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Differential Modulation of Oxidative Stress and Apoptosis by Melatonin and Fucoidan in Doxorubicin-Treated MCF-7 Breast Cancer Cells

Doksorubisin ile Tedavi Edilen MCF-7 Meme Kanseri Hücrelerinde Melatonin ve Fukoidanın Oksidatif Stres ve Apoptoz Üzerindeki Farklılaştırıcı Etkileri

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ABSTRACT

Objective: Doxorubicin (DOX) is a primary chemotherapeutic agent for estrogen receptor (ER)-positive breast cancer. Its cytotoxic effects are largely mediated by mitochondrial reactive oxygen species (ROS) production and the activation of mitochondria-dependent apoptosis. However, it remains unclear how selective redox modulators influence DOX-triggered mitochondrial stress responses and whether these effects intersect with ER-dependent signaling. This study aimed to compare the impacts of melatonin and fucoidan on DOX-induced oxidative stress and apoptosis in ER-positive MCF-7 breast cancer cells.

Materials and Methods: MCF-7 cells were exposed for 24 hours to DOX (1 µM), melatonin (1 mM), fucoidan (100 µg/mL), or their combinations. Cell viability was assessed using the MTT assay. ROS levels were measured via DCFH-DA fluorescence and normalized to controls. Protein expression of Bax, Bcl-2, cleaved caspase-3, and ERα was quantified by ELISA.

Results: DOX significantly decreased cell viability and elevated ROS production ($p<0.001$), while increasing Bax and cleaved caspase-3 levels, reducing Bcl-2 and ERα expression, and shifting the Bax/Bcl-2 balance toward a pro-apoptotic state ($p<0.001$). Both melatonin and fucoidan markedly attenuated DOX-induced ROS generation ($p<0.001$), although neither fully restored viability or normalized apoptotic markers. Fucoidan exhibited a stronger ROS-suppressive effect than melatonin ($p=0.021$). In contrast, melatonin partially restored ERα protein levels ($p=0.008$), whereas fucoidan showed no significant effect on ERα expression.

Conclusions: Melatonin and fucoidan differentially modulate DOX-induced oxidative stress and apoptotic pathways in ER-positive breast cancer cells, highlighting distinct, mechanism-specific roles in regulating chemotherapy-associated cellular stress responses.

Keywords: Doxorubicin, melatonin, fucoidan, oxidative stress, estrogen receptor alpha

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ÖZET

Amaç: Doksorubisin (DOX), östrojen reseptörü (ER)-pozitif meme kanserinin tedavisinde kullanılan temel kemoterapötik ajanlardan biridir. Sitotoksik etkileri büyük ölçüde mitokondriyal reaktif oksijen türleri (ROS) üretimi ve mitokondriye bağımlı apoptozun aktivasyonu aracılığıyla gerçekleşir. Bununla birlikte, seçici redoks modülatörlerinin DOX ile tetiklenen mitokondriyal stres yanıtlarını nasıl etkilediği ve bu etkilerin ER-bağımlı sinyal yolaklarıyla kesişip kesişmediği tam olarak açıklığa kavuşturulmamıştır. Bu çalışmada, melatonin ve fukoidanın DOX kaynaklı oksidatif stres ve apoptoz üzerindeki etkilerinin ER-pozitif MCF-7 meme kanseri hücrelerinde karşılaştırılması amaçlanmıştır.

Materyal ve Metot: MCF-7 hücreleri 24 saat boyunca DOX (1 µM), melatonin (1 mM), fukoidan (100 µg/mL) veya bunların kombinasyonlarına maruz bırakıldı. Hücre canlılığı MTT yöntemi ile değerlendirildi. ROS düzeyleri DCFH-DA floresansı ile ölçülerek kontrol grubuna göre normalize edildi. Bax, Bcl-2, aktif (cleaved) kaspaz-3 ve ERα protein ekspresyonları ELISA yöntemi ile kantitatif olarak analiz edildi.

Bulgular: DOX uygulaması hücre canlılığını anlamlı düzeyde azaltırken ve ROS üretimini artırırken ($p<0.001$), Bax ve aktif kaspaz-3 düzeylerini artırmış, Bcl-2 ve ERα ekspresyonunu azaltmış ve Bax/Bcl-2 dengesini pro-apoptotik yönde değiştirmiştir ($p<0.001$). Hem melatonin hem de fukoidan DOX kaynaklı ROS üretimini belirgin şekilde azaltmıştır ($p<0.001$); ancak her iki ajan da hücre canlılığını tam olarak geri kazandıramamış ve apoptotik belirteçleri normalize edememiştir. Fukoidan, melatonine kıyasla daha güçlü bir ROS baskılayıcı etki göstermiştir ($p=0.021$). Buna karşılık melatonin, ERα protein düzeylerini kısmen geri kazandırmış ($p=0.008$), fukoidan ise ERα ekspresyonu üzerinde anlamlı bir etki göstermemiştir.

Sonuç: Melatonin ve fukoidan, ER-pozitif meme kanseri hücrelerinde DOX ile indüklenen oksidatif stres ve apoptotik yolakları farklı şekillerde modüle etmektedir. Bu bulgular, kemoterapiye bağlı hücresel stres yanıtlarının düzenlenmesinde mekanizma-spesifik ve birbirinden ayrışan rollerini ortaya koymaktadır.

Anahtar Kelimeler: Doksorubisin, melatonin, fukoidan, oksidatif stres, östrojen reseptör alfa

INTRODUCTION

Breast cancer is a molecularly heterogeneous malignancy characterised by subtype-specific biological behaviour (1). Among these subtypes, oestrogen receptor (ER)-positive tumours represent the largest group, with cellular proliferation and survival largely driven by oestrogen-mediated signalling pathways (2,3). Activation of ERα promotes tumour progression by upregulating genes involved in proliferation and anti-apoptotic responses, including cyclin D1 and Bcl-2 (4). Consequently, ER signalling constitutes a central regulatory axis not only in hormonal therapy responsiveness but also in cellular adaptation to stress and chemotherapeutic agents.

Doxorubicin (DOX), an anthracycline derivative, is widely used in the systemic treatment of ER-positive breast cancer. While its antitumour effects have traditionally been attributed to topoisomerase II inhibition and DNA damage, accumulating evidence indicates that oxidative stress-mediated mechanisms play a pivotal role in DOX-induced cytotoxicity (5,6). Within the mitochondrial compartment, DOX participates in redox cycling reactions that markedly enhance intracellular ROS accumulation. This oxidative overload contributes to lipid peroxidation and destabilization of the mitochondrial membrane (7), promoting cytochrome c translocation into the cytosol and subsequent engagement of the mitochondria-dependent apoptotic cascade, which progresses toward caspase-mediated cell death (8).

Bcl-2 family proteins represent a central regulatory node in the mitochondrial apoptotic pathway. The balance between pro-apoptotic Bax and anti-apoptotic Bcl-2 is a key determinant of mitochondrial outer membrane permeabilisation and subsequent apoptotic progression (9). A shift in this ratio toward Bax, together with activation of cleaved caspase-3, provides molecular evidence of apoptosis. Thus, DOX-induced cytotoxicity is mediated not only by DNA damage but also by disruption of mitochondrial redox homeostasis (10).

Chemotherapy-associated oxidative stress exhibits a dual role. While enhanced ROS production contributes to tumour cell cytotoxicity, excessive oxidative stress may also damage non-malignant tissues (11). This duality has led to increasing interest in pharmacological strategies aimed at modulating redox balance during chemotherapy. However, the concomitant use of antioxidants remains controversial. Some studies suggest that antioxidants may attenuate ROS-mediated cytotoxic effects (12,13), whereas others indicate that redox modulation can alter the mode of cell death and reshape apoptotic signalling pathways (14,15). These divergent findings imply that antioxidant effects are highly context-dependent and influenced by the cellular signalling landscape.

Melatonin is a pleiotropic molecule that functions not only as a potent free radical scavenger but also as a regulator of mitochondrial homeostasis (16). Its capacity to activate endogenous antioxidant enzyme systems and preserve mitochondrial membrane integrity distinguishes it from classical antioxidants. In addition, melatonin has been shown to exert antiproliferative effects in ER-positive breast cancer cells and to modulate ERα expression (17). Evidence indicating that melatonin suppresses estrogen-mediated transcriptional activity and alters apoptotic sensitivity suggests that it may act not merely as a redox regulator, but also as a modulator of the hormonal signalling axis.

Fucoidan, a sulphated polysaccharide of natural origin, has gained increasing attention in cancer biology due to its antioxidant and antiproliferative properties (18,19). Previous studies have demonstrated that fucoidan can regulate ROS production and influence mitochondrial apoptotic signalling in various cancer cell lines (20-22). However, its interaction with ER-mediated hormonal signalling in ER-positive breast cancer cells, as well as its comparative impact on chemotherapy-induced mitochondrial stress, remains insufficiently characterised.

Growing evidence highlights the dynamic interplay between ER signaling pathways and oxidative stress as a key regulatory axis in breast cancer biology. Alterations in intracellular ROS levels have been reported to affect ERα stability and transcriptional activity, while ER signalling itself may modulate cellular antioxidant capacity (23). Therefore, elucidating how chemotherapy-induced mitochondrial stress is differentially regulated by distinct redox modulators is of particular relevance for both mechanistic understanding and therapeutic optimisation.

The present study aimed to investigate whether doxorubicin-induced mitochondrial oxidative stress and apoptotic responses in ER-positive MCF-7 breast cancer cells are differentially modulated by melatonin and fucoidan. To address this objective, the effects of targeted redox modulation on mitochondrial apoptotic signaling and ERα expression were investigated by integrating functional viability assessment with quantitative analysis of ROS production and apoptosis-related proteins (Bax, Bcl-2, and cleaved caspase-3).

MATERIALS AND METHODS

Cell culture

The ER-positive human breast adenocarcinoma cell line MCF-7 (ATCC® HTB-22™) was sourced from the American Type Culture Collection (ATCC, USA). Cells were cultured in high-glucose DMEM (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution (100 U/mL penicillin, 100 µg/mL streptomycin).

Cell cultures were maintained at 37°C under 5% CO_2 in a humidified incubator. Experimental procedures were performed using cells in exponential growth phase (passages 5-10). Prior to

use, cultures were screened for mycoplasma contamination and verified as negative.

Experimental groups and drug applications

Six experimental groups were established:
1. Control (vehicle control; 0.1% ethanol):
2. Doxorubicin (DOX, 1 μM)
3. Melatonin (1 mM)
4. Fucoidan (100 μg/mL)
5. DOX (1 μM) + Melatonin (1 mM)
6. DOX (1 μM) + Fucoidan (100 μg/mL)

Doxorubicin (DOX; Sigma-Aldrich, USA; Cat. No. D1515-10MG) concentration was selected based on previously reported 24-hour IC_{50} values for MCF-7 cells (approximately 1–2 μM) (24). Accordingly, cells were exposed to 1 μM DOX for 24 hours to establish a mitochondrial oxidative stress model. This concentration has been shown to induce significant ROS generation and activation of the intrinsic apoptotic pathway without complete loss of cell viability.

Melatonin (Sigma-Aldrich; Cat. No. 444300-1GM) stock solution was prepared in 100% ethanol and protected from light. Fucoidan (MedChemExpress, USA; Cat. No. HY-132179) stock solution was dissolved in sterile distilled water. Stock preparations were passed through sterile filters and preserved at -20°C until use. On the day of treatment, experimental concentrations were freshly diluted in complete culture medium. Ethanol content was kept below 0.1% across all experimental conditions, and vehicle-treated controls were exposed to the corresponding solvent volume.

Melatonin (1 mM) and fucoidan (100 μg/mL) concentrations were selected according to previously reported effective, non-cytotoxic dose ranges in MCF-7 cells (25,26). In combination groups, cells were co-treated with DOX and the respective agent simultaneously for 24 hours. All experimental procedures were conducted using at least three separate biological replicates to ensure reproducibility.

Assessment of cell viability (MTT assay)

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, USA) assay. For the MTT analysis, MCF-7 cells were seeded at 5×10^3 cells per well in 96-well plates and maintained for 24 h to allow proper adherence prior to treatment exposure. Subsequently, cells were subjected to the designated treatment conditions for 24 h. After the exposure period, MTT reagent was introduced into each well to obtain a final concentration of 0.5 mg/mL, followed by incubation at 37°C for 3 h. After incubation, culture medium was carefully aspirated and the formed formazan crystals were solubilized in 100 μL dimethyl sulfoxide (DMSO). Optical density was recorded at 570 nm using a microplate spectrophotometer, , with 630 nm applied as a reference wavelength to correct for background signal. Each condition was independently replicated three times at the biological level, with triplicate technical measurements performed per experiment. Data were normalized to untreated controls and presented as relative cell viability (%).

Determination of cellular ROS levels

ROS production within cells was determined via DCFH-DA fluorescence assay (Sigma-Aldrich), a widely applied method for oxidative stress detection (27). MCF-7 cells were seeded at a density of 5×10^3 cells per well in 96-well plates and maintained for 24 h to ensure proper adherence prior to treatment. At the end of treatment, cells were washed with PBS and exposed to 10 μM DCFH-DA diluted in serum-free DMEM for 30 min at 37°C in the absence of light. Excess probe was removed by washing with PBS. Fluorescent signal intensity was measured with a microplate reader at excitation and emission wavelengths of 485 nm and 535 nm, respectively. Background signal obtained from cell-free wells was subtracted from all measurements. ROS levels were expressed as relative fluorescence units (RFU) and calculated as fold change relative to untreated controls. Each experimental group was independently replicated three times at the biological level, with technical triplicates performed within each replicate.

Preparation of cell lysate

Following completion of the experimental treatments, cells were processed on ice. After removal of the culture medium, cells were washed twice with cold phosphate-buffered saline (PBS). Lysis was performed using ice-cold RIPA buffer (Thermo Fisher Scientific, USA) supplemented with a protease inhibitor cocktail. Briefly, 100-200 μL of lysis buffer was added to each well, and cells were scraped and transferred into microcentrifuge tubes. Lysates were kept at 4°C for 30 min with intermittent vortexing and subsequently centrifuged (12,000 × g, 15 min, 4°C) to remove insoluble debris. The resulting supernatants were carefully harvested and designated as total protein extracts. Protein concentration was quantified using the bicinchoninic acid (BCA) method (Thermo Fisher Scientific). Aliquots were preserved at −80°C pending subsequent analyses.

Quantitative analysis of cellular protein levels (ELISA)

Protein levels of Bax (Abcam, UK; Cat. No. ab199080), Bcl-2 (Abcam; Cat. No. ab119506), cleaved caspase-3 (Asp175) (Abcam; Cat. No. ab220655), and estrogen receptor alpha (ERα) (Thermo Fisher Scientific, USA; Cat. No. EEL146) were quantified in cell lysates using commercially available sandwich ELISA kits according to the manufacturers' protocols. Cell lysate samples were normalised to equal total protein concentrations prior to analysis. Standards provided with each kit were used to generate calibration curves. Appropriately diluted samples and standards were added to 96-well plates, and incubation, washing, and colour development steps were performed as specified

by the respective kit instructions. After development with tetramethylbenzidine (TMB) substrate, optical density was recorded at 570 nm using a microplate spectrophotometer, with 630 nm applied as a reference wavelength to correct for background signal. Each condition was independently replicated three times at the biological level, with triplicate technical measurements performed per experiment. Data were normalized to untreated controls and presented as relative cell viability (%).

Statistical analysis

All experimental conditions were evaluated in a minimum of three independent biological experiments, each including technical triplicates. Data are presented as mean ± standard deviation (SD). Normality of the data was assessed with the Shapiro–Wilk test, and equality of variances was evaluated using Levene's test. Intergroup differences were analyzed by one-way analysis of variance (ANOVA), with Tukey's multiple comparison test applied when appropriate. Statistical computations were conducted with GraphPad Prism (version 9.0; GraphPad Software, USA). A p-value < 0.05 was considered indicative of statistical significance.

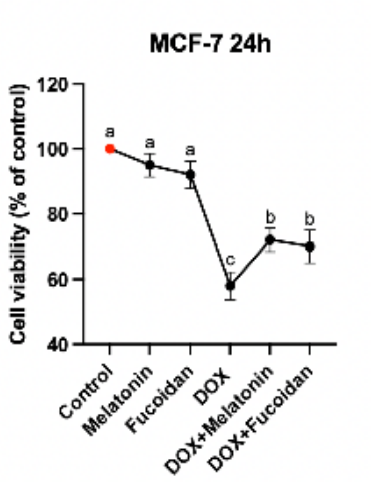
RESULTS

Cell viability

Exposure of MCF-7 cells to 1 µM DOX for 24 hours significantly reduced cell viability (Figure 1). No significant differences were observed between the vehicle control (0.1% ethanol) and untreated control groups ($p > 0.05$), indicating that the solvent used in the study did not affect cell viability.

Cells treated with DOX exhibited a marked reduction in viability (58 ± 6%) relative to the control group (100%), and this difference was statistically significant ($p < 0.001$). Treatment with melatonin (1 mM) or fucoidan (100 µg/mL) alone resulted in cell viabilities of 95 ± 5% and 92 ± 6%, respectively, with no statistically significant difference compared to the control group ($p = 0.312$ and $p = 0.184$, respectively). Combined treatment with melatonin elevated cell viability to 72 ± 5%, indicating a significant increase compared with the DOX-only group ($p = 0.004$); however, viability remained below control levels ($p = 0.018$). A comparable trend was observed in the DOX + fucoidan group, where viability reached 70 ± 7%. This value was significantly higher than DOX alone ($p = 0.006$) yet still lower than the untreated control group ($p = 0.025$). No significant difference was observed between the DOX + melatonin and DOX + fucoidan groups ($p = 0.417$).

Figure 1. Effects of melatonin and fucoidan on DOX-induced reduction in cell viability in MCF-7 cells.

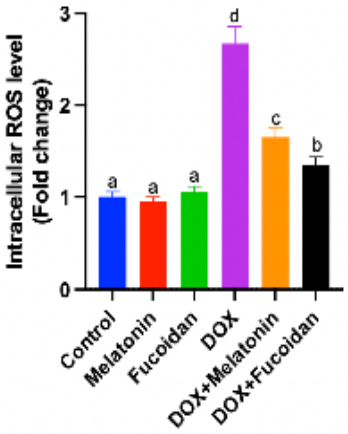


Intracellular ROS levels

Intracellular ROS levels were measured using DCFH-DA fluorescence following 24 h treatment. Data are presented as mean ± SD ($n = 3$). Different lowercase letters indicate significant differences between groups ($p < 0.05$). DOX, doxorubicin.

ROS production in the DOX group reached 2.65 ± 0.21-fold relative to control (1.00 ± 0.08) ($p < 0.001$). Melatonin (1 mM) and fucoidan (100 µg/mL) administered alone resulted in ROS levels of 0.95 ± 0.07-fold and 1.05 ± 0.09-fold, respectively, with no significant difference compared to the control group ($p = 0.428$ and $p = 0.517$, respectively). Co-treatment with melatonin reduced ROS levels to 1.65 ± 0.14-fold, representing a significant reduction relative to the DOX group ($p < 0.001$); however, values remained elevated relative to control ($p = 0.012$). Similarly, the DOX + fucoidan group exhibited ROS levels of 1.35 ± 0.12-fold, which were significantly lower than those of the DOX group ($p < 0.001$) and also significantly lower than the DOX + melatonin group ($p = 0.021$). Despite this reduction, ROS levels in the DOX + fucoidan group remained higher than control values ($p = 0.034$).

Figure 2. Effects of melatonin and fucoidan on DOX-induced intracellular ROS production in MCF-7 cells.



Apoptotic proteins and ERα levels

(A) Bax, (B) Bcl-2, (C) cleaved caspase-3, and (D) ERα protein levels were quantified by ELISA and normalized to total protein content (pg/mg protein). Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences between groups ($p < 0.05$). DOX, doxorubicin.

Bax levels were 94.37 ± 7.82 pg/mg protein in the control group. Treatment with melatonin alone (89.64 ± 6.71 pg/mg) or fucoidan alone (91.83 ± 8.56 pg/mg) did not result in significant differences relative to the control group ($p > 0.05$). In contrast, Bax levels increased statistically significant to 246.85 ± 17.63 pg/mg protein in the DOX group ($p < 0.001$ vs control). Co-treatment with DOX and melatonin reduced Bax levels to 164.42 ± 13.88 pg/mg ($p = 0.004$ vs DOX), while DOX + fucoidan decreased Bax levels to 139.76 ± 11.95 pg/mg ($p = 0.002$ vs DOX). However, both combination groups remained significantly higher than control ($p < 0.05$).

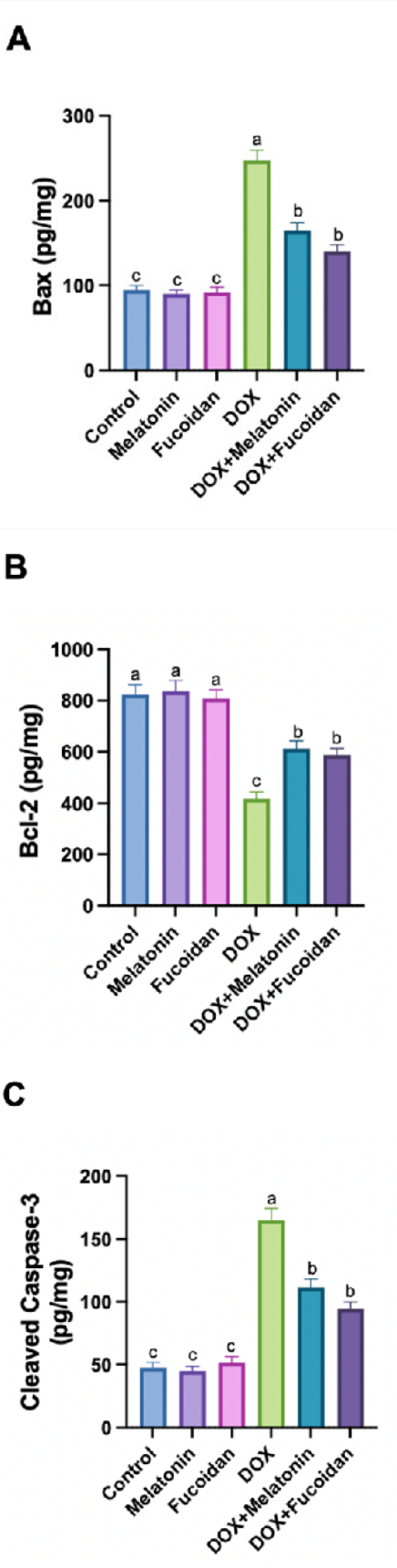
Bcl-2 levels in the control group were 823.46 ± 54.91 pg/mg protein. Melatonin (836.29 ± 61.37 pg/mg) and fucoidan (807.15 ± 49.63 pg/mg) alone did not significantly differ from control ($p > 0.05$). DOX treatment significantly reduced Bcl-2 levels to 418.92 ± 36.74 pg/mg protein ($p < 0.001$ vs control). In the DOX + melatonin and DOX + fucoidan groups, Bcl-2 levels increased to 612.57 ± 43.26 pg/mg ($p = 0.006$ vs DOX) and 587.84 ± 39.58 pg/mg ($p = 0.009$ vs DOX), respectively, but remained significantly lower than control ($p < 0.05$).

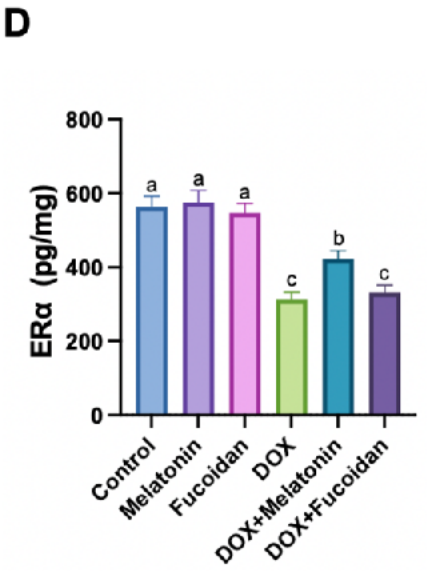
Consistent with these findings, the Bax/Bcl-2 ratio was markedly increased in the DOX-treated group, indicating a shift toward a pro-apoptotic state. Co-treatment with melatonin or fucoidan partially reduced this ratio; however, values remained elevated compared to the control group, supporting the persistence of apoptotic signaling despite antioxidant intervention.

Cleaved caspase-3 levels were 47.58 ± 5.94 pg/mg protein in the control group and were not significantly altered by melatonin (44.92 ± 5.18 pg/mg) or fucoidan (51.36 ± 6.84 pg/mg) alone ($p > 0.05$ vs control). DOX treatment significantly increased cleaved caspase-3 levels to 164.71 ± 13.52 pg/mg protein ($p < 0.001$ vs control). Co-treatment with melatonin or fucoidan partially attenuated this increase, with levels of 111.63 ± 9.27 pg/mg ($p = 0.005$ vs DOX) and 94.28 ± 7.96 pg/mg ($p = 0.003$ vs DOX), respectively. Nevertheless, both values remained significantly higher than control ($p < 0.05$).

ERα levels were 562.73 ± 41.68 pg/mg protein in the control group. Melatonin (574.86 ± 47.25 pg/mg) and fucoidan (547.92 ± 35.74 pg/mg) alone did not significantly affect ERα levels compared to control ($p > 0.05$). DOX significantly reduced ERαlevels to 312.48 ± 27.95 pg/mg protein ($p < 0.001$ vs control). ERα expression in the DOX + melatonin group increased to 421.94 ± 32.87 pg/mg, demonstrating a significant elevation compared with DOX alone ($p = 0.008$); however, levels did not fully recover to those observed in control cells ($p < 0.05$). In contrast, ERα concentrations in the DOX + fucoidan group (331.67 ± 28.41 pg/mg) were comparable to the DOX group, with no statistically meaningful difference detected ($p = 0.214$).

Figure 3. Effects of melatonin and fucoidan on mitochondrial apoptotic proteins and ERα levels in DOX-treated MCF-7 cells.





DISCUSSION

The present study demonstrates that doxorubicin (DOX)-induced mitochondrial oxidative stress and apoptotic signalling in ER-positive MCF-7 breast cancer cells are differentially modulated by melatonin and fucoidan. DOX markedly increased intracellular ROS production, accompanied by upregulation of Bax, downregulation of Bcl-2, and activation of cleaved caspase-3, collectively indicating activation of the intrinsic apoptotic pathway. These findings are consistent with previous reports suggesting that the antitumour activity of DOX is mediated not only through topoisomerase II inhibition but also through mitochondrial redox imbalance (28,29).

DOX undergoes intracellular redox cycling, leading to enhanced generation of superoxide anions and hydrogen peroxide, thereby imposing oxidative stress particularly at the level of the mitochondrial electron transport chain. Elevated ROS levels promote cardiolipin oxidation, increased mitochondrial membrane permeability, and subsequent cytochrome c release (30). In this context, translocation of Bax to the mitochondrial membrane represents a pivotal event, while reduced Bcl-2 expression further facilitates mitochondrial permeabilisation. The increased Bax/Bcl-2 ratio together with caspase-3 activation observed in this study strongly supports the engagement of the intrinsic apoptotic pathway following DOX exposure.

The observed alterations in Bax and Bcl-2 levels also imply a substantial increase in the Bax/Bcl-2 ratio, a widely recognized indicator of mitochondrial apoptotic commitment. The partial normalization of this ratio in the combination treatment groups suggests that both melatonin and fucoidan attenuate, but do not fully reverse, the pro-apoptotic shift induced by doxorubicin. This further supports the notion that redox modulation can influ ence the intensity of apoptotic signaling without completely abolishing it.

When antioxidant modulation was evaluated, both melatonin and fucoidan significantly attenuated DOX-induced ROS elevation. Notably, fucoidan exerted a more pronounced suppressive effect on ROS levels compared to melatonin. Previous studies have attributed the antioxidant capacity of fucoidan to its sulphated structure and direct free radical scavenging properties, which enable reduction of lipid peroxidation and stabilisation of mitochondrial redox balance (31). In addition, fucoidan has been shown to enhance endogenous antioxidant defence mechanisms, partly through activation of the Nrf2 signalling pathway (32,33). Collectively, these findings suggest that fucoidan primarily acts through direct and pathway-mediated redox regulation.

In addition to its antioxidant properties, the more pronounced ROS-suppressive effect of fucoidan may indicate a stronger influence on upstream redox-regulating pathways. Previous studies have suggested that fucoidan can activate Nrf2-dependent antioxidant responses and modulate intracellular signaling cascades such as PI3K/AKT and MAPK pathways. Although these mechanisms were not directly examined in the present study, the observed differences between melatonin and fucoidan raise the possibility that distinct upstream signaling events contribute to their differential effects on mitochondrial stress responses.

Melatonin, in contrast, functions not only as a potent radical scavenger but also as a regulator of mitochondrial physiology. It has been reported to localise within mitochondria and modulate electron transport chain activity (34). Moreover, melatonin can upregulate antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (35). In the present study, although melatonin significantly reduced ROS levels, it did not fully normalise apoptotic markers. This observation indicates that attenuation of oxidative stress alone may be insufficient to completely abrogate the apoptotic cascade, and suggests that melatonin may temper the intensity of apoptosis rather than entirely preventing chemotherapy-induced cell death. This finding is in line with previous reports indicating that reduction of ROS levels does not necessarily abolish apoptosis, as multiple parallel signaling pathways contribute to chemotherapy-induced cell death. For instance, studies have shown that even in the presence of antioxidant treatment, mitochondrial apoptotic signaling can persist due to irreversible damage to mitochondrial components or activation of alternative death pathways. Therefore, the partial attenuation observed in the present study supports the concept that redox modulation influences, but does not fully override, cytotoxic signaling.

A particularly important finding of the present study was the alteration in ERα protein levels. The marked reduction in ERα expression following DOX exposure supports the existence of a functional interplay between oxidative stress and hormonal signalling. Increased ROS levels have been reported to destabilise ERα and enhance its proteasomal degradation (36). Moreover, oxidative stress can suppress ER-mediated transcriptional acti-

vity (37), further linking redox imbalance to disruption of the hormonal axis.

The partial restoration of ERα levels in the DOX + melatonin group indicates that melatonin may exert modulatory effects beyond redox attenuation. Previous studies have shown that melatonin suppresses oestrogen-driven proliferative signalling in ER-positive breast cancer cells, regulates ERα expression, and modulates aromatase activity (38). These observations suggest that melatonin acts not only as a redox regulator but also as a functional modulator of ER-related signalling pathways. In contrast, the absence of a significant restorative effect of fucoidan on ERα levels implies that its action is predominantly confined to the oxidative stress–mitochondrial apoptotic axis rather than direct hormonal modulation.

The bidirectional crosstalk between ER signalling and redox homeostasis represents a critical component of breast cancer biology. ERα activation has been shown to regulate cellular antioxidant defence systems, while oxidative stress can reciprocally influence ER signalling dynamics (39). Within this framework, the partial recovery of ERα expression observed with melatonin suggests an interaction between redox modulation and hormonal signalling networks. Nevertheless, further mechanistic studies examining pathways such as PI3K/AKT, MAPK, and proteasomal degradation are required to delineate the precise molecular basis of this interaction.

A critical observation of the present study is that antioxidant co-treatment did not abolish the cytotoxic effects of DOX. Although partial recovery in cell viability was observed, apoptotic markers did not return to baseline levels, indicating that chemotherapy-induced cell death was attenuated but not eliminated. Previous studies have highlighted that the combination of antioxidants with chemotherapeutic agents may exert context-dependent effects, and excessive suppression of redox signalling does not necessarily confer therapeutic benefit (40). These findings support the concept that redox modulation can fine-tune the cellular response to chemotherapy rather than completely overriding cytotoxic mechanisms.

A key contribution of the present study is the direct comparison of two mechanistically distinct redox modulators under identical experimental conditions in an ER-positive breast cancer model. While both melatonin and fucoidan have been individually investigated in cancer contexts, comparative analyses examining their differential effects on mitochondrial oxidative stress, apoptosis, and ER signaling in the setting of chemotherapy are limited. The present findings therefore provide novel insight into the context-dependent and mechanism-specific roles of redoxmodulating agents in shaping chemotherapy responses.

Despite its strengths, the present study is not without limitations. The use of a single ER-positive breast cancer cell line (MCF-7) limits the generalizability of the findings, and whether similar responses occur across other molecular subtypes remains to be determined. In addition, mechanistic insights were restricted, as parameters such as mitochondrial membrane potential, cytochrome c release, and upstream kinase signaling pathways were not evaluated. The exclusive focus on a 24-hour time point further limits interpretation of potential time-dependent molecular dynamics.

Another important limitation is the use of a single concentration for both melatonin and fucoidan. Although these doses were selected based on previously reported effective and non-cytotoxic ranges in MCF-7 cells, the absence of a dose–response analysis restricts comprehensive evaluation of their concentration-dependent effects. Future studies incorporating multiple concentrations and time-course analyses would provide a more detailed understanding of the pharmacological and mechanistic profiles of these agents.

Nevertheless, the comparative design of the present study enabled direct evaluation of two mechanistically distinct redox modulators under identical experimental conditions, providing valuable insight into their differential roles in chemotherapy-associated cellular stress responses.

Overall, the present findings demonstrate that DOX-induced mitochondrial oxidative stress and apoptotic signalling in ER-positive breast cancer cells are differentially modulated by melatonin and fucoidan. While melatonin exerts partial restorative effects on ERα expression in addition to redox attenuation, fucoidan produces a more pronounced antioxidant response without significant hormonal modulation. These results underscore the importance of evaluating redox-regulating agents in chemotherapy not merely on the basis of their antioxidant capacity, but according to their mechanistic specificity and context-dependent biological actions.

CONCLUSION

This study demonstrates that DOX-induced mitochondrial oxidative stress and intrinsic apoptotic signalling in ER-positive MCF-7 breast cancer cells are differentially modulated by melatonin and fucoidan. DOX exposure triggered a mitochondrial stress response characterised by elevated ROS levels, a pro-apoptotic shift in the Bax/Bcl-2 ratio, and activation of cleaved caspase-3.

Although both melatonin and fucoidan significantly attenuated DOX-associated oxidative stress, neither agent completely abrogated the apoptotic cascade. Fucoidan exerted a more pronounced ROS-suppressive effect, whereas melatonin additionally promoted partial restoration of ERα expression. These findings indicate that melatonin may exert broader biological effects extending beyond redox regulation to include modulation of the hormonal signalling axis.

In conclusion, redox modulation can fine-tune chemotherapy-induced mitochondrial stress responses without fully suppressing cytotoxicity, and distinct antioxidant agents exert context-dependent effects through mechanistically diverse pathways. These results highlight the importance of mechanism-oriented and context-specific evaluation of redox-targeting strategies in combination with chemotherapy.

Ethics Committee Approval: Ethics committee approval was not required for this study, as all experiments were performed solely on commercially available human cell line and did not involve human subjects or animal experimentation.

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