Original Article

Evaluation of Cytotoxicity Effects of Combination Nano-Curcumin and Berberine in Breast Cancer Cell Line

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ABSTRACT

Background: Berberine and Nano-curcumin are two herbal medicines with strong anti-cancer effects on tumor cells, but low toxicity on normal cells, when used alone. Breast cancer is known as the most common cancer in women and second deadly one. In this study, we evaluated the cytotoxicity effects of combination Berberine and Nano-curcumin in breast cancer cell line to see whether they have further synergism cytotoxicity on MCF-7 breast cancer cell line.

Methods: The cytotoxicity effects of Berberine and Nano-curcumin alone and in combination, were evaluated in MCF-7 cell lines using MTT cytotoxicity test. Statistical analysis is done through one-way ANOVA and Tukey multiple range tests.

Results: Analyzing results of this study showed that cytotoxicity of Nano-curcumin was higher than Berberine in a dose-dependent manner. The IC50 of combination Berberine and Nano-curcumin was lower and showed higher cytotoxicity in MCF-7 cells compared with the time we use each of these drugs alone.

Conclusion: In this study co-treatment of Berberine and Nano-curcumin significantly inhibited the growth of MCF-7 breast cancer cell line and resulted in synergism cytotoxicity effects. These results indicated on their potency to further combination of these two drugs with other agents and common chemotherapies to improve breast cancer outcomes.

Key words: Berberine, Breast Cancer, Nano Curcumin.

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INTRODUCTION

Breast cancer is categorized into a group of cancers with high mortality rate. Mortality resulting from this type of cancer is estimated 40,920 deaths in 2018 in the United States and is considered as the second deadly one after lung cancer[1]. High prevalence rate, especially in women, has been a great challenge for years in the area of health services. Common therapeutic methods include surgery and radiotherapy, chemotherapy, hormonal therapy and target therapy. In most of the cases due to the presence of limitations in each method, the combination of these therapies are required [2, 3]. Beside these routine treatments, consumption of natural compounds has been holding of great attention due to their low toxicity on normal cells and fewer side effects in comparison with chemical drugs and better outcomes when combined with other treatments[4]. Curcumin and Berberine are two of these natural compounds. Berberine is isoquinoline alkaloid , and can be isolated from various plants and curcumin is derived from Curcuma longa and being as a phenolic compound make it insoluble in water so different nano forms are constructed to improve its circulation in the blood[5, 6]. In addition to multiple therapeutic effects, anti-cancer effects of both compounds have been evaluated in many cancers, and the efficacy of these compounds on different molecular targets and signaling pathways that interfere in tumor metastasis, recurrence, progression, and angiogenesis have been approved, but results didn't show significant effects when used singly [7-9].

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So in this study, we examine the cytotoxicity effects of combining both Nano-curcumin and Berberine in MCF-7 breast cancer cell line to further evaluate their synergic cytotoxicity and as a hope for combining common drugs with these compounds to improve the breast cancer outcomes.

METHODS AND MATERIALS

Chemicals

Berberine, *Dimethyl sulfoxide (DMSO), trypsin, penicillin, streptomycin, 3-(4, 5-dimethyl- 2-thiazolyl)-2, 5-diphenyl-2-tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA).* Nano-curcumin was *obtained* from Exir Nano Sina Company (Tehran, Iran), Each Nano-curcumin soft gel contained 80 mg of curcumin[10]. RPMI, *Fetal bovine serum* (FBS), *were obtained from* GIBCO (Germany) *Company*.

Cell Culture and Drug Treatment

MCF-7 breast cancer cell lines were obtained from the cell bank of Pasteur Institute, Iran-Tehran. These cells were preserved in RPMI medium containing 10% fetal bovine serum (FBS), penicillin (1% v/v) and streptomycin (1% v/v) and were incubated in 5% CO2 at 37°C.

Determination of Cell Viability

MTT assay known as a colorimetric assay was performed to evaluate cell metabolic activity. In this process, the reduction of MTT in normal cells by mitochondrial dehydrogenase to blue formazan product is detected. The cell suspension containing 1×10^4 cells per 100 µl of complete culture RPMI was added to each well of the plate and incubated in 5% CO2 at 37°C for 48 hours. The second step was washing cell with PBS1X. Then different concentrations of Nano-curcumin and Berberine added to wells and incubated in 5% CO2 at 37°C for 48 hours. It should be mentioned that used concentrations of Nano-curcumin were (0.67, 1.34, 2.5, 5, 10, 20, 40, 50 mg/mL) and for Berberine these ranges were (0, 1, 10, 20, 50, 100, 200, 300, 400 and 500 µg/mL). On the next day, 10 µl of MTT (0/5 mg/mL) added to each well and incubated at 37 ° C for 3 to 4 hours. Then, the whole well contents were evacuated and 100 µl *DMSO* was added to each well and mixed by the shaker for 15 minutes. As the last step, cell viability was determined by the optical density reading of each well at 570 nm by ELISA reader (Organon Teknika, Netherlands), and the IC_{50} was measured as mg/mL. Toxicity level was calculated by the following formula[11]:

$$\text{Cytotoxicity} = 1 - \frac{\text{mean absorbance of toxicant}}{\text{mean absorbance of negative control}} \times 100$$

$$\text{Viability \%} = 100 - \text{Cytotoxicity \%}$$

Statistical Analysis

All assessment for each concentration of drugs performed in triplicate. Data were described as the mean ± standard deviation (SD). Significant difference at P-values < 0.05 microbiological counts assessment conducted by, one-way ANOVA and Tukey multiple range tests (SPSS 19.0 software Package, IBM Inc., Chicago IL, USA).

RESULTS

The effect of treating MCF-7 cells with Berberine and Nano-curcumin, alone and in combination was monitored within 48 h of culturing at different discussed concentrations. In the second step, the MTT assay was performed to detect the cell viability. The results indicate that the 50% decrease in cell viabilities or IC50 of consuming Berberine alone in concentrations containing a wide range between (1µM -100µM) was 63.62mg/ml (Fig1). In treatment with Nano-curcumin alone, IC_{50} was measured 7.348 mg/ml in the increasing concentrations of this drug in a range of (0.67-80mM) (Fig2). Co- treatment with both Berberine and Nano-curcumin in three doses higher and three doses lower than IC50 range of both compound (7.348 mg/ml +63.62mg/ml), resulted in reduction of IC50 values to 61.5178 +/- 13.58 mg/ml (Fig3), so their combination showed synergistic effects compared with treating alone, while control sample didn't show any activity.

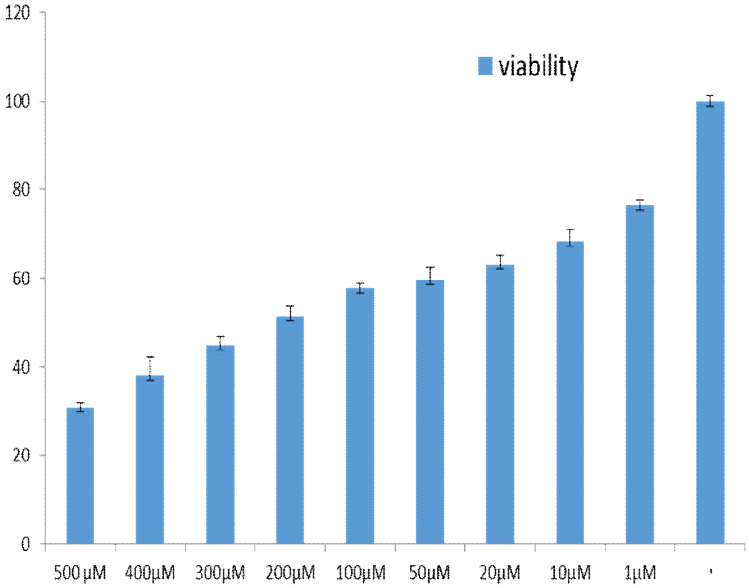

Figure 1. Effect of Berberine (1µM -100µM) on cell viability of MCF-7 cell line.

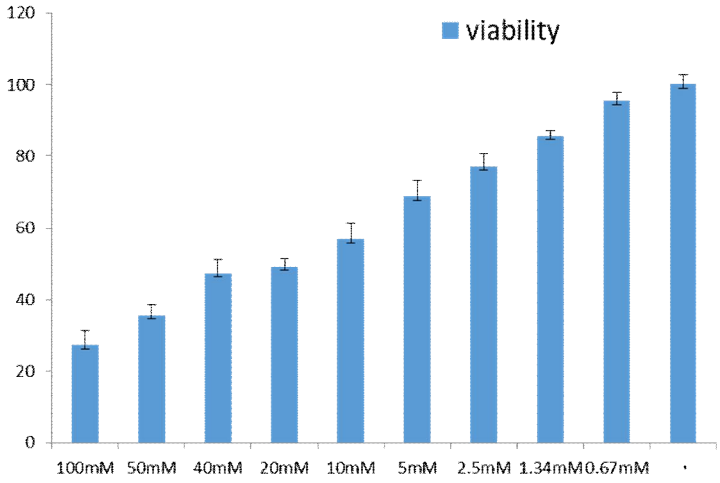

Figure 2. Effect of Nano-curcumin (0.67-80mM) on cell viability of MCF-7 cell line.

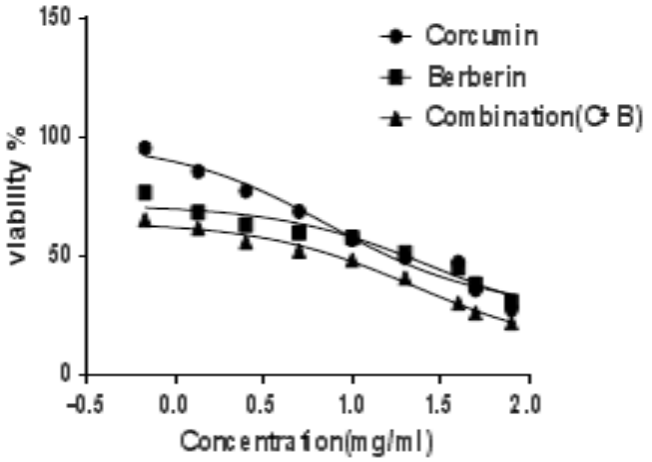

Figure 3. Effect of combination Nano-curcumin and Berberine (7.348 mg/ml +63.62mg/ml) on cell viability of MCF-7 cell line.

DISCUSSION

Breast cancer affects the high majority of women population, herbal drugs showed efficacious results in treating cancers especially this type. Today scientists try examining the combination of different herbal drugs and even their combination with common chemotherapies for cancer treatment. Curcumin is well- known herbal medicine, interfere with various signaling pathways, thus possessing anti-inflammatory, antioxidant and anti-cancer effects[12], this drug has been used in combination with different drugs and numerous cancer cell lines[6, 13-15]. In one study in T47D cells, the combination of curcumin with silibinin (milk thistle) showed remarkable outcomes in reducing cell viability and higher cytotoxicity in comparison with the time we use them alone[16].Combination of curcumin with carnosol in MDA-MB-231 cell lines induced anti-proliferation activity and reduction in cell viability[17]. In combination of curcumin with resveratrol in the HER2 positive JIMT1 cell line, in a study performed by Catania et al, IC50 decrease compared to the liposome form[18]. Anti- cancer effects of Berberine have been approved in many studies indicating its role in apoptosis, cell migration, interaction with DNA and RNA, an effect on tumor metastasis and cell cycle, with mediating different signaling pathways[19, 20]. Co-treatment of this compound with different drugs emphasis on its efficacy especially when used in combination with other treatments [7, 21-24]. In a study performed with Elmira Barzegar et al. the IC_{50} of Berberine in MCF-7 cell line was 25 µM after 48 h, and its combination with doxorubicin showed the increase in cytotoxicity levels[25]. Also in one study combination of Berberine with estrogen receptor (ER) antagonists showed inhibitory effects on growth of MCF-7 cells (ER+)[26].

In our study of treatment MCF-7 cells with Nano-curcumin and Berberine alone or in combination, with different concentrations for 48 h, the cytotoxicity of Nano-curcumin was higher than Berberine with IC50 of 7.348 mg/ml in comparison with 63.62mg/ml (IC50 of Nano-curcumin) Units of measurement have been unified. Our outcomes were synchronized with previous studies and even indicated on the lower IC50 values when these two drugs were co- treated and showed their synergist efficacy in causing better cytotoxicity toward MCF-7 breast cancer cell line. The findings of this study revealed that both Berberine and Nano-curcumin decreased the cell viability of MCF-7 cells in a dose-dependent manner.

CONCLUSION

In conclusion, results of this study indicate that using herbal drugs such as Berberine and Nano-curcumin, alone or in combined form with low toxicity to normal cells, may reduce the cell viability of MCF-7 breast cancer cells and their synergism effects can be efficacious in combination even with common chemotherapies to improve cancer survival outcomes.

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