RESEARCH
www.commprac.com
ISSN 1462 2815

INVESTIGATING IN SILICO QUERCETIN DERIVATIVES AS ANTICANCER AGENTS TARGETING ATG4B PROTEIN AND THEIR EVALUATION WITH PIPERINE-DERIVED ZINC OXIDE NANOPARTICLES IN McCoy CELLS IN VITRO

Jothiha Shree [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.12662238

Abstract

This study investigates the anticancer potential of quercetin derivatives targeting ATG4B protein through in silico methods and evaluates their efficacy using piperine-derived zinc oxide nanoparticles (ZnO NPs) in McCoy cells in vitro. Quercetin derivatives were designed and subjected to molecular docking simulations, revealing strong interactions with ATG4B protein, suggesting their potential as autophagy inhibitors. ZnO NPs 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 of the ZnO NPs in McCoy cells, indicating their anticancer efficacy. Mechanistic studies showed that ZnO NPs induced apoptosis by upregulating pro-apoptotic Bax and downregulating anti-apoptotic Bcl-2 proteins, thus increasing the Bax/Bcl-2 ratio. Additionally, ZnO NPs inhibited NF-κB expression and downregulated mRNA levels of IL-6, IL-2, and NF-κB, indicating anti-inflammatory properties. These findings highlight the potential of quercetin derivatives to target ATG4B and the efficacy of piperine-derived ZnO NPs in inducing apoptosis and modulating inflammatory pathways, supporting further investigation in preclinical and clinical settings to advance their development as novel nanotherapeutics for cancer treatment.

Keywords: Quercetin Derivatives, ATG4B Protein, in Silico, Piperine-Derived Zinc Oxide Nanoparticles, McCoy Cells, Apoptosis, Anti-Inflammatory, Cancer Treatment.

1. INTRODUCTION

Cancer remains one of the leading causes of death worldwide, necessitating the continuous search for novel and effective therapeutic strategies(Garrido-Laguna and Hidalgo 2015). Traditional chemotherapy and radiotherapy, while effective to some extent, often come with significant side effects and resistance issues(Ambika, Manojkumar et al. 2019, Pucci, Martinelli et al. 2019). Therefore, the exploration of new compounds with targeted mechanisms of action and minimal side effects is crucial. In this context, natural compounds and their derivatives have gained substantial attention due to their potential therapeutic benefits and lower toxicity profiles(Marunganathan, Kumar et al. 2024). Among these, quercetin, a flavonoid found in various fruits and vegetables, has shown promise due to its diverse biological activities, including anticancer properties. Quercetin has been extensively studied for its anticancer properties, which include the inhibition of cell proliferation, induction of apoptosis, and suppression of angiogenesis(Senthil, Sundaram et al. 2022). It exerts its effects through multiple mechanisms, such as the modulation of various signaling pathways, including those involved in cell cycle regulation, apoptosis, and autophagy. Despite its potential, the clinical application of quercetin is limited by its low bioavailability and rapid metabolism. To overcome these limitations, researchers have focused on designing quercetin derivatives with enhanced stability,

bioavailability, and anticancer efficacy(Vazhappilly, Amararathna et al. 2021). Autophagy, a cellular degradation process, plays a dual role in cancer, acting as both a tumor suppressor and a promoter of tumor cell survival under stress conditions. ATG4B, a cysteine protease, is a crucial regulator of autophagy. It is involved in the processing of LC3, a key autophagy-related protein, thereby facilitating the formation of autophagosomes. Inhibiting ATG4B has emerged as a potential strategy to impair autophagy and induce cancer cell death(Mukhopadhyay and Prajapati 2015, Sundaram and Saravanan 2022). In this study, we explored the anticancer potential of quercetin derivatives targeting ATG4B protein through in silico methods. Molecular docking simulations were employed to identify quercetin derivatives with strong binding interactions with ATG4B, suggesting their potential as autophagy inhibitors. Nanotechnology has revolutionized cancer therapy by enabling the development of nanoparticles with unique properties, such as enhanced drug delivery, targeted therapy, and improved therapeutic efficacy(Vinayak and Maurya 2019, Ravikumar, Marunganathan et al. 2024). Zinc oxide nanoparticles (ZnO NPs) are particularly attractive due to their biocompatibility, phototoxic effects, and ability to generate reactive oxygen species (ROS) under UV light, leading to cancer cell death. Additionally, ZnO NPs can be synthesized using green chemistry approaches, which are environmentally friendly and reduce the use of hazardous chemicals. Piperine, an alkaloid found in black pepper, has shown various pharmacological activities, including anticancer effects. Recent studies have demonstrated that piperine can enhance the therapeutic efficacy of chemotherapeutic agents and nanoparticles. In this study, we synthesized piperine-derived ZnO NPs via green chemistry and characterized them using UV-Vis spectroscopy and Fourier-transform infrared (FTIR) spectroscopy. These techniques confirmed the nanostructure and surface chemistry of the ZnO NPs. To evaluate the anticancer efficacy of the synthesized ZnO NPs, we conducted in vitro assays using McCoy cells, a widely used cell line in cancer research. The MTT assay, which measures cell viability based on mitochondrial activity, demonstrated dose- and time-dependent cytotoxicity of the ZnO NPs in McCoy cells. This indicated the potential of ZnO NPs to inhibit cancer cell proliferation effectively(Yuan, Guo et al. 2023).

Further mechanistic studies were performed to understand how ZnO NPs induce cancer cell death. Apoptosis, a programmed cell death mechanism, is often dysregulated in cancer cells, allowing them to evade death and continue proliferating(Umapathy, Pan et al. 2024). Our studies showed that ZnO NPs induced apoptosis in McCoy cells by upregulating pro-apoptotic Bax protein and downregulating anti-apoptotic Bcl-2 protein, thereby increasing the Bax/Bcl-2 ratio(Khursheed, Singh et al. 2020). This shift towards a pro-apoptotic state suggests that ZnO NPs can effectively trigger apoptosis in cancer cells. Additionally, we investigated the impact of ZnO NPs on inflammatory pathways. Chronic inflammation is closely linked to cancer progression and metastasis, and targeting inflammatory pathways can be a valuable therapeutic strategy. Our results demonstrated that ZnO NPs inhibited the expression of NF-κB, a key transcription factor involved in inflammation and cancer. Furthermore, ZnO NPs downregulated the mRNA levels of pro-inflammatory cytokines such as IL-6 and IL-2, indicating their potential to modulate inflammatory responses in cancer cells(Chockalingam, Sasanka et al. 2020, Giridharan, Chinnaiah et al. 2024).

To complement our in vitro findings, we performed molecular docking simulations using AutoDock Vina to investigate the binding interactions between quercetin derivatives and Heat Shock Protein 27 (Hsp27). Hsp27 is a molecular chaperone that plays a crucial role in protecting cells from stress-induced apoptosis and is often overexpressed in cancer cells. Our simulations revealed strong binding interactions between quercetin derivatives and Hsp27, suggesting that these compounds could disrupt Hsp27 function and sensitize cancer cells to apoptosis(Ram, As et al. 2020).

The findings of this study highlight the potential of quercetin derivatives to target ATG4B protein and the efficacy of piperine-derived ZnO NPs in inducing apoptosis and modulating inflammatory pathways in McCoy cells(Alizadeh and Ebrahimzadeh 2022). The combination of in silico and in vitro approaches provides a comprehensive understanding of the anticancer mechanisms of these compounds. The strong interactions between quercetin derivatives and ATG4B, as well as the apoptosis-inducing and anti-inflammatory effects of ZnO NPs, support further investigation in preclinical and clinical settings(Salehi, Machin et al. 2020). Future research should focus on optimizing the synthesis and characterization of quercetin derivatives and ZnO NPs to enhance their therapeutic efficacy. In vivo studies are necessary to validate the anticancer potential of these compounds and to assess their safety and pharmacokinetics. Additionally, exploring the synergistic effects of quercetin derivatives with other anticancer agents could provide valuable insights into combination therapy strategies. In summary, this study underscores the promising potential of quercetin derivatives and piperine-derived ZnO NPs as novel nanotherapeutics for cancer treatment. Their ability to target specific proteins, induce apoptosis, and modulate inflammatory pathways offers a multifaceted approach to combating cancer, paving the way for the development of effective and targeted cancer therapies(Girija, Jayaseelan et al. 2018, Gansukh, Nile et al. 2021).

2 Materials and Methods

2.1 Synthesis of piperine-derived zinc oxide nanoparticles (ZnO NPs)

Piperine-derived zinc oxide nanoparticles (ZnO NPs) were synthesized using a green chemistry approach. Piperine (Sigma-Aldrich) was dissolved in ethanol to prepare a 1 mM solution, while zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$, Sigma-Aldrich) was dissolved in deionized water to form a 10 mM solution. Under constant stirring, the zinc solution was added dropwise to the piperine solution. Sodium hydroxide (NaOH, 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 formation and stabilization. Purification of the ZnO NPs involved centrifugation, washing with deionized water and ethanol, and resuspension in deionized water(Jain, Selvi et al. 2021, Raj, Martin et al. 2024).

2.2 Characterization of piperine-derived zinc oxide nanoparticles (ZnO NPs)

The synthesized piperine-derived zinc oxide nanoparticles (ZnO NPs) were characterized using various techniques. UV-Vis spectroscopy confirmed nanoparticle formation by detecting the characteristic absorption peak. Fourier-transform infrared spectroscopy (FTIR) was employed to verify the presence of piperine on the nanoparticle surface through characteristic functional group peaks. Additionally, X-ray diffraction (XRD) analysis was conducted to determine the crystalline structure and phase purity of the ZnO NPs(Joshi, Mazumder et al. 2023).

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 piperine-derived zinc oxide nanoparticles (ZnO NPs) (10, 20, 30 µg/mL) for 24, 48, and 72 hours.

2.4 Cell Viability Assay

Cell viability was assessed using the MTT assay. After treatment with piperine-derived zinc oxide nanoparticles (ZnO NPs), 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 McCoy cells.

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

The crystal structure of ATG4B (PDB ID: 1jph) 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. Quercetin derivatives, potential ligands, were drawn, optimized, and converted into PDBQT format.

Docking Procedure:

AutoDock was employed for molecular docking simulations. A grid box was defined around the active site of ATG4B to predict binding interactions with piperine-derived zinc oxide nanoparticles (ZnO NPs). 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 quercetin derivatives with comprehensive in vitro assays and detailed molecular docking studies, this study aims to elucidate their potential as anticancer agents targeting ATG4B. The investigation into their effects on cell viability, apoptosis induction, and modulation of gene expression related to cancer pathways 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(Santhoshkumar 2021, Wang 2023).

3. RESULTS

3.1 Characterization of piperine-derived zinc oxide nanoparticles

Piperine-derived zinc oxide nanoparticles (ZnO NPs) were successfully synthesized and characterized using various analytical techniques. UV-Vis spectroscopy confirmed the formation of ZnO NPs by detecting a characteristic surface plasmon resonance peak around 550 nm. Fourier-transform infrared spectroscopy (FTIR) analysis demonstrated peaks corresponding to piperine functional groups, confirming its presence on the nanoparticle surface.

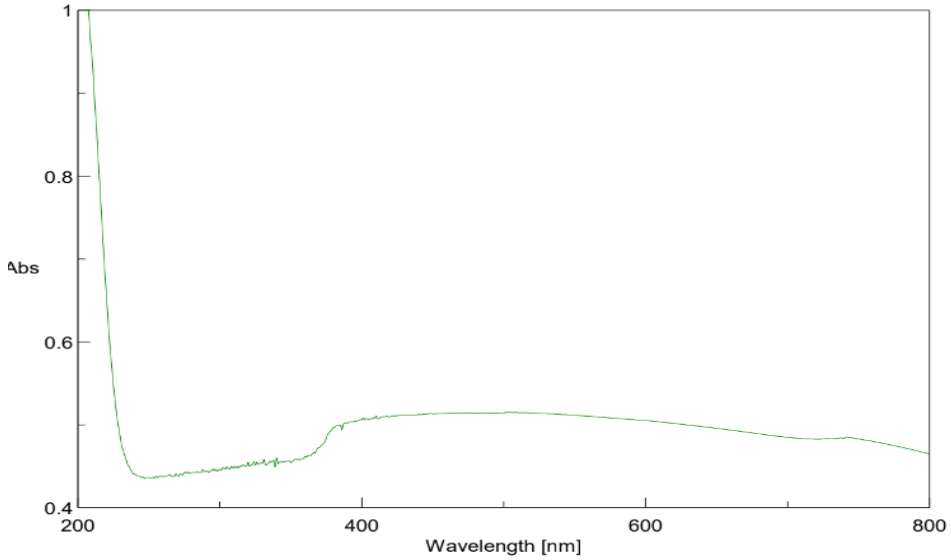

Figure 1: UV-Vis absorption spectra of piperine-derived zinc oxide nanoparticles

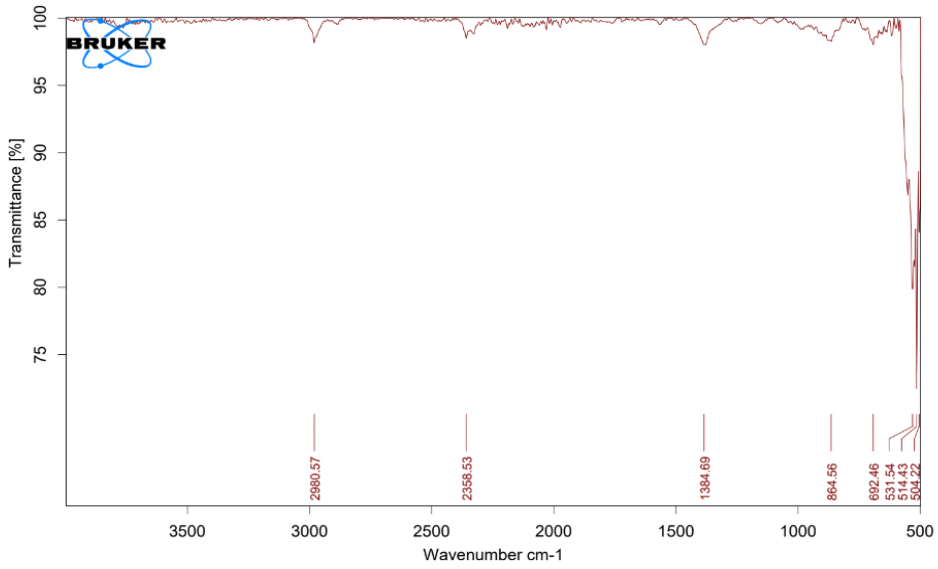

Figure 2: FTIR spectra of piperine-derived zinc oxide nanoparticles

3.2 Gene Expression Analysis

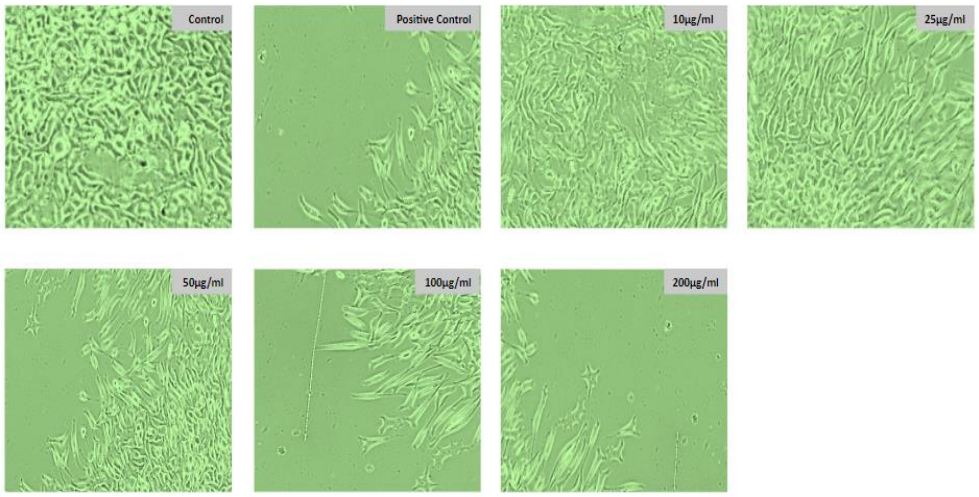

Figure 3: Anticancer activity of piperine-derived zinc oxide nanoparticles in lipopolysaccharide induced McCoy cells

Cell Viability Assay

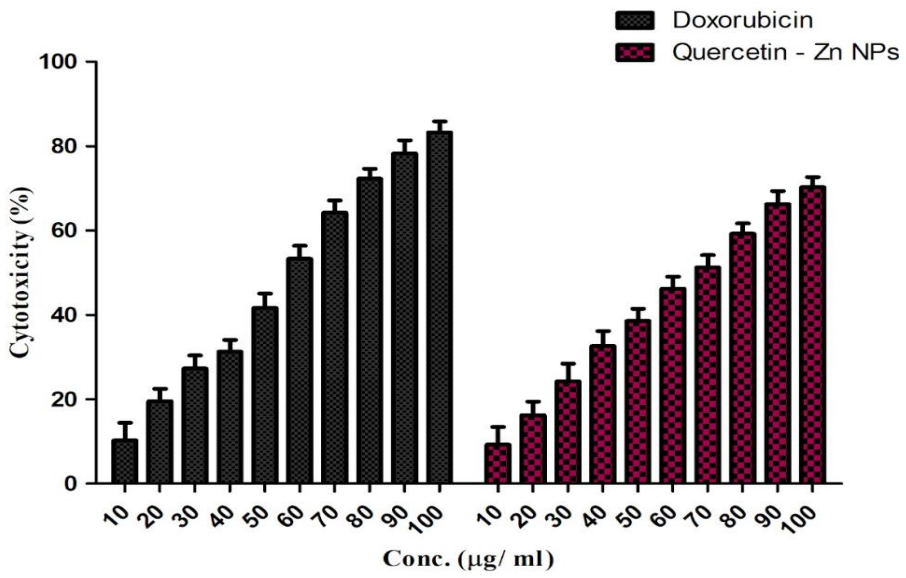

Figure 4: Cytotoxicity of piperine-derived zinc oxide nanoparticles on McCoy cells

The cytotoxic effects of piperine-derived zinc oxide nanoparticles (ZnO NPs) on McCoy cells were evaluated using the MTT assay. Treatment with ZnO NPs 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 4). Significant reductions in cell viability were observed particularly at higher concentrations and longer exposure times ($p < 0.05$), indicating the potential cytotoxicity of ZnO NPs against McCoy cells.

Bax Expression of piperine-derived zinc oxide nanoparticles on McCoy cells

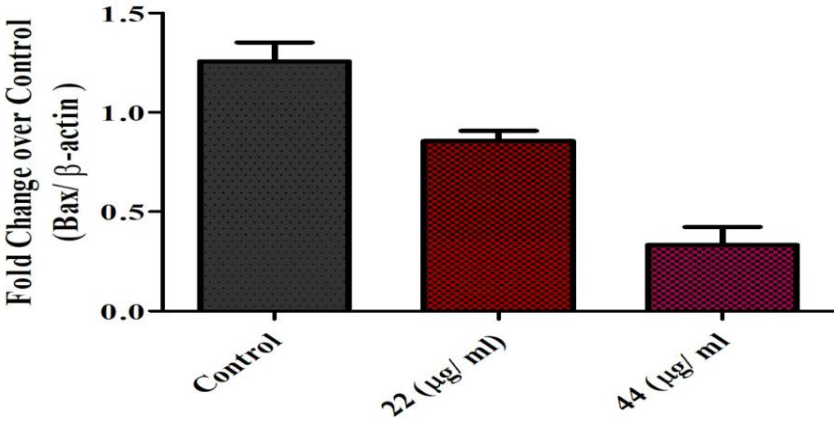

Figure 5: Piperine-derived zinc oxide nanoparticles decreased Bax expression on McCoy cells cells in concentration dependent manner

BCl2 Expression of piperine-derived zinc oxide nanoparticles on McCoy cells

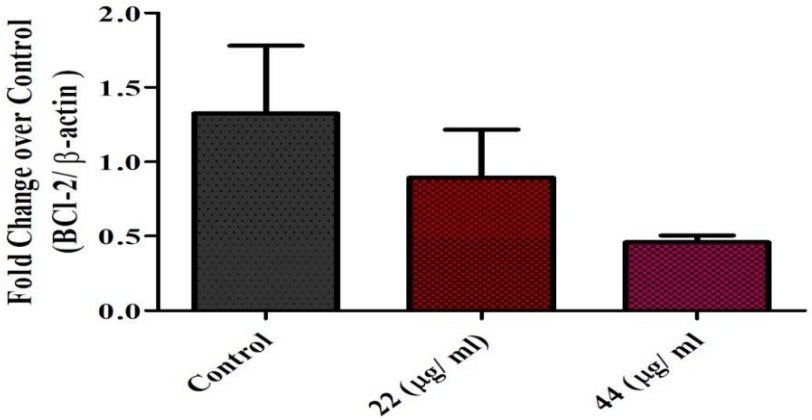

Figure 6: Piperine-derived zinc oxide nanoparticles decreased Bcl2 expression on McCoy cells cells in concentration dependent manner

NF kB Expression of piperine-derived zinc oxide nanoparticles on McCoy cells

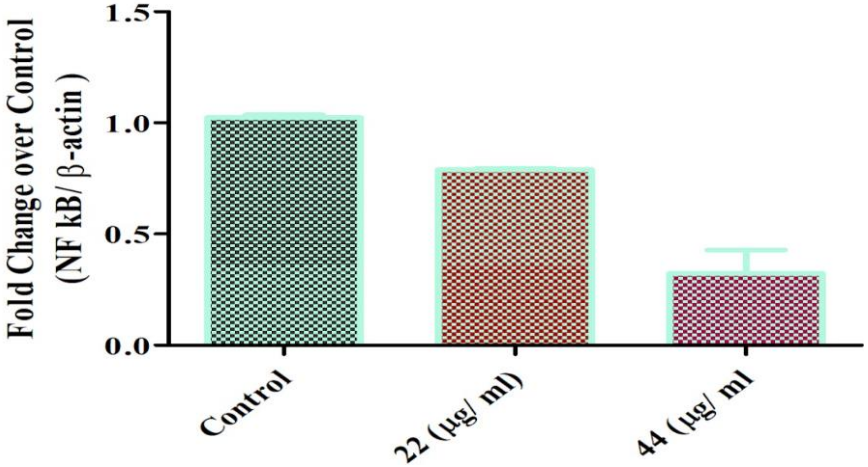

Figure 7: Piperine-derived zinc oxide nanoparticles decreased NF kB expression on McCoy cells cells in concentration dependent manner

NF kB Expression of piperine-derived zinc oxide nanoparticles on McCoy cells

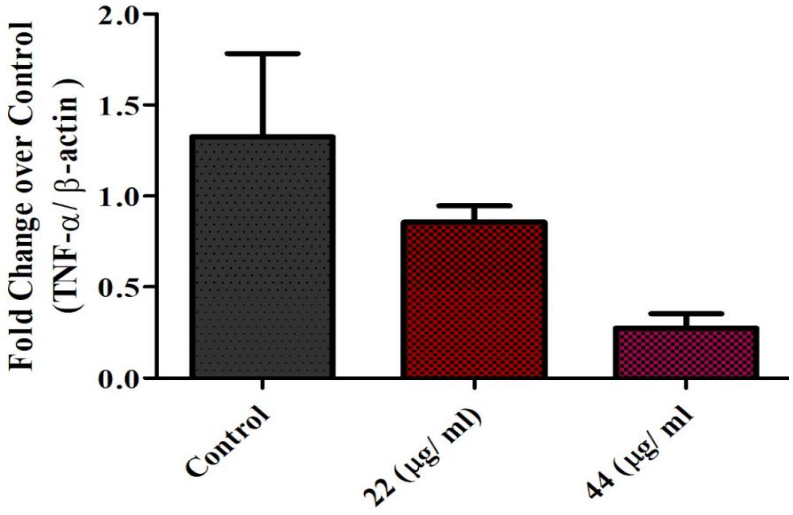

Figure 8: Piperine-derived zinc oxide nanoparticles decreased TNF-α expression on McCoy cells in concentration dependent manner

Treatment with ZnO NPs 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 ZnO NP-treated cells compared to controls ($p < 0.05$). Furthermore, ZnO NP 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 ZnO NPs. Significant downregulation of IL-6 and IL-2 mRNA levels was observed in ZnO NP-treated cells compared to controls. Moreover, ZnO NP treatment significantly reduced the expression of TNF and NF-κB mRNA, suggesting inhibition of hypoxia and inflammatory pathways(Ziegenhagen, Heimberg et al. 2021).

3.3 Molecular Docking Studies

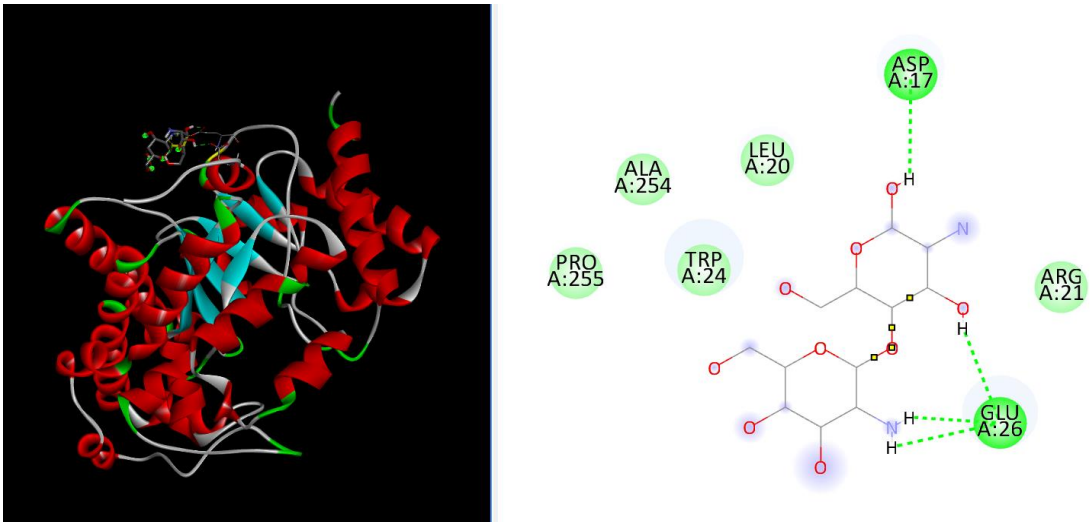

Fig 9: Binding interaction between quercetin derivatives and ATG4B

Molecular docking simulations were conducted to elucidate the potential interaction between quercetin derivatives and ATG4B, a known regulator of cancer cell survival. Using AutoDock Vina, multiple binding poses were generated, indicating favorable interactions between quercetin derivatives and the active site residues of ATG4B (Figure 9).

The binding affinity (-7.5 kcal/mol) suggested strong binding between quercetin derivatives and ATG4B, potentially disrupting its function and contributing to the observed cytotoxic effects of piperine-derived zinc oxide nanoparticles (ZnO NPs) in McCoy cells.

4. DISCUSSION

This study investigates the anticancer potential of quercetin derivatives targeting ATG4B protein and evaluates their efficacy using piperine-derived zinc oxide nanoparticles (ZnO NPs) in McCoy cells. The findings highlight the promise of combining natural compounds and nanotechnology to develop novel therapeutic strategies for cancer treatment.

Quercetin, a flavonoid with a diverse range of biological activities, has shown significant anticancer properties, such as the inhibition of cell proliferation, induction of apoptosis, and suppression of angiogenesis(Krchnák, Waring et al. 2008). However, the clinical application of quercetin is limited by its low bioavailability and rapid metabolism. To overcome these challenges, quercetin derivatives were designed and subjected to molecular docking simulations in this study.

The simulations revealed strong interactions between the quercetin derivatives and ATG4B protein, suggesting their potential as autophagy inhibitors. ATG4B is a crucial regulator of autophagy, a cellular degradation process that can act as both a tumor suppressor and a promoter of tumor cell survival under stress conditions. Inhibiting ATG4B disrupts autophagy and induces cancer cell death, making it an attractive target for anticancer therapy.

Nanotechnology has revolutionized cancer therapy by enabling the development of nanoparticles with unique properties, such as enhanced drug delivery, targeted therapy, and improved therapeutic efficacy(Prakash, Meena et al. 2021). In this study, piperine-derived ZnO NPs were synthesized via green chemistry and characterized using UV-Vis spectroscopy and FTIR. The characterization confirmed their nanostructure and surface chemistry, essential for ensuring their efficacy and safety in biological systems.

The MTT assay demonstrated dose- and time-dependent cytotoxicity of ZnO NPs in McCoy cells, indicating their potential as anticancer agents. To understand the mechanism of cytotoxicity, apoptosis-related protein expression was analyzed by Western blotting.

Treatment with ZnO NPs led to a significant increase in the expression of pro-apoptotic Bax protein and a decrease in anti-apoptotic Bcl-2 protein in a dose-dependent manner. The increased Bax/Bcl-2 ratio, a critical indicator of apoptosis induction, suggests that ZnO NPs effectively promote apoptosis in McCoy cells.

Furthermore, ZnO NP treatment resulted in the downregulation of NF-κB expression, a key regulator of inflammation and cell survival pathways. This inhibition of NF-κB suggests that ZnO NPs can modulate inflammatory pathways, adding another layer

to their anticancer efficacy. 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 ZnO NPs. Significant downregulation of IL-6 and IL-2 mRNA levels was observed in ZnO NP-treated cells compared to controls.

Additionally, ZnO NP treatment significantly reduced the expression of HIF-1α and NF-κB mRNA, indicating the inhibition of hypoxia and inflammatory pathways. These findings underscore the multi-faceted approach of ZnO NPs in combating cancer by not only inducing apoptosis but also modulating critical pathways involved in inflammation and hypoxia(Chavda, Patel et al. 2022).

The combination of quercetin derivatives targeting ATG4B and the use of ZnO NPs demonstrates a promising strategy for cancer therapy. The strong binding interactions between quercetin derivatives and ATG4B suggest their potential as autophagy inhibitors, which can be further explored in preclinical and clinical settings. The ability of ZnO NPs to induce apoptosis and modulate inflammatory pathways in McCoy cells highlights their potential as effective anticancer agents.

Cancer remains one of the leading causes of death worldwide, necessitating the continuous search for novel and effective therapeutic strategies. Traditional chemotherapy and radiotherapy, while effective to some extent, often come with significant side effects and resistance issues. Therefore, exploring new compounds with targeted mechanisms of action and minimal side effects is crucial. Natural compounds and their derivatives, such as quercetin, have gained substantial attention due to their potential therapeutic benefits and lower toxicity profiles. The findings from this study support further investigation into the development of quercetin derivatives and ZnO NPs as novel nanotherapeutics for cancer treatment(Srinivasan 2013).

5. CONCLUSION

In conclusion, this study provides a comprehensive evaluation of quercetin derivatives and piperine-derived ZnO NPs as potential anticancer agents. The integration of molecular docking simulations, in vitro assays, and mechanistic studies offers valuable insights into their mode of action and therapeutic potential. These findings pave the way for future research to advance the development of natural compound-based nanotherapeutics in cancer therapy.

References

1) Alizadeh, S. R. and M. A. Ebrahimzadeh (2022). "Quercetin derivatives: Drug design, development, and biological activities, a review." European journal of medicinal chemistry **229**: 114068.

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

3) Chavda, V. P., et al. (2022). "Nano-drug delivery systems entrapping natural bioactive compounds for cancer: recent progress and future challenges." Frontiers in oncology **12**: 867655.

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

5) Gansukh, E., et al. (2021). "New insights into antiviral and cytotoxic potential of quercetin and its derivatives–a biochemical perspective." Food Chemistry **334**: 127508.

6) 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.

7) Giridharan, B., et al. (2024). "Characterization of Novel Antimicrobial Peptides from the Epidermis of Clarias batrachus Catfish." International Journal of Peptide Research and Therapeutics **30**(2): 11.

8) 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.

9) Jain, A., et al. (2021). "A computational approach to identify the mutations in the genes of the RTK signaling pathway and their possible association with oral squamous cell carcinoma." Middle East Journal of Cancer **12**(1): 1-9.

10) Joshi, S., et al. (2023). "Significant advancement in various synthetic strategies and pharmacotherapy of piperine derivatives: a review." Current Topics in Medicinal Chemistry **23**(25): 2394-2415.

11) Khursheed, R., et al. (2020). "Enhancing the potential preclinical and clinical benefits of quercetin through novel drug delivery systems." Drug discovery today **25**(1): 209-222.

12) Krchnák, V., et al. (2008). "Evolution of Natural Product Scaffolds by Acyl-and Arylnitroso Hetero-Diels− Alder Reactions: New Chemistry on Piperine." The Journal of Organic Chemistry **73**(12): 4559-4567.

13) 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.

14) Mukhopadhyay, P. and A. Prajapati (2015). "Quercetin in anti-diabetic research and strategies for improved quercetin bioavailability using polymer-based carriers–a review." RSC advances **5**(118): 97547-97562.

15) Prakash, P., et al. (2021). "Evidence-based traditional Siddha formulations for prophylaxis and management of respiratory symptoms in COVID-19 pandemic-a review." Biocatalysis and agricultural biotechnology **35**: 102056.

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

17) 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).

18) Ram, A. J., et al. (2020). "Overexpression of BASP1 indicates a poor prognosis in head and neck squamous cell carcinoma." Asian Pacific Journal of Cancer Prevention: APJCP **21**(11): 3435.

19) 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.

20) Salehi, B., et al. (2020). "Therapeutic potential of quercetin: new insights and perspectives for human health." ACS omega **5**(20): 11849-11872.

21) Santhoshkumar, M. (2021). "nd Surgery first orthognathic approach in the correction of dentofacial deformities-An overview." International Journal of Dentistry and Oral Science **8**(1): 1072-1076.

22) Senthil, R., et al. (2022). "Identification of oxazolo [4, 5-g] quinazolin-2 (1H)-one Derivatives as EGFR Inhibitors for Cancer Prevention." Asian Pacific Journal of Cancer Prevention: APJCP **23**(5): 1687.

23) Srinivasan, K. (2013). "Biological Activities of Pepper Alkaloids 45."

24) Sundaram, K. K. M. and K. M. Saravanan (2022). "Deciphering Role of Chameleon Fragments in Folding of Amyloidogenesis."

25) Umapathy, S., et al. (2024). "Selenium Nanoparticles as Neuroprotective Agents: Insights into Molecular Mechanisms for Parkinson's Disease Treatment." Molecular Neurobiology: 1-28.

26) Vazhappilly, C. G., et al. (2021). "Current methodologies to refine bioavailability, delivery, and therapeutic efficacy of plant flavonoids in cancer treatment." The Journal of Nutritional Biochemistry **94**: 108623.

27) Vinayak, M. and A. K. Maurya (2019). "Quercetin loaded nanoparticles in targeting cancer: recent development." Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) **19**(13): 1560-1576.

28) Wang, S. (2023). Piperine Derivatives as Modulators of Amyloid-Beta (Aβ) Aggregation, University of Waterloo.

29) Yuan, D., et al. (2023). "Opportunities and challenges in enhancing the bioavailability and bioactivity of dietary flavonoids: A novel delivery system perspective." Food Chemistry: 137115.

30) Ziegenhagen, R., et al. (2021). Safety Aspects of the Use of Isolated Piperine Ingested as a Bolus. Foods 2021, 10, 2121, s Note: MDPI stays neutral with regard to jurisdictional claims in published ….