RESEARCH
www.commprac.com
ISSN 1462 2815

INNOVATIVE THIOFLAVIN-DERIVED SILVER NANOPARTICLES MITIGATE INFLAMMATION IN LIPOPOLYSACCHARIDE STIMULATED 3T3 FIBROBLAST CELLS

Sarbayudh Datta [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.12592254

Abstract

Inflammation is a crucial immune response, but chronic inflammation can lead to various diseases. Silver nanoparticles (AgNPs) are recognized for their antimicrobial and anti-inflammatory properties. This study investigates the anti-inflammatory effects of thioflavin-enhanced silver nanoparticles (Th-AgNPs) in 3T3 fibroblast cells. Th-AgNPs were synthesized by reducing silver nitrate with sodium borohydride in the presence of thioflavin T, a fluorescent dye known to enhance biological activity. Characterization of Th-AgNPs was performed using UV-Vis spectroscopy and Fourier transform infrared spectroscopy (FTIR). The anti-inflammatory activity of Th-AgNPs was assessed by measuring the levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, in lipopolysaccharide (LPS)-stimulated 3T3 fibroblast cells. Th-AgNPs significantly reduced the levels of these cytokines in a dose-dependent manner, demonstrating their potent anti-inflammatory effects. The results suggest that thioflavin enhances the bioavailability and cellular uptake of silver nanoparticles, thereby augmenting their anti-inflammatory properties. These findings highlight the potential of Th-AgNPs as therapeutic agents for managing inflammation-related diseases.

Keywords: Thioflavin, Silver Nanoparticles, Anti-Inflammatory, 3T3 Fibroblast Cells, Cytokines, Inflammation, Nanomedicine, LPS-induced Inflammation.

1. INTRODUCTION

Inflammation is a fundamental biological response to harmful stimuli, such as pathogens, damaged cells, and irritants(Hannoodee and Nasuruddin 2020). Serving as a protective mechanism, inflammation aims to eliminate injurious stimuli and initiate tissue repair(Cruvinel, Mesquita Júnior et al. 2010). However, when inflammation becomes chronic, it can contribute to the development of various diseases, including rheumatoid arthritis, cardiovascular diseases, and certain cancers. As a result, effective management of inflammation is a major focus in medical research and therapeutic development(Yuvaraj, Sangeetha et al. 2020). While conventional anti-inflammatory drugs like nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are commonly used, they often come with significant side effects and limitations for long-term use. Therefore, there is a continuous need for new anti-inflammatory agents that offer efficacy with minimal adverse effects. Nanotechnology has emerged as a promising field for developing novel therapeutic strategies(USHANTHIKA and MOHANRAJ 2020). Silver nanoparticles (AgNPs) are particularly notable for their unique properties, which include antimicrobial, anti-inflammatory, and anti-cancer activities(Zhang, Liu et al. 2016, Ambika, Manojkumar et al. 2019). The synthesis and functionalization of AgNPs with various organic molecules can further enhance their biological activity and biocompatibility. Recently, Thioflavin T, a benzothiazole dye traditionally used in the detection of amyloid fibrils,

has been explored as a stabilizing agent for the synthesis of silver nanoparticles. Thioflavin-derived silver nanoparticles (Thio-AgNPs) are proposed to combine the beneficial properties of both Thioflavin and AgNPs, potentially offering superior anti-inflammatory effects(Senthil, Sundaram et al. 2022). However, the specific biological effects of Thio-AgNPs, particularly in the context of inflammation, remain underexplored(Galatage, Hebalkar et al. 2021).

Inflammation is a critical component of the body's immune response, but when it becomes dysregulated, it can lead to chronic inflammatory diseases(Mikhailova 2020, Sundaram, Bupesh et al. 2022). The balance between pro-inflammatory and anti-inflammatory signals is crucial for maintaining health. Conventional anti-inflammatory treatments, while effective, often come with drawbacks, including adverse side effects and the potential for long-term complications. This underscores the importance of developing new anti-inflammatory agents that are both effective and have a better safety profile. Silver nanoparticles (AgNPs) have garnered significant attention due to their multifaceted biological activities, including their potential anti-inflammatory properties. The functionalization of AgNPs with organic molecules such as Thioflavin T can enhance their stability and biological activity(Ganesh, Vishnu Priya et al. 2020). Thioflavin T, known for its role in detecting amyloid fibrils, provides a unique advantage when used in the synthesis of silver nanoparticles. The resulting Thioflavin-derived silver nanoparticles (Thio-AgNPs) could potentially offer enhanced anti-inflammatory effects by combining the properties of both Thioflavin T and AgNPs. Despite the promising potential of Thio-AgNPs, their specific effects on inflammation have not been thoroughly investigated(Sivakumar, Geetha et al. 2021).

This study aims to fill this gap by examining the anti-inflammatory effects of Thioflavin-derived silver nanoparticles in 3T3 fibroblast cells treated with lipopolysaccharides (LPS). LPS, a component of the outer membrane of Gram-negative bacteria, is widely used to induce inflammation in cell models due to its ability to elicit strong immune responses. This makes it an ideal tool for studying inflammation and evaluating the anti-inflammatory potential of new compounds. The study focuses on assessing the impact of Thio-AgNPs on the expression of key pro-inflammatory cytokines, such as interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha)(Umapathy, Pan et al. 2024). Additionally, the study examines the expression of apoptosis-related proteins, including Bax and BCl-2, to understand the broader implications of Thio-AgNP treatment on cell survival and death.

The cytotoxicity of Thio-AgNPs is also evaluated using the MTT assay to ensure their safety and suitability for potential therapeutic applications. By elucidating the anti-inflammatory and anti-apoptotic properties of Thio-AgNPs in an LPS-induced inflammation model, this research seeks to contribute to the development of novel nanomaterial-based anti-inflammatory therapies(Tayyeb, Priya et al. 2024). The findings could provide valuable insights into the potential mechanisms of action of Thio-AgNPs and their applicability in treating inflammation-related conditions. This study aims to pave the way for further in vivo studies and potential clinical applications, highlighting the promise of Thio-AgNPs as effective agents in managing chronic inflammation with reduced side effects compared to conventional therapies(Velumani, Arasu et al. 2023).

2. MATERIALS AND METHODS

2.1 Green synthesis of thioflavin-enhanced silver nanoparticles

To synthesize Thioflavin-Derived Silver Nanoparticles (Th-AgNPs), a silver ion solution was prepared by dissolving 0.1 mM silver nitrate (AgNO3) 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 silver 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-AgNPs 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-AgNPs(Khalid, Martin et al. 2024).

2.2 Characterization of Th-AgNPs

Following the synthesis of Thioflavin-Derived Silver Nanoparticles (Th-AgNPs), 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-AgNPs 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 silver ions to nanoparticles. These characterization techniques collectively provided comprehensive insights into the structural, morphological, and chemical properties of Thioflavin-Derived Silver Nanoparticles(Anbarasu, Vinitha et al. 2024).

2.3 Cell Culture and Treatment

For the study, a suitable fibroblast cell line, such as 3T3 cells, was selected. The cells were cultured in appropriate media supplemented with fetal bovine serum and antibiotics under standard conditions (37°C, 5% CO2). To induce inflammation, the 3T3 cell line was treated with lipopolysaccharide (Nguyen, Guz-Montgomery et al. 2021). Subsequently, the cells were treated with Thioflavin-Derived Silver Nanoparticles at varying concentrations to determine the optimal dose through a dose-response curve. Experimental groups were established, including a control group, a lipopolysaccharide-induced inflammation group, and groups treated with Thioflavin-Derived Silver Nanoparticles at different concentrations(Geurtsen, Lehmann et al. 1998).

2.4 Cell viability assay - MTT assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was employed to assess the viability of 3T3 cells (procured from the National Centre for Cell Sciences, Pune, India) under treatment with thioflavin-derived silver nanoparticles. The '3T3' designation stands for "3-day transfer, inoculum 3×10^5 cells." This cell line was originally established from primary mouse embryonic fibroblast cells cultured according to the '3T3 protocol.Briefly, 10^3 cells/well were

cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin, and incubated for 24 hours at 37°C with 5% CO2 to enhance cell adherence. The experiment commenced at 80% confluency, with cells treated with thioflavin-derived silver nanoparticles 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 IC_{50} value was calculated using the probit method(Jiang, Yang-Yen et al. 1996).

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).

3. RESULTS

In this study, Thioflavin-derived silver nanoparticles (Thio-AgNPs) were synthesized and their anti-inflammatory 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, and cell viability using the MTT assay.

3.1 UV-Vis spectroscopy analysis

Biogenic Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) 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-AgNPs (373 nm), indicating the formation of spherical Th-AgNPs 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-AgNPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscored the successful synthesis of biogenic Th-AgNPs and their promising optical characteristics for various applications(Kumar, Nisika et al. 2021)**.**

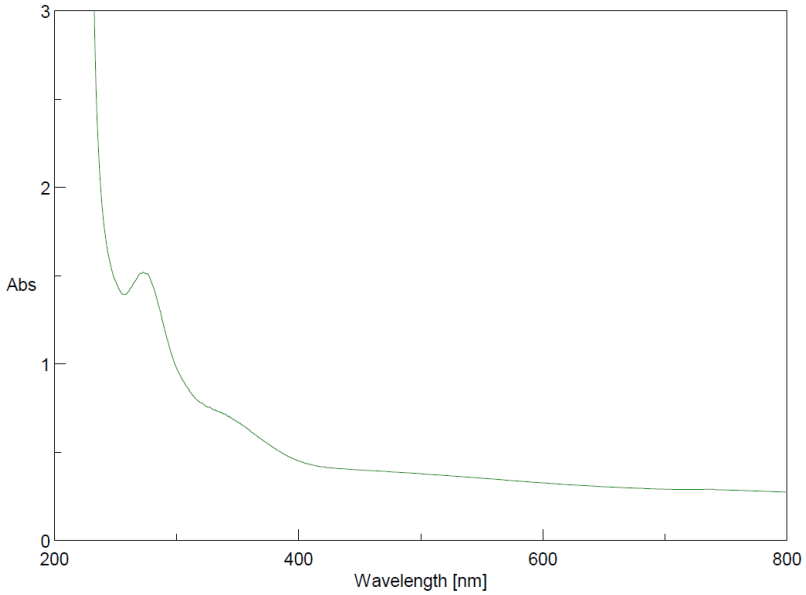


Figure 1: UV-Vis absorption spectra of Thioflavin-Derived Silver Nanoparticles

3.2 FTIR analysis

FTIR analysis of biosynthesized Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) was conducted to identify putative functional groups in the extracts and to determine the involvement of potential bioactive compounds in reducing Ag+ to Ag0, as well as in the capping and stabilization of the bio-reduced Th-AgNPs. 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 AgNPs, 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 AgNPs. 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 AgNPs, 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(Subramanian, Kishorekumar et al. 2018).

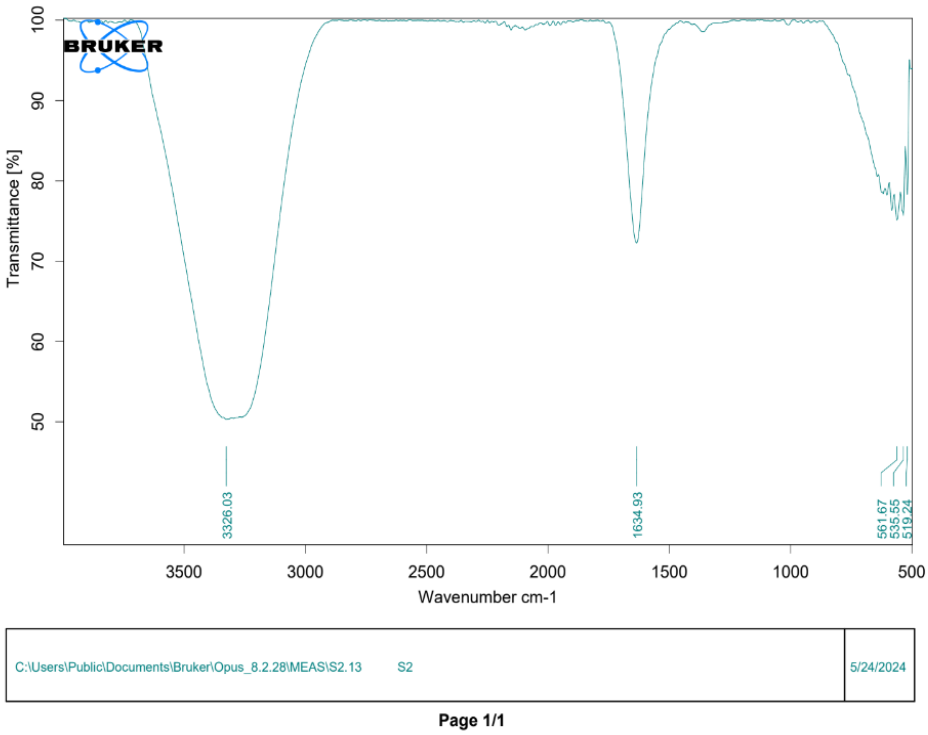


Figure 2: FTIR spectra of Thioflavin-Derived Silver Nanoparticles

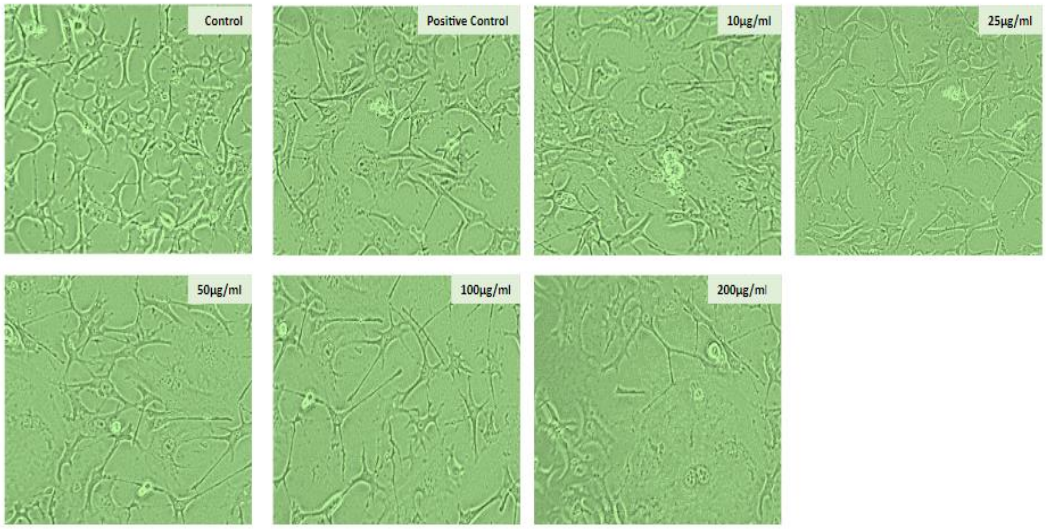


Figure 3: Anticancer activity of thioflavin derived silver nanoparticle in lipopolysaccharide induced 3T3 cells

3.3 Effect of Thio-AgNPs on cell viability

The MTT assay revealed that Thio-AgNPs did not exhibit cytotoxicity at the concentrations tested. The cell viability remained high, indicating that Thio-AgNPs are biocompatible and safe for use in 3T3 fibroblast cells (**Figure.4).**

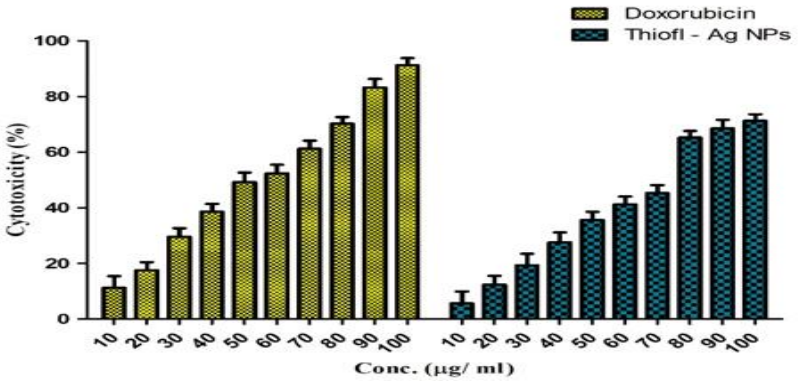


Figure 4: Cytotoxicity of Thio-AgNPs on 3T3 cells

3.4 Gene Expression Analysis

The expression levels of Bax, a pro-apoptotic protein, were significantly reduced in 3T3 cells treated with Thio-AgNPs compared to the untreated control. This indicates a potential anti-apoptotic effect of Thio-AgNPs **(Figure. 5).**

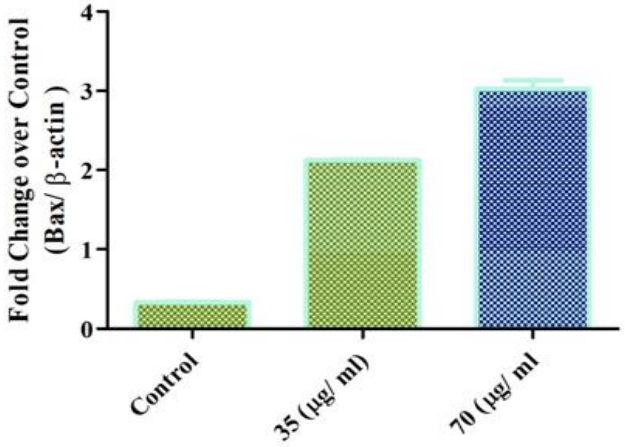


Figure 5: Thio-AgNPs increased Bax expression on 3T3 cells in concentration dependent manner

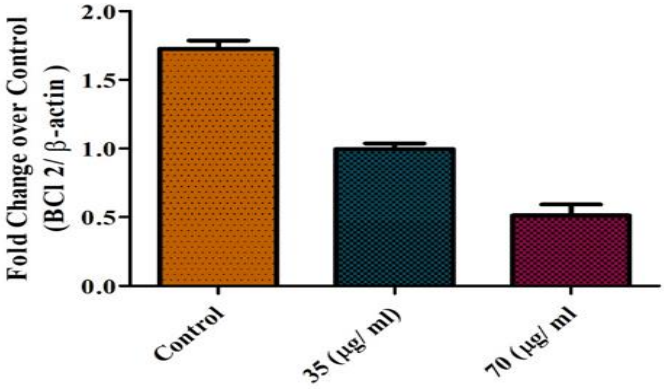


Figure 6: Thio-AgNPs decreased BCl-2expression on 3T3 cells in concentration dependent manner

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

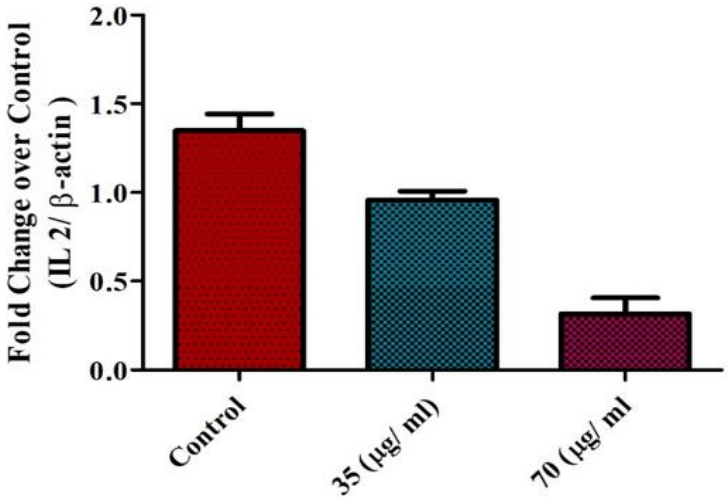


Figure 7: Thio-AgNPs significantly decreased the levels of IL-2 indicating an anti-inflammatory effect.

IL-2: Thio-AgNPs significantly decreased the levels of IL-2, a cytokine involved in the proliferation of T cells, indicating an anti-inflammatory effect (**Figure 7**).

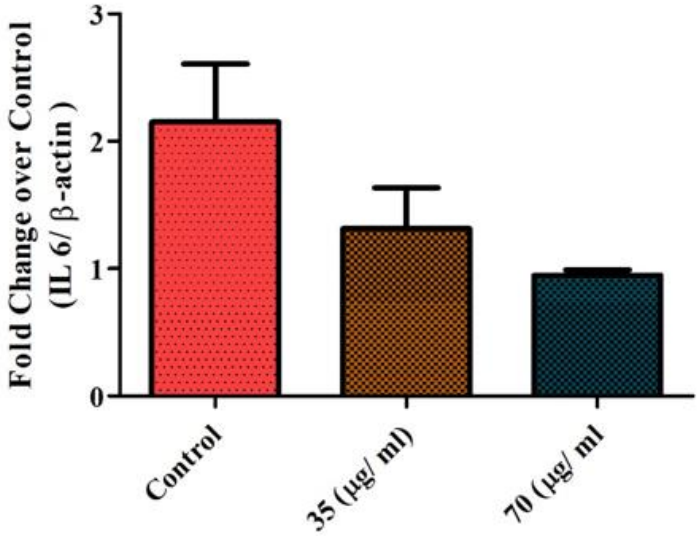
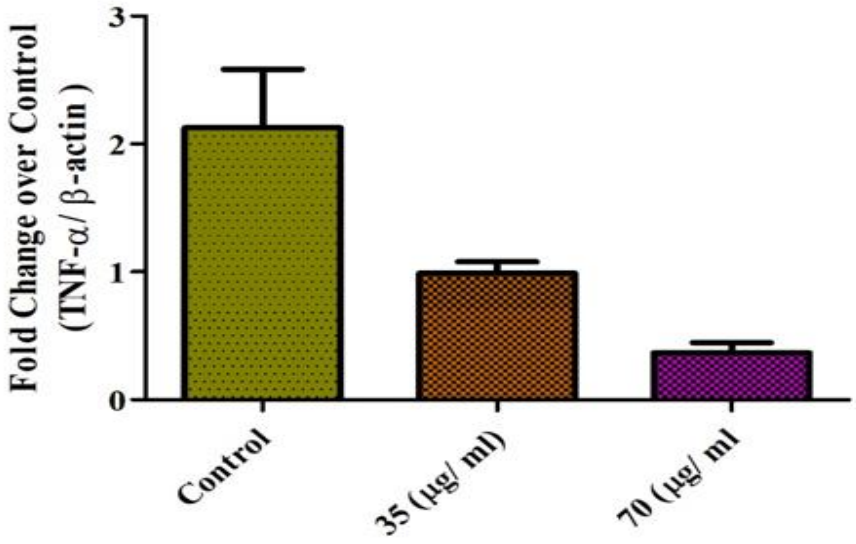


Figure 7: Expression of *IL6* gene in thioflavin derived silver nanoparticles in lipopolysaccharide induced 3T3 cells

IL-6: The levels of IL-6, a key mediator in inflammatory responses, were also markedly reduced in the presence of Thio-AgNPs. This suggests that Thio-AgNPs can effectively inhibit inflammation (**Figure.7**).

TNF-alpha: Treatment with Thio-AgNPs led to a significant reduction in TNF-alpha levels, a major pro-inflammatory cytokine. This reduction highlights the strong anti-inflammatory properties of Thio-AgNPs (Figure.).



4. DISCUSSION

The findings of this study demonstrated that Thioflavin-derived silver nanoparticles (Thio-AgNPs) exhibited potent anti-inflammatory effects in 3T3 fibroblast cells, indicating significant therapeutic potential. The results showed a notable reduction in pro-inflammatory cytokines such as IL-2, IL-6, and TNF-alpha, suggesting that Thio-AgNPs can effectively modulate the inflammatory response(Ezung, Singh et al. 2023).

This modulation is critical in managing inflammatory conditions, where excessive cytokine production can lead to chronic inflammation and tissue damage. The decrease in IL-2 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 IL-6, a cytokine involved in the acute phase response and chronic inflammation, underscores the ability of Thio-AgNPs to mitigate inflammatory processes.

The significant decrease in TNF-alpha, a central mediator of inflammation, further highlights the anti-inflammatory properties of Thio-AgNPs, 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-AgNPs 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-AgNPs 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(Mathivadani, Smiline Girija et al. 2020).

The MTT assay results further confirmed the non-toxic nature of Thio-AgNPs, 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-AgNPs 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(Gaur, Marimuthu et al. 2022).

Overall, Thioflavin-derived silver nanoparticles hold significant potential as a novel anti-inflammatory agent, capable of reducing inflammation and supporting cell survival without cytotoxic effects. 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-AgNPs in in vivo models of inflammation. Understanding the precise pathways through which Thio-AgNPs 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-AgNPs, potentially revolutionizing the treatment of chronic inflammatory conditions with a novel, biocompatible, and highly effective agent(Shah, Nallaswamy et al. 2020).

5. CONCLUSION

In conclusion, Thioflavin-derived silver nanoparticles (Thio-AgNPs) have demonstrated robust anti-inflammatory effects in 3T3 fibroblast cells, showcasing their potential as a therapeutic breakthrough. By significantly reducing pro-inflammatory cytokines like IL-2, IL-6, and TNF-alpha, Thio-AgNPs effectively modulate inflammatory responses critical in managing chronic inflammatory diseases.

Their ability to decrease apoptosis-related proteins such as Bax while increasing BCl-2 levels suggests dual benefits of inflammation suppression and cell survival promotion. Moreover, the MTT assay affirmed their biocompatibility, ensuring safety for therapeutic applications.

These findings highlight Thio-AgNPs as promising candidates for treating conditions like rheumatoid arthritis and inflammatory bowel disease, where conventional therapies often fall short. Further research into their mechanisms and efficacy in vivo will be pivotal in advancing Thio-AgNPs towards clinical applications, potentially transforming how chronic inflammatory diseases are managed.

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) Cruvinel, W. d. M., et al. (2010). "Immune system: Part I. Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response." Revista brasileira de reumatologia **50**: 434-447.

4) Ezung, N. Z., et al. (2023). "A Study of Interspecies Transmission and Reassortment Events in Rotaviruses from Cattle in Pant Nagar, Uttarakhand, India." Int J Hum Genet **23**(2-3): 131-139.

5) Galatage, S. T., et al. (2021). "Silver nanoparticles: properties, synthesis, characterization, applications and future trends." Silver Micro-Nanoparticles—Properties, Synthesis, Characterization, and Applications.

6) Ganesh, S., et al. (2020). "Awareness on dental treatment during COVID-19 among south Indian population." International Journal of Current Research and Review: 165-170.

7) Gaur, S., et al. (2022). "Twin mixed local anesthesia in third molar surgery-randomized controlled trial." Journal of Oral and Maxillofacial Surgery **80**(1): 63-69.

8) 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.

9) Geurtsen, W., et al. (1998). "Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures." Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and the Australian Society for Biomaterials **41**(3): 474-480.

10) Hannoodee, S. and D. N. Nasuruddin (2020). "Acute inflammatory response."

11) Jiang, M. C., et al. (1996). "Curcumin induces apoptosis in immortalized NIH 3T3 and malignant cancer cell lines."

12) Khalid, J. P., et al. (2024). "Exploring Tumor-Promoting Qualities of Cancer-Associated Fibroblasts and Innovative Drug Discovery Strategies With Emphasis on Thymoquinone." Cureus **16**(2).

13) Kumar, V., et al. (2021). "Modified absorption and emission properties leading to intriguing applications in plasmonic–excitonic nanostructures." Advanced Optical Materials **9**(2): 2001150.

14) Mathivadani, V., et al. (2020). "Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach." Acta virologica **64**(1).

15) Mikhailova, E. O. (2020). "Silver nanoparticles: mechanism of action and probable bio-application." Journal of functional biomaterials **11**(4): 84.

16) Sadri, S. and A. J. Khoei (2023). "Ambient salinity affects silver nanoparticles (AgNPs) induced toxicity in the marine bivalve, the rock oyster, Saccostrea cucullata." Aquaculture Reports **30**: 101596.

17) 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.

18) Shah, S., et al. (2020). "Marginal Accuracy of Milled Versus Cast Cobalt Chromium Alloys in Long Span Implant-Supported Frameworks: A Systematic Review and Meta-analysis." Journal of Advanced Oral Research **11**(2): 120-127.

19) 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.

20) Subramanian, U., et al. (2018). "Marine algal secondary metabolites promising anti-angiogenesis factor against retinal neovascularization in CAM model." Res Rev AJ Life Sci **8**: 19-25.

21) Sundaram, K. K. M., et al. (2022). "Instrumentals behind embryo and cancer: a platform for prospective future in cancer research." AIMS Molecular Science **9**(1): 25-45.

22) 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.

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

24) 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).

25) Velumani, K., et al. (2023). "Advancements of fish-derived peptides for mucormycosis: a novel strategy to treat diabetic compilation." Molecular Biology Reports **50**(12): 10485-10507.

26) Yuvaraj, A., et al. (2020). "Perception of general public regarding pros and cons of technological advancements during lockdown due to covid 19 pandemic-an online survey." International Journal of Current Research and Review: 227-233.

27) Zhang, X.-F., et al. (2016). "Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches." International journal of molecular sciences **17**(9): 1534.