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Research

# Investigation of the Effects of Juglone-Selenium Treatments on Epithelial-Mesenchimal Transition and Migration in BxPC-3 and PANC-1 Human Pancreatic Cancer Cells

## Juglon-selenyum Uygulamalarının BxPC-3 ve PANC-1 İnsan Pankreatik Kanser Hücrelerinde Epitelyal-mezenkimal Geçiş ve Migrasyon Üzerindeki Etkilerinin Araştırılması

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## ABSTRACT

**Objective:** Chemotherapy is still the most common and primary treatment option for treating pancreatic cancers because metastasis has already occurred at the time of diagnosis, difficulties of the operation due to its location, and lack of targeted treatment approaches. Moreover, the high chemoteuropatic resistance seen in pancreatic cancer makes research on alternative drug treatments mandatory. Juglone, a natural naphthaquinone found in members of the Juglandaceae family, has various pharmacological effects such as antiviral and antibacterial. It has also been shown to be an effective cytotoxic agent through the production of reactive oxygen species in studies conducted with cancer cell lines. Remarkably, selenium, an important trace element of the cell, was shown to inhibit metastasis, strengthen cell-cell attachments, and reduce angiogenesis. Based on these data, we investigated the effects of juglone-selenium (J/S) combination on epithelial-mesenchymal transition (EMT) and migration in PANC-1 and BxPC-3 cells.

**Methods:** The effects of juglone application in the presence of 2,5 μM NaSe at 5, 10, 15 and 20 μM concentrations on the expression of *FOXL1*, *VIM*, and *MMP-7* genes were determined by qPCR. In addition, the effects of J/S on the migration features of cancer cells were monitored by wound healing tests.

**Results:** According to qPCR results, J/S application showed different effects on *FOXL1*, *VIM*, and *MMP-7* gene expressions, which are critical for EMT, in both cell lines. According to the wound healing assay results, the migration of cancer cells was suppressed in both cell lines compared with the control group.

**Conclusion:** Consequently, our studies have supported that juglone can be an effective therapeutic agent on pancreatic cancer, and our findings also suggest that selenium can strengthen these anticancer effects of juglone.

**Keywords:** Juglone, selenium, pancreatic cancer, epithelial-mesenchymal transition (EMT), wound-healing assay

## ÖZ

**Amaç:** Pankreas kanserinde; tanı sırasında metastazın gerçekleşmiş olması, konumu nedeniyle cerrahi operasyonun zorluğu ve hedefe yönelik tedavi yaklaşımlarının bulunmaması sebebiyle, kemoterapi tedavi için halen en yaygın ve birincil tedavi seçeneğidir. Pankreas kanserinde görülen yüksek kemoterapötik direnç, alternatif ilaç tedavileri geliştirmek için yapılacak araştırmaları zorunlu kılmaktadır. Juglandaceae familyasının üyelerinde bulunan doğal bir naftakinon olan juglon, antiviral, antibakteriyel vb. çeşitli farmakolojik etkilere sahiptir. Kanser hücre hatları ile yapılan çalışmalarda reaktif oksijen türlerinin üretimi yoluyla etkili bir sitotoksik ajan olduğu da gösterilmiştir. Selenyum, hücrenin önemli bir eser elementidir ve dikkat çekici bir şekilde, metastazı inhibe etme, hücre-hücre bağlarını güçlendirme ve anjiyogenezde azalmaya yol açacak etkilerinin olabileceği gösterilmiştir. Bu verilerden yola çıkarak çalışmamızda, juglon-selenyum (J/S) kombinasyonunun PANC-1 ve BxPC-3 hücrelerinde epitelyal-mezenkimal geçiş (EMT) ve göç üzerindeki etkileri araştırıldı.

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**Gereç ve Yöntem:** 2,5 µM NaSe varlığında 5, 10, 15 ve 20 µM konsantrasyonlarda juglon uygulamasının *FOXL1, VIM, MMP-7* genlerinin ekspresyonu üzerindeki etkisi qPCR yöntemi ile belirlendi. Ayrıca J/S'nin kanser hücrelerinin migrasyon özellikleri üzerindeki etkileri yara iyileştirme testleri ile değerlendirildi.

**Bulgular:** qPCR sonuçlarımıza göre, J/S uygulaması EMT için kritik olan *FOXL1, VIM* ve *MMP-7* gen ifadeleri üzerinde her iki hücre hattında farklı düzeyde etki gösterdi. Yara iyileşme testi sonuçlarına göre ise, kontrol grubuna kıyasla her iki hücre hattında da migrasyonun baskılandığı görüldü.

**Sonuç:** Çalışmalarımız juglonun pankreas kanseri üzerinde etkili bir terapötik ajan olabileceğini desteklemektedir ve ayrıca bulgularımız selenyumun juglonun bu etkilerini güçlendirebileceğini düşündürmektedir.

**Anahtar Kelimeler:** Juglon, selenyum, pankreas kanseri, epitelyal-mezenkimal geçiş (EMT), yara iyileştirme testi

## INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of pancreatic cancer (PC), which is known to have poor prognosis and high mortality rates that cannot be detected at an early stage owing to lack of obvious symptoms during its development and metastasis (1,2). The 5-year survival rate is only 9%, and its incidence has been steadily increasing worldwide recently. It is considered as the fourth leading cause of cancer-related deaths in both men and women of all ages in the United States and is expected to be the second leading cause of cancer-related deaths in the western world by 2030 (2,3).

When PC is diagnosed, tumor metastases to regional lymph nodes are already seen in more than 80% of patients (4,5). Metastasis is a multistage and complex process, and can develop because of the interaction of steps such as adhesion, invasion, and angiogenesis (6). Gaining invasive properties of the tumor is the first step for metastasis. This process is called epithelial-mesenchymal transition (EMT) in epithelial-derived cancer cells. EMT is a biological process in which polarized epithelial cells undergo various molecular modifications, resulting in increased migration and mesenchymal characteristics such as invasiveness and resistance to apoptosis. The tumor microenvironment triggers EMT with many factors, including cytokines, growth factors, and chemokines (7,8).

The tumor microenvironment (TME), consisting of cancer cells, stromal cells, and extracellular components, plays a critical role in PC progression. Cancer cells and stromal cells such as pancreatic stellate cells and regulatory T-cells etc. can secrete extracellular components, including extracellular matrix (ECM), matrix metalloproteinase (MMP), growth factors, and transforming growth factor-β to maintain the microenvironment (7,8). ECM enables interactions between structural proteins and other matrix components necessary for the maintenance of tissue integrity (8). Two hallmarks of the PC microenvironment are intense desmoplasia and diffuse immunosuppression. These two features facilitate PC cell proliferation to evade the immune system through direct inhibition of antitumor immunity or induction of immunosuppressive cell proliferation (9). Desmoplasia creates a hypoxic microenvironment by increasing the functions of antiangiogenic factors. Hypoxia caused by an inadequate vascular system is essential for tumor aggressiveness, including metabolic reprograming apoptosis inhibition, continuous proliferation, resistance to treatment, invasion, and metastasis (7,10).

Unfortunately, only about 20% of patients are suitable for surgery, and in most patients, the tumor is very advanced locally or has spread to distant sites, hampering the precluding surgical intervention (11,12). Although radiation and immunotherapy are also potential options, chemotherapy is currently considered the main method to treat patients with PDAC who are not suitable for resection (13).

Gemcitabine, an antimetabolite, is an anticancer drug used to treat PC. Despite extensive research to develop new treatment methods for PC, gemcitabine still remains to be used as a chemotherapeutic agent because resistance to most conventional chemotherapeutic agents such as paclitaxel, doxorubicin, and cisplatin has been observed in clinical management (14,15). Although the combination of FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan) is the commonly used first-line systemic therapy, there is a need to find better combination therapies that can offer better efficacy and less toxicity at lower doses due to the limitations of their toxic effects (15). De novo chemoresistance to chemotherapeutic agents and/or radiotherapy is observed in PC treatment. However, current treatment options are not sufficient for curative outcomes. Therefore, there is a strong need to develop new therapeutic strategies for the treatment of PC (16-18).

Naturally occurring quinones such as thymoquinone, plumbagin, and juglone have been suggested as promising anticancer agents on different cancer cells (19). Juglone has been showed as having effective cytotoxicity through the production of reactive oxygen species (ROS) in studies with cancer cell lines. It is known that the increase in ROS level can contribute to apoptosis in cancer cells (20).

Various forms of selenium, an essential trace element of the cell, have potent antitumor activity by inhibiting the proliferation of various types of cancer, including breast, prostate, lung, melanoma, and cervical cancers (21). Furthermore, there are studies reporting the use of inorganic selenium and synthetic selenium compounds along with gemcitabine, a chemotherapeutic agent used in PC, to increase the growth inhibition of PC cells (22). The effect of sodium selenite (NaSe) on PDAC is considered to be clinically relevant as it is more potent than the other drugs tested. NaSe exerts its anticarcinogenic activity by directly oxidizing cellular free thiols (23). Moro et al. (24) reported that NaSe is a promising candidate for the treatment of PDAC because it has a significant cytotoxic effect on PDAC without any damage to non-malignant tissue components.

The fact that most PC patients are diagnosed while they are in the process of metastasis points to the importance of therapeutically targeting the metastatic stages in particular. For this purpose, in our studies to date, it has been shown for the first time that juglone is an effective agent in the invasion, adhesion, and metastasis processes in PC cell lines (25,26). Investigation of the effects of juglone-selenium (J/S) combination on different molecules involved in invasion and metastasis is necessary to develop new juglone- and selenium-based strategies for the treatment of PC. In our previous study, we showed that J/S has a cytotoxic and dose-dependent suppressive effect on invasion and metastasis in PANC-1 and BxPC-3 cells (27).

In this study, the effects of J/S application on epithelial-mesenchymal transition, and migration properties in PC cells were investigated. For this purpose, the expression levels of *FOXL1*, *vimentin (VIM)* and *MMP-7* were examined. In addition, the effects of J/S on migration properties were evaluated by the wound healing method.

## METHODS

### Cell Culture

PANC-1 and BxPC-3 PC cell lines received from the American Type Culture Collection (Rockville, Md) were cultured according to the manufacturer's instructions. Cells were cultured in DMEM (Gibco, UK) and RPMI (Gibco, UK) medium, respectively, including 10% fetal bovine serum (FBS) (HyClone, Fisherscientific, Canada) and 1% penicillin/streptomycin (Gibco, UK) at 37 °C and 5% $CO_2$ juglone and NaSe were supplied commercially (Sigma-Aldrich Chemical Company, USA).

### Determination of the Cytotoxic Effects of Juglone-selenium and MTT Assay

The MTT (reagent from Sigma-Aldrich, USA) assay used to determine the cytotoxic effects of J/S on human PC cells was performed as described in our previous study (27).

### Wound-healing Assay

A wound-healing assay was used to evaluate the metastatic and proliferative behaviors of PC cells following J/S combination therapy. For this purpose, cells were seeded in 6-well cell culture plates to cover approximately 70-80% of the wells. Cells were cultured in DMEM or RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin for 24 hours (h). One day later, at cell seeding time, the cells were transferred to a starvation medium (starvation medium contains 0% FBS and 1% pen/strep). After 16 h of incubation in the starvation medium, the cells in the wells were scraped with a 200 µL pipette tip to make a straight-line wound. After scratching, the detached cells were washed by gently rinsing the well with PBS, and the determined J/S concentrations were added to the medium and incubated for 48 h in an atmosphere of 5% $CO_2$ at 37 °C. At least 4 repetitions were performed for each group. Wounds were visualized under a microscope at 0, 24, and 48 h.

### Gene Expression Analysis

The effects of juglone application at 5, 10, 15, and 20 µM concentrations in the presence of 2,5 µM NaSe on the expression of *FOXL1*, *VIM*, and *MMP-7* genes were determined by quantitative real-time polymerase chain reaction (qPCR). RNA was isolated from all J/S-treated and control groups and translated into cDNA. Suitable primers for the targeted genes and for the β-actin gene, the housekeeping gene in our study, were used for qPCR.

### Statistical Analysis

The $2^{-\Delta\Delta CT}$ method was used to analyze the relative changes in gene expression. Expression changes as 2-fold increase and decrease were considered significant. For the migration assay, the wound area was calculated using ImageJ software. The area comparison between the control and J/S combination-treated groups was done by imaging cells at 0, 24, and 48 h. Wound closure rates were calculated as a percentage by comparing the remaining areas after 24 h and 48 h.

### Ethics Statement

This study was approved by the Local Ethical Committee of Selçuk University Faculty of Medicine (decision no: 2019/256, date: 16.10.2019) and conducted in accordance with the Declaration of Helsinki.

## RESULTS

### Cytotoxic Effects of Juglone-selenium

For PANC-1 and BxPC-3 cell lines, IC50 doses of J/S were determined as 16.3 µM and 15.17 µM for 24 h, respectively, using the GraphPad Prism 6 program by Arikoglu et al. (27). Based on these IC50 doses, the J/S treatment doses used in our experiments were 5, 10, 15 and 20 µM.

### Wound-healing Assay

The effect of the J/S combination on the invasive ability was investigated using the wound healing assay. According to the wound healing assay, it was observed that migration was suppressed in both J/S-treated cell lines compared with the control group. The results of the wound healing assay are shown as percentage rates in Table 1 and as images in Figures 1 and 2. However, cell losses were also observed with suppression of migration in BxPC-3 cells (Figure 2). In microscopic evaluations, it was observed that these cell losses were not due to cell death; rather, cell loss was due to the loss of their adhesion properties, separating and disrupting tissue integrity.

### Effect of Juglone-selenium Applications on Gene Expression

Gene expression analysis results related to the effects of the J/S combination on gene expression were shown in Figures 3 and 4. While evaluating the results, changes of two fold and above were considered significant. Following J/S treatment, the expression of *FOXL1*, *VIM*, and *MMP-7* genes was analyzed by qPCR. After 24 h of J/S treatment in the PANC-1 cell line, gene expression levels were determined and compared with the control group. *MMP-7* gene expression increased 2.7-fold, 8.2-fold, and 6.4-fold at 10, 15, and 20 µM J/S treatments, respectively; and *VIM* gene expression increased 2.3-fold, 3.3-fold, and 2.4-fold at 10, 15, and 20 µM doses, respectively, while *FOXL1* gene expression decreased 12.3-fold, 8-fold, 11.3-fold, and 4.6-fold in 5, 10, 15, and 20 µM treatments (Figure 3).

**Table 1. Migration properties of PANC-1 and BxPC-3 cells and the percent closure rates of wound healing assay after J/S treatments (%)**

| | PANC-1 | | BXPC-1 | |
|---|---|---|---|---|
| | 24-hour | 48-hour | 24-hour | 48-hour |
| Control | 23.98 | 36.46 | 38.72 | 96.56 |
| 5 µM | 28.17 | 45.14 | -38.93 | -34.56 |
| 10 µM | 5.78 | 9.78 | -16.77 | -4.75 |
| 15 µM | 12.08 | 14.21 | -2.94 | -16.80 |
| 20 µM | 3.77 | 6.34 | -6.23 | -10.77 |

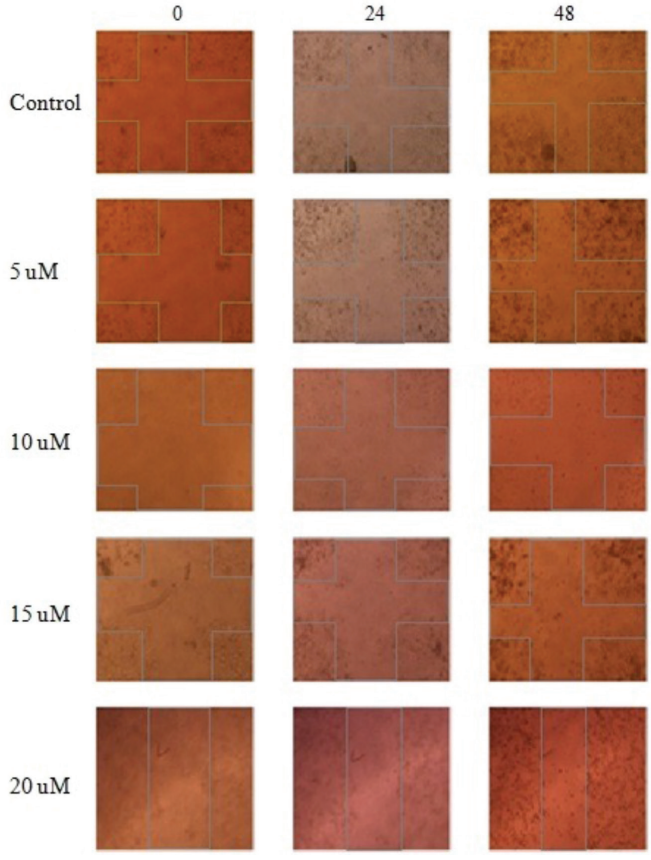


**Figure 1.** Microscopic images of the effects of J/S treatments on the migration properties of PANC-1 cells

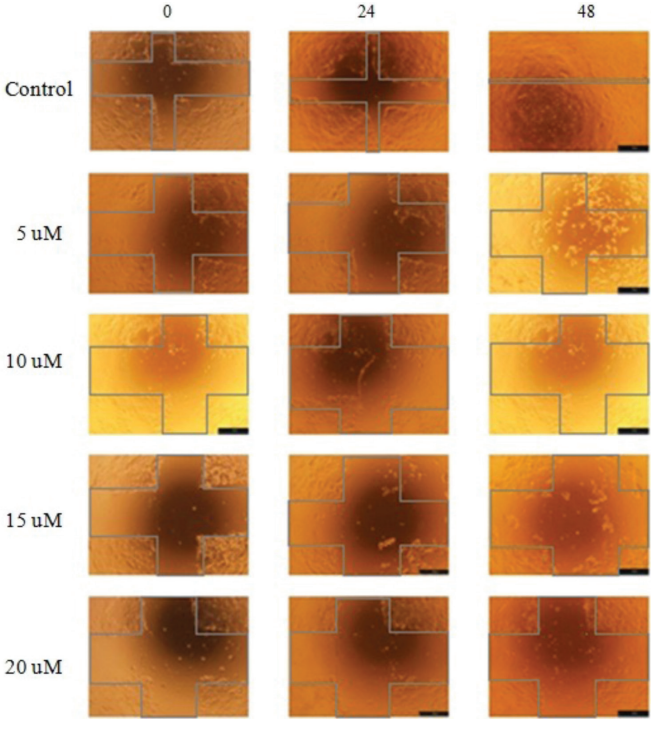


**Figure 2.** Microscopic images of the effects of J/S treatments on the migration properties of BxPC-3 cells

*MMP-7* gene expression in BxPC-3 cells showed a 2.5-fold, 5.5-fold, 6.5-fold increase, and 3.2-fold decrease with 5, 10, 15, and 20 µM treatments, respectively. Unlike PANC-1 cells, after J/S treatment, *VIM* expression decreased at all doses with significant levels as 7.3-fold, 3.9-fold, 4.8-fold, and 6.9-fold, respectively. *FOXL1* gene expression decreased significantly at all doses as 8.5-fold, 3.3-fold, 3.8-fold and 6.6-fold decreases in 5, 10, 15 and 20 µM treatments, respectively, similar to that observed in PANC-1 cells (Figure 4).

## DISCUSSION

Due to the late diagnosis, rapid development, and *de novo* chemoresistance of PC cells against cytotoxic chemotherapeutic agents and/or radiotherapy, difficulties arise for treating PC, leading to a high mortality rate (28). The interaction between cancer stem cells and TME in PC causes poor prognosis in patients with multifactorial chemoresistance (29). Despite the most frequently altered genes (*KRAS, TP53, CDKN2A,* and *SMAD4*) being determined in PC, limited recovery is observed due to the resistance to chemotherapy and radiotherapy as well as immune therapy, which leads to searches for new approaches to treat PC focusing on various phytochemicals (30-32).

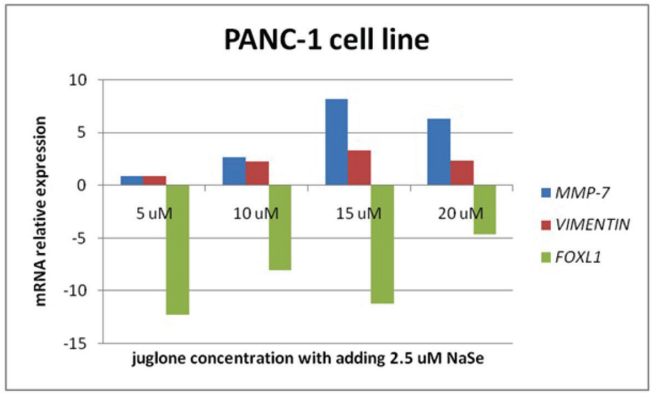


**Figure 3.** Effects of J/S treatments on the expressions of target genes in PANC-1 cells

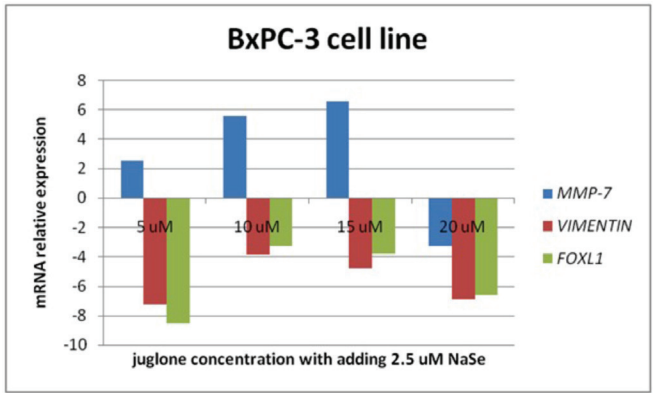


**Figure 4.** Effects of J/S treatments on the expressions of target genes in BxPC-3 cells

Juglone, a secondary metabolite obtained from various parts of Juglandaceae walnut trees, such as leaves, roots, shells, and fruits, is an effective cytotoxic agent that induces ROS and causes a change in redox homeostasis in the cell, leading to apoptosis as well as necrotic cell death. The cytotoxic effects of juglone on cancer cells have been demonstrated by several studies on different cancer cells such as human prostate cancer cells (LNCaP), human gastric cancer cells (SGC-7901), human leukemia cells (HL-60), and human colon carcinoma cells (HCT-8 and HCT-15) (33-37). Juglone is also an effective inhibitor of Pin1 (peptidyl-prolyl isomerase), which plays a role in cell cycle control and is overexpressed in most cancer types (38).

Selenium, an essential trace element, plays an important role in many basic physiological processes by participating in the structure of selenoproteins (39). To date, the therapeutic effects of organic and inorganic forms of selenium components on human cancers have been investigated (22,40-42). Selenium-containing compounds, which participate in basic biological processes ranging from apoptosis to immunity, including cellular antioxidant defense, DNA protection, and repair, also function as antioxidant enzymes such as thioredoxin reductase and glutathione peroxidase (41-42). Selenium has also been reported to have an anti-cancer effect by mechanisms such as accelerating oxidation in cancer cells, modulating carcinogenesis metabolism, regulating the Trx redox system and inhibiting angiogenesis, as well as inducing apoptosis secondary by creating ROS (40-42).

The results indicating that selenium inhibits invasion and metastasis in cancer cells also suggest that selenium is a promising chemotherapeutic agent for treating PC (41). It has been reported that high levels of selenium in the blood of patients who take dietary selenium supplements with PC significantly reduce the incidence and mortality of cancer and increase the DNA damage repair response (40,43). In a Phase I clinical trial evaluating the safety and efficacy of intravenously administered NaSe, it was found that NaSe can be safely tolerated in humans up to 10.2 mg/m (44). Moro et al. (24) revealed that 15 µM NaSe, which they determined as the application dose, is significantly below the maximum tolerated dose reported for humans. In addition, according to transcriptome data analysis, it decreases the expression of genes having metastatic potential (*CEMIP, DDR2, PLOD2, P4HA1*) known to drive PDAC growth. They reported that it significantly increased the expression of genes (*ATF3, ACHE*)

that induce cell death. In the same study, it was determined that NaSe has extraordinary efficacy and specificity against drug-resistant PC by reducing de novo tumor cell growth while protecting non-neoplastic tissues in an organotypic tissue culture model (24).

FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan) and gemcitabine/nab-paclitaxel combinations are commonly used as first-line systemic therapy for PDAC (14,15). Radiation therapy is recommended following the chemotherapeutic treatment of PCs, and new combinations are being investigated to enhance chemotherapeutics (45). Wooten et al. (22) showed that selenium has an antimetastatic effect by creating a synergistic effect with gemcitabine in their study on BxPC-3 cell lines grown on 2D and 3D platforms.

FOX proteins are a large family of transcription factors that have important functions in different biological processes such as cell cycle control, differentiation, epithelial proliferation, and development of the gastrointestinal tract (46,47). It has been hypothesized that low *FOXL1* expression is associated with an increase in cancer-related mortality and therefore affects PC progression. It has been reported that *FOXL1*, especially expressed in the gastrointestinal tract, is a tumor suppressor gene, and studies have shown that the expression of *FOXL1* leads to apoptosis of cancer cells by inducing *TRAIL* expression (48) and triggers apoptosis by disrupting mitochondrial transmembrance depolarization by inducing cytochrome c release (49). In osteosarcoma, overexpression of *FOXL1* has also been reported to suppress proliferation by increasing the expression of p27 and p21, thus stopping the cycle (49). In addition, *FOXL1* represses *ZEB1* transcription by binding directly to the promoter region, which is one of the activators of epithelial -mesenchymal changes that affect cancer cell invasion and aggressiveness (48).

Our previous study (27) reported that J/S has a cytotoxic and dose-dependent suppressive effect on invasion and metastasis in PANC-1 and BxPC-3 cells. Because the increase in *FOXL1* expression was shown in the literature to be associated with suppression of invasion and adhesion ability; an increase in *FOXL1* expression was expected after J/S application in our study. However, contrary to expectations, *FOXL1* expression decreased at all doses in both cell lines. Chen et al. (50) reported that higher *FOXL1* expression in gliomas is associated with a worse prognosis, unlike other malignant tumors. Our study suggests that the decrease in the expression level of *FOXL1* is important in PC, possibly because suppressing the expression of *FOXL1* by J/S may make the poor prognosis of PC more moderate.

EMT is a physiological process in which epithelial cells transform into mesenchymal phenotype cells through certain physiological procedures and under certain conditions. In the EMT process, the epithelial cell loses its characteristic cell-cell connections, polarity changes, keratin intercalation in the cytoskeletal system transforms into VIM intercalation, and becomes isolated from neighboring cells and mobile and anoikis-resistant cells (51). Apart from apoptosis, the most well-known mode of cell death has been anoikis and ferroptosis recently. Ferroptosis is an iron-dependent and non-apoptotic form of cell death that is commonly involved in human pathological conditions, including cancer therapy resistance and brain injury (52). Karki et al. (53) showed that juglone also induces cell death by ferroptosis in the *KRAS*-mutated MIA PaCa-2 pancreatic cell line. *VIM* is an important gene involved in the EMT and metastasis. According to our results, J/S administration increased *VIM* expression in PANC-1 cells and a significant decrease in BxPC-3 cells. This opposite effect of J/S may be because of the different origins and different cell properties and behaviors of the pancreatic cell lines. In addition, unexpected increases in *VIM* expression in PANC-1 cells after J/S application may be caused by different unknown functions of *VIM*.

Another key gene investigated in our study is the *MMP-7* gene, which is a matrix metalloprotease that plays an important role in invasion. MMP-7 is especially secreted from epithelial cells and is overexpressed in many types of cancer (54) including PC (55-57). Therefore, suppression of *MMP-7* expression may be an important strategy for inhibiting PC metastasis. Avcı et al. (25) showed that juglone has suppressive effects on *MMP-2* and *MMP-9* expression in PANC-1 and BxPC-3 pancreatic cells. In our study, it was determined that J/S did not have a suppressive effect on *MMP-7*. There was an increase in *MMP-7* expression in both cells at all doses, whereas there was a decrease in BxPC-3 cells only at the 20 μM dose. This result is almost consistent with the results of Gokturk et al. (26) who reported increased *MMP-7* expression in PANC-1 and BxPC3 cells. We suggest that the increase in the expression of the *MMP-7* gene caused by J/S may create an antimetastatic effect differently than we anticipated. Powell et al. (57) stated that juglone induces apoptosis because of increased expression of the *MMP-7* gene, caused by the production of membrane-bound FAS ligand and binding to the FAS receptor, which is the main mediator of epithelial cell apoptosis. Endothelial and epithelial cells can continue to survive and proliferate when in contact with the ECM. If this connection is broken, it can trigger anoikis both *in vitro* and *in vivo*. Therefore, it is possible that the increase in *MMP-7* gene expression, which is responsible for the degradation of important matrix

proteins such as collagen and fibronectin, may trigger anoikis, leading to tumor cell apoptosis.

According to the wound healing assay, migration was suppressed in both cell lines compared with the control group. Migration in PANC-1 cells decreased in a dose-dependent manner. Although migration of BxPC-3 cells was suppressed at all doses, it was evaluated that this dramatic decrease was because of cell death due to the toxicity of J/S treatment. In the adhesion tests performed in our previous study (27), it was determined that J/S treatment decreased the adhesion levels of PC cells in a dose-dependent manner, consistent with our current wound-healing results.

## CONCLUSION

It has been shown in our previous studies that juglone has cytotoxic and antimetastatic effects on PANC-1 and BxPC-3 cells, and selenium strengthens the cytotoxic and antimetastatic properties by suppressing invasion and metastasis. In this study, we evaluated the effects of J/S treatment on adhesion and invasion by evaluating the expression of *FOXL1*, *VIM,* and *MMP-7*, which are critical genes for metastatic processes and wound healing analysis. The potent antimetastatic effects of juglone on PC cells have been demonstrated in our previous studies and this study. Selenium also strengthens these effects. When all these studies are evaluated together, juglone should be evaluated as an important therapeutic candidate, especially for the development of antimetastatic agents for treating PC, which is known to have silent progress and metastasis at the time of diagnosis. For a more comprehensive evaluation of the juglone and J/S combination, which clearly showed the antimetastatic effects with the invasion, adhesion, and wound healing tests, more other genes and their protein products that have a role in metastatic processes should be investigated.

### ETHICS

**Ethics Committee Approval:** This study was approved by the Local Ethical Committee of Selçuk University Faculty of Medicine (decision no: 2019/256, date: 16.10.2019) and conducted in accordance with the Declaration of Helsinki.

**Informed Consent:** The study does not require patient consent.

### Authorship Contributions

Concept: H.A., Design: D.E.K., Data Collection or Processing: F.G., F.B., Analysis or Interpretation: D.E.K., H.A., Literature Search: F.G., F.B., Interpretation: D.E.K., Technical Studies: F.G., Cell Culture: F.B., Critical Review: H.A., Writing: D.E.K., H.A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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