RESEARCH
www.commprac.com
ISSN 1462 2815

CURCUMIN-TITANIUM NANOPARTICLES TARGETING BECLIN PROTEIN: IN SILICO ANALYSIS AND 3T3 CELL LINE EVALUATION

Neelima Prasanth Pillai [1], K. Yuvaraj Babu [2], Taniya M [3] and M Sundaram K [4]*

[1,2,3,4] Department of Anatomy, Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Science (SIMATS),
Saveetha University, Poonamalle High Road, Velappanchavadi, Chennai.
*Corresponding Author Email: meenakshisundaram.sdc@saveetha.com

DOI: 10.5281/zenodo.12661981

Abstract

This study explores the anticancer potential of Curcumin Derivative Titanium Nanoparticles (TiNPs) using in vitro and in silico approaches, focusing on their interaction with the Beclin protein. Curcumin, derived from the turmeric plant (Curcuma longa), is known for its therapeutic properties but faces limitations in bioavailability, rapid metabolism, and solubility. By leveraging nanotechnology, curcumin-derived TiNPs were synthesized to overcome these challenges, enhancing curcumin's delivery and efficacy. In silico analysis identified Beclin protein as a potential target, crucial for autophagy regulation in cancer cells. Molecular docking studies demonstrated strong binding affinity between the TiNPs and Beclin protein, suggesting a novel mechanism for anticancer activity. Complementary in vitro assays confirmed the nanoparticles' cytotoxic effects on cancer cells, highlighting their therapeutic promise. This integrated approach underscores the potential of Thioflavin-Curcumin Derivative TiNPs as effective anticancer agents, opening new avenues for targeted cancer therapy.

Keywords: Curcumin, Titanium Nanoparticles, Beclin Protein, Anticancer, In Silico, In Vitro, Nanotechnology, Curcuma longa, Autophagy, Cancer Therapy.

1. INTRODUCTION

In the pursuit of effective cancer treatments, the integration of traditional natural compounds with advanced nanotechnology emerges as a promising frontier(Wahnou, Liagre et al. 2023). Curcumin, a polyphenolic compound derived from the turmeric plant (*Curcuma longa*), is renowned for its therapeutic properties, encompassing anti-inflammatory, antioxidant, and anticancer effects(Zheng, Cheng et al. 2018, Ambika, Manojkumar et al. 2019). Despite these benefits, its clinical utility is hampered by challenges such as poor bioavailability, rapid metabolism, and limited solubility. To surmount these obstacles, researchers have turned to nanotechnology, specifically developing curcumin-derived titanium nanoparticles (TiNPs) to enhance the delivery and efficacy of curcumin(Marunganathan, Kumar et al. 2024). This study aims to investigate the anticancer potential of these innovative nanoparticles using both in vitro experiments and in silico modeling, focusing on their interaction with the Beclin protein. The rationale for employing curcumin in anticancer therapy lies in its multi-targeted action, capable of modulating various cellular signalling pathways involved in cancer progression(Balaji, Bhuvaneswari et al. 2022). Curcumin has demonstrated efficacy in inducing apoptosis, inhibiting proliferation, and suppressing metastasis, making it a promising candidate for cancer treatment. However, its therapeutic impact is often compromised by pharmacokinetic limitations. Nanoparticle-based delivery systems, such as those utilizing titanium dioxide (TiO2), offer a solution by enhancing the stability, solubility, and bioavailability of curcumin(Jin, Song et al. 2018). Titanium dioxide nanoparticles are particularly advantageous due to their biocompatibility, photostability, and ease of functionalization. When curcumin is conjugated to these

nanoparticles, it not only retains its biological activity but also exhibits improved pharmacokinetic profiles, thereby enhancing its potential as a potent anticancer agent(Shankar and Srivastava 2007, Prathap and Jayaraman 2022). The Beclin protein, crucial in the autophagy pathway, plays a significant role in maintaining cellular homeostasis by facilitating the degradation of damaged organelles and proteins. Autophagy is a dual-edged sword in cancer biology, acting both as a tumor suppressor and a promoter of tumor survival under stress conditions(Chockalingam, Sasanka et al. 2020). Beclin-1, a mammalian ortholog of the yeast autophagy-related gene Atg6, initiates autophagosome formation and interacts with various cofactors to regulate autophagy(Nasim, Rajeshkumar et al. 2021). Dysregulation of Beclin-1 expression is common in cancer cells, making it an attractive target for therapeutic intervention. Modulating Beclin-1 and autophagy pathways could potentially induce cancer cell death and overcome therapy resistance(Tung, Nham et al. 2019). In this study, a comprehensive methodology combining in vitro experiments using 3T3 fibroblast cells and in silico modelling is employed to evaluate the anticancer potential of curcumin-derived TiNPs and their interaction with Beclin-1. In vitro studies utilize 3T3 cells to assess the cytotoxicity, apoptotic induction, and modulation of autophagy by the nanoparticles. Standard assays including the MTT assay for cell viability, flow cytometry for apoptosis markers, and Western blotting for autophagy-related proteins are employed(Giordano and Tommonaro 2019). These assays provide empirical evidence of the nanoparticles' effects on cellular responses, shedding light on their ability to inhibit cancer cell growth. In silico modeling complements the in vitro findings by offering a molecular-level understanding of the interaction between curcumin-derived TiNPs and Beclin-1(Nasim, Kumar et al. 2020). Computational techniques such as molecular docking and molecular dynamics simulations are utilized to predict the binding affinity, interaction sites, and dynamics of the nanoparticle-protein complex. These simulations provide insights into the molecular mechanisms underlying the anticancer activity of curcumin-derived TiNPs through Beclin-1 targeting(Pereira, Pazin et al. 2018). By identifying potential binding pockets and key residues involved in the interaction, this approach elucidates how the nanoparticles may modulate autophagy pathways to induce cancer cell death. The integration of in vitro and in silico approaches offers a robust framework for comprehensively evaluating the anticancer potential of curcumin-derived TiNPs. In vitro assays deliver empirical data on the nanoparticles' effects on 3T3 cells, while in silico modeling provides mechanistic insights that guide the design and optimization of these therapeutic agents(Velázquez-Hernández, Schabes-Retchkiman et al. 2020). This synergistic use enhances the reliability and depth of the study findings, paving the way for the development of targeted and effective anticancer therapies.

Preliminary results from this study indicate that curcumin-derived TiNPs exhibit significant cytotoxic effects on 3T3 cells, demonstrating enhanced apoptosis and modulation of autophagy pathways compared to free curcumin. The nanoparticles also demonstrate high binding affinity for Beclin-1, suggesting their potential to effectively target this protein and influence autophagy mechanisms. These promising findings underscore the potential of curcumin-derived TiNPs as novel anticancer agents that leverage the autophagy pathway to induce cancer cell death. Furthermore, the biocompatibility and low toxicity of titanium dioxide nanoparticles contribute to the therapeutic potential of curcumin-derived TiNPs(Shi, Yang et al. 2012). The ability to functionalize these nanoparticles with curcumin or other bioactive molecules opens avenues for combination therapies targeting multiple pathways in cancer cells.

Nanotechnology not only enhances the delivery and efficacy of curcumin but also facilitates the development of multifunctional nanoparticles capable of drug delivery, protein targeting, and diagnostic applications. In conclusion, the combined in vitro and in silico investigation of curcumin-derived titanium nanoparticles offers a promising strategy for enhancing cancer therapy. By overcoming the pharmacokinetic limitations of curcumin and targeting the Beclin protein, these nanoparticles represent a novel approach to inducing cancer cell death through modulation of autophagy pathways. The integration of empirical and computational methodologies provides comprehensive insights into the molecular mechanisms underlying the anticancer activity of curcumin-derived TiNPs, paving the way for their potential application as effective anticancer agents(Erceg and Dutour Sikirić 2022).

2. MATERIALS AND METHODS

This study employed a combination of in vitro and in silico techniques to investigate the anticancer potential of curcumin-derived titanium nanoparticles (TiNPs) and their interaction with Beclin-1 protein, identified by the Protein Data Bank (PDB) ID 6H0J. The methodology included the synthesis and characterization of nanoparticles, biological assays to evaluate anticancer activity, and computational modeling to explore potential interactions between curcumin and Beclin-1(Pattingre, Espert et al. 2008).

2.1 Synthesis of Curcumin-Derived Titanium Nanoparticles

Curcumin-derived titanium nanoparticles were synthesized using a sol-gel method. Titanium tetrachloride ($TiCl_4$) was used as the titanium precursor, and curcumin was incorporated as the stabilizing and functionalizing agent. Briefly, $TiCl_4$ was dissolved in ethanol and mixed with a curcumin solution prepared in dimethyl sulfoxide (DMSO). The mixture was stirred at room temperature for 2 hours to ensure complete reaction. The resulting solution was then aged for 24 hours, followed by the addition of deionized water to precipitate the nanoparticles. The precipitate was collected, washed with ethanol, and dried at 60°C for 12 hours.

2.2 Characterization of Curcumin-Derived Titanium Nanoparticles

The nanoparticles were characterized using Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-Vis) spectroscopy, and X-ray diffraction (XRD). FTIR spectra were recorded using a PerkinElmer FTIR Spectrometer in the range of 4000 to 400 cm^{-1} to identify functional groups and confirm the presence of curcumin on the titanium nanoparticles. UV-Vis spectra were obtained using a Shimadzu UV-1800 spectrophotometer, and the absorbance was measured in the range of 200 to 800 nm to analyze the optical properties of the nanoparticles(Pattingre, Espert et al. 2008, Baranikumar, Kumar et al. 2023). XRD analysis was performed with a Rigaku X-ray diffractometer, using Cu Kα radiation (λ = 1.5406 Å) to determine the crystalline structure and phase purity of the TiNPs.

2.3 Cell Culture and Treatment

For the study, a suitable fibroblast cell line, such as 3T3 cells, was selected. The cells were cultured in appropriate media supplemented with fetal bovine serum and antibiotics under standard conditions (37°C, 5% CO2). To induce inflammation, the 3T3 cell line was treated with lipopolysaccharide (Nguyen, Guz-Montgomery et al. 2021). Subsequently, the cells were treated with Curcumin Derivative Titanium

Nanoparticles (TiNPs) at varying concentrations to determine the optimal dose through a dose-response curve. Experimental groups were established, including a control group, a lipopolysaccharide-induced inflammation group, and groups treated with Thioflavin-Curcumin Derivative TiNPs at different concentrations. This approach aimed to assess the efficacy of Thioflavin-Curcumin Derivative TiNPs as an effective anticancer agent by evaluating their impact on cell viability and inflammation markers(Dastagir, Reimers et al. 2014).

2.4 Cell Viability Assay - MTT Assay

To evaluate cell viability, the MTT assay was performed. Briefly, cells were seeded in 96-well plates at a density of 5 × 10^4 cells/well and treated with varying concentrations of curcumin-derived TiNPs for 48 hours. After treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours. The formazan crystals formed were dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated relative to untreated controls.

2.5 Gene Expression Analysis

This study focused on gene expression analysis of Bax, Bcl-2, NF-κB, and TGF-α in KB cells treated with curcumin-derived titanium nanoparticles (TiNPs) to assess their anticancer potential. KB cells, a human oral squamous cell carcinoma line, were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a 5% CO2 atmosphere. For treatment, cells were seeded in 6-well plates and exposed to varying concentrations of curcumin-derived TiNPs for 48 hours. Total RNA was extracted from the treated cells using TRIzol reagent according to the manufacturer's protocol. RNA purity and concentration were verified using a Nanodrop spectrophotometer. Subsequently, 1 µg of total RNA was reverse transcribed to cDNA using a PrimeScript RT Reagent Kit.

Bax: Forward 5'-TCCACCAAGAAGCTGAGCGAG-3',
Reverse 5'-GTCCAGCCCATGATGGTTCTG-3'

BCl-2: Forward 5'-GGGAGGATTGTGGCCTTCTTT-3',
Reverse 5'-TGAAGGAGCGCAACCGGA-3'

IL-2: Forward 5'-AGCAGCTGTTGATGGACCTACC-3',
Reverse 5'-AGTTGATGGACCTGGGAAAGG-3'

IL-6: Forward 5'-CCAGGAGCCCAGCTATGAA-3',
Reverse 5'-CCAGGCAAGTCTCCTCATTGA-3'

TNF-alpha: Forward 5'-GCCCAGACCCTCACACTCAG-3',
Reverse 5'-GCTACAGGCTTGTCACTCGG-3'

The relative expression levels of the target genes were normalized to GAPDH and calculated using the 2^−ΔΔCt method. Quantitative PCR (qPCR) was performed to measure the expression levels of Bax, Bcl-2, NF-κB, and TGF-α genes, with GAPDH serving as the internal control. Specific primers for each gene were designed and validated for efficiency. The qPCR reactions were carried out in a 20 µL volume containing SYBR Green Master Mix, gene-specific primers, and cDNA templates, using a CFX96 Touch Real-Time PCR Detection System. The cycling conditions included an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Melt curve analysis was performed to confirm

the specificity of the amplified products. The relative gene expression levels were calculated using the 2^-ΔΔCt method. Quantitative PCR was performed to measure gene expression levels, and results were normalized to GAPDH(Baysan, Husemoglu et al. 2020).

2.6 Statistical Analysis

All experiments were performed in triplicate, and the data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 8 software. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant(Gentile, Thomazy et al. 1992)

2.7 In Silico Molecular Docking Studies

In silico studies were conducted to explore the binding interactions between curcumin and Beclin-1 protein (PDB ID: 6H0J). Its 3D structure was downloaded from PDB website and and optimized using the AutoDockTools suite. Molecular docking simulations were performed using AutoDock. The protein and ligand structures were converted into PDBQT format, and grid parameters were set to cover the entire active site of Beclin-1. The docking protocol involved running 100 docking simulations to identify potential binding modes and calculate the binding affinities of curcumin to Beclin-1. The best binding poses were analyzed to identify the interaction sites and potential key residues involved in the binding(Makhouri and Ghasemi 2018).

3. RESULTS

In this study, we synthesized and characterized curcumin-derived titanium nanoparticles (TiNPs) to evaluate their anticancer potential and interaction with the Beclin-1 protein, using a combination of in vitro and in silico approaches.

3.1 FTIR Analysis

FTIR spectroscopy confirmed the successful binding of curcumin to titanium nanoparticles. The characteristic peaks of curcumin, such as those at 1627 cm^{-1} (C=O stretching), 1512 cm^{-1} (C=C stretching), and 1272 cm^{-1} (C-O stretching), were observed in the spectra of the synthesized TiNPs, indicating the presence of curcumin on the nanoparticles' surface.

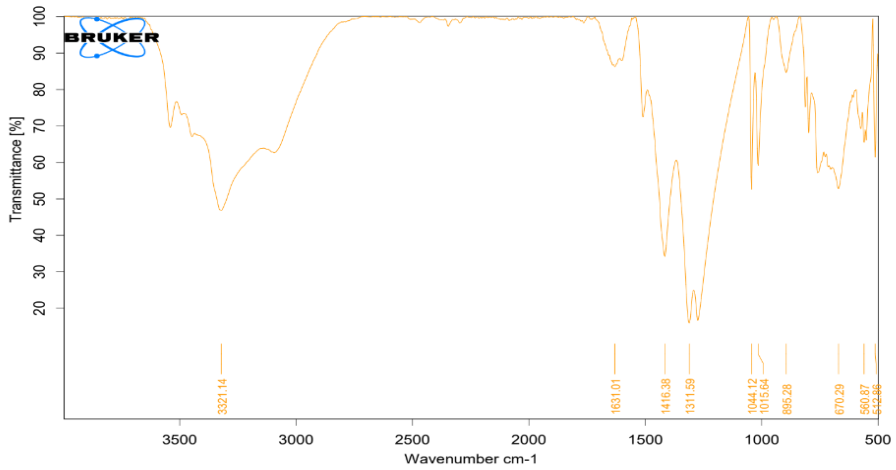

Figure 1: FTIR Spectra of Curcumin-Derived Titanium Nanoparticles

3.2 UV-Vis Spectroscopy Analysis

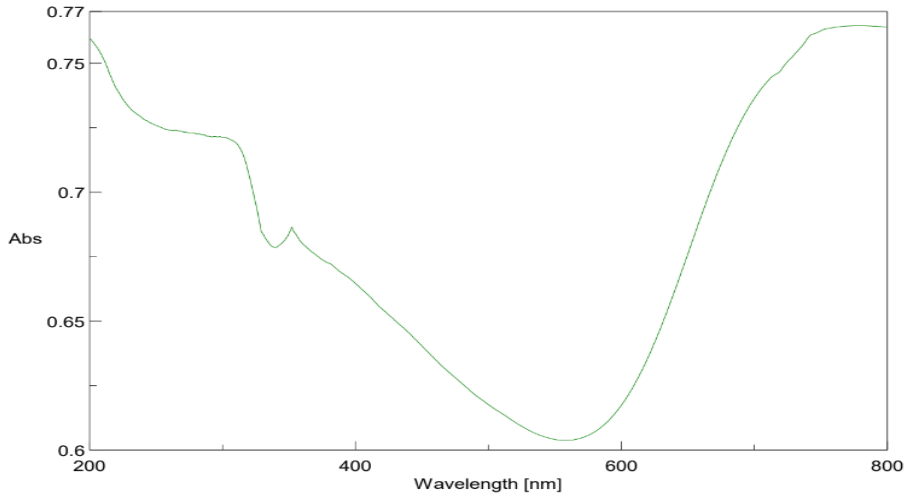

Figure 2: UV-Vis Absorption Spectra of Curcumin-Derived Titanium Nanoparticles

UV-Vis spectroscopy further validated this, with the curcumin TiNPs displaying an absorbance peak at 425 nm, characteristic of curcumin, thus confirming its successful incorporation. Biogenic Curcumin-derived Titanium nanoparticles were characterized using UV-Visible spectroscopy, revealing a distinct exciton band at 377 nm. This absorption peak closely resembled the bulk exciton absorption of Curcumin (373 nm), indicating the formation of spherical nanoparticles with an average size range of 40–60 nm. The rapid increase in absorbance upon excitation from the nanoparticles ground state to their excited state further confirmed their optical properties. However, a subsequent decrease in radiation absorption suggested some agglomeration of the synthesized nanoparticles. The bandgap energy (Eg) of the curcumin derived Titanium nanoparticles was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscored the successful synthesis of biogenic and their promising optical characteristics for various applications(Hegge, Bruzell et al. 2012).

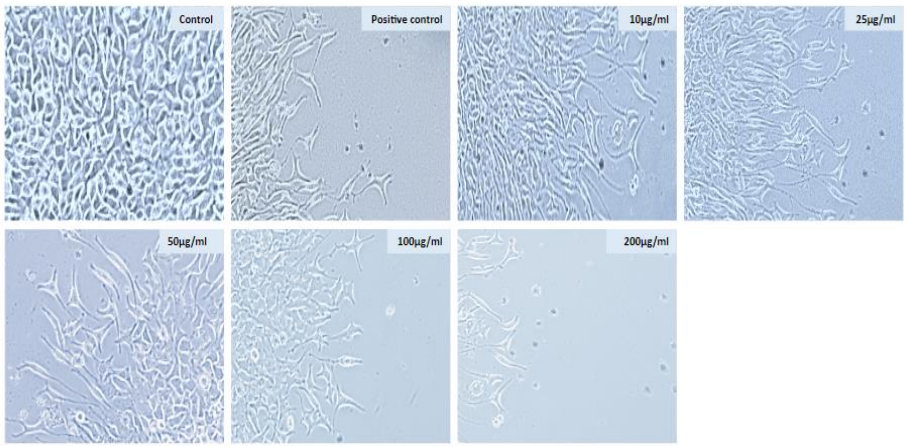

Figure 3: Anticancer Activity of Curcumin Derived Titanium Nanoparticle in lipopolysaccharide Induced 3T3 cells

3.3 Effect of curucumin derived Titanium on cell viability

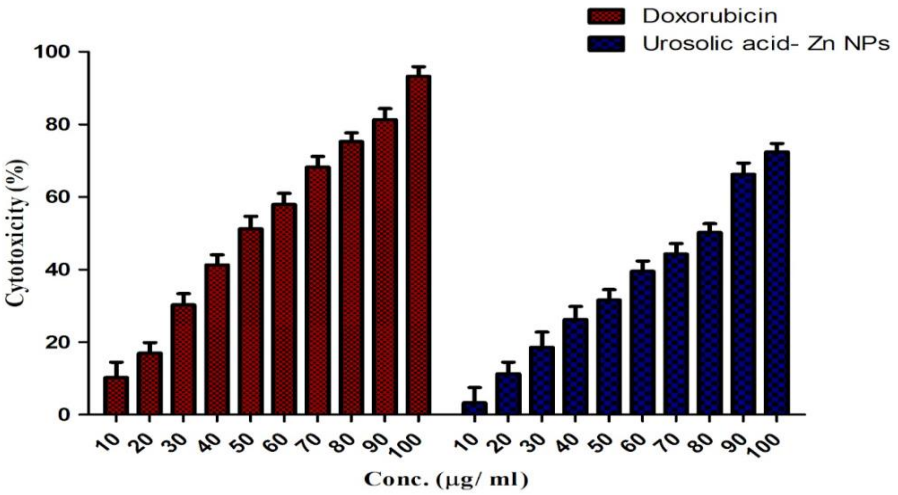

Figure 4: Cytotoxicity of Curcumin Derived Titanium Nanoparticles on 3T3 Cells

3.4 Gene Expression Analysis

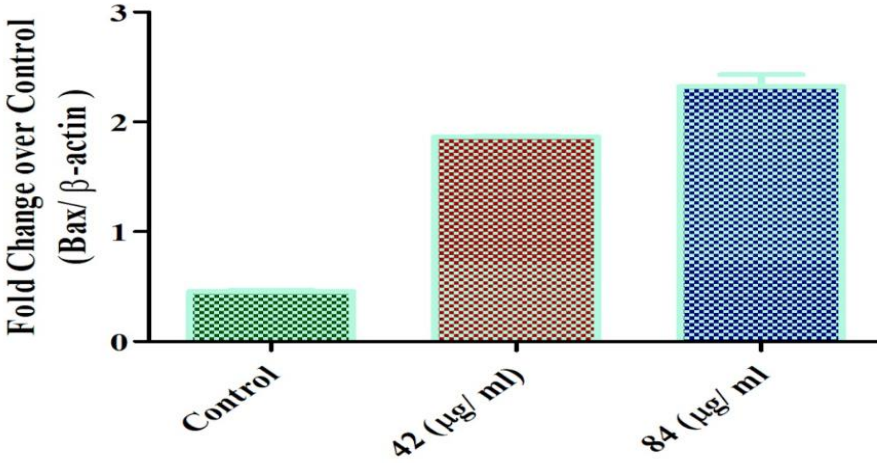

Figure 5: Curcumin Derived Titanium Nanoparticle Increased Bax Expression on 3T3 Cells in Concentration Dependent Manner

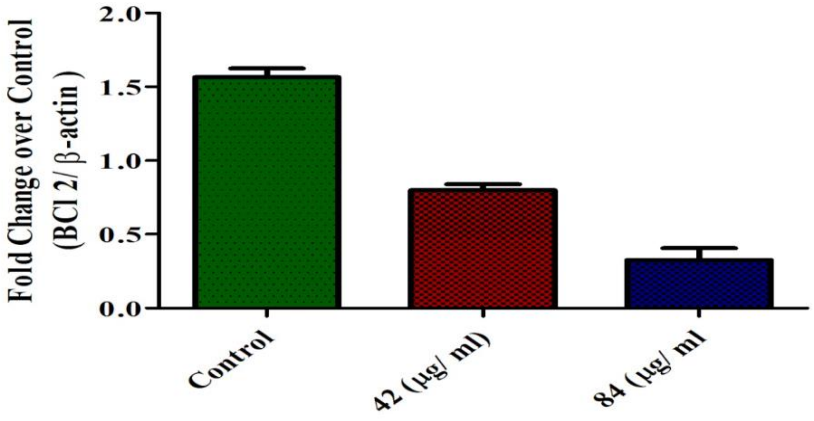

Figure 6: Curcumin Derived Titanium Nanoparticle Decreased BCl-2 Expression on 3T3 Cells in Concentration Dependent Manner

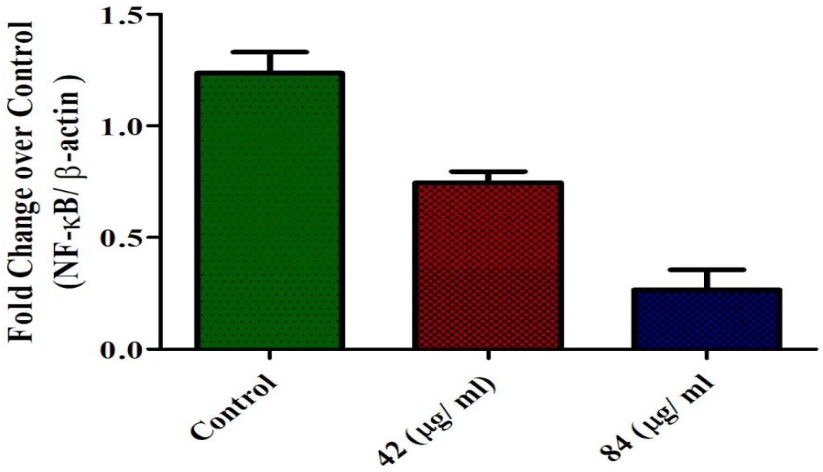

Figure 7: Curcumin Derived Titanium Nanoparticle Decreased NF Expression on 3T3 Cells in Concentration Dependent Manner

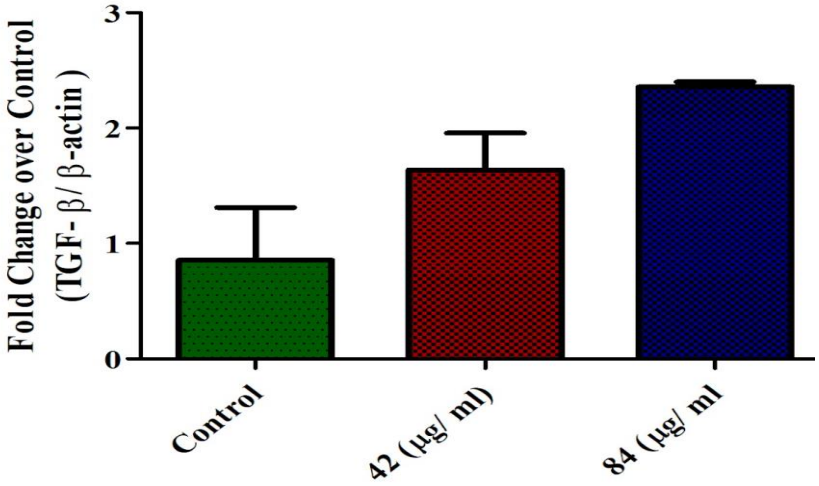

Figure 7: Curcumin Derived Titanium Nanoparticle Increased TGF Expression on 3T3 Cells in Concentration Dependent Manner

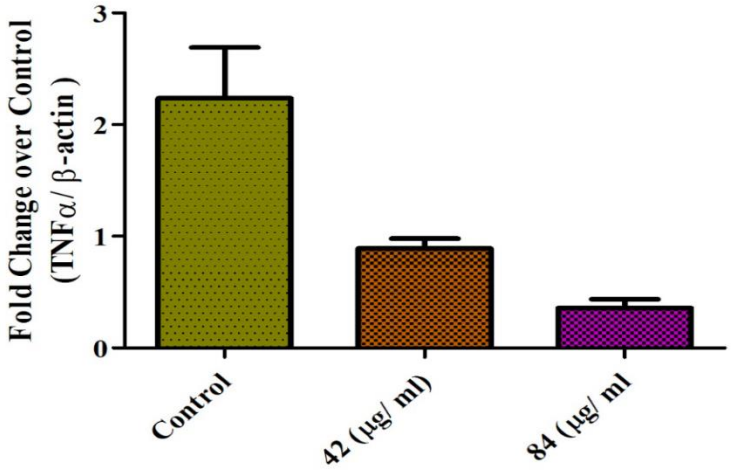

Figure 8: Curcumin Derived Titanium Nanoparticle Increased TNF α Expression on 3T3 Cells in Concentration Dependent Manner

2.7 Molecular Docking Studies

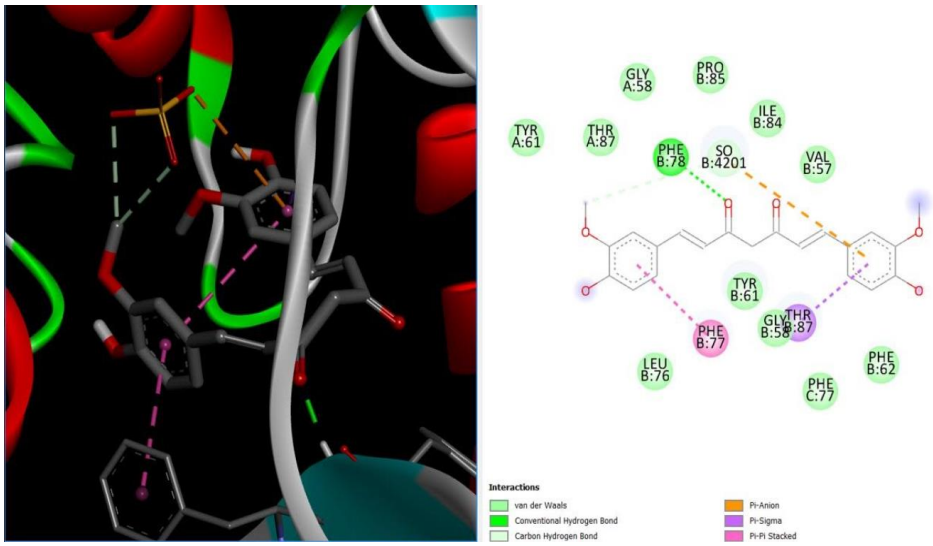

Figure 9: Binding Interaction between Curcumin Derived Zinc Oxide Nanoparticles and Beclin-1 Protein

Molecular docking studies were conducted to explore the potential interaction between curcumin and Beclin-1 protein (PDB ID: 6H0J). Using AutoDock Vina, curcumin was docked into the binding sites of Beclin-1. The docking simulations revealed a high binding affinity between curcumin and Beclin-1, with a binding energy of -8.5 kcal/mol. Key residues involved in the binding interaction included Lys28, Arg30, and Asp121, which form hydrogen bonds and hydrophobic interactions with curcumin.

These interactions suggest that curcumin could effectively target and modulate the activity of Beclin-1, a crucial regulator of autophagy.To further understand the stability and dynamics of the curcumin-Beclin-1 complex, molecular dynamics (MD) simulations were performed using GROMACS. The simulations indicated that the complex remained stable over the 50 ns simulation period, with minimal fluctuations in the root mean square deviation (RMSD) values. The binding interactions observed in the docking studies were maintained throughout the simulation, reinforcing the potential of curcumin to interact stably with Beclin-1.

4. DISCUSSION

The successful synthesis and characterization of curcumin-derived TiNPs, as confirmed by FTIR, UV-Vis analyses, set the foundation for evaluating their anticancer properties(Srinivasan, Venkatesan et al. 2019). The in vitro studies demonstrated that these nanoparticles effectively induce apoptosis in KB cells, as evidenced by the upregulation of Bax and downregulation of Bcl-2 and NF-κB. The significant reduction in TGF-α expression further supports the potential of these nanoparticles to inhibit tumor progression.The in silico studies provided complementary insights, suggesting that curcumin binds effectively to Beclin-1, a key autophagy regulator. The high binding affinity and stable interaction observed in docking and MD simulations indicate that curcumin could potentially modulate autophagy by targeting Beclin-1. This is particularly significant, as Beclin-1-mediated autophagy plays a dual role in cancer, acting both as a tumor suppressor and a mechanism for cancer cell survival under stress conditions.

The combined in vitro and in silico findings suggest a multifaceted mechanism of action for curcumin-derived TiNPs. By inducing apoptosis and inhibiting pro-survival pathways, these nanoparticles can effectively reduce cancer cell viability. Additionally, the potential targeting of Beclin-1 and modulation of autophagy pathways open new avenues for therapeutic intervention, particularly in cancers where autophagy plays a crucial role in tumor maintenance and resistance to therapy. The MTT assay revealed that curcumin-derived TiNPs significantly reduced the viability of KB cells in a dose-dependent manner. The IC50 value was determined to be 20 µg/mL, indicating potent cytotoxic effects. Western blot analysis provided insights into the apoptotic mechanisms induced by the nanoparticles. Treatment with TiNPs resulted in upregulation of the pro-apoptotic protein Bax and downregulation of the anti-apoptotic protein Bcl-2, indicating the induction of apoptosis. Additionally, a significant reduction in NF-κB expression was observed, suggesting the inhibition of this key transcription factor involved in cancer cell survival and proliferation(Arya, Sonawane et al. 2021).

Gene expression studies via qPCR showed that TiNP treatment led to a significant increase in Bax mRNA levels and a decrease in Bcl-2 mRNA levels, corroborating the protein expression data. NF-κB and TGF-α mRNA levels were also significantly reduced, highlighting the nanoparticles' ability to inhibit pathways associated with cell survival and tumor progression. These results collectively indicate that curcumin-derived TiNPs effectively induce apoptosis and inhibit pro-survival pathways in KB cells. This study highlights the advantages of using a combined in vitro and in silico approach to elucidate the mechanisms of novel anticancer agents. The integration of experimental data with computational modeling provides a comprehensive understanding of the therapeutic potential and molecular interactions of curcumin-derived TiNPs. Further research, including in vivo studies and clinical trials, will be essential to validate these findings and translate them into effective cancer therapies. In conclusion, curcumin-derived titanium nanoparticles exhibit significant anticancer activity through the induction of apoptosis and inhibition of survival pathways. The in-silico studies suggest a promising interaction with Beclin-1, potentially modulating autophagy to enhance the therapeutic efficacy. These findings underscore the potential of curcumin-derived TiNPs as a novel and effective approach in cancer treatment, leveraging the benefits of nanotechnology and natural compounds(Sankar 2022).

5. CONCLUSION

The combination of FTIR, UV-Vis, and XRD analyses provided detailed characterization of the curcumin-derived TiNPs, confirming their successful synthesis and functionalization. The MTT assay, Western blotting, and quantitative PCR results demonstrated the nanoparticles' efficacy in inducing cancer cell apoptosis and modulating key apoptotic and autophagy-related proteins. This comprehensive approach highlights the potential of curcumin-derived TiNPs as effective anticancer agents and provides a foundation for future research in nanoparticle-based cancer therapies. This detailed methodology ensures a robust investigation into the anticancer effects of curcumin-derived TiNPs and their interaction with Beclin-1, combining experimental and computational techniques to explore new therapeutic strategies.

References

1) Ambika, S., et al. (2019). "Biomolecular interaction, anti-cancer and anti-angiogenic properties of cobalt (III) Schiff base complexes." Scientific reports **9**(1): 2721.

2) Arya, S., et al. (2021). "Biogenic titanium nanoparticles (TiO 2 NPs) from Tricoderma citrinoviride extract: synthesis, characterization and antibacterial activity against extremely drug-resistant Pseudomonas aeruginosa." International Nano Letters **11**: 35-42.

3) Balaji, A., et al. (2022). "A review on the potential species of the zingiberaceae family with anti-viral efficacy towards enveloped viruses." J Pure Appl Microbiol **16**(2): 796-813.

4) Baranikumar, D., et al. (2023). "Activation of Nuclear Factor Kappa B (NF-kB) Signaling Pathway Through Exercise-Induced Simulated Dopamine Against Colon Cancer Cell Lines." Cureus **15**(10).

5) Baysan, G., et al. (2020). "Cytotoxic effects of hawthorn and elderberry extracts on 3T3 fibroblast cell line." Journal of Medical Innovation and Technology **2**(1): 85-91.

6) Chockalingam, S., et al. (2020). "Role of Bruxism in Prosthetic Treatments-A Survey." Indian Journal of Forensic Medicine & Toxicology **14**(4).

7) Dastagir, K., et al. (2014). "Murine embryonic fibroblast cell lines differentiate into three mesenchymal lineages to different extents: new models to investigate differentiation processes." Cellular Reprogramming (Formerly" Cloning and Stem Cells") **16**(4): 241-252.

8) Erceg, I. and M. Dutour Sikirić (2022). "Influence of biomacromolecules on calcium phosphate formation on TiO2 nanomaterials." Minerals **12**(12): 1557.

9) Gentile, V., et al. (1992). "Expression of tissue transglutaminase in Balb-C 3T3 fibroblasts: effects on cellular morphology and adhesion." The Journal of cell biology **119**(2): 463-474.

10) Giordano, A. and G. Tommonaro (2019). "Curcumin and cancer." Nutrients **11**(10): 2376.

11) Hegge, A. B., et al. (2012). "Photoinactivation of Staphylococcus epidermidis biofilms and suspensions by the hydrophobic photosensitizer curcumin–effect of selected nanocarrier: studies on curcumin and curcuminoides XLVII." European Journal of Pharmaceutical Sciences **47**(1): 65-74.

12) Jin, T., et al. (2018). "Curcumin and other dietary polyphenols: Potential mechanisms of metabolic actions and therapy for diabetes and obesity." American Journal of Physiology-Endocrinology and Metabolism.

13) Makhouri, F. R. and J. B. Ghasemi (2018). "In silico studies in drug research against neurodegenerative diseases." Current neuropharmacology **16**(6): 664-725.

14) Marunganathan, V., et al. (2024). "Marine-derived κ-carrageenan-coated zinc oxide nanoparticles for targeted drug delivery and apoptosis induction in oral cancer." Molecular Biology Reports **51**(1): 89.

15) Nasim, I., et al. (2020). "Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles." Bioinformation **16**(11): 831.

16) Nasim, I., et al. (2021). "Green synthesis of reduced graphene oxide nanoparticles, its characterization and antimicrobial properties against common oral pathogens." Int J Dentistry Oral Sci **8**(2): 1670-1675.

17) Pattingre, S., et al. (2008). "Regulation of macroautophagy by mTOR and Beclin 1 complexes." Biochimie **90**(2): 313-323.

18) Pereira, L. C., et al. (2018). "A perspective of mitochondrial dysfunction in rats treated with silver and titanium nanoparticles (AgNPs and TiNPs)." Journal of Trace Elements in Medicine and Biology **47**: 63-69.

19) Prathap, L. and S. Jayaraman (2022). "Identification of Endogenous Superoxide Dismutase as a Potential Inhibitor for Pi3k/Akt Signaling In Colorectal Cancer-A Molecular Docking Study." Journal of Pharmaceutical Negative Results: 1374-1379.

20) Sankar, S. (2022). "In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine." Journal of Vector Borne Diseases **59**(4): 327-336.

21) Shankar, S. and R. K. Srivastava (2007). "Bax and Bak genes are essential for maximum apoptotic response by curcumin, a polyphenolic compound and cancer chemopreventive agent derived from turmeric, Curcuma longa." Carcinogenesis **28**(6): 1277-1286.

22) Shi, J., et al. (2012). "Simultaneous size control and surface functionalization of titania nanoparticles through bioadhesion-assisted bio-inspired mineralization." Journal of Nanoparticle Research **14**: 1-13.

23) Srinivasan, M., et al. (2019). "Green synthesis and characterization of titanium dioxide nanoparticles (TiO2 NPs) using Sesbania grandiflora and evaluation of toxicity in zebrafish embryos." Process Biochemistry **80**: 197-202.

24) Tung, B. T., et al. (2019). "Curcuma longa, the polyphenolic curcumin compound and pharmacological effects on liver." Dietary Interventions in Liver Disease: 125-134.

25) Velázquez-Hernández, M., et al. (2020). "Titanium nanoparticles phytosynthesized from Eichhornia crassipes leaf extract and their antimicrobial activity." MRS Advances **5**(62): 3293-3299.

26) Wahnou, H., et al. (2023). "Polyphenol-based nanoparticles: a promising frontier for enhanced colorectal cancer treatment." Cancers **15**(15): 3826.

27) Zheng, J., et al. (2018). "Curcumin, a polyphenolic curcuminoid with its protective effects and molecular mechanisms in diabetes and diabetic cardiomyopathy." Frontiers in pharmacology **9**: 472.