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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

# DU145 PROSTAT KANSERİ HÜCRE HATTINDA DOSETAKSEL ve AMİGDALİN TEDAVİSİNİN HÜCRE ÖLÜMÜ, İNTEGRİN-α ve İNTEGRİN-β EKSPRESYONLARI ÜZERİNDEN ETKİLERİNİN KARŞILAŞTIRILMASI

# COMPARISON OF THE EFFECTS OF DOCETAXEL and AMYGDALIN TREATMENT ON CELL DEATH, INTEGRIN-α and INTEGRIN-β EXPRESSIONS IN DU145 PROSTATE CANCER CELL LINE

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**ÖZET**

**AMAÇ:** Prostat kanseri (PK) erkeklerde kansere bağlı ölümler arasında ikinci sırada yer almaktadır ve ölümlerin çoğu metastaz kaynaklıdır. Kanser metastazında hücre yüzey reseptörleri olan integrinler önemli rol oynarlar. İntegrin alfa2beta1'in metastatik prostat kanserlerinde kollajen I'e bağlanmayı artırarak hücre adezyon, migrasyon ve invazyonunda etkili olduğu gösterilmiştir. Prostat kanserinde Dosetaksel kemoterapisi kullanılmakta fakat ileri evrelerde bu tedavi etkisiz kalmaktadır. Amigdalin meyve tohumlarında yaygın olarak bulunan siyanojenik bir glikozittir ve kanser tedavisinde kullanımı konusunda literatürde çelişkiler bulunmaktadır. Çalışmamızda Amigdalin ve Dosetaksel tedavilerinin DU145 prostat kanseri hücre hattına olan etkilerini *integrinalfa2 (ITGA2)* ve *integrinbeta1 (ITGB1)* ekspresyonları, ayrıca hücre ölümü üzerine olan etkilerini Caspase-3 ve Beclin-1 üzerinden karşılaştırmayı amaçladık.

**GEREÇ VE YÖNTEM:** DU145 hücreleri çoğaltılarak dört gruba ayrıldı. Birinci gruba Amygdalin, ikinci gruba Dosetaksel, üçüncü gruba Amygdalin ve Dosetaksel birlikte verilerek 24 saat boyunca aktif maddelere maruz bırakıldı. Dördüncü gruba (Kontrol) herhangi bir madde verilmedi. *ITGA2 ve ITGB1* genlerinin mRNA düzeyleri Real-Time PCR yöntemiyle belirlendi. Hücre ölümünü değerlendirmek için immünositokimyasal olarak Caspase-3 ve Beclin-1 boyamaları yapıldı.

**BULGULAR:** Amigdalin, Dosetaksel uygulanan gruplarda *ITGA2* ve *ITGB*1 ekspresyonlarında artış görüldü (P<0.05). Amigdalin+Dosetaksel verilen grupta *ITGB1* ekspresyonundaki azalma anlamlıydı (P<0,001). Caspase-3 (P<0.05) ve Beclin-1 (P<0.05) immünoreaktivitelerinin her üç grupta kontrol grubuna kıyasla arttığı gözlendi.

**SONUÇ:** DU145 PK hücrelerinde Dosetaksel'in hücre ölümünü Amigdaline göre daha çok artırdığı, Amigdalin ve dosetakselin birlikte kullanıldığında *ITGA2* ve *ITGB1* ekspresyonlarını önemli şekilde azalttığı gözlemlenmiştir. Sonuçlarımız Amigdalin ve dosetakselin ikili tedavisinin prostat kanseri metastazlarının önüne geçebileceğini düşündürmektedir.

**ANAHTAR KELİMELER:** Amigdalin, Dosetaksel, Prostat kanseri, *İntegrin alfa2beta1*.

**ABSTRACT**

**OBJECTIVE:** Prostate cancer (PC) ranks second among cancer-related deaths in men, and most deaths are caused by metastasis. Integrins, which are cell surface receptors, play an important role in cancer metastasis. It has been shown that integrin alpha2beta1 expression is effective in cell adhesion, migration, and invasion by increasing binding to collagen I in metastatic PCs. Docetaxel chemotherapy is used in PC, but it is ineffective in advanced stages. Amygdalin is a cyanogenic glycoside commonly found in fruit seeds, there is conflict in the literature regarding its effectiveness in cancer treatment. We aimed to compare the effects of Amygdalin and Docetaxel treatments on the DU145 prostate cancer cell line on *integrinalfa2 (ITGA2)* and *integrinbeta1 (ITGB1)* expressions, as well as their effects on cell death, Caspase-3, and Beclin-1.

**MATERIAL AND METHODS:** Propagated DU145 cells were divided into four groups. Amygdalin was given to the first group, Docetaxel was given to the second group, and Amygdalin and-Docetaxel were given together to the third group. They were exposed to the active substances for 24 hours. The fourth group (Control) was not given any substance. mRNA levels of *ITGA2* and *ITGB1* genes were determined by the Real-time PCR method. Caspase-3 and Beclin-1 staining were performed immunocytochemically to evaluate cell death.

**RESULTS:** There was an increase in *ITGA2* and *ITGB1* expressions in the groups administered by Amygdalin and by Docetaxel (P<0.05). The decrease in *ITGB1* expression was significant in the group given Amygdalin+Docetaxel (P<0.001). Caspase-3 (P<0.05) and Beclin-1 (P<0.05) immunoreactivities were observed to increase in all three groups compared to the control group.

**CONCLUSIONS:** It was observed that Docetaxel increased cell death more than Amygdalin in DU145 PC cells, and when Amygdalin and Docetaxel were used together, *ITGA2* and *ITGB1* expressions were significantly reduced. Our results suggest that dual treatment of Amygdalin and Docetaxel may prevent prostate cancer metastases.

**KEYWORDS:** Amygdalin, Docetaxel, Prostate cancer, *Integrin alpha2beta1*.

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## INTRODUCTION

Prostate Cancer (PC) is the second most common cause of morbidity and mortality in men after lung cancer due to its high potential for metastasis (1). PC is a type of invasive carcinoma characterized by the expansion of organ volume and the transformation of normal glandular structure into preneoplastic lesions as a result of disruption of the balance between cell proliferation and cell death as a result of genetic and epigenetic changes in the prostate gland (2 - 4). It is known that PC-related deaths are largely due to metastasis (3 - 5). The clinically significant form of this disease, which can occur in highly heterogeneous forms, is locally advanced PC. Surgical intervention is the basic approach in the treatment of locally advanced PC with metastatic potential. In addition to surgery, endocrine therapy (chemical castration approaches) is applied with the use of anti-androgenic agents (6 - 8). Although current treatment has very effective results at the beginning, an aggressive phenotype defined as castration-resistant PC (CRPC), which occurs as androgen insensitivity, is encountered in 80-90% of patients after approximately 18-24 months. In CRPC, for which effective treatment is not currently possible, the average life expectancy is limited to a few months with the most effective chemotherapeutic agents as well as anti-androgenic drugs (9). DU145 is a cell line widely used in in vivo experiments to model CRPC and aggressive PC (10).

Docetaxel (Doce) shows its effect by inhibiting the polymerization of microtubules and nuclear translation of androgen receptors during the metaphase stage of mitosis (11). While Doce-based therapies are increasingly successful in treating PC following early diagnosis, they remain inadequate in patients with advanced and metastatic PC. Both the rapid occurrence of drug resistance and the high rate of toxicity have led researchers to develop new treatment protocols and search for agents that reduce these effects of Doce (such as Doce + angiogenesis inhibitors, + apoptotic agents, + antimetabolites, + proteasome inhibitors, etc.) (12 - 15). Amygdalin is a cyanogenic glycoside found in fruit seeds in nature. It is an agent that is being tested for use in alternative medicine for the treatment of anemia, asthma, high blood pressure, atherosclerosis, diabetes, migraine, and cancer (16 - 18). In human PC cell lines, Amygdalin is effective by increasing apoptosis through *B-cell lymphoma-2 Associated X protein (Bax) and B-cell lymphoma-2 (Bcl-2)* genes (19), and it has been shown to stop the growth of prostate cancer cells by delaying cell cycle progression (20). In these studies, it was determined that Amygdalin showed significant anti-tumor activity on PC cells, and therefore, it was stated that more studies were needed on its use for therapeutic purposes.

Integrins are a large family of proteins that form transmembrane heterodimers on the cell surface and mediate cell-matrix and cell-cell interactions (21). It affects the growth and spread of cancer cells by affecting cell migration, invasion, matrix degradation, and angiogenesis in cancer cells. Integrins also play important roles in the extracellular matrix composition and organization of the tumor stroma, cancer development, metastasis, and treatment resistance (22, 23). There are 24 known members of integrins, and the expression of these members, especially *ITGA2* and *ITGB1*, has been shown to increase in metastatic prostate cancers (24). After the expressions of *ITGA2* and *ITGB1* in many PC cell lines were presented, it was stated that all three of them, together with Integrin α-6, could be studied as PC stem cell markers (25). In another study, it was stated that the use of agents that reduce *ITGA2* expression would be the most appropriate approach in the treatment of this type of cancer (26).

Based on this information, the present study aims to evaluate the effects of the combined use of Doce and Amygdalin in the DU145 PC cell line on metastasis through *ITGA2* and *ITGB1* expressions and on cell death through Beclin-1 and Caspase-3 immunocytochemistry staining.

## MATERIALS AND METHODS

### Cell Passage

The DU145 prostate cancer cell line used in our study was obtained from the American Type Culture Collection. In an atmosphere of 37°C, 5% CO2, and 95%, cells were incubated in DMEM medium (Gibco, 11594486) containing 10% (v/v) fetal bovine serum (FBS, Sigma Aldrich, Germany, F9665), 5 mM glutamine (Capricorn Scientific, Germany, Cat. No: GLN-B), 100 U/ml

penicillin, 100 µg/ml streptomycin (Capricorn Scientific, Germany, Cat. No: PS-B). Cells were planted in a culture dish containing the medium in the bottles and incubated for 48 hours (27).

#### Removal And Preparation of The Cells

After achieving 80% confluency in the cells, 0.25% trypsin (Capricorn Scientific, Germany, Cat. No: TRY-3B) was added and removed. Trypsin is inactivated by adding a medium to the removed cells. The mixture was placed in a 15 ml falcon and then centrifuged at 400g for 5 minutes. After that 2400 µl of fresh medium was added to the pellet and mixed with a pipette. 10 µl of trypan blue was added to the 10 µl cell suspension taken here and mixed. Cell counting was performed by taking 10 µl of the resulting mixture. The total cell count was 6.08 x $10^5$ cells/ml and the number of viable cells was 5.87 x $10^5$ cells/ml. Cells were seeded from this suspension into a 12-well culture dish, with 200 µl per well. The wells were divided into Doce (100 nM, 24h) (Sigma-Aldrich, USA, Cat. No: 01885), Amygdalin (10 mg/ml, 24h) (Sigma, USA, Cat. No: 10050), Doce (100 nM) +Amygdalin (10 mg/ml) and Control groups, and drug applications were made at the specified doses (20, 28).

#### Real-time PCR Analysis

Total RNA was extracted from DU145 cells by using the PureZole reagent according to the manufacturer's protocol. (Biorad, USA, Cat. No: 732-6890). RNA amount and RNA purity were quantified by Nanodrop ND-1000 spectrophotometer V3.7 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). RNA samples were stored at -80°C until use. All the RNA samples were reverse transcribed into cDNA by iScript Reverse Transcription Supermix (Biorad, USA, Cat. No: 1708841) under the following conditions: One cycle at 25°C for 5 minutes, 46°C for 20 minutes, and 95°C for 1 minute. Real-time polymerase chain reaction (PCR) was performed after reverse transcription. *ITGA2* and *ITGB1* gene expression analysis was performed using Rotor-Gene Q (QIAGEN GmbH, Hilden, Germany). cDNAs that belong to each application group were added to iTaq Universal SYBR Green Supermix (Biorad, USA, Cat. No: 1725122) according to the manufacturer's protocol. Oligonucleotide primers were designed by Oligomere Biotechnology (Ankara, Türkiye) based on the following primer sequences **(Table 1)**.

**Table 1:** Primer sequences

| Gene | Primer sequences 5→3' |
|---|---|
| *ITGA2-F* | TGTGGTGAGGACGGACTTTG |
| *ITGA2-R* | CATCAACCGGCAGGGAGAAT |
| *ITGB1-F* | GCGCGGAAAAGATGAATTTACA |
| *ITGB1-R* | ACATCGTGCAGAAGTAGGCA |
| *GAPDH-F* | CATTGCCCTCAACGACCACTTT |
| *GAPDH-R* | GGTGGTCCAGGGGTCTTACTCC |

We used the following RT-PCR protocol for *ITGA2* and *ITGB1* gene mRNA analysis: 95°C for 30 seconds of initial denaturation followed by 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Melting curve analysis was performed for confirmation of single-product amplification at the end of the PCR. 65-95°C, 0.5°C increments at 5 sec/step. Each run has been performed in triplicate (**Figure 1)**.

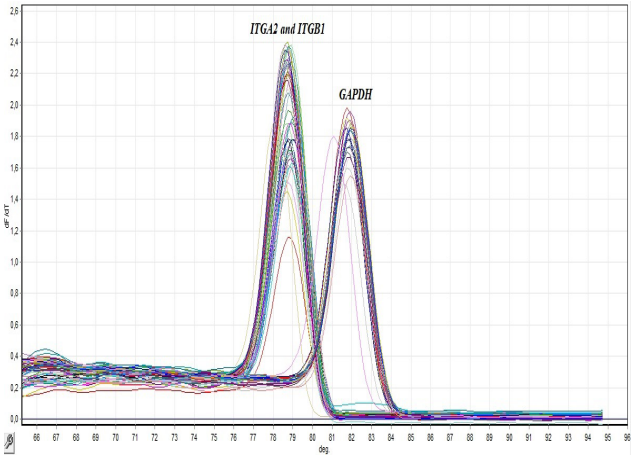


**Figure 1:** Example of *ITGA2*, *ITGB1* and *GAPDH* melting curve

REST 2009 Software is an independent instrument designed for the examination of gene expression data derived from quantitative, real-time PCR experiments. The evaluation or quantification of relative gene expression entails the utilization of reference genes' expression as a means to standardize the expression levels of genes of interest across diverse samples (29).

#### Immunocytochemistry

Cells forming treated with Amygdalin, Doce, Doce+Amygdalin groups, and the Control group were cultured in the 12-well chamber slide (Ibidi cells in focus, 81201). At the end of the 24th hour, the medium was removed. Cells were fixed with paraformaldehyde. After washing with phosphate-buffered saline (PBS). 0.1% Triton-X100 (Bio Basic Inc.) solution was added and kept on ice for 15 minutes. Then, it was incubated with 3% $H_2O_2$ (Emprove, 7722-

84-1) and washed three times with PBS. The blocking solution was then applied for 10 minutes. Cells were incubated with primary antibodies Caspase-3 (1/200, sc-56053, Santa Cruz Biotechnology) and Beclin-1 (1/200, sc-1142, Santa Cruz Biotechnology)) for 1 night at +4 $^{0}$C. The next day, it was washed 3 times with PBS. After treatment with a secondary antibody (Large Volume Anti-polyvalent HRP, Thermo Scientific), AEC chromogen (Thermo Scientific) was applied. Mayer's hematoxylin (Sigma Aldrich) was used for counterstaining, and the preparation was covered with a water-based sealer. Experiments were performed in three repetitions. Cells in the preparations were counted under a light microscope using the Image Analysis Program (NIS Elements, Japan). The evaluation was made by counting 500 different cells at X20 objective magnification. Scoring was done with an H-score, a semi-quantitative method. Stained cells were evaluated in terms of percentage and staining intensity was taken as a second criterion (30).

### Ethical Committee

This study was approved by the Afyonkarahisar Health Sciences University Clinical Ethics Committee with decision number 162 dated 01.06.2018.

### Statistical Analysis

For immunocytochemistry evaluation, all data is represented as mean ± standard error of the mean (SEM) throughout the study. Statistical comparisons were performed using one-way ANOVA followed by an appropriate post-hoc test (Tukey). Comparisons giving P values less than 0.05 were accepted as statistically significant. For PCR evaluation, All the data analyses were performed using REST 2009 V2.0.13 and SPSS v.19 Software (Qiagen, Hilden, Germany) using Pair Wise Fixed Reallocation Randomizasyon test (29) where P<0.05 is deemed to represent a statistically significant result.

## RESULTS

### *ITGA2* and *ITGB1* Genes Expression Levels

*ITGA2* and *ITGB1* gene expression levels in the DU145 cell line exposed to 10 mg/ml Amygdalin, 100 nM Doce, and 10 mg/ml Amygdalin +100 nM Doce for 24h were analyzed by Real-time PCR method using Rotor Gene-Q (Qiagen). *ITGA2* and *ITGB1* genes' mRNA levels for each group were determined according to the mRNA levels of *ITGA2* and *ITGB1* genes expressed in the control group. The *GAPDH* gene was used as a reference gene for normalization. *ITGA2* and *ITGB1* gene expressions were upregulated in 10 mg/ml Amygdalin application (1.473; 1.057, fold change, respectively). Upregulation of the *ITGA2* gene was statistically significant (P<0.05). *ITGA2* and *ITGB1* gene expressions were upregulated in 100 nM Doce application (1.233; 1.186, fold change, respectively). However, these upregulations were not statistically significant (P>0.05). *ITGA2* and *ITGB1* gene expressions were downregulated in 10 mg/ml Amygdalin+100nM Doce application (0.810, 0.710-fold change; respectively). Downregulation of the *ITGB1* gene is statistically significant (P<0.001) **(Figure 2)**.

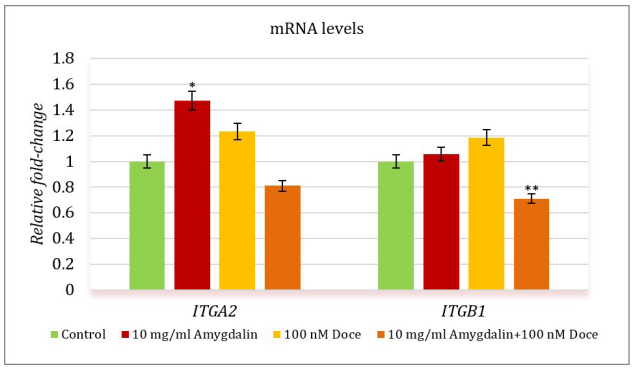


**Figure 2:** Relative mRNA expression of *ITGA2* and *ITGB1* in DU145 cells exposed to 10 mg/ml Amygdalin, 100 nM Doce and 10 mg/ml Amygdalin+100 nM Doce were given as fold regulation levels. *GAPDH* is reference gene for normalization.
*Represents the significance of P<0.05 **Represents the significance of P<0.001

### Immunocytochemistry Results

In the H-score evaluation made by immunocytochemical staining, the intensity of caspase-3 staining was increased in the amygdalin-administered group compared to the control group (P=0.0037). The increase in caspase-3 staining was highest in the Doce group. While the intensity of staining increased in the Amygdalin+Doce group compared to the Control and Amygdalin groups (P<0.001, P<0.001 respectively), its intensity was found to decrease compared to the group given only the Doce group, but it was not found to be statistically significant (P=0076). Beclin-1 staining intensity is parallel to caspase-3 staining intensity. The intensity of staining was seen to be highest in the Doce group. The

intensity of staining in the Amygdalin-administered group increased compared to the Control group (P=0.003). Although the staining intensity was found to be increased in the Amygdalin + Doce group compared to the control and Amygdalin groups (P<0.001, P=0.027 respectively), it was seen to decrease compared to the Doce group (P=0.003) **(Figure 3, 4)**.

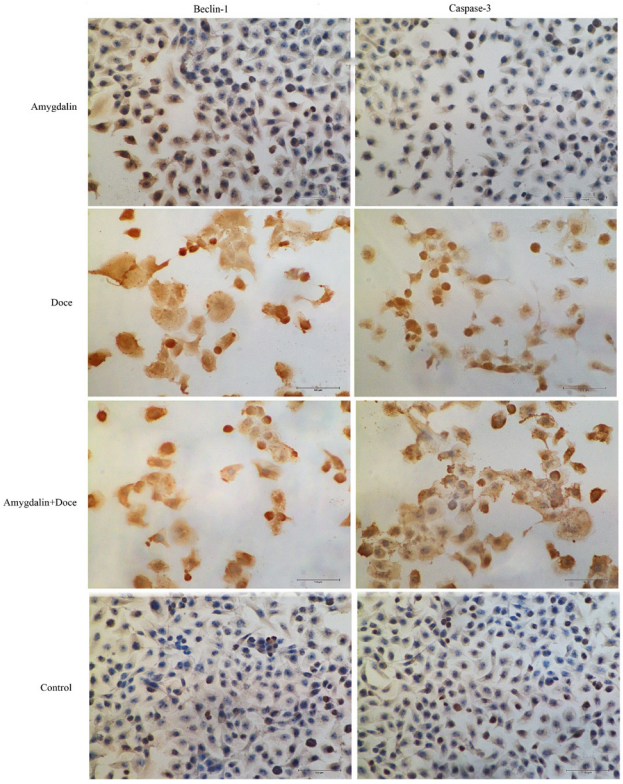


**Figure 3:** Beclin-1 and Caspase-3 immunocytochemical staining in all group. 20X magnification, scale bar 100 µm

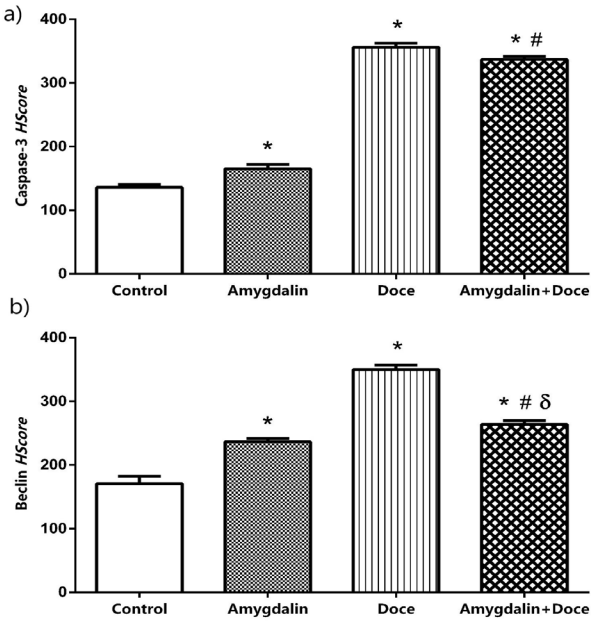


**Figure 4: a)** Graph showing H-score results caspase-3 **b)** Graph showing H-score results beclin-1, *significance compared to control, #significance according to amygdalin, #significance according to Doce* # Represents the significance of P<0.05

## DISCUSSION

PC, the most common solid tissue cancer among men in developed countries, is a serious, life-threatening health problem. While radical prostatectomy and/or radiotherapy are sufficient in the localized type of PC in which tumor cells are limited to the prostate gland, current treatments are not curative in locally advanced, metastatic PC and CRPC (31). PC-related deaths are largely due to metastasis and castration resistance (5). Therefore, new treatment approaches are needed. Preventing metastasis in cancer treatment is an important step in the treatment. The increased expressions of *ITGA2* and *ITGB1* in PC metastases are presented as evidence that these molecules are effective in metastasis (32, 33). Salemi et al. emphasized that the use of ITGA2 and ITGB1 inhibitor BTT-3033 in PC cell lines is an agent that can be used in the treatment of PC by reducing cell viability, proliferation, epithelial-mesenchymal transition, and increasing apoptosis (27). Accordingly, reducing the expressions of *ITGA2* and *ITGB1* is an important step in preventing the spread of cancer cells and in cancer treatment. Our study investigated the effects of Doce, used in the PC treatment, and Amygdalin, whose efficacy in cancer treatment is still under investigation, on *ITGA2* and *ITGB1* expression in DU145 PC cells.

Doce is an agent that is still used effectively in the PC treatment protocol today (12). Studies in PC cell lines have also shown that Doce is cytotoxic to these cells. Cristofani et al. reported that 100 nM doses of Doce reduced cell viability with maximal effect in PC3, DU145, and LNCaP PC cell lines (28). Liu et al. emphasised that it increased apoptosis in PC3 and DU145 PC cells, more so in LNCaP PC cells (34). In the study by Budman et al, Doce was combined with 18 different agents and applied to DU145, LnCaP and PC3 PC cell lines and its synergistic effect with different agents was evaluated. This study emphasised that Doce is the basic agent to be used effectively in PC treatment and that its efficacy can be enhanced with other agents (35). In our study, 100 nM was used for Doce, which is the maximum effective dose in Du145 PC cells in the literature, and it was found that it effectively increased cell death by observing a significant increase in caspase-3 and beclin-1 staining intensities in the Doce-treated groups.

Amygdalin is a compound belonging to the cyanogenic glycoside family. Its use in traditional medicine dates back hundreds of years. It is known that it was used as an expectorant agent in the 1800s (36). Amygdalin regained popularity in the 1970s and 1980s and was seen as a promising agent that could be used in cancer treatment. At this time, Cyanide was thought to be the active cancer-killing component in Amygdalin. It is assumed that Cyanide is released when amygdalin is broken down by enzymes in the cancerous tissue and kills the cancer cell, and that the enzyme called Rhodanese, which can detoxify Cyanide, is present in normal tissues but is missing in cancer cells. Thus, it was thought that cancer cells were selectively eliminated by Cyanide, while normal cells were not damaged (37). On the other hand, there are studies stating that cancer patients treated with high doses of Amygdalin show symptoms of cyanide poisoning, that blood Cyanide levels should be monitored throughout the treatment, and that Amygdalin does not have a therapeutic effect (38). Thereupon, the American Food and Drug Administration (FDA) declared Amygdalin as a toxic product that cannot be used as a medicine, and it was banned in the USA in 1979 (39). In recent years, with the search for supportive agents in cancer treatments, Amygdalin has come to the fore again and the need to investigate its anticancer activities has been emphasized (40). Treatment of metastatic PC is still a matter of research, and the effects of Amygdalin on this type of cancer have been studied in various ways. Makerevic et al. stated that Amygdalin has antitumor effects in PC cell lines by reducing tumor cell growth and suppressing colony formation, depending on the dose (20). Chang et al., in their study, found that the expression of the pro-apoptotic protein Bax increased, the expression of the anti-apoptotic protein Bcl-2 decreased, and the caspase-3 enzyme activity increased in the group treated with Amygdalin, thus showing that Amygdalin increased apoptosis in DU145 and LNCaP cell lines (19). In the present study, a dose of 10 mg/ml of Amygdalin was used, and it was observed that Caspase-3 and Beclin-1 staining intensities increased in the Amygdalin-applied group compared to the Control group, and therefore, Amygdalin increased apoptotic and autophagic cell death. However, this effect was found to be significantly lower than in the group given Doce alone. Again, the fact that cell death was less in the group in which the Amygdalin+Doce combination was applied compared to the group in which only Doce was applied, shows that the cytotoxic effect of Doce is greater than in the Amygdalin and combination treatment groups.

Integrins are structures that regulate intercellular and cell connection with the matrix, so they play important roles in cell migration and adhesion. In the study conducted by Mani et al., it was found that Amygdalin significantly reduced chemotactic activity, cell migration, and adhesion in DU-145 cells (41). In this study, it was found that Amygdalin application increased *ITGA2* and *ITGB1* and decreased *ITGA6* in DU-145 cell lines. Additionally, in this study, it was stated that the increase in *ITGA6* is an important indicator in chemotaxis, that the increase in *ITGA2* and *ITGB1* in the Amygdalin application shows a negative correlation for the DU145 line, and that the decrease in *ITGA6* for this cell line is more effective on metastasis. In another study, Tsaur et al. emphasized that amygdalin moderately increased *ITGβ1* in Taxan-resistant PC cell lines; therefore, its roles in PC are unclear and need to be investigated (42). Additionally, in our study, an increase in *ITGB1* expression, especially in *ITGA2*, was observed in the group to which we applied Amygdalin. These increases suggested that *ITGA6* expression should be supported with other agents, as in the study by Mani et al. (41). Our findings support the literature in this respect. The main target of metastatic PC cells is bone. Bone cells can produce proteins that facilitate the migration and metastasis potential of PC cells. Although it is known that there are increases in the expression levels of *ITGA2, ITGB1*, and *ITGA3* in this process (43, 44), there are still studies in the literature reporting that the effects of *ITGA2* and *ITGB1* on PC cells are not clear (45). It has also been reported that *ITGA2* and *ITGB1* expressions are downregulated in advanced PC (46, 47) In studies on the adhesion of prostate epithelial cells or human prostate carcinoma cells to type I collagen and the stroma of human bone marrow, the majority of studies report that the interactions are predominantly through *ITGA2* and *ITGB1* (48,

49). For this reason, it was thought that agents that treat PC would reduce *ITGA2* and *ITGB1*. Ojalill et al. found that *ITGA2* and *ITGB1* expressions increased in DU145 cells treated with 50 nM Doce. In their study, they attributed the increase in *ITGA2* and *ITGB1* to the increase in resistance in living DU145 cells. Similarly, they noted that surviving cells expressed more CD44 (stem cell marker), and cells with stem cell markers may be more drug-resistant (33). In the present study, there was an increase in *ITGA2* and *ITGB1* expressions in cells administered 100 nM Doce, but this increase was not found to be significant. We think that the fact that more cells die at the 100 nM dose explains this situation. Additionally, a decrease in *ITGA2* and *ITGB1* expressions was observed when 100 nM Doce and 10 mg/ml Amygdalin were administered to DU145 cells together. It shows that the treatment resistance observed when Amygdalin and Doce are administered separately is reversed when the two agents are administered together. The decrease in *ITGA2* and *ITGB1* expressions in combination treatment indicates a decrease in the metastasis ability of DU145 PC cells.

The most important limitation of our study is that the cytotoxicity test could not be performed because our project budget was limited. The cytotoxic effect of Doce was found to be high in accordance with the literature, but the cytotoxic effect of Amygdalin was less than in the literature. If a more optimum dose had been determined by the cytotoxicity test and this dose had been used, we would have been able to observe the optimum cytotoxic effect of Amygdalin and combined treatment. However, even at this dose, the reduction of *ITGA2* and *ITGB1* in combination therapy shows that it may be effective in the treatment by reducing the metastasis of DU145 cells. We mainly looked for clues to the possible synergistic effects of the Doce+Amygdalin combination, in recent studies. The findings of our study need to be supported by three-dimensional cell culture and experimental animal models.

In conclusion, the Amygdalin and Doce combination treatment reduced *ITGA2* and *ITGB1* expressions in the DU145 cell line. Therefore, it is thought that the application of Amygdalin in addition to Doce treatment may prevent metastases in addition, Amygdalin reduces the cytotoxicity of Doce, suggesting that their combined use may reduce the negative effects of Doce in non-cancerous cells, indicating that combination therapy is promising. In this context, more comprehensive studies in vitro and in vivo are needed.

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