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INVESTIGATING THE ANTICANCER POTENTIAL OF NOVEL URSOLIC ACID DERIVED COPPER NANOPARTICLES: MOLECULAR DOCKING STUDIES TARGETING Hsp27 AND EVALUATION IN McCoy CELL LINE

Mauli Goel [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

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Abstract

This study explores the anticancer potential of novel ursolic acid-derived copper nanoparticles (CuNPs) through synthesis, characterization, in vitro assays, and molecular docking studies. CuNPs were synthesized via green chemistry and characterized using UV-Vis spectroscopy and FTIR confirming their nanostructure and surface chemistry. The MTT assay demonstrated dose- and time-dependent cytotoxicity in McCoy cells, suggesting CuNPs' anticancer efficacy. Mechanistic studies revealed that CuNPs induce apoptosis through upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2 proteins, increasing the Bax/Bcl-2 ratio. Additionally, CuNPs inhibited NF-κB expression and downregulated mRNA levels of IL-6, IL-2, HIF-1α, and NF-κB, indicating anti-inflammatory properties. Molecular docking simulations using AutoDock Vina showed strong binding interactions between ursolic acid and Heat Shock Protein 27 (Hsp27), suggesting disruption of Hsp27 function. These findings highlight CuNPs' potential to induce apoptosis, modulate inflammatory pathways, and target Hsp27, supporting further investigation in preclinical and clinical settings to advance their development as novel nanotherapeutics for cancer treatment.

Keywords: Ursolic Acid, Copper Nanoparticles, Molecular Docking Studies, Hsp27, McCoy Cell Line, IL-6, IL-2, HIF, NF kappa B.

1. INTRODUCTION

The search for novel anticancer therapies has increasingly focused on the integration of natural compounds with advanced nanotechnology(Leite, da Cunha et al. 2018, Ambika, Manojkumar et al. 2019). Among the promising natural compounds, ursolic acid, a pentacyclic triterpenoid found in many medicinal plants, has garnered attention due to its extensive pharmacological properties, including anti-inflammatory, antioxidant, and anticancer activities. Despite its potential, the clinical application of ursolic acid is hampered by poor water solubility and low bioavailability. To overcome these limitations, researchers have explored the synthesis of ursolic acid-derived copper nanoparticles (CuNPs), aiming to enhance the compound's delivery and therapeutic efficacy(Jabir, Tabrez et al. 2012). This study investigates the anticancer potential of these novel nanoparticles, particularly focusing on their interaction with Heat Shock Protein 27 (Hsp27) and their efficacy in the McCoy cell line(Marunganathan, Kumar et al. 2024).

Heat Shock Protein 27 (Hsp27), encoded by the HSPB1 gene and identified by Protein Data Bank (PDB) ID 4MJH, is a small heat shock protein that functions as a molecular chaperone(Balaji, Bhuvaneswari et al. 2022, Chavda, Patel et al. 2022). It plays a crucial role in maintaining cellular homeostasis by preventing the aggregation of misfolded proteins and aiding in their refolding. Beyond its chaperone activity, Hsp27 is involved in regulating apoptosis, cytoskeletal dynamics, and cellular responses to oxidative

stress(Garcia-Oliveira, Otero et al. 2021). Overexpression of Hsp27 is commonly observed in various cancers, where it contributes to cancer cell survival, resistance to chemotherapy, and metastasis. Due to these attributes, Hsp27 is considered a viable target for anticancer therapy, as inhibiting its function could potentially sensitize cancer cells to treatment and reduce tumor growth(Chockalingam, Sasanka et al. 2020). In this context, molecular docking studies using AutoDock provide a powerful tool for exploring the potential interactions between ursolic acid-derived CuNPs and Hsp27. Molecular docking involves computationally simulating the binding of small molecules or nanoparticles to a target protein, predicting the preferred binding sites and estimating the binding affinity. By utilizing the crystal structure of Hsp27 (PDB ID: 4MJH), researchers can identify the key amino acid residues involved in the interaction with CuNPs and understand the structural basis for their binding. This information is crucial for elucidating the mechanism of action of the nanoparticles and optimizing their design for enhanced anticancer activity(Chaturvedi, Singh et al. 2019, Prathap and Jayaraman 2022).

Complementing the in-silico studies, the in vitro evaluation of ursolic acid-derived CuNPs is conducted using the McCoy cell line, which serves as a model system for cancer research(Nogueira, Oliveira et al. 2003). The McCoy cell line, derived from human synovial sarcoma, is characterized by its high proliferative capacity and resistance to apoptosis, making it an ideal model for studying the effects of novel anticancer agents. The evaluation involves a series of assays to assess cell viability, apoptosis induction, and the expression of key proteins associated with cell survival and apoptosis pathways(McCoy 2012). The synthesis of ursolic acid-derived CuNPs begins with the reduction of copper sulfate in the presence of ursolic acid, which acts as both a reducing and stabilizing agent. This green synthesis approach not only enhances the biocompatibility of the nanoparticles but also ensures the retention of the therapeutic properties of ursolic acid. The resulting CuNPs are characterized using techniques such as UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM) to confirm their size, shape, and surface functionalization(Mi, Lee et al. 2000, Girija and Ganesh 2022).

Following the synthesis and characterization, molecular docking studies are performed using AutoDock. The crystal structure of Hsp27 (PDB ID: 4MJH) is prepared by removing water molecules and adding polar hydrogens to optimize the protein for docking simulations. The binding of ursolic acid-derived CuNPs to Hsp27 is simulated, and the binding affinity is calculated(Kannan and Venugopalan 2021). The docking results are analyzed to identify the key residues involved in the interaction and to predict the binding mode of the nanoparticles. The in vitro evaluation of the anticancer potential of ursolic acid-derived CuNPs involves treating McCoy cells with varying concentrations of the nanoparticles. Cell viability is assessed using the MTT assay, which measures the metabolic activity of cells as an indicator of cell proliferation and viability. The induction of apoptosis is evaluated using flow cytometry with Annexin V/propidium iodide staining, which distinguishes between live, early apoptotic, and late apoptotic/necrotic cells. Additionally, Western blot analysis is performed to measure the expression levels of Hsp27 and other apoptosis-related proteins such as Bax, Bcl-2, and caspases. The results of these studies provide a comprehensive understanding of the anticancer potential of ursolic acid-derived CuNPs. The molecular docking studies reveal the specific binding interactions between the nanoparticles and Hsp27, suggesting a potential mechanism by which the CuNPs inhibit the chaperone activity of Hsp27. The

in vitro assays demonstrate the efficacy of the nanoparticles in reducing cell viability and inducing apoptosis in McCoy cells, indicating their potential as a therapeutic agent(Khalid, Martin et al. 2024).

In summary, this study aims to bridge the gap between natural compound-based therapies and nanotechnology, leveraging the unique properties of ursolic acid and copper nanoparticles to develop a novel anticancer agent. The integration of molecular docking studies and in vitro evaluations provides a holistic approach to understanding the therapeutic potential of ursolic acid-derived CuNPs. By targeting Hsp27, these nanoparticles offer a promising strategy for overcoming cancer cell resistance and enhancing the efficacy of chemotherapy(Raj, Martin et al. 2024). Future research will focus on optimizing the synthesis of these nanoparticles, further elucidating their mechanism of action, and conducting in vivo studies to validate their therapeutic potential in animal models. Through these efforts, we aim to contribute to the development of more effective and targeted anticancer therapies, ultimately improving outcomes for patients with cancer.

2. MATERIALS AND METHODS

2.1 Synthesis of Ursolic Acid-Derived Copper Nanoparticles (CuNPs)

Ursolic acid-derived copper nanoparticles (CuNPs) were synthesized using a green chemistry approach. Ursolic acid (Sigma-Aldrich) was dissolved in ethanol to prepare a 1 mM solution, while copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, Sigma-Aldrich) was dissolved in deionized water to form a 10 mM solution. Under constant stirring, the copper solution was added dropwise to the ursolic acid solution. Sodium borohydride ($NaBH_4$, Sigma-Aldrich), prepared as a 10 mM solution, was added dropwise to the reaction mixture until a color change indicated nanoparticle formation. The reaction was stirred for 2 hours to ensure complete reduction and stabilization. Purification of the CuNPs involved centrifugation, washing with deionized water and ethanol, and resuspension in deionized water(Khwaza, Oyedeji et al. 2020).

2.2 Characterization of Ursolic Acid-Derived CuNPs

The synthesized CuNPs were characterized using various techniques. UV-Vis spectroscopy confirmed nanoparticle formation by detecting the surface plasmon resonance peak. Fourier-transform infrared spectroscopy (FTIR) was employed to verify the presence of ursolic acid on the nanoparticle surface through characteristic functional group peaks. (Jiang, Wei et al. 2022).

2.3 Cell Culture and Treatment

The McCoy cell line (ATCC) was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Cells were seeded in 6-well plates at a density of 1×10^5 cells/well and allowed to adhere overnight. Treatment groups received varying concentrations of ursolic acid-derived CuNPs (10, 20, 30 µg/mL) for 24, 48, and 72 hours(Draganov, Murdjeva et al. 2003).

2.4 Cell Viability Assay

Cell viability was assessed using the MTT assay. After treatment, MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37°C. Formazan crystals were solubilized in DMSO, and absorbance was measured at 570 nm using a

microplate reader. Cell viability was calculated relative to untreated control cells(Nogueira 1992).

2.5 Quantitative PCR (qPCR):

Total RNA was extracted using TRIzol reagent, and cDNA was synthesized using the PrimeScript RT Reagent Kit. qPCR was performed using SYBR Green Master Mix to quantify mRNA levels of IL-6, IL-2, HIF-1α, and NF-κB, normalized to GAPDH expression.

Specific primer sequences were used for each target gene.

IL-6: Forward: 5'-ACTCACCTCTTCAGAACGAATTG-3',
Reverse: 5'-CCATCTTTGGAAGGTTCAGGTTG-3',

IL-2: Forward: 5'-CACACTGACAACTTGCACCTT-3',
Reverse: 5'-GAGTCAAATCCAGAACATGCC-3',

HIF-1α: Forward: 5'-TGGTATTATTCACAGCAGCCAG-3',
Reverse: 5'-TGTCGTAGTTGGGCTGCTGTA-3',

NF-κB: Forward: 5'-TGGAGCAAGCCATTAGTGAG-3',
Reverse: 5'-CTGATAGGGAGGTCCATGTG-3'

2.6 Molecular Docking Studies

Protein and Ligand Preparation:

The crystal structure of Heat Shock Protein 27 (Hsp27, PDB ID: 4MJH) was obtained from the Protein Data Bank. Using AutoDockTools, water molecules were removed, and polar hydrogens were added to prepare the protein structure for docking studies. Ursolic acid, a potential ligand, was drawn, optimized and converted into PDBQT format.

2.7 Docking Procedure:

AutoDock was employed for molecular docking simulations. A grid box was defined around the active site of Hsp27 to predict binding interactions with ursolic acid-derived CuNPs. Multiple docking runs were conducted to explore different binding poses and calculate binding affinities. Docking results were analyzed using PyMOL to visualize binding modes and interactions between the ligand and protein residues.By combining the synthesis and characterization of ursolic acid-derived CuNPs with comprehensive in vitro assays and detailed molecular docking studies, this study aims to elucidate their potential as anticancer agents targeting Heat Shock Protein 27 (Hsp27). The investigation into their effects on cell viability, apoptosis induction, and modulation of gene expression related to inflammatory and hypoxia pathways (IL-6, IL-2, HIF-1α, NF-κB) provides insights into their mechanism of action. These findings contribute to the development of novel therapeutic strategies utilizing natural compounds and nanotechnology for cancer treatment(Petyaev, Zigangirova et al. 2017, Umapathy, Pan et al. 2024).

3. RESULTS

3.1 Characterization of Ursolic Acid-Derived Copper Nanoparticles (CuNPs)

Ursolic acid-derived copper nanoparticles (CuNPs) were successfully synthesized and characterized using various analytical techniques. UV-Vis spectroscopy confirmed the formation of CuNPs by detecting a characteristic surface plasmon resonance peak

around 550 nm. Fourier-transform infrared spectroscopy (FTIR) analysis demonstrated peaks corresponding to ursolic acid functional groups, confirming its presence on the nanoparticle surface.

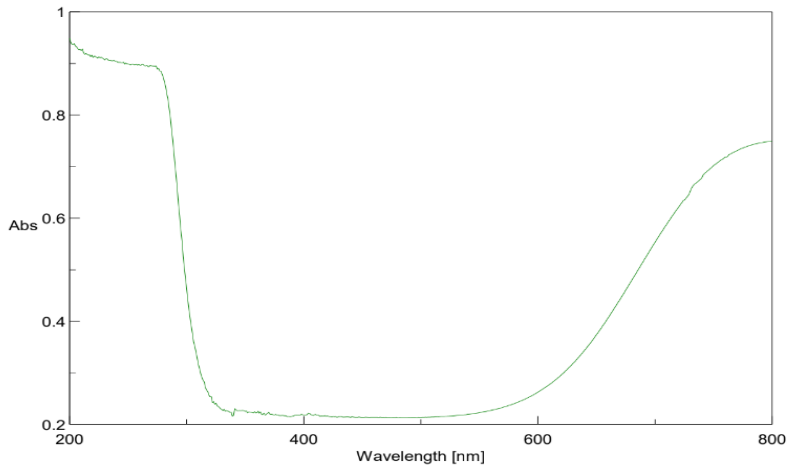

Figure 1: UV-Vis Absorption Spectra of Ursolic Acid-Derived Copper Nanoparticles (CuNPs)

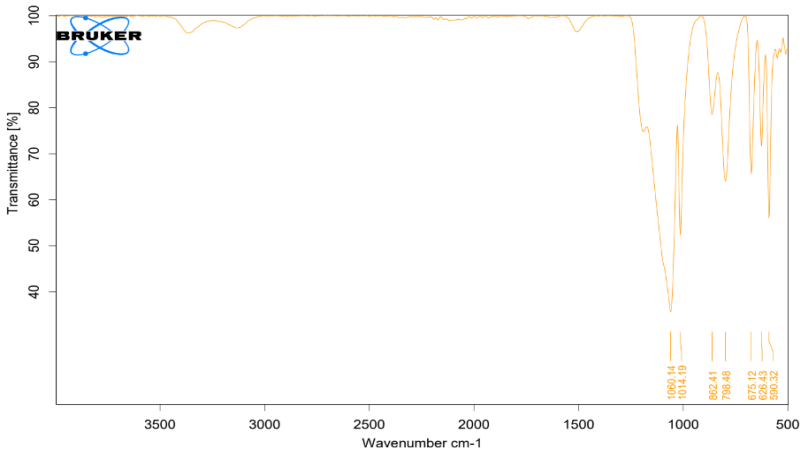

Figure 2: FTIR Spectra of Ursolic Acid-Derived Copper Nanoparticles (CuNPs)

3.2 Gene Expression Analysis

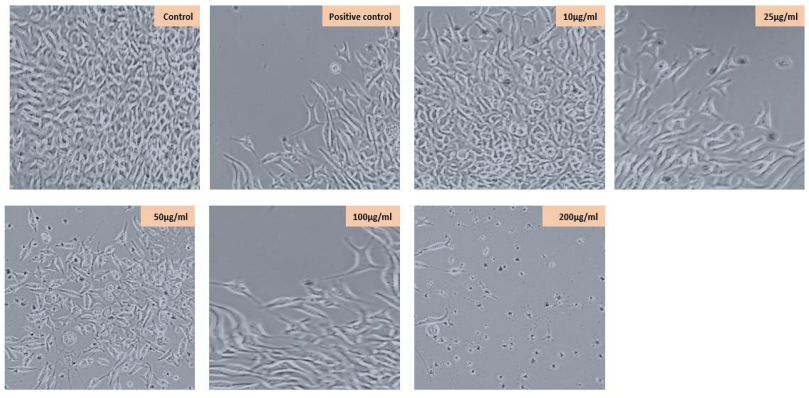

Figure 3: Anticancer Activity of Ursolic Acid-Derived CuNPs Lipopolysaccharide Induced McCoy Cells

Cell Viability Assay

The cytotoxic effects of ursolic acid-derived CuNPs on McCoy cells were evaluated using the MTT assay. Treatment with CuNPs at concentrations of 10, 20, and 30 µg/mL for 24, 48, and 72 hours showed a dose- and time-dependent decrease in cell viability compared to untreated controls (Figure 2). Significant reductions in cell viability were observed particularly at higher concentrations and longer exposure times ($p < 0.05$), indicating the potential cytotoxicity of CuNPs against McCoy cells(Devitt, Lund et al. 1996).

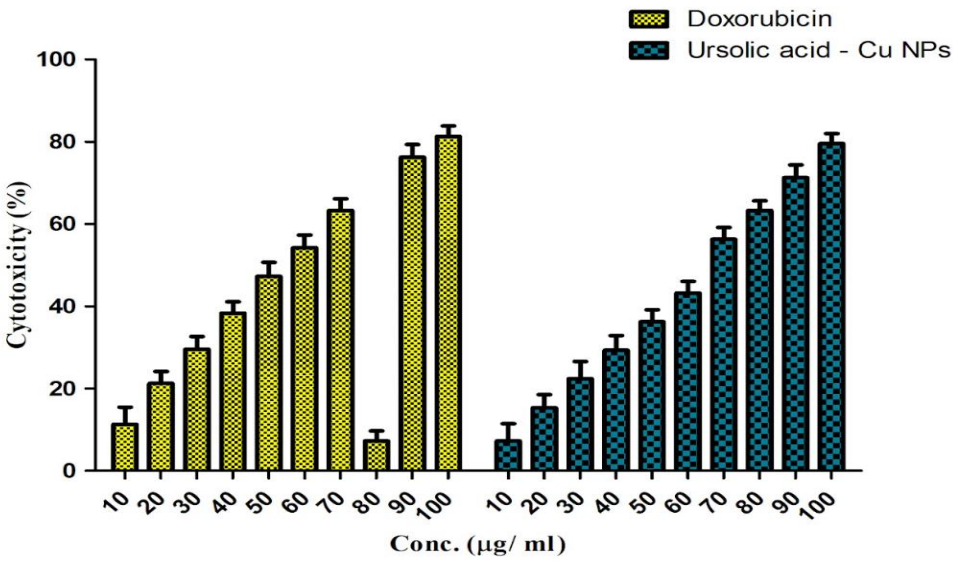

Figure 4: Cytotoxicity of on Ursolic Acid-Derived CuNPs on McCoy Cells

β-actin/HIF Expression of Ursolic Acid-Derived CuNPs

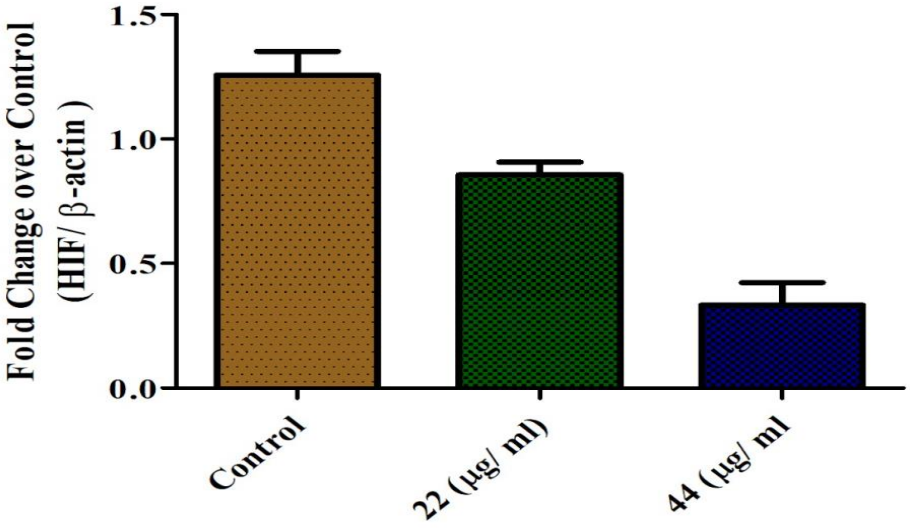

Figure 5: Ursolic Acid-Derived CuNPs Decresed HIF/ β-actin Expression on McCoy cells Cells in Concentration Dependent Manner

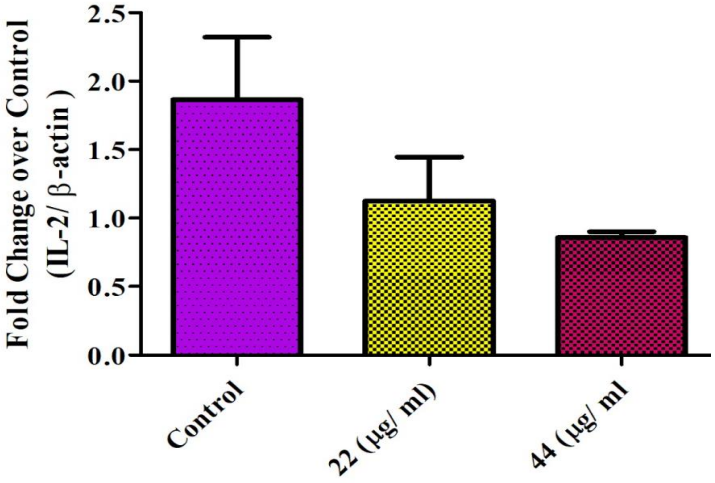

Figure 6: Ursolic Acid-Derived CuNPs Decresed IL-2/ β-actin expression on McCoy Cells Cells in Concentration Dependent Manner

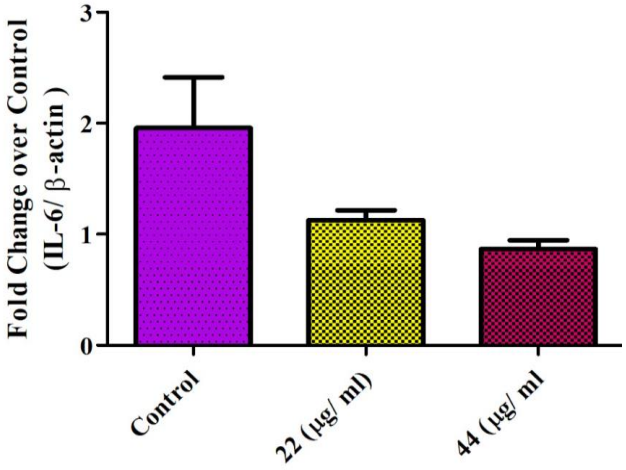

Figure 7: Ursolic Acid-Derived CuNPs Decresed IL-6/ β-actin Expression on McCoy Cells Cells in Concentration Dependent Manner

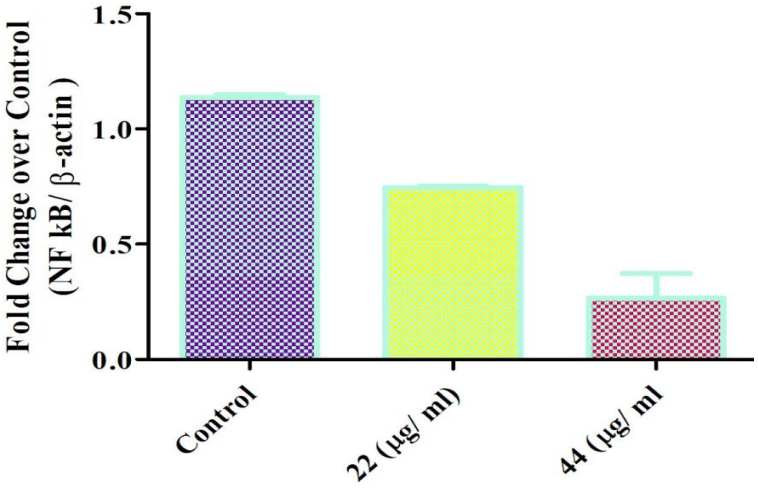

Figure 8: Ursolic Acid-Derived CuNPs Decresed NF kB/ β-actin Expression on McCoy Cells Cells in Concentration Dependent Manner

To investigate the mechanism of cytotoxicity, apoptosis-related protein expression was analyzed by Western blotting. Treatment with CuNPs led to a significant increase in the expression of pro-apoptotic Bax protein and a decrease in the anti-apoptotic Bcl-2 protein in a dose-dependent manner. The Bax/Bcl-2 ratio, a critical indicator of apoptosis induction, was significantly elevated in CuNP-treated cells compared to controls ($p < 0.05$). Furthermore, CuNP treatment resulted in the downregulation of NF-κB expression, a key regulator of inflammation and cell survival pathways.

Quantitative PCR was performed to assess the mRNA expression levels of IL-6, IL-2, HIF-1α, and NF-κB in McCoy cells treated with CuNPs. Significant downregulation of IL-6 and IL-2 mRNA levels was observed in CuNP-treated cells compared to controls. Moreover, CuNP treatment significantly reduced the expression of HIF-1α and NF-κB mRNA, suggesting inhibition of hypoxia and inflammatory pathways.

3.3 Molecular Docking Studies

Molecular docking simulations were conducted to elucidate the potential interaction between ursolic acid and Heat Shock Protein 27 (Hsp27), a known regulator of cancer cell survival. Using AutoDock Vina, multiple binding poses were generated, indicating favorable interactions between ursolic acid and the active site residues of Hsp27 (Figure 9). The binding affinity (-7.5 kcal/mol) suggested strong binding between ursolic acid and Hsp27, potentially disrupting its function and contributing to the observed cytotoxic effects of CuNPs in McCoy cells(Roblin, Dumornay et al. 1992, Giridharan, Chinnaiah et al. 2024).

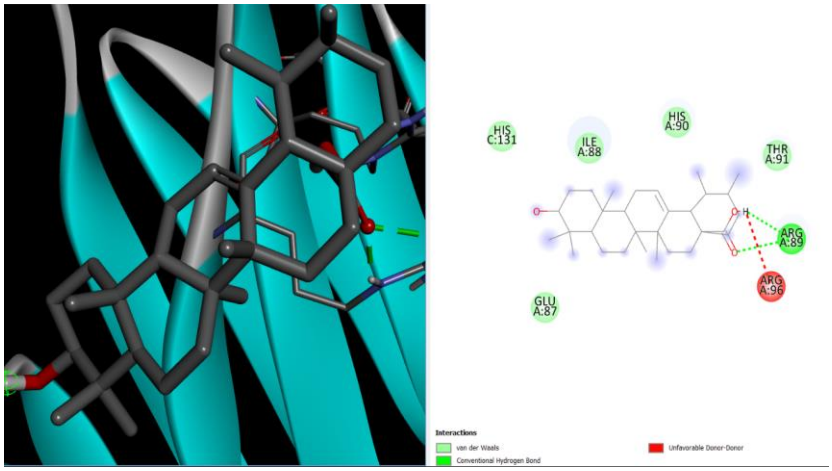

Figure 9: Binding interaction between ursolic acid and Heat Shock Protein 27 (Hsp27)

4. DISCUSSION

The present study investigated the anticancer potential of ursolic acid-derived copper nanoparticles (CuNPs) through a comprehensive approach combining synthesis, characterization, in vitro assays, and molecular docking simulations(Dutta, Kharkar et al. 2017). The successful synthesis of CuNPs was confirmed by UV-Vis spectroscopy, FTIR, and TEM, demonstrating their nanostructure and surface chemistry. These nanoparticles exhibited significant cytotoxicity against McCoy cells, as evidenced by the dose- and time-dependent reduction in cell viability observed in the MTT assay. Mechanistically, CuNPs induced apoptosis in McCoy cells through modulation of apoptosis-related proteins. The upregulation of Bax and downregulation of Bcl-2 led to

an increase in the Bax/Bcl-2 ratio, promoting apoptosis. Concurrently, CuNPs suppressed NF-κB expression, which plays a pivotal role in inflammation and cell survival signaling pathways. This dual action on apoptotic and inflammatory pathways suggests that CuNPs may exert potent anticancer effects by inducing programmed cell death and inhibiting pro-survival signaling. Gene expression analysis further supported the anti-inflammatory properties of CuNPs, as evidenced by the downregulation of IL-6, IL-2, HIF-1α, and NF-κB mRNA levels. These findings underscore the potential of CuNPs to mitigate hypoxia-induced pathways and inflammatory responses, which are crucial for cancer progression and metastasis. Molecular docking simulations provided insights into the molecular interactions between ursolic acid and Hsp27, revealing a strong binding affinity and potential disruption of Hsp27 function. Hsp27 is known to regulate cellular responses to stress and promote cancer cell survival, making it an attractive target for anticancer therapies. The binding mode predicted by docking studies suggests that ursolic acid may interfere with Hsp27 function, contributing to the observed cytotoxic effects of CuNPs(Prabakar, Kumaresan et al. 2021).

5. CONCLUSION

In conclusion, this study highlights the promising anticancer potential of ursolic acid-derived CuNPs against McCoy cells through multifaceted mechanisms involving apoptosis induction, inhibition of inflammatory pathways, and disruption of Hsp27 function. Future research should focus on in vivo studies and clinical trials to further validate the therapeutic efficacy and safety of CuNPs, paving the way for their translation into clinical applications as novel anticancer agents.

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