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Effect of Curcumin on the Viability and Proliferation of Triple-Negative Breast Cancer (MDA-MB-231) Cells

Orhero Moses O [1,2]

[1.]Department of Pharmacology, University of Calabar, Cross River State, Nigeria.

[2.]College of Nursing Sciences, Federal Neuropsychiatric Hospital Calabar, Cross River State, Nigeria.

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ABSTRACT

Triple-negative breast cancer is an aggressive subtype of breast cancer characterised by the absence of oestrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression, a phenotype that severely limits targeted treatment options and is associated with poor clinical outcomes. Curcumin, a polyphenolic compound derived from Curcuma longa, has demonstrated broad anticancer properties in preclinical models; however, its cytotoxic activity specifically against triple-negative breast cancer cells (MDA-MB-231) using standardised in vitro methods remains incompletely characterised. This study evaluated the cytotoxic and anti-proliferative effects of curcumin on MDA-MB-231 cells using a cell viability assay and a cell staining assay, with cisplatin included as a validated positive control. Cells were treated with two-fold serial dilutions of curcumin and cisplatin (0.39–100 micromolar) for 72 hours in the viability assay, and with five fixed concentrations of curcumin (3.75, 7.5, 15, 30 and 60 micromolar) for 72 hours in the staining assay. Curcumin produced a concentration-dependent reduction in cell viability, yielding a half-maximal inhibitory concentration of 27.162 ± 11.0765 micromolar. Cisplatin produced a half-maximal inhibitory concentration of 20.267 ± 26.0745 micromolar, confirming assay validity despite greater variability associated with the positive control. In the staining assay, two independent experiments revealed a threshold, rather than a smoothly progressive, concentration-response relationship: reductions in adherent cell survival at 3.75, 7.5, and 15 micromolar were generally modest and largely non-significant relative to untreated control cells (ranging between approximately 86% and 113% survival), whereas treatment at 30 and 60 micromolar produced marked and highly statistically significant reductions in survival in both independent experiments (falling to between approximately 14% and 45% of control values; $p < 0.0001$). Overall treatment effects were highly significant by one-way analysis of variance in both independent experiments (Experiment 1: $F = 92.17$, $p < 0.0001$; Experiment 2: $F = 251.2$, $p < 0.0001$). These findings provide robust in vitro evidence that curcumin exerts meaningful, concentration-dependent cytotoxic and anti-proliferative activity against MDA-MB-231 triple-negative breast cancer cells, with an apparent transition from a largely sub-threshold effect to pronounced cytotoxicity occurring between 15 and 30 micromolar, supporting its further evaluation as a candidate anticancer agent.

Keywords: triple-negative breast cancer; curcumin; cell viability assay; crystal violet assay; cytotoxicity; MDA-MB-231

INTRODUCTION

Breast cancer is a molecularly heterogeneous disease, and its clinical management is guided principally by the expression status of three key biomarkers: the oestrogen receptor, the progesterone receptor, and human epidermal growth factor receptor 2. Triple-negative breast cancer is defined by the simultaneous absence of all three of these markers, a receptor-negative phenotype that accounts for approximately 15–20% of all breast cancer diagnoses

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worldwide (Sung et al., 2021; Siegel et al., 2023). Unlike hormone receptor-positive or human epidermal growth factor receptor 2-positive disease, triple-negative breast cancer cannot be treated with endocrine therapies such as tamoxifen or aromatase inhibitors, nor with human epidermal growth factor receptor 2-targeted agents such as trastuzumab, leaving cytotoxic chemotherapy as the mainstay of systemic treatment (Harbeck et al., 2019; Bianchini et al., 2022).

Although triple-negative breast cancer is often discussed as a single clinical entity, it is now recognised to comprise a genomically and transcriptionally heterogeneous group of tumours. Integrative multi-omic analyses have identified multiple molecular subtypes with distinct gene expression signatures, mutational landscapes, and immune infiltration profiles, including basal-like, mesenchymal, immunomodulatory, and luminal androgen receptor subtypes (Bareche et al., 2018; Lehmann et al., 2021; Yin et al., 2020). This heterogeneity has important therapeutic implications, as subtypes differ in their sensitivity to chemotherapy, immunotherapy, and targeted agents, and underscores the limitations of studying triple-negative breast cancer using a single representative cell line, such as MDA-MB-231, which itself is classified within the mesenchymal-like subtype.

Triple-negative breast cancer disproportionately affects younger women, women of African ancestry, and carriers of germline BRCA1 mutations, and is associated with a higher rate of visceral and central nervous system metastasis compared with other breast cancer subtypes (Yedjou et al., 2019; Lambertini et al., 2021). Global cancer statistics indicate that breast cancer is now the most commonly diagnosed cancer worldwide, and within this burden, triple-negative disease carries a disproportionately poor prognosis, with five-year survival for metastatic disease remaining as low as 12–15% despite advances in systemic therapy (Sung et al., 2021; Waks & Winer, 2019). Racial and socioeconomic disparities in incidence and outcome have also been well documented, with Black women in the United States experiencing both a higher incidence of triple-negative breast cancer and higher mortality compared with other ethnic groups, reflecting a combination of biological and healthcare access factors (Yedjou et al., 2019).

The aggressive clinical behaviour of triple-negative breast cancer is underpinned by a distinct constellation of molecular alterations. Somatic TP53 mutations are present in the majority of cases, frequently co-occurring with high genomic instability, elevated proliferative indices, and defective DNA damage repair (Shiovitz & Korde, 2020). A substantial proportion of cases also harbour germline or somatic BRCA1/2 mutations, which impair homologous recombination repair and confer both an aggressive phenotype and a therapeutic vulnerability to platinum agents and poly (ADP-ribose) polymerase inhibition (Robson et al., 2017; Tung et al., 2020). Additional oncogenic drivers implicated in triple-negative breast cancer pathophysiology include epidermal growth factor receptor overexpression, activation of the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin signalling axis, constitutive activation of signal transducer and activator of transcription 3, and dysregulation of nuclear factor kappa-light-chain-enhancer of activated B cells signalling, all of which converge to promote proliferation, evasion of apoptosis, invasion, and metastatic spread (Bianchini et al., 2022; Bhatt et al., 2022). The absence of a validated hormonal or human epidermal growth factor receptor 2-directed target, combined with this multiplicity of dysregulated pathways, has historically rendered triple-negative breast cancer difficult to treat with single-agent targeted therapy.

Cytotoxic chemotherapy, most commonly comprising anthracyclines, taxanes, and platinum agents such as cisplatin and carboplatin, remains the backbone of systemic treatment for triple-negative breast cancer (Cardoso et al., 2022). Cisplatin exerts its cytotoxic effect through the formation of intrastrand and interstrand DNA cross-links, which trigger cell cycle arrest and apoptosis via both p53-dependent and p53-independent pathways (Tchounwou et al., 2021; Makovec, 2019). However, the clinical utility of these agents is substantially constrained by dose-limiting adverse drug reactions and toxicity, including myelosuppression, nephrotoxicity, ototoxicity, peripheral neuropathy, and cumulative cardiotoxicity, which frequently necessitate dose reduction or treatment discontinuation (Makovec, 2019; Tchounwou et al., 2021). Furthermore, both intrinsic and acquired chemoresistance are common in triple-negative breast cancer, driven by mechanisms including enhanced drug

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

efflux, upregulation of DNA repair capacity, and epithelial-to-mesenchymal transition, contributing to disease relapse and progression despite initial treatment response (Bianchini et al., 2022). Newer therapeutic modalities, including immune checkpoint inhibition with pembrolizumab (Schmid et al., 2020; Cortés et al., 2021), poly (ADP-ribose) polymerase inhibitors for BRCA-mutated disease (Robson et al., 2017; Tung et al., 2020), and the Trop-2-directed antibody-drug conjugate sacituzumab govitecan (Bardia et al., 2021), have improved outcomes

for selected patient populations; however, these agents are costly, are associated with their own distinct toxicity profiles (including immune-related adverse events and cytopenias), and remain inaccessible in many resource-limited healthcare settings (Bianchini et al., 2022; Cardoso et al., 2022). Collectively, these limitations underscore a persistent and urgent need for novel, better-tolerated therapeutic candidates for triple-negative breast cancer.

Natural products have historically represented a highly productive source of clinically approved anticancer agents, with more than 60% of currently approved oncology drugs derived from, or structurally inspired by, natural compounds (Newman & Cragg, 2020; Atanasov et al., 2021). Within this context, curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; $C_{21}H_{20}O_6$; molecular weight 368.38 g/mol), the principal curcuminoid extracted from the rhizome of Curcuma longa Linn. (turmeric), has attracted considerable scientific interest as a candidate anticancer phytotherapeutic agent. Structurally, curcumin is a polyphenolic diarylheptanoid possessing β-diketone and phenolic hydroxyl pharmacophoric groups that confer potent antioxidant, anti-inflammatory, and anti-neoplastic activity (Moballegh Nasery et al., 2020; Shehzad et al., 2015). Unlike conventional cytotoxic chemotherapy, which typically acts on a single dominant molecular target, curcumin has been reported to simultaneously modulate multiple oncogenic signalling pathways relevant to triple-negative breast cancer biology, including inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells signalling, suppression of the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin axis, inactivation of signal transducer and activator of transcription 3, and modulation of the intrinsic apoptotic cascade through the Bcl-2-associated X protein/B-cell lymphoma 2 axis (Bhatt et al., 2022; Kunnumakkara et al., 2023). A systematic meta-analysis of preclinical studies further confirmed that curcumin consistently inhibits breast cancer cell proliferation and induces apoptosis across multiple triple-negative breast cancer cell line models (Liu et al., 2022), lending weight to the proposition that multi-target natural compounds such as curcumin may offer complementary or synergistic benefit alongside, or ahead of, conventional cytotoxic agents, potentially with a more favourable systemic toxicity profile.

Despite this promising mechanistic profile, curcumin is associated with poor aqueous solubility (approximately 11 ng/mL) and low oral bioavailability owing to rapid hepatic metabolism, which remain significant barriers to its clinical translation (Moballegh Nasery et al., 2020). Nanotechnology-based delivery platforms, including poly (lactic-co-glycolic acid) nanoparticles, have demonstrated substantially enhanced intracellular curcumin concentrations and improved anticancer activity in triple-negative breast cancer models compared with free curcumin (Nair et al., 2020), suggesting that formulation strategies may be able to overcome curcumin's principal pharmacokinetic limitations. Nevertheless, the fundamental cytotoxic activity of curcumin against triple-negative breast cancer remains incompletely characterised in standardised in vitro experimental models incorporating appropriate positive controls and independent methodological endpoints. The present study therefore aimed to evaluate the cytotoxic and anti-proliferative effects of curcumin on MDA-MB-231 triple-negative breast cancer cells using a colorimetric cell viability assay and a cell staining-based proliferation assay, with cisplatin included as a validated chemotherapeutic positive control.

MATERIALS AND METHODS

Cell line and culture conditions

MDA-MB-231 human triple-negative breast cancer cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle Medium (Fisher

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

Scientific, UK) supplemented with 10% foetal bovine serum (Fisher Scientific, UK), 1% L-glutamine (100×; Biosera, UK), and 1% penicillin/streptomycin (Fisher Scientific, UK). Cells were cultured in T75 tissue culture flasks and maintained in a humidified incubator (LEEC Culture Safe CO_2 Touch 190S) at 37°C in a 5% CO_2 atmosphere. All procedures were performed aseptically within a Class II biological safety cabinet (ESCO, Singapore). Cells were routinely passaged upon reaching 70–80% confluency by trypsinisation with 0.25% trypsin-EDTA, pelleted by centrifugation at 1,500 rpm for 5 minutes, and resuspended in complete medium.

MTT cytotoxicity assay

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Fisher Scientific, UK) colorimetric assay. Cells were seeded at 5,000 cells per well in 100 µL complete medium into 96-well plates and allowed to adhere for 24 hours before treatment. Two-fold serial dilutions of curcumin (Sigma-Aldrich, UK) dissolved in dimethyl sulfoxide, and cisplatin (Sigma-Aldrich, UK) dissolved in 0.9% sodium chloride, were prepared spanning 0.39–100 µM in complete medium and added in triplicate to wells containing cells. Vehicle control wells received medium supplemented with 0.1% dimethyl sulfoxide. Cisplatin served as the positive control. After 72 hours of treatment, 50 µL MTT solution (5 mg/mL) was added to each well and incubated for 3 hours. The medium was aspirated, formazan crystals were dissolved in 200 µL dimethyl sulfoxide, and absorbance was measured at 540 nm using a Thermoscientific Multiskan SkyHigh microplate reader. Percentage cell viability was calculated relative to vehicle control wells. Dose-response curves and half-maximal inhibitory concentration (IC_{50}) values were determined by non-linear regression using GraphPad Prism (version 11.0.0, San Diego, CA, USA).

Crystal violet assay

Anti-proliferative activity was assessed using the crystal violet staining assay. Cells were seeded at 80,000 cells per well in 3 mL complete medium into 6-well plates and allowed to adhere for 24 hours. Cells were treated with curcumin at concentrations of 3.75, 7.5, 15, 30 and 60 µM in complete medium for 72 hours, with untreated and dimethyl sulfoxide vehicle controls included. Following treatment, medium was aspirated, cells were fixed with ice-cold methanol for 5 minutes, stained with 0.5% crystal violet solution for 15 minutes at room temperature, and washed repeatedly with distilled water to remove unbound dye. Retained dye was solubilised with 30% acetic acid for 15 minutes, transferred in triplicate to a 96-well plate, and absorbance measured at 540 nm. Percentage cell survival was normalised to untreated control wells. Two independent experiments were performed, each with triplicate measurements. Statistical analysis was performed by one-way analysis of variance followed by Tukey's multiple comparisons test in GraphPad Prism, with Brown-Forsythe and Bartlett's tests applied to assess homogeneity of variance. Data are presented as mean ± standard deviation.

RESULTS

MTT assay with curcumin

Treatment of MDA-MB-231 cells with curcumin for 72 hours across a concentration range of 0.39–100 µM produced a progressive, concentration-dependent reduction in cell viability (Figure 1). The dose-response curve followed a sigmoidal profile consistent with multi-target cytotoxic engagement across the concentration range. The IC_{50} of curcumin was determined to be 27.162 ± 11.0765 µM by non-linear regression.

Overall, curcumin demonstrated clear cytotoxic activity against MDA-MB-231 triple-negative breast cancer cells at low micromolar concentrations.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

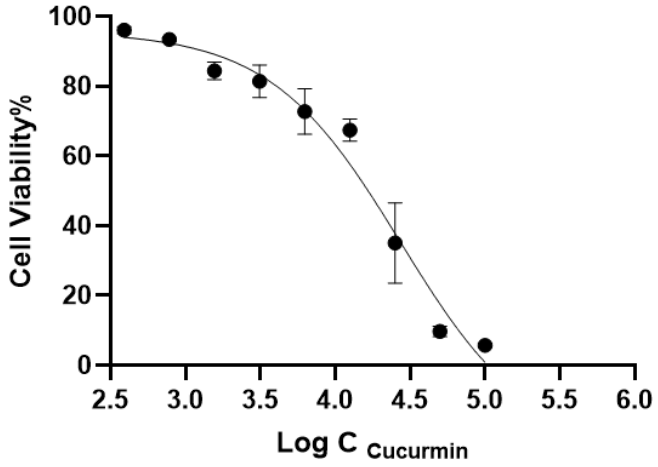


Figure 1. Dose-response curve for curcumin on MDA-MB-231 triple-negative breast cancer cells following 72 hours of treatment, determined by MTT assay. Cell viability (%) is plotted against the logarithm of curcumin concentration (nM). Data are presented as mean ± SD from triplicate measurements across two independent experiments

MTT assay with cisplatin

Cisplatin, included as a validated chemotherapeutic positive control, also produced a concentration-dependent reduction in MDA-MB-231 cell viability over 72 hours (Figure 2). The IC_{50} of cisplatin was determined to be 20.267 ± 26.0745 µM. The substantially larger standard deviation associated with the cisplatin IC_{50}, relative to curcumin, was attributable to inter-replicate variability observed across the mid-concentration range of the dose-response curve. This variability most likely arose from the larger initial stock volume (60 µL) used in preparing cisplatin serial dilutions relative to curcumin (20 µL), which introduced proportionally greater pipetting imprecision at the first dilution step; this error was then propagated through each subsequent two-fold dilution, widening the confidence interval of the fitted non-linear regression. An additional contributing factor may have been partial precipitation of cisplatin at higher concentrations if solubilisation was incomplete. Despite the variability, the cisplatin dose-response curve retained a recognisable sigmoidal profile, confirming the sensitivity of the assay and the cytotoxic activity of the positive control.

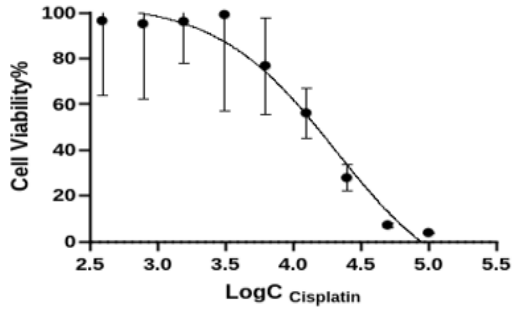


Figure 2. Dose-response curve for cisplatin on MDA-MB-231 triple-negative breast cancer cells following 72 hours of treatment, determined by MTT assay. Cell viability (%) is plotted against the logarithm of cisplatin

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

concentration (nM). Data are presented as mean ± SD from triplicate measurements across two independent experiments.

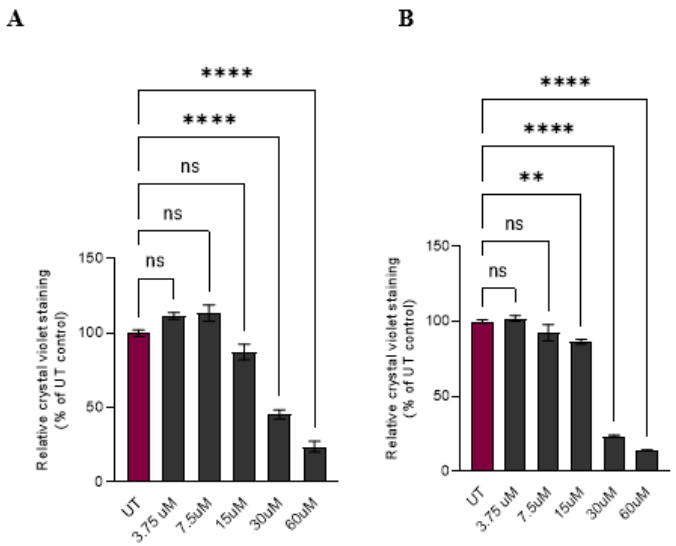
Crystal violet assay

The anti-proliferative activity of curcumin was further assessed in MDA-MB-231 cells by crystal violet staining following 72 hours of treatment at five fixed concentrations (3.75, 7.5, 15, 30, and 60 µM) in two independent experiments (Figures 3A and 3B).

In the first independent experiment (Figure 3A), one-way ANOVA revealed a highly significant overall effect of curcumin treatment on adherent cell survival ($F = 92.17$, $p < 0.0001$, $R^2 = 0.9624$). Treatment at the three lowest concentrations produced modest, non-significant changes in survival relative to the untreated control (3.75 µM, 111% survival, $p = 0.1771$; 7.5 µM, 113% survival, $p = 0.0956$; 15 µM, 87% survival, $p = 0.1139$), whereas treatment at 30 and 60 µM produced marked and highly significant reductions in survival (30 µM, 45% survival, $p < 0.0001$; 60 µM, 24% survival, $p < 0.0001$). Formal assessment of variance homogeneity supported the validity of the ANOVA model applied (Brown-Forsythe test, $p = 0.7284$; Bartlett's test, $p = 0.5565$).

In the second independent experiment (Figure 3B), one-way ANOVA again confirmed a highly significant overall treatment effect ($F = 251.2$, $p < 0.0001$, $R^2 = 0.9859$). Treatment at 3.75 and 7.5 µM produced no statistically significant change in survival relative to the untreated control (102% survival, $p = 0.9654$, and 92% survival, $p = 0.1650$, respectively), whereas treatment at 15 µM produced a small but statistically significant reduction in survival (86% survival, $p = 0.0053$). As in the first experiment, treatment at 30 and 60 µM produced marked and highly significant reductions in survival (30 µM, 24% survival, $p < 0.0001$; 60 µM, 14% survival, $p < 0.0001$).

In both experiments, the dimethyl sulfoxide vehicle control reduced adherent cell survival to approximately 38% relative to the untreated control; accordingly, all statistical comparisons were performed against the untreated control rather than the vehicle control. Overall, both independent experiments demonstrated that curcumin reduces adherent cell survival of MDA-MB-231 cells in a concentration-dependent manner, characterised by a threshold effect in which little or no significant activity was observed at concentrations up to 15 µM, followed by a marked and highly significant reduction in survival at 30 and 60 µM.



Figures 3A and 3B Effect of curcumin on adherent cell survival of MDA-MB-231 triple-negative breast cancer cells following 72 hours of treatment, assessed by crystal violet staining assay. Cells were treated with curcumin at concentrations of 3.75, 7.5, 15, 30 and 60 µM. DMSO served as the vehicle control, and untreated (UT) cells

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represented 100% baseline survival. Figure 3A represents the first independent experiment; Figure 3B represents the second independent experiment performed to confirm reproducibility. Data are presented as mean ± SD from triplicate measurements. Statistical comparisons were made against the untreated control by one-way ANOVA with Tukey's multiple comparisons test. ns = not significant; $p < 0.01$; $p < 0.0001$.

DISCUSSION

The crystal violet assay findings extend the MTT results by providing an independent, metabolic-activity-independent measure of curcumin's anti-proliferative activity. Across the concentration range tested (3.75–60 µM), both independent experiments revealed a clear threshold pattern rather than a smoothly progressive dose-response relationship. At the three lowest concentrations (3.75, 7.5, and 15 µM), reductions in adherent cell survival were generally modest and, in most cases, did not reach statistical significance relative to the untreated control (Experiment 1: 3.75 µM, 111%, $p = 0.1771$; 7.5 µM, 113%, $p = 0.0956$; 15 µM, 87%, $p = 0.1139$; Experiment 2: 3.75 µM, 102%, $p = 0.9654$; 7.5 µM, 92%, $p = 0.1650$). Notably, Experiment 2 did detect a small but statistically significant reduction at 15 µM (86% survival, $p = 0.0053$), whereas the equivalent concentration in Experiment 1 did not reach significance, suggesting that curcumin may begin to exert a mild cytostatic effect somewhere within the 15 µM range, with the precise threshold subject to some inter-experimental variability. In marked contrast, both experiments showed pronounced and highly significant reductions in survival at 30 µM (Experiment 1: 45%; Experiment 2: 24%; both $p < 0.0001$) and 60 µM (Experiment 1: 24%; Experiment 2: 14%; both $p < 0.0001$). This step-change between 15 and 30 µM, rather than a gradual decline across the full concentration range, suggests that curcumin's growth-inhibitory activity in MDA-MB-231 cells may transition relatively abruptly from a largely sub-threshold or weakly cytostatic effect to a pronounced cytotoxic effect within a comparatively narrow concentration window. This is broadly consistent with published evidence that curcumin can induce G_2/M cell cycle arrest via mTOR-mediated suppression of cyclin B1 and Cdk1 at sub-cytotoxic concentrations, before more overt cytotoxic mechanisms dominate at higher doses (Bhatt et al., 2022), although the present data suggest this transition occurs over a narrower concentration range than a purely progressive cytostatic-to-cytocidal continuum would predict.

Both experiments produced highly significant one-way ANOVA results (Experiment 1: $F = 92.17$, $p < 0.0001$, $R^2 = 0.9624$; Experiment 2: $F = 251.2$, $p < 0.0001$, $R^2 = 0.9859$), with the high R^2 values in particular indicating that treatment accounted for the substantial majority of total variance in both datasets. Whilst the F-statistics differed between experiments—reflecting differences in the magnitude of the effect and/or the level of within-group variability—both values were associated with $p < 0.0001$, confirming a robust and reproducible overall treatment effect. Formal testing of the assumption of equal variances (Brown-Forsythe test, $p = 0.7284$; Bartlett's test, $p = 0.5565$) supported the appropriateness of the ANOVA model applied, indicating that the significant differences observed were not artefacts of heteroscedasticity within the dataset. The notable vehicle toxicity observed with DMSO at the concentration employed remains an important methodological finding, reinforcing the rationale for using the untreated control, rather than the vehicle, as the primary reference standard for normalisation, as was correctly applied in this study.

A further point of interest is the apparent plateau in survival reduction observed between 30 and 60 µM in both experiments. Rather than a continued steep decline, the incremental reduction in survival between these two highest concentrations was comparatively modest (Experiment 1: 45% to 24%; Experiment 2: 24% to 14%), suggesting that curcumin's cytotoxic effect may be approaching a maximal or near-maximal response within this upper concentration range in this cell line, with limited additional benefit gained from further dose escalation. This is a notable observation that, together with the threshold effect at lower concentrations, points towards a relatively compressed effective dose-response range for curcumin in MDA-MB-231 cells under these assay conditions, and is consistent with the multi-target, saturable nature of curcumin's engagement with the nuclear factor kappa-light-chain-enhancer of activated B cells, phosphoinositide 3-kinase/protein kinase B/mechanistic

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

target of rapamycin, and Bcl-2-associated X protein/B-cell lymphoma 2 pathways described in the wider literature (Bhatt et al., 2022; Kunnumakkara et al., 2023).

Reassuringly, the overall concentration-response profiles obtained in the two independent crystal violet experiments were highly consistent with one another, both in the qualitative shape of the curve (minimal effect at low concentrations, a transition zone around 15–30 µM, and a plateauing pronounced effect at 30–60 µM) and in the overall conclusion that curcumin reduces MDA-MB-231 cell survival in a concentration-dependent manner. The principal difference between the two runs was the earlier emergence of statistical significance at 15 µM in Experiment 2 relative to Experiment 1, alongside somewhat lower absolute survival values at 30 and 60 µM in Experiment 2. These modest differences in the precise threshold and magnitude of response likely reflect ordinary biological variability between independent experimental runs, potentially arising from minor differences in cell passage number, starting confluency, or inter-experiment variation in the efficiency of cell fixation and staining. Nonetheless, the close agreement between the two experiments in both statistical robustness (high R^2 values, non-significant Brown-Forsythe and Bartlett's tests) and overall trend lends confidence to the reproducibility of the underlying biological effect, whilst still underscoring the value of performing multiple independent biological replicates to more precisely define the concentration at which cytostatic effects transition to pronounced cytotoxic activity.

Considered together, the MTT and crystal violet findings suggest that curcumin's anti-proliferative activity in MDA-MB-231 cells is broad and multi-dimensional, operating through mechanisms that are partially distinct from those of cisplatin. Notably, the half-maximal inhibitory concentrations obtained for curcumin and cisplatin in the MTT assay were broadly comparable (27.162 µM and 20.267 µM, respectively), despite their mechanistically distinct modes of action; cisplatin exerts its cytotoxic effect primarily through DNA platination and interstrand cross-linking, culminating in apoptosis via p53-dependent and p53-independent pathways (Tchounwou et al., 2021; Makovec, 2019), whereas curcumin engages multiple oncogenic signalling nodes simultaneously, including nuclear factor kappa-light-chain-enhancer of activated B cells inhibition, phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin suppression, and modulation of the intrinsic apoptotic cascade (Bhatt et al., 2022; Kunnumakkara et al., 2023). This convergence in potency, combined with their non-overlapping mechanisms, provides a preliminary rationale for investigating curcumin-cisplatin combination regimens in future studies, where synergistic interactions have been previously reported using Chou-Talalay combination index analysis (Bhatt et al., 2022). It should be noted, however, that cisplatin was not directly assessed in the crystal violet assay in the present study; confirming whether this convergence in potency, observed using a metabolic viability endpoint, is also reflected in adherent cell survival represents an important direction for future work.

The present study is limited by its use of a single TNBC cell line, which may not represent the full molecular heterogeneity of TNBC, comprising multiple distinct molecular subtypes (Bareche et al., 2018; Lehmann et al., 2021). MDA-MB-231 cells in particular harbour a gain-of-function TP53 R248W mutation and wild-type BRCA1/2 status, which influence their sensitivity to both curcumin and platinum agents, and findings may not be directly generalisable to BRCA-mutated or other TNBC subtypes (Shiovitz & Korde, 2020). The in vitro monolayer model also does not replicate the complexity of the tumour microenvironment, including stromal and immune cell interactions, hypoxia gradients, or the pharmacokinetic challenges that curcumin would face in vivo owing to its poor oral bioavailability (Moballegh Nasery et al., 2020). Additionally, only two biological replicates were performed for the crystal violet assay, and increasing the number of independent experiments would strengthen confidence in the reproducibility of the findings, particularly in more precisely delineating the apparent threshold between 15 and 30 µM. Future work should address these limitations by extending cytotoxicity assessments to additional TNBC cell lines, employing a finer concentration gradient across the 15–30 µM window to better characterise the transition from cytostatic to cytotoxic activity, conducting mechanistic endpoint experiments including Western blotting and flow cytometry for apoptosis and cell cycle analysis, and

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

investigating aquaporin gene expression modulation as a potentially novel dimension of curcumin's anti-TNBC pharmacological activity.

CONCLUSION

This study demonstrated that curcumin reduces the viability and adherent cell survival of MDA-MB-231 triple-negative breast cancer cells in a concentration-dependent manner, as assessed by MTT and crystal violet assays following 72-hour treatment. An IC_{50} of approximately 27 μM was determined for curcumin using the MTT assay, consistent with values reported in published preclinical studies, while cisplatin produced a comparable IC_{50} of approximately 20 μM, confirming assay validity. The crystal violet assay revealed a threshold concentration-response relationship across five tested concentrations (3.75–60 μM) in two independent experiments, with minimal, largely non-significant activity below 15 μM and pronounced, highly significant reductions in adherent cell survival at 30 and 60 μM, an effect that was highly reproducible between experiments (both $p < 0.0001$). These findings support the potential of curcumin as an anticancer candidate for further investigation in triple-negative breast cancer models, and highlight the 15–30 μM range as a key concentration window warranting closer mechanistic scrutiny. Future work should extend the assessment to additional triple-negative breast cancer cell lines representing distinct molecular subtypes, incorporate mechanistic endpoint experiments to elucidate the pathways involved, and evaluate the selectivity of curcumin towards malignant versus non-malignant breast cells. Investigation of bioavailability-enhanced curcumin formulations and evaluation of combination strategies with standard chemotherapeutic agents represent logical and scientifically justified translational steps building on the present findings.

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