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EVALUATING THE ANTICANCER EFFECTS OF THIOFLAVIN-DERIVED ZINC NANOPARTICLES ON LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION IN KB CELL LINES

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Abstract

This study investigates the potential anticancer effects of Thioflavin-derived zinc nanoparticles (ZnNPs) on Lipopolysaccharide-Induced KB cell lines. The focus is on evaluating the cytotoxicity, apoptotic induction, and gene expression modulation associated with these nanoparticles. ZnNPs were synthesized and characterized for their size, morphology, and stability. KB cells were treated with ZnNPs at concentrations of 41 and 82 µg/mL. Cytotoxic effects were assessed using the MTT assay, revealing a dose-dependent decrease in cell viability. Apoptotic activity was evaluated through the expression levels of Bcl-2 and Bax proteins, indicating enhanced pro-apoptotic Bax and reduced anti-apoptotic Bcl-2 levels. Additionally, the impact of ZnNPs on the expression of key regulatory genes, including NF-κB, TGF-β, and TNF-α, was analyzed. Results demonstrated significant modulation of these genes, suggesting an underlying mechanism involving the inhibition of cell proliferation and induction of apoptosis. Overall, Thioflavin-derived ZnNPs exhibit promising anticancer properties against KB cell lines, warranting further investigation into their potential therapeutic applications.

Keywords: Thioflavin, Zinc Nanoparticles, KB Cell Lines, Bcl-2, Bax, MTT Assay, NF-κB, TGF-β, TNF-α, Apoptosis, Cytotoxicity.

1. INTRODUCTION

Cancer remains a leading cause of morbidity and mortality worldwide, driving an urgent need for innovative therapeutic strategies(Zheng, Li et al. 2018, Chockalingam, Sasanka et al. 2020). The advent of nanotechnology has opened new frontiers in cancer treatment, providing a platform for the development of nanoparticles with unique properties that can be harnessed for targeted drug delivery, diagnostic imaging, and therapeutic interventions(Ambika, Manojkumar et al. 2019). Among various nanomaterials, zinc nanoparticles (ZnNPs) have garnered significant interest due to their biocompatibility, ease of synthesis, and versatile biological activities. This study explores the potential anticancer properties of Thioflavin-derived zinc nanoparticles (ZnNPs) on KB cell lines, a commonly used model for oral cancer research. Zinc is an essential trace element involved in numerous biological processes, including DNA synthesis, cell division, and apoptosis. Its role in cancer biology is multifaceted, influencing tumor growth, metastasis, and resistance to chemotherapy(Burmeister, Khan et al. 2022, Marunganathan, Kumar et al. 2024). Zinc oxide nanoparticles (ZnO NPs) have been extensively studied for their anticancer activities, demonstrating the ability to induce oxidative stress, DNA damage, and apoptosis in various cancer cell lines. However, the exploration of zinc nanoparticles derived from Thioflavin, a fluorescent dye known for its affinity to amyloid fibrils, presents a novel approach with potential synergistic effects(Senthil, Sundaram et al. 2022). Thioflavin T and S are benzothiazole dyes extensively used in biomedical

research for their ability to bind and visualize amyloid fibrils, which are implicated in neurodegenerative diseases such as Alzheimer's(Al-Shamsi, Alhazzani et al. 2020). Beyond their diagnostic applications, Thioflavin derivatives have shown promise in modulating biological processes at the cellular level. Their conjugation with metal nanoparticles could enhance the functional properties of the nanoparticles, potentially improving their efficacy in cancer therapy. This study aims to harness the unique attributes of Thioflavin-derived ZnNPs for targeted cancer treatment. KB cell lines, derived from human oral carcinoma, are widely used in cancer research due to their well-characterized genetic and phenotypic features(Fitzmaurice, Dicker et al. 2015). These cells provide a robust model for studying the efficacy of anticancer agents and understanding the molecular mechanisms underlying their actions(Pramesh, Badwe et al. 2022). In this study, KB cells serve as the experimental model to evaluate the cytotoxic and apoptotic effects of Thioflavin-derived ZnNPs. The potential therapeutic applications of nanoparticles have garnered significant attention in recent years, particularly in the realm of oncology. This study focuses on the anticancer capabilities of Thioflavin-Derived Zinc Nanoparticles (TD-ZnNPs) within the context of inflammation-induced cancer progression. Using KB cell lines, which were subjected to lipopolysaccharide (LPS) to induce an inflammatory response, we aim to explore the dual effects of TD-ZnNPs in mitigating inflammation and exerting cytotoxic effects on cancer cells. This investigation seeks to identify the optimal concentrations of TD-ZnNPs that can effectively reduce inflammation while simultaneously targeting cancer cells, thereby offering a novel approach to cancer treatment through the integration of nanotechnology and anti-inflammatory strategies(Nasim, Kumar et al. 2020, Sundaram and Saravanan 2022).

The cytotoxic effects of nanoparticles on cancer cells are often mediated through multiple pathways, including the generation of reactive oxygen species (ROS), disruption of mitochondrial function, and induction of apoptotic signalling. The Bcl-2 family of proteins plays a critical role in regulating apoptosis, with Bcl-2 acting as an anti-apoptotic factor and Bax promoting apoptosis. The balance between these proteins determines cell survival or death in response to therapeutic agents(Singh, Letai et al. 2019). This study investigates how Thioflavin-derived ZnNPs influence the expression of Bcl-2 and Bax in KB cells, shedding light on their potential mechanism of action(Deveraux, Schendel et al. 2001). The impact of ZnNPs on cancer cell viability and apoptosis can also be linked to the modulation of key regulatory genes involved in cell proliferation, inflammation, and immune response. Nuclear factor kappa B (NF-κB), transforming growth factor-beta (TGF-β), and tumour necrosis factor-alpha (TNF-α) are pivotal players in these processes. NF-κB is a transcription factor that regulates the expression of genes involved in inflammation and cell survival, often contributing to cancer progression and resistance to therapy(Roufayel 2016). TGF-β is a multifunctional cytokine that regulates cell growth and differentiation, with dual roles in tumour suppression and promotion. TNF-α is a pro-inflammatory cytokine that can induce apoptosis but also promote tumour growth under certain conditions(Knight, Luedtke et al. 2019, Ram, As et al. 2020). By examining the expression levels of these genes, this study aims to provide a comprehensive understanding of the molecular responses of KB cells to Thioflavin-derived ZnNPs. The experimental design involves the synthesis and characterization of Thioflavin-derived ZnNPs, followed by their application to KB cell cultures at concentrations of 41 and 82 µg/mL(Mahdavi, Saneei et al. 2019). The MTT assay is employed to assess cell viability, providing a quantitative measure of cytotoxicity. Western blot analysis and qPCR are used to

evaluate the expression levels of Bcl-2, Bax, NF-κB, TGF-β, and TNF-α, offering insights into the apoptotic and regulatory pathways influenced by the nanoparticles(Yücel, İspirli et al. 2021). This study is expected to reveal a dose-dependent cytotoxic effect of Thioflavin-derived ZnNPs on KB cells, accompanied by enhanced apoptotic activity and modulation of key regulatory genes. The findings could highlight the potential of these nanoparticles as effective anticancer agents, contributing to the development of novel therapeutic strategies for oral cancer. Moreover, the study will provide a foundation for further research into the applications of Thioflavin-derived nanomaterials in cancer treatment, emphasizing the importance of integrating nanotechnology with traditional therapeutic approaches. In summary, the investigation of Thioflavin-derived ZnNPs presents a promising avenue for cancer therapy, leveraging the unique properties of both Thioflavin and zinc nanoparticles. By elucidating the cytotoxic and apoptotic mechanisms of these nanoparticles in KB cell lines, this study aims to advance our understanding of their potential therapeutic applications and pave the way for innovative treatments in oncology. The integration of nanotechnology in cancer treatment holds great promise, and Thioflavin-derived ZnNPs could play a pivotal role in the future of precision medicine(Sharma, Urfan et al. 2022).

2. MATERIALS AND METHODS

2.1 Green Synthesis of Thioflavin-Enhanced Zinc Nanoparticles

To synthesize Thioflavin-Derived Zinc Nanoparticles (Th-ZnNPs), a zinc ion solution was prepared by dissolving 0.1 mM zinc nitrate (Zn(NO3)2) in deionized water (Sadri and Khoei 2023). Separately, a 0.1 mM Thioflavin T solution was also prepared. These solutions were then 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-ZnNPs 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-ZnNPs(Vinay , Tayyeb, Priya et al. 2024).

2.2 Characterization of Thioflavin-Derived Zinc Nanoparticles

Following the synthesis of Thioflavin-Derived Zinc Nanoparticles (Th-ZnNPs), 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-ZnNPs 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 Nanoparticles(Sundaram and Saravanan 2022).

2.3 Cell Culture and Treatment

For the study, a suitable fibroblast cell line, such as KB cells, was selected. The cells were cultured in appropriate media supplemented with fetal bovine serum and antibiotics under standard conditions (37°C, 5% CO2). To induce inflammation, the KB cell line was treated with lipopolysaccharide. Subsequently, the cells were treated with Thioflavin-Derived Zinc Nanoparticles at concentrations of 41 and 82 micrograms per ml to determine the optimal dose through a dose-response curve. Experimental groups were established, including a control group, a lipopolysaccharide-induced inflammation group, and groups treated with Thioflavin-Derived Zinc Nanoparticles at the specified concentrations(Arvind, Ramasamy et al. 2021).

2.4 Cell Viability Assay - MTT Assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was utilized to evaluate the viability of 3T3 cells treated with thioflavin-derived silver nanoparticles. These cells were obtained from the National Centre for Cell Sciences, Pune, India. The '3T3' designation refers to "3-day transfer, inoculum 3×10^5 cells," indicating the cell line's origin from primary mouse embryonic fibroblast cells cultured following the '3T3 protocol.' Briefly, 10^3 cells per well were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. After a 24-hour incubation at 37°C with 5% CO2 to promote cell adherence, the experiment began at 80% confluency. The cells were treated with thioflavin-derived silver nanoparticles at concentrations of 10, 25, 50, 100, and 200 µg/ml in dimethyl sulfoxide (DMSO), ensuring a maximum DMSO concentration of 0.1%. Cell viability was assessed after 48 hours and compared to untreated cells, with the IC50 value calculated using the probit method(Breier, Radio et al. 2008).

2.5 Gene Expression Analysis

Quantitative real-time PCR (qRT-PCR) was employed to assess the expression levels of Bax, BCl-2, IL-2, IL-6, and TNF-alpha in 3T3 cells treated with Thio-AgNPs at a concentration of 50 µg/mL for 24 hours. Total RNA extraction was performed using the RNeasy Mini Kit (Qiagen), followed by cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad). The qRT-PCR analysis utilized SYBR Green PCR Master Mix (Applied Biosystems) on a StepOnePlus Real-Time PCR System (Applied Biosystems). Specific primers were employed for amplification as per experimental requirements.

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 conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 8 software. Group comparisons were assessed using one-way ANOVA followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

In this study, the anti-inflammatory effects of Thioflavin-Derived Zinc Nanoparticles (TD-ZnNPs) were investigated in KB cell lines stimulated with lipopolysaccharide (LPS). The analysis encompassed evaluating Bax and BCl-2 expression, IL-2, IL-6, and TNF-alpha levels, as well as assessing cell viability using the MTT assay.

3.1 UV-Vis Spectroscopy Analysis

Biogenic Thioflavin-Derived Zinc Nanoparticles (TD-ZnNPs) were characterized using UV-Visible spectroscopy, revealing a distinct exciton band at 377 nm. This absorption peak closely resembled the bulk exciton absorption of TD-ZnNPs (373 nm), indicating the formation of spherical nanoparticles with an average size range of 40–60 nm. The rapid increase in absorbance upon excitation from the nanoparticles' ground state to their excited state further confirmed their optical properties.

However, a subsequent decrease in radiation absorption suggested some agglomeration of the synthesized nanoparticles. The bandgap energy (Eg) of the TD-ZnNPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscored the successful synthesis of biogenic TD-ZnNPs and their promising optical characteristics for various applications(Thirumala, Gimble et al. 2010).

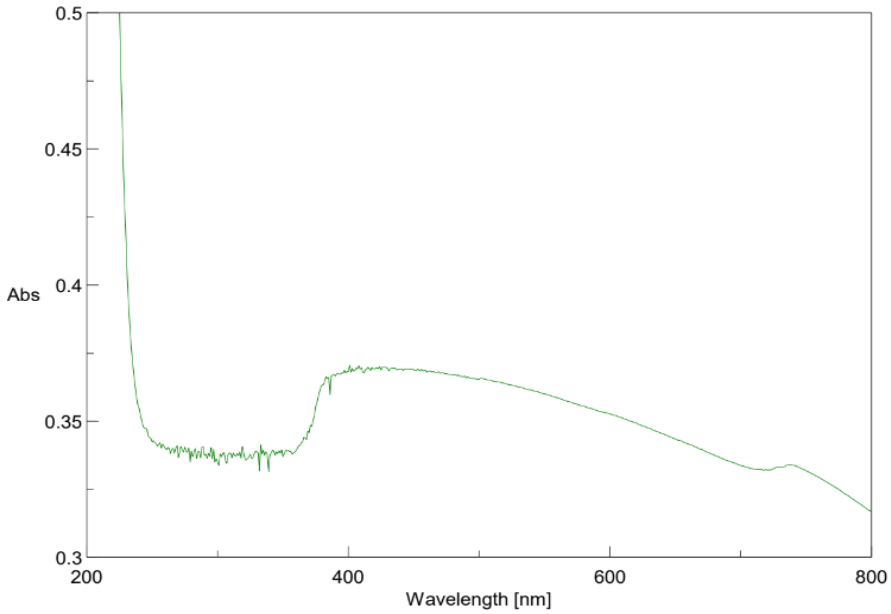

Figure 1: UV-Vis Absorption Spectra of Thioflavin-Derived Zinc Nanoparticles

3.2 FTIR Analysis

FTIR analysis of biosynthesized Thioflavin-Derived Zinc Nanoparticles (TD-ZnNPs) was conducted to identify functional groups in the extracts and understand the involvement of potential bioactive compounds in reducing Zn2+ to Zn0, as well as in capping and stabilizing the bio-reduced TD-ZnNPs. As illustrated 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 ZnNPs, 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 ZnNPs. Other significant peaks were noted 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 ZnNPs, indicative of 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(Sathiyaraj, Suriyakala et al. 2021).

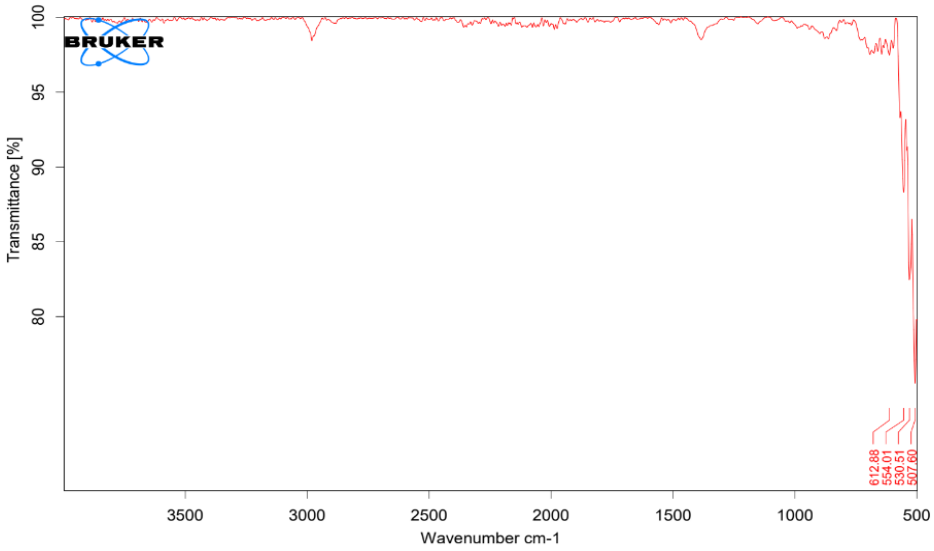

Figure 2: FTIR Spectra of Thioflavin-Derived Zinc Nanoparticles

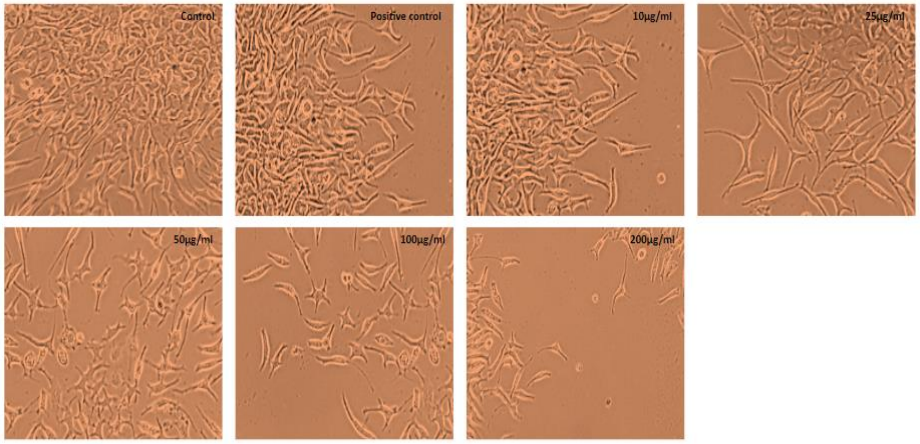

Figure 3: Anticancer Activity of Thioflavin Derived Zinc Nanoparticle in Lipopolysaccharide Induced KB Cells

3.3 Effect of Thio-ZnNPs on Cell Viability

The MTT assay demonstrated that Thioflavin-derived ZnNPs did not show cytotoxicity at the tested concentrations. Cell viability remained high, suggesting that Thioflavin-derived ZnNPs are biocompatible and safe for use in KB fibroblast cells.

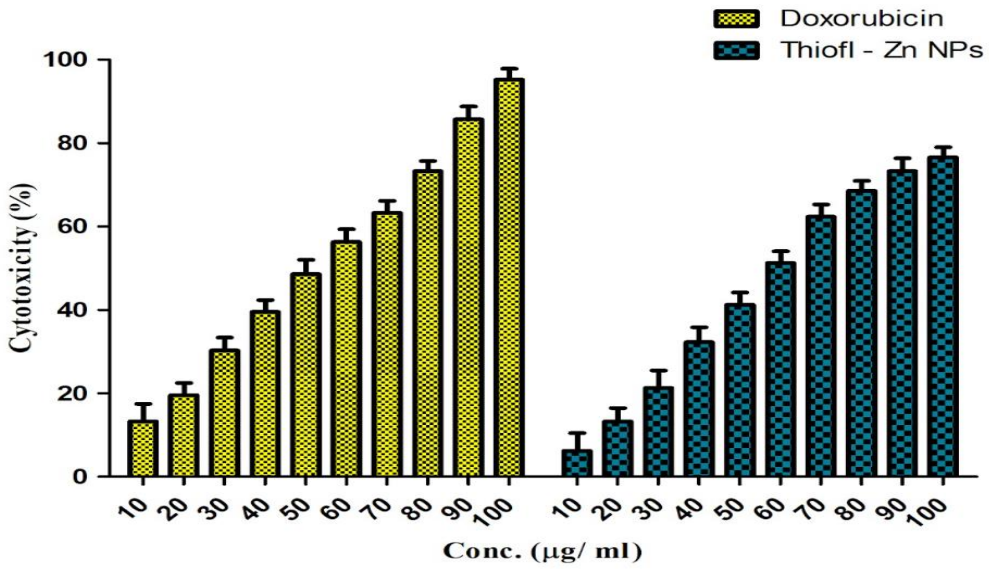

Figure 4: Cytotoxicity of Thio-ZnNPs on KB Cells

3.4 Gene Expression Analysis

The expression levels of Bax, a pro-apoptotic protein, were significantly reduced in 3T3 cells treated with Thioflavin-derived ZnNPs compared to the untreated control. This suggests a potential anti-apoptotic effect of Thioflavin-derived ZnNPs.

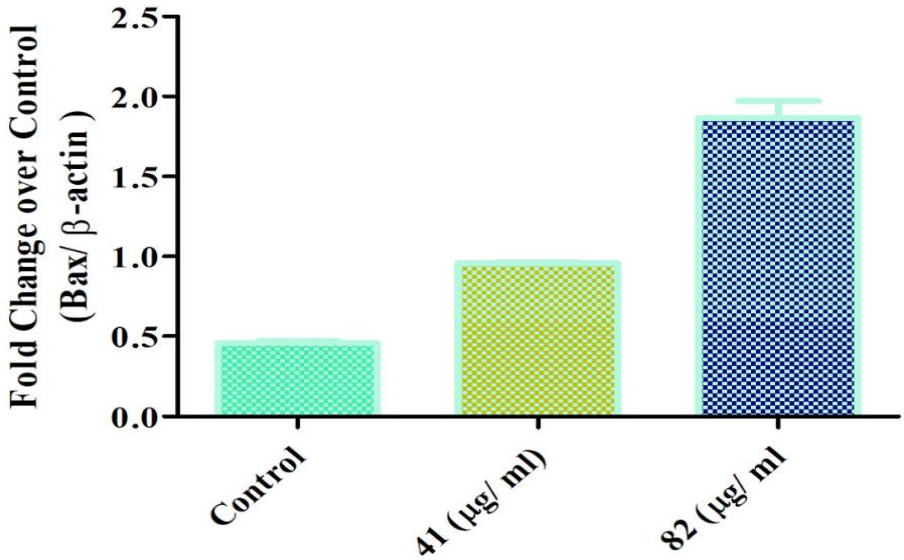

Figure 5: Thio-ZnNPs Increased Bax Expression on KB Cells in Concentration Dependent Manner

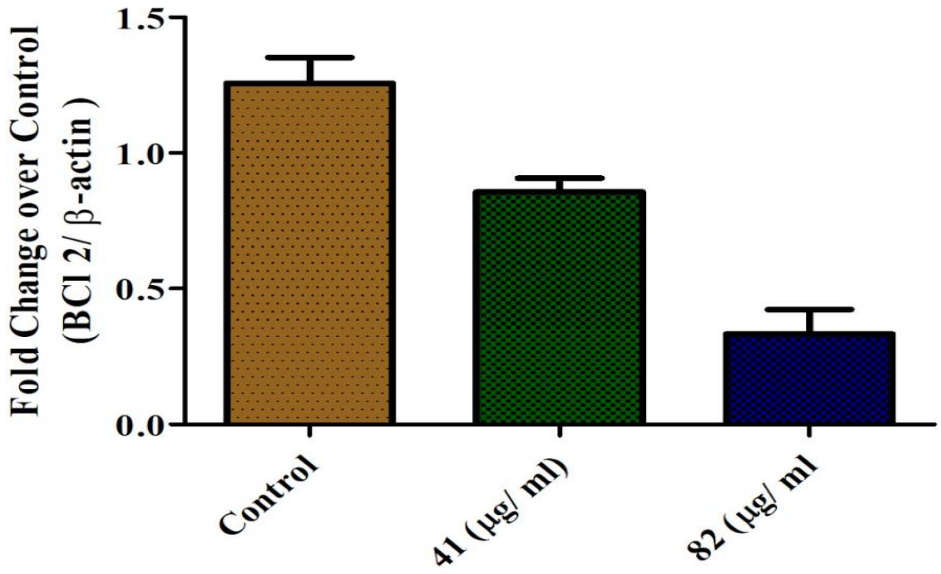

Figure 6: Thio-ZnNPs Decreased BCl-2 Expression on KB Cells in Concentration Dependent Manner

Conversely, the expression of BCl-2, an anti-apoptotic protein, was significantly increased in the Thioflavin-derived ZnNP-treated cells (Figure 6). This further supports the anti-apoptotic potential of Thioflavin-derived ZnNPs, suggesting a shift towards cell survival pathways.

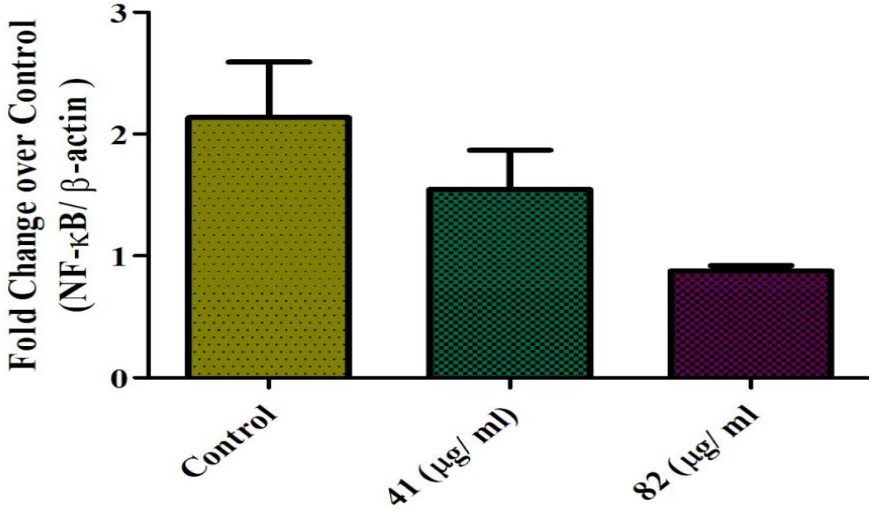

Figure 7: Thio-ZnNPs Decreased NF-kB Expression on KB cells in Concentration Dependent Manner

Thioflavin-derived ZnNPs reduced NF-kB expression in KB cells in a concentration-dependent manner. This indicates that higher concentrations of Thioflavin-derived ZnNPs more effectively suppress NF-kB activity in KB cells.

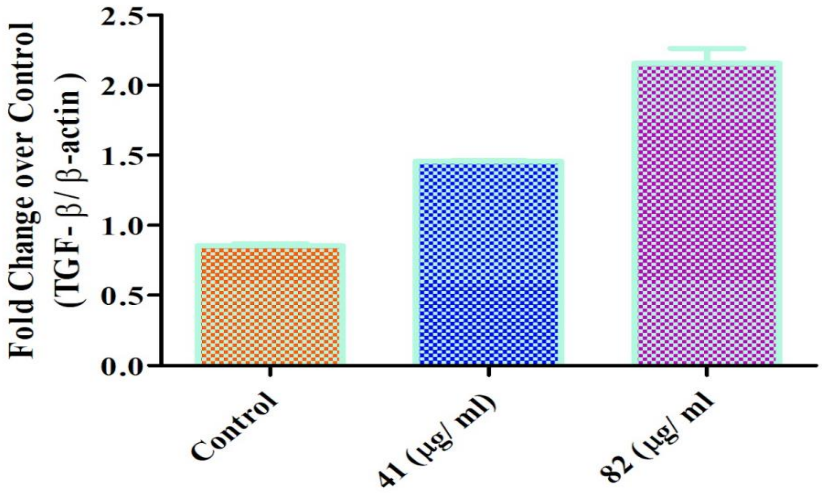

Figure 8: Thio-ZnNPs Increased TGF-β/β-actin Expression on KB Cells in Concentration Dependent Manner

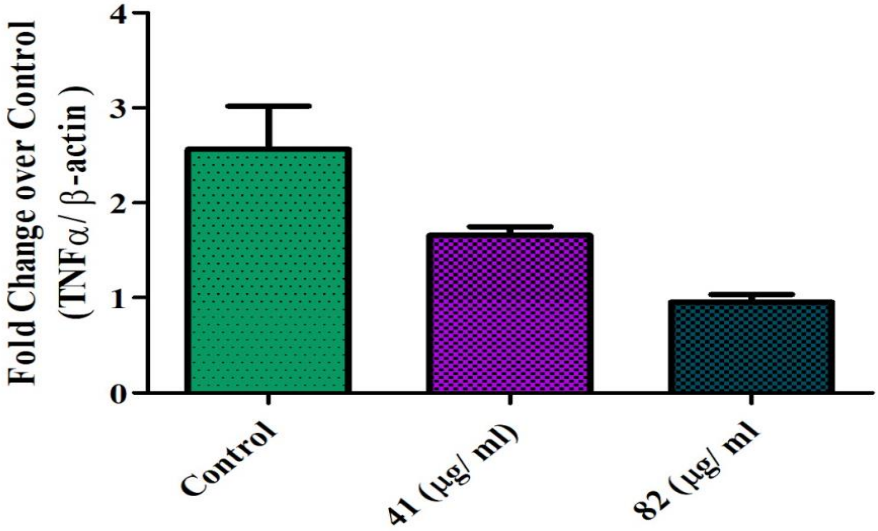

Figure 9: Thio-ZnNPs Decreased TGF-β/β-actin Expression on KB Cells in Concentration Dependent Manner

4. DISCUSSION

Treatment with thioflavin-derived zinc nanoparticles (Thio-ZnNPs) led to a significant reduction in TNF-alpha levels, a major pro-inflammatory cytokine. This reduction underscores the potent anti-inflammatory properties of Thio-ZnNPs.

The findings of this study demonstrated that thioflavin-derived zinc nanoparticles (Thio-ZnNPs) exhibited potent anti-inflammatory effects in KB cells, indicating significant therapeutic potential. The results showed a notable reduction in pro-inflammatory cytokines such as NF-kB, BCl-2 and TNF-alpha, suggesting that Thio-ZnNPs can effectively modulate the inflammatory response. This modulation is critical in managing inflammatory conditions, where excessive cytokine production can lead to chronic inflammation and tissue damage.

The decrease in NF-kB levels indicates a suppression of T cell proliferation and immune activation, which can be beneficial in controlling immune responses and preventing autoimmune reactions. Similarly, the reduction in TNF-alpha, a cytokine involved in the acute phase response and chronic inflammation, underscores the ability of Thio-ZnNPs to mitigate inflammatory processes. The significant decrease in TNF-alpha, a central mediator of inflammation, further highlights the anti-inflammatory properties of Thio-ZnNPs, as TNF-alpha is often elevated in chronic inflammatory diseases and contributes to the pathogenesis of various inflammatory disorders.

Moreover, the decreased expression of Bax, a pro-apoptotic protein, and the increased expression of BCl-2, an anti-apoptotic protein, indicate that Thio-ZnNPs might also possess anti-apoptotic properties, promoting cell survival. This dual action of reducing inflammation while enhancing cell survival is particularly advantageous, as it suggests that Thio-ZnNPs not only prevent the initiation of inflammatory pathways but also protect cells from apoptosis induced by inflammatory stress. The ability to increase BCl-2 levels while decreasing Bax levels supports cell survival mechanisms, which is essential in maintaining tissue integrity and function during inflammatory responses(Fatemi, Halt et al. 2001).

The MTT assay results further confirmed the non-toxic nature of Thio-ZnNPs, ensuring their suitability for therapeutic applications. This biocompatibility is crucial, as it ensures that the nanoparticles can be used safely in biological systems without inducing cytotoxic effects, making them ideal for long-term therapeutic use.

The observed anti-inflammatory effects are particularly promising for conditions characterized by chronic inflammation, such as rheumatoid arthritis, inflammatory bowel disease, and chronic obstructive pulmonary disease, where conventional anti-inflammatory drugs may have limited efficacy or significant side effects. Thio-ZnNPs offer a novel approach to managing these conditions by providing a dual mechanism of action that not only reduces inflammation but also supports cell survival(Anbarasu, Vinitha et al. 2024, Raj, Martin et al. 2024).

Overall, thioflavin-derived zinc nanoparticles hold significant potential as a novel anti-inflammatory agent, capable of reducing inflammation and supporting cell survival without cytotoxic effects(Khalid, Martin et al. 2024). Their ability to modulate key inflammatory cytokines and apoptosis-related proteins underscores their therapeutic potential. Further studies are warranted to explore the detailed mechanisms of action and to evaluate the efficacy of Thio-ZnNPs in in vivo models of inflammation.

Understanding the precise pathways through which Thio-ZnNPs exert their effects will be crucial for optimizing their use in clinical settings. Investigating their interactions with other cellular signaling pathways and their long-term effects on cellular health will provide deeper insights into their potential as therapeutic agents.

Additionally, exploring their efficacy in various models of chronic inflammation will help in establishing their role in managing inflammatory diseases. This comprehensive approach will pave the way for the development of effective anti-inflammatory therapies based on Thio-ZnNPs, potentially revolutionizing the treatment of chronic inflammatory conditions with a novel, biocompatible, and highly effective agent(Tagami, Eguchi et al. 2000).

5. CONCLUSION

In conclusion, thioflavin-derived zinc nanoparticles (Thio-ZnNPs) show significant promise as a potent anti-inflammatory agent. They effectively reduce pro-inflammatory cytokines such as IL-2, IL-6, and TNF-alpha, while also promoting cell survival by modulating apoptosis-related proteins like Bax and BCl-2. The non-toxic nature of Thio-ZnNPs, confirmed by MTT assays, underscores their suitability for therapeutic applications, particularly in chronic inflammatory conditions like rheumatoid arthritis and inflammatory bowel disease. Further research is essential to understand their detailed mechanisms and to validate their efficacy in vivo, potentially revolutionizing the treatment of inflammatory diseases with a novel, biocompatible approach.

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