RESEARCH ARTICLE

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Antiproliferative Effects of Curcumin Different Types of Breast Cancer

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Abstract

Objective: Breast cancer is one of the most frequently diagnosed malignancy among women. Turmeric is isolated from Curcuma longa. Curcumin is main curcuminoid of the turmeric which is a member of Zingiberaceae. In this current study antiproliferative effects of curcumin were investigated in luminal A breast cancer cell line MCF-7 and triple negative breast cancer cell line MDA-MB-231. **Methods:** For this purpose cell viability, cell index values by xCELLigence Real-Time Cell Analysis DP instrument, mitotic index and apoptotic index analysis were used. **Results:** Cell viability and cell index values showed that 75 μM concentration of curcumin was IC_{50} concentration. When IC_{50} concentration was applied to both cell lines, a significant decrease was observed in the mitotic index values, while a significant increase was observed in the apoptotic index values ($p<0.05$). **Conclusion:** Curcumin, which has antiproliferative effects on breast cancer cells, is thought to be effective in cancer treatment.

Keywords: Curcumin- breast cancer- cell kinetics- xCelligence

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Introduction

Curcumin is a compound derived from the rhizome of turmeric (Curcuma longa), a member of the ginger family (Zingiberaceae). Curcumin is a curcuminoid and contains about 2-5% turmeric (Miller et al., 2008). In addition to giving turmeric its yellow color, curcumin is also responsible for most of its therapeutic effects. It is used in alternative medicine due to its anti-inflammatory, anti-tumorigenic, anti-septic and anti-oxidant properties. Curcumin, whose chemical formula is $C_{21}H_{20}O_6$, is a lipophilic polyphenol so it is insoluble in water but soluble in organic solvents such as acetone, ethanol and dimethylsulfoxide (Aggarwal et al., 2003; Priyadarsini, 2013).

Curcumin has the ability to regulate different signaling pathways in different types of cancer. It has antitumor effects on colorectal, pancreatic, breast, lung, hepatic, ovarian, head and neck, prostate (Bimonte et al., 2016). It is thought that curcumin exerts its anticancer effect by acting on multiple signaling pathways that affect the initiation and progression of cancer. These signaling pathways may be involved in cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and inflammation, and curcumin affects various molecules of these signaling pathways (Aggarwal et al., 2003).

Breast cancers are a heterogeneous group of diseases that are quite common but spread widely in terms of biological behavior. The most common type of cancer in women is breast cancer (Güneş et al., 2012; Cetin and Topcul, 2014). One in eight women will be faced with breast cancer in their life. Recent studies state that one in every three new cancer cases will be diagnosed as breast cancer (Jhan and Andrechek, 2017). Breast carcinomas are tumors with heterogeneous groups with different characteristics according to their morphological, clinical, hormone receptor level and response to treatment. The reason for this difference is the difference in the underlying target cell population, different oncogene activation and / or different combinations of tumor suppressor gene function losses (Çiçin, 2008).

Triple negative breast cancer (TNBC) defines a minor group without ER / PR / HER-2 gene expression among breast tumors and constitutes 15-20% of newly diagnosed patients (Gluz et al., 2009; Stevens et al., 2013; Lehmann et al., 2011; Penault-Llorca and Viale, 2012). TNBC tumors exhibit more aggressive behavior biologically. It is the focus of attention of researchers because of its increased tumor size, high grade, lymph node involvement at the time of diagnosis, and poor prognosis (Lehmann et al., 2011 and Penault-Llorca and Viale, 2012). The most important reason for this is the lack of a suitable target for treatment (Nursal, 2015).

It is known that 75% of breast tumors have estrogen and /or progesterone receptors (Yersal et al., 2014). The ER-positive tumors express ER, PR, ER responsive genes and other genes that encode typical proteins of luminal epithelial cells so they are termed the luminal group (Hu et al., 2006).

In this study, we evaluated the antiproliferative effects

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of curcumin on different breast cancer cell lines MCF-7 and MDA-MB-231.

Materials and Methods

Cell culture

The MCF-7 and MDA-MB-231 cell lines used in this study was obtained from European Cell Culture Collection (CCL). MDA-MB-231 derived from a metastatic carcinoma estrogen receptor-negative cells was used as a triple negative breast cancer model. MCF-7 derived from an in situ carcinoma was used as a Luminal A breast cancer model. MDA-MB-231 and MCF-7 cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (high glucose) (Gibco Co) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco Co), Grand Island, NY, USA) at 37°C, in a humidified atmosphere containing 5% CO_2.

Curcumin concentrations

Different curcumin concentrations (75 µM, 100 µM and 125 µM) were obtained by diluting the stock solution.

Cell viability analysis

Both cell lines were seeded into 96-well plates at 104 cells per well. After an overnight incubation, three different concentrations of curcumin (75 µM, 100 µM, 125 µM) were applied to the cells for 24 h. The cells treated with curcumin were incubated for 4 h by applying MTT agent, and formazan salts were dissolved with dimethyl sulfoxide (DMSO) and the absorbance value was measured at 570 nm and 655 nm in a microplate reader at dual wavelengths (Topcul and Cetin, 2016).

Cytotoxicity (CI)

For the experimental procedure, the impedance of gold microelectrodes in a real-time cell analysis system was measured using whole cell media without cells. Then cancer cells were seeded in the E-plate. In all conditions, respectively 5,000-10,000 cells for MDA-MB-231 and MCF-7 were seeded into the E-plates of the real-time cell analysis system. The cells were allowed to adhere to the plate for 20 h. At the end of this period, curcumin in varying concentrations (75 µM, 100 µM, 125 µM) was added to the cells. Cell proliferation measured for 72 h (Cetin and Topcul, 2019).

Mitotic index (MI)

Feulgen method was applied to the prepared preparations to determine the mitotic index. For this process, cells were kept in Schiff separator for 1 h after hydrolysis with 1 N HCl at 60 oC and then stained with Giemsa dye for 2 minutes. Thus, cells in the mitosis phase were enabled to be seen more clearly (Topcul et al., 2018).

Apoptotic index (AI)

DAPI staining was performed to determine the effect of curcumin on apoptotic cell death in MCF-7 and MDA-MB-231 breast cancer cells. $5x10^4$ cells were seeded into a 12-well cell culture petri dish. After the cells were allowed to adhere overnight, the optimum concentration of curcumin was applied to the cells. Following the experimental periods of 24, 48 and 72 h, 1µl / ml DAPI (1mM) dye was applied to each well for 30 minutes (Cetin and Topcul, 2017).

Statistics

Numerical data obtained from all analyzes were evaluated statistically using Graphpad Prism 6. Statistically significant p value was determined as 0.05.

Results

Determination of optimal concentration with cell viability analysis and cell viability

The effect of curcumin administration on cell viability depending on the concentration was evaluated on both MCF-7 and MDA-MB-231 cell lines. The absorbance values of MCF-7 cell line were 637,57 $x10^{-3}$, 327,5$x10^{-3}$, 294,10^5x10^{-3} and 229,85$x10^{-3}$ respectively for control, 75 µM, 100 µM, 125 µM for 24 hrs (Figure 1). The absorbance values of MDA-MB-231 cell line were 596,64 $x10^{-3}$, 293,5$x10^{-3}$, 143,14$x10^{-3}$ and 243,55$x10^{-3}$ respectively for the control, 75 µM, 100 µM, 125 µM for 24 hrs (Figure 2).

Absorbance values showed that compared to the control group which was admitted as 100% for MCF-7 cells, cell viability values were 51,37% for 75 µM, 46,13 for 100 µM and 36,04 for 125 µM (Figure 3). For MDA-MB-231 cells, compared to control group which was admitted as 100%, cell viability values were 49,16% for 75 µM, 40,77 for 100 µM and 38,42 for 125 µM (Figure 4).

xCELLigence Real-Time Cell Analysis (RTCA): cytotoxicity

Different types of breast cancer cells were seeded into the E-plates of the real-time cell analysis system and curcumin was applied on these cancer cells. Cells were exposed to curcumin for 3 days. All concentrations (75 µM, 100 µM, 125 µM) applied showed lower proliferation values than the control for both cell lines. These values also showed that all curcumin concentrations had DNA damaging effects on both of the cell lines (Figures 5 and 6).

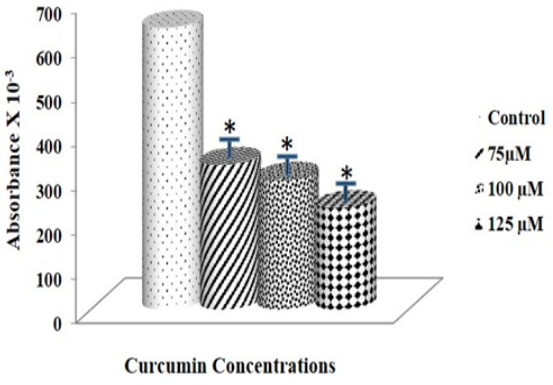

Figure 1. Absorbance Values of Mitochondrial Dehydrogenase Activity (450-690 nm) of MCF-7 Cells Treated with 75 µM, 100 µM, 125 Concentrations of Curcumin for 24 h ($p<0.05$).

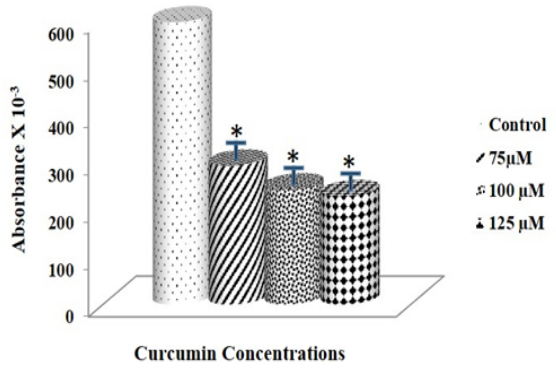

Figure 2. Absorbance Values of Mitochondrial Dehydrogenase Activity (450-690 nm) of MDA-MB-231 Cells Treated with 75 µM, 100 µM, 125 Concentrations of Curcumin for 24 h ($p<0.05$).

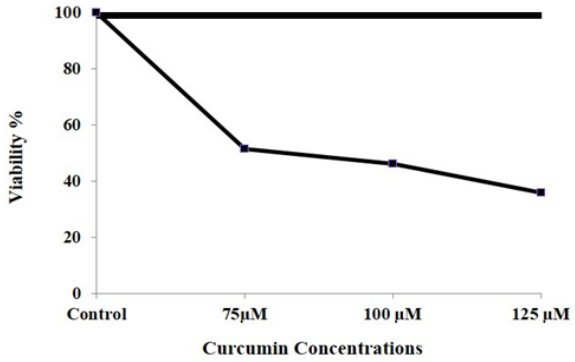

Figure 3. Percent Viability Values of MCF-7 Cells Treated with 75 µM, 100 µM, 125 µM Curcumin for 24 h ($p<0.05$).

Mitotic index (MI)

As a result of the application of curcumin on MCF-7 and MDA-MB-231 cell lines, mitotic index values were determined by applying 75 µM curcumin concentration to both cell lines cultured for 0-72 h. The mitotic index values obtained from the experimental series conducted in parallel with the control group without agent were shown in Tables 1 and 2. As seen in Figures 7 and 8, the mitotic index values of both cell lines decreased significantly from the 24th h depending on the time at 75 µM Curcumin concentration. The statistical significance of this decrease was determined at the level of $p<0.05$ in both cell lines.

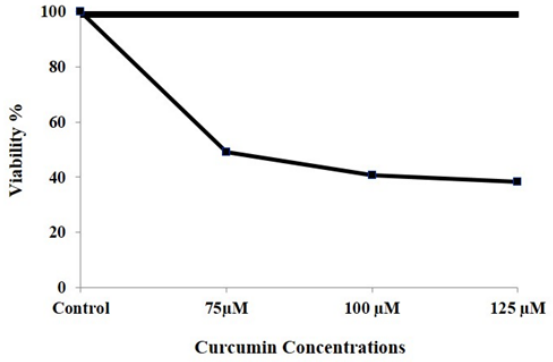

Figure 4. Percent Viability Values of MDA-MB-231 Cells Treated with 75 µM, 100 µM, 125 µM Curcumin for 24 h ($p<0.05$).

Table 1. Mitotic Index Values of MCF-7 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

	Mitotic Index (%)	
Time (Hour)	Control	75 µM
24	5.94±0,05SD	0,46±0.03*
48	6.03±0,04	0,19±0.01*
72	6.53±0,05	0,01±0.01*

Apoptotic index (AI)

As a result of the application of 75 µM curcumin concentration to both cell lines cultured for 0-72 h, apoptotic index values were shown in Tables 3 and 4. As seen in Figures 9 and 10, the apoptotic index values of both cell lines increased significantly. When the data obtained from the experiments were examined, it was found that the time-dependent increase in the apoptotic

Table 2. Mitotic Index Values of MDA-MB-231 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

	Mitotic Index (%)	
Time (Hour)	Control	75 µM
24	6.33±0.04SD	0.33±0.03*
48	6.46±0.05	0.04±0.01*
72	7.27±0.06	0.02±0.01*

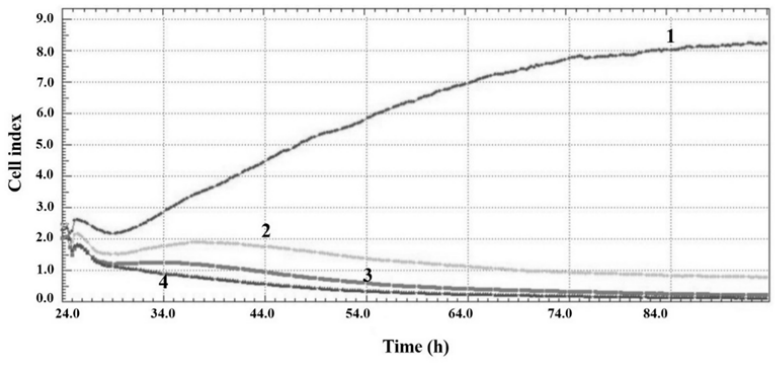

Figure 5. Cell Index Values of MCF-7 Cells Treated with Concentrations of 75 µm, 100 µm, 125 µm Curcumin Obtained from Xcelligence Real-time Cell Analysis (RTCA) System (Line 1, Control; Line 2, 75 µm; Line 3, 100 µm; Line 4, 125 µm).

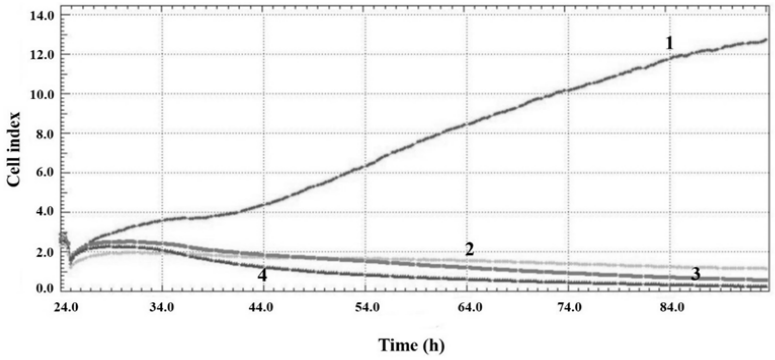

Figure 6. Cell Index Values of MDA-MB-231 Cells Treated with Concentrations of 75 µm, 100 µm, 125 µm Curcumin Obtained from Xcelligence Real-time Cell Analysis (RTCA) System (Line 1, Control; Line 2, 75 µm; Line 3, 100 µm; Line 4, 125 µm).

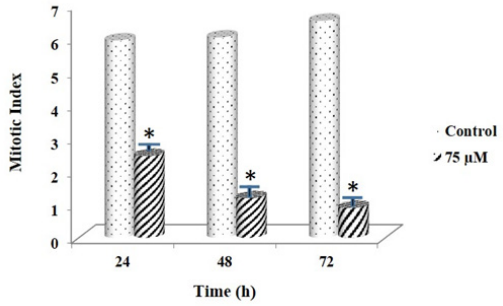

Figure 7. Mitotic Index Values of MCF-7 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

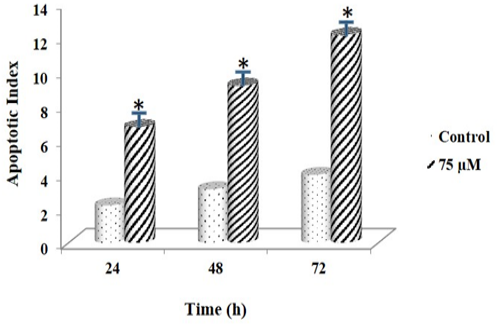

Figure 9. Apoptotic Index Values of MCF-7 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

index values of the cells were statistically significant compared to the control ($p<0.05$).

Discussion

This study was carried out to evaluate the anticancer effects of curcumin on hormone receptor positive MCF-7

Table 3. Apoptotic Index Values of MCF-7 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

	Apoptotic Index (%)	
Time (Hour)	Control	75 µM
24	2,18±0,01SD	6,76±0,04*
48	3,13±0,03	9,18±0,08*
72	3,97±0,02	12,14±0,01*

Table 4. Apoptotic Index Values of MDA-MB-231 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

Time (Hour)	Apoptotic Index (%)	
	Control	75 µM
24	1.97±0.01SD	8.17±0.09*
48	2.21±0.01	12.16±0.01*
72	3.11±0.03	16.18±0.12*

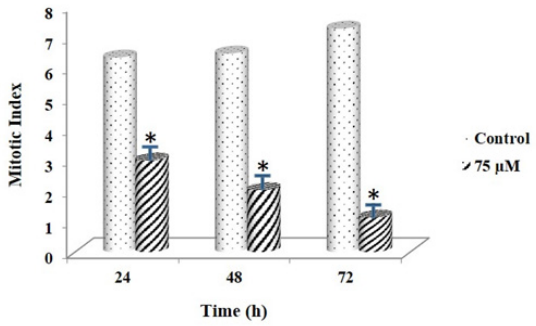

Figure 8. Mitotic Index Values of MDA-MB-231 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

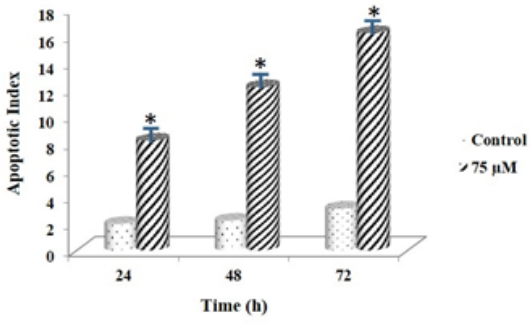

Figure 10. Apoptotic Index Values of MDA-MB-231 Cells Treated with 75 µM Concentration of Curcumin for 0-72 h ($p<0.05$).

and hormone receptor negative MDA-MB-231 cells, which are breast cancers of different molecular subtypes.

Curcumin has been shown to have different effects on endothelial cells, lymphoists, hepatocytes and mammalian epithelial cells. It has been shown to have anti-cancer, chemopreventive and antioxidant properties in various cancers, as well as cellular regulation of growth, apoptotic and autophagic processes of cells (Gupta et al., 2011). It has been observed that the expression of HER2, which is overexpressed in breast cancer, is decreased by curcumin administration. In a study inoculation was performed after 58-60 days in nude mice with breast cancer, and it was found that the incidence of tumor metastasis to the lung was reduced by following the curcumin diet. Lung metastasis was observed in mice in the untreated group (Kunnumakkara et al., 2008; Ravindran et al., 2009). Estrogen receptor is important in tumor development in breast cancer. It expresses the necessary receptors in about a third of breast cancer. Estrogen is an important target for treatments. Curcumin was observed to inhibit ER + and ER– cell proliferation (Verma et al., 1998; Teiten et al., 2010).

One of the reasons why curcumin has become particularly popular in recent years is that there are no side effects that threaten human health, unlike other chemotherapeutic agents. Curcumin is isolated from the root of the turmeric plant, which is both cheap and easily found everywhere. It is native to India. It has also been shown in studies that curcumin also acts in conjunction with other chemotherapeutic drugs and other natural products that are effectively used in cancer treatment, which is not affected, or even enhances the effect of other chemotherapeutic drugs. No concentration-limiting toxicity was observed in human Phase I and Phase II clinical trials (Cheng et al., 2001; Dhillon et al., 2008). In a study in which curcumin was used in combination with 5-FU, it was concluded that curcumin may cause lower toxicity in normal cells and reduce possible side effects (Sarkhosh et al., 2018).

Studies using human bile-duct cancer cells GBC-SD, the MTT assay has been shown to induce a concentration- and time-dependent decrease in cell viability of curcumin, also it was determined that curcumin induced a tyrosine-dependent decrease in colony formation ability in GBC-SD cells (Cheng et al., 2001; Dhillon et al., 2008).

Previous studies have shown that curcumin induces apoptosis in breast cancer cells by regulating expression of genes associated with programmed cell death (Ramachandran et al., 2005). In our study, the apoptotic index values of both MCF-7 and MDA-MB-231 cells were found to be in accordance with the above-mentioned studies, depending on the optimum concentration and time applied. It was shown that phosphorylation of curcumin, increased expression and BRCA1 protein caused DNA damage in association with cytoplasmic retention in triple negative breast cancer cells (Rowe et al., 2009).

In this current study; when both the luminal A and triple negative breast cancer cell lines were treated with different concentrations of curcumin and evaluated with xCelligence RTCA, the obtained cell index values and the comparison of the curves obtained from these values with the standard curve revealed that the curves had a DNA damaging effect for both cell lines.

In a study, it was shown that curcumin induces DNA damage in both mitochondrial and nuclear genomes in high concentrations of G2 cells with human liver cancer, whereas curcumin did not cause DNA damage at low concentrations and plays an antioxidant role in carcinogenesis (Cao et al., 2007).

It is known that curcumin induces cell death by mitotic arrest. Studies on the effect of curcumin on bladder cancer have shown that curcumin delayed mitotic progression in the G2/M phase (Liu et al., 2011). Curcumin has a different effect on mitotic division in each cell, even these effects depend on the time (Martinez-Castillo et al., 2016). Studies have shown that curcumin arrested cell cycle in G0/G1 phase in leukemia cells, it arrested S and G2/M phases in bladder breast cancer (Tuorkey, 2014). In addition to the formation of monopolar mitotic spindles in the MCF-7 cell line treated with curcumin, depolymerization of mitotic microtubules during cell division was also observed (Chakrabarti et al., 2013). The effect of curcumin on the mitotic spindle structure resembles taxol's effect in the MCF-7 cell line (Khafif et al., 2005). Studies have shown that curcumin destroys the organization of mitotic spindles in prostate cancer cells (Gorny and Holy, 2002).

The term 'mitotic catastrophe' is used to describe a delayed mitotic-linked cell death mechanism resulting from the entry of premature or inappropriate cells caused by chemical or physiological stress in mitosis. Mitotic catastrophe is a mode of cell death that occurs during or after a dysregulated/unsuccessful mitosis, and multiple nucleosomes (resulting from chromosomes and/or chromosomal fragments that are not usually distributed equally between the daughter nucleus) and multiple nuclei (two or more nuclei with similar or heterogeneous sizes, due to an inadequate separation during cytokinesis) (Güneydaş and Topçul, 2016). Curcumin also causes mitotic catastrophe consistent with micronucleus formation, as well as malfunctions in cytokinesis and mitotic spindle formation, arresting the cells only between the G2/M phases (O'Sullivan-Coyne et al., 2009). It has been shown that curcumin causes cell death by disrupting mitosis, leading to mitotic catastrophe, leading to fragmented nuclei in cultured cells (Dempe et al., 2008).

In this study, 75 μM curcumin was determined as IC_{50} concentration, MCF-7 and MDA-MB-231 cell lines showed a significant decrease in the concentration of curcumin of 75 μM at 24th h, while at the 48th and 72nd h, both cell lines approached zero. This data supports the idea that curcumin causes mitotic catastrophe and mitotic arrest. It was shown that curcumin causes DNA damage and endoplasmic reticulum (ER) stress through the activation of caspase-3 in human lung cancer A-549 cell line (Lin et al., 2008). It was found that curcumin is a potent inducer of oxidative DNA damage in non-cytotoxic and anti-inflammatory concentrations (Li et al., 2008).

In conclusion, the effect of curcumin on MCF-7 and MDA-MB-231 cell lines was assessed using cell kinetics parameters such as cell viability, cell index, mitotic

index and apoptotic index. The obtained data showed a significant decrease in cell viability, cell index, and mitotic index values depending on the time. There is a significant increase in apoptotic index values for both cell line. This study on hormone receptor negative and positive cell lines shows that curcumin acts independently of hormone receptors and suggests that it may be effective in different types of cancer.

Author Contribution Statement

GG and MT designed the study. GG and MT performed the experiments. GG analyzed the data. Both authors have read and approved the final version of the manuscript.

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Conflicts of interest

The authors state that did not have conflict of interests.

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