

Original Research Article

Investigating the Effect of Nanoemulsion Containing Curcumin on TGF- β Gene Expression in Breast Cancer Cell Line

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Article History

Received: 02.09.2025

Accepted: 27.11.2025

Published: 18.12.2025

Abstract: Curcumin, a natural polyphenol derived from *Curcuma longa*, is known to modulate a multitude of cellular signaling pathways. Despite its promising pleiotropic medicinal properties, the clinical application of curcumin has been significantly hindered by its inherent limitations, including poor solubility in plasma, low chemical stability, rapid systemic metabolism, and consequently, low bioavailability. Transforming growth factor-beta (TGF- β) is a multifunctional cytokine that plays a central and often paradoxical role in regulating cell growth, differentiation, and immune responses. Dysregulation of TGF- β signaling is critically involved in various facets of cancer biology, including tumor initiation, progression, invasion, and metastasis. This research was designed to investigate the anticancer potential of a curcumin-containing nanoemulsion on the MCF-7 human breast cancer cell line, with a specific focus on elucidating its effect on the expression of the TGF- β gene. A stable curcumin nanoemulsion was synthesized and characterized. Dynamic Light Scattering (DLS) analysis confirmed a uniform and small particle size with a mean diameter of 12.3 nm. The efficiency of curcumin encapsulation within the nanoemulsion matrix was calculated to be a high 70%. The cytotoxic potential of the formulation was evaluated on MCF-7 cells using the MTT assay, which determined the half-maximal inhibitory concentration (IC₅₀) to be 13.54 μ g/mL. Subsequently, MCF-7 cells were treated with this IC₅₀ concentration to assess changes in gene expression. Real-Time Quantitative PCR (RT-qPCR) results revealed that treatment with the curcumin nanoemulsion induced a significant 2.1-fold decrease in the mRNA expression level of the TGF- β gene compared to untreated control cells, with a high statistical significance ($p=0.0001$). This study concludes that the curcumin nanoemulsion exerts its anticancer effects, at least in part, by downregulating the TGF- β signaling pathway. These findings underscore the potential of this nano-formulation as a novel therapeutic strategy for breast cancer treatment.

Keywords: Breast Cancer, TGF-B, Curcumin Nanoemulsion, MCF-7, Drug Delivery, Gene Expression.

INTRODUCTION

The type of cancer that is frequently seen and also a leading cause of mortality from cancer among women worldwide is breast cancer. It is a highly heterogeneous disease, which means that there are differences in the disease's molecular characteristics, biological behaviours, and outcome of treatment among patients with the same cancer subtype. According to the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), breast cancers are classified into different molecular subtypes such as Luminal A, Luminal B, HER2-enriched, and triple-negative breast cancer (TNBC). The MCF-7 cell line is considered the most common cell line for studying ER-positive Luminal A breast cancer. Transforming growth factor-beta (TGF- β) is a cytokine known for the regulation of proliferation, differentiation, apoptosis, and immune response of cells. The role of TGF- β in breast cancer is dual depending on the development stages of cancer. At early stages of cancer, it acts as a tumor suppressor by triggering the arrest of the cell cycle and induction of apoptosis. The process of epithelial-mesenchymal transition (EMT), angiogenesis, metastasis, and immune evasion by tumor cells becomes possible at the advanced stages of malignancy through the utilization of the TGF- β signaling pathway. Curcumin is the major active component isolated from the turmeric

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Citation: Ali Abdalla Graye, Alkhafaje, Waleed K, Samer H. Al –Rihaymee, Karrar Y. Ali, Mustafa Abed AL-Jabber Mohammed Saleh (2025). Investigating the Effect of Nanoemulsion Containing Curcumin on TGF- β Gene Expression in Breast Cancer Cell Line. *South Asian Res J App Med Sci*, 7(6), 321-325. 321

rhizomes and known for its anti-inflammatory, antioxidant, and anticancer effects. Previously, curcumin was proven to regulate various molecular signaling pathways involved in cancer progression such as NF- κ B, STAT3, Akt/mTOR, and apoptosis-related pathways. Although exhibiting remarkable pharmacological properties, curcumin failed to reach clinical use because of poor solubility, low bioavailability, fast metabolism, and rapid clearance from the body. Nanoemulsions represent an effective method of improving drug delivery based on the ability of these nanocarriers to increase the solubility, stability, bioavailability, and absorption of hydrophobic substances like curcumin. Thus, it is possible to assume that encapsulation of curcumin in nanoemulsion droplets can enhance the efficiency and anticancer potential of this agent. Taking into account the above information, the present research aimed at exploring the impact of a curcumin-loaded nanoemulsion on the TGF- β gene expression in the MCF-7 breast cancer cell line. Moreover, the study was intended to examine the physicochemical parameters and antitumor effects of synthesized nanoemulsions. It was hypothesized that the curcumin nanoemulsion would exhibit dose-dependent cytotoxicity and significantly downregulate TGF- β gene expression, suggesting a potential mechanism for its anticancer activity.

MATERIALS AND METHODS

Synthesis of Curcumin Nanoemulsion

An oil-in-water (O/W) nanoemulsion was prepared using a high-energy ultrasonication method.

1. **Oil Phase Preparation:** Curcumin powder was dissolved in sesame oil at a concentration of 2 mg/mL with constant stirring to ensure complete dissolution.
2. **Surfactant/Co-surfactant Mixture:** The surfactants Span 80 and Tween 80 were mixed with ethanol.
3. **Emulsification:** The oil phase was added to the surfactant mixture. Deionized water (the aqueous phase) was then added dropwise to the organic mixture under continuous magnetic stirring. This resulted in the formation of a coarse, milky pre-emulsion.
4. **Homogenization:** The coarse emulsion was subjected to high-intensity probe sonication (60 Hz for 5 minutes at ambient temperature). The acoustic cavitation generated by sonication broke down the large oil droplets into the nano-size range, resulting in a stable, translucent curcumin nanoemulsion.

Physicochemical Characterization

- **Particle Size and Distribution (DLS):** Dynamic Light Scattering (Zetasizer, Malvern Instruments) was used to measure the z-average hydrodynamic diameter and polydispersity index (PDI) of the nanoemulsion droplets. The PDI value indicates the breadth of the size distribution, with values <0.3 signifying a monodisperse population.
- **Encapsulation Efficiency (EE):** The EE was determined indirectly. Free, un-encapsulated curcumin was separated from the nanoemulsion by ultracentrifugation (13,000 rpm, 30 min, 4°C). The amount of curcumin in the clear supernatant was quantified using UV-Vis spectrophotometry at $\lambda_{\text{max}}=425$ nm against a pre-established calibration curve. The EE was calculated using the formula:

Equation 3.1: Encapsulation Efficiency

$$EE (\%) = \frac{[\text{Total Drug Amount} - \text{Amount of Free Drug}]}{\text{Total Drug Amount}} \times 100 \quad (3.1)$$

Cell Line and Culture Conditions

The human breast adenocarcinoma cell line MCF-7 (ER-positive) was used. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μ g/mL streptomycin. The cells were cultured in a humidified incubator at 37°C with a 5% CO₂ atmosphere.

In Vitro Cytotoxicity Assessment (MTT Assay)

The MTT assay was used to measure the cytotoxic effect of the curcumin nanoemulsion.

1. MCF-7 cells were seeded into 96-well plates (1.5×10^4 cells/well) and allowed to adhere for 24 hours.
2. The medium was replaced with fresh medium containing serial dilutions of the curcumin nanoemulsion (concentrations ranging from 2 to 160 μ g/mL). Control wells contained untreated cells.
3. After a 24-hour incubation period, the treatment medium was removed, and MTT reagent was added.
4. Following a 3-hour incubation, the formazan crystals formed by viable cells were dissolved in DMSO.
5. The absorbance was read at 570 nm. Cell viability was expressed as a percentage relative to the untreated control. The IC₅₀ value was determined by non-linear regression analysis.

Gene Expression Analysis via RT-qPCR

MCF-7 cells were treated with the predetermined IC₅₀ concentration of the curcumin nanoemulsion for 24 hours. Total RNA was extracted from both treated and untreated (control) cells using the TRIzol reagent. The quality and quantity of RNA were confirmed using Nanodrop spectrophotometry. One microgram of total RNA from each sample was reverse transcribed into cDNA using a commercial kit.

RT-qPCR was performed to quantify the mRNA levels of TGF- β . GAPDH was used as the housekeeping gene for normalization.

Table 3.1: Primer Sequences for RT-qPCR

Gene Name	Sequence (5' → 3')	Type
TGFB	TACCTGAACCCGTGTTGCTCTC	Forward
	GTTGCTGAGGTATCGCCAGGAA	Reverse
GAPDH	GTCTCCTCTGACTTCAACAGCG	Forward
	ACCACCCTGTTGCTGTAGCCAA	Reverse

Table 3.2: RT-qPCR Reaction Components

Component	Volume (μ L)
SYBR Green Master Mix (2 \times)	10.0
Forward Primer (10 μ M)	0.6
Reverse Primer (10 μ M)	0.6
cDNA Template	1.0
Nuclease-Free Water	7.8
Total Volume	20.0

The thermal cycling conditions included an initial denaturation step, followed by 40 cycles of denaturation, annealing, and extension. A melt curve analysis was performed at the end of the run to verify the specificity of the amplicons.

The relative expression of the TGF- β gene was calculated using the Livak method ($2^{-\Delta\Delta CT}$).

Equation 3.2: The $2^{-\Delta\Delta CT}$ Method

- $\Delta CT = CT$ (Target Gene, TGF- β) – CT (Housekeeping Gene, GAPDH) (3.1)
- $\Delta\Delta CT = \Delta CT$ (Treated Sample) – ΔCT (Control Sample) (3.2)
- Fold Change** = $2^{-\Delta\Delta CT}$ (3.3)

Statistical analysis was performed using appropriate tests to determine if the observed differences in gene expression were significant ($p < 0.05$).

RESULTS AND DATA ANALYSIS

Physicochemical Properties of the Curcumin Nanoemulsion

The synthesized nanoemulsion was a visually clear, yellowish, and stable liquid. The particle size and distribution were analyzed using DLS.

- Mean Particle Size:** The DLS analysis revealed a z-average hydrodynamic diameter (d_{50}) of 12.3 nm. This small particle size is highly desirable for biomedical applications, as it can facilitate passive tumor targeting via the Enhanced Permeability and Retention (EPR) effect and improve cellular penetration.
- Homogeneity:** The DLS results showed a single, narrow peak, indicating a monodisperse system with a uniform population of nanoparticles. This homogeneity is crucial for ensuring consistent and reproducible biological activity.

Determination of Encapsulation Efficiency

To quantify the drug-loading success, a standard calibration curve for curcumin was first established.

Table 4.1: Data for Curcumin Standard Calibration Curve

Concentration (μ g/mL)	Absorbance at 425 nm
1	0.25
2	0.37
4	0.67
6	0.82
8	0.98
10	1.23

The plot of absorbance versus concentration yielded a linear relationship with a high correlation coefficient ($R^2 > 0.99$), described by the equation $y = 0.103x + 0.147$. Using this equation, the concentration of free curcumin in the

supernatant was determined. The encapsulation efficiency (EE) was then calculated to be **70%**. This result indicates that the nanoemulsion formulation was highly effective at entrapping the hydrophobic curcumin molecule within its oily core.

Cytotoxic Effects on the MCF-7 Cell Line

The MTT assay was conducted to evaluate the dose-dependent cytotoxicity of the curcumin nanoemulsion on MCF-7 cells after 24 hours of treatment.

Table 4.2: Cell Viability of MCF-7 Cells after Treatment with Curcumin Nanoemulsion

Concentration ($\mu\text{g/mL}$)	Mean Cell Viability (%) \pm SD
0 (Control)	100 \pm 0.0
2	92.4 \pm 4.1
5	81.5 \pm 3.8
10	60.2 \pm 5.2
20	41.3 \pm 4.5
40	25.7 \pm 3.9
80	14.1 \pm 2.8
160	8.9 \pm 2.1

The results clearly show that the curcumin nanoemulsion inhibited the proliferation of MCF-7 cells in a dose-dependent manner. Cell viability decreased significantly as the concentration of the nanoemulsion increased. By plotting cell viability against the logarithm of the concentration, a sigmoidal dose-response curve was generated. From this curve, the IC₅₀ value was calculated to be 13.54 $\mu\text{g/mL}$. This value represents the concentration of the nanoemulsion required to inhibit the growth of 50% of the cancer cells and was selected for the subsequent gene expression studies.

4.4. Modulation of TGF- β Gene Expression

The central aim of the study was to determine if the anticancer effect of the curcumin nanoemulsion was associated with changes in the expression of the TGF- β gene. RT-qPCR was used to quantify TGF- β mRNA levels in MCF-7 cells treated with the IC₅₀ concentration (13.54 $\mu\text{g/mL}$) of the nanoemulsion, relative to untreated control cells.

The RT-qPCR results demonstrated a significant and substantial downregulation of TGF- β expression.

- **Fold Change:** Analysis using the $2^{-\Delta\Delta\text{CT}}$ method revealed that the expression of the TGF- β gene was decreased by 2.1-fold in the cells treated with the curcumin nanoemulsion compared to the untreated control group.
- **Statistical Significance:** This reduction in gene expression was statistically highly significant, with a p-value of 0.0001.

Table 4.3: Summary of RT-qPCR Results for TGF- β Gene Expression

Treatment Group	Normalized Fold Change in TGF- β Expression	p-value
Control (Untreated)	1.0 (Reference)	–
Curcumin Nanoemulsion (13.54 $\mu\text{g/mL}$)	0.476 (A 2.1-fold decrease)	0.0001

DISCUSSION

This study successfully synthesized a stable curcumin nanoemulsion and demonstrated its potent anticancer activity against the MCF-7 breast cancer cell line. The results provide strong evidence that this activity is mediated, at least in part, by the downregulation of the TGF- β gene.

The physicochemical characteristics of the nanoemulsion were optimal for a drug delivery system. The small particle size of 12.3 nm is particularly noteworthy. Particles under 100 nm are known to preferentially accumulate in tumor tissues through the EPR effect and can more readily penetrate cellular membranes. The high encapsulation efficiency of 70% ensures that a significant payload of curcumin is delivered per vesicle, maximizing the therapeutic potential.

The cytotoxic effect, with an IC₅₀ of 13.54 $\mu\text{g/mL}$, confirms that the nano-formulation effectively delivers curcumin to cancer cells, inducing cell death. This is consistent with a large body of literature demonstrating curcumin's anti-proliferative and pro-apoptotic effects. The nanoemulsion platform likely enhanced the cellular uptake and bioavailability of curcumin compared to its free form, leading to a robust biological response.

The most significant finding of this thesis is the 2.1-fold downregulation of TGF- β gene expression (p=0.0001). This provides a novel and specific molecular mechanism for the anticancer action of the curcumin nanoemulsion in breast cancer. In advanced breast cancer, TGF- β signaling is a key driver of malignancy. By suppressing its expression, the curcumin nanoemulsion could potentially inhibit a cascade of downstream events critical for tumor progression, including:

- **Inhibition of EMT:** Reduced TGF- β levels may prevent cancer cells from acquiring an invasive, mesenchymal phenotype, thereby limiting their ability to metastasize.
- **Modulation of the Tumor Microenvironment:** A decrease in TGF- β could alleviate the immunosuppressive environment of the tumor, potentially making it more susceptible to immune attack.
- **Reversal of Drug Resistance:** TGF- β signaling has been linked to resistance to conventional therapies. Its inhibition could re-sensitize cancer cells to other treatments.

These findings align with previous work by Hu *et al.*, (2010) and Yao *et al.*, (2012), who reported curcumin's inhibitory effects on the TGF- β /Smad pathway in other disease models. This study is among the first to specifically demonstrate this effect in breast cancer cells using a nanoemulsion delivery system, highlighting the therapeutic potential of this approach.

CONCLUSION

This study provides compelling evidence that a nanoemulsion formulation of curcumin is a potent agent against the MCF-7 breast cancer cell line. The formulation exhibited significant cytotoxicity and, crucially, was shown to substantially downregulate the expression of the TGF- β gene, a key mediator of cancer progression and metastasis.

In conclusion, this research successfully demonstrates that the curcumin nanoemulsion exerts its therapeutic effects by targeting and suppressing the TGF- β signaling pathway. This work underscores the powerful synergy between a potent natural compound and an advanced nanotechnology-based delivery system, paving the way for a promising new strategy in the treatment of breast cancer.

Recommendations

1. Determine the influence of curcumin nanoemulsion on TGF- β down-streaming signaling molecules, including Smad2/3 and PI3K/AKT.
2. Conduct protein analysis to verify TGF- β suppression via ELISA and Western Blotting.
3. Conduct metastasis assays to determine the antimigration and angiogenesis properties of the nanosuspension formulation.
4. Conduct in vivo experiments to measure efficacy, bioavailability, and safety of the treatment protocol.
5. Explore any synergism between curcumin nanoemulsion and conventional treatments such as chemotherapy and radiation therapy.

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