Research Article

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# Thymoquinone-derived Titanium Nanoparticles Induce Nrf2-mediated Anticancer Activity against KB Cells

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## ABSTRACT

Oral squamous cell carcinoma (OSCC) is a highly aggressive cancer with unfavorable therapeutic responses and high recurrence. KB cells are used as a model to investigate OSCC response to new anticancer drugs. Thymoquinone, a phytochemical obtained from *Nigella sativa*, has high antioxidant and anticancer activities. The purpose of this investigation is the synthesis of titanium nanoparticles based on thymoquinone (TQ-TiNPs) and assessment of their cytotoxicity and antioxidant activity in KB cells with attention towards Nrf2 pathway modulation. TQ-TiNPs were prepared through green chemistry by reducing titanium(IV) isopropoxide using thymoquinone. The nanoparticle characterization was done through UV-Visible spectroscopy, FTIR, XRD, SEM, DLS, and zeta potential analysis. KB cells were treated with TQ-TiNPs at doses of 1, 10, 25, 50, and 100 µg/mL, and the cytotoxicity was evaluated through MTT assay. The DPPH assay was carried out to measure antioxidant activity. The gene expression of Nrf2, Keap1, HO-1, NQO1, and Caspase-3 was evaluated through quantitative real-time PCR. Characterization identified the development of stable, spherical, and crystalline nanoparticles with 300–400 nm absorbance and <100 nm particle size. TQ-TiNPs inhibited KB cell viability in a dose-dependent manner ($p < 0.05$). DPPH assay identified robust radical scavenging activity, especially at high concentrations. Gene expression analysis identified upregulation of Nrf2, HO-1, NQO1, and Caspase-3 and downregulation of Keap1, representing activation of antioxidant and apoptotic pathways. Thymoquinone-derived titanium nanoparticles exerted potent anticancer activity against KB cells by modulating the Nrf2/Keap1 signalling pathway and inducing apoptosis. The results endorse the promise of TQ-TiNPs as a green nanotherapeutic approach towards oral cancer.

**Keywords:** Thymoquinone; Titanium nanoparticles; KB cells; Nrf2; Apoptosis; Antioxidant; Gene expression; Oral cancer.

## 1. INTRODUCTION

Cancer remains one of the leading public health issues globally, marked by uncontrollable cell proliferation, apoptosis resistance, and evasion of immune surveillance. Oral squamous cell carcinoma (OSCC), which includes KB cells as a model, is responsible for most oral cancers and has high therapeutic resistance and recurrence rates (Sanchez *et al.* 2021). Even with the development of chemotherapeutics and targeted therapy, the inherent high toxicity and lack of specificity of traditional drugs have capped their efficiency and tend to produce systemic side effects. Therefore, identification of more efficient and less toxic anticancer agents is an urgent focus of research. Nanotechnology has come to the fore as a revolutionary platform in oncology, which facilitates the delivery of chemotherapeutic agents with improved bioavailability, specificity, and diminished toxicity (Hakim *et al.* 2025). Among different nanocarriers, metal-based nanoparticles have drawn interest because of their prominent physicochemical characteristics, such as high surface area, controlled release properties, and ease of functionalization. Titanium dioxide ($TiO_2$) nanoparticles, specifically, have been thoroughly examined because of their biocompatibility, photocatalytic potential, and ROS generation ability under oxidative stress conditions. The higher surface area and smaller size make them as efficient materials on cellular uptake, and surface functionalization with other adjuvants such as ligand and or antibodies, enhance their active tumor targeting. Besides, their deliverable properties, they are also acting as anticancer agents, to potentiate the DNA damage, disrupting the mitochondrial function, and inhibiting the angiogenesis on tumor sites. Due to these features, titanium-based nanoparticles can be potential anticancer candidates (Afrin *et al.* 2025; Rajasekar *et al.* 2024).

Thymoquinone (TQ), a bioactive compound isolated from *Nigella sativa*, is being studied intensely for its pharmacological actions with a broad spectrum of antioxidant, anti-inflammatory, anti-microbial, and

anticancer effects. Its anticancer activity is postulated to be due to various mechanisms like inhibition of cell growth, induction of apoptosis, suppression of angiogenesis, and modulation of the central signalling cascades like P13K/AKT, NF-κB, and MAPK. Most recently, the nuclear factor erythroid 2-related factor (Nrf2) pathway has been identified as an essential target of TQ for controlling oxidative stress and keeping cellular redox homeostasis. The Nrf2 signaling pathway plays a crucial role in the cellular antioxidant defence mechanism against oxidative injury by regulating the expression of numerous antioxidant and cytoprotective genes (Sebastian *et al.* 2025). Under basal conditions, Nrf2 is retained in the cytoplasm by its inhibitory protein Keap 1, which facilitates its ubiquitination and proteasomal degradation. On encountering oxidative stress or electrophilic substances, Nrf2 separates from Keap1, translocates to the nucleus, and activates antioxidant response elements within the promoter regions of target genes like heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1), and glutathione S transferases. Nrf2 activation promotes resistance to oxidative stress, increases detoxification and suppresses cancer development (Fathima *et al.* 2025).

Though the Nrf2 pathway is largely cytoprotective in non-cancer cells, modulation thereof in cancer treatment is a double-edged sword. At the initial stages of carcinogenesis, Nrf2 activation inhibits tumour initiation by preventing DNA damage and inducing apoptosis. In other advanced cancers, however, constitutive activation of Nrf2 could enhance chemoresistance and tumour survival. Therefore, finely tuned activation of Nrf2 *via* natural compounds or nanocarriers presents a rational approach to make use of its tumour suppressive action without inducing unwanted resistance mechanisms (Chartoumpekis *et al.* 2015).

Recent studies showed that TQ efficiently activated the Nrf2/ ARE axis and conferred the protection on neurodegeneration, inflammation, drug-induced organ injury, and anti-cancer activity. Most of these studies employed free TQ and generally used intra-peritoneal administered on toxin models. Besides, free TQ suffers from inadequate bioavailability, widespread distribution on systemic circulation, rapid metabolism and clearance. Further, their susceptible physiochemical instability might lead to the destruction under pH shifts, heat and light variations. Most of the available literatures had limited attention to its pharmacokinetics, tissue distribution, or long-term stability. Integrating the TQ with TiNPs would ensure physiochemical stability and selectivity towards tumor. Further, it may also confirm the reduced systemic toxicity, and improved clinical translational potential, by addressing the major limitations of the TQ alone. Merging the therapeutic activity of TQ and nanotechnology, the present study set out to formulate a thymoquinone derived titanium nanoparticle (TQ-TiNPs) for assessing its antioxidant and cytotoxic impacts on KB oral carcinoma cells. Merging the redox modulating activity of TQ and the delivery benefits of titanium nanoparticles, the present study hypothesized that this nanoformulation would enhance the anticancer activity of TQ by activating the targeted Nrf2 pathway along with oxidative stress modulation (Khalid *et al.* 2024; Umapathy *et al.* 2025).

In the present research work TQ-TiNPs were prepared through green, bio-inspired reduction process and were examined by employing different analytical methodologies to validate their physicochemical characteristics, such as particle size, shape, surface charge, and functional groups. The cytotoxic activity of the nanoparticles was evaluated using the MTT assay to check their inhibitory potential to suppress KB cell viability in a dose-dependent manner. Concurrently, their antioxidant capacity was measured through the DPPH free radical scavenging assay to identify their ROS scavenging activity and redox modulation capacity. To explore the mechanisms involved at the molecular level, the present research addressed the expression levels of genes that play a crucial role in oxidative stress and apoptosis. Expression levels of Nrf2, Keap1, HO-1, NQO1, and caspase-3 were determined by real time quantitative PCR to ascertain the relative mRNA concentration. Nrf2 and its downstream targets HO-1 and NQO1 were chosen to measure the redox-sensitive defence mechanism, with Keap1 being the negative regulator whose suppression reflects Nrf2 activation. Caspase-3, one of the most essential executors of apoptosis, was chosen to determine if the treatment initiated programmed cell death via intrinsic apoptotic pathways (Wen *et al.* 2012).

The rationale for this panel of genes is based on the interplay of redox equilibrium and apoptosis in tumour development and response to treatment. Upregulation of Nrf2 and its targets due to TQ-TiNPs would signify an induction of antioxidant defense, which could result in oxidative stress-mediated apoptosis in cancer cells. At the same time, the upregulation of Caspase-3 would validate the induction of apoptosis as an active process, verifying the dual action of TQ-TiNPs in both cryoprotection and the elimination of cancer cells (Jayaraman and Veeraraghavan, 2025).

This study was aimed at investigating the synthesis, characterization, and therapeutic application of TQ-TiNPs against oral cancer cells in a multidimensional manner. By disrupting the Nrf2 signalling pathway and examining both oxidative stress responses and apoptotic pathways, this research presents new findings on the potential of natural compound derived nanotherapeutics in the treatment of oral cancer. The results are likely to pave the way for future preclinical testing of TQ-TiNPs as a novel adjunct or alternative to standard chemotherapy treatments, especially for redox imbalanced cancers such as OSCC. This research not

only highlights the importance of the conjugation of phytochemicals with nanotechnology but also reiterates the therapeutic significance of the Nrf2 pathway in cancer research. Future studies can involve examining the impact of TQ-TiNPs in three-dimensional tumour models, determining their selectivity against normal cells, and investigating combinational therapy with established chemotherapeutic agents to enhance anticancer activity with less toxicity (Shafi *et al.* 2025).

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

All the chemicals and reagents used in this study were obtained in their analytical grades and utilized without purification. TQ with purity ≥98% was the bioactive agent used for the green synthesis of titanium nanoparticles and was obtained from Sigma-Aldrich (St Louis, MO, USA). Titanium(IV) isopropoxide ($Ti[OCH(CH_3)_2]_4$) was used as a precursor for titanium while ethanol as a solvent for the reaction was obtained from Merck (Germany). Sodium hydroxide, used for pH adjustment in the synthesis of nanoparticles, was purchased from Himedia Laboratories (Mumbai, India). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, and phosphate buffered saline (PBS) for *in vitro* assays were from Gibco (Thermo Fisher Scientific, USA). Total RNA was extracted with the assistance of TRIzol reagent (Invitrogen, USA), reverse transcription with the aid of a First Strand cDNA Synthesis Kit (Applied Biosystem), and gene-specific primers for Nrf2, Keap1, HO-1, NQO1, Caspase-3, and the housekeeping gene β-actin were explicitly ordered by Eurofins Genomics (India). In antioxidant determination, antioxidant capacity was assessed with the assistance of the 2,2-diphenyl-1-pricrylhydrazyl (DPPH) radical scavenging assay purchased from Sigma-Aldrich. The experiments were conducted under aseptic conditions for reproducibility and authenticity of data. TQ alone showed development of turbid/milky yellow-brown, at highest concentration (100 µg/ mL, 0.01% DMSO, comparably, but there was no such precipitation with 0.1% DMSO. Hence, 0.1% DMSO was used as the vehicle throughout the study. Meanwhile, TQ-TiNPs showed clear solution on 100 µg/ mL.

### 2.2 Preparation of Precursor Solutions

The TQ-TiNPs were synthesized by green synthesis with TQ as both a reducer and stabilizer. In a typical procedure, a Titanium (IV) isopropoxide (TTIP) solution was obtained by dissolving TTIP in ethanol with constant magnetic stirring. TQ was dissolved in ethanol in another flask and slowly added dropwise to the TTIP solution at constant stirring at room temperature. The reaction mixture was then subjected to mild heating (approximately 60-70°C) to enable the reduction of titanium ions and promote nanoparticle growth. To initiate hydrolysis and control the pH of the reaction medium, a dilute NaOH solution was slowly added until a stable colloidal suspension was observed. The mixture was stirred continuously for 3-4 hours, during which a visible colour change indicated the successful synthesis of TQ-TiNPs. The synthesized nanoparticles were then obtained by centrifugation at 10,000 rpm for 15 minutes, washed several times with ethanol and deionized water to remove unreacted chemicals and byproducts, and later dried under vacuum or lyophilized for further characterization and biological studies (Ali *et al.* 2025).

### 2.3 Detailed Characterization of Green-Synthesized Titanium Nanoparticles

TQ-TiNPs were characterized and authenticated for their chemical, structural, and morphological properties by the use of Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). FTIR was used using a KBr pellet method for the identification of TQ-related functional groups and the TiNPs and for the confirmation of phytochemical moieties capping the nanoparticle's surface. Spectra were recorded in the range 4000–400 $cm^{-1}$. Crystal structure and phase were evaluated through XRD; patterns were recorded in a range of $2\theta = 10°–80°$ using Cu-Kα radiation (1.5406 Å). The peaks of diffraction were studied to analyze the crystalline nature, average crystallite size, and phase purity of the nanoparticles. Particle size distribution and surface morphology were evaluated by SEM analysis. Dry TQ-TiNP samples were mounted on a carbon-coated grid and deposited with a thin gold coating to enhance conductivity for imaging. Micrographs were examined to determine nanoparticle shape, dispersion, and agglomeration. These characterization techniques confirmed successful synthesis, crystallinity, and phytochemical-assisted surface functionalization of TQ-TiNPs (Kasem *et al.* 2025).

### 2.4 Cell Culture and Treatment of KB Cells

Human oral squamous carcinoma KB cells were provided by a quality-control cell bank and cultured under standard cell culture conditions. The cells were maintained in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and incubated at 37°C in a humidified atmosphere that comprised 5% $CO_2$. For assay experiments, cells were seeded at $1 \times 10^4$ cells per well in 96-well plates and left for 24 hours to attach. The medium was then replaced with a new medium supplemented with TQ-TiNPs at concentrations of 1, 10, 25, 50, and 100 µg/mL. Control groups included untreated cells and a positive control treated with doxorubicin. Treatment was carried out for 24 hours, then cytotoxicity and gene expression assays were performed on the cells. All treatments were carried out in triplicate to enhance statistical precision (Roy *et al.* 2025).

### 2.5 MTT Assay

The cytotoxic potential of TQ-TiNPs was assessed in KB cells using MTT assay. Following 24 hours of incubation with TQ-TiNPs at doses of 1, 10, 25, 50, and 100 µg/mL, the culture medium was removed and replaced with 100 µL of fresh serum-free medium. About 20 µL of MTT reagent (5 mg/mL in PBS) was added to every well and the plate was placed in an incubator at 37°C for 4 hours to provide sufficient time for the formation of formazan crystals by the metabolically active cells. After incubation, the medium was discarded and 100 µL of DMSO was added to dissolve the formazan crystals. The plate was shaken gently for 10 minutes and absorbance at 570 nm was measured with a microplate reader (BioTek, USA). Cell viability was calculated as:

$$\text{Viability (\%)} = (OD_{sample} / OD_{control}) \times 100 \quad \ldots (1)$$

and cytotoxicity was calculated as:

$$\text{Cytotoxicity (\%)} = 100 - \text{Viability (\%)}. \quad \ldots (2)$$

All experiments were carried out in triplicate, and the values are reported as mean ± standard deviation (SD). The statistical comparison was carried out using one-way ANOVA followed by Tukey's post hoc test, and $p < 0.05$ was considered significant.

### 2.6 DPPH Assay for Estimation of Free Radical Scavenging Activity

Antioxidant activity of TQ-TiNPs was estimated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A fresh stock solution of 0.1 mM DPPH was prepared in methanol and left in the dark for 30 minutes to stabilize. TQ-TiNPs were also diluted in methanol to a test concentration of 1, 10, 25, 50, and 100 µg/mL. In a 96-well plate, 100 µL of each nanoparticle dilution was mixed with 100 µL of the DPPH solution. Methanol was used exclusively as the negative control and ascorbic acid as the positive control. The samples were incubated at room temperature in the dark for 30 minutes to permit the reaction. After incubation, absorbance was measured at 517 nm using a microplate reader (BioTek, USA). Free radical scavenging activity was calculated by the following formula: Scavenging (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$, where $A_{control}$ is the absorbance of DPPH solution without nanoparticles and $A_{sample}$ is the absorbance in the presence of TQ-TiNPs. Experiments were conducted in triplicate and the values were calculated as mean ± standard deviation (SD). Statistical comparison was performed by one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ being statistically significant (Kedare and Singh, 2011).

### 2.7 Gene Expression Analysis using Quantitative Real-time PCR (qRT-PCR)

Quantitative real-time PCR (qRT-PCR) was employed to assess the molecular effect of TQ-TiNPs on oxidative stress and apoptosis pathways by gene expression analysis. Total RNA was extracted from the TQ-TiNPs-treated periodontal ligament (PDL) cells at 1, 10, 25, 50, and 100 µg/mL for 24 hours using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol. The concentration and purity of the RNA were assayed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). About 1µg of RNA was reverse-transcribed to complementary DNA (cDNA) with First Strand cDNA Synthesis Kit (Applied Biosystems, USA). Quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems) on a real-time PCR instrument (StepOnePlus™, Applied Biosystems). Gene-specific primers were used to study the expression levels of Nrf2, Keap1, HO-1, NQO1, and Caspase-3, with β-actin as the endogenous control. The thermal cycling procedure included an initial denaturation of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds denaturation and 60°C for 1 minute annealing/extension. The relative levels of gene expression were normalized with the control and ascertained by the $2^{-\Delta\Delta C_T}$ method. The reactions were conducted in triplicate, and the results were expressed in terms of fold changes relative to untreated controls. The inter samples analysis showed no variation on the β-actin expression. Statistical comparison was established by one-way ANOVA followed by Tukey's post hoc test, where $p < 0.05$ was considered statistically significant (Ambika *et al.* 2019; Lv *et al.* 2022).

**Table 1: Primers used**

| Gene | Forward Primer (5'→3') | Reverse Primer (5'→3') |
|---|---|---|
| Nrf2 | AGCAGGACTGGAGAAGTTCA | TTTTCCAGGTTGCCCAGACT |
| Keap1 | GCGATCCTTCTGCCATAGAA | CTCAGGAGGCTGCTTCTTGA |
| HO-1 | GAGCGTCTGCACAGATGGTG | AGCTCCTGCAACTCCTCAAA |
| NQO1 | AGGATTGGACCGAGCTGGAA | GAATTCGGGCGTCTGCTTGA |
| Caspase-3 | TGTCATCTCGCTCTGGTACG | AAATGACCCCTTCATCACCA |
| β-actin | AGAGCTACGAGCTGCCTGAC | AGCACTGTGTTGGCGTACAG |

## 3. RESULTS

### 3.1 Functional Group Analysis by Fourier Transform Infrared Spectroscopy

FTIR analysis was carried out to determine the functional groups participating in the reduction and stabilization of titanium nanoparticles using TQ. FTIR spectrum of TQ-TiNPs showed some characteristic peaks that confirmed successful phytochemical-mediated synthesis. A broad band centered at ~3400 $cm^{-1}$ was associated with O–H stretching vibrations indicating the presence of hydroxyl groups either from TQ or adsorbed water. The highest peak at around ~1635 $cm^{-1}$ was

attributed to C=O stretching vibration of the quinone moiety, verifying TQ participation in the capping process. Peaks ranging from ~1450 $cm^{-1}$ was attributed to C=C stretching of the aromatic ring. Moreover, the bands at 690 and ~525 $cm^{-1}$ was attributed to Ti–O–Ti and Ti–O bond stretches, verifying the formation of titanium oxide nanoparticles (Fig. 1).

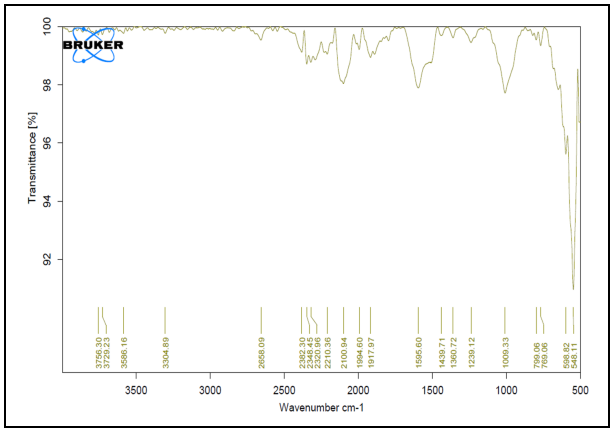



**Fig. 1: FTIR spectrum of TQ-TiNPs**

## 3.2 Crystalline Structure Analysis by X-ray Diffraction

X-ray diffraction (XRD) analysis was used to investigate the crystallographic structure of the synthesized TQ-TiNPs. The diffraction pattern exhibited multiple sharp and intense peaks that are characteristic of the anatase crystalline phase of titanium dioxide. The presence of well-defined peaks indicated a high degree of crystallinity, while the absence of secondary or unassigned peaks suggested high purity of the synthesized nanoparticles. The average crystallite size of the TQ-TiNPs was calculated using the Debye–Scherrer equation based on the full width at half maximum (FWHM) of the most intense diffraction peak and was estimated to be in the range 20–30 °. These results confirm the successful formation of crystalline titanium nanoparticles (Fig. 2).

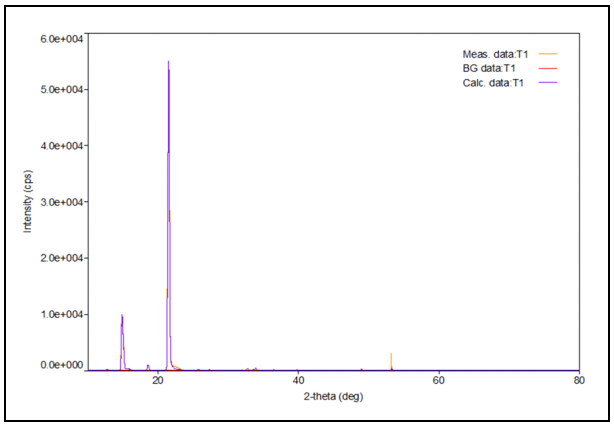



**Fig. 2: X-ray diffraction pattern of TQ-TiNPs**

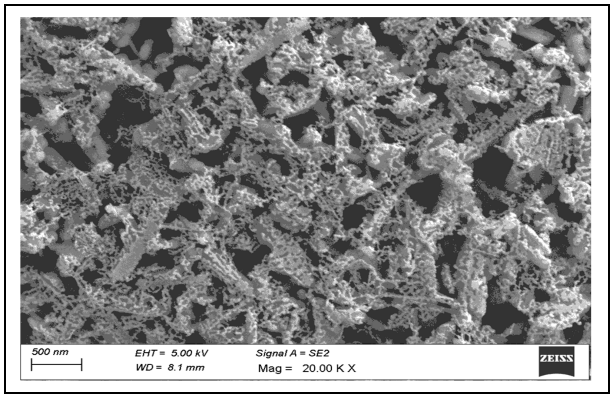



**Fig. 3: Scanning electron microscopy image of TQ-TiNPs**

## 3.3 Surface Morphology Analysis by Scanning Electron Microscopy

The surface morphology of the synthesized TQ-TiNPs was examined using SEM with the JEOL JSM-IT800 system. SEM images revealed that the nanoparticles were predominantly spherical in shape with a smooth surface texture and minimal agglomeration. The particles appeared to be well dispersed and uniformly distributed across the field, indicating effective capping and stabilization by TQ during synthesis. The average particle size observed was below 100 nm, supporting their classification within the nanoscale range. The homogeneity in morphology and nanoscale dimensions further confirmed the success of the green synthesis approach and the potential suitability of TQ-TiNPs for biomedical applications, particularly in targeted cancer therapy. No signs of irregular aggregation or morphological collapse were observed, suggesting good stability of the nanoparticle formulation (Fig. 3).

## 3.4 Dose-dependent Effect of TQ-TiNPs on KB Cell Viability

The cytotoxic activity of doxorubicin, TQ, titanium, and TQ-TiNPs against KB cells was determined with MTT assay over a concentration range of 1 to 100 µg/mL. All test groups demonstrated dose-dependent reduction in cell viability. The positive control doxorubicin was highly cytotoxic with a reduction in mean cell viability from 94.59% at 1 µg/mL to 6.03% at 100 µg/mL. Treatment with TQ showed moderate cytotoxicity, with viability decreasing from 97.68% in 1 µg/mL to 12.37% in 100 µg/mL. Each group of titanium nanoparticles without any drug showed comparatively low cytotoxicity, with more than 60% viability even at the highest concentration. On the other hand, TQ-TiNPs exhibited significantly improved cytotoxicity, as mean cell viability decreased from 87.64% in 1 µg/mL to 4.66% in 100 µg/mL, exhibiting synergistic actions between TQ and titanium. These findings indicate that TQ-TiNPs are more potent in suppressing the viability of KB cells than TQ or titanium alone, and their cytotoxic

activity is close to that of doxorubicin at higher concentrations (Fig. 4).

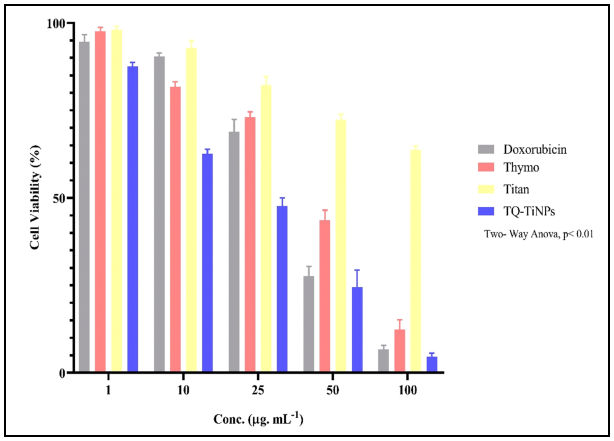



**Fig. 4: Cytotoxic effects of doxorubicin, TQ, titanium nanoparticles, and TQ-TiNPs on KB cells after 24 hours of treatment**

## 3.5 Antioxidant Activity Assessment by DPPH Assay

The antioxidant activity of $\alpha$-tocopherol, TQ, titanium nanoparticles, and TQ-TiNPs was determined by the DPPH free radical scavenging assay at 1, 10, 25, 50, and 100 µg/mL. All samples showed a dose-dependent increase in the radical scavenging activity. $\alpha$-tocopherol showed maximum antioxidant activity with the scavenging activity ranging from a mean value of 8.31% at 1 µg/mL to 96.83% at 100 µg/mL. TQ-TiNPs exhibited greater antioxidant activity than TQ and titanium in isolation. At 100 µg/mL, TQ-TiNPs had a scavenging activity of 96.91%, which was very close to that of $\alpha$-tocopherol, and significantly greater than titanium (mean of 22.74%) and TQ (mean of 65.25%). Even at lower concentrations (25 and 50 µg/mL), TQ-TiNPs consistently exhibited greater free radical neutralization, suggesting a synergistic effect between TQ and titanium. These results indicate that TQ-TiNPs have strong antioxidant activity and might provide better redox modulation capacity compared to their individual components.

## 3.6 Mechanism of Action of TQ-TiNPs *via* Nrf2 Pathway and Apoptosis Induction

Quantitative real-time PCR was carried out to explore the molecular pathways behind the antioxidant and apoptotic activities of TQ-TiNPs in KB cells. Cells exposed to TQ-TiNPs displayed a prominent upregulation of Nrf2, the master control of oxidative stress response, when compared with the untreated control. This was followed by the corresponding downregulation of Keap1, the cytoplasmic suppressor of Nrf2, suggesting activation of the Nrf2 pathway. In addition, major downstream antioxidant genes HO-1 and NQO1 were considerably upregulated, indicating increased cellular protection against oxidative stress. Notably, Caspase-3, which is a core effector in the apoptotic pathway, was significantly upregulated in TQ-TiNP-treated cells, reflecting induction of apoptosis. These molecular observations affirm that TQ-TiNPs induce antioxidant defense mechanisms through the Nrf2/Keap1 signaling pathway while, at the same time, inducing programmed cell death through caspase-dependent mechanisms. This dual behavior validates the curative potential of TQ-TiNPs as both antioxidant and anticancer in oral carcinoma.

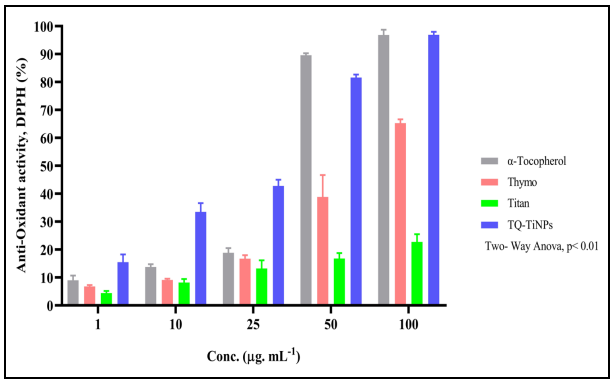



**Fig. 5: DPPH radical scavenging activity of $\alpha$-tocopherol, TQ, titanium nanoparticles, and TQ-TiNPs measured at concentrations of 1, 10, 25, 50, and 100 µg/mL**

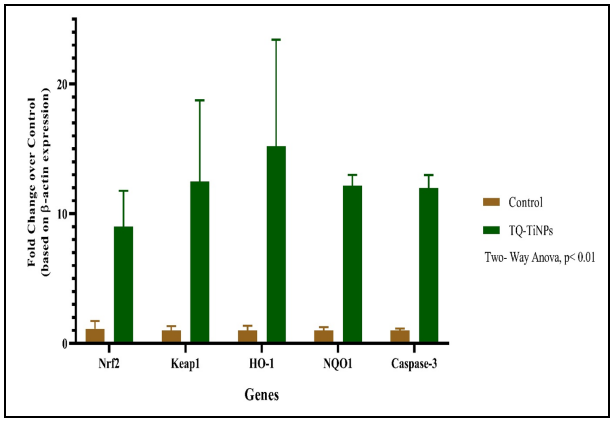



**Fig. 6: Relative mRNA expression levels of Nrf2, Keap1, HO-1, NQO1, and Caspase-3 in KB cells following treatment with TQ-TiNPs, analyzed by quantitative real-time PCR**

## 4. DISCUSSION

The current research illustrates that TQ-TiNPs have strong anticancer and antioxidant activity against human oral carcinoma KB cells. This activity was assessed by thorough characterization, MTT cytotoxicity assay, DPPH radical scavenging, and gene expression analysis of important markers involved in oxidative stress response and apoptosis. The findings present strong evidence that TQ-TiNPs act by triggering the Nrf2 signaling pathway and inducing caspase-mediated cell

death, providing a dual action for their applicability as a nanotheranostic platform in oral cancer therapy. Green synthesis of TQ-TiNPs through TQ reduction and stabilization was successfully established and verified by various characterization methods (Tachakittirungrod *et al.* 2007). SEM micrographs showed that the nanoparticles were spherical, well-dispersed, and in the nanoscale range (<100 nm), which is suitable for cellular uptake and biomedical applications. FTIR spectra gave an indication of the role of hydroxyl and carbonyl groups from TQ in stabilizing the process, while XRD analysis proved the crystalline anatase-phase titanium dioxide formation. Such physicochemical characteristics are crucial to bioavailability, cell interaction, and reactivity (Zhu *et al.* 2013).

In the MTT assay, TQ-TiNPs demonstrated a clear dose-response cytotoxic effect on KB cells with much greater efficacy than TQ or titanium alone. This indicates a synergistic effect that has improved cytotoxicity when both ingredients are presented as a composite nanoparticle system. Notably, the cytotoxic activity of TQ-TiNPs was almost the same as that of the chemotherapeutic drug doxorubicin at higher concentrations, illustrating their therapeutic potential. These results are in line with earlier findings establishing that plant-mediated metallic nanoparticles can break through drug resistance and increase cancer cell selectivity by better redox modulation and intracellular delivery. Antioxidant activity of TQ-TiNPs, as determined by the DPPH radical scavenging assay, also displayed robust dose-dependent activity. Though TQ alone exhibited moderate scavenging activity, nanoparticle formulation greatly promoted free radical neutralization, possibly because it had a greater surface area and better dispersion. Enhanced antioxidant activity is relevant in the case of cancer treatment, as oxidative stress has a dual role—both in causing cancer and in controlling cell death pathways. Controlled oxidative modulation, like that provided by TQ-TiNPs, can be utilized to induce selective killing of tumor cells while sparing normal tissues. To determine the molecular mechanisms involved in these effects, we examined the expression of genes involved in the Nrf2 antioxidant pathway and apoptosis regulation. We observed considerable upregulation of Nrf2, HO-1, and NQO1, with a simultaneous downregulation of Keap1, the cytoplasmic repressor of Nrf2. These findings validate that TQ-TiNPs stimulate the Nrf2/Keap1 pathway, which results in increased transcription of detoxifying and cytoprotective genes. Stimulation of this pathway was previously documented to be of significant importance in inhibiting tumor growth and enhancing cellular protection against oxidative stress (Gandhimathi *et al.* 2025).

Notably, Caspase-3 was also highly upregulated in TQ-TiNP-treated KB cells, suggesting the induction of apoptosis. This implies that although TQ-TiNPs bestow antioxidant effects through Nrf2 activation, they at the same time induce programmed cell death pathways through caspase activation. The bimodal regulation of oxidative stress and apoptosis represents a very desirable quality for anticancer drugs, especially for tumors like OSCC that are notoriously resistant to monotherapy. Our findings suggest that TQ-TiNPs can function as apoptosis-inducing, redox-sensitive agents that can selectively target cancer cells (He *et al.* 2025).

The dual action of TQ-TiNPs in this study is most likely due to the distinct characteristics of TQ and titanium in nanoscale form. TQ, a phytochemical with established anti-inflammatory, antioxidant, and anticancer activities, has been reported to modulate several signaling pathways such as NF-κB, PI3K/AKT, and MAPK. Titanium dioxide, however, is a long-established biocompatible nanomaterial possessing photocatalytic and ROS-modulating properties. When used in the form of a green-synthesized nanoparticle, their mutual properties seem to reinforce one another, providing synergistic biological efficacy and lesser systemic toxicity. While, Caspase-2 up-regulation and reduced viability, suggest the occurrence of apoptosis, the absence of functional assays for apoptosis, such as Annexin V/PI, Caspase-3/7 activity, would limit definitive conclusions in the present study. Hence, further molecular level confirmation is required.

From a translation viewpoint, TQ-TiNPs have several benefits. Their green synthesis avoids toxic chemicals, making them more biocompatible. Their small size and surface functionalization enable effective cellular uptake, and their potent cytotoxic and antioxidant activities identify them as potential candidates for adjunctive cancer therapy. In addition, their ability to modulate redox pathways without inducing overwhelming oxidative damage has the potential to afford a protective effect on non-cancerous tissues. The pharmacokinetic and biodistribution studies would establish the best dose and delivery mechanism for clinical use (Harrington *et al.* 2025).

## 5. CONCLUSION

In conclusion, the present study effectively proves that TQ-TiNPs prepared through a green approach are effective anticancer and antioxidant agents against KB oral carcinoma cells. TQ-TiNPs caused a dose-dependent inhibition of cell viability, increased free radical scavenging activity, and affected major molecular pathways by upregulating Nrf2, HO-1, NQO1, and Caspase-3, whereas downregulating Keap1. These findings indicate that TQ-TiNPs had their therapeutic actions by triggering antioxidant defense systems and apoptosis. The dual mechanism of redox regulation and induction of cell death points towards their potential as a safe and efficient nanotherapeutic agent against oral cancer. Although the present study did not calculate the

therapeutic index/ selectivity index at present, incorporation of such assays would be beneficial for to quantitatively benchmark the safety–efficacy window of TQ-TiNPs. Clinical translation and further *in vivo* studies will facilitate further development of TQ-TiNPs as a new intervention for oncology.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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