Pharmacogn. Res., 2025; 17(3):850-858.
https://www.phcogres.com

Original Article

Thymoquinone-Loaded Zinc Nanoparticles Mitigate Inflammation and Inhibit Glioblastoma Progression: A Novel Therapeutic Approach

Sofia Sebastian[1], Taniya Mary Martin[2], Meenakshi Sundaram Kishore Kumar[2,*]

[1]Department of Oral and Maxillofacial Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, INDIA.
[2]Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: Glioblastoma is the most aggressive and lethal primary brain tumour, characterized by rapid proliferation, high invasiveness, and resistance to conventional therapies. Chronic inflammation plays a crucial role in its progression, with pro-inflammatory cytokines contributing to tumour growth and therapy resistance. Nanotechnology based approaches, such as Thymoquinone derived Zinc Nanoparticles (TQ-ZnNPs) offer a promising strategy to enhance drug bioavailability and target inflammation driven glioblastoma more effectively. **Objectives:** Thymoquinone, a naturally occurring substance with anti-inflammatory and anticancer qualities, has drawbacks such as low bioavailability and solubility. To overcome these, TQ-ZnNPs were synthesized using nanotechnology. **Materials and Methods:** TQ-ZnNPs were synthesized using a green nanotechnology approach and characterized for their physicochemical properties through techniques like, Scanning Electron Microscopy (SEM), Fourier-Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD) and Ultraviolet-visible (UV-vis) spectroscopy examination to verify their optical and structural characteristics. Glioblastoma cells were treated with Lipopolysaccharide to induce inflammation, followed by exposure to TQ-ZnNPs to assess their anti-inflammatory and anti-cancer effects. RT-PCR method was used to assess gene expression. **Results:** TQ-ZnNPs exhibited enhanced stability and bioavailability, significantly reducing oxidative stress and suppressing pro-inflammatory cytokine expression in glioblastoma cells. They effectively inhibited cell proliferation and induced apoptosis, suggesting potent anti-inflammatory and anti-cancer properties. These findings highlight the therapeutic potential of TQ-ZnNPs in targeting inflammation driven glioblastoma. **Conclusion:** TQ-ZnNPs demonstrate significant anti-inflammatory and anti-cancer potential, making them a promising nanotherapeutic approach for combating inflammation driven glioblastoma.

Keywords: Anti-inflammatory, Glioblastoma, Inflammation, Lipopolysaccharide, Nanotechnology, Thymoquinone, Zinc nanoparticles.

Correspondence:
Dr. Meenakshi Sundaram Kishore Kumar
Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, INDIA.
Email: meenakshisundaram.sdc@saveetha.com

Received: 19-02-2025;
Revised: 29-04-2025;
Accepted: 02-06-2025.

INTRODUCTION

Glioblastoma Multiforme (GBM) stands as one of the most formidable and fatal brain cancers, notorious for its aggressive behaviour, rapid progression, and poor prognosis. Despite advancements in medical technology and treatment protocols, the survival rate for GBM patients remains dismally low, often limited to just 12-15 months post-diagnosis. Traditional treatment regimens, encompassing surgical resection, radiotherapy, and chemotherapy, face significant hurdles such as the tumour's infiltrative nature, intrinsic resistance mechanisms, and the blood-brain barrier, which collectively impede therapeutic efficacy.[1] Consequently, there is a pressing need for innovative therapeutic strategies that can effectively target both the tumour cells and the inflammatory milieu that supports tumour progression. A key factor in the pathogenesis of glioblastoma is inflammation. Numerous pro-inflammatory cytokines, chemokines, and growth factors contribute to the persistent inflammation that characterizes the tumor microenvironment in GBM. This inflammatory environment contributes to immunosuppression and treatment resistance in addition to encouraging tumor development and invasion. Targeting the inflammatory pathways in glioblastoma, therefore, represents a promising therapeutic avenue.[2]

DOI: 10.5530/pres.20252143

Black seed (*Nigella sativa*) contains Thymoquinone (TQ), a bioactive component that has been extensively studied for its potential as a treatment for a variety of diseases, including cancer. TQ has an array of pharmacological characteristics such as anti-inflammatory, antioxidant and anticancer activities.[3] Its ability to change several key molecular pathways associated with inflammation and cancer in glaucoma including Bcl-2 associated X protein (BAX), B cell lymphoma-2 (Bcl-2), Interleukin-2 (IL-2), Interleukin-6 (IL-6), and Transforming Growth Factor (TGF-α) in glioblastoma cells explains these features. Although TQ has a promising therapeutic profile, its rapid physiological breakdown, low bioavailability, and poor solubility hinder its clinical use. By improving the effectiveness and distribution of medicinal drugs, nanotechnology presents a strong answer to these problems.[4] The special physiochemical characteristics of Zinc Nanoparticles (ZnNPs), such as their high surface area-to-volume ratio, improved cellular absorption, and biocompatibility, make them especially appealing in this situation. Additionally, ZnNPs have anti-oxidant and anti-inflammatory properties of their own that can complement the medicinal benefits of encapsulated substances. Thus, TQ-ZnNPs has a lot of potential to increase TQ's bioavailability, stability, and therapeutic effectiveness in the treatment of cancer. Lipopolysaccharide (LPS), a significant part of gram-negative bacteria's outer membrane, is frequently utilized in a variety of experimental contexts to stimulate inflammation. LPS triggers a strong inflammatory response by activating immune cells' Toll-like Receptor 4 (TLR4), which causes the production of numerous pro-inflammatory cytokines, including TGF- α, Bax, Bcl-2, IL-2, and IL-6. LPS- induced inflammation in glioblastoma can worsen the tumor growth and treatment resistance. Thus, it is essential to examine the anti-inflammatory properties of possible treatments in models of LPS-induced glioblastoma in order to create efficient therapies. The goal of this work is to investigate TQ-ZnNPs' anti-inflammatory and anticancer properties in LPS-induced glioma cells. We predict that TQ-ZnNPs can successfully reduce inflammation and stop the growth of glioblastoma cells by utilizing the combined attributes of TQ and Zn NPs. Particularly, this study will concentrate on assessing how TQ-ZnNPs affect the expression of important inflammatory cytokines, the initiation of inflammatory signalling pathways, and the triggering of glioblastoma cell death by apoptosis.[2,5]

LPS-induced glioma cell lines will be exposed to varying concentrations of TQ-ZnNPs as part of the experimental plan, and the impact of these treatments on molecular signalling pathways and cell viability will be assessed.[6] Using TQ-derived zinc nanoparticles to treat LPS-induced glioma cells is a novel and promising therapeutic strategy. TQ-ZnNPs may be able to overcome the limitations of currently available glioblastoma treatments and improve patient outcomes by addressing tumor development and inflammation. This study aims to pave the way for potential future therapeutic applications in glioblastoma therapy by better understanding the mechanisms underlying TQ-ZnNPs' anti-inflammatory and anticancer effects.[7]

MATERIALS AND METHODS

Synthesis of TQ-ZnNPs

All chemicals used in this study were of standard grade and procured from HiMedia, Mumbai, India. TQ-ZnNPs were synthesized using the sol gel method with Zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) as the zinc precursor and TQ serving as both stabilizing and functionalizing agent. The synthesis involved dissolving $Zn(CH_3COO)_2.2H_2O$ in ethanol, followed by gradual addition of TQ solution prepared in Dimethyl Sulfoxide (DMSO). The mixture was continuously stirred at room temperature for 2 hr to facilitate complete reaction. After allowing the solution to settle for 24 hr, deionized water was added to promote nanoparticle precipitation. The resulting precipitate was carefully collected, thoroughly washed with ethanol to eliminate residual impurities, and dried at 60°C for 12 hr. This method produces TQ-ZnNPs with enhanced bioavailability and functional attributes, making them suitable for further characterization and biological studies.[2,8]

Characterization of TQ-ZnNPs

The structural and optical properties of TQ-ZnNPs were analysed by Scanning Electron Microscopy (SEM), Fourier-Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD) and Ultraviolet-visible (UV-vis) spectroscopy. FTIR spectra was recorded in the range of 4000-400 cm^{-1} by using a PerkinElmer FTIR spectrometer (USA, Waltham) to identify functional groups and conform the successful incorporation of TQ onto the ZnNPs. UV-vis spectral analysis was conducted using a Shimadzu UV-1800 spectrophotometer (Japan, Kyoto), with absorbance measurements taken between 200 and 800 nm to evaluate the nanoparticles' optical properties. The phase purity and crystalline structure of TQ-ZnNPS were determined through XRD analysis using a Rigaku X-ray diffractometer (Japan, Tokyo) with Cu Kα radiation (λ=1.5406 Å). The characterisation techniques confirmed stability, functionalization, and crystalline nature of the synthesized nanoparticles ensuring a suitability for further biological investigations.[9,10]

Cell culture and treatment

Glioblastoma cell lines, obtained from National Centre for Cell Science (Pune, India) were cultured under standard conditions at 37°C with 5% CO_2 in a suitable growth medium supplemented with foetal bovine serum (Gibco, India) and penicillin-streptomycin (Gibco). To stimulate glioblastoma associated inflammation, cells were pretreated with Lipopolysaccharide (LPS) before exposing varying concentration of TQ-ZnNPs. The study included multiple experimental groups; LPS-induced inflammatory cells treated with different doses of TQ-ZnNPs, and LPS-induced

inflammation group without treatment, and a control group of untreated cells.[11]

Cell Viability Assay

The MTT assay (HiMedia, India) was employed to assess the viability of glioblastoma cells. Cells were seeded in 96-well plates at a density of $5x10^4$ cells per well and treated with varying concentration of TQ-ZnNPs for 48 hr. After treatment period, 10 µL of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well to facilitate formazan crystal formation, followed by incubation for 4 hr. The resulting crystals were then dissolved in DMSO (HiMedia, India) and absorbances were measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control group. Statistical analysis was performed using two-way Analysis of Variance (ANOVA) followed by Bonferroni *post hoc* test to assess the difference between treated groups.[12]

Gene expression analysis

This study examined the gene expression of Bax, BCl-2, TGF-α, IL-2 and IL-6 in glioblastoma cells treated with TQ-ZnNPs to evaluate their anticancer potential. Glioblastoma cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin under standard conditions (5% CO_2, 37°C). for gene expression analysis cells were seeded in 6-well plates and treated with different concentrations of TQ-ZnNPs for 48 hr. Total RNA was isolated using TRIzol reagent (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions, RNA concentrsatin and purity were determined using nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Subsequently 1 µg of total RNA was reverse transcribed into complementary DNA (cDNA) using PrimeScript RT reagent Kit (Takara Bio Inc.) for further gene expression analysis (Table 1).

Quantitative Polymerase Chain Reaction (qPCR) was performed to measure the expression levels of Bax, Bcl-2, TGF-α, IL-2, IL-6 with GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) serving as a reference gene for normalisation. Specific primers were designed for each target gene and their amplification efficiency was validated prior to qPCR. Reactions were conducted using CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, USA, Hercules) in a 20 µL reaction mixture containing gene specific primers, cDNA templates, and SYBR Green Thermo Fisher Scientific's Master Mix. The qPCR protocol included an initial denaturation at 95°C for 10 min, followed by 40 amplification cycles at 95°C for 60 sec. Melt curve analysis was performed to conform the specificity of amplified products. Gene expression levels were normalized to GAPDH and analysed using $2^{-\Delta\Delta Ct}$ method. Statistical significance was determined using Mann-Whitney U test and *t*-test to compare differenced between groups.

Statistical Analysis

Each experiment was run in triplicate, and the mean±Standard Deviation (SD) represents the way the data are shown. Software called GraphPad Prism 8 (USA, San Diego, GraphPad Software) was used for statistical analyses. The MTT assay data was analysed using Bonferroni *post hoc* test and two-way ANOVA, while the gene expression results were assessed using Mann-Whitney U test and a *t*-test. A *p*-value of less than 0.05 indicated that the difference was statistically significant.

RESULTS

The characterization results confirm that TQ-ZnNPs possess distinct chemical and structural properties. SEM analysis reveals an irregular morphology suggesting non uniform crystal formation which may contribute to enhance surface area, potentially benefiting applications such as drug delivery or catalysis (Figure 1). FTIR spectroscopy identifies characteristic

Table 1: Primer sequences used for amplifying the specific genes in gene expression analysis.

Gene	Primer type	Sequence
Bax	Forward	5'-TCCACCAAGAAGCTGAGCGAG-3'
	Reverse	5'-GTCCAGCCCATGATGGTTCTG-3'
BCl-2	Forward	5'-GGGAGGATTGTGGCCTTCTTT-3'
	Reverse	5'-TGAAGGAGCGCAACCGGA-3'
TNF-alpha	Forward	5'-GCCCAGACCCTCACACTCAG-3'
	Reverse	5'-GCTACAGGCTTGTCACTCGG-3'
IL-2	Forward	5'-AGCAGCTGTTGATGGACCTACC-3'
	Reverse	5'-AGTTGATGGACCTGGGAAAGG-3'
IL-6	Forward	5'-CCAGGAGCCCAGCTATGAA-3'
	Reverse	5'-CCAGGCAAGTCTCCTCATTGA-3'

peaks at 1062.95, 1013.14, 861.78, 799.23, 675.18, and 590.26 indicating the presence of functional groups associated with both TQ and zinc oxide (Figure 2). These peaks correspond to specific molecular vibrations and chemical bonds, providing insight into the composition and interaction within the nanoparticles. XRD analysis conforms a crystalline phase of approximately 30.1% and an amorphous phase of around 79.9%, reflecting the nanoparticles' atomic arrangement, which may influence their physical and chemical behaviour (Figure 3). Additionally, UV-vis spectroscopy exhibits a peak at 268 nm, indicating electronic transition within the TQ-ZnNPs and conforming their unique optical properties.

The findings of the characterisation reveal that TQ-ZnNPs have unique chemical and structural characteristics. Scanning Electron Microscopy (SEM) reveals an irregular morphology, indicative of non-uniform crystal formation, which may enhance specific properties such as drug delivery or catalysis by increasing the surface area (Figure 1). This is suggestive of non-uniform crystal formation. The presence of functional groups linked to both TQ and zinc oxide is indicated by distinctive peaks at wave numbers 1062.95, 1013.14, 861.78, 799.23, 675.18, and 590.26 that are identified by Fourier-Transform Infrared (FTIR) spectroscopy (Figure 2). The composition and interactions within the TQ-ZnNPs are revealed by these peaks, which correlate to different chemical bonds and molecular vibrations. The nanoparticles' ordered atomic organisation, which might affect their physical and chemical behaviours, is reflected in their crystalline phase of about 30.1% and amorphous phase of about 79.9%, according to X-Ray Diffraction (XRD) study. Furthermore, a peak at 268 nm is shown by UV spectroscopy, indicating the presence of electronic transitions within the TQ-ZnNPs and verifying their distinct optical characteristics.

Invitro anti-inflammatory and antioxidant analysis

Effect of TQ-ZnNPs on cell viability

The MTT assay, a popular technique for determining cell viability, was performed to examine the cytotoxicity of TQ-ZnNPs in glioblastoma cells. Cell viability was assessed in this assay by treating glioblastoma cells with varying concentrations (10, 20, 40, 60, 80, and 100 µg/mL) of TQ-ZnNPs for 24 and 48 hr. When MTT reagent is reduced to formazan crystals, it indicates the existence of metabolically active cells. The findings showed that cell viability decreased in a dose-dependent manner, with larger TQ-ZnNP concentrations resulting in a noticeably lower number of viable cells. This implies that glioblastoma cells are significantly cytotoxically affected by TQ-ZnNPs. The MTT assay (Figure 3) validates these nanoparticles' continued investigation as therapeutic agents in the treatment of glioblastoma by confirming their potential as efficient agents for identifying and destroying malignant brain tumour cells. In glioma cells, TQ-ZnNPs enhanced Bax expression in a concentration-dependent manner (Figure 4). Bax levels increased significantly between 33 and 66 µg/mL, suggesting a favourable relationship between pro-apoptotic signalling and TQ-ZnNP concentration. TQ-ZnNPs may be able to successfully induce cell death in glioblastoma, as evidenced by the correlation between decreased cell viability and an increase in Bax, a crucial protein that regulates apoptosis. In glioma cells, TQ-ZnNPs reduced Bcl-2 expression in a concentration-dependent manner (Figure 5). There was an apparent reduction in Bcl-2 levels with increasing concentrations of 33 and 66 µg/mL, indicating an inverse link between TQ-ZnNPs concentration and anti-apoptotic protein production (BCl2). This implies that by downregulating important survival pathways, TQ-ZnNPs successfully shift the scales in favour of apoptosis. Although more research is required to maximise their clinical application, these findings demonstrate the promise of TQ-ZnNPs in upsetting survival systems and improving the therapeutic potential against glioblastoma.

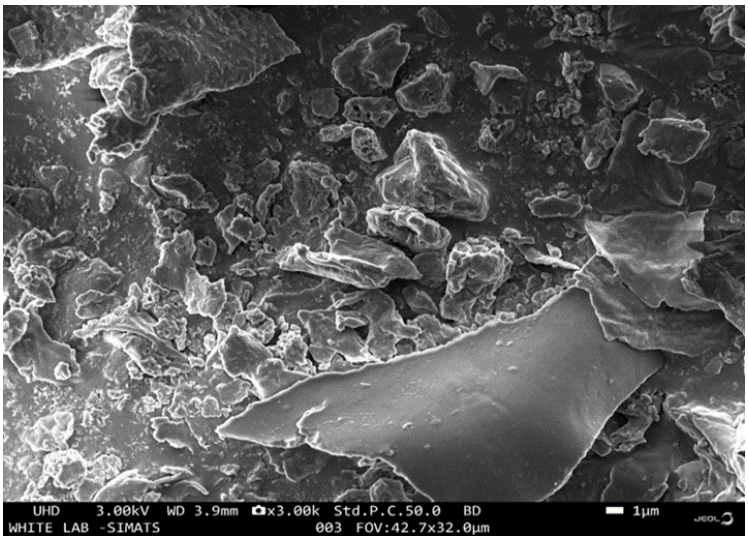

Figure 1: SEM analysis of TQ-ZnNPs revealed crystal-like structure morphology.

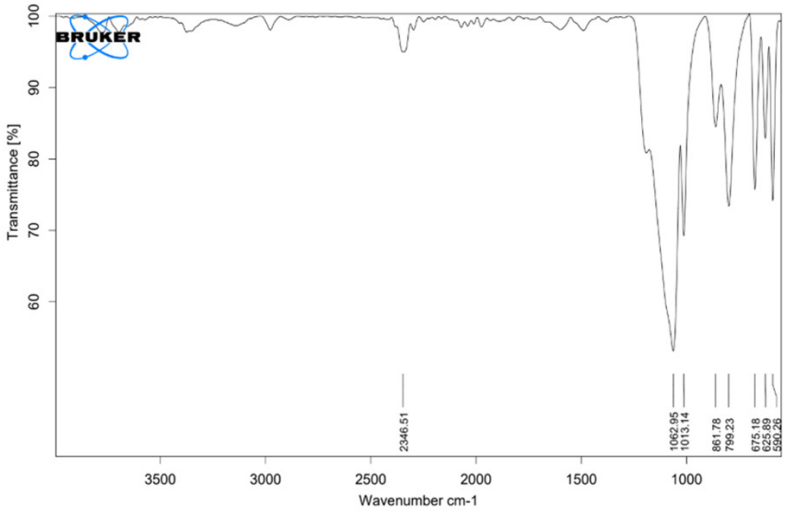

Figure 2: FTIR spectrum represent specific vibrational modes corresponding to functional groups present in the TQ-ZnNPs.

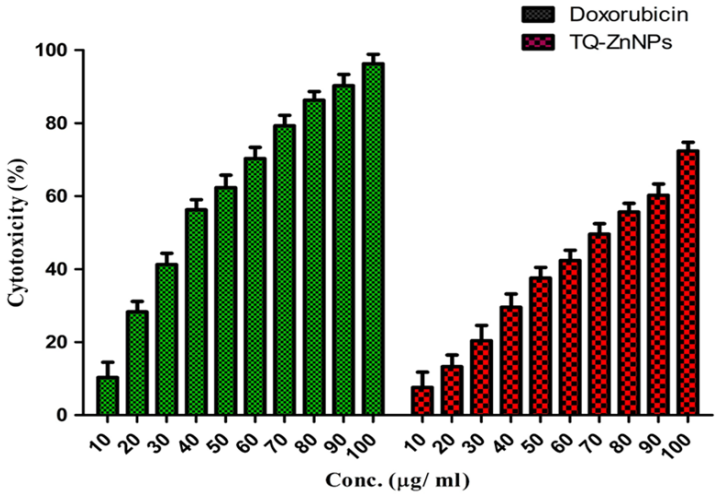

Figure 3: Cytotoxicity of TQ-ZnNPs in glioblastoma cells.

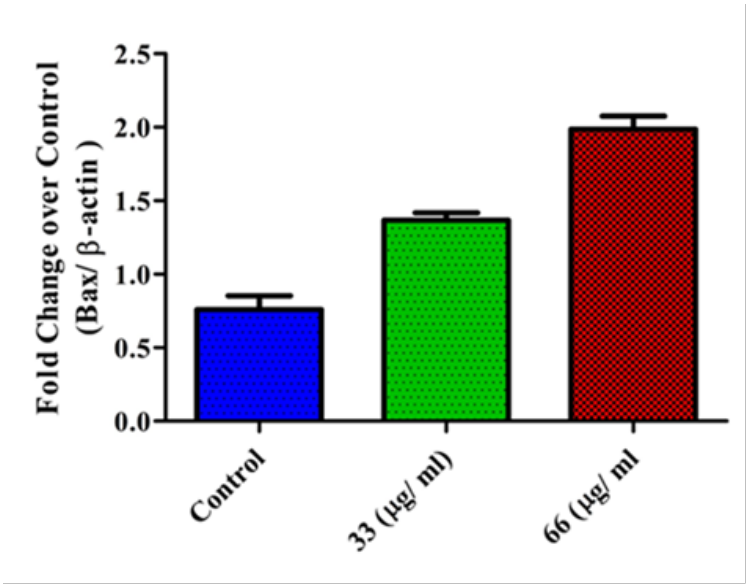

Figure 4: TQ-ZnNPs increased Bax expression on glioblastoma cells in concentration-dependent manner.

In glioma cells, TQ-ZnNPs were observed to reduce TNF-α expression in a concentration-dependent manner (Figure 6). While the decrease in TNF-α expression was extremely statistically significant at 66 µg/mL, it was not statistically significant at 33 µg/mL. One important pro-inflammatory cytokine that affects the tumour microenvironment and regulates immune responses is TNF-α. With their ability to alter cytokine profiles and reduce inflammatory responses in glioblastoma, these findings demonstrate the dose-dependent effect of TQ-ZnNPs on inflammatory pathways. More research is required to determine whether decreased TNF-α expression could be used to enhance glioblastoma therapies by reducing inflammation and creating a microenvironment that is less conducive to tumour growth. In glioma cells, TQ-ZnNPs reduced IL-2 expression in a concentration-dependent manner (Figure 7). The levels of IL-2, a cytokine essential for immunological response and T-cell activation, decreased when TQ-ZnNP concentrations raised to 33 and 66 µg/mL. The decline in IL-2 expression was very significant at 66 µg/mL, but not statistically significant at 33 µg/mL. This suggests that at greater concentrations, TQ-ZnNPs can inhibit immune-regulatory signals. TQ-ZnNPs may modify immunological pathways, impacting tumor-immune interactions and reducing immune-mediated cytotoxicity in glioblastoma, according to the modulation of IL-2. To fully comprehend the potential consequences of IL-2 inhibition and its possible function in the therapeutic application of TQ-ZnNPs, more research is necessary.

IL-6's expression using TQ-ZnNPs decreased in glioblastoma cells in a concentration-dependent manner (Figure 8). IL-6, a pro-inflammatory cytokine involved in tumour progression and survival, was significantly reduced with increasing concentrations of TQ-ZnNPs. This reduction suggests that TQ-ZnNPs effectively suppress IL-6-mediated inflammatory pathways, which are often associated with tumour progression and resistance. By downregulating IL-6, TQ-ZnNPs could potentially disrupt tumour-promoting inflammation, making them a promising candidate for glioblastoma therapy. Further studies are required to elucidate the mechanisms of IL-6 suppression and its therapeutic implications.

DISCUSSION

GBM is a highly aggressive and lethal brain tumor with limited therapeutic options due to its complex tumor microenvironment, infiltrative nature, and resistance to conventional therapies. Targeting both the tumour cells and the inflammatory milieu that supports tumor progression is critical for improving treatment outcomes.[13] In this context, the integration of TQ and Zinc Nanoparticles (ZnNPs) offers a promising avenue for addressing these challenges. However, its clinical application has been hindered by poor solubility and bioavailability. Nanotechnology, particularly ZnNPs, enhances TQ's therapeutic potential by improving stability, solubility, and cellular uptake.[14] The study findings demonstrate that TQ-ZnNPs exhibit dose-dependent cytotoxic effects against glioblastoma cells. Through MTT assays, significant reductions in cell viability were observed, confirming their potential as effective therapeutic agents. Mechanistically, TQ-ZnNPs modulate apoptotic signalling by upregulating Bax (pro-apoptotic) and downregulating Bcl-2 (anti-apoptotic), thereby inducing apoptosis in glioblastoma cells. This shift in the apoptotic balance underlines their ability to disrupt tumor survival mechanisms. Moreover, TQ-ZnNPs significantly reduced the expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-2 in a concentration-dependent manner. These cytokines are integral to the tumor-promoting inflammatory pathways in GBM.[15,16] TNF-α suppression highlights a potential reduction in immune evasion and microenvironment-mediated tumor

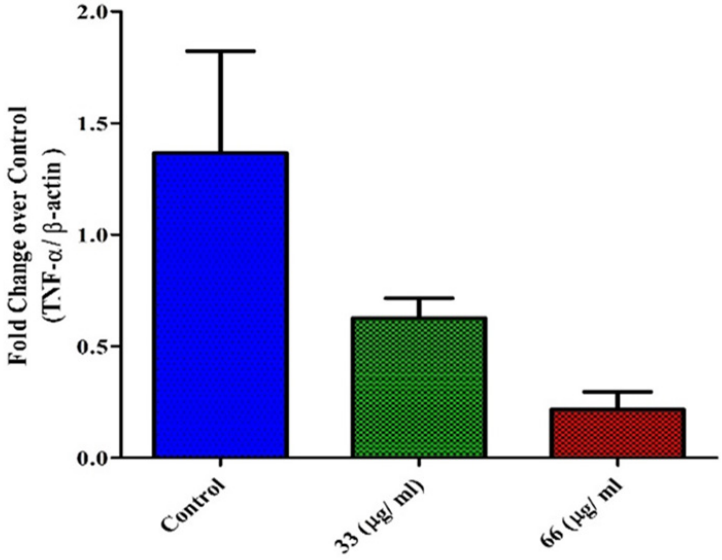

Figure 5: TQ-ZnNPs decreased BCl2 expression on glioblastoma cells in concentration-dependent manner.

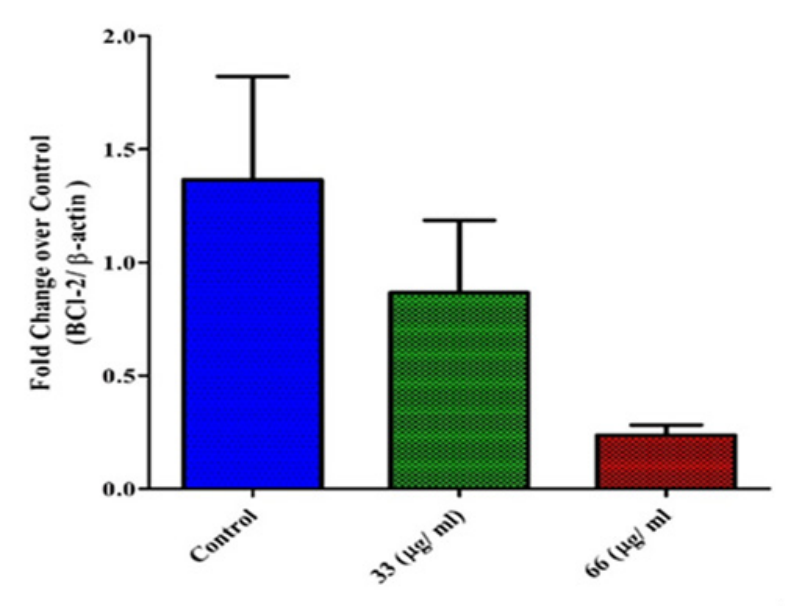

Figure 6: TQ-ZnNPs decreased TNF-α expression on glioblastoma cells in concentration-dependent manner.

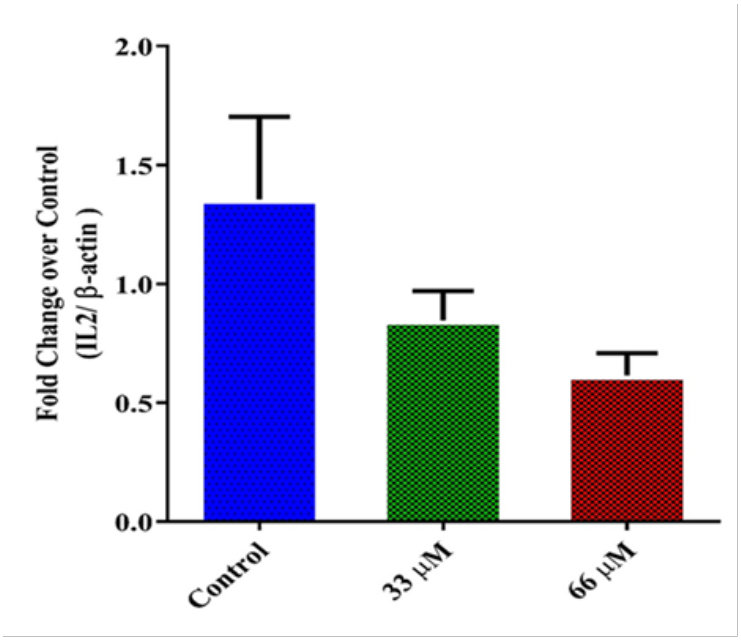

Figure 7: TQ-ZnNPs decreased IL-2 expression on glioblastoma cells in a concentration-dependent manner.

progression, while IL-6 downregulation may mitigate tumor survival and resistance pathways. Interestingly, the reduction in IL-2 levels suggests an influence on immune modulation, which warrants further exploration to understand its dual role in tumor immunity and inflammation. These findings underscore the potential of TQ-ZnNPs to target both tumor cells and the inflammatory microenvironment, addressing critical aspects of GBM pathophysiology. However, several challenges remain, including optimizing the dosage for maximal therapeutic efficacy while minimizing off-target effects. Furthermore, *in vivo* studies and clinical trials are necessary to validate these findings and determine safety profiles. TQ-ZnNPs represent a promising therapeutic strategy for GBM, with the potential to overcome existing treatment barriers by targeting both apoptotic and inflammatory pathways. Their ability to modulate the tumor microenvironment and disrupt tumor progression holds promise for future advancements in glioblastoma therapy.[17]

The unique physicochemical properties of ZnNPs, such as their high surface area-to-volume ratio and enhanced cellular uptake, contribute significantly to their therapeutic efficacy. These properties enable better penetration of the blood-brain barrier, a critical obstacle in GBM treatment, and facilitate targeted delivery of TQ to tumor sites. By improving the bioavailability of TQ, ZnNPs address a major limitation in its clinical

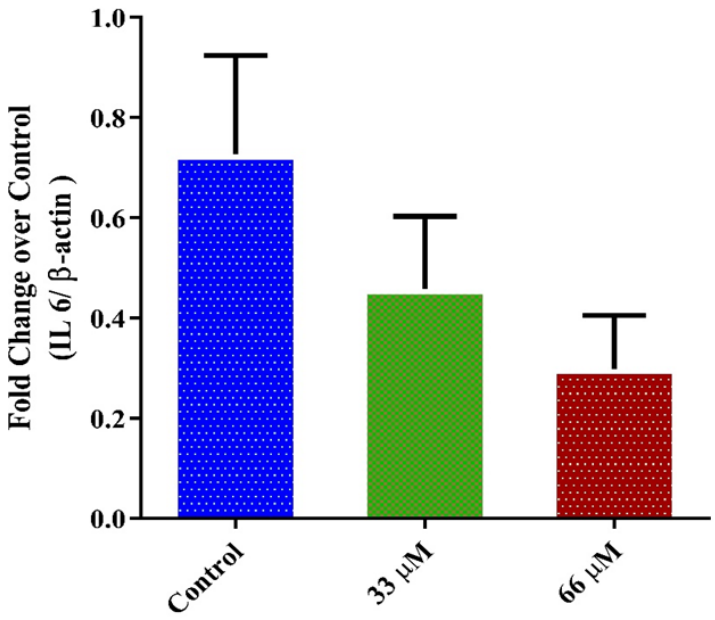

Figure 8: TQ-ZnNPs decreased IL-6 expression on glioblastoma cells in a concentration-dependent manner.

application, ensuring sustained and effective therapeutic action. Another noteworthy aspect of TQ-ZnNPs is their potential to reduce therapy resistance in GBM.[2,5,18] By downregulating pro-inflammatory cytokines like IL-6 and TNF-α, which are implicated in tumor progression and treatment resistance, TQ-ZnNPs may enhance the efficacy of existing therapeutic modalities, including radiotherapy and chemotherapy. This multi-faceted approach not only suppresses tumor growth but may also prevent recurrence by altering the tumor-supportive microenvironment. Future studies could explore the combination of TQ-ZnNPs with other therapeutic agents, such as immune checkpoint inhibitors or targeted molecular therapies, to develop synergistic treatment strategies. Additionally, evaluating the long-term effects of TQ-ZnNPs in preclinical models and conducting pharmacokinetic studies will provide insights into their safety, biodistribution, and metabolism, paving the way for clinical trials. Overall, the development of TQ-ZnNPs represents a paradigm shift in glioblastoma treatment, offering a dual-action approach that combines direct tumor cytotoxicity with modulation of the inflammatory microenvironment. By addressing multiple facets of GBM pathogenesis, TQ-ZnNPs hold the promise of improving patient outcomes and advancing the field of nanotechnology-based oncology.[19,20]

CONCLUSION

This study concludes by demonstrating the anti-inflammatory properties of TQ-ZnNPs in Glioblastoma cells induced by LPS. The findings show that the expression of TNF- α, a crucial pro-inflammatory cytokine implicated in immune modulation and tumor microenvironment dynamics decreases in a concentration dependent manner. At higher concentrations, TQ-ZnNPs significantly suppressed TNF-α expression, suggesting their ability to modulate inflammatory pathways effectively. This anti-inflammatory effect positions TQ-ZnNPs as a promising therapeutic candidate for glioblastoma, a highly aggressive and treatment-resistant brain tumour often associated with inflammation-driven progression. The findings underscore the potential of TQ-ZnNPs to target inflammation-driven tumorigenesis, offering a dual advantage of mitigating inflammation and modulating the tumour microenvironment.

These characteristics may open the door to the creation of novel therapeutic approaches meant to increase the effectiveness of glioblastoma therapy by reducing the negative effects of traditional therapies. To understand the molecular processes behind TQ-ZnNPs' anti-inflammatory effects, more investigation is necessary. Thorough preclinical and clinical research must be performed to evaluate their safety, bioavailability, and *in vivo* therapeutic effectiveness. TQ-ZnNPs may become a novel, tailored treatment for glioblastoma with future research making a substantial contribution to the field of cancer nanomedicine.

ACKNOWLEDGEMENT

I would like to extend my sincere appreciation to Saveetha Dental and Medical College for their invaluable support and resources that made this research possible. Their commitment to excellence in education and research has significantly contributed to the advancement of knowledge in our field.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TQ-ZnNPs: Thymoquinone derived zinc nanoparticles; **TQ:** Thymoquinone; **GBM:** Glioblastoma multiforme; **BAX:** BCL_2 associated X protein; **Bcl-2:** B cell lymphoma-2; **IL-2:** Interleukin-2; **IL-6:** Interleukin-6; **TGF-α:** Tumor Growth Factor; **LPS**: Lipopolysaccharide; **TLR-4:** Toll-like receptor 4; **SEM:** Scanning Electron Microscopy; **FTIR:** Fourier-transform infrared spectroscopy; **XRD:** X-ray diffraction; **UV-vis:** Ultraviolet-visible spectroscopy; **qPCR:** Quantitative Polymerase Chain Reaction; **GAPDH:** Glyceraldehyde-3-phosphate dehydrogenase.

SUMMARY

Glioblastoma, a highly aggressive brain tumor, is driven by chronic inflammation, which contributes to its growth and resistance to treatment. Thymoquinone (TQ), a compound with anti-inflammatory and anticancer properties, has limitations such as low bioavailability. To enhance its effectiveness, TQ was combined with Zinc Nanoparticles (TQ-ZnNPs) using green nanotechnology. The TQ-ZnNPs were synthesised and characterized for their physical and chemical properties. *In vitro* studies on glioblastoma cells showed that TQ-ZnNPs reduced oxidative stress, suppressed pro-inflammatory cytokines, inhibited cell proliferation, and induced apoptosis. These results suggest that TQ-ZnNPs offer significant anti-inflammatory and anti-cancer potential, making them a promising strategy for treating inflammation-driven glioblastoma.

REFERENCES

1. Bijalwan G, Shrivastav AK, Mallik S, Dubey MK. Glioblastoma multiforme-a rare type of cancer: A narrative review. Cancer Res Stat Treat. 2024;7(3):340-51. doi: 10.4103/crst.crst_250_23.
2. Dandagi P, K YB, Mary Martin T, K MS. *In silico* and glioblastoma cell line evaluation of thioflavin-derived zinc nanoparticles targeting beclin protein. Cureus. 2024;16(9):e69319. doi: 10.7759/cureus.69319, PMID 39398744.
3. Priyadharshini Jr R, Yuwanati M, Sekaran S, Senthilmurugan M, Priyadharshini R, Senthil Murugan S. miRNA associated with glucose transporters in oral squamous cell carcinoma: a systematic review. Cureus. 2023;15(9). DOI:10.7759/cureus.46057.
4. Krishnan RP, Pandiar D, Ramani P, Jayaraman S, Subramanian R, Nandhakumar R. Comparison of clinico-demographic and histological parameters between young and old patients with oral squamous cell carcinoma. Cureus. 2023;15(11). DOI: 10.7759/cureus.48137.
5. Qiang L, Yang Y, Ma YJ, Chen FH, Zhang LB, Liu W, *et al.* Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. Cancer Lett. 2009;279(1):13-21. doi: 10.1016/j.canlet.2009.01.016, PMID 19232461.
6. Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2021;78(4):1233-61. doi: 10.1007/s00018-020-03656-y, PMID 33057840.
7. Russo V, Falco L, Tessitore V, Mauriello A, Catapano D, Napolitano N, *et al.* Anti-inflammatory and anticancer effects of anticoagulant therapy in patients with malignancy. Life (Basel). 2023;13(9):1888. doi: 10.3390/life13091888, PMID 37763292.
8. Jamkhande PG, Ghule NW, Bamer AH, Kalaskar MG. M, *et al* nanoparticles synthesis: an overview on methods of preparation, advantages and disadvantages, and applications. J Drug Deliv Sci Technol. 2019;53:101174.
9. Payra M, Mohanraj KG, Mary Martin T, K MS. Modulation of inflammation in McCoy cells by zinc nanoparticles conjugated with β-chitosan. Cureus. 2024;16(9):e68653. doi: 10.7759/cureus.68653, PMID 39371713.
10. Challa D, Pandi C, Kannan B, Priyadharsini VJ, Arumugam P. Exploring the Expression of BCAS3 in Head and Neck Squamous Cell Carcinoma and Its Association With Prognosis. Cureus. 2023;15(12). DOI: 10.7759/cureus.50995.
11. Zhang WJ, Wei H, Hagen T, Frei B. α-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. Proc Natl Acad Sci U S A. 2007;104(10):4077-82. doi: 10.1073/pnas.0700305104, PMID 17360480.
12. Yin K, Zhu R, Wang S, Zhao RC. Low level laser (LLL) attenuate LPS-induced inflammatory responses in mesenchymal stem cells via the suppression of NF-κB signaling pathway *in vitro*. PLOS One. 2017;12(6):e0179175. doi: 10.1371/journal.pone.0179175, PMID 28594941.
13. Yalamarty SS, Filipczak N, Li X, Subhan MA, Parveen F, Ataide JA, *et al.* Mechanisms of resistance and current treatment options for glioblastoma multiforme (GBM). Cancers. 2023;15(7):2116. doi: 10.3390/cancers15072116, PMID 37046777.
14. Ali T, Hussain F, Naeem M, Khan A, Al-Harrasi A. Nanotechnology approach for exploring the enhanced bioactivities and biochemical characterization of freshly prepared *Nigella sativa* L. nanosuspensions and their phytochemical profile. Front Bioeng Biotechnol. 2022;10:888177. doi: 10.3389/fbioe.2022.888177, PMID 35656198.
15. Dharini S, Ramalingam K, Ramani P, Krishnan M. A concordance between clinical and pathological tumor staging of oral squamous cell carcinoma: an institutional study. Cureus. 2024;16(6).
16. Senthil R, Meenakshi Sundaram KK, Bupesh G, Usha S, Saravanan KM. Identification of oxazolo [4, 5-g] quinazolin-2 (1H)-one derivatives as EGFR inhibitors for cancer prevention. Asian Pac J Cancer Prev. 2022;23(5):1687-97. doi: 10.31557/APJCP.2022.23.5.1687, PMID 35633554.
17. Tayyeb JZ, Priya M, Guru A, Kishore Kumar MS, Giri J, Garg A, *et al.* Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells. Mol Biol Rep. 2024;51(1):423. doi: 10.1007/s11033-024-09407-7, PMID 38489102.
18. Maletínská L, Blakely EA, Bjornstad KA, Deen DF, Knoff LJ, Forte TM. Human glioblastoma cell lines: levels of low-density lipoprotein receptor and low-density lipoprotein receptor-related protein. Cancer Res. 2000;60(8):2300-3. PMID 10786698.
19. Liu Q, Li G, Li R, Shen J, He Q, Deng L, *et al.* IL-6 promotion of glioblastoma cell invasion and angiogenesis in U251 and T98G cell lines. J Neurooncol. 2010;100(2):165-76. doi: 10.1007/s11060-010-0158-0, PMID 20361349.
20. Lampronti I, Martello D, Bianchi N, Borgatti M, Lambertini E, Piva R, et al. *In vitro* antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. Phytomedicine. 2003;10(4):300-8. doi: 10.1078/094471103322004794, PMID 12809360.

Cite this article: Sebastian S, Martin TM, Kumar MSK. Thymoquinone-Loaded Zinc Nanoparticles Mitigate Inflammation and Inhibit Glioblastoma Progression: A Novel Therapeutic Approach. Pharmacog Res. 2025;17(3):850-8.