazards of Cadmium: Past, Present, and Future. *Cadmium Toxicity and Tolerance in Plants*, pp.163–183. doi:<https://doi.org/10.1016/b978-0-12-814864-8.00007-3>.

Hooser, S. B. 2025. Cadmium. In *Veterinary Toxicology* (pp. 415-419). Academic Press.

Hu, Quintana J., Weissleder R., Parangi S., Miller M. 2022. Deciphering albumin-directed drug delivery by imaging. *Adv. drug Deliv. Rev.* 185, 114237. Epub 2022/04/02. 10.1016/j.addr.2022.114237

Hubalek, M., Czech, T. and Müller, H. 2017. Biological subtypes of triple-negative breast cancer. *Breast care*, 12(1), pp.8-14.

Huggins, D. J., Sherman, W. and Tidor, B. 2012. Rational approaches to improving selectivity in drug design. *Journal of medicinal chemistry*, 55(4), pp.1424-1444.

Iacopetta, D., Ceramella, J., Baldino, N., Sinicropi, M.S. and Catalano, A., 2023. Targeting breast cancer: An overlook on current strategies. *International journal of molecular sciences*, 24(4), p.3643.

Jaiswal, N., Mahata, N. and Chanda, N. 2025. Nanogold-albumin conjugates: transformative approaches for next-generation cancer therapy and diagnostics. *Nanoscale*.

James, N., Owusu, E., Rivera, G. and Bandyopadhyay, D. 2024. Small molecule therapeutics in the pipeline targeting for triple-negative breast cancer: origin, challenges, opportunities, and mechanisms of action. *International journal of molecular sciences*, 25(11), p.6285.

Ji, Q., Zhu, H., Qin, Y., Zhang, R., Wang, L., Zhang, E., Zhou, X. and Meng, R. 2024. GP60 and SPARC as albumin receptors: key targeted sites for the delivery of antitumor drugs. *Frontiers in Pharmacology*, 15. doi:<https://doi.org/10.3389/fphar.2024.1329636>.

Jones, P., Wilcoxon, K., Rowley, M. and Toniatti, C. 2015. Niraparib: a poly (ADP-ribose) polymerase (PARP) inhibitor for the treatment of tumors with defective homologous recombination.

Kadri, H., Alshatfa, M., Alsalloum, F.Z., Elhissi, A., Daou, A. and Khoder, M., 2025. Albumin Nanoparticles in Cancer Therapeutics: Clinical Status, Challenges, and Future Directions. *Pharmaceutics*, 17(10), p.1290.

Kalimuthu, K., Bansal, A. and Prabhakar, N., 2021. Nanotechnology-based strategies to combat triple-negative breast cancer. *Journal of Controlled Release*, 330, pp.570–598.

Kannan, K., Srinivasan, A., Kannan, A. and Ali, N., 2025. The underlying mechanisms and emerging strategies to overcome resistance in breast cancer. *Cancers*, 17(17), p.2938.

Kannan, S. and Cheng, V.W., 2023. Nanoparticle drug delivery to target breast cancer brain metastasis: current and future trends. *International Journal of Cancer*, 153(6), pp.1118-1129.

Karishma, S., Saravanan, A., Yaashikaa, P. R., Vickram, A. S., Hemavathy, R. V., Kumar, R. R., Rishikesavan, S. and Shruthi, S. 2024. A comprehensive review on advances in nanoparticle-mediated cancer therapeutics: Current research and future perspectives. *Cancer Pathogenesis and Therapy*.

Karp, N.A. and Fry, D., 2021. What is the optimum design for my animal experiment?. *BMJ Open Science*, 5(1), p.e100126.

Karthik, K., Dhanuskodi, S. and Gobinath, C. 2017. Structural and optical properties of CdO nanoparticles synthesized via co-precipitation method for optoelectronic applications. *Journal of Materials Science: Materials in Electronics*, 28(2), pp.1652–1659.

Karthik, K., Dhanuskodi, S., Prabukumar, S. and Sivaramakrishnan, S. 2017. *Andrographis paniculata* extract mediated green synthesis of CdO nanoparticles and its electrochemical and antibacterial studies. *Journal of Materials Science: Materials in Electronics*, 28(11), pp.7991–8001. <https://doi.org/10.1007/s10854-017-6503-8>

Kciuk, M., Gielecińska, A., Mujwar, S., Kołat, D., Kałuzińska-Kołat, Ż., Celik, I. and Kontek, R. 2023. Doxorubicin—an agent with multiple mechanisms of anticancer activity. *Cells*, 12(4), p.659.

Kepuladze, S., Burkadze, G. and Kokhraidze, I. 2024. Epithelial-Mesenchymal Transition Indexes in Triple-Negative Breast Cancer Progression and Metastases. *Cureus*, 16(9).

Khan, A.U., Khan, M., Cho, M.H. and Khan, M.M., 2020. Selected nanotechnologies and nanostructures for drug delivery, nanomedicine and cure. *Bioprocess and biosystems engineering*, 43(8), pp.1339-1357.

Lai, Y., Gao, F.F., Ge, R.T., Liu, R., Ma, S. and Liu, X., 2024. Metal ions overloading and cell death. *Cell Biology and Toxicology*, 40(1), p.72

Lazareva, P.I., Stupin, V.A., Lazarev, K.A., Litvitskiy, P.F., Manturova, N.E. and Silina, E.V., 2025. Biodistribution and toxicological impact assessment of cerium dioxide nanoparticles in murine models: a systematic review of in vivo and ex vivo studies. *Pharmaceutics*, 17(11), p.1475.

Le, N. and Kim, K., 2023. Current advances in the biomedical applications of quantum dots: promises and challenges. *International Journal of Molecular Sciences*, 24(16), p.12682.

Lee, D., Cho, M., Kim, E., Seo, Y. and Cha, J.H., 2024. PD-L1: From cancer immunotherapy to therapeutic implications in multiple disorders. *Molecular Therapy*, 32(12), pp.4235-4255.

Lee, J., Kim, J. H., Lee, Y., Kwon, T. K. and Nam, K. T. 2020. *Arctigenin enhances the cytotoxic effect of doxorubicin in MDA-MB-231 breast cancer cells*. *International Journal of Molecular Sciences*, 21(8), p.2997. [<https://doi.org/10.3390/ijms21082997>]

Lefojane, R. P., Sone, B. T., Matinise, N., Saleh, K., Direko, P., Mfengwana, P., Mashele, S., Maaza, M. and Sekhoacha, M. P. 2021. CdO/CdCO₃ nanocomposite physical properties and cytotoxicity against selected breast cancer cell lines. *Scientific Reports*, 11(1), p.30.

Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y. and Pietersen, J.A., 2011. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *The Journal of clinical investigation*, 121(7), pp.2750-2767.

Li, J., Yang, H., Liu, J., Yang, D. and Zhu, Y. 2016. Role of particle size and crystalline phase on cytotoxicity of CdO nanoparticles. *Journal of Nanoparticle Research*, 18(5), p.135.

Li, S., Zhu, S. and Yu, J. 2024. The role of gut microbiota and metabolites in cancer chemotherapy. *Journal of Advanced Research*, 64, pp.223-235.

Li, Y., Meng, Q., Yang, M., Liu, D., Hou, X., Tang, L., Wang, X., Lyu, Y., Chen, X., Liu, K. and Yu, A.M., 2019. Current trends in drug metabolism and pharmacokinetics. *Acta Pharmaceutica Sinica B*, 9(6), pp.1113-1144.

Lian, S., Yang, W., Zeng, Y., Tang, R. and Wang, K., 2025. Targeted nano-drug delivery systems for tumor immunotherapy. *Journal of Pharmaceutical Analysis*, p.101408.

Liliopoulos, S. G., Stavrakakis, G. S. and Dimas, K. S. 2025. Linear and Non-Linear Optimal Control Methods to Determine the Best Chemotherapy Schedule for Most Effectively Inhibiting Tumor Growth. *Biomedicines*, 13(2), p.315.

Linders, L., Mihailova, D. and Meyer, T. 2024. *Doxorubicin-induced cardiotoxicity and aging: a pathophysiological review*. **npj Aging**, 10, Article No.17.

Liu, Y., Li, Y., Shen, W., Li, M., Wang, W. and Jin, X. 2024. Trend of albumin nanoparticles in oncology: a bibliometric analysis of research progress and prospects. *Frontiers in Pharmacology*, 15, p.1409163.

Loizides, S. and Constantinidou, A., 2023. Triple negative breast cancer: Immunogenicity, tumor microenvironment, and immunotherapy. *Frontiers in genetics*, 13, p.1095839.

Maeda, H. and Khatami, M. 2018. Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. *Clinical and translational medicine*, 7, pp.1-20.

Meric-Bernstam, F., Lloyd, M.W., Koc, S., Evrard, Y.A., McShane, L.M., Lewis, M.T., Evans, K.W., Li, D., Rubinstein, L., Welm, A. and Dean, D.A., 2024. Assessment of

patient-derived xenograft growth and antitumor activity: the NCI PDXNet consensus recommendations. *Molecular cancer therapeutics*, 23(7), pp.924-938.

Miao, Y., Mahdavi, B. and Zangeneh, M. M. 2021. Anti-leukemia properties of cadmium nanoparticles in vitro and in vivo conditions: a chemobiological study. *Arch Med Sci*.

Mirhadi, E., Mashreghi, M., Askarizadeh, A., Mehrabian, A., Alavizadeh, S. H., Arabi, L., Badiee, A. and Jaafari, M. R. 2022. Redox-sensitive doxorubicin liposome: A formulation approach for targeted tumor therapy. *Scientific reports*, 12(1), p.11310

Mohamed, A. I., Abdellatif, A. A. H., Tawfeek, H. M. and Abdel-Mottaleb, M. M. A. 2017. Albumin-based nanoparticles as potential controlled release drug delivery systems. *International Journal of Pharmaceutics*, 516(1–2), pp.292–303.

Mohsenipour, Z. and Hassanshahian, M. 2016. Antibacterial activity of Euphorbia hebecarpa alcoholic extracts against six human pathogenic bacteria in planktonic and biofilm forms. *Jundishapur Journal of Microbiology*, 9(6), p.e34701.

Molitoris B. A., Sandoval R. M., Yadav S. P. S., Wagner M. C. 2022. Albumin uptake and processing by the proximal tubule: physiological, pathological, and therapeutic implications. *Physiol. Rev.* 102, 1625–1667. Epub 2022/04/06. 10.1152/physrev.00014.2021

Moman, R. N., Varacallo, M. and Gupta, N. 2022. *Physiology, albumin*. [online] Nih.gov. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK459198/>.

Morganti, S., Marra, A., De Angelis, C., Toss, A., Licata, L., Giugliano, F., Salimbeni, B. T., Giachetti, P. P. M. B., Esposito, A., Giordano, A. and Bianchini, G. 2024. PARP inhibitors for breast cancer treatment: a review. *JAMA oncology*, 10(5), pp.658-670.

Mourdikoudis, S. ed., 2021. *Reducing agents in colloidal nanoparticle synthesis*. Royal Society of Chemistry.

Mustafa, M., Abbas, K., Alam, M., Ahmad, W., Moinuddin, Usmani, N., Siddiqui, S. A. and Habib, S. 2024. Molecular pathways and therapeutic targets linked to triple-

negative breast cancer (TNBC). *Molecular and cellular biochemistry*, 479(4), pp.895-913.

Nakamura, H. and Takada, K., 2021. Reactive oxygen species in cancer: Current findings and future directions. *Cancer science*, 112(10), pp.3945-3952.

Nanoshel 2025. <https://www.nanoshel.com/product/cadmium-oxide-nanoparticles>

Nasrullah, M., Sajjad, U., Iqbal, M. and Raza, M. A. 2020. Influence of synthesis parameters on structural and optical properties of CdO nanoparticles. *Journal of Inorganic and Organometallic Polymers and Materials*, 30(9), pp.3421–3430. <https://doi.org/10.1007/s10904-020-01532-4>

Nasrullah, M., Yousef, A., Ahmad, M., Awan, S., Mahmood, Z. and Nadeem, A. 2020. *Green and chemical zses of CdO NPs: a comparative study for yield attributes, biological characteristics, and toxicity concerns*. *ACS Omega*, 5(9), pp.5739–5747.

Newton, E.E., Mueller, L.E., Treadwell, S.M., Morris, C.A. and Machado, H.L., 2022. Molecular targets of triple-negative breast cancer: where do we stand?. *Cancers*, 14(3), p.482.

Niland, S., Riscanevo, A.X. and Eble, J.A., 2021. Matrix metalloproteinases shape the tumor microenvironment in cancer progression. *International journal of molecular sciences*, 23(1), p.146.

Nunnery, S. E., Mayer, I. A. and Balko, J. M. 2021. Triple-negative breast cancer: breast tumors with an identity crisis. *The Cancer Journal*, 27(1), pp.2-7.

Obidiro, O., Battogtokh, G. and Akala, E. O. 2023. Triple negative breast cancer treatment options and limitations: future outlook. *Pharmaceutics*, 15(7), p.1796.

Oh, S. S. and Narver, H. L. 2024. Mouse and rat anesthesia and analgesia. *Current Protocols*, 4(2), p.e995.

Orrantia-Borunda, E., Anchondo-Nuñez, P., Acuña-Aguilar, L. E., Gómez-Valles, F. O. and Ramírez-Valdespino, C. A. 2022. Subtypes of breast cancer. *Breast Cancer [Internet]*.

Pal, S., G, B.R., Mohny, F.P., Choudhury, S.G., Karmakar, A., Gupta, S. and Ganguli, M., 2023. Albumin nanoparticles surface decorated with a tumor-homing peptide help in selective killing of triple-negative breast cancer cells. *ACS Applied Materials & Interfaces*, 15(40), pp.46721-46737.

Panico, S., Capolla, S., Bozzer, S., Toffoli, G., Dal Bo, M. and Macor, P., 2022. Biological features of nanoparticles: protein corona formation and interaction with the immune system. *Pharmaceutics*, 14(12), p.2605

Park, S.J., 2020. Protein–nanoparticle interaction: corona formation and conformational changes in proteins on nanoparticles. *International journal of nanomedicine*, pp.5783-5802.

Patil, J. and Bhattacharya, S. 2024. Exploring the potential of quantum dots and plasmonic nanoparticles for imaging and phototherapy in colorectal neoplasia. *Results in Chemistry*, p.101689.

Peng, Y., Yang, Z., Sun, H., Li, J., Lan, X. and Liu, S., 2024. Nanomaterials in medicine: understanding cellular uptake, localization, and retention for enhanced disease diagnosis and therapy. *Aging and Disease*, 16(1), p.168.

Qasim, M. and Akram, Z., 2013. SYNTHESIS AND CHARACTERIZATION OF CADMIUM SULFIDE NANOPARTICLES-BASED INK USING A CHEMICAL PRECIPITATION TECHNIQUE. *Nanoscience and Technology: An International Journal*, 4(3).

Qiao, S., Kang, Y., Tan, X., Zhou, X., Zhang, C., Lai, S., Liu, J. and Shao, L. 2024. Nanomaterials-induced programmed cell death: Focus on mitochondria. *Toxicology*, p.153803.

Qu, N., Song, K., Ji, Y., Liu, M., Chen, L., E, R. and Teng, L. 2024. Albumin Nanoparticle-Based Drug Delivery Systems. *International journal of nanomedicine*, Volume 19, pp.6945–6980. doi:<https://doi.org/10.2147/ijn.s467876c>

Rampado, R., Crotti, S., Caliceti, P., Pucciarelli, S. and Agostini, M. 2020. Recent advances in understanding the protein corona of nanoparticles and in the formulation of “stealthy” nanomaterials. *Frontiers in bioengineering and biotechnology*, 8, p.166.

Ratuski, A. S. 2023. *Environmental enrichment for rats and mice housed in laboratories*. Doctoral dissertation, University of British Columbia.

Retecki, K., Seweryn, M., Graczyk-Jarzynka, A. and Bajor, M., 2021. The immune landscape of breast cancer: strategies for overcoming immunotherapy resistance. *Cancers*, 13(23), p.6012.

Rodríguez-Bautista, R., Caro-Sánchez, C.H., Cabrera-Galeana, P., Alanis-Funes, G.J., Gutierrez-Millán, E., Ávila-Ríos, S., Matías-Florentino, M., Reyes-Terán, G., Díaz-Chávez, J., Villarreal-Garza, C. and Hernández-Pedro, N.Y., 2021. Immune milieu and genomic alterations set the triple-negative breast cancer immunomodulatory subtype tumor behavior. *Cancers*, 13(24), p.6256.

Savage, D.T., Hilt, J.Z. and Dziubla, T.D., 2018. In vitro methods for assessing nanoparticle toxicity. In *Nanotoxicity: Methods and protocols* (pp. 1-29). New York, NY: Springer New York.

Saxena, R., Kotnala, S., Bhatt, S. C., Uniyal, M., Rawat, B. S., Negi, P. and Riyal, M. K. 2025. A review on green synthesis of nanoparticles toward a sustainable environment. *Sustainable Chemistry for Climate Action*, p.100071.

Schubert, J. and Chanana, M. 2018. Coating matters: Review on colloidal stability of nanoparticles with biocompatible coatings in biological media, living cells and organisms. *Current medicinal chemistry*, 25(35), pp.4553-4586.

Sharma, N., Verma, A. and Mehta, R. 2022. Crystallographic and morphological impact of synthesis temperature on CdO nanoparticles. *Ceramics International*, 48(9), pp.12785–12794.

Sheikh, M., Khobragade, D., Sakore, A. and Telrandhe, U., 2026. Harnessing albumin's natural tumor-targeting properties: nanoplatform strategies for triple-negative breast cancer therapy. *Discover Nano*, 21(1), p.3.

Singh, J., Dutta, T., Kim, K. H., Rawat, M., Samddar, P. and Kumar, P. 2018. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental

remediation. *Journal of Nanobiotechnology*, 16(1), p.84.
<https://doi.org/10.1186/s12951-018-0408-Ah>

Sivalingam, P., Prakash, K. and Kumar, R. 2024. Green synthesis of metal oxide nanoparticles using plant extracts: A review of recent developments. *Materials Today: Proceedings*. <https://doi.org/10.1016/j.matpr.2024.04.203>

Sivalingam, S., Sekar, M., Prakash, P., Maruthamuthu, S. and Ramasamy, G. 2024. *Green synthesis of cadmium oxide nanoparticles using Syzygium cumini: exploring industrial applications*. *RSC Advances*, 14, pp.7932–7939.

Small, L., Ehrlich, A., Iversen, J., Ashcroft, S. P., Trošt, K., Moritz, T., Hartmann, B., Holst, J. J., Treebak, J. T., Zierath, J. R. and Barrès, R. 2022. Comparative analysis of oral and intraperitoneal glucose tolerance tests in mice. *Molecular metabolism*, 57, p.101440.

Smith, B. R., Cheng, Z., De, A., Koh, A. L., Sinclair, R. and Gambhir, S. S. 2008. Real-Time Intravital Imaging of RGD–Quantum Dot Binding to Luminal Endothelium in Mouse Tumor Neovasculature. *Nano Letters*, 8(9), pp.2599–2606.
doi:<https://doi.org/10.1021/nl080141f>.

Smith, Z. L., Wang, Y., Butterworth, S. and Tirella, A. 2022. Sustained drug release from smart nanoparticles in cancer therapy: a comprehensive review. *Micromachines*, 13(10), p.1623.

Solomon, L. 2011. *Synadenium cupulare*. [online] Kumbula Indigenous Nursery. Available at: <https://kumbulanursery.co.za/plants/synadenium-cupulare> [Accessed 6 Nov. 2024].

Sood, A., Roy, A., Mahajan, S., Han, S.S., Khan, R. and Sahoo, S., 2025. Microwave chemistry and microwave-assisted synthesis of nanomaterials in biomedicine: a focused review. *Nanoscale*.

Spada, A., Emami, J., Tuszynski, J.A. and Lavasanifar, A., 2021. The uniqueness of albumin as a carrier in nanodrug delivery. *Molecular pharmaceuticals*, 18(5), pp.1862-1894.

The PIK3CA gene and its pivotal role in tumor tropism of triple-negative breast cancer. *Translational Oncology*, 50, p.102140.

Theivendren, P., Pavadai, P., Veerachamy, S., Palanisamy, P. and Kunjiappan, S. 2025. Surface receptor-targeted protein-based nanocarriers for drug delivery: advances in cancer therapy. *Nanotechnology*.

Thompson, D.M., Kuchta, K., Simovic, S. and Yao, K.A., 2025. Breast cancer trends and outcomes among young black women: a national cancer database analysis. *The American Journal of Surgery*, p.116577.

Tian, H., Zhang, T., Qin, S., Huang, Z., Zhou, L., Shi, J., Nice, E. C., Xie, N., Huang, C. and Shen, Z. 2022. Enhancing the therapeutic efficacy of nanoparticles for cancer treatment using versatile targeted strategies. *Journal of Hematology & Oncology*, [online] 15(1), pp.1–40. doi:<https://doi.org/10.1186/s13045-022-01320-5>.

V, H., Kim, Y., Kim, J., Cho, H. and Kim, K. 2024. Overcoming Cancer Drug Resistance with Nanoparticle Strategies for Key Protein Inhibition. *Molecules*, 29(17), p.3994.

Vectorstock 2025. available at <https://www.vectorstock.com/royalty-free-vector/diagram-representation-of-the-element-cadmium-vector-6009432>, accessed Jan, 2025

Vtorushin, S., Dulesova, A. and Krakhmal, N. 2022. Luminal androgen receptor (LAR) subtype of triple-negative breast cancer: molecular, morphological, and clinical features. *Journal of Zhejiang University-SCIENCE B*, 23(8), pp.617-624.

Wang, C., Xu, J., Zhang, Y. and Nie, G. 2023. Emerging nanotechnological approaches to regulating tumor vasculature for cancer therapy. *Journal of Controlled Release*, 362, pp.647-666.

Wang, Y., Cui, H., Zhou, J., Li, F., Wang, J. and Chen, M. 2018. Toxicity of metal oxide nanoparticles in mammalian cells. *Journal of Applied Toxicology*, 38(1), pp.3–24.

Wang, Y., Li, X., Pu, S., Wang, X., Guo, L., Zhang, L. and Wang, Z. 2022. Ameliorative effects of arctigenin on pulmonary fibrosis induced by bleomycin via the antioxidant activity. *Oxidative Medicine and Cellular Longevity*, (1), p.3541731.

Wijesinghe, H.D., Fernando, J., Senarath, U., Wijesinghe, G.K. and Lokuhetty, M.D., 2020. A clinicopathological study of triple-negative breast carcinoma in a patient cohort from a tertiary care center in Sri Lanka. *Indian Journal of Pathology and Microbiology*, 63(3), pp.388-396

World Health Organisation 2024. Global Burden growing, Amidst Mounting Need for services. [www.who.int.Available](https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing—amidst-mounting-need-for-services) at:https://www/who/int/news/item/01-02-2024-global-cancer-burden-growing—amidst-mounting-need-for services.

Wu, J. 2021. The enhanced permeability and retention (EPR) effect: the significance of the concept and methods to enhance its application. *Journal of personalized medicine*, 11(8), p.771.

Xiao, Y., Hong, H. and Matson, V. Z. 2016. Surface modification of nanoparticles for targeted drug delivery. *Nanomedicine*, 11(18), pp.2391–2402.

Xiong, N., Wu, H. and Yu, Z. 2024. Advancements and challenges in triple-negative breast cancer: A comprehensive review of therapeutic and diagnostic strategies. *Frontiers in Oncology*, 14, p.1405491.

Yadav, K. S., Chuttani, K., Mishra, A. K. and Sawant, K. K. 2011. Effect of size on the biodistribution and blood clearance of etoposide-loaded PLGA nanoparticles. *PDA J Pharm Sci Technol*, 65(2), pp.131-139.

Yameen, B., Choi, W.I., Vilos, C., Swami, A., Shi, J. and Farokhzad, O.C., 2014. Insight into nanoparticle cellular uptake and intracellular targeting. *Journal of controlled release*, 190, pp.485-499.

Yan, L.J. and Allen, D.C., 2021. Cadmium-induced kidney injury: oxidative damage as a unifying mechanism. *Biomolecules*, 11(11), p.1575.

Yao, Y., Zhou, Y., Liu, L., Xu, Y., Chen, Q., Wang, Y., Wu, S., Deng, Y., Zhang, J. and Shao, A., 2020. Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. *Frontiers in molecular biosciences*, 7, p.193.

Yasir, M. Y., Jaspreet Singh, J. S., Tripathi, M. K., Pushpendra Singh, P. S. and Rahul Shrivastava, R. S. 2017. Green synthesis of silver nanoparticles using leaf extract of common arrowhead houseplant and its anticandidal activity.

Yin, L., Duan, J. -J., Bian, X. -W. and Yu, S. 2020. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Research*, 22(1).

Zadehnazari, A. 2025. Chemical synthesis strategies for metal oxide nanoparticles: A comprehensive review. *Inorganic and Nano-Metal Chemistry*, 55(6), pp.734-773.

Zafar, A., Khatoon, S., Khan, M.J., Abu, J. and Naeem, A., 2025. Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy. *Discover oncology*, 16(1), p.607.

Zagami, P. and Carey, L. A. 2022. Triple negative breast cancer: Pitfalls and progress. *npj Breast Cancer*, 8(1). doi:<https://doi.org/10.1038/s41523-022-00468-0>

Zang, Y., Odwin-DaCosta, S. and Yager, J. D. 2009. Effects of cadmium on estrogen receptor-mediated signaling and estrogen-induced DNA synthesis in T47D human breast cancer cells. *Toxicology Letters*, 184(2), pp.134–138. doi:<https://doi.org/10.1016/j.toxlet.2008.10.032>.

Zhang, B., Georgiev, O., Hagmann, M., GünesC., Cramer, M., Faller, P., VasákM. and Schaffner, W. 2003. Activity of Metal-Responsive Transcription Factor 1 by Toxic Heavy Metals and H₂O₂ In Vitro Is Modulated by Metallothionein. *Molecular and Cellular Biology*, 23(23), pp.8471–8485. doi:<https://doi.org/10.1128/mcb.23.23.8471-8485.2003>.

Zhang, D., Ma, X. L., Gu, Y., Huang, H. and Zhang, G. W. 2020. Green synthesis of metallic nanoparticles and their potential applications to treat cancer. *Frontiers in Chemistry*, 8, p.799.

Zhao, L., Islam, R., Wang, Y., Zhang, X. and Liu, L.Z., 2022. Epigenetic regulation in chromium-, nickel-and cadmium-induced carcinogenesis. *Cancers*, 14(23), p.5768.

Zhen, Y., Ge, L., Chen, Q., Xu, J., Duan, Z., Loo, J.J. and Wang, M., 2022. Latent benefits and toxicity risks transmission chain of high dietary copper along the livestock–environment–plant–human health axis and microbial homeostasis: a review. *Journal of Agricultural and Food Chemistry*, 70(23), pp.6943–6962.

Zhong, Q., Zhou, Y., Tsang, D. C., Liu, J., Yang, X., Yin, M., Wu, S., Wang, J., Xiao, T. and Zhang, Z. 2020. Cadmium isotopes as tracers in environmental studies: A review. *Science*

7.0 Appendices

Appendix A: Plant (*Synadenium cupulare*) Collection permit

PERMIT TO COLLECT NATURAL RESOURCES MATERIAL IN THE KRUGER NATIONAL PARK
In compliance with section 4(1) of the National Environmental Management: Protected Areas Act No. 57 of 2003, permission is hereby granted to the person named below to collect natural resources material in the Kruger National Park, as detailed below.

South African NATIONAL PARKS

Permit No: SK2141
TOPS No: 62

Title: Miss Initials and Surname: R. LEFOJANE ID or Passport No: RC638622
Institution (or residential address, if collecting in personal capacity): University of the Free State

DESCRIPTION OF MATERIAL	QUANTITY
<u>Euphorbia Cupularis (Branches)</u>	<u>4</u>
NO ANY ALTERATION	

In (area of the KNP): Skukuza
For the purpose of: Scientific Research
This permit is valid until (date): 09/07/2022

Conditions under which this permit is issued:

1. This permit must always be available for presentation, should be requested by any Section Ranger or any other SANParks official.
2. This permit is non-transferable, i.e. may not be used by any person other than the person whose name appears above.
3. This permit is not valid without an official stamp and the signature of the permit-holder.
4. Unless specifically authorized to do so, no live material may be collected and removed from the KNP.
5. The material may only be utilised for the purposes agreed to with SANParks.
6. The material may not be used for the purposes of propagation or enhancement in any manner whatsoever, without the written approval of SANParks.
7. All intellectual property rights relating to the material remain the property of SANParks, unless otherwise agreed.
8. Misuse of the permit will result in its withdrawal and cancellation, and may also result in criminal prosecution.
9. The authorization of the State Veterinary Office in Skukuza is a statutory requirement for the removal of material from the KNP.

Additional conditions: State Veterinary permit not required

Official stamp: 

I understand and accept the above conditions and any other conditions which may have been set for the collection of the above material. Furthermore, I certify that the information provided by me for the issuing of this permit is true and correct.

Signature of permit-holder: [Signature] Date: 08/07/2022

Authorised by (name): Stephen. A. Moko

Signature: [Signature] Date: 08/07/2022

GENERAL MANAGER: CONSERVATION MANAGEMENT KRUGER NATIONAL PARK

Appendix B: Biostatistician Approval

Maryn Viljoen
Statistics Consulting Services

082 823 5731
maryn.viljoen1@gmail.com

Protocol & research methodology consultation | Ethical consultation | Database construction & capturing of data
Analysing data using statistical software packages (SAS Version 9.2) | Statistics consultation services to analyse and interpret data
Conveys results with statistical tables & figures where needed

28 April 2022

Title: "Targeted delivery of albumin-coated cadmium oxide nanoparticles as
a triple-negative breast cancer anti-agent."

Researcher: Relebohile Lefojane

To whom is my concern,

I have seen and read through this protocol. I shall be the biostatistician
responsible for the analysis of the data.

Maryn Viljoen
M.Sc. Risk Analysis (UFS)
maryn.viljoen1@gmail.com
082 82 35 731

Appendix C: Environment and Biosafety Research Ethics Committee Ethical Clearance



Environment & Biosafety Research Ethics Committee

15-Aug-2023

Dear Miss Relebohile Lefojane

Project Title: **Targeted delivery of albumin-coated cadmium oxide nanoparticles as a triple-negative breast cancer anti-agent**

Department: **Biomedical Technology - CUT**

APPLICATION APPROVED

This letter confirms that this research proposal was given ethical clearance by the Environment & Biosafety Research Ethics Committee of the University of the Free State.

Your ethical clearance number, to be used in all correspondence is: **UFS-ESD2022/0258**

Please note the following:

1. This ethical clearance is valid for two years from the issuance of this letter.
2. If the research takes longer than two years to complete, please submit a Continuation Report to the Ethics Committee before ethical clearance expires.
3. If any changes are made during the research process (including a change in investigators), please inform the Ethics Committee by submitting an Amendment.
4. When the research is concluded, please submit a Final Report to the Ethics Committee.

Thank you for your application and we wish you well in all of your research endeavours.

Yours Sincerely



Prof. RR (Robert) Bragg

Chairperson: Environment & Biosafety Research Ethics Committee
University of the Free State

Directorate: Research Development
T: +27 (0)51 401 9398 | +27 (0)51 401 2075 | E: smitham@ufs.ac.za
Johannes Brill Building, Room 106D, First Floor
205 Nelson Mandela Drive | Park West, Bloemfontein 9301 | South Africa
P.O. Box 339 | Bloemfontein 9300 | South Africa | www.ufs.ac.za



Appendix D: Animal Research Ethics Training Certificate



**SCIENTIFIC VETERINARY
CONSULTING**

CERTIFICATE

OF SUCCESSFUL COMPLETION

ANIMAL RESEARCH ETHICS

Relebohile Lefojane

Has attended the following in-person (onsite) workshops hosted at the University of the Free State, Bloemfontein, South Africa:

Activity	Date	Attended	Assessment	CPD earned
Animal Research Ethics: Foundational level	13 February 2023	5 of 5 hrs	Passed	6 CPD points
Animal Research Ethics: Intermediate level	15 February 2023	5 of 5 hrs	Passed	6 CPD points

Accreditation
 South African Veterinary Council (SAVC)
 Continuing Professional Development (CPD)
 Accredited activities (structured CPD points):
 - Foundational level: AC/0811/21
 - Intermediate level: AC/0921/22

Certificate number: SVC-C-883

Date
of Issue

14 April 2023



DR BERT MOHR
Director

Appendix E: University of the Free State Animal Research Ethical Clearance



Animal Research Ethics

31-Oct-2022

Dear Miss Relebohile Lefojane

Student Project Number: UFS-AED2021/0039/22

Project Title: Targeted delivery of albumin-coated cadmium oxide nanoparticles as a triple-negative breast cancer anti-agent invivo

Department: Biomedical Technology - CUT

You are hereby kindly informed that, the UFS Animal Research Ethics Committee approved the above project.

Kindly take note of the following:

1.
A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.
2.
Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.
3.
Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

Yours Sincerely



Appendix F: North-West University Animal Research Ethical Clearance



Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: <http://www.nwu.ac.za/>

**North-West University Animal Care, Health and
Safety Research Ethics Committee
(NWU-AnimCareREC)**

Tel: 018 299-1208
Email: Ethics-AnimCare@nwu.ac.za (for animal
studies)

1 December 2023

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC) on 01/12/2023, the NWU-AnimCareREC hereby approves your study as indicated below. This implies that the NWU-AnimCareREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Targeted delivery of albumin coated cadmium oxide nanoparticles as a TNBC anti-cancer agent																														
Principal Investigator/Study Supervisor/Researcher: Prof R Hayeshi																														
Student: -																														
Ethics number:	<table border="1"> <tr> <td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>2</td><td>0</td><td>1</td><td>5</td><td>-</td><td>2</td><td>3</td><td>-</td><td>A</td><td>5</td> </tr> <tr> <td colspan="3">Institution</td> <td colspan="3">Study Number</td> <td colspan="3">Year</td> <td colspan="3">Status</td> </tr> </table>			N	W	U	-	0	2	0	1	5	-	2	3	-	A	5	Institution			Study Number			Year			Status		
N	W	U	-	0	2	0	1	5	-	2	3	-	A	5																
Institution			Study Number			Year			Status																					
<u>Status:</u> S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation																														
Application Type: Single study	Risk:	<table border="1"> <tr> <td>Category 5</td> </tr> </table>		Category 5																										
Category 5																														
Commencement date: 01/12/2023																														
Expiry date: 28/02/2025																														
Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of a six-monthly monitoring report and the concomitant issuing of a letter of continuation. Monitoring reports are required at the end of June and February annually until completion of the study.																														

- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility, the NWU-AnimCareREC reserves the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected;
 - it becomes apparent that any relevant information was withheld from the NWU-AnimCareREC or that information has been false or misrepresented;
 - submission of the six-monthly monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or
 - new institutional rules, national legislation or international conventions deem it necessary.
- NWU-AnimCareREC can be contacted for further information via Ethics-AnimCare@nwu.ac.za or 018 299 1208

NWU-AnimCareREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-AnimCareREC for any further enquiries or requests for assistance.

Yours sincerely,



Prof. C. Weldon

Vice-Chairperson: NWU-AnimCareREC

Current details: C:\Users\22654704\OneDrive - North-West University\NWU-AnimCareREC\9.1.5.1_NWU-AnimCareREC Ethics\NWU-00015-23-55 (R Hayeshi)\9.1.5.1_FVG.1.5.4.2_Approval letter
File Reference: 9.1.5.4

Appendix G: North-West University Ammended Animal Research Ethical Clearance



NWU
NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT
YUNIBESITHI YA BORORONE BOPIHAKA

Private Bag X6001, Potchefstroom
South Africa 2520
Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

**Health Sciences Ethics Office for Research,
Training and Support**

**North-West University Animal Care, Health and
Safety Research Ethics Committee
(NWU-AnimCareREC)**
Tel: 018 299 2234
Email: Tiaan.Brink@nwu.ac.za

Prof R Hayeshi
Pharmaceutical Sciences
DSI/NWU-Preclinical Drug Development Platform

9 April 2024

Dear Prof Hayeshi

**APPROVAL OF YOUR AMENDMENT REQUEST BY THE
NWU-ANIMCAREREC OF THE FACULTY OF HEALTH SCIENCES**

Ethics number: NWU-02015-23-A5

Kindly use the ethics reference number provided above in all future correspondence or documents submitted to the administrative assistant of the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC).

Study title: Targeted delivery of albumin coated cadmium oxide nanoparticles as a TNBC anti-cancer agent

Study leader/Researcher: Prof R Hayeshi

Student: -

You are kindly informed that your amendment request (Change in cell density and addition of team members) to the aforementioned project has been approved. Any future amendments to the proposal or other associated documentation must be submitted to the NWU-AnimCareREC, Faculty of Health Sciences, North-West University, prior to implementing these changes. These requests should be electronically submitted to Ethics-AnimCare@nwu.ac.za, for review BEFORE approval can be provided, with a cover letter with a specific subject title indicating, "Amendment request: NWU-XXXX-XX-A1". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted. The amendments made should be indicated in **yellow highlight** in the amended documents. The *e-mail*, to which you attach the documents that you send, should have a *specific subject line* indicating that it is an amendment request e.g. "Amendment request: NWU-XXXX-XX-XX". This e-mail should indicate the nature of the amendment. This submission will be handled via the expedited process.

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-AnimCare@nwu.ac.za.

Yours sincerely



Digitally signed by Chairperson R
Brink (Tiaan)
DN: cn=Chairperson R Brink, o=NWU, ou=Faculty of Health Sciences, email=Chairperson.R.Brink@nwu.ac.za, c=ZA

Chairperson: NWU-AnimCareREC

Appendix H: Researchers publication that influenced the current study

CdO/CdCO₃ nanocomposite physical properties and cytotoxicity against selected breast cancer cell lines (<https://www.nature.com/articles/s41598-020-78720-5>)

scientific reports

 Check for updates

OPEN CdO/CdCO₃ nanocomposite physical properties and cytotoxicity against selected breast cancer cell lines

R. P. Lefojane¹, B. T. Sone⁴, N. Matinise³, K. Saleh³, P. Direko¹, P. Mfengwana¹, S. Mashele¹, M. Maaza³ & M. P. Sekhoacha²✉

Cadmium Oxide nanoparticles have the lowest toxicity when compared to nanoparticles of other semiconductors and they are not detrimental to human and mammalian cells, thereby making them candidates for targeting cancer cells. *Synadenium cupulare* plant extracts were used to synthesize CdO/CdCO₃ nanocomposite using cadmium nitrate tetrahydrate 98% as a precursor salt. The resultant nanoparticles were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy, ultraviolet visible spectroscopy, and Fourier transform infrared spectroscopy (FTIR). The nanoparticles were then screened for effect on breast cancer cell lines (MCF-7 and MDA MB-231) and Vero cell line to determine their growth inhibition effect. Cytotoxicity effect was evaluated using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. XRD showed the peaks of monteponite CdO and otavite CdCO₃ nanoparticles. TEM results showed irregular and spherical particles of varying sizes, whilst SEM revealed a non-uniform morphology. FTIR results showed peaks of functional groups which are present in some of the phytochemical compounds found in *S. cupulare*, and point to the presence of CdO. Annealed CdO/CdCO₃ NPs showed selectivity for MCF7 and MDA MB231 in comparison to Vero cell line, thereby supporting the hypothesis that cadmium oxide nanoparticles inhibit growth of cancerous cells more than non-cancerous cells.

Appendix I: Researcher's co-authored Review publication on medicinal plants. *Synadenium cupulare*, the plant in the study is also in the review.

Pharmacological Significance, Medicinal Use, and Toxicity of Extracted and Isolated Compounds from *Euphorbia* Species Found in Southern Africa: A Review
(<https://www.mdpi.com/2223-7747/14/3/469>)

Pharmacological Significance, Medicinal Use, and Toxicity of Extracted and Isolated Compounds from *Euphorbia* Species Found in Southern Africa: A Review

Ipeleng Kopano Rosinah Kgosiemang ¹, Relebohile Lefojane ¹, Ayodeji Mathias Adegoke ^{2,3},
Oludare Ogunyemi ⁴, Samson Sitheni Mashele ¹ and Mamello Patience Sekhoacha ^{2,*}

¹ Unit for Drug Discovery Research, Department of Health Sciences, Faculty of Health and Environmental Sciences, Central University of Technology, Bloemfontein 9301, South Africa; kopanokgosiemang@gmail.com (I.K.R.K.); lefojanerelebohile@outlook.com (R.L.); smashele@cut.ac.za (S.S.M.)

² Department of Pharmacology, Faculty of Health Sciences, University of the Free State, Bloemfontein 9301, South Africa; adegoke.am@ufs.ac.za

³ Cancer Research and Molecular Biology Laboratories, College of Medicine, University of Ibadan, Ibadan 200005, Nigeria

⁴ Nutritional and Industrial Biochemistry Research Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan 200005, Nigeria; om.ogunyemi@mail.u.i.edu.ng

* Correspondence: sekhoachamp@ufs.ac.za; Tel.: +27-051-4017969

Abstract: This study documents the Euphorbiaceae family of plants in Southern Africa, with a focus on their traditional medicinal applications, pharmacological properties, toxicity, and active secondary metabolites. A review of the literature from scientific journals, books, dissertations, and conference papers spanning from 1962 to 2023 was conducted for 15 *Euphorbia* species. Recent findings indicate that specific compounds found in *Euphorbia* plants exhibit significant biological and pharmacological properties. However, the white sticky latex sap they contain is highly toxic, although it may also have medicinal applications. Phytochemical analyses have demonstrated that these plants exhibit beneficial effects, including antibacterial, antioxidant, antiproliferative, anticancer, anti-inflammatory, antiviral, antifungal, and anti-HIV activities. Key phytochemicals such as euphol, cycloartenol, tirucallol, and triterpenoids contribute to their therapeutic efficacy, along with various proteins like lectin and lysozyme. Despite some Euphorbiaceae species undergoing screening for medicinal compounds, many remain insufficiently examined, highlighting a critical gap in the research literature. Given their historical usage, further investigations are essential to evaluate the medicinal significance of *Euphorbia* species through detailed studies of isolated compounds and their pharmacokinetics and pharmacodynamics. This research will serve as a valuable resource for future inquiries into the benefits of lesser-studied *Euphorbia* species.

Keywords: *Euphorbia* species; toxicity; secondary metabolites; pharmacological properties; medicinal plants; cancer



Academic Editors: Dejan D. Baskić and Nenad L. Vuković

Received: 21 November 2024

Revised: 30 December 2024

Accepted: 19 January 2025

Published: 5 February 2025

Citation: Kgosiemang, I.K.R.; Lefojane, R.; Adegoke, A.M.; Ogunyemi, O.; Mashele, S.S.; Sekhoacha, M.P. Pharmacological Significance, Medicinal Use, and Toxicity of Extracted and Isolated Compounds from *Euphorbia* Species Found in Southern Africa: A Review. *Pharmaceuticals* 2025, 18, 469. <https://doi.org/10.3390/ph18030469>

Appendix J: Researcher's articles on nanoparticles Synthesis and characterization

Green Synthesis of Nickel Oxide (NiO) Nanoparticles Using *Spirostachys africana* Bark Extract

Research Article

Green Synthesis of Nickel Oxide (NiO) Nanoparticles Using *Spirostachys africana* Bark Extract

²R. Lefojane, ²P. Direko, ²P. Mfengwana, ²S. Mashele, ³N. Matinise, ³M. Maaza and ¹M. Sekhoacha

¹Department of Pharmacology, University of the Free State, Bloemfontein, South Africa

²Department of Health and Environmental Science, Central University of Technology, Bloemfontein, South Africa

³Department of Material Research, Nano sciences Laboratory, iThemba LABS, Cape Town, South Africa

Abstract

Background and Objectives: Applications of nanoparticles in the biomedical field are diverse. Metal-based nanoparticles are attracting many researchers in biomedicine. Whilst various methods of nanoparticle synthesis are being explored, green synthesis is considered an environmentally friendly, safer and cost-effective method. The objectives of the study encompass the green synthesis of nickel oxide nanoparticles (NiO NPs) using bark extract of *Spirostachys africana* and their characterization using various techniques.

Materials and Methods: Distilled water, *S. africana* bark and nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were some of the materials used. The methodologies employed were extraction, phytochemical analysis, green synthesis, oven drying, annealing and characterization of the synthesized nanoparticles using different techniques. **Results:** In the phytochemical analysis test, seven phytochemical constituents tested positive. In X-ray photoelectron spectroscopy (XPS), nickel and oxygen spectra were observed. X-ray diffraction (XRD) confirmed the presence of nickel and oxygen elements. Fourier transform Infrared spectroscopy (FTIR) showed peaks that are associated with the presence of C-O and C=C. Scanning Electron Microscopy (SEM) revealed agglomerated and amorphous morphology. Ultraviolet-visible spectroscopy (UV-VIS) showed a maximum peak of 240 nm. **Conclusion:** NiONPs were successfully synthesized using the green chemistry route (green synthesis). Physical properties of NiONPs were investigated and *S. africana* bark extract showed the presence of secondary metabolites.

Appendix H: Researcher's co-authored article on nanoparticles Synthesis and characterization

Green synthesis of magnesium and cobalt oxide nanoparticles using *Euphorbia tirucalli*: Characterization and potential application for breast cancer inhibition

Green synthesis of magnesium and cobalt oxide nanoparticles using *Euphorbia tirucalli*: Characterization and potential application for breast cancer inhibition

Ipeleng Kopano Kgosiemang , Relebohile Lefojane, Paballo Direko, Zandile Madlanga, Samson Mashele & Mamello Sekhoacha 

Pages 1070-1080 | Received 01 Oct 2019, Accepted 15 Feb 2020, Published online: 17 Mar 2020

 Cite this article
  <https://doi.org/10.1080/24701556.2020.1735422>
 Check for updates

[Full Article](#)
[Figures & data](#)
[References](#)
[Supplemental](#)
[Citations](#)
[Metrics](#)
[Reprints & Permissions](#)
[Read this article](#)
[Share](#)

Abstract

The current study reports on the synthesis of magnesium oxide (MgONPs) and cobalt oxide (CoONPs) nanoparticles using the aerial parts of *Euphorbia tirucalli* and further characterizing and evaluating the anti-proliferative effect of the nanoparticles (NPs) on MCF-7 breast cancer cell line. The synthesized NPs were characterized using ultraviolet-visible spectrophotometry (UV-vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive spectroscopy (EDS), X-ray photoelectron

Related Research

People also read

Recommended articles

Cited by 41

Green synthesis of Iron oxide and Iron dioxide nanoparticles using *Euphorbia tirucalli*: characterization and antiproliferative evaluation against three breast c... >

Ipeleng Kopano Rosinah Kgosiemang et al.