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Synergistic Growth Inhibitory Effects of *Lyciumbarbarum*(Gojiberry) Extract with Doxorubicin against Human Breast CancerCells

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Abstract

Goji berries, or Lyciumbarbarum L., have been used medicinally for thousands of years. The purpose of this research was to examine the cytotoxic impact of Lyciumbarbarum fruit extract (LBE) on MCF-7 and MDA-MB-231 breast cancer cells, as well as the effect of LBE in combination with doxorubicin. The MTT test demonstrated that doxorubicin had a substantial cytotoxic impact on the MCF-7 and MDA-MB-231 cell lines after a single treatment. Fractional effect analysis (FA) and the estimated combination index (CI) demonstrated that LBE plus doxorubicin had a synergistic cytotoxic impact on MCF-7 and MDA-MB-231 cells. L. barbarum fruits are chemosensitizing and chemoprotective because they increase anticancer effects while also protecting against dose-limiting effects such cardiotoxicity caused by anthracycline antibiotics.

Keywords:*L.barbarum*;Gojiberry;Breastcancer;Pharmacodynamicsdruginteractions;Combinationtherapy

1. Introduction

Goji berries, or Lyciumbarbarum L. (Solanaceae), have been used as a traditional Chinese medicine or dietary supplement for centuries. The fruit is the most important portion of the plant for therapeutic uses.Polysaccharides and proteoglycans make up around 23% of the dry mass of L. barbarum fruit, while other components include carotenoids (mostly zeaxanthindipalmitate), vitamins (riboflavin, thiamin, and ascorbic acid), fatty acids, free amino acids, flavonoids, phenolic acids, and anthocyanins [1, 2]. The fruits of L. barbarum have been linked to several health benefits, the most prominent of which are antioxidant and anticancer activity [3-6]. The anthracycline antibiotic doxorubicin is often used to treat a variety of cancers, including breast cancer. Intercalation in the structure of DNA and RNA, which results in inhibition of synthesis, is the primary mechanism of action. Free radical production and subsequent inhibition of topoisomerase II lead to chain breakage [7]. Doxorubicin's detrimental impact on the heart limits how much of the drug may be used to treat cancer. It may prevent the therapy from working or possibly cause death [8]. It has been hypothesized that elevated oxidative stress [9] is the major mechanism through which cardiotoxicity occurs. The primary goal of this paper is to assess how well two breast cancer cell lines respond to doxorubicin in combination with L. barbarum fruit extract (LBE).

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2. MaterialsandMethods

2.1 Reagents

*L. barbarum*fruits (Lot №: L05042017) were provided by Paula Fruits Ltd-an official importer of Goji berries forBulgaria with guaranteed Chinese origin. Preparation of total *L. barbarum*extract: 200 g of dried Goji berries weresoaked in cold distilled water for 30 minutes and then homogenized in a laboratory blender. The obtained mixturewas extracted for 1 hour at 60°C and shaking on thermostatic water bath (NUVE, Turkey). After that, extract wascentrifuged (6000 g) and supernatant was denoted as *L. barbarum*total extract (LBE). Doxorubicin and all otherused chemicals were of analytical grade and were purchased from the local representatives of Merck (Darmstadt,Germany)andSigma (St. Louis,USA), unlessotherwise indicated.

2.2 Cellcultures

Both the estrogen receptor (ER)-positive and progesterone receptor (PR)-negative MCF-7 and HER2 null MDA-MB-231 breast cancer cell lines were maintained in a 5% CO2 and 95% air atmosphere at 37°C in DMEM supplemented with 10% (v/v) fetal bovine serum (Gibco, Austria), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Lonza, Belgium). The cells were cultured in Greiner, Germany-supplied 25 cm2 plastic flasks. Cells were seeded onto 96-well plates (100 l/well) at a density of 1x104 cells/ml when they were in the exponential phase of growth after treatment with trypsin-EDTA (FlowLab, Australia).

2.3 Cell survival test, version 2.3

The MTT dye reduction test was used to determine the viability of MCF-7 and MDA-MB-231 cells using the modified standard technique [10]. Exponentially developing MCF-7 and MDA-MB-231 cell cultures were seeded onto 96-well plates. After 72 hours of incubation with the compounds of interest, cell viability was assessed. After three hours incubation in the treatment medium, the cells were switched to DMEM supplemented with 0.5 mg/mL MTT. After washing the plates with DMSO to remove the MTT solution, the formazan crystals could be decomposed. The samples' optical densities were evaluated at 540 nm using a microplate reader (TECAN, Sunrise TM, Groedig/Salzburg, Austria). Results were reported as a percentage relative to an untreated control group.

2.4 Analysisofdrugcombinations

We used two methods for determining the pharmacodynamics drug interactions. The first of them is the most widelyusedand isbased onthemultipledrugeffectmethodofChou–Talalay[11],represented asthefollowingequation:

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where A_1 and B_2 are percentage of inhibitionwith single agents, expressed as percentage of the untreated control.For each concentration we calculated the theoretical values, which we further compared with those we receivedactually: for $CE_{measured}$= $CE_{calculated}$we conceded the effect as additive; for $CE_{measured}$<$CE_{calculated}$as synergistic; andfor$CE_{measured}$>$CE_{calculated}$asantagonistic.

2.5 Statisticalanalysis

The statistical evaluation was performed using Graph Pad Prism 8.01 software, and significant differences betweengroups were analyzed using ANOVA. All results are expressed as arithmetic means ± standard deviation (SD) of themeans of three separate experiments (each experiment was done with three parallels). A difference at $P < 0.05$ wasconsidered statisticallysignificant.

3. Results

Our initial series of experiments characterized the doxorubicin susceptibility of the tested cell lines. Cell viability in both cell lines was shown to decrease with increasing dosage compared to the control (untreated cells). When tested on MCF-7 cells, the IC50 was 0.07 M, whereas when tested on MDA-MB-231 cells, it was 0.79 M. Figure 1 displays the dose-response curves that were produced. Figure 1 shows that MDA-MB-231 (triple negative) cells are far more resistant to the effects of doxorubicin than other cell types. We cultivated cells with doxorubicin at concentrations ranging from 0.02 to 0.6 M, alone or in combination with fixed 100, 120, or 140 g/ml LBE doses, for 72 hours to see whether L. barbarum extract (LBE) might sensitize MCF-7 and MDA-MB-231 to treatment with doxorubicin. MTT test was used to measure cell growth. The findings demonstrated that the combined therapy significantly reduced cell viability compared to doxorubicin alone. The Bliss method's predicted and observed combination effects are shown in Figures 2 and 3, respectively.

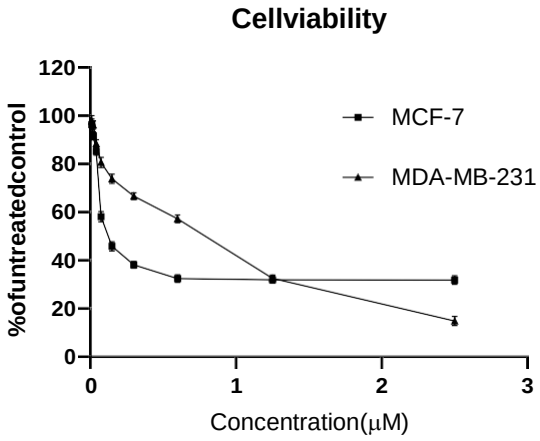

Figure1: CellviabilityofMCF-7andMDA-MB-231cellculturesincubatedwithdoxorubicinfor72h.EstimatedIC_{50}valueswere0.07μMand 0.79μMrespectively.

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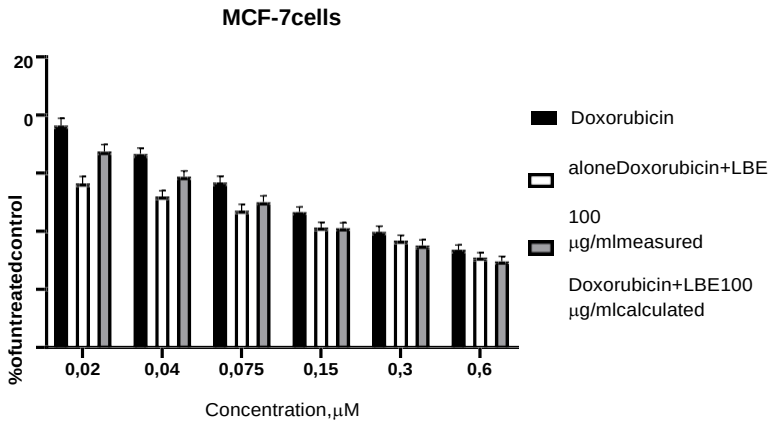

Figure2:MCF-7cellstreatedwithdoxorubicinaloneandincombinationwithfixed100µg/ml LBE.

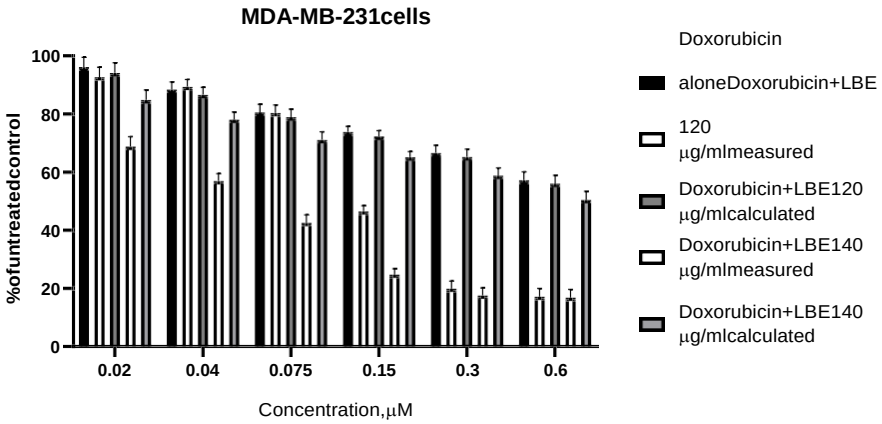

Figure3: MDA-MB-231cellstreatedwithdoxorubicinaloneandincombinationoffixedether120or140µg/mlLBE.

UsingthesameresultsandtheCalcuSyn®software,wecalculatedcombinationindex.TheresultsarepresentedinTable1andTable2.

Doxorubicinconcentration(µM)	*LBEconcentration(µg/ml)*	*CI*
0.02	100.0	0.75862
0.04	100.0	0.83698
0.075	100.0	0.89938
0.15	100.0	0.97530
0.3	100.0	1.19463
0.6	100.0	1.32293

Table 1: Calculated combination index (CI) after treatment of MCF-7 cells with combination doxorubicin (0,02-0,6µM)+ LBE (100µg/ml).

Doxorubicinconcentration (µM)	*LBEconcentration (µg/ml)*	*CI*	*LBEconcentration (µg/ml)*	*CI*
0.02	120.0	1.51415	140.0	0.92343
0.04	120.0	1.66865	140.0	0.89044
0.075	120.0	1.42208	140.0	0.82990
0.15	120.0	0.84412	140.0	0.74387
0.3	120.0	0.64732	140.0	0.72160
0.6	120.0	0.69030	140.0	0.76843

Table2: Calculatedcombinationindex(CI)aftertreatment ofMDA-MB-231cells withcombinationdoxorubicin(0.02-0.6µM)+ LBE(120and140µg/ml).

4. Discussion

In vitro pharmacodynamic drug interactions are based on the pursuit of useful medication combinations and techniques for evaluating them. The current research set out to assess the most popular techniques for testing in vitro combinations that may then be put to the test in in vivo settings or later clinical investigations. Treatment procedures for breast cancer often include the use of anthracyclines and taxanes, two of the most prevalent types of conventional chemotherapeutic drugs. One of the most successful antineoplastic medications in the treatment of solid tumors (including breast cancer) is the antibiotic doxorubicin from the anthracycline group, although it has severe dose-limiting toxicities, including as damage to the bone marrow and the heart. Therefore, we are trying to find methods to maintain its high effectiveness, or perhaps to augment it in certain circumstances, without making it more likely that it would cause harm. Many studies have demonstrated that L. barbarum extract has anticancer efficacy, exhibited selectivity on tumor cells, organ protective effects (such as cardioprotective), and may be used with chemotherapy [15–17].

Our earlier research showed that the polyphenolic component of L. barbarum has significant antioxidant properties, which correspond with its cytotoxicity against breast cancer cell lines [4]. We have also shown that L. barbarum's polysaccharide fraction plays a role in its antiproliferative properties. Since L. barbarum fruit extract includes both polyphenols and polysaccharides, it was employed in this investigation. Figure 1 shows that doxorubicin sensitivity is increased 10-fold in MCF-7 cells when compared to MDA-MB-231 cells. We found synergistic effects at low doxorubicin concentrations (0.02-0.075 M) and additive effects with the rise of doxorubicin concentrations while utilizing a fixed 100 g/ml LBE. Combinational index (CI) values derived by the CalcuSyn® program revealed substantial overlap with fractional analysis (table 1). The only distinction is that 100 g/ml LBE and 0.6 mM doxorubicin are evaluated as antagonistic rather than additive.

It is also worth noting that the combination has no effect on the IC50 value (0.069 M), which is the same as when using doxorubicin alone (0.07 M). As one of the most aggressive subtypes of breast cancer, triple-negative breast cancer cells like MDA-MB-231 are the topic of much study. The doxorubicin concentrations employed with MDA-MB-231 cells were lower than the IC50 values used in the prior batch. The observed combination reactions were substantial synergistic effects (Figure 3), while at low doses (0.02-0.075 M), doxorubicin and 120 g/ml LBE demonstrated rather antagonistic than additive effects. Results are consistent when looking at the combination index (Table 2), with antagonism at low concentrations and synergy at high ones. We boosted the LBE content to

140 g/ml to see any alterations. In all of the doxorubicin doses tested, synergistic effects were evident using both assessment techniques.

The IC50 values of doxorubicin on the MDA-MB-231 cell line were 0.09369 M and 0.07231 M, respectively, after addition of 120 or 140 g/ml LBE. Conventional chemotherapeutic drugs, such as doxorubicin, have been shown to have synergistic effects with natural compounds [18, 19]. Furthermore, our prior work in rats demonstrates that doxorubicin-induced cardiotoxicity may be mitigated by using isolated fractions of L. barbarum [20]. Other articles [21, 22] corroborate the reduced damage seen in the groups pretreated with L. barbarum isolated fractions, as measured by biochemical cardiac indicators of cardiotoxicity and histology. Pharmacodynamic drug interactions under in vitro settings are often characterized using the Chou-Talalay Combination Index (CTCI) approach and Fractional Effect Analysis (FA) [23, 24]. Our findings indicated a strong correlation between the two approaches (r=0.764559) and no significant difference between the two approaches (p=0.637). That's why it's possible to use either technique to assess pharmacodynamic interactions.

5. Conclusion

Combination therapy with anthracycline antibiotics (like doxorubicin) and the extract of the Lyciumbarbarum fruit (Goji berry) has shown promising results. The anthracycline therapy regimen for breast cancer has the dual benefit of increasing anticancer activity (the therapeutic effect) and decreasing the risk of dose-dependent cardiotoxicity (the adverse impact)..

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