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Research Article

Cellular Response of Cancer to Music: Mirror, Mirror, on the Wall, Which is the Most Effective of Them All?

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Abstract

Aim: Cancer is one of the most common health problems in the world. Music is a therapy method that creates positive changes in human behavior. This study aims to examine the effects of Turkish and Western classical music on MCF-7, SKOV-3, PC-3, U87, and COLO741 cancer cell lines.

Material and Methods: Group 1: No sound, Group 2: Turkish classical music (ney), Group 3: Western classical music (piano), and Group 4: Western classical music (violin). After listening to the cancer cells for 40 min, the audio files were interrupted for 1h, and then music was played for another 40 min. The effect of music on the proliferation of cancer cells was determined by WST-1 cell viability analysis. Statistical analyzes were performed with the SPSS program.

Results: There was no statistically significant difference between the groups that were applied music once in only the MCF-7 breast cancer cells and the control group ($p>0.05$). A statistically significant decrease was detected in cell viability when the control group and the other groups that were applied to music twice were compared ($p<0.05$). For other cancer cell lines except (please the SKOV-3 ovarian cancer cells, it was determined that music application once also caused a statistically significant decrease in cell viability ($p<0.05$).

Conclusion: We determined that classical music effectively reduced and suppressed the number of cancer cells. According to the data, Turkish and Western classical music can be used to support the treatment in of cancer patients.

Keywords: Breast cancer, prostate cancer, colon cancer, ovarian cancer, glioblastoma, turkish classical music, western classical music

INTRODUCTION

Cancer is one of the most important clinical issues in the world. According to the Turkey Cancer Statistics 2017 report, prostate and colon cancer in men and breast, colon, ovarian, and brain cancer in women are the most common cancer types in all age groups (1). According to the World Health Organization (WHO) data, 10 million people died of cancer worldwide in 2020 (2).

Cancer patients experience physical problems such as pain, loss of appetite, cachexia, hair loss, nausea, vomiting, fatigue, and psychological problems such as sleep disorders, depression, and anxiety due to the side effects of chemotherapy and radiotherapy. Music is a therapy method that changes human behavior and reduces pain and anxiety (3). Music has been used in various clinical treatments from the past to the present. In recent years, music therapy has been used as an option in many medical fields such as relieving symptoms associated with chemotherapy in cancer patients (4). Turkish-Islamic physicians such as Er-Razi, Farabi, Ibn-i Sina (Avicenna) were used music therapy for psychological diseases. They used medicine and music treatment, and these methods were developed by both Seljuk and Ottoman physicians until the 18th century (5).

Studies have shown that Western classical music can reduce the number of cancer cells (6), and there is no publication in the literature examining the effects of

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Turkish classical music on cancer cells using in vitro cell culture techniques. In our study, we aimed to find out whether Turkish classical music has a lethal effect on cancer cells, and if so, on which cancer cell type it is more effective. In vitro studies in the literature have been carried out only with Western classical music, and no publication that examines Turkish classical music comparatively.

MATERIAL AND METHOD

Cell culture

This study was carried out in the cell culture laboratory of Mugla Sitki Kocman University Faculty of Medicine, Department of Histology and Embryology. Before starting the laboratory experiments, "Laboratory Safety Rules" were explained to the lead student by the advisors and all experiments were performed under the supervision of the advisors. In this study, MCF-7 human breast cancer cell line (HTB-22™), PC-3 human prostate cancer cell line (CRL-1435™), SKOV-3 human ovarian cancer cell line (HTB-77™), U87 human glioblastoma cell line (HTB-14™), and COLO741 human colon cancer cell line (93052621) were used (Figure 1). These cells were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA) and the European Collection of Authenticated Cell Cultures (ECACC, UK Health Security Agency, Salisbury, UK).

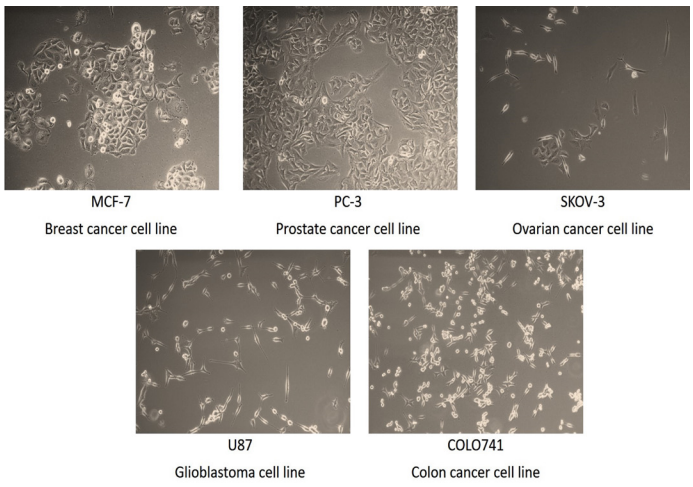
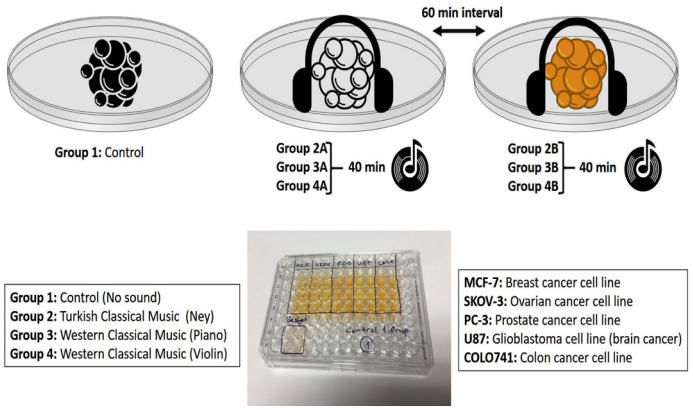


Figure 1. Inverted light microscope images of the cell lines used in the study. X20 magnification

Study design

Cancer cell lines used in the study were grown by culturing in a humid incubator at 37°C and 5% CO_2 conditions using Roswell Park Memorial Institute1640 (RPMI1640) medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% amphotericin B, and 1% L-Glutamine. After the cells seeded in cell culture dishes were incubated overnight, four experimental groups were formed. Group 1 (Control): No sound. Group 2 (Ney): Pesrevs played with ney from Turkish classical music were chosen for this group. An instrument called "Ney" is one of the musical instruments in Turkish history. Ney is the reed flute, especially played in Mevlevi (Sufi) music (7). Group 3 (Piano): The compositions of Western classical music played with the piano were selected for this group. Group 4 (Violin): The compositions of Western classical music played with the piano were selected for this group. The audio files of the compositions were limited to 40 min and combined for each group. The combined audio files were played for 40 min (Group 2A, Group 3A, Group 4A), after a break of 1 hour, they were played again for 40 min for the second time (Group 2B, Group 3B, Group 4B). Audio files were played in the Class II Biosafety cabinet using LG speakers, in the range of 70-100 dB (6,8).



Frequency spectra

The frequency spectra of all combined audio files were determined using Audacity version 3.1.3 software for Windows (Figure 2). Sound pressure levels were kept between 70 and 100 dB for all configurations. The compositions used in this study are shown in Table 1.

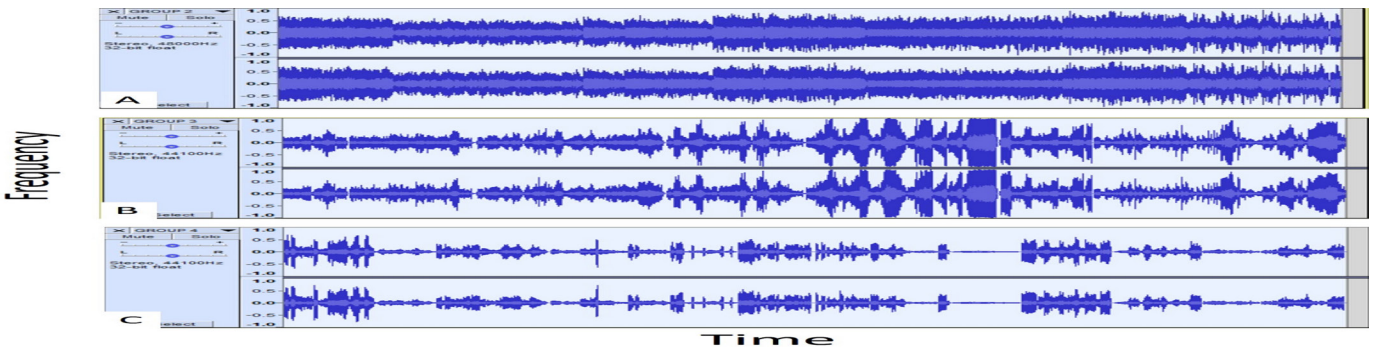


Figure 2. Frequency spectra of the compositions used in the study, obtained by the software Audacity version 3.1.3 for Windows. A. Group 2: Turkish classical music (ney), B. Group 3: Western classical music (piano), C. Group 4: Western classical music (violin)

Table 1. Compositions of music used as treatment on cancer cells

Groups	Compositions	Compositions
Group 1 (Control)	No sound	No composer
	Turkish Classical Music	
Group 2 (Ney)	Buselik Peşrev	Kemençeci Nikolaki (e. -1915)
	Segah Peşrev	Neyzen Yûsuf Paşa (e. 1821-1884)
	Yegah Peşrev	Râuf Yektâ Bey (e. 1871-1935)
	Nihavend Peşrev	Tanbûrî Refik Fersan (e. 1893-1965)
	Uşşak Peşrev	Neyzen Salih Dede (e. 1823-1886)
	Hüseyni Peşrev	Kemençeci Nikolaki (e. -1915)
	Hüzzam Peşrev	Şehzade Seyfeddin Osmanoğlu (e. e1874-1927)
	Western Classical Music	
Group 3 (Piano)	Prelude I in C Major	Johann Sebastian Bach (e. 1685-1750)
	Rondo Alla Turca "Turkish March"	Wolfgang Amadeus Mozart (e.1756-1791)
	Bagatelle No. 25 in A Minor "Für Elise"	Ludwig van Beethoven (e. 1770-1827)
	Swan Lake, Op. 20: Scene	Pyotr Ilyich Tchaikovsky (e. 1840-1893)
	Nocturne No. 2 in E-Flat Major, Op. 9 No. 2	Frédéric François Chopin (e. 1810-1849)
	Schwanengesang "Serenade"	Franz Schubert (e. 1797-1828)
	Arabesque No. 1 in E Major	Claude Achille Debussy (e. 1862-1918)
	Hungarian Rhapsody No. 2	Franz Liszt (e. 1811-1886)
Group 4 (Violin)	The Four Seasons (Spring, Summer, Autumn, Winter)	Antonio Vivaldi (e. 1678-1741)

Measurement of cell viability

MCF-7, PC-3, SKOV-3, U87, and COLO741 cancer cell lines were grown in 25 cm^2 flasks in an appropriate medium at 37°C and a 5% CO_2 environment, and the medium was changed 3 times a week. When the cells grown in the flask were 90% confluent, they were separated from the flasks with the trypsin-EDTA solution and counted. Cells counted at ~1x10^5 cells/ml in each well were transferred to 96-well culture dishes and incubated at 37°C and 5% CO_2 to be confluent. After the confluent cells were taken into the Class II Biosafety cabinet according to the order of the experimental groups. WST-1 measurements were made at 24h. Stock WST-1 at pH 7.4 by adding 25 mg of WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium] into 5 ml of sterile Phosphate-Buffered Saline (PBS) solution was prepared. The media on the cells were withdrawn without touching the cells, and the cells were washed with 100 µl of PBS. 10 µl of the prepared WST-1 solution was put into each well and kept in the incubator (37°C, 5% CO_2) for 2-4h. After incubation, 420-460 nm absorbance was measured in a microplate reader. Each experiment was repeated 3 times. Proliferation was determined by proportioning the absorbance values compared to the control group. The absorbance values of the control group were accepted as 100% viable cells.

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean cell viability values of the groups were compared using the Student's t-test. The statistical significance value was accepted as $p<0.05$.

RESULTS

Effects of music on MCF-7 human breast cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 97.307%, Group 3A was 95.918%, and Group 4A was 96.199%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 85.728%, Group 3B was 86.593%, and Group 4B was 80.728%. When the control group and Group 2A, Group 3A, and Group 4A were compared, there was no statistically significant difference ($p>0.05$). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected ($p=0.001$, $p=0.002$, $p=0.002$, respectively). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was detected in Group 2B, Group 3B, and Group 4B, compared to the groups

that applied music once (p=0.038, p=0.045, p=0.15, respectively). When Group 2, Group 3, and Group 4 were compared with other, there was no statistically significant difference (p>0.05) (Figure 3).

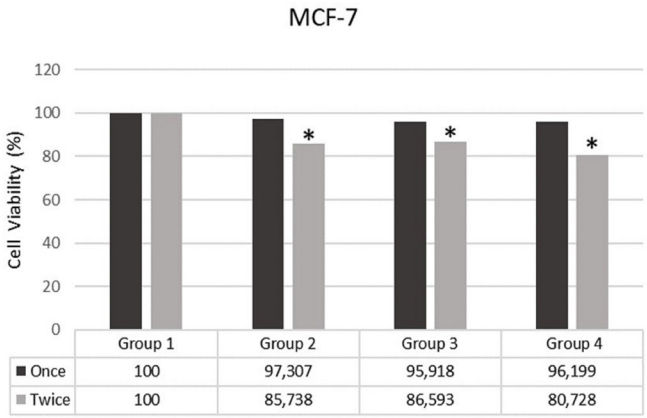


Figure 3. Effects of Turkish and Western classical music on MCF-7 breast cancer cell line after 24h. *Significant difference when compared with control p<0.05

Effects of music on PC-3 human prostate cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 93.371%, Group 3A was 86.772%, and Group 4A was 87.048%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 89.112%, Group 3B was 77.443%, and Group 4B was 75.291%. When the control group and Group 2A, Group 3A, and Group 4A were compared, there were statistically significant differences (p=0.01, p=0.000, p=0.000, respectively). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected (p=0.002, p=0.000, p=0.015, respectively). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was detected in Group 3B and Group 4B, compared to the groups that applied music once (p=0.000, for all). When the application numbers for Group 2 were compared, there was no statistically significant difference (p>0.05). When Group 2, Group 3, and Group 4 were compared with each other, there was a statistically significant difference between Group 2B and Group 3B, and between Group 2B and Group 4B (p=0.000, for all) (Figure 4).

Effects of music on SKOV-3 human ovarian cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 125.312%, Group 3A was 120.72%, and Group 4A was 125.574%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 105.306%, Group 3B was 92.943%, and Group 4B was 91.542%. When the control group was compared with Group 2A, Group 3A, and Group 4A, a statistically significant increase was detected (p=0.000, for all). When the control group and Group 2B were compared, a statistically significant increase was detected (p=0.038). When the control group was compared with Group 3B and Group 4B, a statistically significant decrease was detected (p=0.003, for all). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease was detected in Group 2B, Group 3B, and Group 4B compared to the groups that applied music once (p=0.000, for all). When Group 2, Group 3, and Group 4 were compared with each other, there was a statistically significant difference between Group 2B and Group 3B, and between Group 2B and Group 4B (p=0.000, for all) (Figure 5).

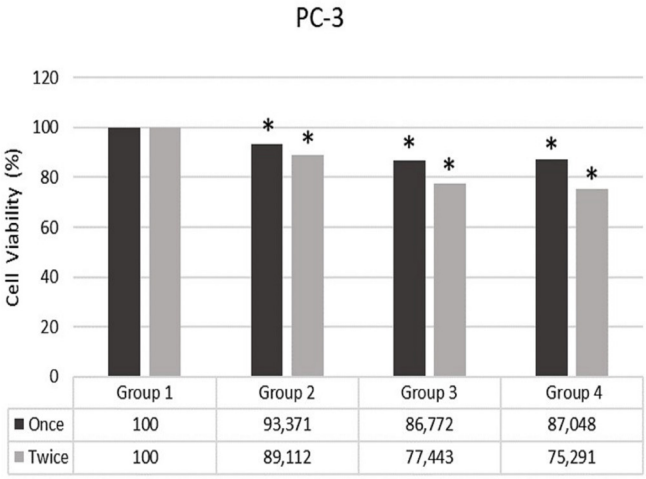


Figure 4. Effects of Turkish and Western classical music on PC-3 prostate cancer cell line after 24h. *Significant difference when compared with control p<0.05

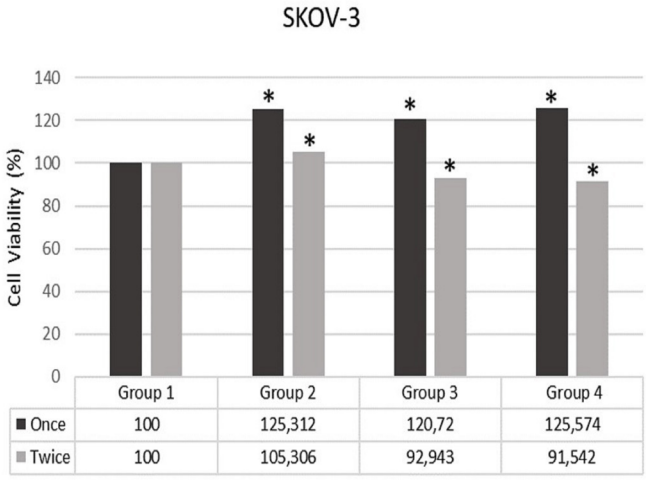


Figure 5. Effects of Turkish and Western classical music on SKOV-3 ovarian cancer cell line after 24h. *Significant difference when compared with control p<0.05

Effects of music on U87 human glioblastoma cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 92.817%, Group 3A was 89.179%, and Group 4A was 90.178%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the

control group was 100%, Group 2B was 74.396%, Group 3B was 70.054%, and Group 4B was 83.321%. When the control group was compared with Group 2A, Group 3A, and Group 4A, a statistically significant decrease was detected (p=0.003, p=0.000, p=0.000, respectively). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected (p=0.000, p=0.000, p=0.005, respectively). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was found in Group 2B, Group 3B, and Group 4B, compared to the groups that applied to music once (p=0.000, p=0.000, p=0.038, respectively). When Group 2, Group 3, and Group 4 were compared with each other, there were statistically significant differences between Group 2A and Group 3A, and between Group 3B and Group 4B were detected (p=0.028, p=0.001, respectively) (Figure 6).

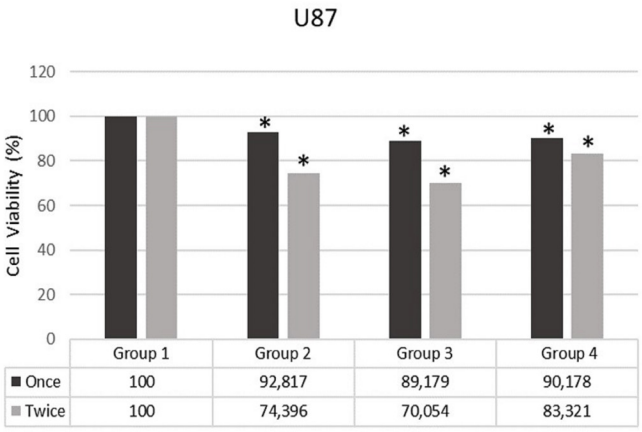


Figure 6. Effects of Turkish and Western classical music on U87 glioblastoma cell line after 24h. *Significant difference when compared with control p<0.05

Effects of music on COLO741 human colon cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 76.850%, Group 3A was 76.417%, and Group 4A was 84.958%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 73.647%, Group 3B was 69.631%, and Group 4B was 72.006%. When the control group was compared with Group 2A, Group 3A, and Group 4A, a statistically significant decrease was detected (p=0.000, for all). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected (p=0.000, for all). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was detected in Group 3B and Group 4B, compared to the groups that applied music once (p=0.002, p=0.007, respectively). There was no statistically significant difference for Group 2 (p>0.05). When Group 2, Group 3, and Group 4 were compared, a statistically significant difference was detected between Group 3A and Group 4A (p=0.021) (Figure 7).

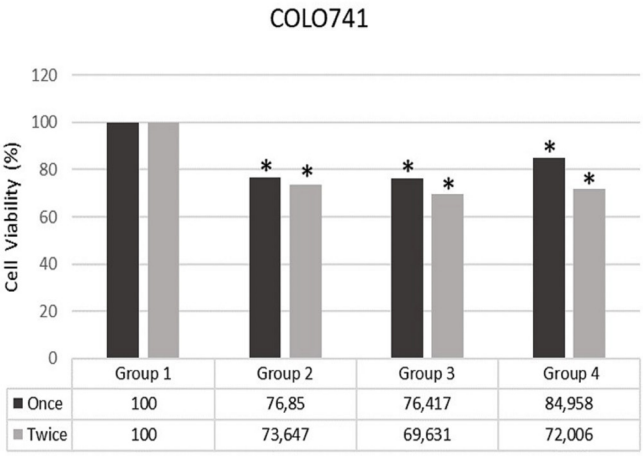


Figure 7. Effects of Turkish and Western classical music on COLO741 colon cancer cell line after 24h. *Significant difference when compared with control p<0.05

DISCUSSION

Music has physiological and psychological effects on individuals. It can help to restore physiological markers such as pulse, blood pressure, and breathing (3,4). Studies have shown that music has a positive effect on hormones such as serotonin, dopamine, and adrenaline, which regulate people's emotions (9). Marshall and Tomcala (1981) studied the effects of different types of music on stress. They found that subjects had varying stress levels and that all types of music had the same psychotherapeutic effects (10). Akpinar et al. (2020) investigated the effects of Turkish and Western classical music on the level of examination stress of university students. They found that students who listened to music experienced a significant decrease in exam anxiety. They concluded that Turkish and Western classical music can be used to reduce stress (4). Yıldırım and Gurkan (2007) reported that music reduced anxiety levels in patients undergoing chemotherapy (11).

Music also has antiproliferative properties in cancer cells (6,8,12). Ramirez-Rivera and Bernal (2019) applied Western classical music (Ludwig Van Beethoven) and metal music (Cannibal Corpse) to the AGS human gastric cancer cell line. The cell viability analysis was performed 12 h after the music applications. The researchers reported that metal music increased the number of cancer cells, but this increase was not seen in Western classical music. There was an increase in gene signals that caused cell death in Western classical music, thus reducing the number of AGS gastric cancer cells. In this study, high sound frequency (0-15 kHz) and sound peaks in metal music, low and medium sound frequencies (~3 kHz) in classical music were determined. The researchers suggested that high frequency and peaking sound may cause an increase in cell viability (12). Lestard (2013) et al. studied that MCF-7 breast cancer cells were applied to compositions of Mozart, Beethoven, and Ligeti for 30 min. While the compositions of Beethoven and Ligeti reduced cell viability, Mozart did not make any changes to cancer cells (8). In another study, Lestard (2016) et al. reported

that music activates cell death signaling pathways 48h after being applied to the Mozart, Beethoven, and Ligeti on MCF-7 and MDA-MB-231 breast cancer cell lines for 30 min. In Lestard's studies, while the compositions of Beethoven and Ligeti had a high frequency of sound (15 and above kHz), it was determined that the compositions of Mozart had a low frequency of sound (0-5 kHz). It was reported that the most effective group on breast cancer cells was the Beethoven group (6). It is an expected finding that more cells will die due to the negative physical effect of high sound frequency on cells. It is a remarkable finding that music with more sound peaks also increases the number of cancer cells.

In our study, we examine the effects of Turkish and Western classical music on cell viability in different cancer cells. First, there was no statistically significant difference between the groups that applied music once only in the MCF-7 breast cancer cells and the control group ($p>0.05$). A statistically significant decrease in cell viability was detected when the control group and the other groups that were applied to music twice were compared ($p<0.05$). It was determined that the most effective application time in breast cancer cells was 40 min twice. For other cancer cell lines, except for SKOV-3 ovarian cancer cells, it was determined that music application once also caused a statistically significant decrease in cell viability ($p<0.05$). However, in these cell lines, it was determined that the application of music twice for 40 min was more effective in reducing the number of cells. On the basis of these findings, we believe that cancer cells exposed to sound frequency for a longer period of time are physically affected more negatively and react in the form of increased cell death. Turkish and Western classical music were effective in reducing the number of cells on the MCF-7 breast cancer cells. Although the Western classical music violin group had the highest decrease in cell number, there was no statistically significant difference when the groups were compared ($p>0.05$). Second, it was determined that the most effective application time on PC-3 prostate cancer cells was 40 min twice for the groups in which Western lassical music was applied. Turkish and Western classical music were effective in reducing the number of cells. When Turkish and Western classical music were compared, Western classical music was statistically more effective in reducing cell number ($p>0.05$). The Western classical music violin group was the most effective type of music. On the contrary, the application of music did not have reducing effects on cell viability in SKOV-3 ovarian cancer cells. Turkish and Western classical music increased the number of SKOV-3 cancer cells. It was observed that the cell increase was less when music was applied twice. So, it is possible to suppress the number of cells with more repetitive music applications.

On the other hand, it was determined that the most effective application time on U87 glioblastoma cells was 40 min twice for all groups. Turkish and Western classical music were effective in reducing the number of cells. It was the Western classical music piano group that reduced the number of cells the most. Turkish classical music was most effective in U87 brain cancer cells. The U87 glioblastoma cell line consists of neurons and glial cells. Compared to other cell lines used in our study, this is the only cancer cell line associated with the nervous system. Because the sound is perceived by the sensory neurons in the inner ear, it is not surprising that U87 glioblastoma cells is also more sensitive to the sound we applied in this study. Finally, it was determined that the most effective application time on the COLO741 colon cancer cells was 40 min twice for the groups in which Western classical music was applied. Turkish and Western classical music were effective in reducing the number of cells on the colon cancer cell line. The groups with the highest decrease in the number of cells were Turkish and Western classical music piano group.

In conclusion, activities that support non-pharmacological treatment such as dance therapy, music therapy, art therapy, meditation have been recommended for cancer patients in recent years. Music is known as a potential tool to improve the quality of life of cancer patients. In developed countries, music therapy in cancer treatment is an emerging field. We suggest that the use of music therapy together with pharmacological applications for cancer patients can increase the response to treatment by reducing the number of cancer cells. Despite the limitations, this study is the first to demonstrate a reduction in the number of cancer cells using Turkish classical music. It is also the first study to examine the effects of music on ovarian, prostate, colon, and brain cancers. We determined that Turkish and Western classical music were effective in reducing and suppressing the number of cancer cells. Turkish classical music showed the highest antiproliferative effects on U87 glioblastoma and COLO741 colon cancer cell lines. Considering that intestinal cells also produce hormones in the nervous system and communicate with them, it is not surprising that the cells most sensitive to music are those that are in constant communication. According to the data, Turkish and Western classical music can be used to support the treatment in cancer patients.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Ethical approval is not required, because this article does not contain any studies with human or animal subjects. The cancer cell lines used in this study were obtained from an accredited commercial provider.*

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