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# Differential Cytotoxic and Morphological Effects of Curcumin and Etoposide on MCF-7 Breast Cancer Cells

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## ABSTRACT

Curcumin, a turmeric-derived polyphenol, modulates apoptotic signaling and cell-cycle control, whereas etoposide, a topoisomerase II inhibitor, exerts cytotoxicity by inducing DNA strand breaks. To delineate their relative actions on tumor cell viability, the objective was to directly compare the dose-dependent cytotoxic and anti-proliferative effects of curcumin and etoposide in ER-positive MCF-7 breast cancer cells, quantifying changes in viability and survival across graded concentrations and characterizing attendant morphological alterations. MCF-7 breast cancer cells were seeded at $1 \times 10^4$ cells per well in 96-well plates and into two groups (three technical replicates each): control DMSO (100µL), curcumin (31.2, 62.5, 125, 500, and 1000 µg/mL), etoposide (31.2, 62.5, 125, 500, and 1000 µg/mL). Treatments were applied for 72 h at 37°C. Cell viability was quantified by the MTT assay while morphological observations were documented by inverted microscope. Significantly, at the maximum concentration evaluated (1000 µg/mL), both drugs elicited pronounced morphological changes in MCF-7 cells. MTT analysis demonstrated a clear dose-dependent reduction in cell viability. Curcumin therapy diminished viability to 51%, whereas etoposide treatment led to a 75% reduction in viability at the identical quantity (1000 µg/mL). The determined half-maximal inhibitory concentration ($IC_{50}$) values were 165.4 µg/mL for curcumin and 89.2 µg/mL for etoposide, corroborating the significant cytotoxicity of both therapies.

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## INTRODUCTION

Breast cancer is the most common cancer diagnosed worldwide, with an estimated 2.3 million new cases occurring in 2020, causing approximately 685 000 deaths, it was responsible for 16% of cancer deaths in female individuals in 2020 [1]. Standard treatment for breast cancer, including chemotherapy, radiation therapy, and targeted therapies has

significantly improved patient outcomes. However, these treatments are often associated with severe side effects and the development of drug resistance, which limit their long-term [2]. The MCF-7 cell line is a widely used model for hormone-responsive (luminal) breast cancer. It was established in 1970 from a malignant pleural effusion of a 69-year-old patient with metastatic disease [3]. Natural compounds like curcumin show promise as chemotherapeutic adjuvants by enhancing efficacy and reducing systemic toxicity, with studies demonstrating their ability to mitigate drug resistance and protect normal tissue [4]. Notably, curcumin is a safe, multi-targeting agent that modulates key signaling pathways such as NF-κB and STAT3, underpinning its relevance in cancer prevention and adjunct therapy [5]. Etoposide exerts its cytotoxic effect by stabilizing the topoisomerase II-DNA cleavage complex, preventing DNA relegation and causing double-strand breaks that trigger apoptosis in cancer cells [6]. Curcumin ($C_{21}H_{20}O_6$), the primary bioactive compound in turmeric, contains functional groups like hydroxyl and methoxy that influence its chemical reactivity, including interactions with reactive oxygen species (ROS) [7]. It is a non-toxic compound with historic medicinal use and diverse therapeutic properties. Its potent anti-cancer activity is primarily attributed to its inhibition of the NF-κB pathway and subsequent downregulation of genes involved in proliferation, tumorigenesis, and metastasis [8]. Etoposide (VP-16) derives from podophyllotoxin, a toxin found in the American Mayapple. It was first synthesized in 1966 and approved for cancer therapy in 1983 by the U.S. Food and Drug Administration [9]. Etoposide is used as an anti-cancer drug for various types of cancer, including lung cancer, lymphoma, leukemia, neuroblastoma, ovarian cancer, and breast cancer [10]. Classified under the group of ‘‘topoisomerase II poisons’’ Topoisomerase II (Topo II) is an essential enzyme that controls the topology of DNA by passing an intact double helix through a transient double-stranded DNA break in an ATP-dependent reaction [11].

## MATERIALS AND METHODS

### Drugs and reagents

Curcumin (Cat. No. CS-1490; CAS 458-37-7; purity 98%) and etoposide (Cat. No. CS-1774; CAS 33419-42-0; purity 98%) were from ChemScene (USA). MTT reagent was from Elabscience (China); DMSO from HiMedia (India). DMEM and trypsin-EDTA were from US Biological (USA); FBS and penicillin–streptomycin from Capricorn Scientific (Germany). The estrogen-responsive MCF-7 breast-cancer cell line was obtained from the Iraq Center for Cancer and Medical Genetic Research (Mustansiriyah University, Baghdad). Cells were maintained in DMEM + 10% FBS at 37 °C and 5% $CO_2$, passaged at 80–90% confluence, and counted by trypan-blue exclusion. Classification of MCF-7 as $ER^+/PR^+$ and generally $HER2^-$ (luminal phenotype) guided its selection as a hormone-responsive model [12].

### MTT Cytotoxicity Assay

The estrogen-responsive breast-cancer cell line MCF-7 were obtained from the Iraq Center for Cancer and Medical Genetic Research (Mustansiriyah University, Baghdad). Cell lines were propagated in DMEM supplemented with 10 % fetal bovine serum at 37 °C in 90 % confluence. Cells were detached with trypsin-EDTA, neutralized with complete medium, and viable cells were enumerated by trypan-blue exclusion using a hemocytometer.

For cytotoxicity testing, $1 \times 10^4$ viable cells per well were seeded into 96-well plates (100 µL per well) and allowed to attach for 24 h. Curcumin and etoposide were then added at 1000, 500, 250, 125, 62.5, 31.2 µg/ml [13][14] and cultures were incubated for a further 72 h.

Cell viability was quantified by adding 28 µL of MTT solution (2 mg/ ml), incubating for 3h, removing supernatant, and solubilizing the resulting formazan crystals in 100 µL DMSO for 15 min. Absorbance was read at 492 nm with a microplate reader.

**Statistical Analysis**

The obtained data were statically analyzed using ANOVA, Tukey's multiple comparisons test with GraphPad prism 8 [15]. The values were presented as the means ± SD of triplicate measurements [16].

**RESULTS**

Phase contrast inverted microscopy was employed to evaluate the morphological effects of curcumin and etoposide on MCF-7 breast cancer cells after 72 hours of treatment. In the DMSO control group, MCF-7 cells formed a uniform epithelial-like monolayer with tight cell–cell contacts and firm adherence to the substrate, as shown in Figure (1.A). In curcumin-treated cells (Fig. 1.B), only subtle morphological changes were observed; the monolayer remained largely intact, with limited evidence of cell shrinkage, occasional cell detachment, and mild irregularities in cellular outlines. By contrast, etoposide exposure produced clear concentration-dependent alterations (Fig. 1.C), At the highest concentration (1000 µg/mL), cells exhibited marked morphological changes, including cell shrinkage, membrane blebbing, and the formation of apoptotic bodies, indicating cell death.

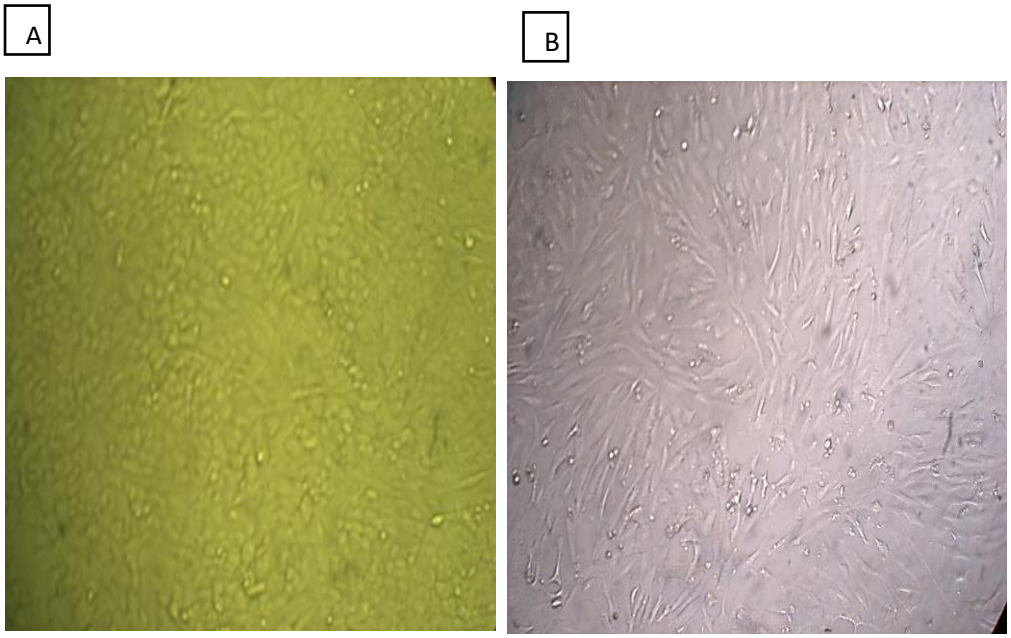



**Fig. 1.A** DMSO control MCF-7 monolayer after 72 h, imaged with an inverted light microscope at (40×).

**Fig. 1.B** MCF-7 cells after 72 h exposure to curcumin (1000 µg/mL) under an inverted microscope (40×).

C

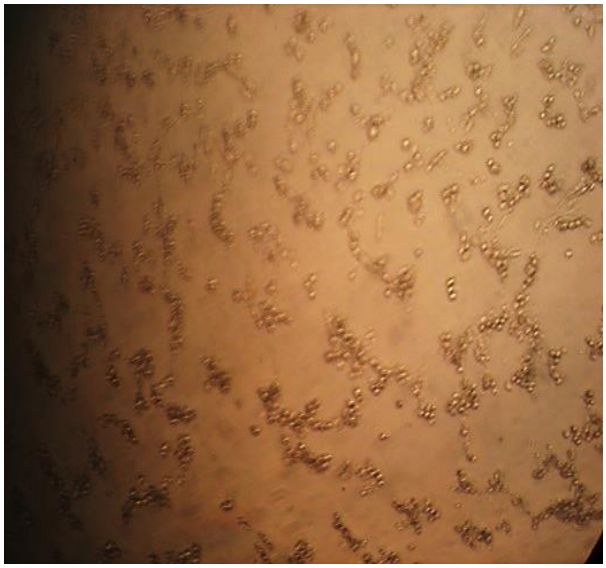
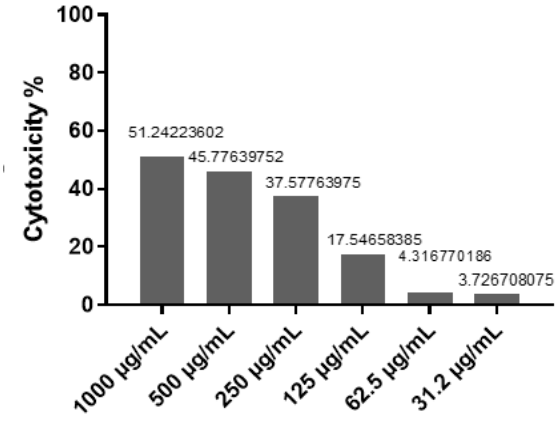


**Fig. 1.C** MCF-7 cells after 72 h exposure to etoposide (1000µg/mL) under an inverted microscope (40×).

The MTT assay revealed a clear dose-dependent decline in MCF-7 viability after 72 h of exposure (31.2, 62.5, 125, 250, 500, and 1000 µg/mL). Curcumin reduced viability significantly from 250 µg/ml upward **(Fig. 2.A)**. Etoposide produced the steepest response, pronounced inhibition beginning at 62.5 µg/ml **(Fig. 2.B)**.

A

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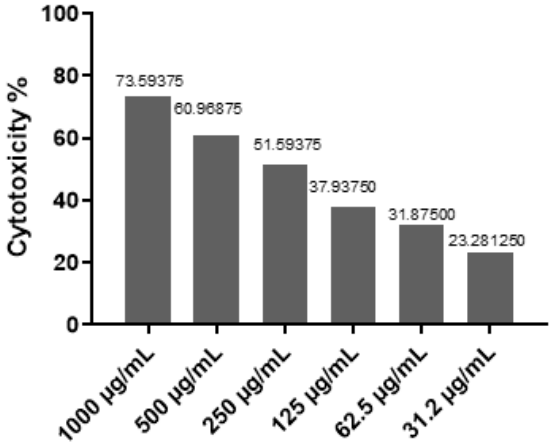


**Fig. 2.A** Dose-response curve of curcumin (31.2–1000 µg/mL) on MCF-7 cells following 72 hours of treatment. Bars show % cytotoxicity calculated from OD492 readings relative to control; numeric labels indicate the mean at each dose. The profile demonstrates a monotonic increase in cytotoxicity with dose, with the largest effect at 1000 µg/mL.

**Fig. 2.B** Dose-response curve of etoposide (31.2–1000 µg/mL) on MCF-7 cells following 72 hours of treatment. Bars show % cytotoxicity computed from OD492 readings relative to control; numeric

labels indicate the mean at each dose. Cytotoxicity increases monotonically with concentration, with the largest effect at 1000 µg/mL.

Curcumin demonstrated a higher $IC_{50}$ value (165.4 µg/mL) as showing **Fig. (3.A)** compared to etoposide (89.21 µg/mL) as showing **Fig. (3.B)** after 72 h of treatment on MCF-7 cells, indicating that curcumin is less potent, whereas etoposide exerted stronger cytotoxic activity at nearly half the concentration.

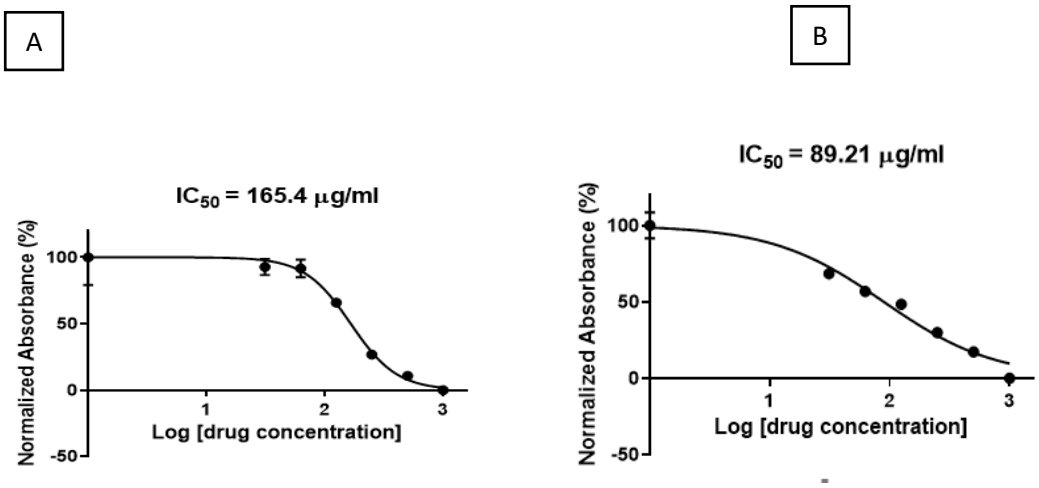


**Fig. 3.A** The IC50 value of curcumin is presented on MCF-7 cells. The fitted curve yields $IC_{50}$ = 165.4 µg/mL (≈ 449 µM; MW = 368.38 g/mol), confirming a clear dose–response relationship consistent with MTT assay principles.

**Fig. 3.B** The IC50 value of etoposide is presented on MCF-7 cells. The fit yields $IC_{50}$ = 89.21 µg/mL (≈ 151.6 µM; MW = 588.56 g/mol), confirming a robust concentration-dependent decrease in viability consistent with the MTT assay principle.

The cytotoxic effects of curcumin and etoposide on MCF-7 breast cancer cells were evaluated using the MTT assay after 72 h of exposure at different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/mL). The dose–response relationship is illustrated in (**Fig. 4)**. The x-axis represents the applied concentrations of each compound (µg/mL), while the y-axis indicates the mean optical density (OD) values measured at 570 nm, which directly reflect cell viability. Lower OD values correspond to reduced cell survival, indicating stronger cytotoxic effects.

Etoposide treatment resulted in a significant, concentration-dependent decline in OD values compared to curcumin, with the lowest readings observed at 1000 µg/mL (****$p < 0.0001$). In contrast, curcumin-treated cells maintained relatively higher OD values across all concentrations, indicating a weaker inhibitory effect on cell proliferation.

The numerical data presented as show **Table (1)** represent the raw OD values obtained from three independent replicates (triplicates) for each concentration. For example, at the maximum concentration (1000 µg/mL), etoposide-treated cells yielded OD values of 0.302, 0.291, and 0.252, confirming marked cytotoxicity, while curcumin-treated cells

recorded higher OD values (0.521, 0.532, and 0.517), reflecting less pronounced growth inhibition. These results clearly demonstrate that etoposide exerts stronger cytotoxic activity against MCF-7 cells compared to curcumin.

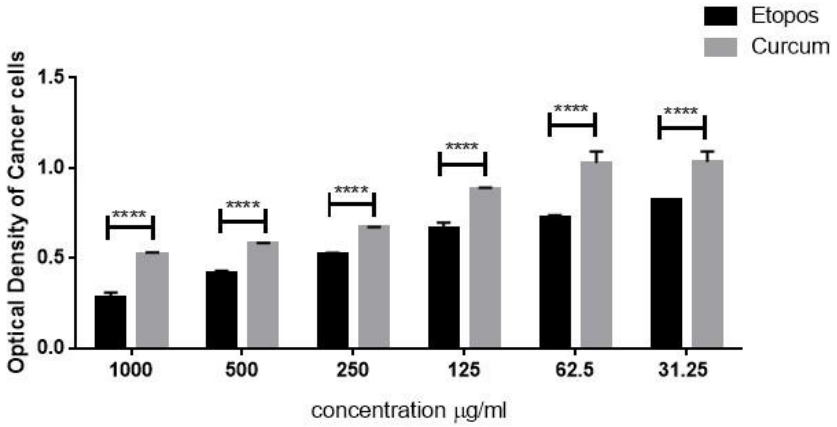


**Fig. 4.** Optical density (OD) values of MCF-7 cells treated with curcumin and etoposide at different concentrations for 72 h, measured by MTT assay

**Table 1.** Data represent the mean of three replicates (triplicates)

| | Etopos | | | Curcum | | |
|---|---|---|---|---|---|---|
| 1000 | 0.302 | 0.291 | 0.252 | 0.521 | 0.532 | 0.517 |
| 500 | 0.419 | 0.428 | 0.402 | 0.581 | 0.580 | 0.585 |
| 250 | 0.528 | 0.518 | 0.503 | 0.671 | 0.671 | 0.668 |
| 125 | 0.668 | 0.693 | 0.625 | 0.882 | 0.881 | 0.892 |
| 62.5 | 0.724 | 0.738 | 0.718 | 0.992 | 0.989 | 1.100 |
| 31.25 | 0.823 | 0.817 | 0.815 | 1.000 | 1.100 | 1.000 |

## DISCUSSION

In the present study, curcumin exhibited moderate cytotoxic activity against MCF-7 breast cancer cells, with an $IC_{50}$ value of 165.4 µg/mL after 72 hours of treatment. This value was notably higher than that of etoposide (89.21 µg/mL), reflecting a comparatively lower potency. Such findings align with previous evidence suggesting that curcumin can modulate cell cycle regulators and apoptotic pathways in breast cancer cells [17].

Although p21 (WAF1/CIP1) expression was not directly assessed in this work, earlier studies have shown that curcumin upregulates this cyclin-dependent kinase inhibitor, thereby influencing both the G1/S and G2/M checkpoints and promoting apoptosis via mitochondrial signaling [17]. Importantly, p21 functions to prevent the G2/M transition rather than inducing mitotic arrest directly. Thus, the observed cytotoxicity may partially result from cell cycle delay and apoptosis triggered through p21-associated suppression of CDK activity [17].

Curcumin has also been reported to disrupt mitotic spindle formation and induce micronucleation in MCF-7 cells [18]. These mitotic defects likely involve dysregulation of the CDK1/Cyclin B1 complex, leading to abnormal cell division and death. Although CDK1 was not measured in the current study, such mechanisms are consistent with our observed dose-dependent inhibition of cell viability [18].

Morphological observations supported the biochemical evidence of cytotoxicity. Cells treated with curcumin exhibited mild apoptotic features, including shrinkage, irregular membranes, and scattered apoptotic bodies. These alterations were less pronounced than those induced by etoposide but remain consistent with intrinsic apoptotic processes reported in other studies [19]. Curcumin has also been shown to inhibit NF-κB and Akt/mTOR signaling pathways, contributing to apoptosis and growth suppression in MCF-7 sublines [19]. Taken together, these findings indicate that curcumin exerts multi-targeted anticancer effects but demonstrates limited potency, suggesting its potential use as an adjunct rather than a standalone chemotherapeutic agent.

Etoposide, in contrast, produced marked cytotoxicity and distinct morphological alterations, including membrane blebbing, shrinkage, and detachment. Such characteristics are typical of apoptosis and correspond with previous reports describing early morphological responses to etoposide exposure [20]. Morphological evaluation therefore represents a sensitive early indicator of chemotherapeutic activity, preceding measurable declines in metabolic assays [20].

The $IC_{50}$ value of etoposide reported here is higher than that found in several previous studies. For instance, lower $IC_{50}$ values have been reported using alternative assays and longer exposure durations [21,22]. These variations likely arise from methodological differences such as assay type, incubation time, and cell density. Because the MTT assay measures mitochondrial activity that may persist in metabolically impaired cells, it tends to yield slightly elevated $IC_{50}$ estimates compared with assays assessing membrane integrity or proliferation [21,22]. Nevertheless, both our findings and prior reports consistently confirm the dose-dependent cytotoxicity of etoposide and support its established efficacy as a topoisomerase II inhibitor in hormone receptor–positive breast cancer [22].

## CONCLUSION

1- Both curcumin and etoposide reduced MCF-7 cell viability in a clear, dose-dependent manner after 72 hours of exposure.

2- Curcumin produced moderate cytotoxicity, with significant inhibition appearing from 250 µg/mL and an $IC_{50}$ of 165.4 µg/mL, indicating relatively lower potency.

3- Etoposide caused a steeper decline in viability, with significant effects starting at 62.5 µg/mL and an $IC_{50}$ of 89.21 µg/mL, confirming stronger cytotoxic activity at lower concentrations compared with curcumin.

4- Morphological evaluation showed only subtle changes in curcumin-treated cultures, whereas etoposide induced marked apoptotic features (cell shrinkage, membrane blebbing, apoptotic bodies), supporting its higher cytotoxic impact.

5- Overall, the findings demonstrate that while curcumin exerts measurable but moderate cytotoxic effects on MCF-7 cells, etoposide acts as a more potent chemotherapeutic agent under the tested conditions.

## CONFLICT INTEREST

Regarding the publication of this article, the authors have declared that they have no conflicts of interest.

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