RESEARCH ARTICLE

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Curcumin Enhanced the Chemosensitivity of Breast Cancer Cells through the Intrinsic Pathway of Apoptosis

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Abstract

Introduction: Over-expression of the anti-apoptotic proteins like *Mcl-1* is linked to the development of resistance in tumor cells against the *Bcl-2* inhibitor ABT- 737. Combination therapy involving *Bcl-2* inhibitors and *Mcl-1* inhibitors has been suggested as a promising approach to address the issue of drug resistance. In this study, we examined the impact of curcumin on the expression of *Mcl-1* and the sensitivity MDA-MB-231 and MCF-7 breast cancer cells to ABT-737. **Methods:** In this experimental study, cell toxicity was evaluated using MTT assay. The cell growth assay and colony formation assay were used to evaluate the effect of treatments on cell proliferation. The mRNA levels of *Mcl-1* and *MMP-2* were determined by qRT-PCR. Cell migration was assessed by wound healing assay. Apoptosis was detected by Hoechst 33342 staining, ELISA cell death assay and caspase-3 activity assay. **Results:** Our data demonstrated that combination treatment with curcumin and ABT-737 synergistically lowered the IC50 values and reduced colony formation, cell migration, and cell survival compared with curcumin or ABT-737 alone. ABT-737 increased the expression level of *Mcl-1* mRNA, while curcumin suppressed the expression of both *Mcl-1* and *MMP-2* mRNA. Moreover, suppression of *Mcl-1* expression by curcumin was associated with enhanced apoptosis induced by ABT-737 in breast cancer cells. **Conclusion:** In conclusion, curcumin demonstrates anti-tumor activity in human breast cancer cells by inhibiting colony formation, cell migration, and cell survival. Furthermore, curcumin can augment the apoptotic effect of ABT-737 by suppressing the expression of *Mcl-1*.

Keywords: ABT-737- Apoptosis- Breast cancer- Curcumin- *Mcl-1*

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Introduction

Breast cancer is the most common and the second leading cause of cancer deaths in women [1]. The most common treatment options for breast cancer include surgery, radiation therapy, chemotherapy, hormone therapy and targeted therapy [1, 2]. Today, chemoresistance is the one of the main obstacles to breast cancer treatment [1]. Therefore, understanding the molecular mechanisms and developing new methods to overcome chemoresistance is essential.

Mitochondrial pathway of apoptosis is regulated by two groups of *Bcl-2* family proteins including pro- and anti-apoptotic members of *Bcl-2* family which consist of 1-4 homologs domains of *Bcl-2* (B-H), including BH1, BH2, BH3 and BH4 [3]. Over-expression of anti-apoptotic members of *Bcl-2* family is often observed in different types of cancer cells, which is associated with increased cell growth, cell survival and chemoresistance [4, 5]. Moreover, previous studies show that the expression levels of *Bcl-2* protein is increased in breast cancer cells that strongly linked to the pathological stages and survival rate of breast cancer patients [6, 7]. Therefore, targeting *Bcl-2* anti-apoptotic protein members, such as *Bcl-2* protein, can be a potential strategy for breast cancer treatment.

ABT-737 is a small BH3-mimetic specifically binds to the hydrophobic groove of Bcl-xL, *Bcl-2* and to a lesser extent Bcl-w. Binding of ABT-737 to anti-apoptotic proteins lead to release of pro-apoptotic proteins and induction of apoptosis [8, 9]. ABT-737 showed the anti-cancer potential as a single agent or in combination with chemotherapeutic drugs in many types of cancers [8-12]. Studies have shown that ABT-737 does not target *Mcl-1*, and increased expression of *Mcl-1* is linked to the ABT-737 resistance in tumor cells [7, 9, 12]. Therefore, inhibiting *Mcl-1* represents a acceptable strategy to sensitize tumor cells to ABT-737 [13-15].

Curcumin (diferuloylmethane) is a hydrophobic polyphenol derived from Curcuma longa. Curcumin has been shown to have anti-cancer potential in different types of tumors, including breast cancer [16-18]. Previous findings have shown that curcumin can effectively

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suppress the proliferation of breast cancer cells in vitro and in vivo. Moreover, it significantly improves the anti-tumor properties of some traditional chemotherapeutic agents and targeted therapies [19, 20]. However, the effect of curcumin and ABT-737 combination on cancer cells has not been investigated so far. In this research we hypothesized that curcumin can enhance the therapeutic effect of ABT-737 in breast cancer cells by suppression of *Mcl-1* expression. Therefore, we examined the effect of curcumin on *Mcl-1* expression and sensitivity of breast cancer cells to ABT-737 in vitro.

Materials and Methods

Cell culture

In this experimental study, human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from the Pasteur Institute (Tehran, Iran). The cells were cultured in RPMI-1640 medium (Gibco, Invitrogen, Life technologies, Germany) supplemented with 100 U/ml penicillin-streptomycin (Gibco), 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2 mM of glutamine, and 1% sodium pyruvate, at 37 °C in a 95% humidified atmosphere containing 5% CO2.

Cytotoxicity assay

The effect of curcumin on the sensitivity of MDA-MB-231 and MCF-7 cells to ABT-737 was assessed using MTT assay. Curcumin (C21H20O6) and ABT-737 (C42H45ClN6O5S2), both obtained from Cayman (Ann Arbor, MI, USA). The experiment was subdivided into five groups: ABT-737, curcumin, ABT-737 + curcumin, blank control and solvent control. In brief, cells were seeded in 96-well tissue culture plates at a density of 3×10^3 cells/well. After overnight incubation, the cells were treated with different concentrations of ABT-737 (0-32 μM), curcumin (0-32 μM) for 48 hours. Next, the cytotoxic effect of the compounds was evaluated by using a cell proliferation MTT kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions. The absorbance (A) of each well was measured on a multi-plate reader (Awareness Technology, Palm City, FL, USA) at a wavelength of 570 nm with a reference wavelength of 650 nm. The survival rate (SR) was determined by the equation as follows: SR (%) = (A Test /A Control) ×100%. IC_{50} (half max inhibitory concentration) values were calculated using Prism 6.01 software (GraphPad Software Inc., San Diego, CA, USA).

Analysis of combined drug effects

The combination effect of ABT-737 and curcumin was evaluated for its impact on the survival of breast cancer cells using the Cho-Talalay method [21], which employs the combination index (CI) analysis. The data obtained from MTT assay were transformed into Fraction affected (Fa values ranging from 0 to 1, where Fa=0 represents 100% cell survival and Fa=1 represents 0% cell survival. The CompuSyn program (ComboSyn Inc., Paramus, NJ, USA) was used to calculate Fa values. The CI value were used to determine the type of interaction, with CI less than 1 indicating synergism, a CI equal to 1 indicating an additive effect, and a CI greater than 1 indicating antagonism.

Cell proliferation assay

The antiproliferative effects of ABT-737 and curcumin were assessed by trypan blue staining. Briefly, the cell lines were cultured in six-well plates at a density of 5×104 cells/well and treated with ABT-737 and curcumin, alone and in combination. After the treatment, the cells were allowed to incubate for duration of 5 days. At different times, total cells were collected by trypsinization. Subsequently, cell suspensions were subjected to staining with 0.4% trypan blue (Merck KGaA, Darmstadt, Germany) for 3 min. The viable cells, or stained cells, were enumerated using an inverted microscope (Nikon Instrument Inc., Melville, NY, USA) and a hematocytometer. Cell viability was evaluated by comparing the percentage of viable cells to the blank control group.

Quantitative real-time PCR (qRT-PCR)

After the 48 h treatments, total RNA was isolated from the cell lines using the RNA extraction kit (Parstous, Tehran, Iran) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 2 μg of total RNA using Easy cDNA Synthesis Kit (Parstous) and oligo-dT primer, as described by the manufacturer. The qRT-PCR analysis was conducted utilizing the LightCycler 96 System (Roche Diagnostics GmbH, Mannhein, Germany) and SYBR Green qPCR MasterMix (Parstous). The specific component of qRT-PCR reactions were 1 μl of cDNA template, 10 μl of SYBR Green qRT-PCR MasterMix, 0.2 μM of each of the primers, and 7 μl of distilled water. The specific primers used for qRT-PCR analysis were as follows: forward, 5'-ATACCATCGAGACCATGCG-3', reverse, 5'-CCAATGATCCTGTATGTGATCTG-3', for *MMP-2*, forward, 5'- TAGTTAAACAAAGAGGCTGGGA-3', reverse, 5'- CCTTCTAGGTCCTCTACATGG -3', for *Mcl-1*, and forward, 5'-GACATCCGCAAAGACCTGTA-3', and reverse, 5'- GGAGCAATGATCTTGATCTTCA-3', for β-actin. The experimental conditions were as follows: an initial incubation step at a temperature of 95 oC for 10 min, followed by 35 cycles at 95 oC for 15 seconds, 57 oC for 20 sec, and 72 oC for 20 sec. To determine the relative gene expression levels, the comparative CT methods [22] were used. To ensure accurate and reliable results, the expression levels were normalized using β-actin as an endogenous control gene.

Wound healing assay

Breast cancer cells were cultured into 6-well plates in complete growth medium. An artificial scratching was made using 2 mm wide pipette tips when the cells had grown to 90%. The plates were washed 2 times with sterile PBS and then filled with complete medium containing IC_{50} doses of ABT-737 and curcumin, alone and in combination. After 48 h, the wound closure was photographed using an inverted microscope.

Colony formation assay

MCF-7 and MDA-MB-231 cells (5×10^3 cells/well)

were seeded in 6-well plates. Then cells were allowed to attach overnight and then treated with IC_{50} doses of ABT-737 and curcumin for 48h. After 10 days, the colonies were fixed with 3.7% paraformaldehyde and stained with 0.4% crystal violet. The number of grown colonies was measured and assessed as the ratio of the number of treated cells to control cells

Hoechst 33342

The two breast cancer cell lines were separately plated at the density of 4×10^4 cells/well in 12-well plates for 24 h. Then the cells were exposed to ABT-737 and curcumin. After 48 h of incubation, the cells were washed twice with PBS, fixed in 3.7% formaldehyde and stained with Hoechst 33342 (Beyotime, Jiangsu, China) for 10 minutes. Under an inverted fluorescence microscope, cells showing condensed or fragmented nuclei were defined as apoptotic cells.

Apoptosis ELISA assay

The measurement of the total amount of cytosolic mono- and oligonucleosomes that were released by apoptotic cells was conducted using the cell death detection ELISA plus kit (Roche Diagnostics GmbH). The cultivation of breast cancer cells was carried out at a density of 1×105 cells/well in 6-well plates. Cells were collected and resuspended in a lysis buffer 48 h after treatments. The resulting suspension was then subjected to centrifugation at 200 g. Subsequently, 20 µl of resulting supernatant was combined with 80 µl of a solution containing anti-histone-biotin and anti-peroxidase. The combined mixture was then transferred to individual wells of a sreptoavidin-coated plate. After 2 h of incubation, the wells were washed and 100 µl of 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) solution was added to each well. The ABTS stop solution was utilized to terminate the reactions, and the absorbance was immediately determined at 405 nm using ELISA microplate reader. The fold change in apoptosis was obtained by dividing the absorbance value of treatment group by the absorbance value of the control group.

Caspase-3 activity assay

The in vitro activity of the caspase-3 assay was measured according to the colorimetric caspase-3 activity assay Kit (Abnova Corporation, Taipei, Taiwan). After treatment, the cells were collected and lysed in a cell lysis buffer. The cell lysate was centrifuged at 10,000 g for 1 min. Next, the supernatant was transferred to a fresh tube. Then, 50 µl of 2X reaction buffer containing 10 mM DTT and 5 µl of 4 mM caspase-3 (DEVD-pNA) colorimetric substrate were added to each sample. Following a 2 h incubation period at 37 oC, the absorbance was measured using a microplate reader at a wavelength of 405 nm.

Statistical analysis

Results in this study are shown as mean ± standard deviation (SD) of at least three independent experiments. Statistical comparisons between groups were evaluated using one-way analysis of variance (ANOVA) and Student's t-test. The statistical analyses were performed by GraphPad Prism software (GraphPad, San Diego, CA, USA). P-values less than or equal to 0.05 were considered statistically significant.

Results

Curcumin enhanced the sensitivity of breast cancer cells to ABT-737

To assess whether curcumin could increase the sensitivity of the breast cancer cells to ABT-737, a combination treatment of curcumin and ABT-737 on MDA-MB-231 and MCF-7 cells was performed. The results of MTT assay showed that treatment with curcumin and ABT-737 alone, markedly reduced the cell survival rate in a dose-dependent way (relative to the blank control) (Figure 1). Moreover, combination treatment further decreased the cell survival rate relative to curcumin or ABT-737 alone ($p<0.05$). The IC_{50} values of curcumin and ABT-737 for 48 h treatment were 12.8 and 2.6 µM in MDA-MB-231 cells, and 23.9 and 4.1 µM in MCF-7 cells, respectively (Table 1). As illustrated in Table 1, the combination treatment with curcumin and ABT-737 led to a significant decrease in the IC_{50} value, relative to the monotreatment. These data propose that curcumin can increase the sensitivity of breast cancer cells to ABT-737.

The combination effect of curcumin and ABT-737 on breast cancer cells was synergistic

To evaluate whether the effect of curcumin and ABT-737 on cell survival is responsible for their synergistic impact, we performed the combination index analysis, based on the non-constant method of Chou-Talalay. The CI-Fa plot demonstrated that the combination of curcumin (0-32 µM) with ABT-737 (0-32 µM) on breast cancer cells resulted in a synergistic interaction (CI<1) combination. Notably, the pronounced synergistic effect was observed after 48 h of treatment, with a combination of 4 µM curcumin and 4 µM ABT-737 (CI=0.70), with Fa level of 0.93 in MDA-MB-231 cell line (Figure 1). Moreover, in MCF-7 cells the strongest synergistic effect was obtained at 4 µM curcumin in combination with 4 µM ABT-737 (CI=0.71), with Fa level of 0.78 (Figure 1).

Curcumin increased the proliferation inhibition effect of ABT-737

We then studied the effect of curcumin and ABT-737 on the proliferation of the breast cancer cells. The cells were treated with curcumin and ABT-737, alone and in combination. Cell viability was then evaluated

Table 1. IC_{50} Values of the Curcumin and ABT-737, Alone and in Combination, in MCF-7 and MDA-MB-231 Cell Lines.

	IC_{50} (µM)	
	MCF-7	MDA-MB-231
Curcumin	23.9	12.8
ABT-737	4.1	2.6
Combination	1.3*	0.8*

Data are expressed as the mean±SD (n=3). *p<0.05 versus single treatment

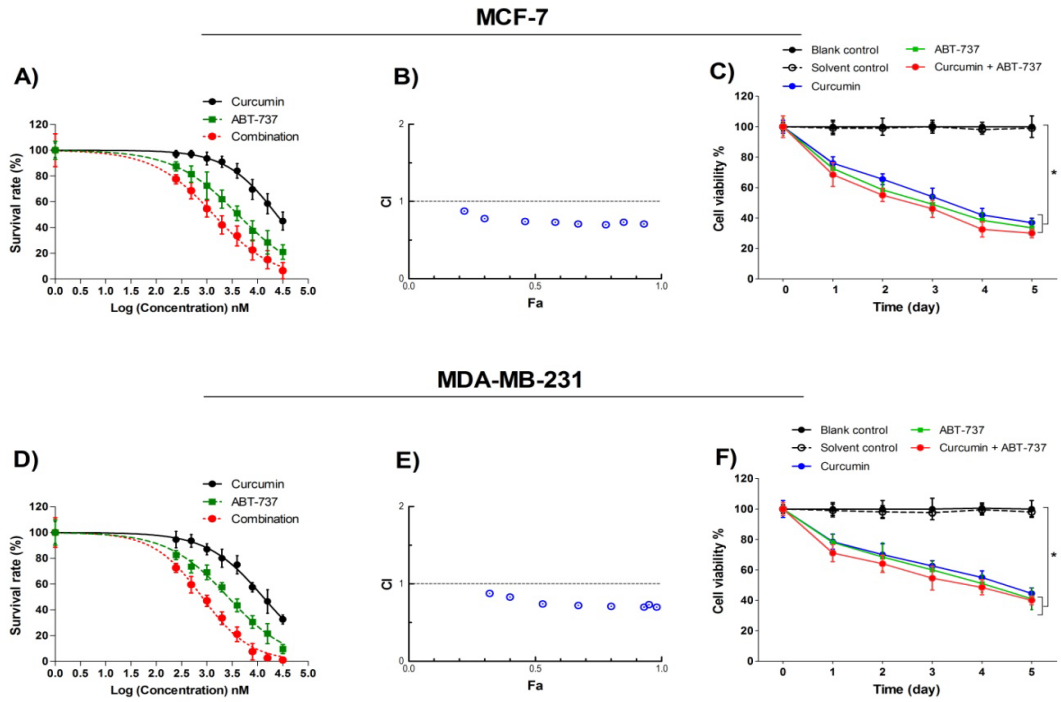

Figure 1. Effect of Curcumin and ABT-737 on Cell Proliferation and Survival. The breast cancer cells were treated with curcumin and ABT-737 at concentrations indicated in the study. After a period of 24 h, the MTT assay was conducted to assess the survival of the cells. The resulting data was then utilized to plot the cell survival curves using GraphPad software (A and D). The data presented in this study represents the mean±SD of three independent experiments. The combination index (CI) values were determined by analyzing the fractional affected (Fa) values obtained from the MTT assay using CalcuSyn software (B and E). C and F show the proliferation curves of the breast cancer cells.

by trypan blue assay during a period of 5 days. The cell proliferation curve showed that compared with the control groups, the viability of the cells in curcumin, ABT-737 and combinatorial group significantly reduced in a time-dependent way. In MDA-MB-231 cells, the cell viability in curcumin, ABT-737 and combinatorial group decreased to 78 %, 77 % and 71 % respectively, after 24 h of treatment, and then to a further 44 %, 41 % and 40 %, at the end of the experiment ($p<0.05$; Figure 1). Similar results were observed in the MC-7 cell line. The IC_{50} value in combined group was lower than the IC_{50} value where the reagents were used individually. These results indicate that the combined effect of two agents on inhibiting cell proliferation is more potent than their individual effects.

Curcumin reduced the expression of Mcl-1 (apoptotic) and MMP-2 (Metastatic) genes

qRT-PCR method was used to analysis the effect of curcumin and ABT-737 on gene expression. The results demonstrated that 48 h after the treatment of MDA-MB-231 and MCF-7 cells with curcumin, the mRNA levels of *Mcl-1* and *MMP-2* decreased relative to the blank control group ($p<0.05$). Treatment with ABT-737 did not cause a significant change in the expression of *MMP-2* and enhanced the expression of *Mcl-1* (Figure 2). Moreover, combination treatment significantly lowered the expression of *MMP-2* compared to the blank control and ABT-737 treated cells. The effect of combination treatment and *MMP-2* expression was markedly less

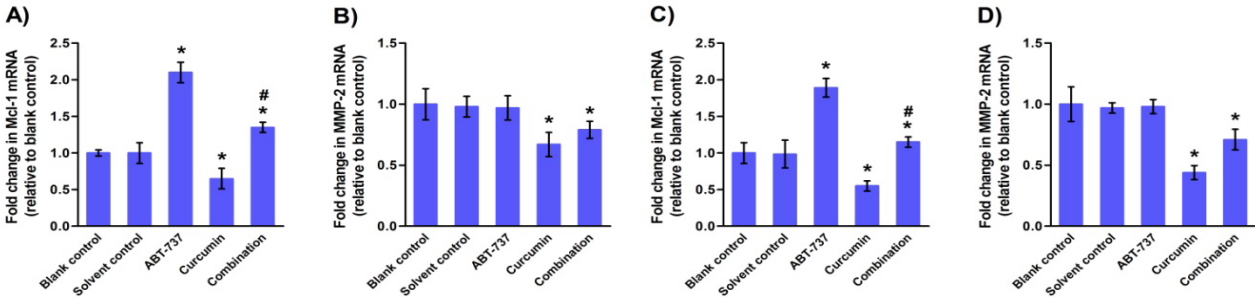

Figure 2. RT-qPCR Analysis of Breast Cancer Cells. The MCF-7 and MDA-MD-231 cells underwent a 24 h treatment with curcumin and ABT-737 (IC50 doses). The expression levels of *Mcl-1* and *MMP-2* mRNA in MCF-7 (A and B) and MDA-MD-231 (C and D) cells were subsequently assessed using the RT-qPCR technique and the 2-(ΔΔCt) method. Results are presented as mean±SD (n=3). #$p<0.05$ relative to single treatment; *$p<0.05$ relative to blank control.

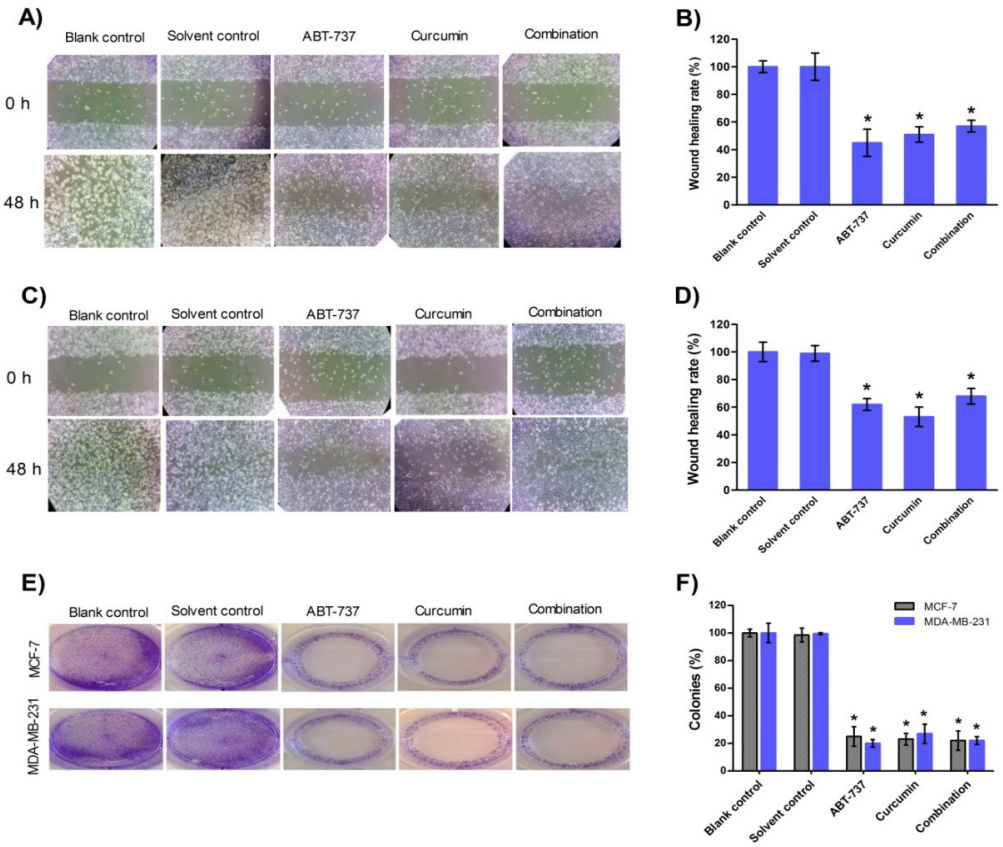

Figure 3. Effect of Curcumin and ABT-737 on Colony Formation and Migration of Breast Cancer Cells. The cells were treated with IC50 doses of curcumin and ABT-737 for 48 h. The migration of MCF-7 (A and B) and MDA-MB-231 (C and D) cells was quantified by measuring wound closure areas 48 h after treatments. E and F show the Effect of curcumin and ABT-737 on colony formation in breast cancer cells. The cell colonies were stained with crystal violet and the number of colonies was observed after 48 h. Data were representative of three independent experiments.

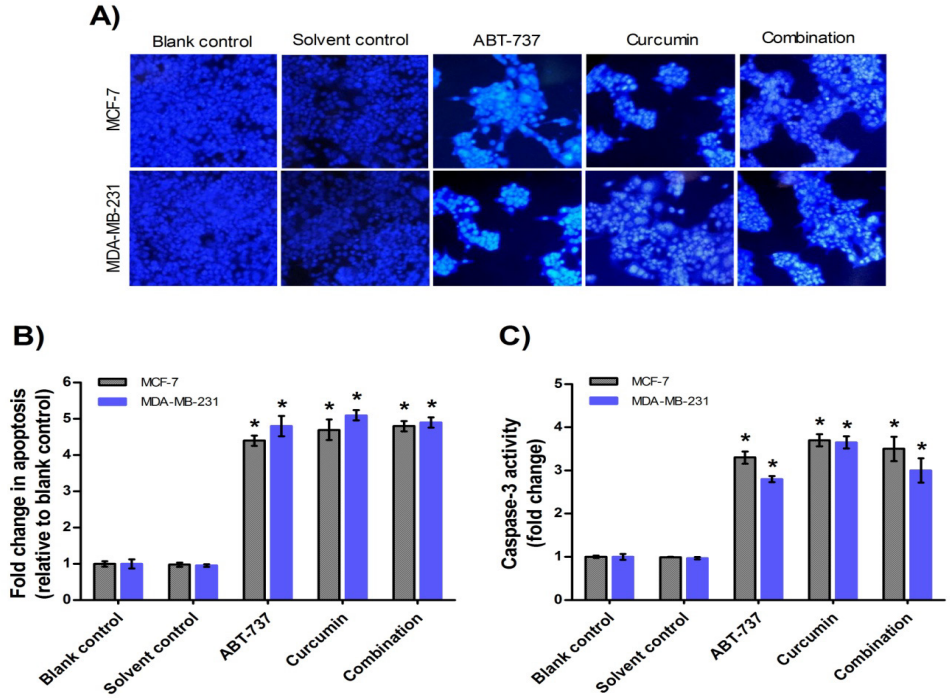

Figure 4. Induction of Apoptosis by Curcumin and ABT-737 in Breast Cancer Cells. MCF-7 and MDA-MB-231 breast cancer cells were treated with IC50 doses of curcumin and ABT-737 for 48 h. Nuclear morphological changes were observed using Hoechst33342 staining and a fluorescence microscope (A). Fold change in apoptosis was measured using ELISA cell death assay (B). C show caspase-3 activity of breast cancer cells after 48 h treatment.

than curcumin treated cells. *Mcl-1* gene expression in the combination group demonstrated a clear difference with the single treatment and blank control groups (Figure 2). These findings suggest that curcumin may have the potential to neutralize the effect of ABT-737 on *Mcl-1* expression. The mRNA expression levels in solvent control group were found to be similar to these in the blank control group, indicating no significant difference ($p>0.05$).

Curcumin enhanced the ABT-737-mediated inhibition of breast cancer cell migration

Wound-healing assay was used to assess the effects of a combination treatment with curcumin and ABT-737 on breast cancer cell migration. Wound-healing assay showed that treatment with curcumin and ABT-737 alone inhibited breast cancer cell migration, while, combined treatment showed the same effect at a lower dose (Figure 3). Quantitative analysis of cell migration in wound healing was executed (Figure 3). These results indicate that curcumin enhances the ABT-737-mediated inhibition of breast cancer cell migration.

Curcumin decreased the colony formation rate of breast cancer cells

Antitumor activities of curcumin and ABT-737 in MDA-MB-231 and MCF-7 cells were also determined using the colony formation assay. Results showed that the colony formation ability of MDA-MB-231 and MCF-7 cells was significantly reduced after the exposure to curcumin and ABT-737 (Figure 3). Moreover, in the combination treatment group, a significant reduction in colony formation of both cell lines was observed compared to control cells ($p<0.05$).

The apoptotic effect of ABT-737 in breast cancer cells was potentiated by the addition of curcumin

To analysis whether the inhibitory effect of compounds on cell survival was related to the induction of apoptosis, the MDA-MB-231 and MCF-7 cells were exposed to the IC_{50} doses of curcumin, ABT-199 and their combination for 48 h. Then, we carried out Hoechst 33342 nuclear staining and ELISA cell death assay. Results clearly demonstrated the occurrence of apoptotic cells containing nuclear fragments in cells treated with curcumin and ABT-737. Conversely, the control cell did not exhibit this particular characteristic (Figure 4). Moreover, the combination treatment did not result in a significant change in the number of apoptosis cells compared to the cells that were treated with a single agent.

The ELISA cell death assay results indicated a notable increase in apoptosis after 48 hours of exposure to curcumin and ABT-737. The extent of apoptosis in MDA-MB-231 cells were found to be 5.1 and 4.8 times higher in curcumin or ABT-737 treated cells, respectively (compared to the blank control group, $p<0.05$). Moreover, the extent of apoptosis was determined to be 4.9 times greater in the combination group at the specified time point ($p<0.05$). Conversely, no significant changes in apoptosis were observed in the solvent control group when compared to the blank control group ($p>0.05$). Similar results were observed in the MCF-7 cell line.

The results indicate that the combination of two agents has a more potent effect in inducing apoptosis compared to the individual agent alone. This is evident from the fact that IC_{50} dose of the combination group is lower than that of either agent alone. The enhanced apoptotic effect observed aligns with the results obtained from the MTT assay.

Caspase-3 activity enhanced after treatment of the breast cancer cells with curcumin and ABT-737

In order to investigate the underlying nuclear process of apoptosis triggered by curcumin and ABT-737, a caspase-3 activity assay was performed. The results demonstrated a significant increase in caspase-3 activity in the cell exposed to curcumin or ABT-737 ($p<0.05$). Moreover, the level of caspase-3 activity in breast cancer cells treated with the combination of curcumin and ABT-737 did not differ when compared to the caspase-3 activity in cells treated with either curcumin or ABT-737 alone ($p>0.05$, Figure 4).

Discussion

The management of breast cancer requires a multifaceted approach that includes both local and systemic strategies. These may encompass procedures like surgical tumor removal, the use of targeted antibody treatments, chemotherapy, and small inhibitory molecules. Nevertheless, the efficacy of chemotherapy in addressing breast cancer is frequently impeded by the intricate and varied characteristics of the illness, leading to the development of drug resistance [1-3]. It is crucial to emphasize the significance of creating innovative and enhanced approaches for treating breast cancer in a more efficient manner. Up-regulation of anti-apoptotic proteins have been correlated with heightened cellular proliferation, resistance to pharmaceutical therapies, diminished cell apoptosis, and an unfavorable prognosis for individuals suffering from breast cancer. It has been reported that cancer cells with increased *Mcl-1* levels show resistance to ABT-737 [4, 5, 7]. The concept of combining ABT-737 with *Mcl-1* inhibitors represents a promising strategy for overcoming resistance to ABT-737 in cancer treatment [9, 15, 23]. Thus, in this study, we investigated the impact of curcumin on the expression of *Mcl-1* and the sensitivity of breast cancer cells to ABT-737.

The results of our study demonstrated that the administration of either curcumin or ABT-737 individually resulted in a notable decrease in cell survival and triggered apoptosis. However, when curcumin and ABT-737 were used in combination, there was a significant reduction in the IC_{50} value and a synergistic decrease in the cell survival rate compared to using formononetin or ABT-737 alone. The IC_{50} dose required for the combination treatment was lower than the IC_{50} dose needed for either compound on its own. Therefore, our data clearly indicate that the combined use of these two agents has a more pronounced impact on cell survival and apoptosis than the treatment with each agent separately. So far,

numerous investigations have been conducted to explore the correlation between the expression level of the *Mcl-1* gene in cancer cells and their resistance to ABT-737. For example, Konopleva et al. [24] in a study investigated the factors influencing the sensitivity and resistance of AML cells to ABT-737. Their research revealed that AML cells exhibiting elevated levels of anti-apoptotic proteins such as Bcl-xL, *Mcl-1*, or *Bcl-2*, along with decreased levels of Bim, a pro-apoptotic protein, is associated with the resistance of AML cells to ABT-737. Furthermore, the activation of survival pathways, like the PI3K/Akt pathway, was identified as a contributing factor to the resistance of AML cells to ABT-737. Tahir et al. [25] in another study investigated the contribution of *Bcl-2* family member proteins to the cellular response of different small-cell lung cancer cells to ABT-737. They found that small-cell lung cancer cell lines that exhibited heightened expression of *Bcl-2, Bcl-xL*, Bim, and Noxa, along with diminished levels of *Mcl-1*, demonstrated susceptibility to ABT-737. Wang et al. [26] demonstrated that A-1210477, a specific inhibitor of *Mcl-1*, exhibited the ability to overcome resistance to ABT-737 in AML cells that had up-regulated *Mcl-1*. By combining A-1210477 with ABT-737, a synergistic effect was observed, leading to the induction of apoptosis in AML cells. In their study, Woo et al. [15] conducted research on the effect of YM155, a survivin inhibitor, and ABT-737 on cellular apoptosis in lung cancer, glioma, and renal cell carcinoma cells. They found that YM155 played a crucial role in enhancing the sensitivity of tumor cells to ABT-737 by reducing the expression of *Mcl-1*. In this study, we demonstrated that curcumin decreases the expression of the *Mcl-1* mRNA and enhances the apoptosis caused by ABT-737 in MCF-7 and MDA-MB-231 breast cancer cells. Our results are in agreement with the above previous reports and confirm that curcumin can increase the sensitivity of the breast cancer cells to ABT-737 by suppressing the *Mcl-1*expression.

We also investigated the effect of curcumin and ABT-737 on gene expression. The result of qPCR revealed that ABT-737 enhanced the expression of *Mcl-1* mRNA without affecting the expression of *MMP-2* mRNA. Moreover, curcumin lowered the expression of both *MMP-2* and *Mcl-1* mRNA in tumor cells. In combination treatment, curcumin lowered the increased *Mcl-1* mRNA induced by ABT-737, which was associated with inhibition of cell proliferation, colony formation, and cell migration, and enhanced apoptotic sensitivity to ABT-737. In accordance with our study, various studies have been conducted regarding the effect of curcumin on gene expression and cellular process of tumor cells. A previous study showed that curcumin inhibits tumor proliferation and induces apoptosis by inhibition of prosurvival pathways, including *STAT3, AKT*, and *NF-kB* in CLL B cells. Moreover, curcumin suppressed expression of the anti-apoptotic proteins *Mcl-1* and XIAP, and up-regulated the pro-apoptotic protein Bim [27]. In another study, the effect of the dual PI3K/Akt and mTOR inhibitor NVP-BEZ235 and curcumin on human renal carcinoma Caki cells was investigated. The results of this study revealed that curcumin markedly induced apoptosis in NVP-BEZ235-treated cells. Moreover, combined treatment with NVP-BEZ235 and curcumin induces apoptosis through p53-dependent *Bcl-2* mRNA down-regulation at the transcriptional level and *Mcl-1* protein down-regulation at the post-transcriptional level [28]. Zou et al. [29] investigated the role of Flap endonuclease 1 (FEN1) in cisplatin resistance and the chemosensitizing effects of curcumin in breast cancer cells. They showed that FEN1 over-expression enhances cisplatin resistance in breast cancer cells, and that FEN1 knockdown by curcumin enhances cisplatin sensitivity. In a study, Attia et al. [30] investigated the effect of curcumin in combination with paclitaxel on MCF-7 breast cancer cells. Results showed that curcumin in combination with paclitaxel have synergistic cytotoxic interaction in MCF-7 breast cancer cells. Moreover, they showed that drug combination enhanced the expression of *Bax, Caspase 7* and 9, and down-regulated the expression of multidrug resistance complex (MDR-1 or P-glycoprotein). Aggarwal et al. [31] in another study demonstrated that paclitaxel activates NF-kB in breast cancer cells and curcumin inhibits it. Curcumin also suppressed the paclitaxel-induced expression of anti-apoptotic (XIAP, IAP-1, IAP-2, *Bcl-2*, and *Bcl-xL*), proliferative (*COX-2, c-Myc,* and *cyclin D1*), and metastatic proteins (VEGF, MMP-9, and ICAM-1). It also enhanced apoptosis. The results presented in the above reports are consistent with our research, suggesting that treatment involving curcumin has the potential to increase the susceptibility of cancer cells to chemotherapeutic agents, such as ABT-737, through the activation of apoptosis.

Two primary signaling pathways regulate cellular apoptosis: the extrinsic pathway and the intrinsic or mitochondrial pathway [32]. The intrinsic pathway is activated by harmful stimuli within the cell, leading to the release of cytochrome c and the activation of caspases-9. In contrast, the extrinsic pathway is triggered by ligands binding to death receptors on the cell's surface, resulting in the activation of caspase-8. Both pathways converge at caspase-3, a central player in the apoptotic process. Caspase-3 activation initiates a proteolytic cascade, activating other caspases and initiating a series of apoptotic events [32]. The regulation of intrinsic pathway is performed by the pro- and anti-apoptotic members of the *Bcl-2* family proteins [33]. In apoptotic conditions, the pro-apoptotic members such as Bak and Bax are activated. Activated Bak and Bax cause the mitochondrial outer membrane permeability (MOMP), release of cytochrome c into the cytoplasm, and subsequently activation of caspases. The anti-apoptotic proteins such as *Bcl-2* and *Mcl-1*, when not sequestered by pro-apoptotic members, inhibit apoptosis [34]. ABT-737, a synthetic BH3-mimic, exhibits strong binding affinity towards *Bcl-2*, Bcl-Xl, and Bcl-w proteins. However, its affinity towards *Mcl-1* is relatively low. Research indicates that the up-regulation of *Mcl-1* is linked to a decrease in the toxicity of ABT-737 and the development of secondary resistance. Consequently, inhibiting the *Mcl-1* protein has been suggested as a potential approach to enhance the sensitivity to ABT-737 [8-10, 12, 35].

Curcumin has been shown to cause changes the

expression of Bcl-xL, XIAP, *Mcl-1* and *Bcl-2* through AKT, NF-kB and STAT3 signaling pathways by which inhibits cell proliferation and triggers apoptosis [36]. Studies have shown that curcumin can induce apoptosis through both the extrinsic and intrinsic pathways. However, the exact mechanism of action is still unclear [37]. In this study, we demonstrated that curcumin decreases the expression *Mcl-1*. The observed change in gene expression was found to be closely correlated with the initiation of cellular apoptosis. Additionally, down-regulation of *Mcl-1* was correlated with heightened responsiveness to ABT-737. The findings of our study indicate that curcumin has the potential to not only induce cell death but also increase the susceptibility of breast cells to chemotherapeutic drugs like ABT-737. This effect is achieved by altering the expression of *Mcl-1*, a protein involved in cell survival.

We also examined the effect of the compounds on cell migration. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that play a crucial role in the progression of cancer, particularly in tumor invasion, neoangiogenesis, and metastasis. The MMPs are involved in the degradation of the extracellular matrix, which allows for the spread of cancer cells. High levels of MMPs have been correlated with poor overall survival in various types of solid tumors, including breast, lung, colon, gastric, pancreatic, and prostate cancer [38]. Our results showed that treatment with each of ABT-737 or curcumin inhibits the rate of cell migration. Inhibition of cell migration by curcumin was associated with suppression of *MMP-2* gene expression in breast cancer cells. Our findings confirm the results of several past studies and show that curcumin plays a role in inhibiting cell migration and metastasis by reducing the expression of *MMP-2* [36].

Curcumin exhibits extremely poor oral bioavailability (<1%) due to low water solubility, rapid metabolism (glucuronidation/sulfation), and chemical instability at physiological pH. These pharmacokinetic limitations result in subtherapeutic plasma concentrations despite high oral doses (up to 12 g/day), creating a significant gap between promising preclinical results and clinical efficacy. While in vitro studies show activity at μM concentrations, human trials achieve only nM levels, with >90% of circulating compounds being inactive metabolites. Translational challenges are compounded by inconsistent clinical trial designs and insufficient focus on bioavailability-enhanced formulations. Solutions include nanoparticle delivery (e.g., Theracurmin increasing bioavailability 185-fold), phytosome complexes (Meriva), and piperine adjuvants though manufacturing scalability and rigorous clinical validation remain critical for therapeutic translation.

In conclusion, our study revealed that the combined administration of curcumin and ABT-737 resulted in a significant decrease in the IC_{50} value. Furthermore, this combination treatment exhibited a synergistic effect in reducing colony formation, impeding cell growth, and enhancing cell death compared to individual treatments. Notably, curcumin demonstrated its ability to inhibit cell migration and induce apoptosis in breast cancer cells by down-regulating *MMP-2* and *Mcl-1*, respectively. Interestingly, the expression of *Mcl-1* was observed to increase following treatment with ABT-737. Additionally, curcumin exhibited the potential to augment the apoptotic impact of ABT-737 in breast cancer cells by suppressing *Mcl-1*.

Author Contribution Statement

Study concept and design: HK and AA; Acquisition of data: ZSM, HB and HK; Analysis and interpretation of data: ZSM, HK and AA; Drafting of the manuscript: ZSM, HB, HK and AA; Critical revision of the manuscript for important intellectual content: HK and AA; Funding recipients: HK and AA..

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Ethical approval

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Conflict of interest

The authors have no conflict of interest to declare

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