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SYNERGISTIC APOPTOTIC EFFECTS OF CISPLATIN AND ARBUTIN IN MCF-7 BREAST CANCER CELLS

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Abstract

Objective: Cisplatin is a key component in cancer treatment, but its effectiveness can be limited by dose-related toxicities. Combining it with natural compounds such as arbutin offers a promising approach to improve treatment outcomes while reducing side effects. This study aimed to explore the combined apoptotic effects of arbutin and cisplatin in MCF-7 breast cancer cells, specifically focusing on mitochondrial gene expression.

Methods: MCF-7 cells were treated for 48 hours with arbutin, cisplatin, or a combination of both at fixed ratios. Cytotoxicity and synergy were evaluated using the Chou-Talalay median-effect method. Nuclear morphology, indicative of apoptosis, was assessed through Hoechst 33342 staining. Gene expression analysis targeted mitochondrial dynamics (*DRP1*, *Fis1*, *MFN1*, *MFN2*), oxidative stress markers (*SOD2*, *GPx*), apoptosis indicators (*Bcl2*), autophagy (*Beclin1*), and prostaglandin pathways (*PGF2α*, *PGF2β*), with results normalized to β-actin.

Results: The combination therapy significantly enhanced cytotoxicity compared to individual treatments (Combination Index <1). Hoechst staining revealed increased nuclear condensation and fragmentation, clear indicators of apoptosis. Among the genes analyzed, only PGF2β showed a significant downregulation in cells treated with the combination (p<0.05). Trends indicated elevated levels of DRP1 and Fis1, while MFN1 and MFN2 levels were decreased, suggesting a shift towards mitochondrial fragmentation, despite the results not reaching statistical significance.

Conclusion: The combination of arbutin and cisplatin promotes apoptosis in MCF-7 cells, potentially due to changes in mitochondrial dynamics. These findings indicate that arbutin may enhance the efficacy of cisplatin, potentially allowing for reduced cisplatin doses and a lower risk of side effects.

Keywords*: Cisplatin, arbutin, MCF-7, apoptosis, mitochondria*.

Introduction

Cancer encompasses a complex array of diseases marked by abnormal cell growth and proliferation, significantly impacting global health and mortality rates. Current treatment options, including chemotherapy, surgery, and radiotherapy, are commonly employed; however, these methods often result in severe side effects and can lead to treatment resistance over time.[1,2] Therefore, there is an urgent need for innovative treatment strategies that are more effective, less toxic, and reduce the risk of resistance. In this regard, research into the combination of natural compounds with chemotherapeutic agents is gaining importance.[3,4] This study explores the anticancer effects and potential combination strategy involving arbutin, a natural compound derived from hydroquinone, and cisplatin, a well-established chemotherapeutic agent.

Arbutin is a compound that consists of hydroquinone and a glucose molecule. It is available in two isomeric forms, α- and β-arbutin, which have different configurations of the glycosidic bond between hydroquinone and glucose.[5,6] Arbutin has garnered considerable scientific attention for its antioxidative, anti-inflammatory, antimicrobial, and more recently, anticancer properties.[5,7] Its most recognized function is the inhibition of tyrosinase, which is why it is commonly used in skin-whitening products.[6] Notably, α-arbutin demonstrates approximately ten times stronger tyrosinase inhibition compared to β-arbutin, making it the preferred choice in the cosmetic industry.[6]

Natural compounds are increasingly studied for their ability to induce apoptosis and modulate key signaling pathways in cancer.[7] The anticancer properties of arbutin are mainly attributed to its ability to modulate intracellular signaling pathways, rather than directly interacting with DNA.[5,9] It can inhibit cancer cell growth by decreasing oxidative stress, stopping the cell cycle, and triggering apoptosis.[5,10,11] In vitro studies have indicated that arbutin reduces cell proliferation and increases the expression of apoptotic genes in breast, colon, and liver cancer cell lines.[5,10,12] Despite these encouraging results, the specific mechanisms behind arbutin's anticancer effects are not yet completely understood and are primarily observed in in vitro studies. Given its low cytotoxicity, arbutin is considered as a promising complementary agent in cancer treatment.[5,10] Further in vivo studies and clinical trials are necessary to confirm its clinical efficacy.

Cisplatin, discovered in the 1960s, is a platinum-based chemotherapy drug that has significantly changed cancer treatment.[13] Cisplatin induces apoptosis by creating DNA cross-links that inhibit replication and transcription. It continues to be a standard chemotherapeutic agent for various solid tumors, including testicular, ovarian, bladder, lung, and head and neck cancers.[13] However, the clinical use of cisplatin is constrained by considerable toxicities, such as nephrotoxicity, ototoxicity, gastrointestinal distress, and myelosuppression.[13,14] Supportive strategies like hydration, antiemetics, and nephroprotective agents are employed to mitigate these side effects.[14] Furthermore, long-term use can lead to drug resistance, presenting a significant therapeutic challenge. Therefore, there is an urgent need for new strategies to enhance the efficacy of cisplatin and minimize its adverse effects.

Combination therapies are a fundamental aspect of modern oncology, designed to create synergistic effects by targeting multiple molecular pathways. This strategy improves efficacy, lowers drug dosages and side effects, and helps delay resistance.[3] Combining cisplatin with natural bioactive compounds has emerged as a promising research area.[3,4] Among these natural compounds, polyphenols like curcumin, resveratrol, epigallocatechin gallate (EGCG), and quercetin have shown considerable potential.[3,4,15,16] These compounds can enhance the antitumor effects of cisplatin by modulating oxidative stress, regulating the cell cycle, and promoting apoptosis. For instance, curcumin has been observed to reduce nephrotoxicity associated with cisplatin while boosting its tumor cytotoxicity.[4]

Although there is limited research specifically on the combination of arbutin and cisplatin, the findings in literature suggest the potential of the combination therapy in cancer treatment. Arbutin's antioxidant properties, low toxicity, and ability to suppress growth may enhance the cytotoxic effects of cisplatin.[15,16] Additionally, arbutin may help reduce organ damage caused by cisplatin by counteracting oxidative stress, thereby improving treatment tolerability.[16,17] This study was conducted to explore the anticancer effects and potential combination strategy involving arbutin and cisplatin, where the findings revealed a promising anti-cancer effect. Future research should aim to optimize dose ratios, treatment timing, molecular mechanisms, and the in vivo effectiveness of the arbutin–cisplatin combination. This approach could provide a promising therapeutic alternative, especially within personalized treatment strategies that integrate complementary medicine with standard oncology practices.

This study aims to clarify the molecular mechanisms behind the cytotoxic and synergistic effects of arbutin and cisplatin by examining the expression profiles of genes involved in various cellular pathways. We first determined the IC50 values for both compounds and analyzed their interactions in MCF-7 cells using the Chou-Talalay method. Following the confirmation of the synergistic effect, we evaluated the expression levels of genes related to apoptosis, autophagy, mitochondrial dynamics, and oxidative stress. Bcl-2 and Beclin-1 were chosen as markers for apoptosis and autophagy, respectively, due to their crucial roles in cell survival and programmed cell death. To assess mitochondrial health, we examined PGC-1α and PGC-1β, which are transcriptional coactivators essential for mitochondrial biogenesis and energy metabolism. Additionally, we analyzed the genes Drp1, Fis1, Mfn1, and Mfn2, which are key regulators of mitochondrial fission and fusion, considering their significant role in the life-death balance of cancer cells. We also included antioxidant defense genes Sod2 and Gpx1, which indicate the cellular response to oxidative stress, a critical factor in cisplatin-induced toxicity. Furthermore, we investigated potential apoptotic activity across groups through nuclear morphology analysis using Hoechst 33342 staining. These findings provide valuable insights into how arbutin and cisplatin, whether individually or in combination, regulate cellular processes. mitochondrial function, oxidative stress and cell fate in cancer cells.

Methods

Cell Culture

MCF-7 cells (ATCC, HTB-22) were maintained following the supplier's guidelines. The cells were initially seeded into 75 cm² culture flasks (Corning) and passaged at a 1:3 ratio once they reached 80–90% confluence. During the experiments, the cells were cultured in complete DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS) and standard concentrations of glutamine, penicillin, and streptomycin.

Cytotoxicity Assay, IC_{50}/ED_{50} Determination, and Combination Index Analysis

We sourced β-Arbutin (Catalog No. S2263) from Selleckchem (Houston, TX, USA) and cisplatin from Sigma-Aldrich (Darmstadt, Germany). A 5 mM working stock solution of cisplatin was prepared by diluting a 250 mM DMSO stock, resulting in a final DMSO concentration of 2%. Although this working stock contained 2% DMSO, the highest final concentration of DMSO in the treatment groups (corresponding to 100 µM cisplatin) was only 0.04%, a level considered non-cytotoxic for MCF-7 cells. Meanwhile, β-arbutin was dissolved directly in complete culture medium to prepare a 500 mM stock solution.

We assessed the cytotoxic effects of each compound using the MTT assay (Sigma-Aldrich, Cat. No. 5015944001) following the manufacturer's instructions. MCF-7 cells were seeded in 96-well plates at a density of 2×10^4 cells per well and incubated overnight for attachment. The cells were then exposed to serial dilutions of β-arbutin (from 500 to 1.8 mM) and cisplatin (from 100 to 0.195 µM) for 24 and 48 hours. After treatment, MTT reagent was added to each well and incubated for 1 hour. Absorbance was measured at 450 nm with a reference at 620 nm using a microplate reader (Thermo Scientific, Multiskan FC, Finland). Cell viability was calculated relative to untreated controls and expressed as a percentage. IC_{50} and ED_{50} values were determined using CalcuSyn software (Biosoft, Cambridge, UK). In combination studies, we utilized fixed-ratio dosing based on concentrations derived from each compound's IC_{50}. Combination Index (CI) values were calculated using the Chou–Talalay method with the same software, where CI <1 indicates synergy, CI=1 represents additive effects, and CI >1 suggests antagonism.

Nuclear Morphology Assessment via Hoechst 33342 Staining

To assess nuclear morphology and detect apoptotic changes, MCF-7 cells were incubated with Hoechst 33342 (Thermo Fisher Scientific, Cat. No. H3570, Waltham, MA, USA), a DNA-specific fluorescent dye that readily permeates live cells. After 48 hours of treatment with β-arbutin, cisplatin, or their combination, cells were gently rinsed twice with phosphate-buffered saline (PBS) to eliminate residual culture medium. Staining was performed by adding Hoechst 33342 at a final concentration of 5 µg/mL in PBS, followed by a 15-minute incubation at 37 °C in the dark. Subsequently, excess dye was removed by additional PBS washing. Fluorescent images were acquired using a Zeiss Axio inverted fluorescence microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with a DAPI filter set. Nuclei exhibiting condensed, brightly stained, or fragmented structures were identified as apoptotic, while cells with round, evenly stained nuclei were considered viable.

RT-PCR

MCF-7 cells were treated with β-arbutin, cisplatin, and their combination at determined concentrations. Total RNA was isolated from all cell groups using the Trizol method, following the manufacturer's instructions. RNA purity and concentration were assessed using a Nabi Spectrophotometer (MicroDigital Co., Ltd., South Korea). Complementary DNA (cDNA) synthesis was performed from total RNA using the OneScript® cDNA Synthesis Kit (ABM, Canada) according to the manufacturer's protocol. Quantitative PCR was carried out using SYBR™ Green Universal Master Mix (Applied Biosystems, Thermo Fisher Scientific, USA) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). Thermal cycling conditions consisted of an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 55–60°C for 30 seconds (annealing), and 72°C for 30 seconds (extension). A final extension was performed at 72°C for 5 minutes. Melting curve analysis was conducted from 60°C to 95°C with a 0.1°C/s ramp to confirm product specificity. Gene expression levels were analyzed using the relative quantification method, and statistical significance was determined by one-way ANOVA; p-values less than 0.05 were considered statistically significant. The primer sequences used were as follows: For *MFN1*, the forward primer was 5′-CCTGTTTCTCCACTGAAGCAC-3′ and the reverse was 5′-CCTCACCAATGATGGAAAGC-3′; *MFN2* was amplified using forward 5′-ACACATGGCTGAGGTGAATG-3′ and reverse 5′-CGTCCAGAACCTGTTCTTCTG-3′; *BCL2* primers were forward 5′-CATGTGTGTGGAGAGCGTCAA-3′ and reverse 5′-GCCGGTTCAGGTACTCAGTCA-3′; *Beclin1* used forward 5′-TCGCCCTTTTCTACTTTGCC-3′ and reverse 5′-AGTCTCACCCAACCACCCT-3′. *PGF2α* primers were forward 5′-GGATTGTGCCTGACATTGTG-3′ and reverse 5′-AAGGCTTTCAACAATCTTGTCA-3′, while *PGF2β* used the same pair as *BCL2*. *DRP1* primers were 5′-CAGTGTGCCAAAGGCAGTAA-3′ (forward) and 5′-GATGAGTCTCCCGGATTTCA-3′ (reverse); *FIS1* primers were 5′-CTTGCTGTGTCCAAGTCCAA-3′ (forward) and 5′-GCTGAAGGACGAATCTCAGG-3′ (reverse); *SOD2* was amplified with 5′-AAGGGAGATGTTACAGCCCAGATA-3′ (forward) and 5′-TCCAGAAAATGCTATGATT-3′ (reverse); *GPX1* with 5′-GGGACTACACCCAGATGAA-3′ (forward) and 5′-TCTCTTCGTTCTTGGCGTTC-3′ (reverse). Finally, *β-actin* was used as an internal control using forward primer 5′-AACTGGGACGACATGGAGAA-3′ and reverse primer 5′-GAAGGTCTCAAACATGATCTGG-3′.

Statistical Analysis

MTT assay experiments were performed in triplicate, and results are expressed as mean±standard error of the mean (SEM). Each treatment group in the qPCR analysis was assessed in duplicate. Statistical evaluation was conducted using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test to compare treated groups against control. A p-value below 0.05 was considered statistically significant. Drug interactions and synergistic effects were analyzed with the Chou–Talalay method, and both combination index (CI) and dose reduction index (DRI) values were computed using CompuSyn software (Biosoft, Cambridge, UK).

Results

Determination of Toxicity of Cisplatin and Arbutin and IC_{50} and ED50 Values

Arbutin and cisplatin treatments induced time- and concentration-dependent changes in MCF-7 cell viability, as assessed by one-way ANOVA followed by Dunnett's post hoc analysis with p-value adjustment. For arbutin, at 24 hours, no significant differences were observed at concentrations of 1.8–62.5 mM (p>0.05). Significant reductions in viability were detected at 125 mM (p=0.0321), 250 mM (p=0.0001), and 500 mM (p<0.0001) (top left panel of Figure 1). At 48 hours, statistically significant decreases began at 3.90 mM (p<0.05) and continued at all higher concentrations, with p-values <0.0001 for 15.62 mM and

above (top right panel of Figure 1). For cisplatin, after 24 hours, significant effects started at 3.12 µM (p<0.05), with further reductions at 6.25 µM, 12.5 µM, 25 µM, 50 µM, and 100 µM (all p<0.0001) (bottom left panel of Figure 1). At 48 hours, statistically significant reductions in cell viability were observed across all concentrations starting from 0.195 µM (p<0.05) (bottom right panel of Figure 1). These results confirm a dose- and time-dependent response for both compounds, with cisplatin exhibiting stronger cytotoxicity than arbutin, particularly at lower doses and longer exposure times.

The IC_{50} value for cisplatin was determined to be 13.16 µM after 24 hours and 3.77 µM after 48 hours of treatment. In comparison, arbutin showed IC_{50} values of 105.36 mM and 47.11 mM at the same time points. These values were calculated using the Chou–Talalay method, which accurately quantifies drug potency based on dose–response curves. The 48-hour time point was chosen to evaluate both time-dependent cytotoxic effects and cumulative changes in mitochondrial dynamics, given their critical role in apoptosis and response to chemotherapy. Since mitochondria mediate cisplatin-induced cytotoxicity and arbutin may affect mitochondrial function through antioxidant mechanisms, longer exposure durations were necessary to fully observe their biological impact.

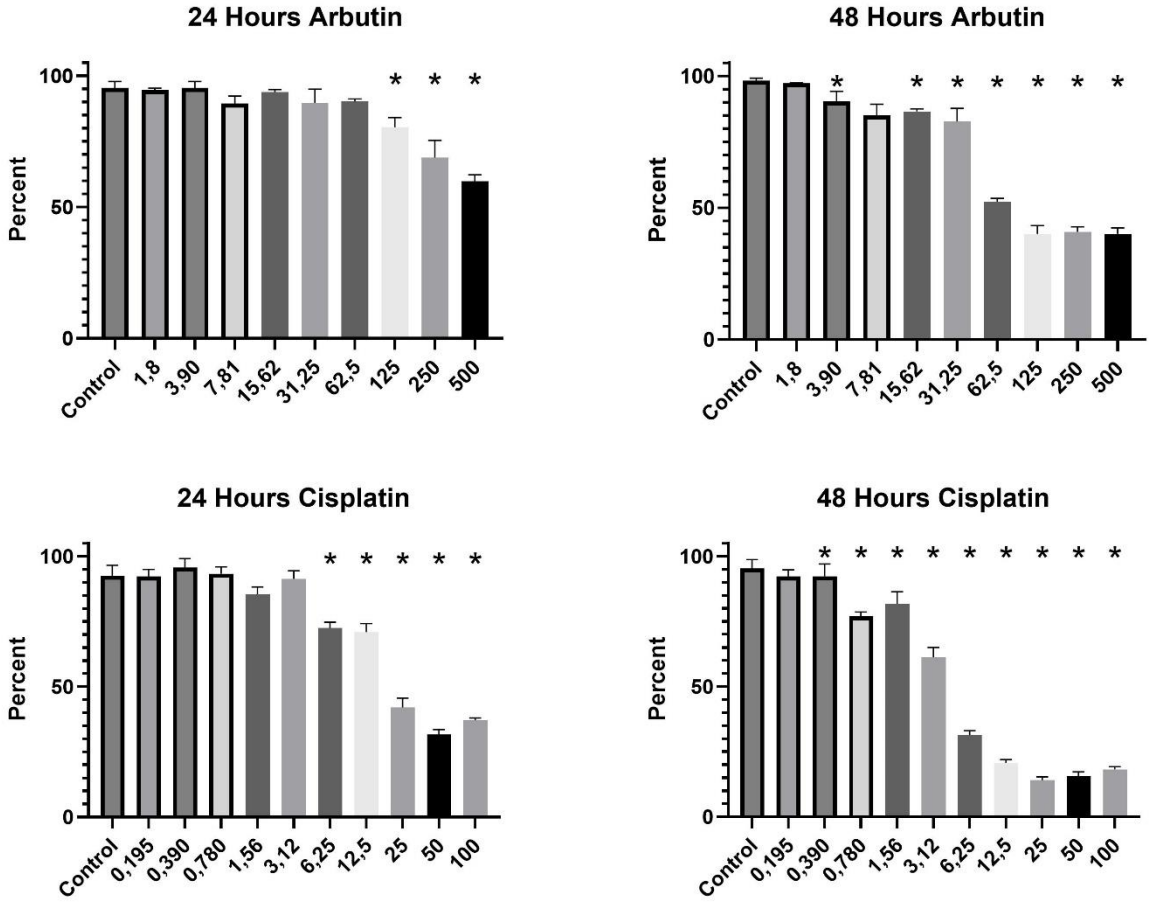

Figure 1. Cytotoxic effects of β-arbutin and cisplatin on MCF-7 cells at 24 and 48 hours. MCF-7 cells were treated with increasing concentrations of β-arbutin (1.8–500 mM) and cisplatin (0.195–100 µM). Cell viability was assessed by MTT assay, and absorbance was measured at 450 nm. Data represent mean ± SD (n=3).

β-Arbutin, Cisplatin, or their combination inhibits the proliferation of MCF-7 cells

For the combination assay, both compounds were tested at concentrations starting from twice their 48-hour IC_{50} values and diluted stepwise by 20%. Arbutin was used at 94.22, 75.37, 45.22, 18.09, and 3.61 mM, while cisplatin was tested at 7.54, 6.032, 3.61, 1.44, and 0.28 µM. In the combination groups, the agents were administered simultaneously at corresponding percentage concentrations. The cytotoxic interaction between arbutin and cisplatin was evaluated using the Chou–Talalay method through fixed-ratio combination analysis (12496:1, arbutin:cisplatin) over a 48-hour treatment period. The IC_{50} values for single-agent treatments were calculated as 47.11 mM for arbutin and 3.77 µM for cisplatin. Based on the median-effect analysis, arbutin alone exhibited a Dm of 137.17 mM, with a shallow dose–response curve ($m = 0.47$, $r = 0.95$), while cisplatin showed higher potency with a Dm of 6.77 mM ($m = 0.94$, $r = 0.97$) (Figure 2, middle). In the fixed-ratio combination assay, the calculated Dm values were 31.32 mM for arbutin and 2.51 mM for cisplatin, indicating a reduction in the effective dose of cisplatin required to reach 50% inhibition (Figure 2, left). The combination also displayed an improved dose–response slope ($m = 0.79$, $r = 0.96$), suggesting enhanced cooperative activity (Figure 2, right).

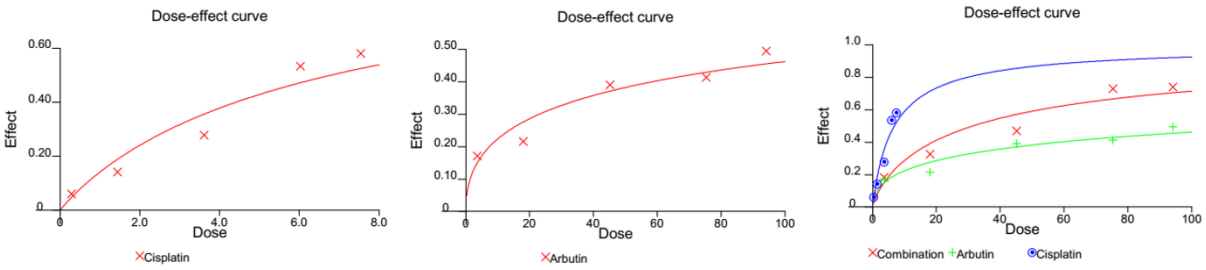

Figure 2. Dose–response curves of arbutin, cisplatin, and their combination in MCF-7 cells. Cisplatin (left) and arbutin (middle) showed dose-dependent cytotoxicity. The fixed-ratio combination (right, 12496:1 arbutin:cisplatin) enhanced the cytotoxic effect compared to either agent alone, indicating synergism.

Quantitative Gene Expression Analysis of Treated MCF-7 Cells

In this experiment, gene expression levels were evaluated across four treatment groups: control, arbutin (47.11 mM), cisplatin (3.77 µM), and a combination group receiving 2.51 µM cisplatin with 31.32 mM arbutin. Quantitative PCR analysis revealed a significant increase in PGF2β expression in the arbutin-treated group (2.015 ± 0.1061) compared to cisplatin (0.5250 ± 0.4455, p=0.0159) and the combination treatment (0.9250 ± 0.0919, p=0.0461). No statistically significant differences were observed for other genes. Bcl-2 expression ranged from 0.7895 ± 0.0439 (cisplatin) to 1.141 ± 0.0149 (combination), with control at 1.002 ± 0.064. Beclin-1 showed slight variation, with values between 0.815 ± 0.175 (cisplatin) and 1.345 ± 0.185 (arbutin). PGF2α expression was highest with arbutin (2.102 ± 0.4697) and lowest with cisplatin (0.8951 ± 0.0351). MFN1 expression notably increased in the combination group (3.34 ± 1.91), while other groups remained near control (1.005 ± 0.055). MFN2 ranged from 1.000 ± 0.0062 (control) to 1.273 ± 0.406 (combination). Fis1 and DRP1, both involved in mitochondrial fission, showed elevated expression in the combination (1.995 ± 0.7566) and arbutin (2.465 ± 1.068) groups, respectively. SOD2 and GPx levels were moderately variable, with SOD2 ranging from 0.7600 ± 0.1414 (arbutin) to 1.090 ± 0.1838 (cisplatin), and GPx from 0.9000 ± 0.7212 (arbutin) to 1.095 ± 0.4313 (cisplatin) (Figure 3). Despite numerical differences, these changes were not statistically significant.

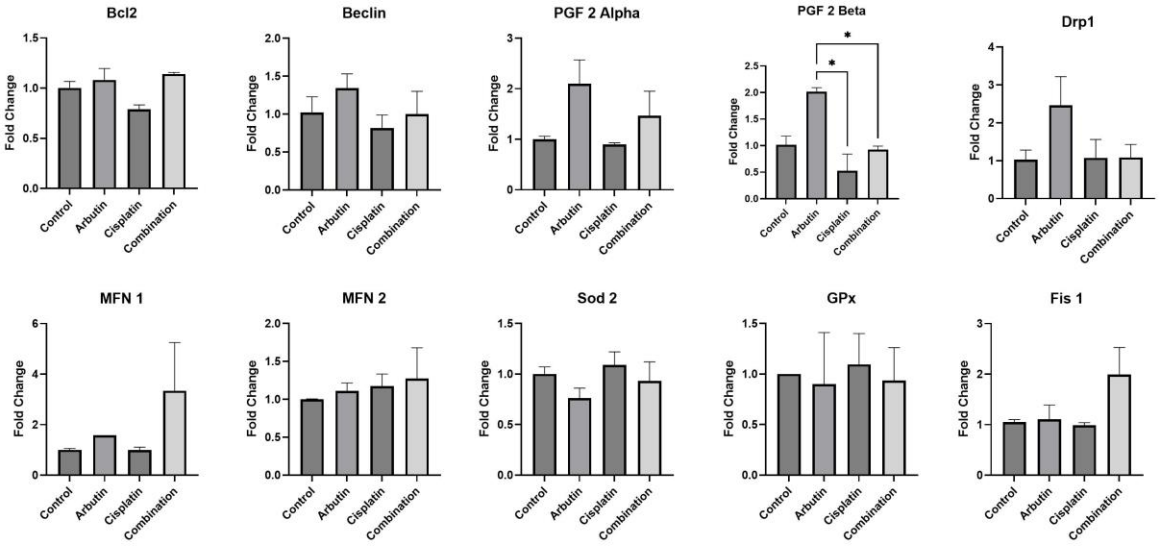

Figure 3. Relative gene expression levels in MCF-7 cells after 48-hour treatments with arbutin (47.11 mM), cisplatin (3.77 µM), and their combination (31.32 mM arbutin + 2.51 µM cisplatin). Quantitative PCR was used to assess the fold changes in genes related to apoptosis (Bcl-2), autophagy (Beclin-1), prostaglandin signaling (PGF2α, PGF2β), mitochondrial dynamics (Drp1, MFN1, MFN2, Fis1), and oxidative stress (SOD2, GPx). Data are presented as mean±SEM. A statistically significant increase in PGF2β expression was observed in the arbutin group compared to cisplatin and combination treatments (p<0.05). No other comparisons reached statistical significance.

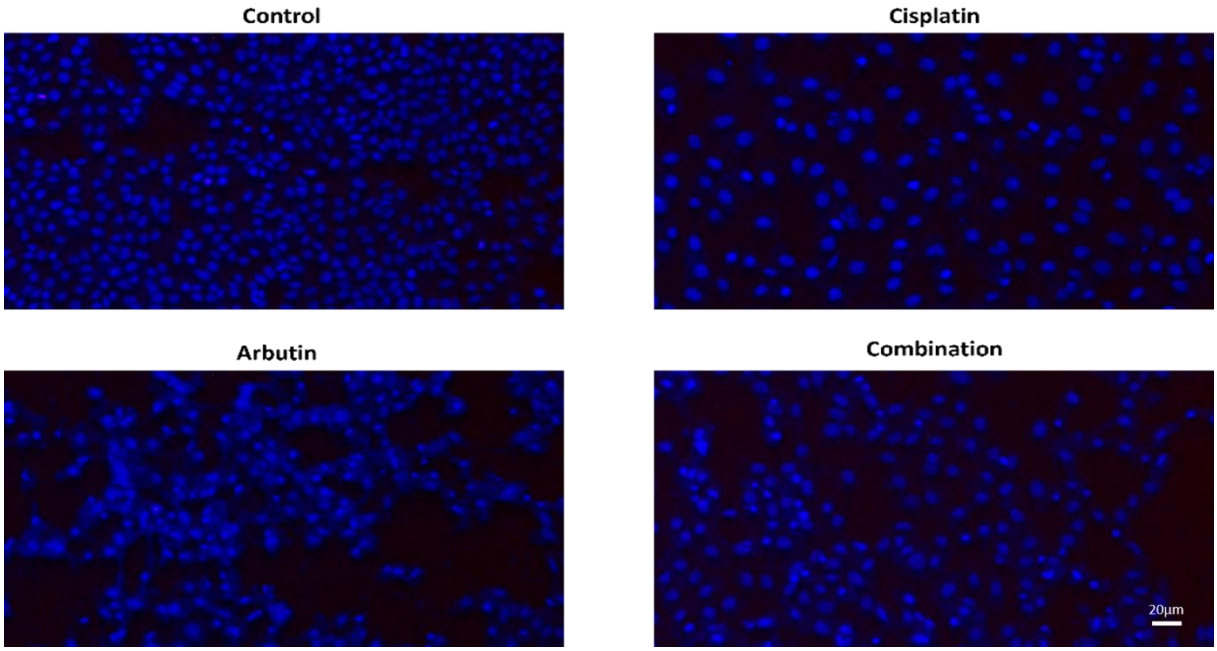

Figure 4. Nuclear morphology of MCF-7 cells after 48-hour treatments visualized with Hoechst 33342 staining. Representative fluorescence images show control, arbutin (47.11 mM), cisplatin (3.77 µM), and combination (31.32 mM arbutin + 2.51 µM cisplatin) groups. Increased nuclear condensation and fragmentation, indicative of apoptosis, were most evident in the combination-treated cells.

Nuclear Morphology (Hoechst 33342 / DAPI images)

Fluorescence microscopy analysis utilizing DAPI staining revealed distinct nuclear morphological changes across treatment groups, reinforcing the pro-apoptotic effects observed in gene expression and combination index data. Control cells displayed high confluency with uniformly round, intact nuclei, indicating healthy proliferation (top left panel of Figure 4). In contrast, Arbutin-treated cells exhibited a moderate decrease in cell number with occasional nuclear condensation, suggesting early signs of stress or apoptosis (bottom left panel of Figure 4). Cisplatin treatment resulted in significant nuclear condensation and fragmentation, accompanied by a notable reduction in cell density, indicative of apoptosis (top right panel of Figure 4). Remarkably, the combination treatment (Arbutin + Cisplatin) led to the most severe nuclear alterations, including extensive fragmentation, condensed chromatin, and the lowest observed cell density, consistent with an enhanced apoptotic response (bottom right panel of Figure 4).

Discussion

The present study investigated the combined effects of arbutin and cisplatin on MCF-7 breast cancer cells, focusing on cytotoxicity, nuclear morphology, and mitochondrial gene expression. While cisplatin is a widely used chemotherapeutic agent, its clinical use is often limited by dose-dependent toxicity and resistance. Arbutin, a natural compound with reported antioxidant and anticancer properties, was evaluated for its potential to enhance cisplatin efficacy. Our findings demonstrate that the arbutin–cisplatin combination induces synergistic cytotoxicity and amplifies apoptotic responses through modulation of mitochondrial dynamics and apoptotic signaling pathways.

Hoechst 33342 staining revealed pronounced nuclear condensation and fragmentation in MCF-7 cells after 48 h of combined arbutin (mM) and cisplatin (µM) treatment (Figure 4). In control and single-agent treatments, most nuclei remained intact and uniformly stained, whereas the combination led to many pyknotic, fragmented nuclei, a hallmark of apoptosis. These nuclear changes—brightly fluorescent, shrunken or irregular nuclei with chromatin clumping—indicate enhanced apoptotic cell death in the combination group. Such chromatin condensation and nuclear fragmentation are characteristic features of apoptosis, supporting that arbutin augments cisplatin-induced apoptotic nuclear damage in MCF-7 cells. Consistent with this, the combination-treated cells showed an increased proportion of apoptotic bodies and nuclear debris compared to either treatment alone, confirming that the 48h combination triggers robust apoptosis at the nuclear level (Figure 4).[18]

Dose–response curves for arbutin and cisplatin (48 h exposure) demonstrated dose-dependent cytotoxic effects on MCF-7 viability (Figure 1). Cisplatin alone exhibited a steep dose-effect curve characteristic of a potent chemotherapeutic, while arbutin alone produced a more modest cytotoxic effect at millimolar concentrations. Notably, co-treatment produced greater-than-additive growth inhibition across a range of doses. Combination Index (CI) analysis via the Chou–Talalay method showed CI values <1, indicating synergistic cytotoxicity (Figure 2).[19] In practice, the IC_{50} of cisplatin was effectively lowered in the presence of arbutin. CI values at ED50, ED75, and ED90 consistently demonstrated synergy (CI<1), suggesting that arbutin enhances cisplatin's efficacy, potentially allowing dose reduction of cisplatin while maintaining anti-proliferative effect.

Quantitative PCR at 48 h provided insight into mitochondrial fission–fusion dynamics, including dynamin-related protein 1 (*DRP1*). *DRP1* expression remained near baseline in controls and was mildly affected by arbutin alone. In contrast, cisplatin alone upregulated *DRP1* mRNA, and the arbutin+cisplatin combination triggered a greater increase in *DRP1* expression. Fission 1 (*Fis1*) showed similar trends, with higher expression in the combination group than in single treatments, indicating enhanced mitochondrial fission (Figure 3).[20]

Meanwhile, mitofusin 1 (*MFN1*) and mitofusin 2 (*MFN2*)—key fusion protein gene expressions—showed reduced or unchanged transcript levels in cisplatin-treated cells and were further decreased in the combination group. Although *MFN1* generally facilitates mitochondrial fusion and stability, its elevated levels may indicate a compensatory mechanism in

response to heightened mitochondrial fragmentation and stress. Mitochondrial fragmentation, driven by fission proteins like Drp1 and Fis1, is closely associated with the initiation of apoptosis.[21] This suggests that the combination boosts pro-fission (*DRP1*, *Fis1*) and suppresses pro-fusion (*MFN1/2*) signals, favoring mitochondrial fragmentation, a known precursor to apoptosis (Figure3).[22] Notably, the *DRP1* and *Fis1* changes appear synergistic, correlating with the enhanced cytotoxic effects of the combination treatment.

A notable increase in *PGF2β* expression in cells treated with arbutin, indicating that prostaglandin-mediated signaling pathways are activated as a stress response. Interestingly, this upregulation was diminished in the group receiving both arbutin and cisplatin. This suggests that cisplatin may inhibit cell survival signals that are regulated by prostaglandins.[23] This finding highlights a significant molecular mechanism that explains how the combination of these two compounds can enhance cell death processes.

Additionally, *BCL2* mRNA, an anti-apoptotic gene, was significantly downregulated in the combination group, further supporting increased apoptosis. *BCL2* reduction facilitates mitochondrial outer membrane permeabilization and cytochrome c release (Figure 3).[24] The analysis revealed a moderate decrease in *BCL2* expression levels in the cisplatin group, with a more significant reduction observed in the combination group of arbutin and cisplatin. This suggests that the suppression of the anti-apoptotic *BCL2* may trigger mitochondrial-based apoptosis mechanisms. Previous studies indicate that arbutin can shift the balance between the pro-apoptotic *BAX* and the anti-apoptotic *BCL2*, promoting apoptosis. This molecular regulation is expected to enhance the sensitivity of cells to the cytotoxic effects of cisplatin.[25]

A slight increase in *Beclin-1* expression levels was noted following arbutin application. This rise in the gene expression, a key regulator of autophagy, may have initially activated the cell's survival mechanisms. However, existing literature indicates that prolonged or excessive activation of autophagy, particularly when paired with signals that induce apoptosis, can lead to cell death.[26] These findings suggest that in the presence of both arbutin and cisplatin, cells may transition from autophagic survival to apoptotic processes. This shift could be one of the mechanisms contributing to the synergistic effect of the two treatment agents.

The 48-hour arbutin+cisplatin combination induces potent apoptosis in MCF-7 cells. Morphologically, nuclear fragmentation and chromatin condensation confirm apoptotic death (Figure 4). The synergy in cytotoxicity (CI<1) aligns with mitochondrial fission data, where *DRP1* and *Fis1* are upregulated and *MFN1/2* are downregulated (Figure 2 and 3).[27] Fragmented mitochondria are more susceptible to outer membrane permeabilization, facilitating cytochrome c release and caspase activation.

DRP1, a GTPase involved in mitochondrial division, and *Fis1*, a membrane adaptor, act together to promote fission.[28] Enhanced expression of both under combination treatment supports a mechanistic link between mitochondrial fragmentation and apoptosis, as also demonstrated in other stress-related models involving mitochondrial dynamics.[29] This shift toward fission, combined with reduced *BCL2* and insufficient autophagy, leads to a cellular environment primed for intrinsic apoptotic signaling.

The limited changes observed in *SOD2* and *GPx1* gene expressions in this study highlight the redox imbalance and oxidative stress induced by cisplatin. Notably, while arbutin is known for its antioxidant properties under normal conditions, it demonstrated a pro-oxidant effect in cancer cells, leading to increased production of reactive oxygen species (ROS) and the initiation of apoptotic processes.[30]

The data indicate that the combination of arbutin and cisplatin affects several interconnected cellular processes, including mitochondrial dysfunction, heightened oxidative stress, and the activation of apoptosis. This multifaceted mechanism significantly diminishes the survival capacity of cancer cells and enhances treatment efficacy. Therefore, arbutin may serve as a potential chemosensitizing agent that amplifies the therapeutic effects of cisplatin while allowing for a reduced dosage. These findings provide valuable insights for developing combination treatment strategies.

Although MCF-7 cells lack caspase-3, caspase-7-mediated apoptosis proceeds, as evidenced by nuclear morphological changes (Figure 4).[31] Thus, arbutin appears to potentiate cisplatin's efficacy by amplifying mitochondrial dysfunction and reducing resistance mechanisms such as fusion, antioxidant defense, and survival signaling. This study has several limitations. First, the experiments were conducted solely on the MCF-7 breast cancer cell line, which lacks caspase-3 and may not fully represent apoptosis mechanisms in other cancer types. Additional validation in caspase-3-positive cell lines or in vivo models would help confirm the generalizability of the observed synergistic effects. Second, although mitochondrial gene expression changes were evaluated, no protein-level validation (e.g., Western blotting or immunostaining) was performed to confirm changes in DRP1, Fis1, or BCL2 expression. Third, the qPCR analyses were conducted in duplicate, which may limit statistical robustness. Finally, while the Chou–Talalay method demonstrated synergy, further mechanistic studies such as mitochondrial membrane potential assays or cytochrome c release quantification would strengthen the apoptotic pathway findings.

Conclusion

In conclusion, arbutin enhances cisplatin-induced apoptosis in MCF-7 cells through the modulation of mitochondrial dynamics and suppression of survival signals. This strategy may enable reduced cisplatin dosing, thereby minimizing systemic toxicity while preserving therapeutic efficacy. Future studies should explore the efficacy of this combination in other breast cancer cell lines, particularly those with functional caspase-3 expression, and validate these results in animal models to assess in vivo antitumor activity and toxicity profiles. Protein-level confirmation of key mitochondrial regulators, along with functional assays such as mitochondrial membrane potential, caspase activity, and cytochrome c release, would further clarify the underlying mechanisms. Clinically, this combination holds promise for reducing cisplatin's dose-limiting side effects, including nephrotoxicity and neurotoxicity. If confirmed in preclinical models, the arbutin–cisplatin combination could serve as the basis for novel adjuvant regimens in breast cancer therapy, especially in patients with mitochondrial dysfunction or resistance to conventional chemotherapy.

Conflict of Interest

The authors declare no conflicts of interest.

Compliance of Ethical Statement

This study involved only commercially available human cell lines (MCF-7; ATCC HTB-22). No experiments were conducted on human participants or animals; therefore, ethical ap-proval was not required.

Financial Disclosure
None

Author Contributions
M.A.K. Ö.B.G., U.K.K., B.G.B.: Study idea/Hypothesis; M.A.K. Ö.B.G., U.K.K., B.G.B.: Design; M.A.K.: Data Collection; Ö.B.G., U.K.K., B.G.B.: Analysis; M.A.K.: Literature review; M.A.K.: Writing; Ö.B.G, B.G.B.: Critical review.

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