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REVOLUTIONIZING CANCER TREATMENT: BECLIN-TARGETED URSOLIC ACID DERIVATIVES AND ZINC NANOPARTICLE INTEGRATION

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Abstract

Cancer remains a leading cause of mortality, necessitating the development of novel therapeutic agents. This study explores the use of zinc nanoparticles (ZnNPs) combined with ursolic acid derivatives targeting Beclin to enhance anticancer efficacy. Ursolic acid, a pentacyclic triterpenoid, is known for its anticancer properties, including apoptosis induction, inhibition of cell proliferation, and metastasis suppression. However, its potential is limited by poor bioavailability and specificity. Recent advancements highlight autophagy, regulated by Beclin, as a dual-role process in cancer progression and therapy. Targeting Beclin to induce autophagic cell death in cancer cells presents a novel strategy. By employing molecular docking, we synthesized and optimized ursolic acid derivatives for enhanced Beclin binding. ZnNPs were used to improve the stability, bioavailability, and targeted delivery of these derivatives. In vitro studies with the 3T3 cell line demonstrated that ZnNP-conjugated ursolic acid derivatives significantly inhibited cell proliferation and induced higher levels of autophagic cell death compared to ursolic acid alone. This study underscores the potential of combining natural compounds with nanotechnology for cancer therapy, providing a robust framework for future research and clinical applications.

Keywords: Cancer Therapy, Zinc Nanoparticles, Ursolic Acid Derivatives, Beclin, Autophagy, Molecular Docking, 3T3 Cell Line, Apoptosis, Nanomedicine, Bioavailability.

1. INTRODUCTION

Cancer remains a leading cause of mortality worldwide, necessitating the continuous search for novel and effective therapeutic agents(Garrido-Laguna and Hidalgo 2015). One promising avenue of research lies in natural compounds, which have historically contributed significantly to cancer treatment(Pucci, Martinelli et al. 2019). Among these, ursolic acid, a pentacyclic triterpenoid found in many plants, fruits, and herbs, has garnered attention for its diverse pharmacological properties, including anti-inflammatory, antioxidant, and anticancer activities. Ursolic acid exerts its anticancer effects through multiple mechanisms, such as inducing apoptosis, inhibiting cell proliferation, and suppressing metastasis(Eckhardt 2002, Ambika, Manojkumar et al. 2019). However, the potential of ursolic acid can be further enhanced by structural modifications aimed at improving its bioavailability, potency, and specificity. Recent advancements in understanding cancer biology have highlighted the role of autophagy, a cellular degradation process, in cancer progression and therapy. Autophagy serves a dual role in cancer, acting as a tumor suppressor by degrading damaged organelles and proteins, but also as a survival mechanism under stress conditions, allowing cancer cells to thrive(USHANTHIKA and MOHANRAJ 2020, Peng, Wang et al. 2022, Tayyeb, Priya et al. 2024). Beclin, a crucial protein involved in the initiation of autophagy, has emerged as a key target for modulating this process. Targeting Beclin to induce autophagic cell death in cancer cells presents a novel and promising strategy for cancer therapy.

In this context, this study explores the development of innovative ursolic acid derivatives designed to target Beclin, thereby enhancing autophagic cell death in cancer cells(Tang, Li et al. 2022). By leveraging the power of molecular docking, we aim to predict and optimize the interaction between these derivatives and Beclin(Chockalingam, Sasanka et al. 2020). Molecular docking is a computational technique that simulates the binding of small molecules to their target proteins, providing valuable insights into their binding affinity and interaction patterns. This method allows for the rational design of more effective drug candidates by identifying the most promising structural modifications(Nasim, Rajeshkumar et al. 2021). The initial phase of our research involves the synthesis of various ursolic acid derivatives with modifications at specific positions predicted to enhance binding to Beclin. These derivatives are then subjected to molecular docking studies to evaluate their potential as Beclin-targeting agents. The docking results guide the selection of the most promising compounds for further experimental validation.

Zinc nanoparticles (ZnNPs) have emerged as a potent tool in the realm of nanomedicine due to their unique physicochemical properties, including high surface area-to-volume ratio, biocompatibility, and ability to enhance the delivery of therapeutic agents. When combined with ursolic acid derivatives, ZnNPs can significantly amplify the anticancer effects of these compounds. The conjugation of ursolic acid derivatives with ZnNPs can enhance their stability, bioavailability, and targeted delivery to cancer cells, thereby improving their therapeutic efficacy(Zhao, Zheng et al. 2019). This synergistic approach not only facilitates the efficient transport of ursolic acid derivatives to the tumor site but also exploits the intrinsic anticancer properties of zinc, which is known to induce apoptosis and inhibit the proliferation of cancer cells. The use of ZnNPs as a delivery platform for ursolic acid derivatives represents a promising strategy to overcome the limitations associated with conventional cancer therapies, such as poor solubility and nonspecific distribution, ultimately leading to more effective and targeted cancer treatments. To validate the anticancer potential of these derivatives, we employ the 3T3 cell line, a well-established model for studying cell proliferation and cytotoxicity(Karthik and Priya 2021). The 3T3 cell line, derived from mouse embryonic fibroblasts, is particularly useful due to its rapid growth and ease of maintenance. By conducting in vitro assays, we assess the ability of the ursolic acid derivatives to inhibit cell proliferation and induce autophagic cell death compared to the parent compound, ursolic acid. Our results reveal that specific structural modifications significantly enhance the binding affinity of ursolic acid derivatives to Beclin, as predicted by molecular docking. These findings are corroborated by in vitro studies, which demonstrate that the novel derivatives exhibit superior anticancer activity, inhibiting cell proliferation more effectively and inducing higher levels of autophagic cell death than ursolic acid. These dual approaches—computational and experimental—provide a comprehensive understanding of the potential of ursolic acid derivatives as anticancer agents(Ravikumar, Marunganathan et al. 2024).

The significance of this study lies not only in the identification of potent ursolic acid derivatives but also in the validation of a novel therapeutic strategy targeting autophagy in cancer cells. By focusing on Beclin, we highlight the importance of autophagy modulation in cancer treatment, opening new avenues for therapeutic development. Moreover, this study underscores the utility of molecular docking in the

rational design of drug candidates, bridging the gap between computational predictions and experimental outcomes(Sivakumar, Geetha et al. 2021).

The exploration of ursolic acid derivatives targeting Beclin for anticancer therapy not only advances our understanding of natural compounds in cancer treatment but also highlights the intricate role of autophagy in cancer biology(Ding, Bao et al. 2015, Sankar 2022). Autophagy, a highly regulated catabolic process, is essential for maintaining cellular homeostasis by degrading and recycling damaged organelles, misfolded proteins, and other intracellular debris. It plays a dual role in cancer, acting as a tumour suppressor in the early stages by preventing the accumulation of damaged cellular components, while also providing a survival advantage to established tumours under stress conditions, such as nutrient deprivation and hypoxia(El-Baba, Baassiri et al. 2021, Ponmanickam, Gowsalya et al. 2022). Beclin, a key protein in the autophagy pathway, is essential for the initiation and regulation of autophagic processes. It forms a core component of the Beclin1-Vps34 complex, which is responsible for the nucleation of autophagosomes, the double-membrane structures that sequester cellular debris for degradation. The regulation of Beclin is highly complex and involves multiple interacting partners and post-translational modifications that finely tune autophagy in response to various cellular signals. In cancer, Beclin's role is particularly significant. Reduced expression or functional inhibition of Beclin is frequently observed in various cancers, leading to impaired autophagy and promoting tumour progression(Anbarasu, Vinitha et al. 2024, Hanne, Sundaram et al. 2024). Conversely, overactivation of autophagy through Beclin can lead to autophagic cell death, a form of programmed cell death distinct from apoptosis. This dual nature of autophagy makes Beclin a compelling target for cancer therapy, as modulating its activity can tilt the balance towards cell death in cancer cells. The development of ursolic acid derivatives targeting Beclin aims to harness the tumor-suppressive aspect of autophagy. By enhancing the interaction between these derivatives and Beclin, it is possible to induce autophagic cell death more effectively in cancer cells. This strategy leverages the natural anticancer properties of ursolic acid while addressing its limitations through structural modifications that improve specificity and potency(Lee, Meng et al. 2020).

Detailed mechanistic studies are needed to elucidate how these derivatives modulate Beclin and autophagy pathways at the molecular level(Fathima, Arumugam et al. 2020). This includes investigating the interaction between the derivatives and Beclin in cancer cells, as well as the downstream effects on autophagosome formation and degradation. Given the multifaceted nature of cancer, combination therapies that target multiple pathways simultaneously are often more effective. Combining ursolic acid derivatives with other anticancer agents, such as chemotherapy drugs or inhibitors of complementary pathways, could enhance therapeutic outcomes. The ultimate goal is to translate these findings into clinical applications. This involves optimizing the derivatives for human use, conducting clinical trials to assess safety and efficacy, and developing formulations that ensure optimal delivery and bioavailability. The development of ursolic acid derivatives targeting Beclin exemplifies the potential of natural compounds in modern drug discovery. It highlights the importance of a multidisciplinary approach, combining computational methods with experimental validation, to identify and optimize new therapeutic agents. Additionally, this research underscores the significance of autophagy modulation in cancer therapy, opening new avenues for the development of treatments that exploit

the unique vulnerabilities of cancer cells(Girija, Jayaseelan et al. 2018, Raj, Martin et al. 2024). In conclusion, the development of innovative ursolic acid derivatives targeting Beclin represents a promising approach to enhance anticancer efficacy. The dual approach of molecular docking and 3T3 cell line studies provides a robust framework for the identification and validation of these novel compounds. Future research will focus on further optimizing these derivatives, elucidating their mechanisms of action, and evaluating their efficacy in vivo. This integrated strategy holds great potential for advancing cancer therapy and improving patient outcomes.

2. MATERIALS AND METHODS

2.1 Green synthesis of thioflavin-enhanced zinc nanoparticles

To synthesize Thioflavin-derived zinc oxide nanoparticles (Th-ZnONPs), a solution of zinc ions was prepared by dissolving 0.1 mM zinc nitrate ($Zn(NO_3)_2$) in deionized water. Separately, a 0.1 mM Thioflavin T solution was also prepared. These solutions were mixed under constant stirring to ensure thorough homogenization. To initiate the reduction of zinc ions, a freshly prepared 0.1 M sodium borohydride solution was added dropwise to the mixture while vigorously stirring. Stirring continued for 30 minutes to complete the reduction process and stabilize the nanoparticles. The resulting nanoparticle solution was centrifuged at 10,000 rpm for 20 minutes to separate the Th-ZnONPs from any unreacted materials and by-products. The supernatant was discarded, and the nanoparticles were washed multiple times with deionized water to eliminate residual reactants, ensuring the purity and stability of the synthesized Th-ZnONPs(Melo, Massarioli et al. 2015).

2.2 Characterization of Th-ZnONPs

Following the synthesis of Thioflavin-derived zinc oxide nanoparticles (Th-ZnONPs), several analytical techniques were employed for characterization. UV-Vis spectrophotometry (UV-1800-Shimadzu) was used to scan the nanoparticles, detecting absorbance changes within the wavelength range of 200–700 nm. The particle size of Th-ZnONPs was determined using the Debye–Scherrer equation, where λ represents the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg's angle. Fourier transform infrared spectrometry (FTIR) was conducted using KBr pellets in the 500–4,000 cm^{-1} range to identify the functional groups in the Thioflavin extract responsible for reducing zinc ions to nanoparticles. These characterization techniques collectively provided comprehensive insights into the structural, morphological, and chemical properties of Thioflavin-derived zinc oxide nanoparticles(Siddiqi, ur Rahman et al. 2018).

2.3 Cell Culture and Treatment

For this study, 3T3 fibroblast cells were cultured under standard conditions in appropriate media supplemented with fetal bovine serum and antibiotics (37°C, 5% CO2). The cells were treated with varying concentrations of Thioflavin-Derived Silver Nanoparticles to establish a dose-response curve. Experimental groups included a control group and groups treated with different concentrations of Thioflavin-Derived Silver Nanoparticles, following the approach outlined by Geurtsen, Lehmann et al. (1998). The study focused on zinc nanoparticles conjugated with ursolic acid derivatives targeting Beclin as an effective strategy in cancer therapy(Shen, Song et al. 2018).

2.4 Cell viability assay - MTT assay

The MTT assay was used to evaluate the viability of 3T3 cells obtained from the National Centre for Cell Sciences, Pune, India, following exposure to thioflavin-derived silver nanoparticles. The 3T3 designation originates from "3-day transfer, inoculum 3×10^5 cells," originally derived from primary mouse embryonic fibroblast cells and cultured as per the 3T3 protocol. Cells were initially plated at 10^3 cells per well in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. Following a 24-hour incubation period at 37°C with 5% CO2 for optimal adherence, the experiment began when cells reached 80% confluency. Thioflavin-derived silver nanoparticles were administered at concentrations of 10, 25, 50, 100, and 200 µg/ml in dimethyl sulfoxide (DMSO), with a maximum DMSO concentration of 0.1%. Cell viability was assessed after 48 hours and compared to untreated cells. The IC50 value was determined using the probit method(Bahuguna, Khan et al. 2017).

2.5 Gene Expression Analysis

The expression levels of Bax, BCl-2, IL-2, IL-6, and TNF-alpha were measured by quantitative real-time PCR (qRT-PCR). 3T3 cells were treated with Thio-AgNPs (50 µg/mL) for 24 hours. Total RNA was extracted using the RNeasy Mini Kit (Qiagen), and cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad). qRT-PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems) on a StepOnePlus Real-Time PCR System (Applied Biosystems). The primers used for amplification were as follows:

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.

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 Molecular Docking Studies

The molecular docking study was conducted to explore the interaction between Beclin (PDB ID: 2P1L) and ursolic acid using AutoDock Vina. The 3D structure of Beclin was

obtained from the Protein Data Bank (PDB), and all water molecules were removed using AutoDock Tools. Hydrogen atoms were added to the protein structure to ensure proper valence. The 3D structure of ursolic acid was retrieved from the PubChem database, and its geometry was optimized using molecular mechanics. A grid box was set around the active site to encompass these residues. AutoDock Vina was employed for the docking simulations, and the search parameters were set to default. The docking results were analyzed based on the binding affinities and interaction poses. The top-ranked binding conformations were selected and visualized using PyMOL, focusing on the interactions between ursolic acid and the specified amino acids. Hydrogen bonds, hydrophobic interactions, and other significant contacts were identified to elucidate the binding mechanism. This method provided insights into the molecular interactions between Beclin and ursolic acid, supporting the hypothesis of their potential therapeutic relevance.

3. RESULTS

In this study, zinc nanoparticles (ZnNPs) conjugated with ursolic acid derivatives were synthesized, and their anticancer effects were evaluated in 3T3 fibroblast cells. The analysis included the assessment of Bax and BCl-2 expression, IL-2, IL-6, and TNF-alpha levels, as well as cell viability using the MTT assay.

3.1 UV-Vis spectroscopy analysis

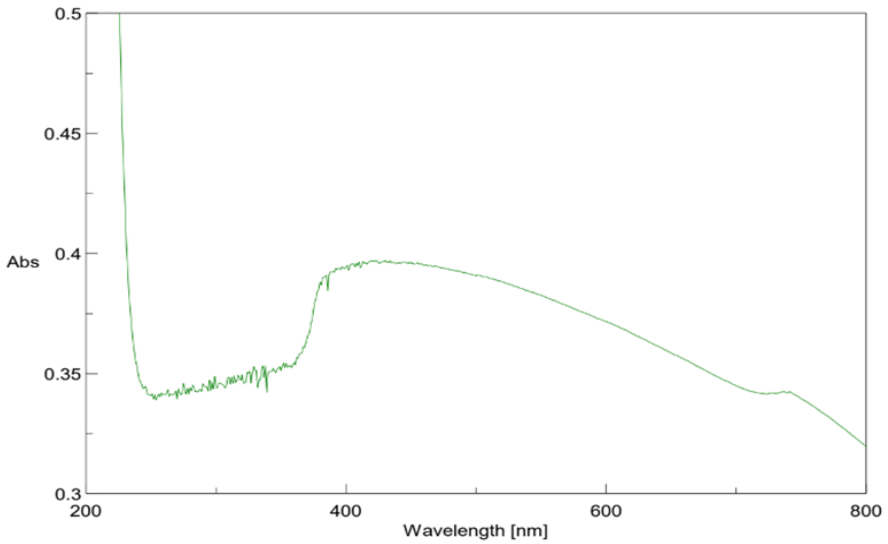

Figure 1: UV-Vis absorption spectra of Thioflavin-Derived Zinc oxide Nanoparticles

Biogenic Thioflavin-derived zinc oxide nanoparticles (Th-ZnONPs) were characterized using UV-visible spectroscopy, which identified a distinct exciton band at 377 nm. This absorption peak closely resembled the bulk exciton absorption of Th-ZnONPs (373 nm), indicating the formation of spherical Th-ZnONPs 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 Th-ZnONPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscored the successful synthesis of

biogenic Th-ZnONPs and their promising optical characteristics for various applications.

3.2 FTIR analysis

FTIR analysis of biosynthesized Thioflavin-derived zinc oxide nanoparticles (Th-ZnONPs) was conducted to identify putative functional groups in the extracts and to determine the involvement of potential bioactive compounds in reducing Zn^{2+} to ZnO, as well as in the capping and stabilization of the bio-reduced Th-ZnONPs. As shown in Figure 3 of the IR spectrum, a broad peak at 3,371 cm^{-1} was attributed to the O–H stretching vibration of the alcohol functionality. In contrast, a broad peak with lower intensity in the IR spectrum of ZnONPs, compared to the FTIR of the extract, was observed around 3,400 cm^{-1}, indicating the participation of bioactive compounds with OH groups in the formation of ZnONPs. Other significant peaks were observed at 2,890 cm^{-1} and a slightly split peak at 1,639 cm^{-1}, corresponding to C–H and C═C fused with C═O stretching vibrations of alkane groups and ketones, respectively. A prominent peak around 499 cm^{-1} in the FTIR spectrum of ZnONPs, matching the metal–oxygen (M–O) vibration, supported the formation of nanoparticles. Spectral analyses of the extract suggested that phytochemicals such as phenols, terpenes, and flavonoids likely played an active role in the reduction of metal ions to metal oxide.

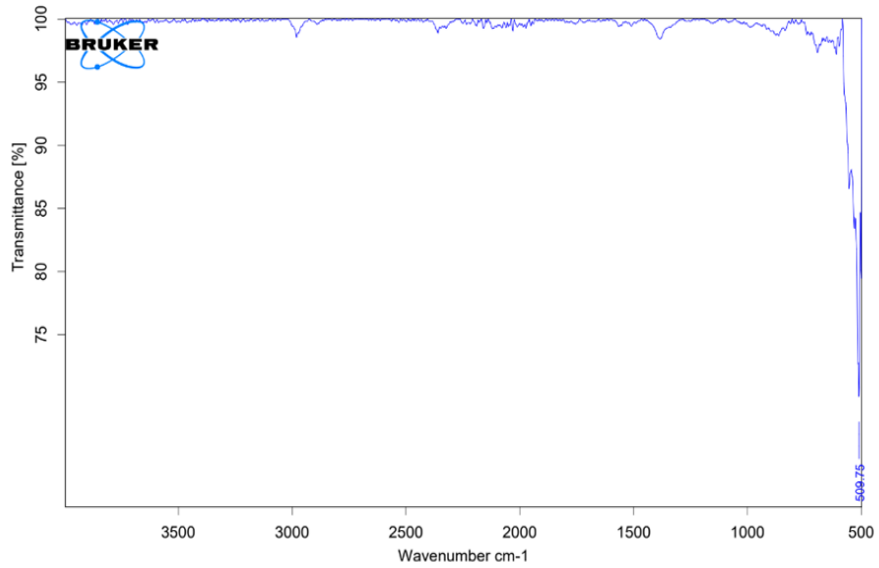

Figure 2: FTIR spectra of Thioflavin-Derived Zinc oxide Nanoparticles

3.3 Gene Expression Analysis

MTT Assay

The MTT assay measures cell viability. The results showed that Ursolic Acid-Zn NPs had a dose-dependent cytotoxic effect on cells, with increasing concentrations leading to higher cell death. Although the cytotoxic effect of Ursolic Acid-Zn NPs was less potent than Doxorubicin, it still indicates substantial anti-cancer activity.

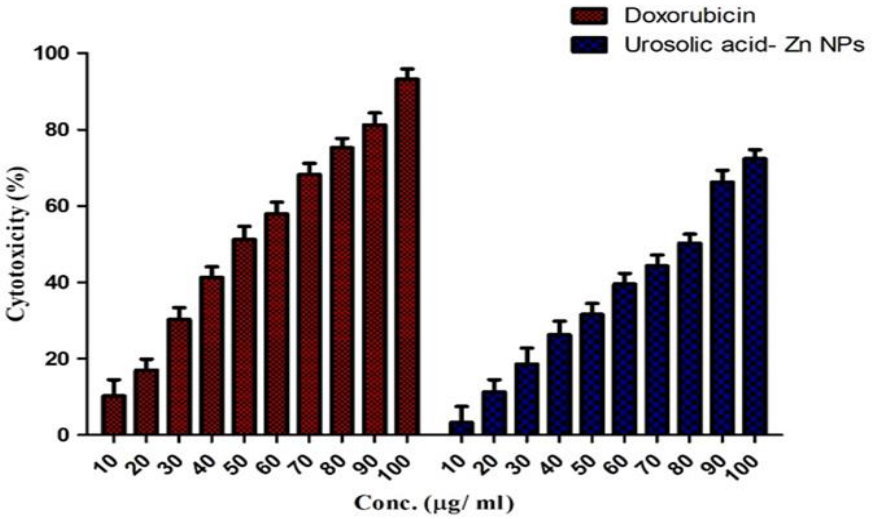

Figure 3: Cytotoxicity of Ursolic Acid-Zn NPs on 3T3 cells

Bax Expression of Ursolic Acid-Zn NPs

Bax is a pro-apoptotic protein, which promotes cell death. The significant increase in Bax expression at both 42 µg/ml and 84 µg/ml concentrations of Ursolic Acid-Zn NPs suggested that these nanoparticles may induce apoptosis in cells.

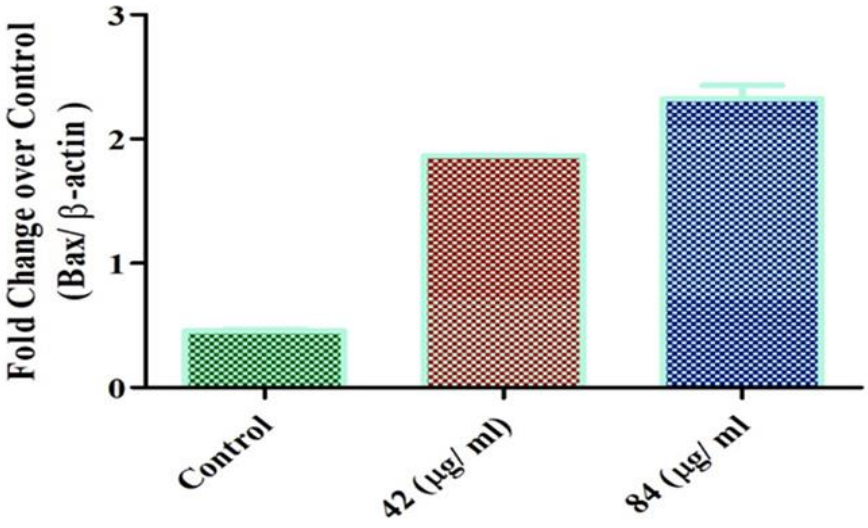

Figure 4: Ursolic Acid-ZnNPs increased Bax expression on 3T3 cells in concentration dependent manner

Nuclear Factor Kappa B (NF-κB) Expression of Ursolic Acid-Zn NPs

NF-κB is a protein complex that controled the transcription of DNA and plays a key role in regulating the immune response to infection. The decrease in NF-κB expression at both concentrations indicateed that Ursolic Acid-Zn NPs have an anti-inflammatory effect by potentially suppressing the NF-κB pathway.

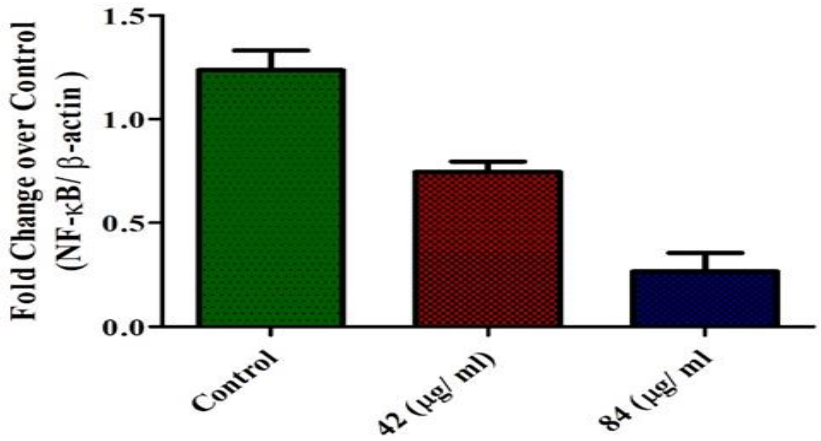

Figure 5: Ursolic Acid-ZnNPs decreased NF-κB expression on 3T3 cells in concentration dependent manner

Bcl-2 Expression of Ursolic Acid-Zn NPs

Bcl-2 is an anti-apoptotic protein that helps in cell survival. The decrease in Bcl-2 expression at both concentrations of Ursolic Acid-Zn NPs suggests that these nanoparticles promote apoptosis by downregulating anti-apoptotic pathways.

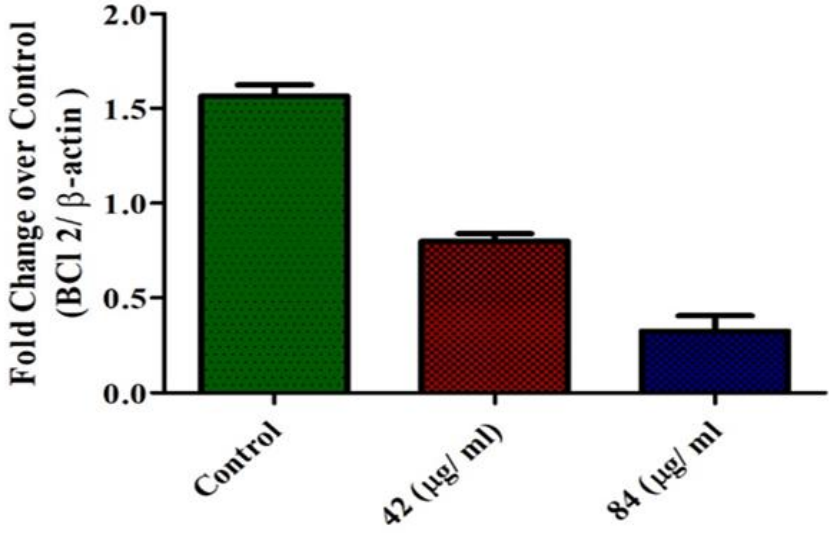

Figure 6: Ursolic Acid-ZnNPs decreased Bcl-2 expression on 3T3 cells in concentration dependent manner

TGF-α Expression of Ursolic Acid-Zn NPs

TGF-α is a cytokine involved in cell proliferation and differentiation. The increase in TGF-α expression with higher concentrations of Ursolic Acid-Zn NPs indicates that these nanoparticles may have a role in promoting cell proliferation or differentiation, which might be context-dependent based on the cell type and environment.

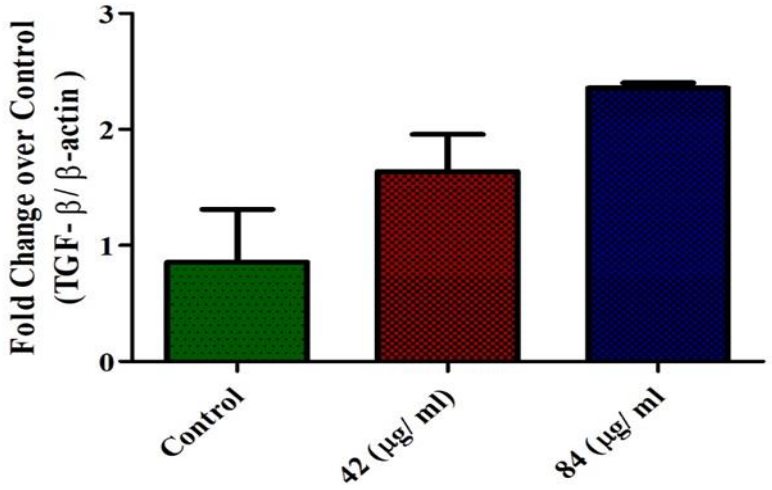

Figure 7: Ursolic Acid-ZnNPs increased TGF-α expression on 3T3 cells in concentration dependent manner

TNF-α/β Expression of Ursolic Acid-Zn NPs

TNF-α/β actin is a pro-inflammatory cytokine. The significant decrease in TNF-α expression at both concentrations of Ursolic Acid-Zn NPs suggests that these nanoparticles have anti-inflammatory properties by reducing the levels of this pro-inflammatory cytokine.

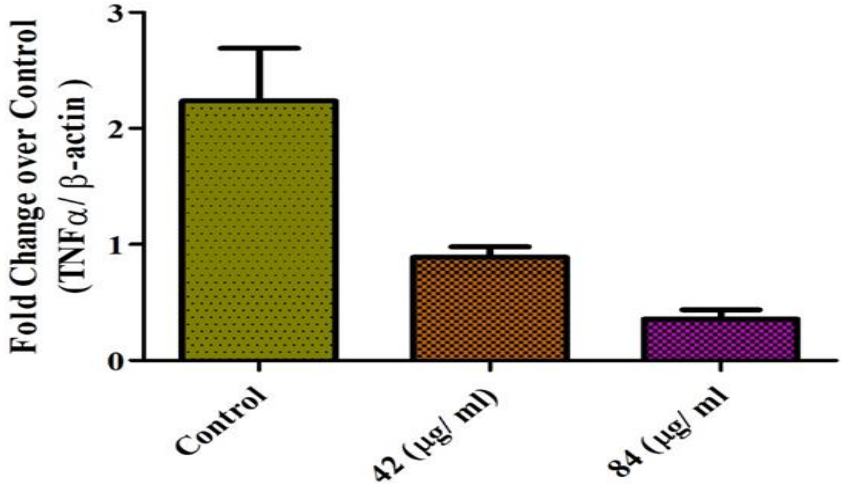

Figure 8: Ursolic Acid-ZnNPs decreased TGF-α expression on 3T3 cells in concentration dependent manner

The expression levels of various markers indicate the potential effects of Ursolic Acid-Zn NPs. Specifically, Bax expression significantly increased with higher concentrations, indicating enhanced pro-apoptotic activity. Conversely, NF-κB expression decreased, suggesting anti-inflammatory effects. The MTT assay results show a dose-dependent cytotoxic effect of Ursolic Acid-Zn NPs, though less potent compared to Doxorubicin. Additionally, the expression of the anti-apoptotic protein Bcl-2 decreased with higher concentrations of Ursolic Acid-Zn NPs, aligning with the increased Bax expression. The pro-inflammatory cytokines, TGF-α and TNF-α,

showed varying trends; while TGF-α expression increased, TNF-α expression decreased with higher concentrations, indicating complex regulatory effects on inflammatory pathways.

2.7 Molecular Docking Studies

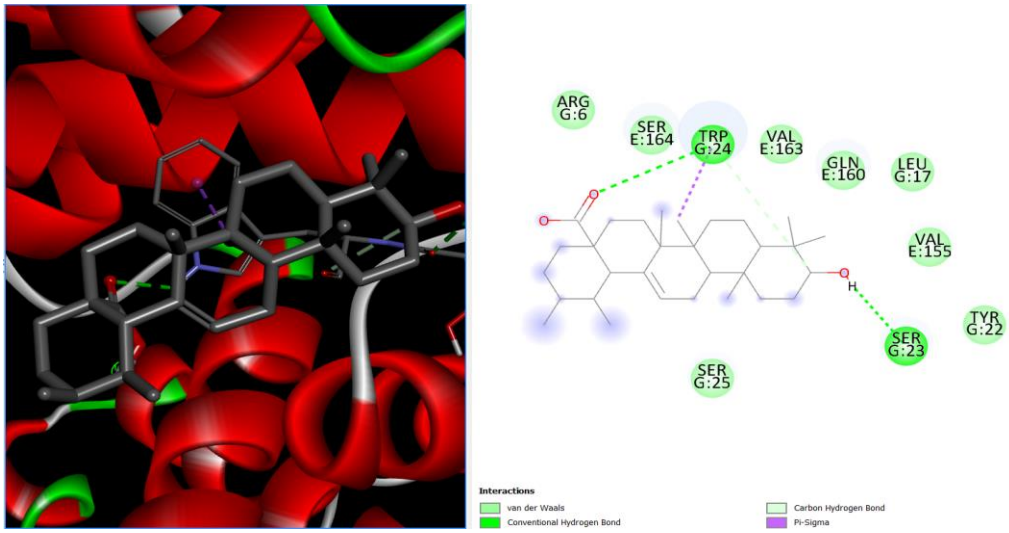

Fig 9: Binding interaction between Beclin and Ursolic Acid-ZnNPs

Molecular docking studies were performed to investigate the binding interaction between Beclin, a crucial protein in autophagy regulation, and ursolic acid, a naturally occurring triterpenoid known for its anti-inflammatory and anticancer properties. The crystal structure of Beclin (PDB ID: 2P1L) was utilized as the receptor, and the binding site was defined based on key interacting amino acids, Serine 164 (Ser164) and Tryptophan 24 (Trp24).

The docking simulation revealed that ursolic acid binds favorably within the active site of Beclin, forming stable interactions with the specified residues. The docking score indicated a significant binding affinity, suggesting a strong interaction between the ligand and the protein. Specifically, the hydroxyl groups of ursolic acid formed hydrogen bonds with the side chains of Ser164, enhancing the stability of the ligand-protein complex.

Additionally, hydrophobic interactions between the hydrophobic core of ursolic acid and the aromatic ring of Trp24 further contributed to the binding affinity.These interactions potentially influence the conformational state of Beclin, thereby impacting its role in autophagy. The binding of ursolic acid to Beclin may modulate its activity, offering insights into the mechanism by which ursolic acid exerts its therapeutic effects.

This finding provides a foundation for further experimental validation and development of ursolic acid as a potential therapeutic agent targeting autophagy pathways in cancer and inflammatory diseases. Overall, the molecular docking results highlight the promising interaction between ursolic acid and Beclin, opening avenues for future research in drug design and development(Taslimi, Erden et al. 2021).

4. DISCUSSION

Cancer therapy continues to be a significant challenge despite advances in treatment options(Zheng, Li et al. 2018). The approach of combining zinc nanoparticles (ZnNPs) with ursolic acid derivatives targeting Beclin represents a promising strategy to enhance anticancer efficacy. Ursolic acid, known for its multifaceted anticancer properties, including apoptosis induction and inhibition of cell proliferation, serves as a potent natural compound in this study(Huang, Shen et al. 2014). However, its clinical utility has been hampered by issues of poor bioavailability and lack of specificity. By conjugating ursolic acid derivatives with ZnNPs, we aimed to address these limitations, leveraging the unique physicochemical properties of nanoparticles to improve drug delivery and stability(Deeksheetha, Vadivel et al. 2020).

Autophagy, regulated by Beclin, plays a pivotal role in cancer progression. The dual nature of autophagy in cancer—acting both as a tumor suppressor and a survival mechanism—underscores the importance of targeting Beclin to induce autophagic cell death. Molecular docking facilitated the rational design of ursolic acid derivatives optimized for enhanced Beclin binding, potentially enhancing their therapeutic efficacy. Our findings in the 3T3 cell line demonstrate that ZnNP-conjugated ursolic acid derivatives not only inhibited cell proliferation more effectively than free ursolic acid but also induced higher levels of autophagic cell death, highlighting the synergistic effects of nanoparticle delivery.

The expression analysis of various molecular markers provides insights into the mechanisms underlying the anticancer effects of Ursolic Acid-Zn NPs. The significant increase in Bax expression with higher concentrations indicates enhanced pro-apoptotic activity, suggesting a mechanism by which these nanoparticles induce apoptosis in cancer cells. Concurrently, the decrease in NF-κB expression suggests potential anti-inflammatory effects, which are crucial for suppressing inflammatory pathways often dysregulated in cancer(Devi and Duraisamy 2020).

The MTT assay results demonstrated a dose-dependent cytotoxic effect of Ursolic Acid-Zn NPs, albeit less potent compared to the standard chemotherapeutic agent, Doxorubicin. This comparative efficacy highlights the potential of Ursolic Acid-Zn NPs as a viable alternative or adjunctive therapy in cancer treatment. The downregulation of the anti-apoptotic protein Bcl-2 further supports the pro-apoptotic effects observed, reinforcing the notion that Ursolic Acid-Zn NPs induce apoptosis through multiple pathways. Interestingly, the expression patterns of pro-inflammatory cytokines TGF-α and TNF-α showed distinct responses to Ursolic Acid-Zn NPs.

While TGF-α expression increased with higher concentrations, suggesting a complex regulatory effect on cell proliferation and differentiation, TNF-α expression decreased, indicating potential modulation of inflammatory responses. These findings underscore the multifaceted nature of Ursolic Acid-Zn NPs in influencing both apoptotic and inflammatory pathways in cancer cells. The integration of natural compounds like ursolic acid derivatives with nanotechnology represents a promising approach in cancer therapy. By enhancing drug delivery and bioavailability, ZnNPs not only improve the efficacy of ursolic acid derivatives but also mitigate their limitations, such as poor solubility and non-specific distribution. The molecular insights gained from this study pave the way for future research into optimizing nanoparticle formulations and exploring combination therapies that target complementary pathways in cancer biology(Farinati, Cardin et al. 2006).

5. CONCLUSION

In conclusion, this study demonstrates the potential of zinc nanoparticles conjugated with ursolic acid derivatives targeting Beclin as an effective strategy in cancer therapy. The enhanced anticancer efficacy observed in vitro, including increased apoptosis and modulation of inflammatory markers, highlights the therapeutic promise of this novel approach. Moving forward, further preclinical and clinical studies are warranted to validate these findings and optimize the therapeutic potential of Ursolic Acid-Zn NPs for clinical application in cancer treatment.

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) Anbarasu, M., et al. (2024). "Depolymerization of PET Wastes Catalysed by Tin and Silver doped Zinc oxide Nanoparticles and Evaluation of Embryonic Toxicity Using Zebrafish." Water, Air, & Soil Pollution **235**(6): 433.

3) Bahuguna, A., et al. (2017). "MTT assay to evaluate the cytotoxic potential of a drug." ||| Bangladesh Journal of Pharmacology **12**(2): 115-118.

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

5) Deeksheetha, P., et al. (2020). "Qualitative analysis of styloid process in OPGs of patients who do not have symptoms of Eagle's syndrome." Int J of Dent Oral Sci **7**: 892-897.

6) Devi, S. and R. Duraisamy (2020). "Crestal bone loss in implants Postloading and its association with age, gender, and implant site: a retrospective study." Journal of long-term effects of medical implants **30**(3).

7) Ding, Q., et al. (2015). "Natural autophagy regulators in cancer therapy: a review." Phytochemistry Reviews **14**: 137-154.

8) Eckhardt, S. (2002). "Recent progress in the development of anticancer agents." Current medicinal chemistry-anti-cancer agents **2**(3): 419-439.

9) El-Baba, C., et al. (2021). "Terpenoids' anti-cancer effects: Focus on autophagy." Apoptosis **26**(9): 491-511.

10) Farinati, F., et al. (2006). "Oxidative damage, pro-inflammatory cytokines, TGF-α and c-myc in chronic HCV-related hepatitis and cirrhosis." World Journal of Gastroenterology: WJG **12**(13): 2065.

11) Fathima, T., et al. (2020). "Decoding the genetic alterations in genes of DNMT family (DNA Methyl-Transferase) and their association with head and neck squamous cell carcinoma." Asian Pacific journal of cancer prevention: APJCP **21**(12): 3605.

12) Garrido-Laguna, I. and M. Hidalgo (2015). "Pancreatic cancer: from state-of-the-art treatments to promising novel therapies." Nature reviews Clinical oncology **12**(6): 319-334.

13) 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.

14) Girija, S. A., et al. (2018). "Prevalence of VIM-and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection." Acta microbiologica et immunologica Hungarica **65**(4): 539-550.

15) Hanne, L. S., et al. (2024). Retinal Authentication for E-Voting. 2024 IEEE 9th International Conference for Convergence in Technology (I2CT), IEEE.

16) Huang, M., et al. (2014). "Molecularly targeted cancer therapy: some lessons from the past decade." Trends in pharmacological sciences **35**(1): 41-50.

17) Karthik, E. V. G. and V. Priya (2021). "Gayathri. R, Dhanraj Ganapathy. Health Benefits Of Annona Muricata-A Review." Int J Dentistry Oral Sci **8**(7): 2965-2967.

18) Lee, N.-R., et al. (2020). "Reactive oxygen species-mediated autophagy by ursolic acid inhibits growth and metastasis of esophageal cancer cells." International Journal of Molecular Sciences **21**(24): 9409.

19) Melo, P. S., et al. (2015). "Winery by-products: Extraction optimization, phenolic composition and cytotoxic evaluation to act as a new source of scavenging of reactive oxygen species." Food Chemistry **181**: 160-169.

20) 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.

21) Peng, Y., et al. (2022). "PI3K/Akt/mTOR pathway and its role in cancer therapeutics: are we making headway?" Frontiers in oncology **12**: 819128.

22) Ponmanickam, P., et al. (2022). "Biodiversity of butterflies in Ayya Nadar Janaki Ammal College Campus, Sivakasi, Tamil Nadu, India." International Journal of Entomology Research **7**(5): 175-182.

23) Pucci, C., et al. (2019). "Innovative approaches for cancer treatment: Current perspectives and new challenges." ecancermedicalscience **13**.

24) Raj, P. S. M., et al. (2024). "Anti-psychotic Nature of Antibiotics: Vancomycin and Omadacycline Combination Ameliorating Stress in a Zebrafish Model." Cureus **16**(3).

25) Ravikumar, O., et al. (2024). "Zinc oxide nanoparticles functionalized with cinnamic acid for targeting dental pathogens receptor and modulating apoptotic genes in human oral epidermal carcinoma KB cells." Molecular Biology Reports **51**(1): 352.

26) 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.

27) Shen, Z., et al. (2018). "Emerging strategies of cancer therapy based on ferroptosis." Advanced Materials **30**(12): 1704007.

28) Siddiqi, K. S., et al. (2018). "Properties of zinc oxide nanoparticles and their activity against microbes." Nanoscale research letters **13**: 1-13.

29) Sivakumar, N., et al. (2021). "Gayathri R, Dhanraj Ganapathy. Targeted Phytotherapy For Reactive Oxygen Species Linked Oral Cancer." Int J Dentistry Oral Sci **8**(1): 1425-1429.

30) Tang, Z.-Y., et al. (2022). "Anticancer activity of oleanolic acid and its derivatives: Recent advances in evidence, target profiling and mechanisms of action." Biomedicine & Pharmacotherapy **145**: 112397.

31) Taslimi, P., et al. (2021). "The biological activities, molecular docking studies, and anticancer effects of 1-arylsuphonylpyrazole derivatives." Journal of biomolecular structure and dynamics **39**(9): 3336-3346.

32) Tayyeb, J. Z., et al. (2024). "Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells." Molecular Biology Reports **51**(1): 423.

33) USHANTHIKA, T. and K. G. MOHANRAJ (2020). "Knowledge, Awareness and Risk Factors of Smartphone Addiction in Young Adult Population." International Journal of Pharmaceutical Research (09752366) **12**(3).

34) Zhao, J., et al. (2019). "Ursolic acid exhibits anti-inflammatory effects through blocking TLR4-MyD88 pathway mediated by autophagy." Cytokine **123**: 154726.

35) Zheng, P. P., et al. (2018). "Breakthroughs in modern cancer therapy and elusive cardiotoxicity: Critical research-practice gaps, challenges, and insights." Medicinal research reviews **38**(1): 325-376.