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RESVERATROL SUPPRESSES CELL VIABILITY AND INVASION IN PANCREATIC CANCER CELLS

RESVERATROL, PANKREAS KANSERI HÜCRELERINDE HÜCRE CANLILIĞINI VE INVAZYONUNU BASKILAR

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ABSTRACT

Introduction: Pancreatic cancer is a challenging disease to diagnose and treat due to its asymptomatic progression and high mortality rate. It is often identified at an advanced stage, where it typically metastasizes to other organs. To address these challenges, scientific studies explore various treatments, including natural and anticancer compounds like resveratrol. This study investigates the effects of resveratrol on pancreatic cancer cells.

Methods: The study used PANC-1 pancreatic cancer cells cultured *in vitro*. Cell viability in resveratrol-containing media was measured using the MTT assay. Safe, toxic, and IC50 doses for a 24-hour period were determined. Morphological changes were analyzed using hematoxylin-eosin staining. In invasion experiments, a wound-healing assay was performed by scraping cells in a 6-well plate and photographing their movement in resveratrol media.

Results: Resveratrol increased cell viability at concentrations up to 10 µM, showed similar viability to the control at 50 µM, and reduced viability below 50% at 200 µM. The IC50 dose was calculated as 189.5 µM. At concentrations above 100 µM, significant disruptions in nuclear and cytoplasmic structures were observed. Additionally, cell movement ceased at 100, 150, and 189 µM doses, showing dose-dependent inhibition of cell invasion ($p<0.05$).

Conclusions: Resveratrol, a naturally occurring compound found in many fruits and plants, demonstrated cytotoxic and anti-metastatic effects on PANC-1 cells. This study highlights its potential role in pancreatic cancer treatment, emphasizing the importance of continued research to combat this aggressive disease effectively.

Keywords: PANC-1 cells, cell viability, anti-metastatic, resveratrol, anti-proliferative

ÖZET

Giriş: Pankreas kanseri, asemptomatik ilerlemesi, teşhis ve tedavisinin zor olması nedeniyle yüksek ölüm oranlarına neden olan bir hastalıktır. Genellikle ileri bir aşamada tanımlanır ve bu nedenle başka organlara metastaz yapar. Bu hastalığın tedavisindeki zorluklar nedeniyle araştırmacılar bazı antikanser özelliği yüksek moleküllerin de pankreas kanserine etkisini araştırmışlardır. Çalışmamızda, bu moleküllerden biri olan resveratrolün pankreas kanseri hücrelerine olan potansiyel etkilerini araştırdık.

Yöntemler: Çalışmamızda, *in vitro* kültüre edilmiş PANC-1 pankreas kanseri hücreleri kullanılmıştır. Resveratrol içeren midyumlarda hücre canlılığı, MTT testi kullanılarak ölçülmüştür. Resveratrolün 24 saatlik güvenli, sitotoksik ve IC50 dozları belirlenmiştir. Morfolojik değişiklikler hematoksilin-eozin boyama kullanılarak analiz edilmiştir. İnvazyon deneylerinde, 6 kuyucuklu plakalarda yara iyileştirme testi gerçekleştirilmiştir.

Bulgular: Resveratrol 10 µM'a kadar hücre canlılığını arttırmış, 50 µM'da kontrole benzer canlılık göstermiş ve 200 µM'da hücre canlılığını %50'nin altına düşürmüştür. IC50 dozu 189,5 µM olarak hesaplanmıştır. 100 µM'nin üzerindeki konsantrasyonlarda, nükleer ve sitoplazmik yapılarda önemli bozulmalar gözlenmiştir. Ek olarak, resveratrol hücre hareketini 100, 150 ve 189 µM'larda durdurarak hücre hareketinde doza bağlı inhibisyon göstermiştir ($p<0,05$).

Sonuç: Bu çalışma ile kanser proliferasyonunu ve hareketini inhibe edici etkiye sahip bir bileşikle pankreas kanseri tedavisine katkıda bulunma amaçlanmıştır. Birçok meyve ve bitkide bulunan ve doğal olarak oluşan bir bileşik olan resveratrol, PANC-1 hücreleri üzerinde sitotoksik ve anti-metastatik etkiler göstermiştir. Çalışmamız sonuçlarına göre, bu alanda daha fazla derinlemesine çalışmalara ihtiyaç olduğu açıktır.

Anahtar Kelimeler: PANC-1 hücreleri, hücre canlılığı, anti-metastatik, resveratrol, anti-proliferatif

INTRODUCTION

Pancreatic cancer is a type of cancer characterized by its aggressive nature, late-stage diagnosis, and poor prognosis. More than 200,000 people die from pancreatic cancer each year. According to the American (US) Cancer Society, approximately 56,000 pancreatic cancer cases were diagnosed in the USA in 2019, and approximately 45,000 of them died. This rate ranks third after lung cancer and colorectal cancer. Increases in pancreatic cancer-related death rates have been reported in America and European countries, indicating that the disease is widespread worldwide. Pancreatic cancer incidence varies by gender, with the incidence being 50% higher in men than in women. This cancer is more common in older adults. The etiology of

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pancreatic cancer includes familial transmission, smoking, chronic pancreatitis, diabetes, excess weight, and heavy exposure to certain chemicals (carcinogens). Currently, surgery, chemotherapy, radiotherapy and palliative care methods are used in the treatment of pancreatic cancer (1, 2). Increasing and developing diagnostic and treatment options for this type of cancer, which is very difficult to diagnose and treat, is a very important goal for scientists. More basic and clinical research is needed on these issues. Many scientific studies have shown that eating foods high in antioxidants has significant effects on reducing cancer risk and progression (3, 4).

Resveratrol is a polyphenol compound found in many fruits, especially in grapes. It has antioxidant, anti-cancer, chemo-preventive, antiviral, cardio-protective, anti-aging, anti-nociceptive, and life-prolonging properties that have been shown in many studies. Recently, many studies have shown that Resveratrol has inhibitory effects on the proliferation and metastasis of cancer cells (5, 6). Resveratrol has also been shown to have significant results in pancreatic cancer. Resveratrol is effective on pancreatic cancer by acting on many pathways and steps in cell physiology (proliferation, metastasis, apoptosis, pancreatic cancer stem cells, chemoradiosensitization, etc.). For example, Jiang et al. found resveratrol has been shown to inhibit the proliferation and metastasis of pancreatic cancer cells via fibroblasts in cancerous tissue. (7, 8).

Our aim in this study is to investigate the efficacy of resveratrol, which has been shown to have anticancer properties in many studies, in pancreatic cancer, which is very difficult to treat, on the basis of cell viability, morphology and cell movement, which is an important feature in metastasis, on the PANC-1 cell line.

METHODS

Cell Culture

PANC-1 cells (ATCC, CRL-1469) were cultured in 75 cm2 flasks (TPP, 90076) in DMEM high glucose, with L-glutamine (Capricorn, DMEM-HA) containing 10% fetal bovine serum (Sigma, F9665) and 1% penicillin/streptomycin (Sigma, P4333) at 37°C in an incubator (PHCbi) containing 5% CO2. When the cells in the flasks reached 70% density, the cells were subcultured or taken into the experiment.

Preparation of Resveratrol Doses

For preparing experimental resveratrol (Sigma, R5010) doses, resveratrol was dissolved in dimethyl sulfoxide (Sigma, 41640) to create a stock solution. Experimental doses were prepared by diluting from this stock solution. The experimental doses were as follows; 2, 2.5, 5, 10, 40, 50, 100, 150, 200 μM. The stock solution of resveratrol was stored at -20 degrees.

Cell viability assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, M5655) test was used to determine cell viability in the experiments. In MTT tests, 9 doses of resveratrol (2, 2.5, 5, 10, 40, 50, 100, 150, 200 μM) were used. When the cells in the flasks reached sufficient maturity, they were collected from the flasks, counted with a thoma slide (Marienfeld Superior) and seeded into 96-well cell culture plates at 5000 cells per well (TPP, 92096). After allowing the cells to adhere for 24 h, the media were removed by inverting the plates. And then the cells were exposed to different resveratrol concentrations for 24 h. After 24 hours, the resveratrol-containing media were removed and media containing 0.5 mg/ml MTT were added to the wells and incubated for 2 hours. After the MTT-containing media were removed, 0.1 ml DMSO was added to the wells. The optical density of the wells was measured at 570 nm using an ELISA reader (BioTek 800TS). The cell viability rate of the control group that did not receive resveratrol was accepted as 100%. MTT analyses were repeated three times to increase the reliability of the results.

Morphological examination of PANC-1 cells

Hematoxylin-eosin (Moslab) staining was performed to examine the morphological changes of PANC-1 cells. First, sterile coverslips were placed on the bottoms of 6-cell culture flasks (TPP, 92006). Then, the cells collected from the flasks were counted and 3x105 cells seeded into these 6-well plates. After the cells adhered to the coverslips, different doses of resveratrol (40, 50, 100, 150 and 189 μM) were added to the cells' media and waited for 24 hours. At the end of the period, the media with resveratrol were removed and the cells were fixed in 10% formaldehyde (Sigma, F8775) (15 min at 37 °C). After fixation, the coverslips were washed three times with phosphate buffer (PBS) (Sigma, D5652). Cells were incubated with Triton X-100 (0.2%) (Sigma, T8787) for 5 min and then rinsed three times with PBS. After the cells were stained with hematoxylin-eosin, they were covered with a water-based mounting medium. The photographs of the coverslips were captured under a light microscope.

Cell migration assay

Equal numbers of PANC-1 cells (3x105 cells) were seeded in 6-well plates. After the cells covered the base, cells were scraped in a straight line at a certain point on the base of the plate with a 200 μL pipette tip (Eppendorf, 0030000870). Mediums containing doses of resveratrol (40, 50, 100, 150 and 189 μM) were added to the wells. Images of the striped area were taken at 0 and 48 hours with a 10X objective under an inverted microscope (IX71 mikroskop ve DP70 kamera, Olympus).

Statistical analysis

The experiments were conducted in triplicate, and data analysis was performed using statistical package software (IBM SPSS Statistics 21). Significance was determined at p<0,05. The normal distribution of data was assessed using the Shapiro–Wilk test. For normally distributed data, one-way ANOVA was applied, followed by Tukey's multiple comparison test.

RESULTS

Viability Results

In the MTT experiment, the 24-hour effectiveness of resveratrol on PANC-1 cells was examined. Resveratrol increases cell viability at low doses. Cell viability decreases with increasing doses. When we examine in terms of dose, the highest cell viability was determined at 10 μM (p<0.05). Cell viability at 50 μM was similar to the control group (p>0.05). Cell viability in the 100, 150 and 200 μM treated groups was as follows: 81%, 73%, 36%, respectively (p<0.05). The 24-hour IC50 dose of resveratrol in PANC-1 cells was determined as 189.5 μM. (Figure 1).

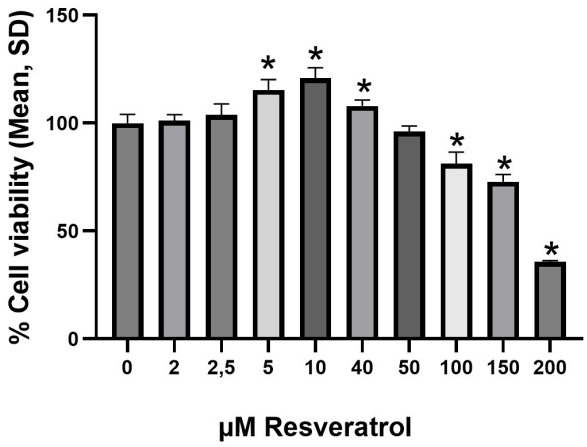

Figure 1. MTT results. Resveratrol increases cell viability up to certain doses (10μM). After 10μM, viability decreases in a dose-dependent manner. After 40μM, viability falls below the control group (p<0.05).
*Significantly different from untreated cells (Control group).

Effects of resveratrol on PANC-1 cell morphology

When the effects of resveratrol on PANC-1 cell morphology were examined, it was observed that resveratrol had no effect on cell morphology and density at 40 and 50 μM, and the cells had euchromatic nuclei and eosinophilic cytoplasm. The cells had similar cell sizes. At these two doses, cell morphology was similar to the control group. It was determined that cell morphology was impaired in a dose-dependent manner at 100, 150 and 189 μM. It was determined that the cells physically shrank and contracted, their nuclei became heterochromatin and cell density decreased (Figure 2).

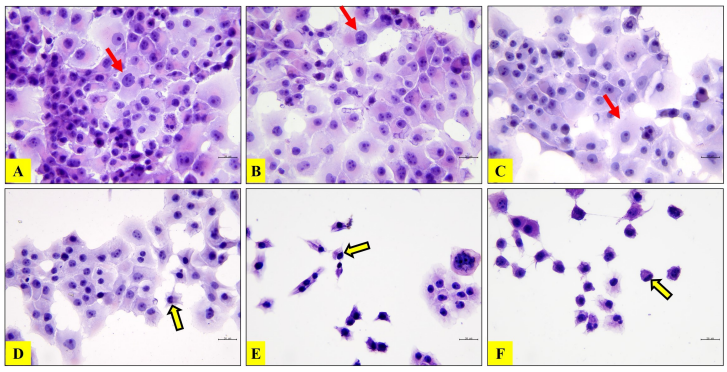

Figure 2. HE staining of PANC-1 cancer cells treated with resveratrol at different deses. The groups in the figure are as follows: A:Control, B:40μM, C:50μM, D:100μM, E:150μM and F:189μM resveratrol treated. Cells treated with 40 and 50 μM resveratrol were similar to the control group in terms of morphology, and cells were observed to have normal appearance (Thin arrow). Cells treated with 100, 150 and 189 μM were found to have shrunk, and in terms of nuclear appearance, the formation of pyknotic nuclei (Thick arrow) increased in a dose-dependent manner. Scale bars are 30.0 μm in all figures.

Cell migration assay

We evaluated cell movement of PANC-1 cells in a resveratrol environment with a wound closure experiment. This is a cheap, effective, and easy-to-apply method. When we evaluated our results; it was calculated that after 48 hours, the cells in the group without any application had closed the wound area by 73.37% and showed rapid movement. The wound closure amounts in the groups with 40 and 50 μM doses were as follows; 34.77% and 8.76%, respectively. It was determined that the movement of the cells stopped in the groups with 100, 150 and 189 μM doses. (Figure 3).

DISCUSSION

This study showed that resveratrol, which is found in many plants, especially grapes, and has many beneficial physiological properties, has cytotoxic and anti-metastatic properties on PANC-1 pancreatic cancer cells.

Pancreatic cancer is considered one of the most malignant cancer types in terms of cancer types. Despite advances in surgical techniques and the use of local and systemic adjuvant treatments, the mortality rate of patients with pancreatic cancer is still very high. In order to determine treatment technologies and strategies, the process from the beginning of the cancer type to its growth and metastasis must be understood in detail. In addition to existing ones, natural compounds must also be considered in the creation of new treatment strategies. Every day, a new compound is found in this field, and we can say that resveratrol is one of the oldest and most biologically active in this field (9, 10).

Resveratrol is a polyphenolic compound found naturally in many plants, including grapes, peanuts, and blueberries. Resveratrol has a wide range of biological functions, including antioxidant, anti-inflammatory, anti-diabetic, and

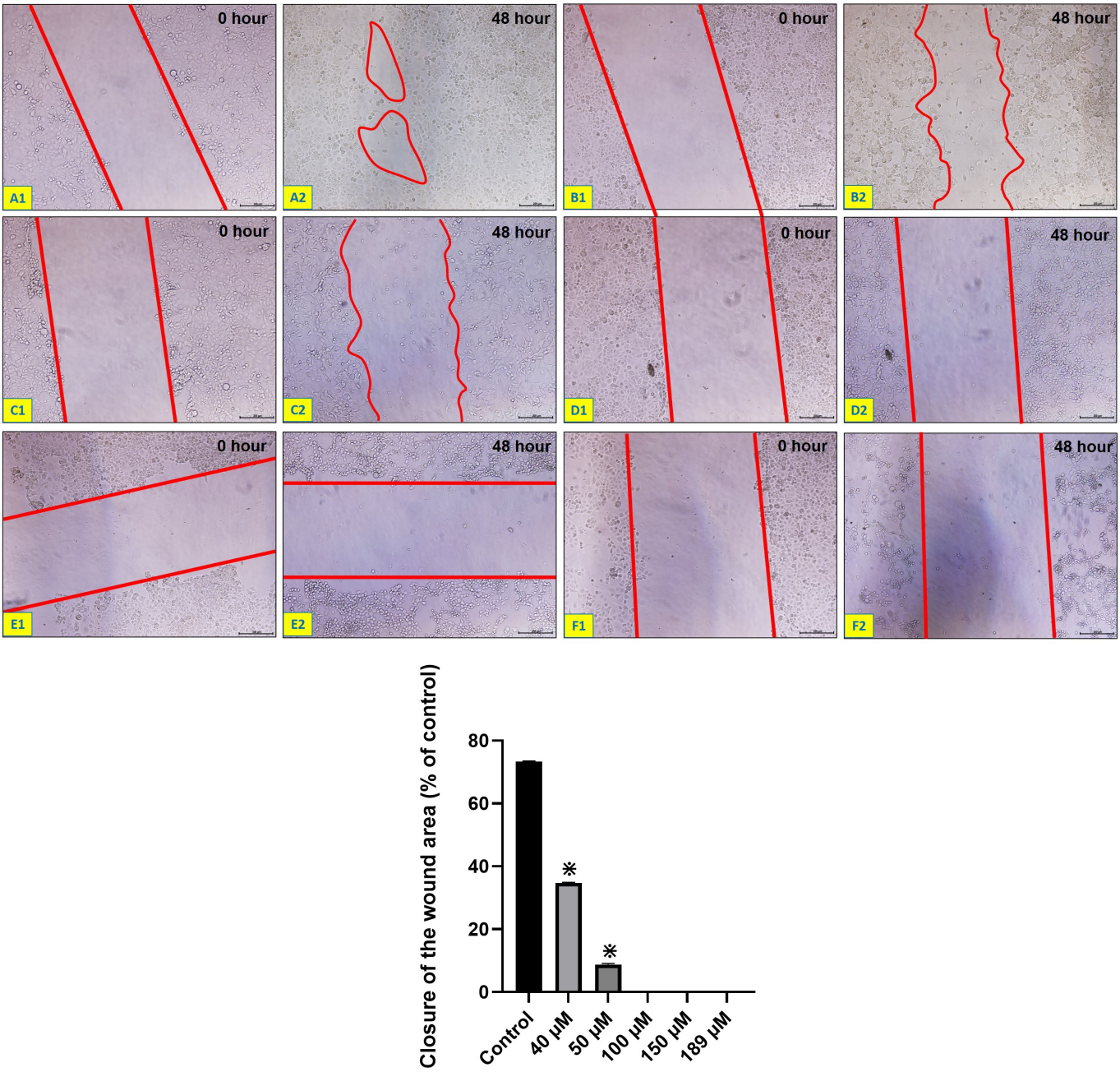

Figure 3. Results of cell migration of PANC-1 cancer cells. At 48 hours, the cells in the control group (A1-A2) covered 73% of the wound area, while the area covered by the cells in the 40 (B1-B2) and 50 µM (C1-C2) applied groups was 34% and 8%, respectively. It was determined that the movement of the cells stopped at other doses. Other doses: 100 µM (D1-D2), 150 µM (E1-E2) and 189 µM (F1-F2). *Significantly different from untreated cells (Control group). Scale bars are 200 µm in all figures.

anti-cancer (11). With the discovery of resveratrol, many studies have been conducted and are still being conducted to investigate its biological activity on different cancer cell types and lines and its detailed mechanism of action. Its activity on cancer in particular is a very important topic of curiosity (12). With developing diagnostic methods, cancer types are diversifying. Although there are many studies on resveratrol on cancer types, this issue needs to be detailed and resolved in the finest detail. We tested increasing doses of resveratrol in PANC-1 cancer cell lines in an in vitro experimental setting to demonstrate the effects of resveratrol on pancreatic cancer. We examined possible changes in cell viability, cell morphology and cell invasion. We observed that resveratrol increased cell viability in cells at low doses compared to control. The fact that low doses of resveratrol support viability show how important dose studies are in cancer treatments. Viability was above control up to 40 µM. Several studies have indicated that antioxidants can enhance the viability of cancer cells by mitigating oxidative stress, which may inadvertently support tumor growth and resistance to treatment (13-15). In the studies in the literature studying resveratrol and pancreatic cancer, it is generally emphasized that viability is below control at these doses (16, 17). Such differences may be due to many

reasons. For example, the cell used may be affected by the experimental conditions and experimental materials used and may produce different results. Cancer cells are dynamic cells that are highly adaptable to different environments. These characteristics may be the source of differences between experiments. We can consider cell morphology as an important end product that provides information about the internal dynamics of the cell. We can obtain information about the cell's membrane, cytoplasm, nucleus and cell volume through morphology examinations. In our experiment, we made the cell examinable in terms of cell compartments with hematoxylin-eosin staining. We observed that the cell lost its volume with increasing resveratrol doses, the cell shrank and its nucleus became pyknotic. Studies in the literature have shown that resveratrol disrupts cell morphology (18, 19). We differed with other studies in the literature regarding the dose. Molecules like resveratrol can affect almost all physiological processes of the cell. Cell movement is the most important weapon of cancer cells. Cancer cells are carried to nearby and distant organs, disrupting, slowing down and eventually stopping the normal functioning of the organism. When pancreatic cancer is diagnosed, it is usually found to have metastasized. As in other types of cancer, focusing on cell movement in pancreatic cancer is very important in the treatment of this disease. In our study, resveratrol first slowed down and then stopped PANC-1 cells with increasing doses. It was seen that cell migration was completely inhibited at doses of 100 µM and above. Similar results were also seen in the literature at different doses (7, 20, 21).

Like other types of cancer, pancreatic cancer is a major health problem that needs to be solved in society. There are many clinical, in vivo and in vitro studies on pancreatic cancer in the literature. We approached this field from the PANC-1 pancreatic cancer cell and resveratrol window. We contributed to the literature with new doses we detected in cell viability, morphological changes of cells and cell movement experiments. If our study had been supported by intracellular pathways, experimental animal models and additional molecular techniques, we could have explained the result with sharper boundaries.

CONCLUSION

As a result, we determined with this study that resveratrol reduces cell viability, disrupts cell morphology and first slows down and then stops cell movement in a dose-dependent manner. Finding anti-cancer molecules that are easily obtainable, highly beneficial, competitive with commercial equivalents and less damaging to normal tissue and determining their effectiveness are of great importance today in terms of both the country's economy and patient welfare. Resveratrol, on which thousands of studies have been conducted for years, is a strong candidate in this sense. There are many drug studies of resveratrol that are preclinical and candidates to reach clinical levels. We wanted to contribute to this pool with this study we conducted. Of course, as long as a disease is not completely cured, researching treatment options is an important goal. Although the path to the treatment of this cancer disease is difficult and long, the goal will be closer as long as we work with determination.

Ethics Committee Approval: This study is an in vitro study. Since no human or animal material was used in this study, an ethics committee report is not required.

Informed Consent: Since this study was not conducted on humans, we do not have an informed consent form.

Authorship Contributions: Idea/Concept:ES, Design:ES, Supervision:ES, Data Collection and Processing:ES, Analysis or Interpretation:ES, Literature Search:ES, Writing:ES, Critical Review:ES, References and Fundings: - , Materials:ES.

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

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