Does Melatonin Kill Human Brain Cancer Cells?

Introduction

School biology, like no other academic discipline, allows you to demonstrate the cognitive power of the unity of a systematic, structural-level and historical approach to natural phenomena.

In the process of teaching biology in unity with the development of the dialectical thinking of schoolchildren, they are exposed to the scientific picture of the organic world, the historicity of life and its place in the system of forms of movement, the contradictory way of knowing wildlife. Biology is one of the leading subjects of the natural science cycle in the school system, since it is of great importance in the formation and development of the individual. Without it, it is impossible to ensure a healthy lifestyle and preserve the environment - the place of life of all mankind.school biology began to be guided by the following learning objectives: Fundamental (General):

- mental and emotional-volitional development; - the formation of a scientific worldview;

- labor training;

- physical development [2].

For these purposes, the educational and developmental significance of biological education is reflected.

Biological (special):

✓ mastery of knowledge about living nature, general methods of its study, educational skills;

✓ the formation on the basis of this knowledge of a scientific picture of the world;

✓ hygienic education and the formation of a healthy lifestyle that contributes to the preservation of the physical and moral health of a person;

✓ the formation of environmental literacy of people who know biological patterns, the relationship between living organisms, their evolution, causes of species diversity;

✓the establishment of harmonious relations with nature, society, ourselves, a reflection of the humanistic significance of nature;

✓ maintaining the positive experience of the biology education process accumulated in the school .I like study biology and do research

Now I will explain my research project about melatonin and cancer cells.

Summary

Melatonin is a hormone secreted by the pineal gland that regulates the sleep-wake cycle. Melatonin acts as an antioxidant directly as a radical scavenger and indirectly through the regulation of antioxidant enzymes, and its protection against cancer has been shown in cancer types such as breast cancer.

It is a subject that has been researched by scientific studies that cancer is less common in patients who experience blindness from an early age, and that this may be due to increased melatonin secretion. This situation has been studied mostly on breast cancer.

In our study, it is suggested that the protective effect of melatonin may also be effective in different types of cancer. For this reason, the effect of 3 different doses of melatonin applied on human brain cancer cells for 6 hours on cell viability and cell death was investigated in our project.

1. Introduction:

According to the results written by Maria Feychting, Bill Österlund, and Anders Albom and supported by many subsequent articles, the risk of cancer in visually impaired people is lower. According to the articles, the reason for this is the hormone called melatonin secreted from the pineal gland [1-4].

Melatonin level decreases during the day and rises at night. The darker the environment we sleep in, the healthier our melatonin secretion. Research has shown that melatonin, which is secreted during the night, sends signals to the cells and organs of the body to maintain homeostasis and to regulate metabolic rhythms such as the biological clock and regulating our body's biorhythm. However, it also has effects on the immune system [5].

1. What is Melatonin?

Melatonin is a pineal gland hormone known for a long time. Basically, it has immune-modulatory, daytime and seasonal rhythm-regulating, sleep-regulating effects. There are widespread melatonin receptors throughout the body. Melatonin, which is also used against sleep disorders and as an antidepressant, is being tried in the treatment of many diseases [6].

The pineal gland (pineal gland) was described by Herophilus of Alexandria (325-280 BC) in 300 BC. Galen of Pergamum used the name conareion (Latin conarium) for the pineal gland because it resembles a pine cone. Vesalius (1514-1564) described the topography of the pineal gland and

2nd

Rene Descartes (1596-1650), the famous philosopher, physician and mathematician of the middle age, defined its structure as "the place where the soul resides" and emphasized its importance in memory functions [7]. In 1850 Kolliker observed the presence of nerve fibers in the pineal gland of mammals [7]. Cajal found bundled nerve fibers in the mouse pineal gland and claimed to be of sympathetic origin [7].

The most important development is that Lerner et al. isolated the potential pineal hormone found in pineal extracts, which, when administered to amphibians, causes skin lightening. However, it has been found that this hormone has no effect on pigment in mammals. Lerner named this substance “melatonin” by combining the Greek words “molasses” meaning black and “tosos” meaning work [6,9].

2. Melatonin and Cancer

In the studies carried out by Bergmann and Engel between 1935-1952, it was revealed that pineal gland extracts retarded growth in experimental animals, giving rise to the idea that this could also delay cancer growth. The authors reported that, in addition to the cancer being under control in patients with prostate cancer, the patients' pain also decreased and their general condition improved.

have observed [10].
Today, the majority of melatonin and cancer studies are performed on breast cancer models. Studies have shown that nighttime applications of melatonin give more successful results in cancer. Data have been obtained suggesting that the disruption of melatonin secretion at night is important in cancer development. It has been revealed that the incidence of cancer increases especially in women who work under the light at night. In fact, studies showing the existence of a direct correlation between the degree of light intensity and tumor growth rate have been reported [11-13].
The effect of seasons on cancer development is proportional to melatonin. Melatonin production is high during the winter months, when the nights are long, and tumor development slows down during this period [14].
In cancer treatment, melatonin is used together with IL-2. In the combination of IL-2, which has many side effects, with melatonin, melatonin increases the desired effect of IL-2, thus reducing the effective dose of IL-2 [15].
Melatonin reduces the receptors of linoleic acid, which is one of the cancer cell growth factors, that allow it to enter the cancer cell. It was determined that melatonin binds to Ca2+ activated calmodulin with high affinity. Thus, it has been suggested that melatonin will prevent the growth of the tumor by slowing down the cell cycle by removing calmodulin from calcium. Melatonin also has the ability to prevent the formation of apoptosis in healthy cells [13].
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The balance between protooncogenes and tumor suppressor genes (TSG) is important in cancer formation. For the continuity of this balance, sensitive and balanced expression of melatonin genes in cells is required. Factors that disrupt this balance increase the tendency of cells to cancer [17].
Melatonin has also been reported to promote the repair of damaged DNA. As it is known, linoleic acid, which is an energy source and growth factor for the development, division and proliferation of cancer cells, cannot be produced in the body and therefore has to be taken from outside with food. At this stage, melatonin prevents linoleic acid from entering the cancer cell and suppresses its metabolism [18]. Melatonin reduces the synthesis of adhesion molecules and proinflammatory cytokines [19].
In addition to its strong antioxidant effect, melatonin has another function, such as increasing glutathione peroxidase activity (GPA) in neural tissues. Higher brain GPA at night is closely related to high melatonin levels. Glutathione peroxidase is known as the main enzyme that removes peroxides in the brain [20].
As studies in the literature have shown, melatonin stimulates antioxidant enzymes, prevents lipid peroxidation and protects brain tissue from oxygen-derived free radicals.

3. Hypothesis

Based on this, it was hypothesized in this study that melatonin would also be effective in brain cancer cells and would kill cancer cells.
2.Materials and Methods
The process of determining the method started with a literature search. Many publications were scanned in the research. The determined method was determined and the suitability was discussed with Prof. Dr. Şermin Genç. After the approval of its suitability, the research was started with Prof. Dr. Şermin Genç. For the studies that lasted 3-4 months, and preliminary literature research was completed.

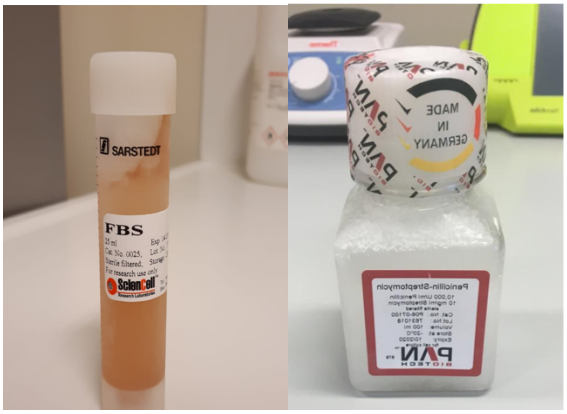
2.1.Materials

U87mg cells: These commercially available cells are human-derived brain cancer (neuroblastoma) cells. They cling to the surface. These cells are immortalized (immortal) cells

by biotechnological methods. In this way, it can be kept alive for the desired time, when the experiment is not going to be done, it can be frozen in liquid nitrogen and thawed again and the experiment can be done.

Cell nutrient medium:

Contains the nutrients required for the healthy growth of cells. It is the most basic need for growing cells in cell culture. The media needs of each cell type are different. The basic cell nutrient medium must be amino acids, vitamins, inorganic salts, glucose and serum. In addition to providing nutrients, a nutrient medium must also be appropriate in terms of pH and osmolality. For the U87mg cells in our study, Gibco Brand Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g/L D-glucose and L-glucose was used. D-glucose is food for cells. L-glutamine is an essential amino acid.



Fetal bovine serum and Penicillin/streptomycin:

10% fetal bovine serum and 1% penicillin/streptomycin antibiotics were added to the basic medium for cell growth. While the serum promotes growth, the antibiotic mixture provides protection against contamination in cell culture.

Trypsin: Cells grown in cell culture, like our cells, are removed from the surface using the chemical trypsin. This chemical ensures that the surface proteins that allow the cells to attach to the surface are detached from the surface. The cells, which are removed and collected with trypsin, are precipitated with a centrifuge machine for an appropriate time and speed and turned into a pellet. These pellet cells are counted under the microscope and inoculated into new containers at a density suitable for the study.

Melatonin: The melatonin substance used in our study was mixed into the cell nutrient medium in 3 different doses and given to the cells.

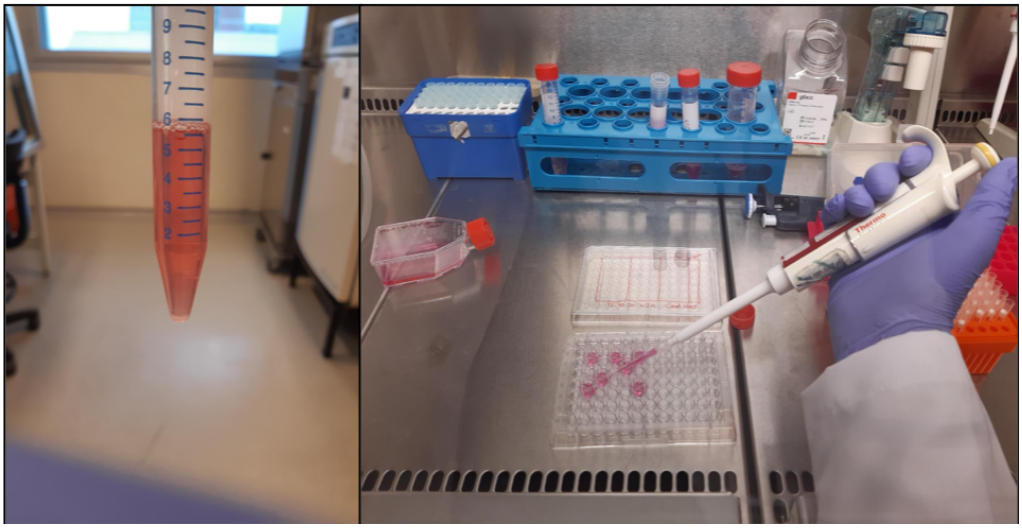
Propidium iodide dye: This dye, briefly referred to as PI, stains the DNA that becomes accessible in dead cells and gives a red color under a fluorescent microscope. In this way, we can see how many dead cells are in the Cell cup.

Presto blue: This dye provides data about the viability of our cells by making use of the metabolic activity of living cells. This dye is the substance resazurin. This substance is metabolized by living cells and converted to resorufin and released out of the cell. Thus, the cell environment changes from blue to pink.

2.2. Methods

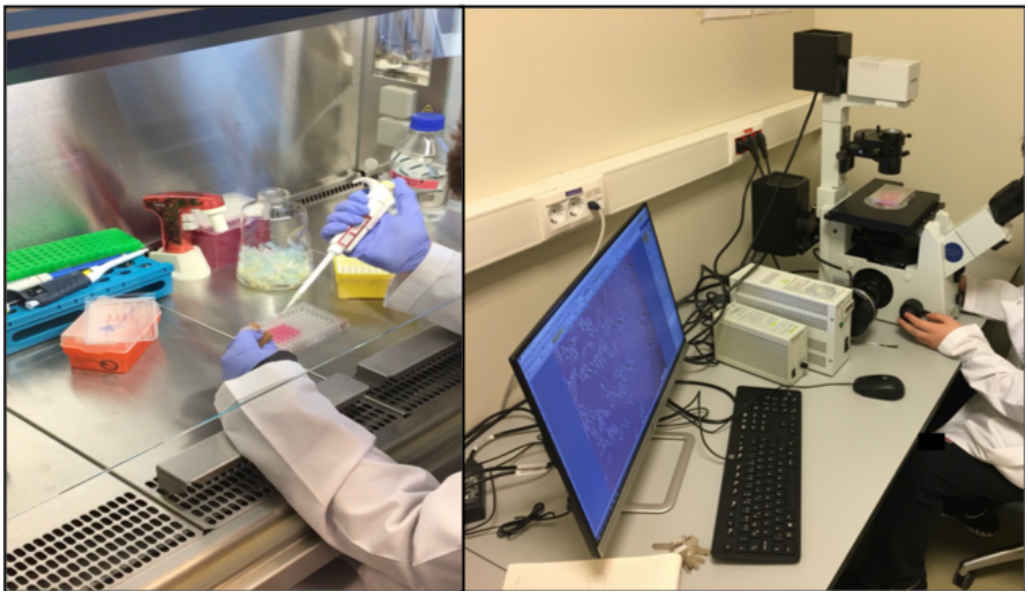
2.2.1. Cell Culture Method

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 an 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.



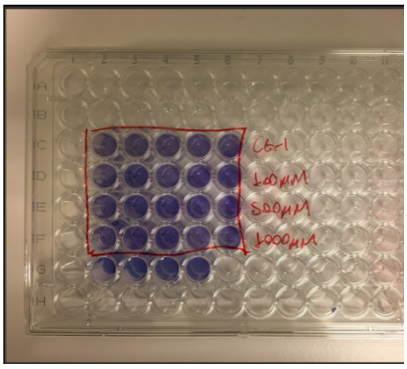
2.2.2. Propidium Iodide (PI) Staining Method

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 a fluorescent microscope. 20X objective is used.



2.2.3. Presto Blue 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 in the incubator for 15 minutes, they were read at 560 and 590 nm wavelengths in the Thermo brand Varioskan Flash, a multiplex reader device, and the values were analyzed.



2.2.4. Analysis of Data

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

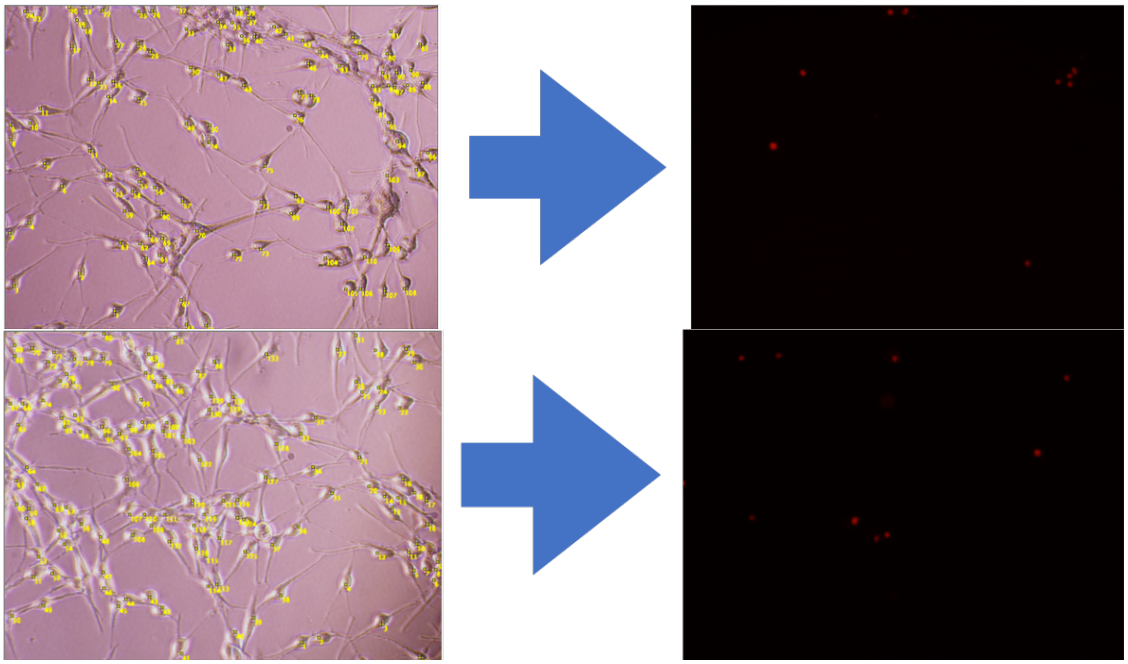
Data obtained from PI staining and Presto blue staining were analyzed by statistical methods, using the Graphpad program. Non-parametric Mann-Whitney U test was used for 2-group comparison in the analysis. This test evaluated whether the results we obtained occurred randomly when assessing the accuracy 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 the two 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.

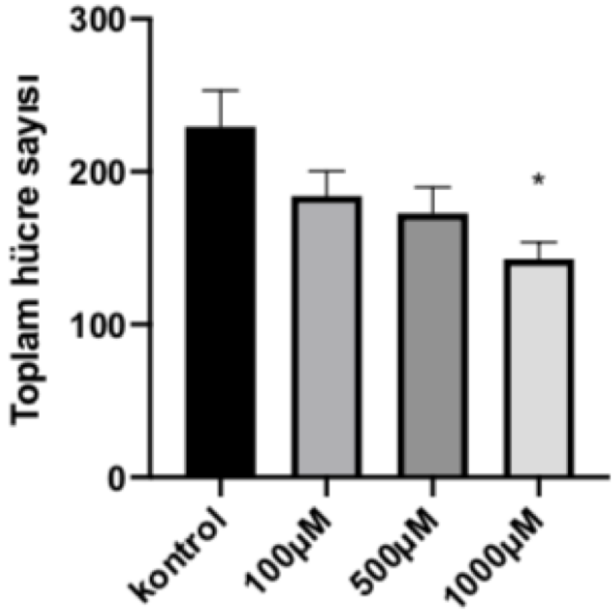
3. **Findings**

3.1.PI Staining Findings

As a result of PI staining, the total number of cells 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: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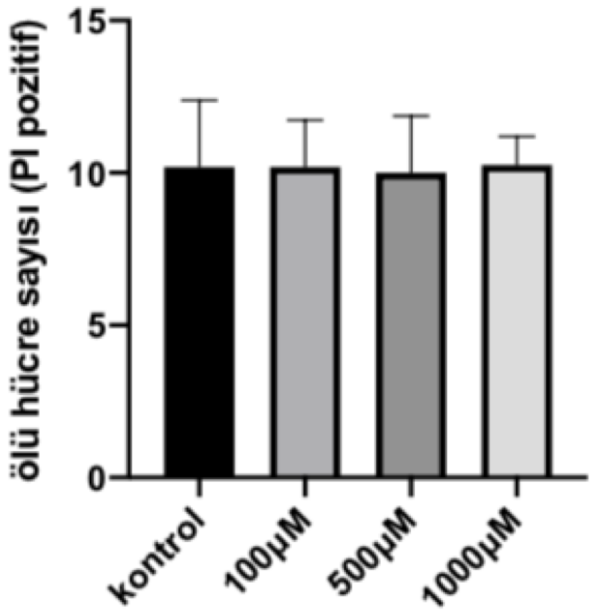
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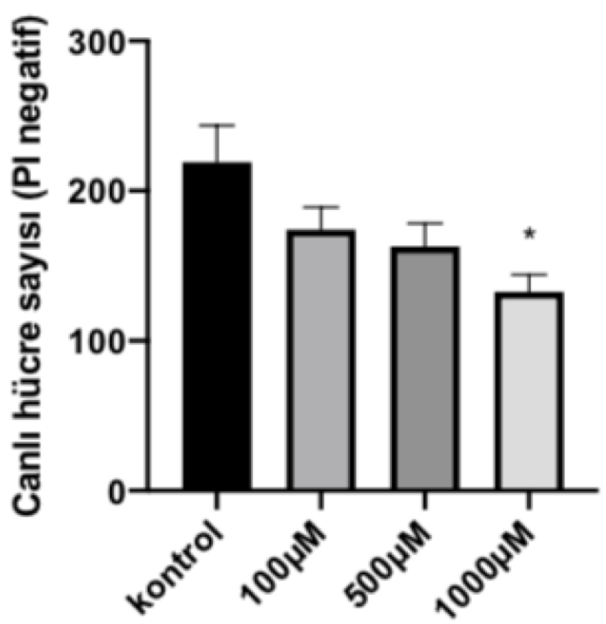
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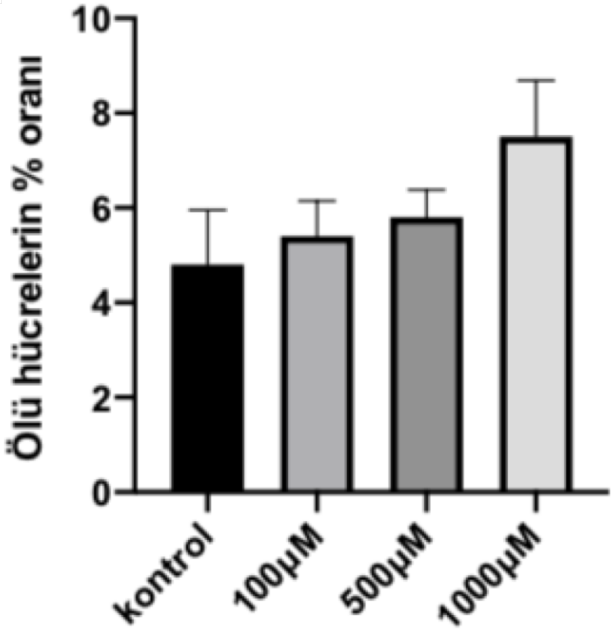
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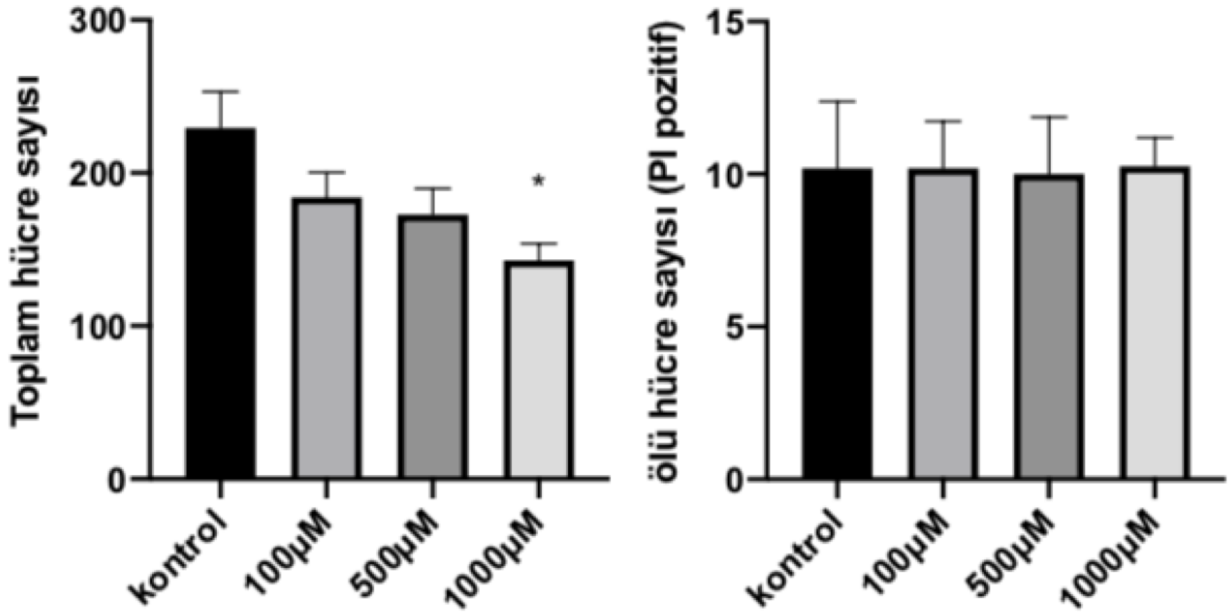
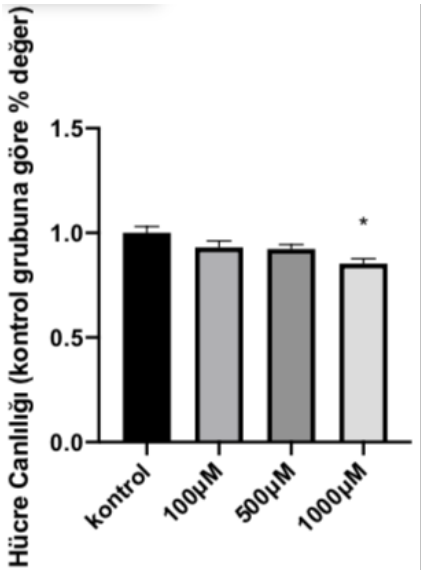


According to these analyses, melatonin at a dose of 1000 µM significantly reduces the number of viable cells and the total number of cells ($p>0.05$). This effect was not observed for other doses. Similarly, no effect was observed in the number of dead cells and the percentage of dead cells. As we see in the graphs, there is a decreasing 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.

3.2. 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.



4. **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 the application, so they are not painted. A study can be done by doing experiments at different times (time course).

5. **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.

Sources

1. Coleman, M. P. and R. J. Reiter (1992). "Breast cancer, blindness and melatonin." Eur J Cancer 28(2-3): 501-503.
2. Feychting, M., et al. (1998). "Reduced cancer incidence among the blind." Epidemiology 9(5): 490-494.
3. Flynn-Evans, E. E., et al. (2009). "Total visual blindness is protective against breast cancer." Cancer Causes Control 20(9): 1753-1756.
4. Kliukiene, J., et al. (2001). "Risk of breast cancer among Norwegian women with visual impairment." Br J Cancer 84(3): 397-399.
5. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: Distribution and functions. Mol Cell Endocrinol. 2012; 351:152-166.
6. Grant SG, Melan MA, Latimer JJ, Witt-Enderby PA. Melatonin and breast cancer: Cellular mechanisms, clinical studies and future perspectives. Expert Rev Mol Med. 2009; 11:e5.
7. Palaoğlu ÖS, Beşkonaklı E. Pineal bez ve yaşlanma. Geriatri Turkish Journal of Geriatrics 1998;1:13-8.
8. Çam A, Erdoğan MF. Melatonin. Ankara Üniversitesi Tıp Fakültesi. Mecmuası 2003; 56:103-12.
9. Lerner AB. Hormones and Skin color. Scientific American 1961 (July) 456-60.
10. Bergmann, W. and Engel, P. Uber den Einfluss von Zirbelextrakten auf Tumoren bei weissen Mäusen und bei Menschen. Wien. klin. Wschr 1950;62:79–82.
11. Hotchkiss AK,Nelson RJ.Melatonin and immune function:hyper hypothesis?Crit Rev Immunol 2002;22:351-71
12. Çetin E. Melatonin ve bağışıklık sistemi. Erciyes Üniv Vet Fak Derg 2005;2:119-23.
13. Reiter RJ, Tan DX, Erren TC, Fuentes-Broto L, Paredes SD. Light-mediated perturbations of circadian timing and cancer risk: a mechanistic analysis. Integr Cancer Ther 2009;8:354-60
14. Choi, D. (2013). "Potency of melatonin in living beings." Dev Reprod 17(3): 149-177.
15. Lissoni P, Barni S, Tancini G, Mainini E, Piglia F, Maestroni GJ, et al. Immunoendocrine therapy with low-dose subcutaneous interleukin-2 plus melatonin of locally advanced or metastatic endocrine tumors. Oncology 1995;52:163-6.
16. Maestroni GJ. The photoperiod transducer melatonin and the immune-hematopoietic system. J Photochem Photobiol B 1998;43:186-92.
17. Turgut T, Şükrü Ö, Ahmet K. Melatonin ve kanserle ilişkisi. Genel Tıp Dergisi 2009;19:137-43.
18. Kerman M, Cirak B, Ozguner MF, Dagtekin A, Sutcu R, Altuntas I, et al. Does melatonin protect or treat brain damage from traumatic oxidative stress? Exp Brain Res 2005;163:406-10.

19. Reiter RJ. Melatonin: clinical relevance. Best Pract Res Clin Endocrinol Metab 19.
2003;17:273-85.
20. Kuş D, Sarsılmaz M. Pineal bezin morfolojik yapısı ve fonksiyonları. T Klin J Med 20.
Sci 2002;22:221-6.