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ORIGINAL ARTICLE

Anti-proliferative and Cytotoxic Effects of Curcumin in MCF-7 Human Breast Cancer Cells

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KEYWORDS

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ABSTRACT: Curcumin is the effective constituents of *Curcuma longa*, which inhibit the growth of cancer cells. Prevention of breast cancer, as one of the common cancer type with serious health problem, is an area of interest in breast cancer research. In this study, we have evaluated the effects of curcumin on the cell proliferation of human breast cancer cell line (MCF-7) compared to non-cancer line (MCF-10A). The cell lines were subjected to increasing doses of curcumin ranging from 0 to 30 µg/ml. Cell viability was quantified by MTT assay. In vitro clonogenic survival assay was performed on MCF-7 cells. Curcumin inhibited the growth of malignant cells in a time and dose-dependent manner. Calculated IC_{50} value for MCF-7 cells in 48 h was 12 µg/ml. Overall, 45%–70% decrease in colony formation was observed in MCF-7 cells treated by 30-60 µg/ml curcumin respectively. The result of our study confirms the potential cytotoxic effects of curcumin in breast cancer cell line. It could be considered as a potential chemopreventive agent in breast cancer treatment.

INTRODUCTION

Curcumin, as a polyphenolic compound derived from turmeric (*Curcuma longa*), is an effective anticancer compound [1]. Curcumin widely used as spices, preservatives and food-coloring agent and in folk medicine in Asia [2]. In Iran, it is also one of the most used spices. In traditional medicine, turmeric is used for many illnesses, especially as an anti-inflammatory agent [3]. Curcumin is effective in the treatment of inflammatory diseases such as cardiovascular disease, respiratory diseases and metabolic diseases as well asdiseases of the nervous system [2]. Curcumin can play a role in the prevention and treatment of cancer and several clinical trials have demonstrated its effectiveness. Curcumin inhibits the growth of cancer cells at very low concentrations. Curcumin induces apoptosis (programmed cell death) and inhibit the proliferation of cancer cells [4]. This cell death occurs by fragmentation of DNA and cell cycle arrest in G2/M [5-7]. The availability and multiple therapeutic effects of curcumin made it as a potential compound to treat and prevent a wide range of human diseases.

Breast cancer is the most common cancer in women, so that is the second cause of death among women worldwide [8]. Since breast cancer, especially in estrogen-negative type, is resistant to chemotherapy, other therapeutic options should be considered [9]. Many researchers have attempted to find safe preventive and therapeutic agents that have multiple

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effects and can influence several signaling pathways [8, 10, 11]. Metabolites and compounds of plants can be a good candidate in the case of breast cancer prevention.

In this study, the effect of curcumin on cell viability and colony formation unit of breast cancer cell line (MCF-7) in comparison with breast normal cell line (MCF 10A) was evaluated.

MATERIALS AND METHODS

Regents, cell lines and culture medium

Curcumin was purchased from Sigma (St, Louis, MO, USA), dissolved in dimethyl sulfoxide (DMSO) to a 10-mM stock solution, and stored at -20°C. Human breast cancer cell lines (MCF-7) and a normal breast cell line (MCF-10A) were obtained from Pasteur Institute (Tehran, Iran). The cells were cultured in 25 cm^2 flasks with RPMI-1640 (RPMI Sigma) and supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin solutions, at 37°C with 5% CO_2 and 95% humidity. The medium was exchanged every day and the cells were passaged every 3-4 days.

Cytotoxicity assay

The cytotoxic activity of curcumin was determined using MTT assay (2, 3, 5 diphenil tetrazolium bromide). Cells were plated with density of 3× 104 cells/dish in 96 well plates and were incubated for 24 h at 37 °C. Then, the cells were treated with different concentrations of curcumin (0, 10, 20 and 30 µg/ml) and were incubated for 24, 48 and 72 h. A 2 mM MTT solution was added to each well and plate was incubated for 4 h. The medium was then discarded, 100 ml DMSO (Dimethyl sulfoxide) was added, and the plate was shaken for 10 min. Finally, optical density was determined in 540 nm using ELISA microplate reader (Awareness, Palm City, FL, USA). Each experiment was performed in triplicate. Results are expressed as the percentage of viable cells with respect to the untreated cells.

Colony formation assay

The effect of curcumin on MCF7 cells was investigated by colony formation assay. Briefly, the cells (500 cells/ml) were allowed to grow in 60 mm Petri dishes for 12 h. Subsequently, the cells were treated for 48 h with curcumin (10, 20 and 30 µg/ml), or 0.1% DMSO. The colonies were fixed and stained with 0.2% crystal violet and counted under stereomicroscope.

RESULTS

Cytotoxicity of Curcumin

To evaluate the cytotoxic activity of curcumin, human breast cell lines MCF-10A (Normal) and MCF-7 (cancer) were exposed to various concentrations of curcumin for 24, 48 and 72 h, and viability of cells were measured by MTT assay. The results demonstrated that curcumin significantly decreased the viability of malignant cells in a time- and dose-dependent manner. Curcumin in the same concentrations caused less toxicity to MCF-10A than tumor cells indicating a degree of specificity for malignant cell lines (Figure 1). By 48 hours, 30 µg/ml curcumin had produced a growth inhibitory effect of approximately 40% in MCF-7 and 40% in MCF-10A cells. Concentrations inducing 50% cell growth inhibition (IC_{50}) against MCF-7 cells were 12 and 7.5 µg/ml for 48 and 72 h, but IC_{50} against MCF-10A cells was 20.31 µg/ml for 72 h.

Figure 2 shows the photomicrographic images of MCF-7 cell line treated by various concentration of curcumin. Cytotoxicity effects were coupled with morphological changes including decrease in cell volume and shrinking of the cells. Treated cells especially by high concentration of curcumin became round and shrunken, while the untreated cells remained normal in size and shape.

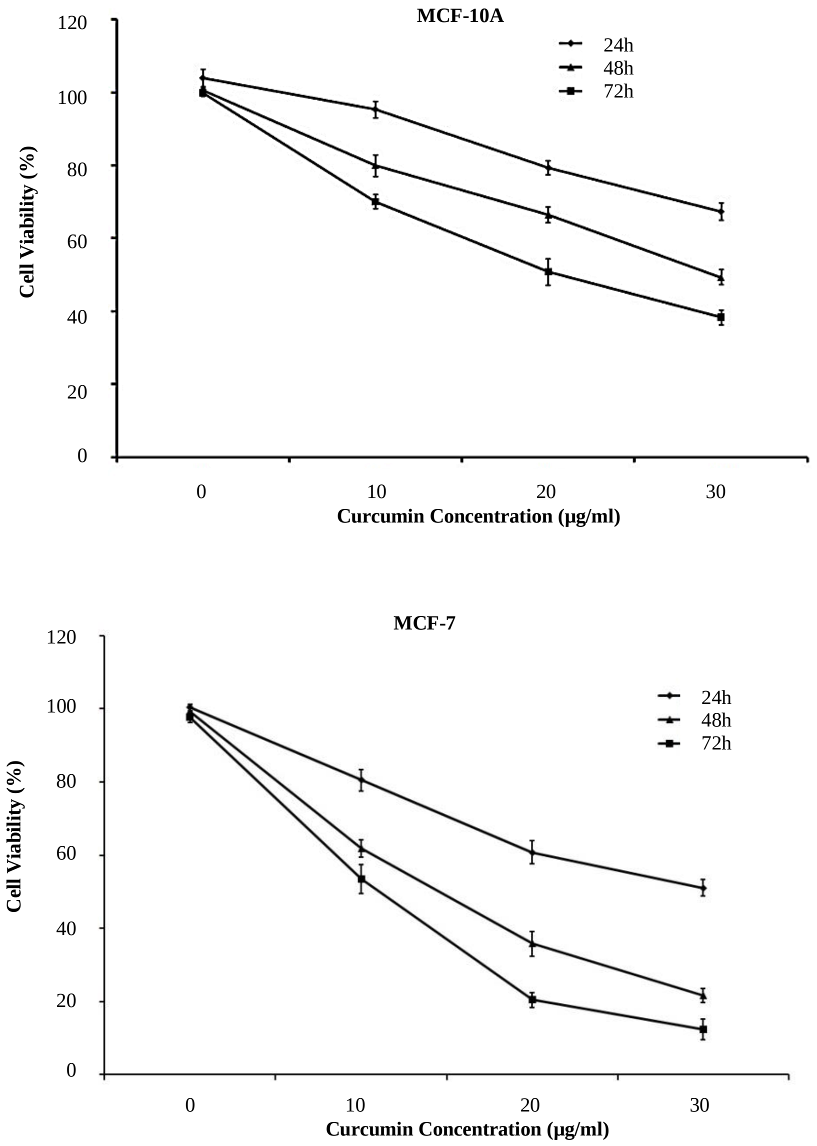

Figure 1. Cytotoxic effects of curcumin on MCF-10A (Normal) and MCF-7 (cancer) human breast cell lines. Cells were treated for 24, 48 and 72 h with different concentrations of curcumin. Cytotoxicity was determined by MTT assay. Results are the mean ± SEM of three independent experiments.

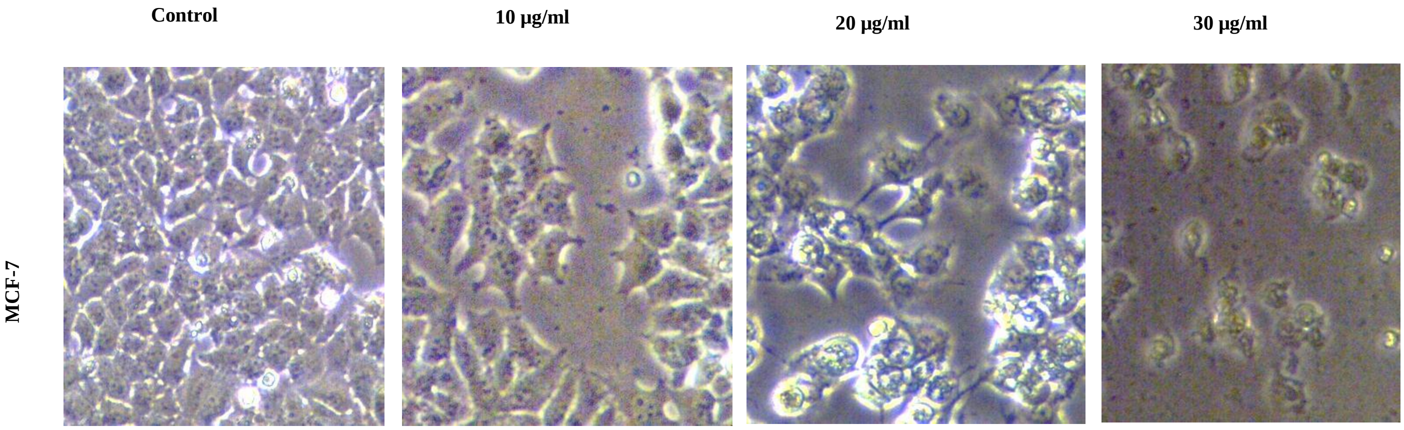

Figure 2. Morphological changes of MCF-7 cells treated with different concentrations of curcumin for 48 h viewed under inverted phase-contrast microscope (200× magnification). Reduce in cell population was noted with the increase in the concentration of the treatment as compared to the control (untreated cells).

In vitro clonogenic survival assay

As given in Figure 3 there was a significant dose dependent inhibition in colony formation in MCF-7 after curcumin treatments ($p< 0.01$). By 30 µg/ml and 60 µg/ml curcumin, 45%–70% decrease in colony formation was observed in MCF-7 cells respectively.

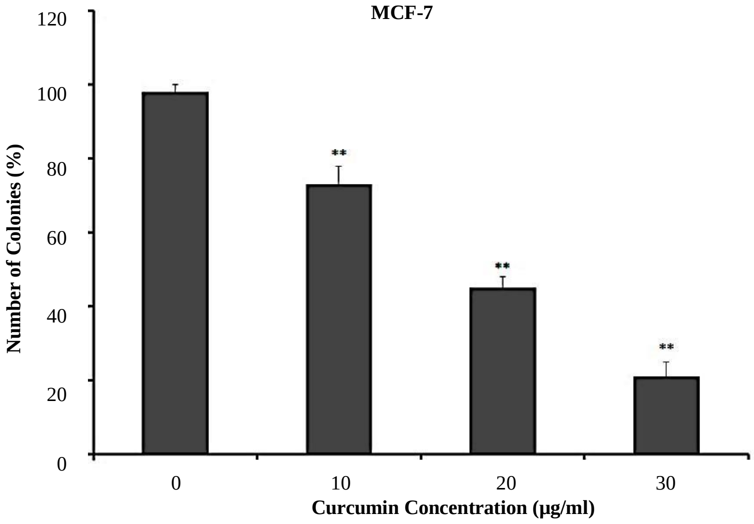

Figure 3. Colony formation assay shows a significant reduction in colony formation in MCF-7 cell line treated with different concentration of curcumin. The results were presented as mean±SEM, n= 3 (** $p < 0.01$).

DISCUSSION

Despite progresses in cancer research in recent decades, the number of cancer-related deaths has not diminished [10]. Current cancer drugs are less effective, and cause many side effects and are expensive. Finding agents or compounds that have not been these disadvantages have always been of great interests [12]. Curcumin is one of these compounds widely is explored in the last four decades due to the potential anti-inflammatory and anti-cancer effects [1, 13 and 14]. Curcumin can inhibit cancer initiation, promotion and progression. These anti-cancer effects generally mediated by down-regulation of transcription factors, growth factors, inflammatory cytokines and protein kinases [9, 15]. Curcumin also inhibits cell proliferation through cell cycle slows down at certain stages and apoptosis [16]. Therefore, further analysis of the mechanisms of action of curcumin could be effective in its more precise application.

In the present study, the cytotoxic effects of curcumin on cancer cell line MCF-7 and non-cancerous MCF-10A cell line was investigated. Cytotoxicity and growth inhibition effects were investigated by MTT assay. Growth inhibition of cancerous cell treated by curcumin confirmed in a time- and dose-dependent manner. The results indicate that curcumin have cytotoxic effects on both cancerous and non-cancerous cells in the applied concentration range but the cytotoxic effects on MCF-7 cells were greater than MCF-10A cells.

After treatment of cells with curcumin, by increasing the curcumin concentration the viability of cells is decreased. However, in cancer cells a significant reduction in the viability of cells from concentration of 10 to 20 and 20 to 30 μg/ml curcumin in the normal cells are observed. Furthermore, by increasing exposure time of cells with curcumin increased cell death rate is observed. As in cancer cells in 48h, percentage of living cells decrease below 50% and this reduction in normal cells occur in 72 h. However, in this study anticancer effect of curcumin is also confirmed.

Several studies have been conducted in conjunction with the anticancer effect of curcumin that could note to the study Lv et al., in which anticancer effects of an analog of curcumin on prostate cancer cells studied and showed that curcumin via increased reactive oxygen and inducing apoptosis cause death of tumor cells [17]. In another study, the treatment of breast cancer cell line MDA-MB-231 with curcumin revealed that cell proliferation inhibited by arresting cell cycle in G2 phase (Table 1) [18]. Curcumin through inhibition of microtubules polymerization and thereby prevent spindle assembly and activation of mitotic checkpoints diminished MCF-7 cell proliferation [19].

Curcumin by induction of programmed cell death causes reduced proliferation of MCF-7 cancer cells and apoptosis could be induce through p53 reduction and Bax protein elevation [5]. Therapeutic effects of curcumin partly attributed to the cell proliferation inhibition and induction of apoptosis. Curcumin cause cell cycle arrest by down-regulation of several genes such as cyclins [20, 21]. Cascades of protein phosphorylation by cyclin/cdk complexes promote cell cycle progression. Activation of CDK inhibitors by curcumin especially G1/S inhibitors, stopped the cancer cells in the G1 phase of cell cycle [22, 23].

Curcumin as a natural compound have diverse therapeutic effects that this variation is due to its ability to interact with different molecules and regulation of different pathways [2, 14]. Curcumin by activating or inhibitory effects on the molecules involved in various biological processes regulate their function [15, 20]. Curcumin's multi-targeting ability may be the key to its therapeutic potential against cancer and other diseases [9].

In the current study, also curcumin significantly reduced the proliferation of cancer cells compared to normal cells in time and dose dependent manner that this growth inhibition seems to be through cell cycle arrest and apoptosis.

Table 1. Anti-proliferative effects of curcumin on two common human breast cancer cell lines

Cell lines	Mechanism	References
MCF-7	Inhibition the activities of protein kinases (C and/or protein tyrosine kinase)	[24]
	Inhibition of telomerase activity	[25]
	Disruption of mitotic spindle structure	[19, 26]
	Downregulation of the expression of Bcl-2 by upregulating miR-15a and miR-16, Apoptosis	[5, 27]
	cell cycle arrest by inhibition of cyclins and CDKs	[20-22, 28]
MDA-MB-231	arresting cell cycle in G2 phase	[18]
	Cycle arrest and apoptosis	[29-32]

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Conflict of interest

The authors declare that there is no conflict of interest.

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