DOES MELATONIN KILL BRAIN CANCER CELLS

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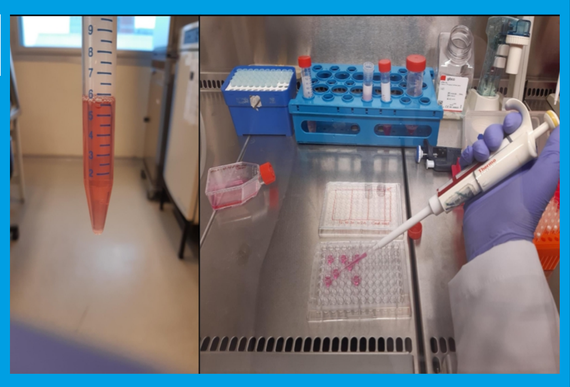
CELL CULTURE METHOD

Cell culture makes it possible to grow cell lines obtained from tissues or commercially available by providing the necessary conditions for use in research. Each cell type may need different conditions in cell culture. General minimum conditions are controlled temperature, sterile cell culture dishes in which cells can grow, a surface for cells to attach (for adherent cells), the most suitable medium for growth, and an incubator that provides the appropriate pH and osmolality.

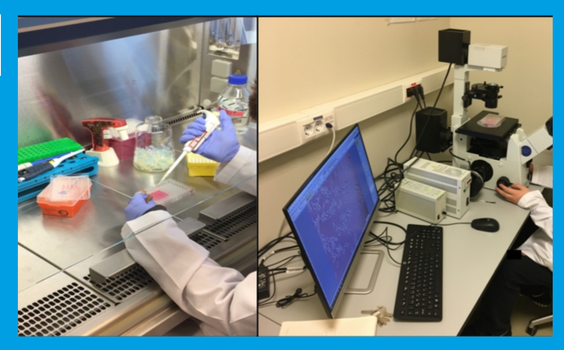
After a while, the cells are grown under suitable environmental conditions, and since they multiply by dividing, the cell fills the entire culture vessel. At this stage the cells need to be transferred to a new culture dish. For experimental studies, cells should be transferred to culture dishes of appropriate size for the experiment.



In our study, U87mg cells were removed from the normal cell culture dish and seeded into 96- well cell dishes at a density of 50000/ml. A volume of 200 µl medium was used for these dishes. The seeded cells were kept for 24 hours at 37°C and in a incubator containing 5% CO2. After 24 hours, it was added to the melatonin groups at a concentration of 100-500-1000 µM. After the melatonin was kept in the cell medium for 6 hours, other methods were used to get the results.

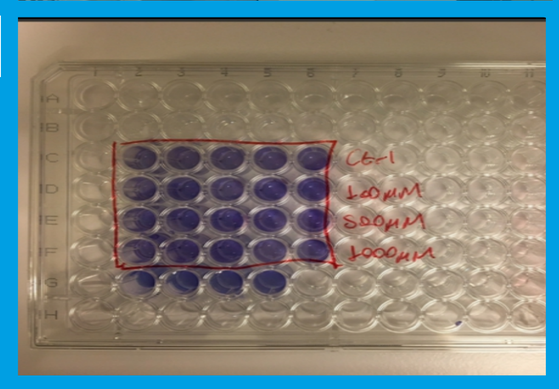
PROPIDIUM IODIDE (PI) STAINING METHOD

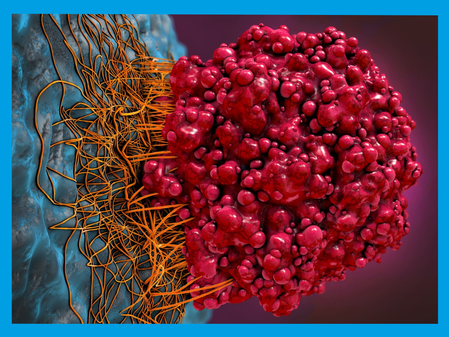
The PI dye stains the DNA of dead cells, giving a red color under a fluorescent microscope. For this method, PI dye was added to the cell wells at 1/20 of the cell medium volume (5 µl). The cells were then kept in the incubator for 15 minutes. At the end of 15 minutes, pictures of the cells were taken with afluorescent microscope. 20X objective is used.



PRESTO METHOD

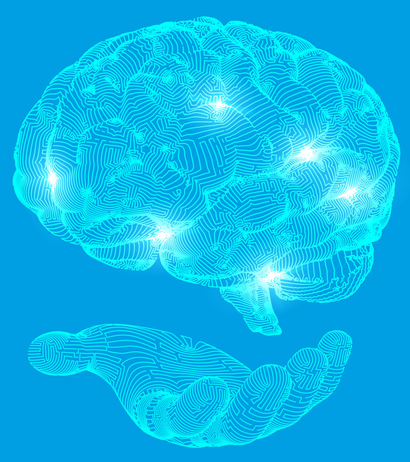
- **After the pictures taken with PI staining, the cell medium was decanted and presto blue dye was added to the new cell medium at a dilution of 1/10 (20 µl). After the cells were left inthe incubator for 15 minutes, they were read at 560 and 590 nm wavelengths in the Thermo brand Varioskan Flash, amultiplex reader device, and the values were analyzed.**



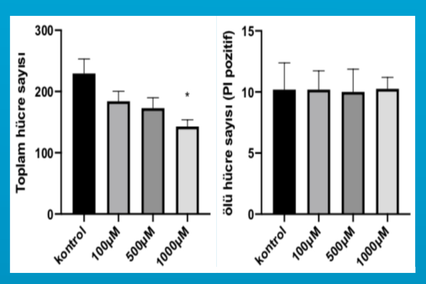


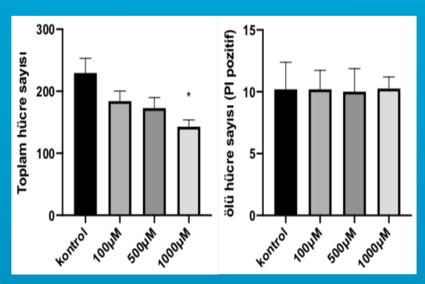
ANALYSIS OF DATA

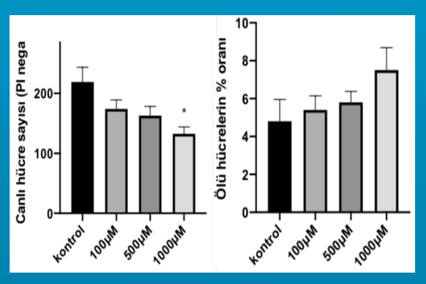
- **In our study, we worked with 5 samples in each group. The data obtained for these 5 samples were counted using Image J and Graphpad programs, and the cells in the PI staining photographs were counted using the ImageJ program.**

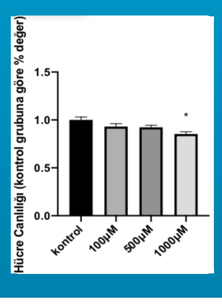


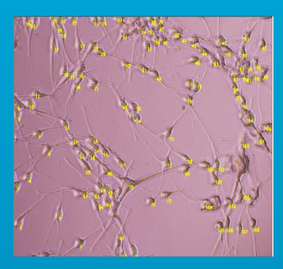
- **Data obtained from PI staining and Presto blue staining were analyzed by statistical methods, using the Graphpad program. Non-parametric Mann-Whitney U test was used for 2-group comparison in the analysis. This test evaluated whether the results we obtained occurredrandomly when assessing the accuracy of our hypothesis. Our confidence interval determined in this test is 95%. That is, if the test gives us a meaningful result, it means that the result is 95% reliable and has been obtained at random with a 5% probability. Thus, when we compare thetwo groups, we can say that these groups are different from each other with a probability of 95% and this difference has emerged as a result of our application. This is called a significant difference and is expressed as (p>0.05). The distance of the numbers in the groups from the group mean is called the standard error of the mean. This was calculated in the Graphpad program and indicated by the error bars in the graphs.**

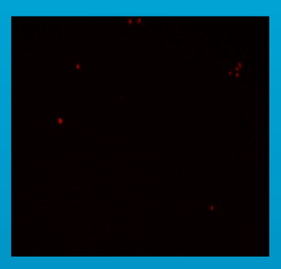


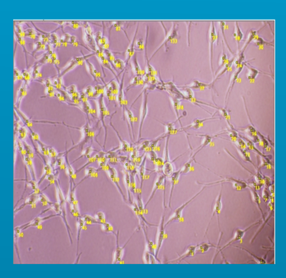


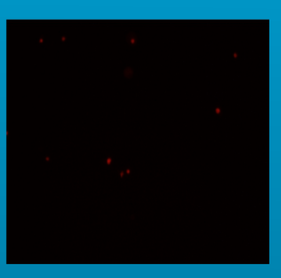












FINDINGS 3.1 PI STAINING FINDINGS

- **As a result of PI staining, the total number ofcells and dead cells were counted using the Image J program. The number of viable cells was obtained by subtracting the number of dead cells from the total number of cells.**
- **These numbers were analyzed by intergroup comparison. Then, the percentage of death was obtained by dividing the number of dead cells by the total number of cells and these were compared between groups. The graphs obtained as a result of these analyzes are as follows:**
- **According to these analyses, melatonin at a dose of 1000 µM significantly reduces the number of viable cells and the total number of cells (p>0.05). This effect was not observed for other doses. Similarly, no effect was observed in the number of dead cells and the percentage of dead cells. As we see in the graphs, there is adecreasing number of viable cells and total cell number with increasing dose. Although the number of dead cells does not change, the percentage of dead cells increases with increasing dose.**

PRESTO BLUE FINDINGS

- **In Presto blue analysis, the 590 nm wavelength is the control wavelength and data is obtained by subtracting it from the measurements at the 560 nm wavelength. Then, the melatonin group values were normalized by proportioning to the control group value. As a result, we see the control group value as 1 and we rate the other groups to this number. Then, the results of this measurement were analyzed with the comparison analysis between groups. The resulting graph is as follows:**
- **According to this graph, there is a significant decrease in the 1000µM dose. At other doses, melatonin did not affect cell viability.**

CONCLUSION AND DISCUSSION

- **Our study was inspired by studies investigating the reasons for the reduced incidence of cancer in people who experience blindness from an early age. Although the protection of melatonin in this regard is mostly tested in breast cancer studies, we thought that it may also be effective on different cancer cells. For this reason, we examined the effects on cell death and cell viability with 3 different doses of melatonin using neuroblastoma (human brain cancer) cells. According to our results, only 1000uM dose of melatonin showed a negative effect on brain cancer cells and reduced viable cell count, according to our study. This is a result that supports the correctness of our hypothesis. It is also compatible with the results of previous studies with other cancer types. Other doses were not effective in our study. The study can be enriched by repeating with a larger number of samples, including different times, and including different cell activities and types of death.**
- **We found no significant difference in the number of dead cells and the percentage of dead cells. PI dye is a dye that determines apoptotic death, these cells may be dying from apoptosis in different ways. To explain this better, it might be good to look at different types of cell death and different cell activities. Or they may have died during the application period, disintegrated and may no longer be there at the end of theapplication, so they are not painted. A study can be done by doing experiments at different times (time course).**

SUGGESTIONS

- **According to the results of our study, we can list the future studies that can be suggested as follows:**
- **1. In our study, it was found that melatonin caused high dose cell death in U87 Glioblastoma cells. Melatonin administration was performed at a single time point. Longer administration may also be effective at lower doses.**
- **2. Melatonin was used alone in our study. Its use with drugs used in the treatment of glioblastoma should be tried.**
- **3. High doses of melatonin can be harmful to normal cells. It should be determined whether it is harmful in normal cells.**
- **4. In our study, the effect of melatonin was examined in a single cell type. It should be tested on other cell lines or human tumor cells.**
- **5. The effect of melatonin on tumor should be tested in animal studies.**

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