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Investigation of the Effects of Sodium Phenylpyruvate on Pulmonary Adenocarcinoma (A549) and Mammary Adenocarcinoma (MDA-MB-231) on Cell Lines

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Öz

Bu araştırma akciğer (A549) ve meme kanseri (MDA-MB-231) hücre hatlarında sodyum fenilpirüvatın antitümöral etkilerinin belirlenmesi amacıyla yapılmıştır. Araştırmada distile suyla sodyum fenilpirüvatın değişik yoğunluklarda çözeltileri hazırlandı. Bu çözeltilerden içerisinde $1x10^3$ adet kanser hücresi bulunan mikropleyt kuyucuklarına sırasıyla kontrol, 0.0375 mM, 0.075 mM, 0.15 mM, 0.3 mM, 0.6 mM, 1.2 mM ve 2.4 mM'lik konsantrasyonlarından 100 mikrolitre hacimlerde ilave edildi. Aynı doz sağlıklı hücrelere de uygulandı (HUVEC). Her kuyucuğa 24, 48 ve 72. saatlerde CVDK-8 cell viability test kitinden (Eco-Tech) 10'ar µL eklendi ve 1 saat sonra mikropleytlerin 450 nm dalga boyunda verdikleri absorbansları spektrofotometrede ölçülerek hücrelerin yoğunlukları belirlendi. Kontrol ve deney gruplarından elde edilen sonuçlar istatistiki yönden değerlendirildi. Deney gruplarda kontrole göre kanser hücre sayılarının azaldığı, hücre katlanma süreleriin ise arttığı tespit edildi. Kontrole göre en fazla azalma akciğer karsinom hücre hattında 2.4 mM dozda 24. saatte, meme adenokarsinom hücre hattında 2.4 mM dozda 72. saatte ve human umbilical vein endothelial cells (HUVEC) hattında 1.2 mM dozda 48. saatte olduğu tespit edildi. Sodyum fenilpirüvatın akciğer ve meme kanseri hücreleri üzerine *in vitro* şartlarda sitotoksik etki gösterdiği sonucuna varıldı.

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Abstract

This study was conducted to determine the antitumoral effects of sodium phenylpyruvate on cell lines in lung (A549) and breast cancer (MDA-MB-231). In the study, solutions of different concentrations of sodium phenylpyruvate with distilled water were prepared. In these solutions, 100 µL volumes of control, 0.0375 mM, 0.075 mM, 0.15 mM, 0.3 mM, 0.6 mM, 1.2 mM and 2.4 mM concentrations were added to the microplate wells containing 1x103 cancer cells, respectively. The same dose was applied to healthy cells (HUVEC). 10 µL of CVDK-8 cell viability test kit (Eco-Tech) was added to each well at 24, 48 and 72th hours, and after 1 hour, the absorbance of the microplates at 450 nm wavelength was measured through a spectrophotometer and the density of the cells was determined. The results obtained from the control and experimental groups were evaluated statistically. It was determined that the number of cancer cells decreased and the time of cell folding increased in the experimental groups compared to the control group. Compared to the control groups, the highest decrease was observed in the lung carcinoma cell line with a dose of 2.4 mM at the 24th hour, in the breast adenocarcinoma cell line with a dose of 2.4 mM at the 72nd hour, and in the human umbilical vein endothelial cells (HUVEC) line with a 1.2 mM dose at the 48th hour. It was concluded that sodium phenylpyruvate had an cytotoxic effect on lung and breast cancer cells *in vitro*.

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1. INTRODUCTION

Cancer is a disease that is characterized by the uncontrolled and abnormal reproduction of cells. 70-75% of cancer is caused by chemical carcinogens, and the remainder by irritation (physical) and viruses (Dogan, 2017; Dogan, 2016). Genetic predisposition is also important in the formation of cancer. In 2018, 2.09 million people worldwide were diagnosed with lung cancer. This number constitutes 11.6% of all the detected cancer cases. 1.76 million people died of lung cancer worldwide in 2018 (Herreros-Pomares et al., 2021). Breast cancer is the second deadliest type of cancer in women in the United States. Thanks to the treatments applied for breast cancer, the survival rate has increased from 75% to 90%. In 10-15% of diagnosed breast cancer cases, there are no estrogen and progesterone receptors and HER2 protein is negative. The 10-year recurrence-free rate in this type of breast cancer is 97% (Bussard et al., 2021). There is a close relationship between the survival rate of breast cancer and socioeconomic status in Singapore (Wong et al., 2021). Delayed diagnosis and development of resistance to drugs in cancer stem cells increase the death rate in cancer (Herreros-Pomares et al., 2021). Detection of mutated genes serves a function to understand the biology of cancer. However, the investigation of the cancer treatments in terms of genes alone may not solve the problem completely.

Phenylpyruvic acid is chemical 2-oxo-3-phenylpropanoic acid. It is chemically synthesized. Microorganisms such as *Proteus* that synthesize the deaminase enzyme, from alpha-keto acid from amino acids (phenylalanine is converted to phenylpyruvic acid). *P. vulgaris* strains have been shown to have high deaminase activity (Coban et al., 2014). Recombinant *Escherichia coli BL21* (DE3) strain was obtained by transferring the membrane bound L-amino acid deaminase (L-AAD) gene in *Proteus mirabilis KCTC 2566*. With the help of this recombinant *E. coli*, phenylpyruvic acid could be produced from L-phenylalanine in high yield. This method does not release toxic products for the environment (Hou et al., 2015). Phenylpyruvic acid could be produced with 98% yield by oxidizing D,L-phenylalanine with D-amino acid oxidase (DAAO)/catalase enzyme obtained from *Trigonopsis variabilis* (Ferandez-Lafuente et al., 1998). The presence of 13-D-glucopyranoside, which is an enolic derivative of phenylpyruvic acid, has been demonstrated in the water extract of *Aspalathus linearis*. Bacteria and plants can perform the biosynthesis of important aromatic amino acids L-phenylalanine and L-tyrosine by using phenylpyruvic acid in the shikimic acid pathway (Marais et al., 1996). Phenylpyruvic acid is found in the urine of patients with phenylketonuria (Marais

et al., 1996; Blau et al., 2010). Phenylketonuria, an autosomal recessive disease, has not the enzyme phenylalanine hydroxylase. Since phenylalanine cannot be converted to tyrosine, the level of tyrosine in the body decreases (Blau et al., 2010; Rosa et al., 2012). The levels of phenylalanine and its metabolites such as phenylpyruvic acid, phenyllactic acid and phenylacetic acid increase in blood and tissues. These metabolites are excreted in the urine (Blau et al., 2010; Rosa et al., 2012).

Alpha-keto acids (R-COCOOH) are compounds used in the production of amino acids, food additives, medicine and pesticides. Phenylpyruvic acid is an alpha-keto acid (Coban et al., 2014). Today, more than 10.000 tons of phenylpyruvic acid is produced in the world. Phenylpyruvic acid is used in the pharmaceutical, food and chemical industries (phenylalanine and phenyllactic acid synthesis, etc.). Aspartame, a sweetener, is obtained from phenylalanine (Hou et al., 2015; Li et al., 2017). Phenylpyruvic acid and other alpha-keto acids are added to foods as flavor enhancers. Phenylpyruvic acid is used to develop specific odor and taste in cheese and wine production. It is included in the diets of the patients with renal failure to reduce the accumulation of urea in the body. It is also recommended to be added to poultry feeds to prevent excessive nitrogen excretion with fertilizers. Phenylpyruvic acid is searched in the urine for the diagnosis of phenylketonuria (Coban et al., 2014). High oral doses of phenylpyruvic acid can cause irritation and inflammation in the digestive tract.

Phenylalanine is transaminated into phenylpyruvic acid (with pyruvic acid). Phenylpyruvic acid is reduced to phenyllactic acid by forming a hydroxy group with the enzyme lactate dehydrogenase. Phenylpyruvic acid synthesis could be increased by transamination by adding phenylalanine to the growth medium of *L. plantarum*. Transamination is an important step in the conversion of phenylalanine to phenyllactic acid by *L. sanfranciscensis DSM20451T* and *L. plantarum TMW1.468* (Li et al., 2017; Valerio et al., 2016). Phenylpyruvic acid and phenyllactic acid are oxidized to phenylacetic acid in the organism. This product is conjugated with amino acids and excreted from the body (Fernandez-Lafuente et al., 1998). A portion of phenyllactic acid that is not excreted can be used in protein synthesis after conversion to phenylalanine via phenylpyruvic acid (Eıdusont & Dunn, 1956). The reduction of aromatic alpha-keto acids to aromatic lactic acids in mammalian tissues is accomplished by 80% aromatic alpha-keto acid reductase and 20% lactate dehydrogenase. Lactate dehydrogenase also catalyzes the reduction of p-hydroxyphenylpyruvic acid and phenylpyruvic acid (Webert & Zannonı, 1966).

Warburg effect is seen in cancer cells. Pyruvic acid, which is produced as a result of glucose metabolism, is converted to lactic acid by the enzyme lactate dehydrogenase. The lactic acid is converted to alanine and glucose. In this way, cancer cells obtain the building blocks (amino acids and DNA) and energy they need. Since phenylpyruvic acid uses the same enzyme in its metabolism, it can inhibit the production of lactic acid that occurs in cancer cells. Therefore, the formation of glycine, glutumate and aspartate synthesized from alanine and glucose can be suppressed. This may cause an antitumoral effect. In this study, it was aimed to investigate the antitumoral effects of sodium phenylpyruvate in cell cultures of lung adenocarcinoma (A549) and breast adenocarcinoma (MDA-MB-231).

2. MATERIALS AND METHODS

Study design

Sodium phenylpyruvate (MA 186.14 g) was obtained from Sigma (CAS 114-76-1). In the study, the cell culture method by Doğan and Mutlu (Doğan & Mutlu, 2019, s. 361-370). was used by modifying it to the laboratory.

Lung adenocarcinoma (A549), mammary adenocarcinoma (MDA-MB-231), and normal healthy primary cell (HUVEC) lines (Kafkas University Central Research Laboratory) were inoculated into DMEM (Dulbecco's Modified Eagle Medium, Sıgma) containing 10% FBS (Fetal Bovine Serum, Sıgma) and 1% antibiotic (Penstrep, Thermo Fisher) and incubated at 37 °C in a free humidity incubator containing 5% CO_2. After incubation, cells that grew at the rate of 70% were removed by trypsin (Sigma) and each well was inoculated into microplates containing $1x10^3$ cells. Cell lines were incubated for 48 hours under the same conditions. At the end of the period, control, 0.0375 mM, 0.075 mM, 0.15 mM, 0.3 mM, 0.6 mM, 1.2 mM and 2.4 mM doses and 100 µL volumes from daily prepared solutions of sodium phenylpyruvate were added to the microplate wells containing 1x103 cells, respectively. The same inoculation was done on normal healthy cells. Low doses of phenylpyrvate were tested in cell culture (lung cancer cell) and effective doses used were determined. To determine the densities of the cells in the wells at 24, 48 and 72^{th} hours, 10 µL of CVDK-8 Cell Viability Test kit (Eco-Tech) was added to each well and 1 hour later, the absorbance of the microplates was measured at 450 nm in the spectrophotometer.

Determination of doubling times of cells

Cell densities at the inoculation time of cell lines inoculated into wells were determined using the formula time · log (2) / log (final concentration) – log (first concentration) together with the cell densities obtained at the end of 72 hours of experimental applications (Roth, 2006).

Statistical analysis

Data set was created by transferring absorbance values and cell folding times to the software IBM SPSS 26.0.0 (IBM SPSS, 2017). Shapiro-Wilk normality test first was applied to the created data set, and then variance homogeneity test was applied to the obtained results. According to the normality results of the data, Post-Hoc tests were performed in accordance with the parametric data in line with the results of the One-Way ANOVA test (Bonferroni or Tamhane's T2 PostHoc). $P<0.05$ was considered significant in all statistical analyzes. The absorbance values of the cell densities obtained from the experimental groups were calculated compared to the control groups. Mean ± standard deviation values were used because the obtained data are parametric in cell density percentage and doubling time figures.

3. RESULTS

The densities of lung adenocarcinoma cell (A549), breast adenocarcinoma cell (A549) and normal healthy primary cell (HUVEC: Human umbilical vein endothelial cells) 24, 48 and 72 hours after the administration of sodium phenylpyruvate are presented in Figures 1, 2 and 3, respectively. It was determined that the cell densities of all groups decreased according to the dose and time compared to the control group.

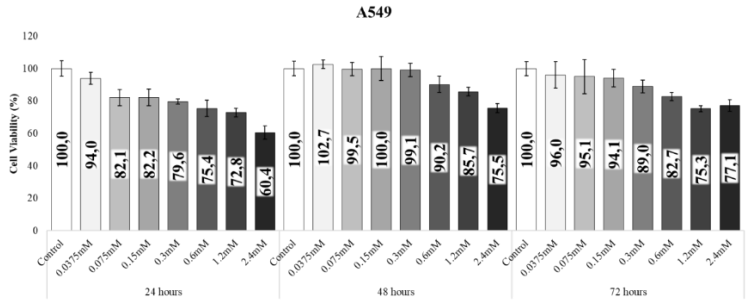

Figure 1. Effects of sodium phenylpyruvate on the densities of lung adenocarcinoma cell (A549) 24, 48 and 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (the cell density of each hour group is compared with its control group).

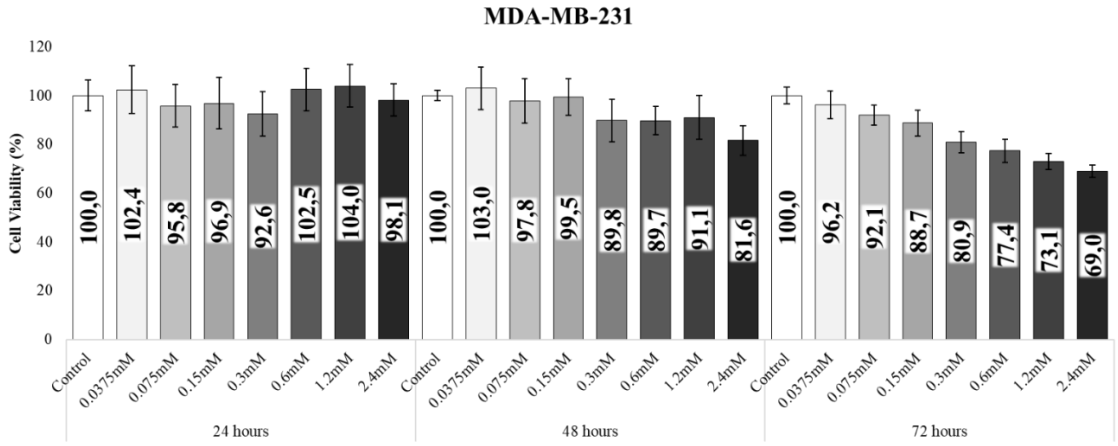

Figure 2. Effects of sodium phenylpyruvate on the densities of breast adenocarcinoma cells (MDA-MB-231) 24, 48 and 72 hours after the application at concentrations varying between 0.0375-2.4 mM (the cell density of each hour group is compared with its control group).

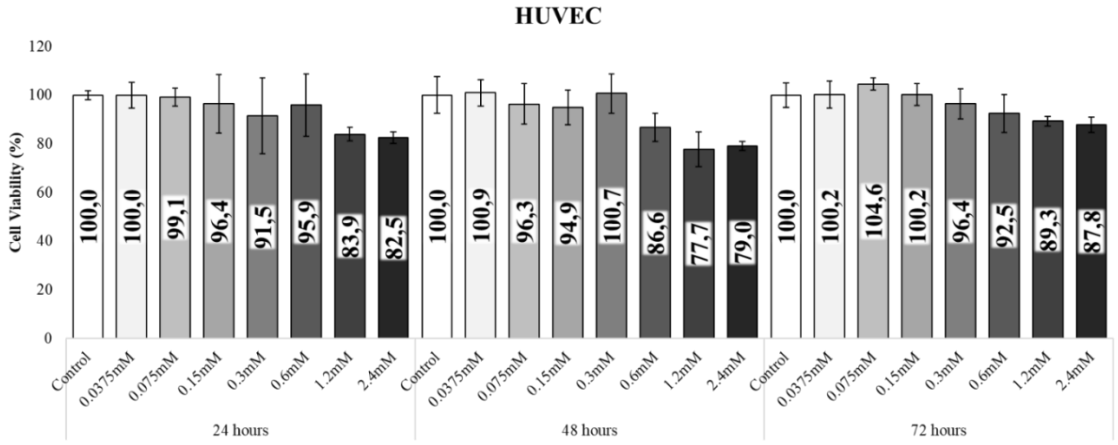

Figure 3. Effects of sodium phenylpyruvate on the densities of HUVEC 24, 48 and 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (the cell density of each hour group is compared with its control group).

The effects on the folding of Lung (A549), breast adenocarcinoma and normal healthy primary cells 24, 48 and 72 hours after the administration of sodium phenylpyruvate are

presented in Figures 4, 5 and 6, respectively. In general, the rise of the dose increases the doubling times in the groups.

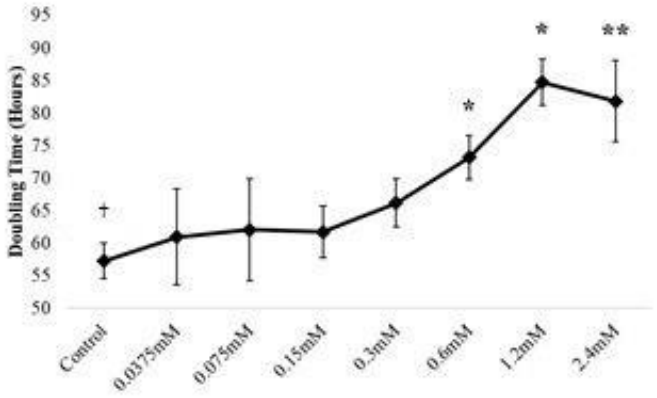

Figure 4. Effects of sodium phenylpyruvate on the folding times of lung adenocarcinoma cells (A549) 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (One-Way ANOVA: $p<0.001$, PostHoc Tests (Bonferroni/Tamhane's T2): †-*$p<0.001$, †-**$p<0.01$).

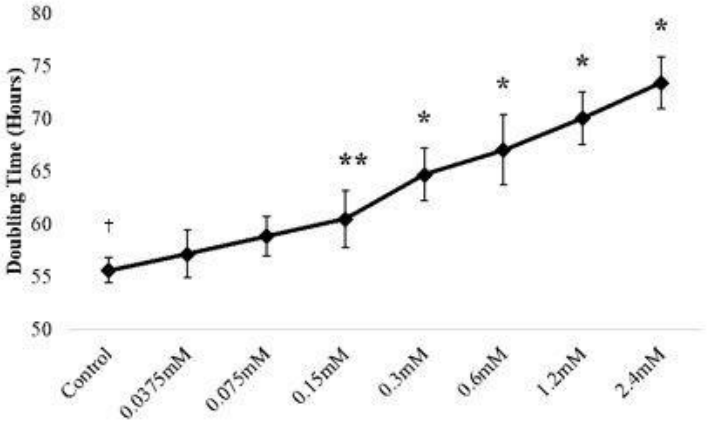

Figure 5. Effects of sodium phenylpyruvate on the folding times of mammary adenocarcinoma cells (MDA-MB-231) 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (One-Way ANOVA: $p<0.001$, PostHoc Tests (Bonferroni/Tamhane's T2): †-*$p <0.001$, †-**$p<0.01$).

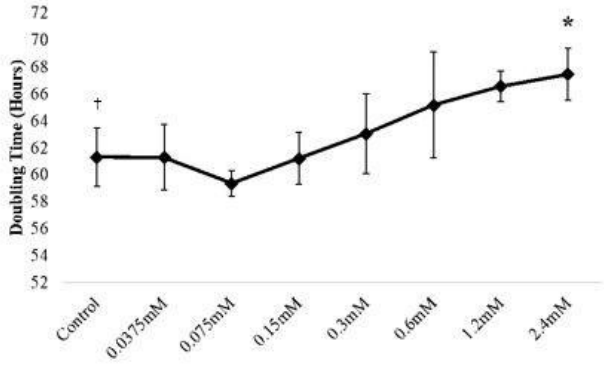

Figure 6. Effects of sodium phenylpyruvate on the folding times of HUVEC 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (One-Way ANOVA: p<0.001, PostHoc Tests (Bonferroni/Tamhane's T2): †-*p<0.001).

A statistical comparison of the effects of sodium phenylpyruvate on the densities of lung adenocarcinoma, breast adenocarcinoma (A549) and normal healthy primary cell 24, 48 and 72 hours after the application of its concentrations ranging from 0.0375-2.4 mM is presented in Table 1, 2 and 3.

Table 1. Statistical evaluation of the effects of sodium phenylpyruvate doses on densities in lung carcinoma cell lines.

Dose (mMol)	Lung adenocarcinoma (A549) densities			P-values
	24 Hours	48 Hours	72 Hours	
Control (0)	†0.479±0.026	†0.444±0.022	†0.446±0.021	
0.0375	0.450±0.020	0.456±0.013	0.428±0.040	*NSD*‡
0.075mM	*0.393±0.027	0.442±0.020	0.424±0.052	*NSD*‡
0.150 mM	*0.382±0.010[a]	0.444±0.036[d]	0.420±0.026	*p<0.05*‡
0.3 mM	*0.381±0.008[a]	0.440±0,020[c]	0.397±0,020	*p<0.001*‡
0.6 mM	*0.361±0.027[a]	0.400±0,024[d]	**0.369±0.012	*p<0.05*‡
1.2 mM	*0.349±0.015[a]	**0.381±0.013[c]	*0.336±0.009	*p<0.001*‡
2.4 mM	*0.289±0.022[a]	*0.335±0.014[c]	*0.344±0.017[c]	*p<0.001*‡
p-values	*p<0.001*‡	*p<0.001*‡	*p<0.001*‡	

‡One-Way ANOVA, PostHoc Tests (Bonferroni/Tamhane's T2): [ab, af, bf, eb, df]p<0.001, [ac, ae]p<0.01, [ad, bc, ce, cd]p<0.05, †-*p<0.001, †-**p<0.01, †-***p<0.05, NSD: No significant difference (Dots were used to indicate statistical differences for horizontal direction and letters for vertical direction). Symbols also apply to Tables 1, 2 and 3.

Table 2. Statistical evaluation of the effects of sodium phenylpyruvate doses on densities in breast adenocarcinoma cell lines.

Dose (mMol)	Breast adenocarcinoma (MDA-MB231) densities			P-values
	24 Hours	48 Hours	72 Hours	
Control (0)	0.851±0.058	†1.055±0.025	†1.038±0.038	
0.0375	0.871±0.090[a]	1.086±0.099[b]	0.999±0.063[d]	*p<0.001*‡
0.075mM	0.815±0.079[a]	1.031±0.104[b]	***0.956±0,045[c]	*p<0.001*‡
0.150 mM	0.824±0.095[a]	1.049±0.085[b]	*0.921±0.058[c]	*p<0.001*‡
0.3 mM	0.787±0.083[a]	0.947±0.098[c]	*0.840±0.049[e]	*p<0.01*‡
0.6 mM	0.872±0,083	0.946±0066[a]	*0.803±0,053[c]	*p<0.01*‡
1.2 mM	0.884±0,079[a]	0.960±0.101[d]	*0.759±0.036[b]	*p<0.001*‡
2.4 mM	0.835±0.060[c]	**0.860±0.069[b]	*0.717±0.029[a]	*p<0.001*‡
p-values	*NSD*‡	*p<0.001*‡	*p<0.001*‡	

‡One-Way ANOVA, PostHoc Tests (Bonferroni/Tamhane's T2): [ab, af, bf, eb, df]p<0.001, [ac, ae]p<0.01, [ad, bc, ce, cd]p<0.05, †-*p<0.001, †-**p<0.01, †-***p<0.05, NSD: No significant difference (Dots were used to indicate statistical differences for horizontal direction and letters for vertical direction). Symbols also apply to Tables 1, 2 and 3.

Table 3. Statistical evaluation of the effects of sodium phenylpyruvate doses on densities in HUVEC cell lines.

Dose (mMol)	HUVEC densities and hours			P-values
	24 Hours	48 Hours	72 Hours	
Control (0)	0.360±0,008	†0.529±0.044	†0.717±0.040	
0.0375	0.360±0.022[a]	0.534±0,032[b]	0.718±0,045[f]	*p<0.001*‡
0.075mM	0.357±0.015[a]	0.510±0.050[e]	0.750±0.019[b]	*p<0.001*‡
0.150 mM	0.347±0.050[a]	0.502±0.042[b]	0.719±0.037[f]	*p<0.001*‡
0.3 mM	0.329±0.063[a]	0.533±0.047[b]	0.691±0.050[f]	*p<0.001*‡
0.6 mM	0.345±0.053[a]	0.459±0.034[d]	0.663±0.063[f]	*p<0.001*‡
1.2 mM	0.302±0.011[a]	*0.411±0.042[e]	0.640±0.016[b]	*p<0.001*‡
2.4 mM	0.297±0.010[a]	**0.418±0.011[b]	***0.630±0025[f]	*p<0.001*‡
p-values	*NSD*‡	*p<0.001*‡	*p<0.001*‡	

‡One-Way ANOVA, PostHoc Tests (Bonferroni/Tamhane's T2): [ab, af, bf, eb, df]p<0.001, [ac, ae]p<0.01, [ad, bc, ce, cd]p<0.05, †-*p<0.001, †-**p<0.01, †-***p<0.05, NSD: No significant difference (Dots were used to indicate statistical differences for horizontal direction and letters for vertical direction). Symbols also apply to Tables 1, 2 and 3.

4. DISCUSSION

Cell culture and animal experiments are used in drug development studies for cancer. Cell cultures reduce the number of animals to be used in research. This is one of the important advantages of two-dimensional (2D) cell culture that grows in flat layers on plastic surfaces (Herreros-Pomares et al., 2021; Foglietta et al., 2020). However, two-dimensional cell cultures cannot fully simulate the natural environment in studies with cancer stem cells. For this reason, three-dimensional cell cultures (3D) taken from cancer cell lines and enriched with cancer stem cells have been developed. 3D cell cultures are better at imitating tumor cells than 2D cell cultures. However, 2D cell cultures are easier to prepare and cost-effective (Herreros-Pomares et al., 2021). In this study, 2D cell culture was used due to its advantages.

Phenylpyruvic acid can be produced by some microorganisms. In plants and bacteria, it is used in the shikimic acid pathway for the biosynthesis of L-phenylalanine and L-tyrosine (Coban et al., 2014; Hou et al., 2015; Fernandez-Lafuente et al., 1998; Marais et al., 1996). Shikimic acid hardly penetrates into bacteria. Therefore, rumen mıcroorganism (*Aerobacter aerogenes, Pseudomonas aeruginosa, Bacillus subtilis, Saccharomyces cerevisiae*) synthesize small amounts of aromatic amino acids. It has been reported that phenylalanine from phenylpyruvic acid and tryptophan from indolpyruvic acid can be produced by rumen bacteria, protozoa and their mixtures (Khan et al., 2002).

Phenylalanine is an essential amino acid that constitutes 4-6% of the amino acid content of proteins. Phenylalanine is primarily metabolized by hydroxylation (75%) to tyrosine. Phenylalanine is converted to phenylethylamine by quadradic decarboxylation. This substance is converted to phenylacetylglutamate over phenylacetate and excreted in the urine. Phenylalanine can also be metabolized by transamination. Phenylpyruvate emerges as a result of this reaction. Phenylpyruvate is converted to either o-hydroxyphenylacetate or phenylactate. It can be excreted by conversion to phenylacetylglutamate directly through phenylacetate (Erdem, 2013). Significant amounts of phenylpyruvic acid and phenylacetic acid were detected in the urine of rabbits after oral or subcutaneous administration of 2.0 g of phenylalanine or equivalent amount of phenylpyruvic acid. In the metabolism of phenylalanine, oxidation events occur primarily in the side chains. The benzene ring is less oxidized. Benzene is then decomposed in a small amount (Chandler & Lewis, 1932). It has been shown that some microorganism (*Lactobacillus plantarum CECT-221*) produce phenyllactic

acid (antibacterial) when phenylpyruvic acid is added to the medium (Li et al., 2007; Valerio et al., 2016; Rodríguez-Pazo et al., 2013).

Significant intrinsic targets (epigenetic changes, transcriptional and signal transduction dysregulation, abnormal pathway and metabolic activity) and extrinsic targets (changes in the tumor microenvironment, and differentiation and composition of immune cells and fibroblasts) of drugs have been identified in the tumor cell. Detection of the characterization of cancer genomes has made great advances in the development of drug targets. However, the function of most cancer genes has not been fully elucidated. Persistent cells are usually able to escape chemotherapeutic stress and in some cases become reresponsive to therapy after drug discontinuation. This means that there is a non-genetic mechanism of drug tolerance (Hahn et al., 2021). Therefore, targeting genes alone may not be sufficient for most cancer treatments. Metabolic vulnerabilities are important among non-gene therapeutic targets. In this study, non-gene metabolic vulnerabilities of cancer cells were targeted.

Since cancer cells can constantly reproduce, their metabolism is fast. Therefore, cancer cells are addicted to glucose, glutamine, glycine, serine and aspartate. These amino acids (glutamine, glycine, aspartate) are involved in the biosynthesis of structures such as protein, pyrimidine, purines, phospholipids and glutathione. In some tumors, increased expression of serine synthetase and phosphoglycerate dehydrogenase has been known. The suppression of serine metabolism in these tumor cells leads to tumor regression (Hahn et al., 2021).

Aromatic keto acid reductase and lactate dehydrogenase enzymes are involved in the reduction of alpha-keto acids such as phenylpyruvic acid (eg, to phenyllactic acid) in mammalian tissues. The lactate dehydrogenase enzyme is inhibited by sodium dodecyl sulfate (Webert & Zannonı, 1966). NADH-dependent L-lactate dehydrogenase enzyme has been detected in *L. casei*. This enzyme catalyzes phenylpyruvic acid to phenyllactic acid. Their genes have been able to be transferred to *E. coli*. It has been shown that the recombinant *E. coli BL21* converts phenylpyruvic acid to L-phenyllactic acid (Li et al., 2018). Lactate dehydrogenase has also been demonstrated in Lactic acid bacteria *Pediococcus acidilactici DSM 20284* and lactic acid producing *Bacillus coagulans* (Mu et al., 2012). Phenyllactic acid is excreted in the body by conjugation with aliphatic amino acids.

Tumor cells metabolize glucose to pyruvic acid with the effect of Warburg, and then convert it to lactic acid through the enzyme lactate dehydrogenase. The resulting lactic acid is converted to alanine (pyruvic acid and glucose) and enters into the synthesis of some aliphatic

amino acids. These amino acids are used in DNA and glucose synthesis. When phenylpyruvic acid is given, the enzyme lactate dehydrogenase uses phenylpyruvate instead of pyruvic acid to produce phenylactate. The resulting phenylacetate is excreted by conjugation with aliphatic amino acids. Due to the decrease in the level of aliphatic amino acids, DNA synthesis can be suppressed and the growth of tumor cells can be inhibited. In this study, it was determined that phenylpyruvic acid inhibites the growth of tumor cells in lung and breast cancer cell lines. The findings strengthen the suspicion that phenylpyruvic acid can suppress the Warburg effect. Sodium phenylpyruvate showed the most tumorostatic effect in the Lung Adenocarcinoma cell line after 24 hours and in the Breast Adenocarcinoma cell line after 72 hours at a dose of 2.4 mM (Figure 1, 2, 3). It was also seen that the cell folding times increase (Figure 4, 5, 6). Statistical differences were significant between suppressions at some doses (Table 1, 2, 3). Therefore, the results obtained in the study support the hypothesis. In the study, phenylpyruvic acid reduces the density of normal and cancer cells. These effects may be due to its chemical structure. In the study, a linear curve could not be obtained in cell densities due to dose increases in some groups. This can be caused by operating errors.

4-Phenylbutyrate, similar to phenylpyruvic acid, affects various cellular processes. This substance is known as a biological response modulator that can cause cellular differentiation or apoptosis in various neoplasms such as prostate, ovarian, melanoma, glioma and leukemia. Phase I and II are on trial in treatment studies of Phenylbutyrate and its metabolic product, phenylacetate, and Hodgkin and non-Hodgkin acute myeloid leukemia (Liu et al., 2004). The chemical resemblance of phenylpyruvic acid to phenylbutyrate and its conversion to phenylacetate as a result of metabolism is consistent with the results of this study. DNA degradation initiates cancer. However, impaired metabolic functions can have a significant impact on cancer progression. The results of these studies raise the suspicion that aromatic amino acid deficiency or the inability to metabolize aromatic amino acids to alpha-keto acids may play a role in the development of tumors.

Exposure of human fibroblasts to UV in a medium with phenylpyruvic acid increases single-stranded breaks in DNA (Hargreaves et al., 2007). It was determined that phenylpyruvic acid significantly reduces the glucose-6-phosphate dehydrogenase activity in rats (Rosa et al., 2012). Cytotoxic effects of phenylpyruvic acid detected in this study may also be due to its local chemical or oxidant effects. Phenylpyruvic acid can damage cells by reacting with cell structures. Since some amount of phenylpyruvic acid is converted to phenylalanine, it can be

used in the proliferation of cells. Since some of the phenylpyruvic acid is converted to phenylalanine, it can be used as an amino acid in the proliferation of cells.

As a result, it can be suggested that sodium phenylpyruvate has an cytotoxic effect in cell culture. This may bring up the use of aromatic amino acids and phenylpyruvate (or other aromatic alfa-keto acids and their derivatives) in the treatment or the synthesis of a new group of antitumoral drugs. For this reason, it is of great importance to support or confirm the results of research with studies on many cancer cell lines and experiments on animals.

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