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# Investigation of Synergistic effect of Tamoxifen and Usnic Acid on Breast Cancer Cell Line

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**Abstract**

The presence of unwanted side effects of many drugs used in cancer treatment brings about research for alternative therapeutic approaches. Lichens, symbiotic organisms of fungi and algae, synthesize metabolites with remarkable biological activities. Usnic acid, one of the lichen secondary metabolite, has been extensively studied in cancer research. The aim of the study was to screen the synergistic effect of tamoxifen and usnic acid on MCF-7 breast cancer cell line. The inhibitory effect of usnic acid on breast cancer cells was determined by MTT assay. Usnic acid has been mainly investigated on cell proliferation of different cancer cell lines in recent years. However, no research has been made to look into the effect of both tamoxifen and usnic acid on breast cancer cell lines.

## INTRODUCTION

Breast cancer is the most frequent cancer type in women and it has the highest mortality rate after lung cancer. Breast cancer development may be associated with some risk factors such as age, race, menopausal age, childbearing age, familial and genetic factors, especially mutations in PTEN, p53 and BRCA1/2 genes and environmental factors [1]. In addition, estrogen, one of the steroid hormones has been shown to be a stimulatory effect on the proliferation of cancer cells, although it is not directly identified as a risk factor for breast cancer development [2]. Gholamreza et al., also demonstrated that estrogen receptors are expressed in the majority of hormone dependent breast cancers types [3]. Tamoxifen as a drug that has agonistic and antagonistic effects on estrogen receptors is used hormone dependent breast cancer for therapeutic purposes [4]. Tamoxifen can be applied to all stages of breast cancer due to its anti-estrogenic effects on the cell of breast tissue. In addition to synthetically produced drugs such as tamoxifen, secondary metabolites obtained from different biological organisms are also promising as a candidate pharmaceutical agent in the treatment of cancer. Secondary metabolites are effective on the disease treatment therefore these compounds can be used with some drugs. Usnic acid, which is a lichen secondary metabolite, has some properties such as antibacterial, anti-inflammatory, antiviral and anti-proliferative activities [5]. Besides all of these properties, usnic asid is also known as an cytotoxic activity on cancer cells [6,7]. It was aim to study the determination of antiproliferative effect both usnic acid and tamoxifen on breast cancer cells.

## MATERIALS AND METHODS

### Drugs (Tamoxifen and Usnic acid)

Usnic acid was purchased from Sigma and was prepared in 14 mM DMSO. The stock solutions were prepared to 200 µM concentration. In previous studies, $EC_{50}$ value of usnic acid was determined as 13.11 µM on MCF-7 breast cancer cell line in our laboratory. 1M tamoxifen stock solution (Sigma-Aldrich) was prepared with ethanol and serially diluted as 100, 80, 40, 20, 10, 5, 2, 1 µg/ml in DMEM medium. To determine the synergistic effect of tamoxifen and usnic acid, 1M tamoxifen stock solution was serially diluted as 100, 80, 40, 20, 10, 5, 2, 1 µg/ml in EC 50 value of usnic acid solution (13.11 µM).

### Maintenance of MCF-7 Cell Line

Human breast cell line MCF-7 was obtained from the ATCC. MCF-7 cell line was cultured in DMEM containing 10% FBS, 2 mM L-glutamine and 1% penicillin and streptomycin. Cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$.

### Cell viability assay for tamoxifen

MCF-7 cells ($1x10^4$) were seeded in each well of 96-well plate and incubated overnight. The cells treated various doses of tamoxifen (100, 80, 40, 20, 10, 5, 2, 1 µg/ml) for 48h. After 48 hours drug administrations, 20 µl of 5 mg/ml MTT reagent was added into each well and incubated for 4 h at 37°C 100 µl isopropanol was added into each well and the samples were measured by spectrophotometer at 570 nm. The results represent

the average values of three experiments. Each experiment was performed at least three times.

### Cell viability assay for tamoxifen /usnic acid

MCF-7 cell line was plated into a 96 well culture plate at a density of 1 x $10^4$ cells per well. Tamoxifen concentrations (100, 80, 40, 20, 10, 5, 2, 1 µg/ml) was diluted to appropriate value of usnic acid (13.11 µM) and immediately applied to the cells for 48h. Controls included ethanol and DMEM treated cells for tamoxifen treatment, and ethanol and DMSO treated cells for both tamoxifen and usnic acid treatment. Concentration dependent cytotoxicity were assessed by MTT assay. The absorbance was recorded at 570 nm on microplate reader. All experiments were performed in three independent biological and technical replicates.

### Statistical analyses

Statistical significance for cell viability was determined using Student's *t*-test, using a confidence level of 95 % ($p < 0.05$).

## RESULTS AND DISCUSSION

The breast cancer cell line, MCF-7 was treated with different concentrations of tamoxifen and with usnic acid (13.11 µM)/ tamoxifen concentrations (100, 80, 40, 20, 10, 5, 2, 1 µg/ml). The viability of treated MCF-7 cell was evaluated by MTT assay after 48h treatment. The $EC_{50}$ concentration of cell death were observed in 37.6 µg/ml concentration tamoxifen for 48 h on MCF-7 cancer cell line ($p < 0.05$) (Figure). The value of the $EC_{50}$ of usnic acid and different concentrations of tamoxifen application was determined to be 24.9 µg/ml for MCF-7 cancer cell line (Figure). As shown Figure 1, MCF-7 cell was found to be sensitive to $EC_{50}$ value of tamoxifen (approximately 62.43% reduction in cell viability, $p < 0.05$), but tamoxifen+usnic acid treatment was more effective on cell death ratio (75.12% reduction in cell viability, $p < 0.05$). This result suggests that tamoxifen+usnic acid treatment significantly inhibited the proliferation of MCF-7 breast cancer cell lines ($p < 0.05$). In this study, tamoxifen+usnic acid treatment showed the promising effect on MCF-7 cancer cell line. As a result of Papanikolaou et al., study MCF-7 cells were found to be sensitive to tamoxifen (40 % reduction in cell viability) while tamoxifen plus leptin treatment partially restored cell viability (22 % reduction in cell viability, $p < 0.05$).

Natural products have been potentially sources as novel drugs in cancer treatment [9]. Some researchers have recently demonstrated the effects of natural metabolites obtained from biological organisms on cancer cells. Among them, some metabolites have been used in the pharmaceutical industry in recent years and many potential sources of drug therapies still need to be investigated [10]. Lichen species and their related metabolites can now be used in the pharmaceutical field. One of the secondary metabolites obtained from lichen is usnic acid. Previous our study also demonstrated that usnic acid is strongly effective in cancer cell lines [11,12].

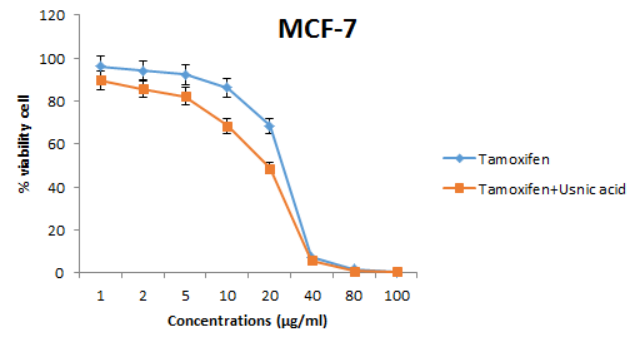


**Figure 1** Dose-responce curves for the effect of tamoxifen and tamoxifen+usnic acid on MCF-7 cell line for 48 h.

The aim of this study was to investigate the effect of usnic acid administration on MCF-7 breast cancer cell line treated with tamoxifen. Our study provides novel evidence indicating the synergy between tamoxifen and usnic acid on treatment of breast cancer for the first time. Papanikolaou et al., (2005) investigated that the effect of tamoxifen/leptin treatment on breast cancer cells by using MTT assay [8]. They have demonstrated that tamoxifen and leptin has relatively anti-proliferative effect on breast cancer cell lines. Our study indicated a difference in MCF-7 breast cancer cell in their response to tamoxifen and usnic acid. The study by Papanikolaou et al., and our study provide similar results.

## CONCLUSION

To the best of our knowledge, this is the first study on anti-proliferative effect of tamoxifen and usnic acid against breast cancer cell line. This study revealed that combination of tamoxifen and usnic acid seemed to be suitable agent for anticancer therapy in clinical applications.

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