RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(1):2-9

Investigation of the Cytotoxicity of Arbutin Combined with Doxorubicin *In Vitro*

Mehmet Halil Kılınç[1], Furkan Aydın[2], Başak Aru[2]

[1] 4th-Year Medical Student, Yeditepe University Faculty of Medicine, İstanbul, Türkiye; [2] Department of Immunology, Yeditepe University Faculty of Medicine, İstanbul, Türkiye.

Abstract

Objective: The aim of this study was to investigate the anti-cancer effects of arbutin on doxorubicin-induced cytotoxicity in the double-positive estrogen receptor +/ progesterone receptor +/ human epidermal growth factor receptor 2 negative (ER+/PR+/HER2-) breast cancer (BC) cell line MCF-7 *in vitro*.

Materials and Methods: Viability screening was performed with colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. Intracellular reactive oxygen species (ROS) accumulation was evaluated by dihydrorhodamine 123 (DHR123) staining. Apoptosis, necrosis and viability to arbutin, doxorubicin and their combination were assessed by Annexin V/7-AAD (7-aminoactinomycin D) staining. Cell cycle phase distribution was analyzed by DNA content analysis.

Results: Arbutin alone, at concentrations up to 500 μM, did not reduce MCF-7 cell viability over incubation periods ranging from 6 to 48 hours. Arbutin at concentrations above 20 μM transiently decreased intracellular ROS levels at 6 hours but had no significant effect at 24 and 48 hours. When combined with doxorubicin, arbutin partially reversed doxorubicin-induced reductions in cell viability, decreased late apoptosis and necrosis rates, and regulated doxorubicin-induced cell cycle disruptions.

Conclusions: These results suggest that while arbutin does not exhibit direct cytotoxicity in MCF-7 cells, it modulates doxorubicin-induced cellular responses. Future studies with arbutin at higher concentrations investigating the molecular mechanisms underlying this effect, particularly at the gene and protein expression levels, are necessary to further elucidate the potential role of arbutin in BC therapy.

Keywords: Breast cancer, arbutin, doxorubicin, apoptosis, DNA content analysis

Received February 29, 2024 **Accepted** March 21, 2025 **Published** April 25, 2025 **DOI** 10.36519/yjhs.2025.615

Suggested Citation Kılınç MH, Aydın F, Aru B. Investigation of the cytotoxicity of arbutin combined with doxorubicin *in vitro*. Yeditepe JHS. 2025;1:2-9.

Correspondence Başak Aru **E-mail** basak.aru@yeditepe.edu.tr

INTRODUCTION

Worldwide, breast cancer (BC) is the most common malignancy in women, with an estimated 2.3 million new cases and 685,000 deaths, accounting for 16% of female cancer deaths in 2020 (1). Breast cancer is a heterogeneous disease with several molecular subtypes, each associated with different prognoses. Routine evaluation of BC includes estrogen receptor alpha (ERα), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression (2); therefore, the primary subtypes are classified as hormone receptor (HR)-positive/HER2-negative, HR-positive/HER2-positive, HR-negative/HER2-positive, and triple-negative (HR-negative/HER2-negative), all of which have different survival outcomes (3). BC classification aims to provide data for oncologic decisions that would lead to the successful treatment of the disease. In this context, BC is evaluated according to its type, grade, and the stage of the tumor. The type and grade of breast tumors are based on histological subtypes and grades and are defined by the World Health Organization (WHO). BC stages are associated with tumor size, node invasion, and metastasis. These prognostic markers provide important predictive data for hormone therapies and anti-HER2-therapies (2).

Arbutin is a naturally occurring glycoside found mainly in the leaves of various plant species, most notably bearberry (*Arctostaphylos uva-ursi*) of the *Ericaceae* family, although it has also been identified in plants of the *Asteraceae*, *Proteaceae*, and *Rosaceae* families (4). Chemically, arbutin is a β-glucoside derived from hydroquinone, with the molecular formula $C_{12}H_{16}O_7$. Its structure consists of a hydroquinone molecule linked to a glucose moiety (4). Traditionally, arbutin has been used in herbal medicine, particularly in the treatment of urinary tract infections, and extracts of bearberry leaves, which are rich in arbutin, have been used for their antimicrobial properties (5). Being a competitive inhibitor of the enzyme tyrosinase, which plays a crucial role in melanin production, arbutin is widely used in cosmetic products for its skin whitening properties in addition to its medical applications (6). To date, arbutin has demonstrated potential anticancer properties in several cancer types through induction of apoptosis, inhibition of inflammatory markers, and suppression of the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) signaling pathway (7-9). However, to the best of our knowledge, the impact of arbutin in combination with standard chemotherapeutic agents on BC has not been reported. When considering studies suggesting enhanced therapeutic efficacy and diminished drug resistance when doxorubicin was combined with natural compounds such as resveratrol (10), we aimed to determine the efect of arbutin use on the anticancer activity of doxorubicin on MCF-7 double-positive BC cell line.

MATERIALS AND METHODS

Cell Culture Conditions

In this study, the MCF-7 double-positive BC cell line (HTB-22™, passage 14) (American Type Culture Collection - ATCC) was used. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Cat. No: DMEM-HPA; Capricorn Scientific, Germany) supplemented with 10% Fetal Bovine Serum (FBS) (Cat. No: FBS-16B; Capricorn Scientific, Germany) and 100 U/mL penicillin-streptomycin antibiotic solution (Cat. No: 15140122; Thermo Fisher Scientific, USA). Upon reaching 80% confluence, cells were detached from the flasks using trypsin-EDTA solution (Cat. No: 25300054; Thermo Fisher Scientific, USA) counted with the JuLI™ Br Cell Counting Station (NanoEnTek Inc., South Korea) and adjusted to a concentration of 1×10^6 cells per mL. For viability screening and assessment of intracellular ROS levels, 5×10^3 cells were seeded in triplicate into 96-well plates. For flow cytometric analysis of apoptosis and viability and DNA content analysis, cells were seeded at 5×10^5 cells per dish in 60 mm cell culture dishes. All experiments were performed as triplicates.

Colorimetric Evaluation of Viability

Cellular viability of MCF-7 cells after treatment with arbutin and doxorubicin was assessed with MTS assay, which is based on the reduction of the reagent to formazan salt upon reduction with cellular enzymes (11). For treatment, stock solution of arbutin was prepared by dissolving it in complete culture medium at 500 mM concentration. After seeding, the plates were incubated overnight to allow for cell attachment, and then treated with arbutin at concentrations of 0.8, 4, 20, 100, and 500 µM for 6, 12, 24, and 48 hours. At the end of the incubation, MTS reagent (Cat. No: ab197010; Abcam Limited, UK) (10% v/v) was added to the wells and the samples were incubated for 2 hours. Absorbance was read at 490 nm wavelength. Untreated cells were included as negative control, and plain culture medium was used as blank. Cytotoxicity was calculated by subtracting the blank absorbance from both the test and control groups. The corrected absorbance of the test group was then divided by the corrected absorbance of the control group and multiplied by 100 to obtain the percentage of viability after treatments.

Fluorometric Evaluation of Reactive Oxygen Species

Dihydrorhodamine 123 (DHR123) is a non-fluorescent probe commonly used to detect the production of reactive nitrogen and oxygen species in cells. Upon oxida-

tion, DHR123 is converted to fluorescent rhodamine (12). Cells were incubated with arbutin at concentrations of 0.8, 4, 20, 100, and 500 µM for 6, 12, 24, and 48 hours, after which the medium was discarded and the cells were incubated with 100 µL DHR123 solution for 40 minutes at room temperature (5 µM in DPBS). At the end of incubation, DHR123 solution was discarded, 100 µL fresh Dulbecco's Phosphate Buffered Saline (DPBS) was added to wells, and plates were read with the Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, USA) at an excitation/emission (ex/em) wavelength of 510/530 nm (±5 nm). Values were obtained as relative fluorescent unit (RFU).

Annexin V/7-Aminoactinomycin Staining

Annexin V/7-aminoactinomycin (7-AAD) staining is a common method used to evaluate apoptosis. In the presence of Ca^{2+} ions, annexin V binds specifically to phosphatidylserine residues, membrane phospholipids that translocate from the inner to the outer leaflet of the cell membrane during apoptosis. Meanwhile, 7-AAD, a DNA-binding dye, can only penetrate necrotic or late apoptotic cells, enabling to distinguish between different stages of cell death (13). To evaluate the apoptotic effect of arbutin, doxorubicin, and arbutin in combination with doxorubicin, cells were incubated with the compounds for 48 hours and collected by trypsinization. After centrifugation, supernatant was discarded, cells were suspended in 1 mL V Binding Buffer (Cat. No: 422201; Biolegend, USA), and labelled with Annexin V-Pacific Blue/7-AAD (Cat. No: 640926; Biolegend, USA) according to the kit instructions. After incubation at room temperature under dark for 15 minutes, 2.5×10^4 cells per tube were evaluated with DxFLEX Flow Cytometry System (Beckman Coulter, USA). Analysis was performed with Kaluza analysis software (Beckman Coulter, USA).

DNA Content Analysis

DNA content analysis is a widely used method for evaluating cell cycle distribution, identifying apoptotic cells, and determining DNA ploidy status. In this method, cellular DNA content is evaluated to distinguish the major phases of the cell cycle; G0/G1, S, and G2/M, in addition to apoptotic cells based on their fractional DNA content resulting from DNA fragmentation (14). Here, the effects of arbutin, doxorubicin, and arbutin in combination with doxorubicin on cell cycle progression were evaluated by DNA content analysis by incubating cells with the compounds, either alone or in combination, for 48 hours. At the end of the incubation, the cells were trypsinized and collected by centrifugation. The cells were fixed with 5 mL of 70% ethanol solution by incubating the tubes at 4 °C for one hour. At the end of fixation, the ethanol solution was discarded and the cells were resuspended in 500 µL of Cell Cycle Kit (Cat. No: C03551; Beckman Coulter, USA). After incubation at room temperature under dark for an hour, 5×10^4 cells per tube were evaluated with DxFLEX Flow Cytometry System (Beckman Coulter, USA). Analysis was performed with Modfit software (Verity Software House, USA).

Statistical Analysis

Statistical analysis was performed with GraphPad Prism 8.0.2 (GraphPad Software Inc, USA). One-way ANOVA followed by Dunnett's multiple comparison test was used to determine the effect of arbutin on MCF-7 cell line. For evaluating viability, apoptosis and necrosis, one-way ANOVA followed by Tukey's multiple comparison test was employed. DNA content analysis was evaluated with two-way ANOVA followed by Tukey's multiple comparison test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Arbutin Does Not Decrease Viability or ROS Accumulation in MCF-7 Cell Line

Our results indicated that within the given dose interval, arbutin did not reduce the viability of MCF-7 cells compared to the control group (Figure 1). Only a slight increase in viability was observed in the 4 µM group compared to the control group at 6 hours ($p<0.05$) (Figure 1a), although this finding was not observed at longer incubation periods (Figure 1b-d). When intracellular ROS accumulation was evaluated with DHR123 staining, even though no alterations was observed in 6 hours (Figure 1e), arbutin over 20 µM concentration led to significant decreases in ROS levels compared to the control group in 12 hours (20 µM, $p<0.05$; 100 and 500 µM, $p<0.01$) (Figure 1f). Yet, similar to 6 hours incubation duration, arbutin did not alter ROS levels in 24 (Figure 1g) and 48 hours (Figure 1h). Altogether, these results indicate that arbutin up to 500 µM concentration do not exert cytotoxicity on MCF-7 cell line but decrease ROS levels in relatively short incubation interval. Since the compound did not decrease viability at all incubation periods, further studies were conducted with 500 µM arbutin at 48 hours. The IC_{50} value of doxorubicin on 48 hours was calculated for a previous study as 2.32 µM for MCF-7 cell line (15), which was also applied either alone or in combination with arbutin for mechanistic studies.

Arbutin Partially Reverses Doxorubicin Induced Cell Death

When the effect of arbutin in combination with doxorubicin was evaluated, doxorubicin significantly decreased viability compared to the control and arbutin groups ($p<0.0001$), while when its combined with arbutin, viability was increased in comparison with the doxorubicin group ($p<0.0001$). Yet, the viability levels of

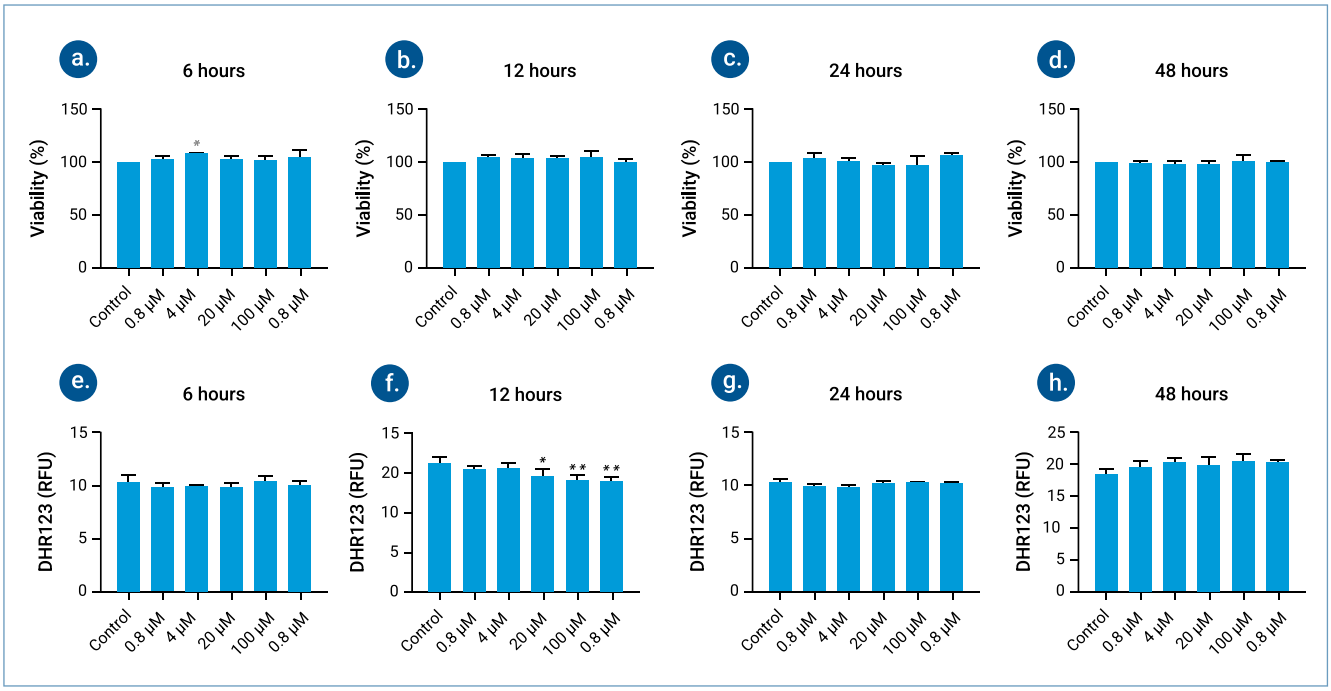

FIGURE 1. Bar graphs regarding the effects of arbutin at 0.8, 4, 20, 100 and 500 µM concentrations on MCF-7 cell line in terms of viability and intracellular reactive oxygen species at different time points. **a.** Viability upon arbutin treatment for 6 hours, **b.** Viability upon arbutin treatment for 12 hours, **c.** Viability upon arbutin treatment for 24 hours, **d**. Viability upon arbutin treatment for 48 hours, **e.** Intracellular ROS accumulation upon arbutin treatment for 6 hours, **f.** Intracellular ROS accumulation upon arbutin treatment for 12 hours, **g.** Intracellular ROS accumulation upon arbutin treatment for 24 hours, **h.** Intracellular ROS accumulation upon arbutin treatment for 48 hours. *p*-values lower than 0.05 were considered statistically significant. *p<0.05, **p<0.01.

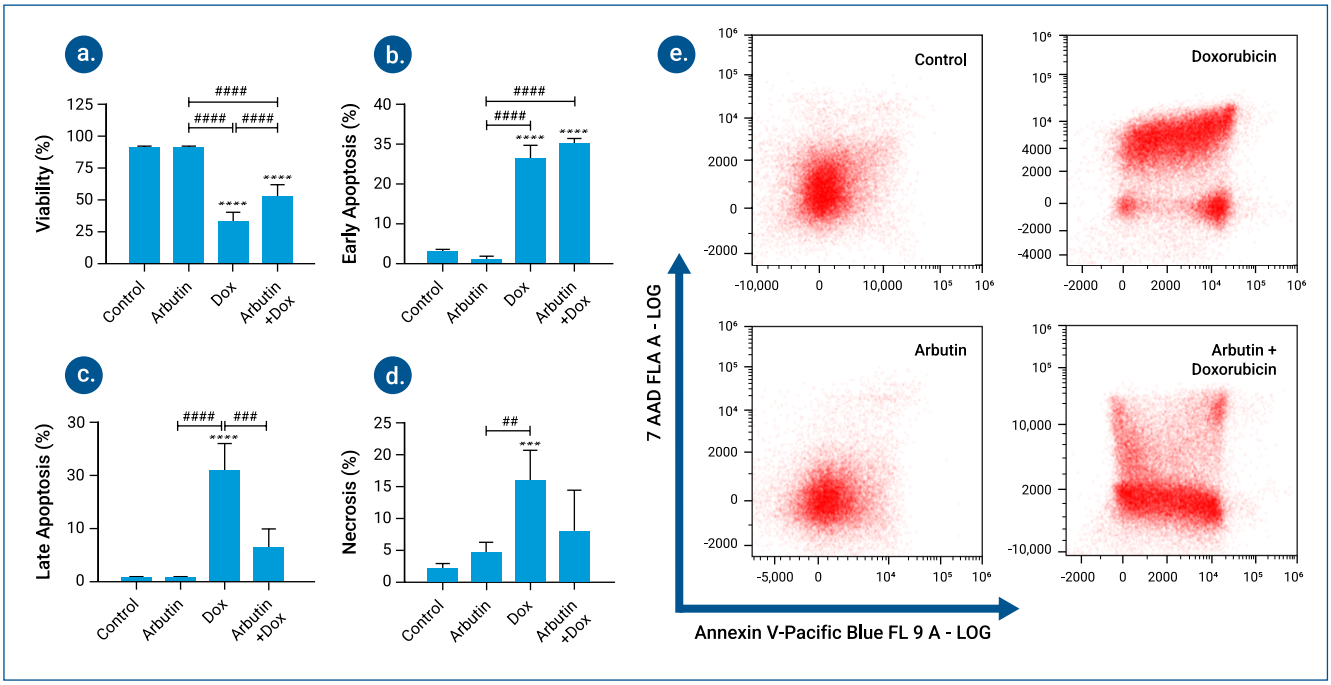

FIGURE 2. Comparisons between arbutin, doxorubicin and arbutin in combination with doxorubicin treatment in terms of viability and apoptosis evaluated by Annexin V/7-AAD staining. Bar graphic revealing **a.** Viability, **b.** Early apoptosis, **c.** Late apoptosis and d. Necrosis. **e.** Representative flow cytometry dot plots. *p*-values lower than 0.05 were considered statistically significant.

* indicates differences between the control and the treatment groups, and # indicates differences between treatment groups. ***p<0.001, ****p<0.0001; ##p<0.01, ###p<0.001, ####p<0.0001.

the arbutin+doxorubicin group were still significantly lower compared to the control and the arbutin groups (p<0.0001) (Figure 2a). On the other hand, doxorubicin increased early apoptosis levels compared to the control group (p<0.0001), but arbutin could not reverse this effect as this group also had higher early apoptosis levels when compared to both control and the arbutin groups (p<0.0001) (Figure 2b). Doxorubicin treatment significantly increased late apoptosis rates (p<0.0001), which is decreased when doxorubicin was combined with arbutin (p<0.001) (Figure 2c). Lastly, similar to late apoptosis, in the doxorubicin group, necrosis rates wereincreased compared to the control and the arbutin groups (p<0.001); though in the arbutin+doxorubicin group, no difference compared to the control or only arbutin group was observed (p>0.05) (Figure 2d). Representative flow cytometry dot plots are given in Figure 2e.

Arbutin Exhibits Regulatory Effects on Doxorubicin-Induced Cell Cycle Arrest

Evaluations regarding G0/G1 phase revealed that arbutin did not cause a significant alteration compared to the control group (p>0.05). Doxorubicin significantly decreased in this phase compared to both the control and arbutin groups (p<0.0001). The G0/G1 phase was higher in the doxorubicin+arbutin group compared to the arbutin and control groups (p<0.0001) (Figure 3a). Interestingly, arbutin decreased S phase in comparison with the control group (p<0.05). On the contrast, doxorubicin treatment increased this phase (p<0.01). In comparison with the doxorubicin group, the combination of arbutin with doxorubicin decreased the S phase (p<0.01), although this decrease did not lower its levels to the control level (p<0.01) (Figure 3a). Lastly, doxorubicin led to a significant increase in G2/M phase compared to the control group (p<0.05), and arbutin treatment did not have an impact on this accumulation as arbutin+doxorubicin group had higher G2/M phase levels compared to both control (p<0.0001) and the arbutin (p<0.01) alone group (Figure 3a). Representative flow cytometry histogram plots are provided in Figure 3b.

DISCUSSION

Arbutin, a glycosylated hydroquinone, has been extensively studied for its skin depigmenting properties due to its ability to inhibit melanin synthesis. Research indicates that arbutin effectively reduces hyperpigmentation by inhibiting melanosomal tyrosinase activity, thereby decreasing melanin production. To enhance its stability and transdermal delivery, various formulations and biotechnological methods have been developed, including enzymatic bioconversion techniques to produce α- and β-arbutin derivatives. These derivatives have demonstrated significant antimelanogenic effects, making them valuable in treating conditions characterized by hyperactive melanocyte function (16, 17).

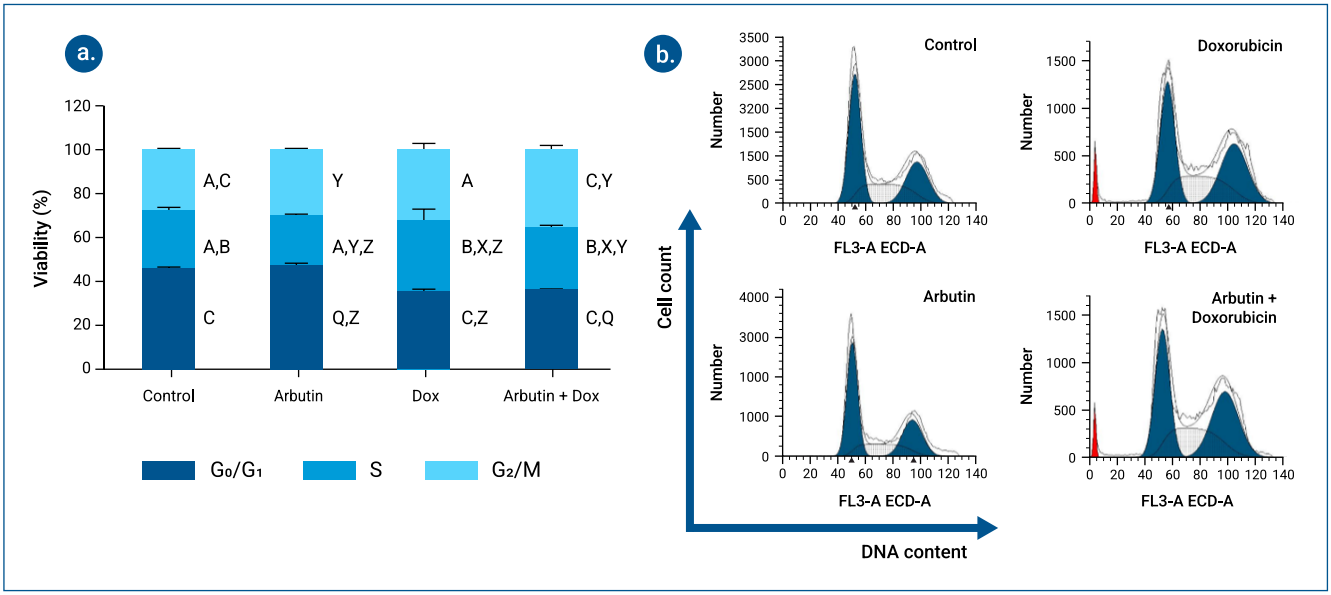

FIGURE 3. Comparisons between arbutin, doxorubicin and arbutin in combination with doxorubicin treatment in terms of cell cycle phases' distribution evaluated by DNA content analysis. **a.** Bar graphics, **b.** Representative flow cytometry histograms. p-values lower than 0.05 were considered statistically significant.

Letters A (p<0.05), B (p<0.01) and C (p<0.0001) indicate comparisons between the control and the treatment groups, letters X (p<0.05), Y (p<0.01) and Z (p<0.0001) indicate comparisons between treatment groups.

Preliminary studies investigating the anticancer activity of arbutin focused on melanoma. In a study published in 2009, Nawarak et al. aimed to investigate the anticancer effects of arbutin on A375 human malignant melanoma cells by elucidating changes in protein expression profiles following arbutin treatment (18). Proteomic analysis revealed that arbutin treatment at the concentration of 8 μg/mL resulted in significant changes in the expression of proteins associated with various cellular processes, including apoptosis, cell cycle regulation, and oxidative stress response. In particular, proteins involved in the apoptotic pathway were upregulated, suggesting that arbutin may induce programmed cell death in melanoma cells. In addition, proteins related to cell proliferation were downregulated, suggesting a potential inhibitory effect on tumor growth. These findings provide insight into the molecular mechanisms underlying the anticancer activity of arbutin against malignant melanoma cells. Also, Ma et al. used a combination of network pharmacology and experimental validation to identify compounds in *Prinsepia utilis* with potential antimelanoma activity where the authors utilized bioinformatics databases to predict compounds and their targets, followed by molecular docking to assess interactions between these compounds and key targets (19). Subsequent *in vitro* studies were performed on human melanoma A375 cells to evaluate the biological activities of the identified compounds. This study identified oleanolic acid, ursolic acid, and arbutin as active ingredients in *P. utilis*, and in vitro studies revealed that oleanolic acid and ursolic acid significantly inhibited the growth and migration of A375 melanoma cells, induced apoptosis, and reduced both tyrosinase activity and melanin synthesis yet arbutin did not exhibit significant effects. In order to enhance its efficacy, Jian et al. synthesized an acetylated derivative of arbutin to enhance its biological activity and compared its effects to those of the parent compound on B16 murine melanoma cells (8). According to this study, both arbutin and its acetylated derivative significantly reduced cell viability, promoted apoptosis, caused G1 phase cell cycle arrest, and induced mitochondrial disruption in B16 melanoma cells. These pro-apoptotic effects were associated with decreased expression of anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra-large (Bcl-xL), indicating involvement of the mitochondrial pathway. However, it should be noted that the lowest dose of arbutin used for the mechanistic evaluations was 1.4 mM, which is approximately three times higher than the dose (500 μM) used in our current study.

Arbutin has previously been studied in the treatment of various cancers in vitro beside melanoma. In a study published in 2020, the authors aimed to investigate the antioxidant effects of arbutin on LNCaP (lymph node carcinoma of the prostate) cancer cells (20). Researchers treated the cells with arbutin up to 2 mM and evaluated intracellular ROS levels, induction of apoptosis, and expression of pro-inflammatory cytokines IL-1β and tumor necrosis factor alpha (TNF-α). The authors showed that arbutin significantly decreased intracellular ROS levels in a dose-dependent manner and significantly induced apoptosis in LNCaP cells, in addition to reducing IL-1β and TNF-α expression levels at 1 mM concentration. In a study published by Ciğerci et al., the authors investigated the anticarcinogenic potential of high concentrations of arbutin and its protective effects against cisplatin-induced toxicity at low concentrations on HepG2 human hepatocellular carcinoma cell line (21). In this study, high concentrations of arbutin exhibited anticarcinogenic effects by reducing HepG2 cell viability while low concentrations of arbutin provided protective effects against cisplatin-induced toxicity, likely through its antioxidant and anti-inflammatory properties. Similarly, our results indicate that arbutin in combination with doxorubicin reduces the apoptotic efficacy of this chemotherapeutic drug, a finding consistent with a previous report. With a similar study design, Terzi et al. evaluated the effects of arbutin, both alone and in combination with cisplatin, on HT-1376 bladder cancer cells. In this study, cell viability, apoptosis induction, and cell migration were assessed, and the IC_{50} dose for arbutin was calculated as 4317 mM on 24th hour (22). The authors revealed that arbutin, both alone and in combination with cisplatin, significantly increased apoptosis and inhibited migration in HT-1376 cells, suggesting that β-arbutin may enhance the anticancer effects of cisplatin, making it a potential therapeutic candidate for bladder cancer treatment. Both of these studies highlight the fact that arbutin exerts its anti-cancer effect over 1 mM concentration, a rather high dose compared to our study.

In 2021, Yang et al. evaluated the anticancer effect of arbutin on C6 glioma cells, where the authors treated the cells with 10-60μM arbutin for 24 hours, and analyzed cell viability, apoptosis, ROS generation, mitochondrial membrane potential disruption, and expression of inflammatory markers and components of the PI3K/Akt/mTOR signaling pathway (23). The results revealed that arbutin decreased viability in C6 cell viability in a dose-dependent manner, induced apoptosis by increasing ROS production and disrupting mitochondrial membrane potential. Additionally, arbutin inhibited the expression of inflammatory markers and downregulated the PI3K/Akt/mTOR signaling pathway, suggesting its potential as a therapeutic agent against gliomas. In contrast, although much higher concentrations of arbutin were evaluated in our study, we did not observe a decrease in viability of MCF-7 cells in response to arbutin, which may reflect the relative insensitivity of this cell line to this compound. In MCF-7 cell line, arbutin was

demonstrated to exert its effect *via* inhibition of ER-α, although much higher doses were required to reduce cell viability compared to our study (9).

In addition to studies evaluating its cytotoxic effect on cancer cells, a recent study assessed arbutin's effect on the expression of programmed cell death ligand 1 (PD-L1) on tumor cells (24). Involving B16F10 melanoma and LL2 lung cancer cell lines, the primary focus of this study was to determine whether arbutin could modulate PD-L1 expression and thereby influence tumor immune tolerance. The authors reported that arbutin treatment led to a significant reduction in PD-L1 expression on both B16F10 and LL2 tumor cells, which was associated with the inhibition of the AKT/mTOR signaling pathway. *In vivo* studies further demonstrated that arbutin reduced tumor growth and decreased PD-L1 expression in tumor tissues of mice compared to control group. Altogether, these results suggested that even if arbutin cannot exert toxicity, it can effectively diminish tumor-induced immune tolerance by targeting PD-L1 expression, offering potential therapeutic implications for enhancing antitumor immunity.

In our study, arbutin at concentrations up to 500 μM did not reduce MCF-7 cell viability across 6 to 48 hours incubation periods. Regarding intracellular ROS accumulation, arbutin concentrations above 20 μM significantly decreased ROS levels at 6 hours; though no significant changes were observed at 24 and 48 hours, indicating the compound's short-term effect on this cell line. When combined with doxorubicin, arbutin partially reversed doxorubicin-induced reductions in cell viability and decreased late apoptosis and necrosis rates, and cell cycle analysis revealed that arbutin regulated doxorubicin-induced disruptions. These findings suggest that arbutin alone does not exhibit cytotoxic effects on MCF-7 cells but can modulate doxorubicin-induced cytotoxicity and cell cycle changes. Nevertheless, given that studies in the literature suggest regulatory effects of arbutin in cancer cells even at non-cytotoxic doses, a detailed evaluation of the effects of low dose arbutin treatment on gene and protein expression levels in BC may contribute to the assessment of the anticancer potential of this compound.

Ethics Committee Approval: This *in vitro* study was conducted using a commercially available cell line; therefore,ethical approval was not required.

Informed Consent: N.A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.H.K., B.A.; Design – M.H.K., B.A.; Supervision – F.A., B.A.; Fundings – M.H.K.; Materials – B.A. Data Collection and/or Processing – M.H.K., F.A., B.A.; Analysis and/or Interpretation – B.A.; Literature Review – M.H.K., F.A., B.A.; Writer – M.H.K., B.A.; Critical Reviews – B.A.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: This study was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under the 2209-A Research Grant Program for Undergraduate Students in 2023, awarded to Mehmet Halil Kılınç and supervised by Prof. Dr. Gülderen Yanıkkaya Demirel.

Scientific Presentation: This study was presented in YÜTBAT 17th National Medical Student Congress by Mehmet Halil Kılınç.

Acknowledgements: This study was conducted in the HLA Typing Laboratory of Yeditepe University Hospital. We would like to thank Prof. Dr. Gülderen Yanıkkaya Demirel, M.D., Ph.D., Head of the Department of Immunology, Yeditepe University Faculty of Medicine, for her supervision. We also thank Prof. Dr. Hasan Kırmızıbekmez from the Faculty of Pharmacy, Yeditepe University, for providing arbutin.

REFERENCES

1 Benitez Fuentes JD, Morgan E, de Luna Aguilar A, Mafra A, Shah R, Giusti F, et al. Global stage distribution of breast cancer at diagnosis: A systematic review and meta-analysis. JAMA Oncol. 2024;10(1):71-8. [CrossRef]

2 Vuong B, Darbinian J, Savitz A, Odele P, Perry LM, Sandhu L, et al. Breast cancer recurrence by subtype in a diverse, contemporary cohort of young women. J Am Coll Surg. 2023;237(1):13-23. [CrossRef]

3 Hwang KT, Kim J, Jung J, Chang JH, Chai YJ, Oh SW, et al. Impact of breast cancer subtypes on prognosis of women with operable invasive breast cancer: A population-based study using SEER Database. Clin Cancer Res. 2019;25(6):1970-9. [CrossRef]

4 Nahar L, Al-Groshi A, Kumar A, Sarker SD. Arbutin: Occurrence in plants, and its potential as an anticancer agent. Molecules. 2022;27(24):8786. [CrossRef]

5 Migas P, Krauze-Baranowska M. The significance of arbutin and its derivatives in therapy and cosmetics. Phytochem Lett. 2015;13:35-40. [CrossRef]

6 Hori I, Nihei K, Kubo I. Structural criteria for depigmenting mechanism of arbutin. Phytother Res. 2004;18(6):475-9. [CrossRef]

7 Zhang S, Yao K, Pi Y, Yang S, Huang Z, Pan X, et al. Role of arbutin in the inhibition of FBXO5 in hepatocellular carcinoma. Discov Oncol. 2024;15(1):827. [CrossRef]

8 Jiang L, Wang D, Zhang Y, Li J, Wu Z, Wang Z, et al. Investigation of the pro-apoptotic effects of arbutin and its acetylated derivative on murine melanoma cells. Int J Mol Med. 2018;41(2):1048-54. [CrossRef]

9 Hazman Ö, Sarıova A, Bozkurt MF, Ciğerci İH. The anticarcinogen activity of β-arbutin on MCF-7 cells: Stimulation of apoptosis through estrogen receptor-α signal pathway, inflammation and genotoxicity. Mol Cell Biochem. 2021;476(1):349-60. [CrossRef]

10 Jin X, Wei Y, Liu Y, Lu X, Ding F, Wang J, et al. Resveratrol promotes sensitization to doxorubicin by inhibiting epithelial-mesenchymal transition and modulating SIRT1/β-catenin signaling pathway in breast cancer. Cancer Med. 2019;8(3):1246-57. [CrossRef]

11 Wang Y, Nguyen DT, Yang G, Anesi J, Kelly J, Chai Z, et al. A modified MTS proliferation assay for suspended cells to avoid the interference by hydralazine and β-mercaptoethanol. Assay Drug Dev Technol. 2021;19(3):184-90. [CrossRef]

12 Crow JP. Dichlorodihydrofluorescein and dihydrorhodamine 123 are sensitive indicators of peroxynitrite *in vitro*: implications for intracellular measurement of reactive nitrogen and oxygen species. Nitric Oxide. 1997;1(2):145-57. [CrossRef]

13 Kumar R, Saneja A, Panda AK. An annexin V-FITC-propidium iodide-based method for detecting apoptosis in a non-small cell lung cancer cell line. Methods Mol Biol. 2021;2279:213-23. [CrossRef]

14 Darzynkiewicz Z, Huang X. Analysis of cellular DNA content by flow cytometry. Curr Protoc Immunol. 2004;Chapter 5:Unit 5.7. [CrossRef]

15 Çelik C, İdiş ÖD, Özhan Y, Tirpanlar D, Unal N, Gungor B, et al. Isolation of anti-inflammatory and cytotoxic secondary metabolites from *Valeriana phu* and evaluation of their mechanisms of action. Fitoterapia. 2025;181:106377. [CrossRef]

16 Seo DH, Jung JH, Lee JE, Jeon EJ, Kim W, Park CS. Biotechnological production of arbutins (α- and β-arbutins), skin-lightening agents, and their derivatives. Appl Microbiol Biotechnol. 2012;95(6):1417-25. [CrossRef]

17 Boo YC. Arbutin as a skin depigmenting agent with antimelanogenic and antioxidant properties. Antioxidants (Basel). 2021;10(7):1129. [CrossRef]

18 Nawarak J, Huang-Liu R, Kao SH, Liao HH, Sinchaikul S, Chen ST, et al. Proteomics analysis of A375 human malignant melanoma cells in response to arbutin treatment. Biochim Biophys Acta. 2009;1794(2):159-67. [CrossRef]

19 Ma J, Wang J, Tao Y, Zeng G, Yang X, Lai Q. Discovery of active ingredients oleanolic acid, ursolic acid, and arbutin for *in vitro* anti-melanoma: Network pharmacology and experimental validation. Nat Prod Commun. 2025;20(1). [CrossRef]

20 Safari H, Zabihi E, Pouramir M, Morakabati P, Abedian Z, Karkhah A, et al. Decrease of intracellular ROS by arbutin is associated with apoptosis induction and downregulation of IL-1β and TNF-α in LNCaP; prostate cancer. J Food Biochem. 2020;44(9):e13360. [CrossRef]

21 Hazman Ö, Evin H, Bozkurt MF, Ciğerci İH. Two faces of arbutin in hepatocellular carcinoma (HepG2) cells: Anticarcinogenic effect in high concentration and protective effect against cisplatin toxicity through its antioxidant and anti-inflammatory activity in low concentration. Biologia (Bratisl). 2022;77(1):225-39. [CrossRef]

22 Terzi E, Oz-Bedir BE, Ercan E, Ozdemir-Sanci T, Jafarova S, Aydin T. β-Arbutin and cisplatin: A combined approach to modulating apoptosis, cell viability, and migration in bladder cancer cells. Toxicol In Vitro. 2025;104:105985. [CrossRef]

23 Yang Z, Shi H, Chinnathambi A, Salmen SH, Alharbi SA, Veeraraghavan VP, et al. Arbutin exerts anticancer activity against rat C6 glioma cells by inducing apoptosis and inhibiting the inflammatory markers and P13/Akt/mTOR cascade. J Biochem Mol Toxicol. 2021;35(9):e22857. [CrossRef]

24 Liu CH, Weng JR, Wu LH, Song RY, Huang MD, Wu XH, et al. Arbutin overcomes tumor immune tolerance by inhibiting tumor programmed cell death-ligand 1 expression. Int J Med Sci. 2024;21(15):2992-3002. [CrossRef]