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Research Article

Agonistic Effects of Deinoxanthin on Tamoxifen Antiproliferative Activity on HER2 Positive Breast Cancer: An In vitro Study on MDA-MB-453

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Abstract

Tamoxifen, an estrogen receptor competitive and nonsteroidal drug, has been used for nearly 20 years to treat patients with hormone receptor-positive breast cancer. Deinoxanthin is a xanthophyll derivative purified from the cell wall of a radiation-resistant bacterium, *Deinococcus radiodurans*, has been shown in some studies to have proapoptotic and antiproliferative effects on some types of cancer.

Within the study's scope, it aimed to increase the effectiveness of Tamoxifen with deinoxanthin. The combination of 4.21 µM tamoxifen (TMX) and 3.125 µM deinoxanthin showed the best synergistic effect (CI:0.052) at 24 h incubation in MDA-MB-453 cells according to calculation of XTT findings using CompuSyn software. In this concentration combination, gene expression levels and protein levels of BCL2 Associated X protein (BAX), Caspase 3 (CASP-3), B cell leukemia/lymphoma 2 protein (BCL-2) and Human Epidermal Growth Factor Receptor 2 (HER2) were determined by RT-qPCR and ELISA method, respectively, and according to the results, it is thought that the intrinsic apoptotic pathway is activated.

Keywords: Breast cancer, Deinoxanthin, Tamoxifen, MDA-MB-453.

Deinoksantinin HER2 Pozitif Meme Kanserinde Tamoksifenin Antiproliferatif Aktivitesi Üzerindeki Agonistik Etkileri: MDA-MB-453 Üzerine Bir In vitro Çalışma

Öz

Östrojen reseptörü yarışmalı ve steroid olmayan bir bileşik olan Tamoksifen, hormon reseptör pozitif meme kanseri olan hastaları tedavi etmek için yaklaşık 20 yıldır kullanılmaktadır. Deinoxanthin, radyasyona dirençli bir bakteri olan *Deinococcus radiodurans*'ın hücre duvarından saflaştırılan bir ksantofil türevi olup, bazı kanser türleri üzerinde proapoptotik ve antiproliferatif etkilere sahip olduğu bazı çalışmalarda gösterilmiştir.

Çalışma kapsamında Tamoksifenin (TMX) deinoksantin (DNX) ile etkinliğinin artırılması hedeflendi. 4.21 µM TMX ve 3.125 µM DNX kombinasyonu, CompuSyn yazılımı kullanılarak XTT bulgularının hesaplanmasına göre MDA-MB-453 hücrelerinde 24 saatlik inkübasyonda en iyi sinerjistik etkiyi (CI:0.052) gösterdi. Bu konsantrasyon kombinasyonunda BCL-2 ilişkili X proteini (BAX), Kaspaz 3 (CASP-3), B hücreli lösemi/lenfoma 2 proteini (BCL-2) ve İnsan Epidermal Büyüme Faktörü Reseptörü 2'nün (HER2) gen ifade seviyeleri ve protein düzeyleri sırasıyla RT-qPCR ve ELISA yöntemi ile belirlenmiş olup sonuçlara göre intrensek apoptotik yolağın aktifleştiği olduğu düşünülmektedir.

Anahtar Kelimeler: Meme Kanseri, Deinoksantin, Tamoksifen, MDA-MB-453.

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1. Introduction

Cancer, which has become a global threat and causes millions of deaths, is among the causes of mortality after heart diseases [1] Breast cancer, which is the most common type of cancer in women, is malignant and metastatic tumors. Hormone receptor-positive breast cancer accounts for 80% of all breast cancers [2].

Cancer caused an estimated 9.6 million deaths in 2018. About 1 out of 6 deaths in the world and 1 out of every 5 deaths in Türkiye are caused by cancer [3]. According to 2013 data, breast cancer is the most common type of cancer in women in our country, and it is known that approximately 25% of women with this disease have HER2 positive breast cancer [4].

The exact cause of the onset of cancer is not known. In addition, studies are carried out to characterize cancer and prevent its formation and progression [5]. Since the determination of the type of treatment is of great importance, scientists have used different parameters to define the types of breast cancer. Rare breast cancers, special type; the common breast cancers were named as non-specific types. There are cells with specific characteristics in rare special types of breast cancers.

Combination therapy has also gained momentum in oncology in recent years, with higher response rates with drug combinations compared to monotherapy in several studies, the FDA has recently approved therapeutic combination regimens that show superior safety and efficacy to monotherapy [6,7].

In therapeutic agent development research, the identification of membrane receptors and the creation of ligands with high affinity for them have been identified as the most important end goal. Advances in molecular biology and genetics have brought along important innovations in drug development. As a result of the joint studies of these fields with the science of bioinformatics, it has been an important step in the discovery of new therapeutic targets by investigating the genetic causes of many diseases [8].

Tamoxifen is a non-steroidal antiestrogen drug commonly used in the treatment of hormone-sensitive breast cancer. More than 70% of all breast cancer patients are estrogen receptor-positive breast cancer patients [9]. Tamoxifen is used in the prevention and treatment of ER+ breast cancer in pre- and post-menopausal women [10]. It reduces breast cancer recurrence by 50% and the annual death rate by 31%. Despite this success, approximately 25% of tumors develop resistance to Tamoxifen treatment within a few years in addition to its side effects [11].

Estrogen hormone, which is a growth factor, binds to the estrogen receptor on the cell surface, also called its name, and ensures the growth of normal breast cells. In the case of breast cancer, estrogen receptors work for the growth and proliferation of cancer cells. It has been determined that in the majority of breast cancer types, tumor cells become dependent on estrogen for growth by significantly increasing the number of estrogen receptors in their membranes. Tamoxifen, designed to block estrogen receptors on the breast cancer cell surface, exerts its anti-proliferative effect by competitively binding to the estrogen receptor, thereby blocking the mitogenic effect of estrogen [12].

Evidence is presented that Tamoxifen induces a response in tumors lacking *ER* gene expression [13,14]. Adjuvant Tamoxifen treatment has been shown to reduce the risk of recurrence even in lesions without *ER* gene expression [15].

These clinical findings propose that Tamoxifen may have some ER-independent anticancer properties. Additionally, Blackwell et al. (2000) found that Tamoxifen was able to inhibit angiogenesis in the ER-negative fibrosarcoma model [16].

Liu et al., in a study they conducted on five different ER-negative human breast cancer cell lines to understand the antiproliferative effect of Tamoxifen on ER-negative breast cancer cells; a dose- and time-dependent induction of apoptosis was observed in MDA-MB-468, MDA-MB-231, MDA-MB-453, and SK-BR3 cells, while no significant apoptotic effect was observed in HCC-1937 cell line [17, 18].

There are more than 600 different carotenoids in nature, and they are common natural pigments [19]. Although some natural carotenoids, such as astraxanthin, β-carotene, and lycopene have been used as food coloring for many years, it has recently been an area of intense study in investigating its potential to prevent epithelial cancer and chronic diseases, with the discovery of antioxidant activity [20, 21]. Carotenoids are found in many bacteria and are found in a wide variety of phyla, not only photosynthetic but also non-photosynthetic species [19].

Deinococcus radiodurans is a red-pigmented, non-sporeforming, non-pathogenic bacteria species 1.5-3.5 μm in diameter, in clusters of two or four cells, highly resistant to stress and UV rays [19, 22, 23, 24, 29]. *D. radiodurans* synthesizes a unique ketocarotenoid, deinoxanthin (DNX) [25, 26]. Deinoxanthin has a much higher antioxidant effect than other xanthine derivatives and therefore shows a stronger ROS scavenging ability [27, 28].

Some carotenoids such as fucoxanthin, lycopene, lutein, and β-carotene suppress carcinogenesis in animals and cause apoptosis of various cancer cell lines [29]. Also, some studies show that taking carotenoids reduces the risk of cancer. It protects against DNA damage as deinoxanthin is a more effective reactive oxygen species scavenger than lutein, lycopene, or β-carotene [30, 31]. Therefore, it is noteworthy to investigate the effect of deinoxanthin on cancer cells.

Deinoxanthin has a pro-oxidative effect as well as an antioxidant effect under certain conditions. Many studies have shown that oxidative stress plays an important role in the process of inducing apoptosis. [32, 33, 34, 35]

Studies have shown that this pigment inhibits the growth of cancer cells by inducing apoptosis in various cancer cells. Within the scope of the study, it was aimed to increase the antiproliferative activity of Tamoxifen with deinoxanthin on MDA-MB-453, a HER-2 positive breast cancer cell line.

Deinoxanthin is a carotenoid derivative that has a pro-oxidant effect against cancer cells, on which studies have not been fully deepened. This xanthine derivative, which helps Deinococcus radiodurans survive in the highly oxidative environments it is exposed to, also plays an

antioxidant role. In the study, it was aimed that tamoxifen, which is used against ER-positive cancer types, with deinoxanthin at lower doses, has a better anti-cancer effect against ER-negative breast cancer. Deinoxanthin, which makes tamoxifen more effective in vitro with lower IC50 values than known, can also reduce side effects with its antioxidant property in healthy cells.

2. Materials and Methods

2.1. Materials

The MDA-MB-453 (HTB-131) breast cancer cell line was purchased commercially from ATCC.

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, heat-inactivated fetal bovine serum, penicillin-streptomycin, trypsin-EDTA, phosphate buffer saline (PBS), and XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) cell proliferation kit was obtained from Biological Industries Ltd (Catalog number:20-300-1000).

TMX was obtained from Sigma Aldrich.

DNX was supplied from the purified stock of Seda KILINÇ, produced in the joint venture of Erzincan Binali Yıldırım University and Marmara University, Türkiye.

RNA isolation (Catalog number:K0732) and cDNA synthesis (Catalog number:4368814) were performed according to the manufacturer's instructions (Thermo Scientific, US). Human BAX (Catalog number: E-EL-H0562), CASP-3 (Catalog number: E-EL-H0017), BCL-2 (Catalog number: E-EL-H0114), and HER2 (Catalog number: E-EL-H6083) ELISA kits were purchased from Elabscience Biotechnology Co. (Wuhan, China). All other chemical reagents were purchased from Merck and Sigma Aldrich.

2.2. Cell line and culture

MDA-MB-453 breast cancer cell line; was cultured in DMEM containing 1% penicillin-streptomycin, 1% L-glutamine, and 10% FBS. Incubation was carried out in an incubator at 37° C and 5% CO_2 sterile air.

2.3. Cell proliferation assay

Cell cultivation was inoculated into each well of 96-well TC-treated plates in 200 µl DMEM at $5x10^4$ cells and incubated for 24 hours at 37° C, 5% CO_2, and 95% humidity.

To determine the cytotoxic effects depending on time and concentration, a study was conducted to determine the combined and separate concentrations of TMX and DNX.

Table 1. Application concentrations of TMX and DNX.

Concentration Sets	TMX (µM)	DNX (µM)	TMX (µM) + DNX (µM)
1	270	200	270+200
2	135	100	135+100
3	67,5	50	67,5+50
4	33,75	25	33,75+25
5	16,87	12,5	16,87+12,5
6	8,43	6,25	8,43+6,25
7	4,21	3,125	4,21+3,125

At the end of the incubation periods, 50 µl of XTT reagent was added to each well to determine cell viability. After 3 hours, the absorbance was measured at 450 nm with a microplate reader to calculate the percent cell viability versus negative control.

2.4.Combinational Index Value

TMX and DNX interactions and combination index (CI) values were determined with the CompuSyn program.

2.5. RT-qPCR Analysis

In study, It was determined that the best synergistic effect in the calculated CI values was the 24 hours incubation period and the combination of 4.21 µM TMX and 3.125 µM DNX. RNA isolation and cDNA synthesis were performed by culturing the MDA-MB-453 breast cancer cell line applied with these parameters. The expression levels of *BAX*, *CASP-3*, *BCL2*, and *HER2* genes of the MDA-MB-453 cell line were determined. The *ACTB* gene was used as the reference gene. Real-time PCR with SYBR green was performed using Bio-rad iTaq™ universal SYBR® Green supermix (Catalog number: 1725125) and a Qiagen Rotor-Gene Q 6plex Real-Time PCR System. Primers designed by using NCBI Primer-BLAST program were synthesized by Metabion Company according to the sequences specified in (Table 2.).

Table 2. Primer sequences.

Gene	Primer sequence	Accession Number
BAX	F: 5-AGCAGATCATGAAGACAGGG R: 5-GAAGTTGCCGTCAGAAAACA	NM 138764
CASP-3	F: 5-GCGCTCTGGTTTTCGTTAAT R: 5-ACCCATCTCAGGATAATCCATTT	NM 004346
BCL2	F: 5-TATCTGGGCCACAAGTGAAG R: 5-ATTCGACGTTTTGCCTGAAG	NM 000657
HER2	F: 5-GTGAAGCTGAGATTCCCCTC R: 5-GCAGCTTCATGTCTGTGC	NM 001005862
ACTB	F: 5-CACCATGGATGATGATATCGC R: 5-GAATCCTTCTGACCCATGCC	NM 001101

2.6. Protein extraction

TMX (4.21 µM), DNX (3.125 µM), and TMX + DNX (4.21 µM + 3.125 µM) drug applications, which were the lowest CI values obtained in the study, levels of BAX, CASP-3, BCL-2 and HER2, on MDA-MB-453 cell line and to determine the expression proteins, commercial ELISA kits were used. Also, the total protein concentration in the sample was measured using the Bradford protein assay.

2.7. Statistical analysis

BAX, *CASP-3*, *BCL-2*, and *HER2* genes expression levels were determined using the primary normalization $2^{-\Delta\Delta CT}$ method developed by Livak and Schmittgen using *ACTB* as the reference gene (Livak & Schmittgen, 2001). Statistical analysis was evaluated using SPSS 17.0 program. The significant percentage of the results is $p < 0.05$.

3. Results and Discussion

3.1.Cell Viability Test

XTT studies were carried out on MDA-MB-453 cells at 24 and 48 hours incubation times where the indicated concentrations of TMX and DNX were applied separately and in combination. With the absorbance data obtained at 450 nm, "cell survival percentages" were calculated and as a result of these calculations, the findings in the graphs shown in (Figure 1) and (Figure 2) were obtained.

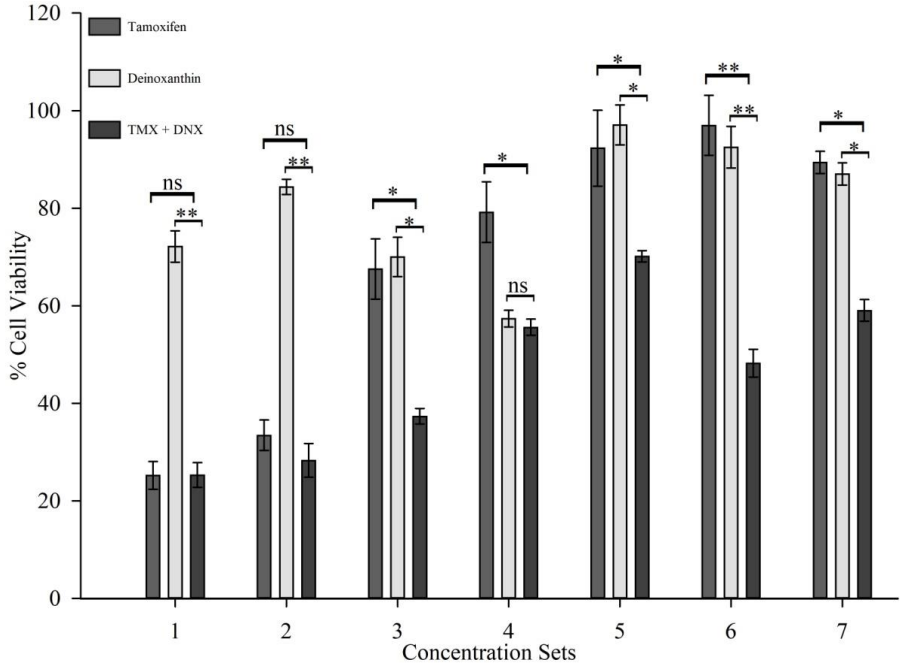

Figure 1. Antiproliferative activity of TMX, DNX and TMX + DNX on MDA-MB-453 at 24 hours. Values are represented as the mean ± standard error of mean. "ns" indicates not significant ($p>0.05$); *$p < 0.05$; **$p < 0.01$.

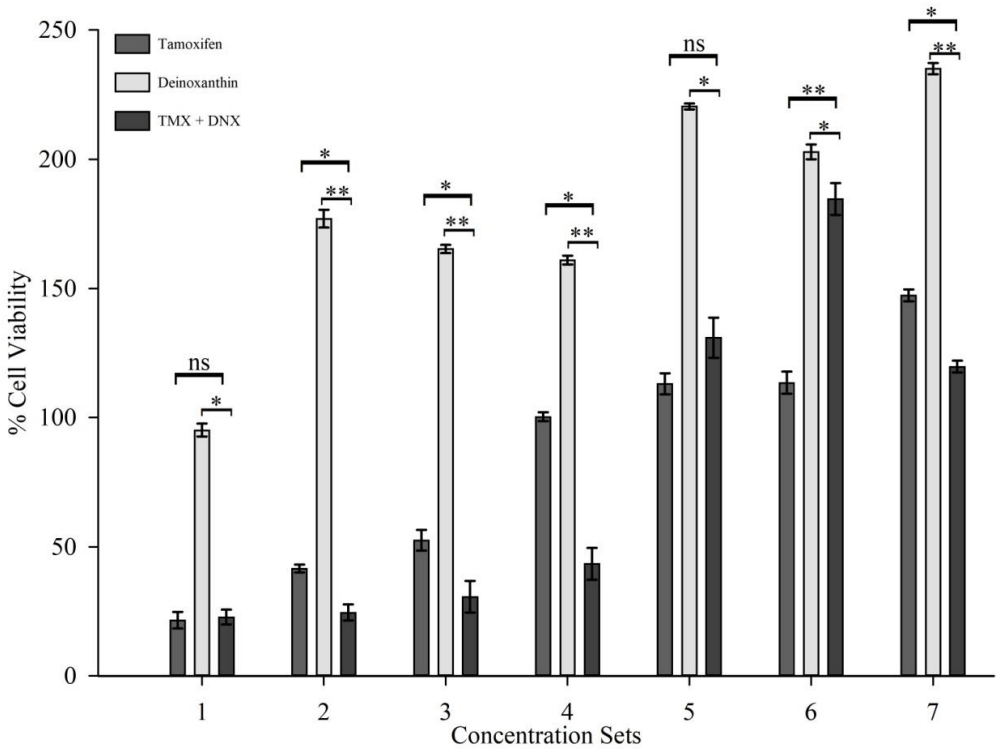

Figure 2. Antiproliferative activity of TMX, DNX and TMX + DNX on MDA-MB-453 at 48 hours. Values are represented as the mean ± standard error of mean. "ns" indicates not significant ($p>0.05$); *$p < 0.05$; **$p < 0.01$.

The combination effect value (CI) was calculated using the CompuSyn program using the absorbance values measured at the 24 and 48 hours in the MDA-MB-453 cell line where TMX, DNX and their combinations were administered.

Table 3. CI values calculated after the application of TMX, DNX and TMX + DNX on MDA-MB-453 at the 24 hours.

TMX+DNX (μM)	**CI**	**Effect**
270+200	1,048	Medium Antagonistic
135+100	0,589	Synergistic
67,5+50	0,407	Synergistic
33,75+25	0,373	Synergistic
16,87+12,5	0,327	Synergistic
8,43+6,25	0,072	Very strong synergistic
4,21+3,125	0,052	Very strong synergistic

Table 4. CI values calculated after the application of TMX, DNX and TMX + DNX on MDA-MB-453 at the 48 hours.

TMX+DNX (μM)	CI	Effect
270+200	1,228	Medium Antagonistic
135+100	0,652	Synergistic
67,5+50	0,394	Synergistic
33,75+25	0,286	Strong synergistic
16,87+12,5	0,943	Additive
8,43+6,25	9,033	Strong Antagonistic
4,21+3,125	0,159	Strong synergistic

Within the scope of the studies carried out, as a result of 24 and 48 hours incubation on MDA-MB-453 cell line; according to the results of XTT studies conducted with the application of TMX, DNX and their combinations at the determined concentrations; it has been determined that TMX has a higher antiproliferative effect than DNX, except for the 4th, 6th and 7th concentrations of the 24 hours, and when applied together at all concentrations, it has a better antiproliferative effect than when applied separately.

It was observed that at the 48th hour, compared to the 24th hour, TMX, DNX, or both lost their half-lives because they were present in a longer period or fluctuations in the vitality rates were observed due to probabilities such as deterioration of their molecular structure.

3.2.RT-qPCR Analysis and ELISA

The expression levels of *BAX*, *CASP-3*, *BCL-2*, and *HER2* genes of the MDA-MB-453 cell line were determined at the lowest CI value determined in the study. *ACTB* (beta-actin) gene was used as the reference gene. Graphics of the results obtained; (Figure 3, Figure 4, Figure 5, and Figure 6.

When looking at the findings, it was observed that TMX applied alone on the MDA-MB-453 breast cancer cell line did not have a very high effect on the expression level of *BAX* and *CASP-3* genes, but when applied together with DNX, it was observed that it caused a significant increase in the expression levels of *BAX* and *CASP-3* genes.

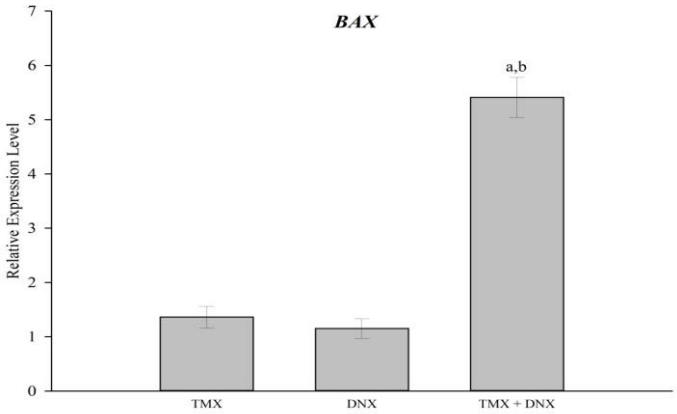

Figure 3. The effect of TMX, DNX, and their combinations on *BAX* gene expression level. Values are represented as the mean ± standard error of mean. [a]$p < 0.05$ versus TMX-treated group. [b]$p < 0.05$ versus DNX-treated group.

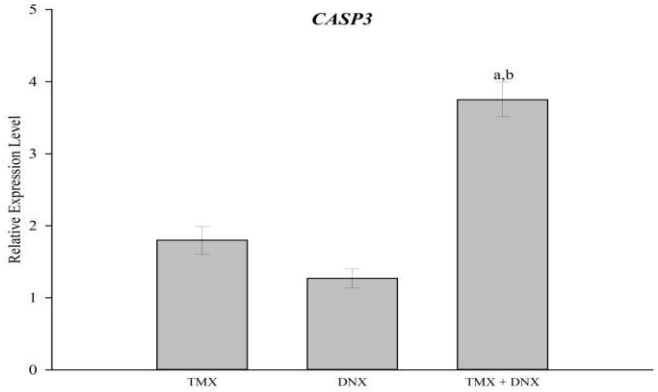

Figure 4. The effect of TMX, DNX, and their combinations on *CASP-3* gene expression level. Values are represented as the mean ± standard error of mean. [a]$p < 0.05$ versus TMX-treated group. [b]$p < 0.05$ versus DNX -treated group.

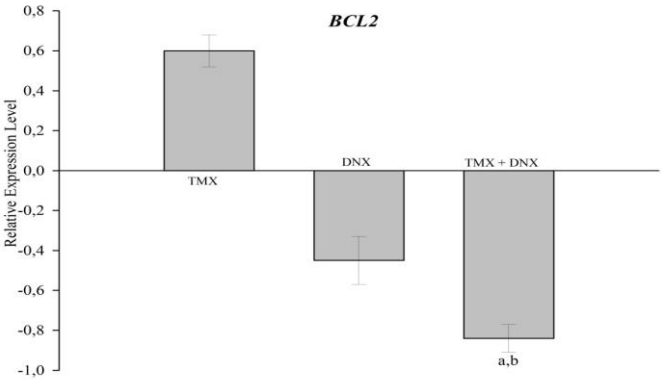

Figure 5. The effect of TMX, DNX, and their combinations on *BCL2* gene expression level. Values are represented as the mean ± standard error of mean. [a]$p < 0.05$ versus TMX-treated group. [b]$p < 0.05$ versus DNX-treated group.

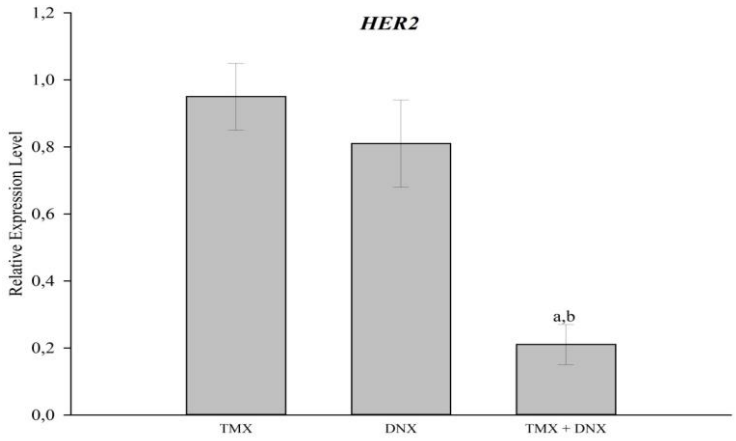

Figure 6. TMX, DNX, and their combination effects on *HER2* gene expression. Values are represented as the mean ± standard error of mean. $^{a}p < 0.05$ versus TMX-treated group. $^{b}p < 0.05$ versus DNX-treated group.

The expression level of BAX and CASP-3 genes in the DNX-group did not increase at a very high rate, and had less effect on *BCL-2* and *HER2* alone. It is not considered to have a greater effect as an intrinsic apoptotic stimulus alone.

According to the results of RT-qPCR in all samples where 4,21 µM TMX + 3,125 µM DNX combination was applied; *BAX* and *CASP3* genes; It was observed that higher expression levels were reached compared to the control group and TMX-group. In determining the expression levels of *BCL-2* and *HER2* genes, it was revealed that the combination of drugs was more effective than individual drugs on MDA-MB-453.

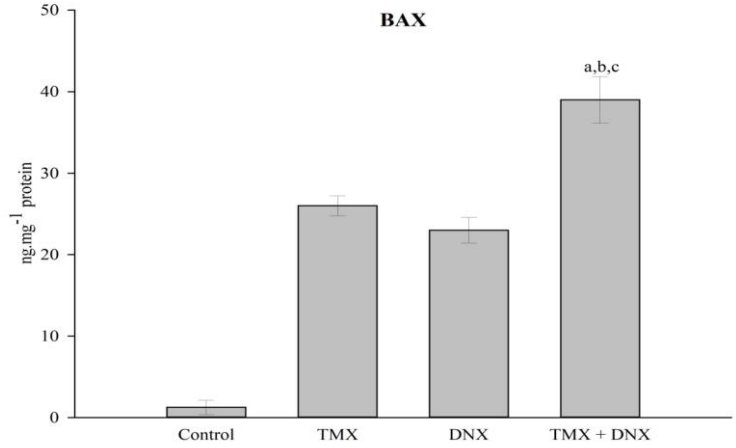

Figure 7. The effect of TMX, DNX, and their combinations on BAX protein expression level. Values are represented as the mean ± standard error of mean. $^{a}p < 0.05$ versus TMX-treated group. $^{b}p < 0.05$ versus DNX-treated group.

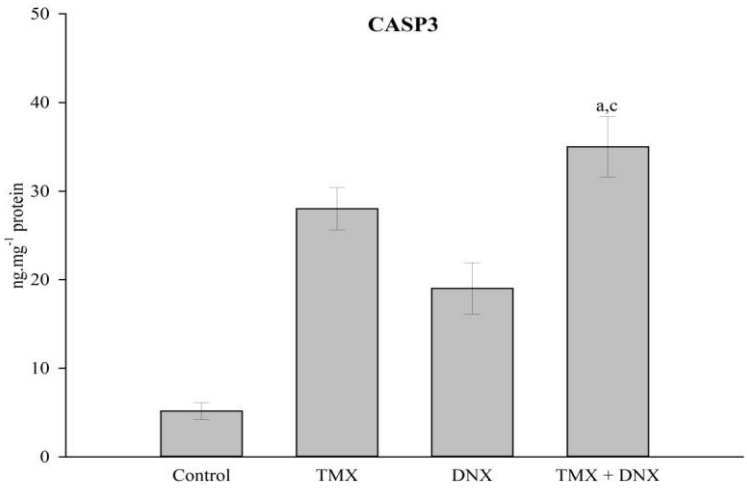

Figure 8. The effect of TMX, DNX, and their combinations on CASP-3 protein expression level. Values are represented as the mean ± standard error of mean. $^{a}p < 0.05$ versus TMX-treated group. $^{b}p < 0.05$ versus DNX-treated group.

In many cancer types, while pro-apoptotic protein levels such as BAX and CASP-3 decrease, anti-apoptotic protein levels such as BCL-2 increase. It was determined that BAX and CASP-3 protein levels differed significantly in the drug concentration administered in combination compared to the control and separately administered drug concentrations. This significant increase in BAX and CASP-3 protein levels suggests that apoptosis was induced in cancer cells.

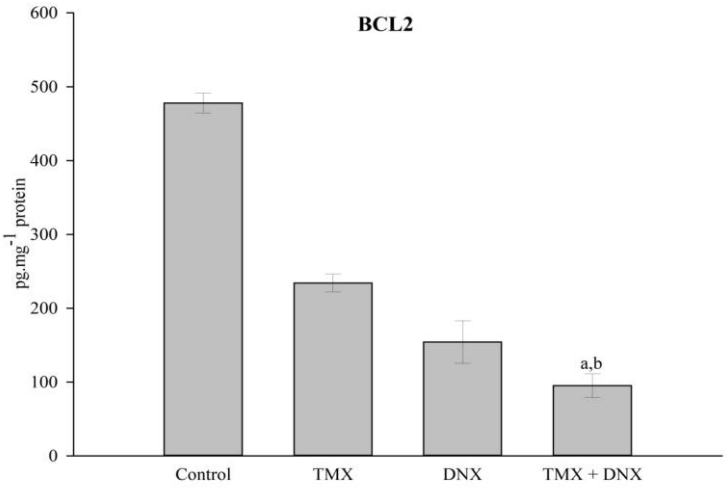

Figure 9. The effect of TMX, DNX and their combinations on BCL-2 protein expression level. Values are represented as the mean ± standard error of mean. $^{a}p < 0.05$ versus TMX-treated group. $^{b}p < 0.05$ versus DNX-treated group.

The Bcl-2 family plays an important role in the regulation of apoptosis. The intensity of Bcl-2 production in various cancer cells shows a positive correlation with the lifespan of the cell [36]. In addition to BAX and CASP-3 protein levels, a significant decrease was observed in the determined BCL-2 and HER-2 protein levels, and the suppression of apoptosis in cells decreased. And it is thought that the cell is more easily dragged into apoptosis.

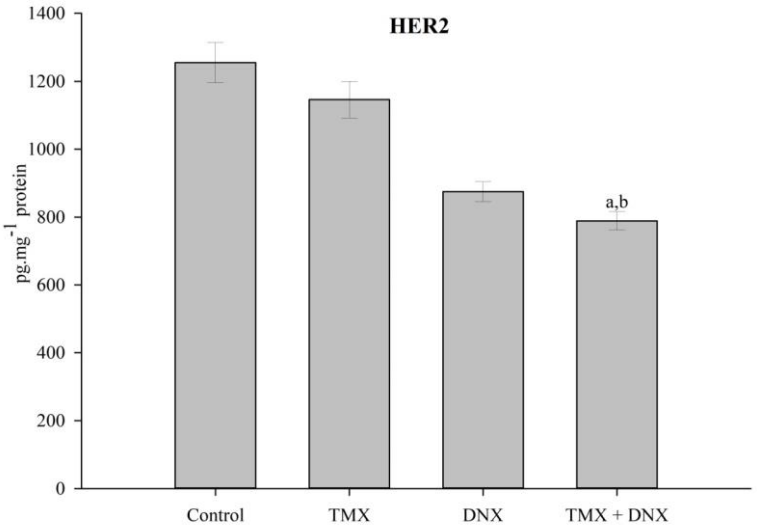

Figure 10. The effect of TMX, DNX and their combinations on HER-2 protein expression level. Values are represented as the mean ± standard error of mean. [a]$p < 0.05$ versus TMX-treated group. [b]$p < 0.05$ versus DNX-treated group.

Table 5. The amount of BAX, BCL-2, CASP-3, and HER2 in MDA-MB-453 cell line exposed to DNX (3,125 μM), TMX (4,21μM) separately and also in combination (4,21μM+3,125μM) for 24 h.

	BAX (ng/mg protein)	BCL2 (pg/mg protein)	CASP3 (ng/mg protein)	HER2 (pg/mg protein)
Control	1,25 ± 0,87[b,c,d,*]	478 ± 13,5[b,c,d]	5,16 ± 0,96[b,c,d]	1255 ± 59[c,d]
TMX	26,15 ± 1,23[a,d]	234 ± 12[a,c,d]	28 ± 2,4[a,c]	1146 ± 54[c,d]
DNX	22,65 ± 1,58[a,d]	154 ± 29[a,b]	19 ± 2,9[a,b,d]	875 ± 29[a,b]
TMX+DNX	39,24 ± 2,87[a,b,c]	95 ± 16[a,b]	35 ± 3,4[a,c]	789 ± 27[a,b]

*:Results given as the mean±SD. One-way ANOVA with post-hoc LSD test. Different letters show statistical differences. P<0.05 is the level of significance.

The results were as follows BAX protein was found to be increased 31 times more compared to control and 1.5 and 1.7 times more compared to TMX-group and DNX-group, respectively; CASP-3 protein was found to be increased approximately 7 times compared to control and 1.25; 1.8 times more compared to TMX-group and DNX-group, respectively.

The combination of 4.21 μM TMX + 3.125 μM DNX at the *BCL2* expression level decreased 5 times compared to the control and approximately 2.5 and 1.5 times, respectively, compared to TMX-group and DNX-group; In the expression level of HER2, it was determined that the

combination of 4.21 μM TMX + 3.125 μM DNX decreased 1.6 times compared to the control and 1.4; 1.1 times more compared to TMX-group and DNX-group.

In a 2020 study by Maqbool et al., they showed that the synergistic combination of tamoxifen with the novel synthesis product di-2-pyridylketone4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC), a thiosemicarbazone derivative, could be a promising new therapeutic strategy to overcome tamoxifen resistance in ER-positive breast cancer. Using a tamoxifen-resistant cell line, MDA-MB-453 and MDA-MB-231, in the study creates the potential to provide a different treatment strategy with a similar combinatorial approach. As a matter of fact, in previous bioinformatic studies, it was determined that deinoxanthin's ability to bind to testosterone and estrogen receptors may be high. In addition, studies are continuing that deinoxanthine increases the expression levels of estrogen and testosterone receptors.

Tamoxifen, a selective ER modulator, can drive cancer cells into apoptosis through mechanisms other than ER antagonism. In a study by Liu et al. in 2014, the drug mechanism was examined by testing the efficacy of tamoxifen by testing the ER-negative breast cancer cell lines HCC-1937, MDA-MB-231, MDA-MB-468, MDA-MB-453 and SK-BR-3. As a result, it was determined that Tamoxifen significantly induced apoptosis in MDA-MB-231, MDA-MB-468, MDA-MB-453 and SK-BR-3 cells, but was not effective in HCC-1937 cells. They suggested that tamoxifen-induced induction of apoptosis is associated with tamoxifen dose-dependent inhibition of protein phosphatase 2A (CIP2A) and phospho-Akt (p-Akt).

According to the research conducted by Ibrahim et al. in 2019, the combination of Tamoxifen and Simvastatin was treated with different concentrations of TAM and/or SIM for 72 hours in the estrogen receptor positive (ER+) breast cancer cell line T47D. In the study, cytotoxicity, oxidative stress markers, apoptosis, angiogenesis and metastasis analyzes were performed and as a result, higher BAX/BCL-2 ratio and CASP3 activity were observed in the combination groups compared to the others.

In the study, it was observed that the apoptotic pathway was more active in the combination of deinoxanthin with tamoxifen than when tamoxifen was administered alone. Although the MDA-MB-453 cell line is estrogen receptor-negative, it became more sensitive to tamoxifen by triggering inhibition of CIP2A and p-Akt or by a different pathway. More detailed metabolomic analysis of the effect of deinoxanthin with tamoxifen is planned in future studies.

4. Conclusion

In line with these results, it has been shown that Tamoxifen may be to combined with deinoxanthin, which has high antioxidant properties, to increase its effectiveness and further strengthen its antiproliferative activity. In addition, Tamoxifen, which is used in hormone receptor sensitive breast cancer treatments, has also been reported to have a significant effect on cell lines that also show triple negative breast cancer characteristics [37]. We aimed to obtain a more effective combination therapy that can be used in Tamoxifen treatment by using this cell line that can show both features.

Significant antiproliferative activity was found in our study as a result of the combination of Tamoxifen, which was confirmed to have a strong effect on breast cancer, by benefiting from the strong antioxidant effect of deinoxanthin and its ability to drive cancer cells to apoptosis. It is planned to increase the effectiveness of this cure by making much more detailed analyzes in future studies.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

With the joint contribution of Nihan GÜNAY and Mehmet KUZUCU, the literature was searched, the study was designed, the experiments were carried out, the study was analyzed and interpreted, the results were determined and interpreted and the article was written.

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