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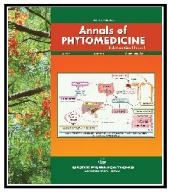

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Optimizing melatonin dosage for neuroprotection in HT22 hippocampal neuronal cells

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Abstract

Melatonin is widely studied for its neuroprotective properties in various cellular models, but the optimal dosage for therapeutic effects remains poorly defined, especially in neuronal cell lines like HT22. Discrepancies in the literature regarding effective melatonin concentrations necessitate a systematic investigation to establish a standardized therapeutic window that balances efficacy and safety. The aim is to identify the optimal dosage of melatonin that promotes cell viability and neuroprotection in the HT22 hippocampal neuronal cell line over various exposure times. A quantitative analysis using the MTT assay measured cell viability in HT22 cells treated with increasing concentrations of melatonin (0.1 mM to 5 mM) over time intervals of 24, 48, and 72 h. This approach allowed for the assessment of both the neuroprotective and cytotoxic effects of melatonin across a range of dosages. The study revealed a dose-dependent impact on cell viability, with higher concentrations leading to significant cytotoxic effects. Cell viability was relatively high at lower concentrations (0.1 mM to 0.5 mM) across all time points but showed a marked decline at concentrations exceeding 0.75 mM. The optimal balance between neuroprotection and minimal cytotoxicity was found at doses from 0.1 mM to 0.25 mM. The half-maximal inhibitory concentration (IC_{50}) values indicated increased sensitivity to melatonin over time, decreasing from 0.83 mM at 24 h to 0.39 mM at 72 h. This study successfully defines a more precise therapeutic range for melatonin in HT22 cells, suggesting that lower concentrations (0.1 mM to 0.25 mM) optimize cell viability and neuroprotection without inducing significant cytotoxic effects. These findings contribute to the ongoing effort to standardize melatonin dosages in neurobiological research and clinical applications, potentially influencing.

1. Introduction

Cell culture experiments are commonly conducted in biological research to study the behaviour and characteristics of cells under controlled conditions (Zatulovskiy *et al*., 2020). These cell lines can be derived from various tissues and organisms, including humans, mice, insects, and others such as HEK293, HeLa, NIH/3T3, and HT22 (Zhang *et al*., 2018). They are used extensively in biological and medical research for various purposes, such as studying cellular mechanisms, drug development, and understanding disease (Shekh *et al*., 2023). HT22 cells are an immortalized mouse hippocampal cell line (Wang *et al*., 2019A). They exhibit a high susceptibility to oxidative stress and excitotoxicity, which makes them a dependable, consistent, and an appropriate model for investigating neurodegenerative diseases (Cesarini *et al*., 2018; Fernández-Acosta *et al*., 2023). During any experimentation, it is vital to consider the concentration of the test compound (Fukui *et al*., 2009). Cell viability assays are crucial in determining the health and function of cells in various experimental conditions. They help to assess different aspects of cell viability, such as metabolic activity, membrane integrity, and enzyme activity (Casañas-Sánchez *et al*., 2016). There are too many common assays and techniques used for cell viability measurements, such as MTT assay, ATP assay, assessment based on membrane integrity, dye exclusion tests, esterase activity, flow cytometry, western blot, and mitochondrial membrane potential assays (He *et al*., 2013; Park *et al*., 2020; Zhang *et al*., 2021).

MTT assay utilizes the yellow tetrazolium salt, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), which is reduced by the action of mitochondrial dehydrogenases in living cells to an insoluble purple formazan product. The amount of formazan produced is directly proportional to the number of viable cells (Karatop *et al*., 2022). It is important to note that dose

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concentrations of compounds used in experiments can affect the viability of cells and increasing the concentrations could lead to decreased viability (Dludla *et al*., 2018). Therefore, dose-response curves are essential to understanding the relationship between dose and cell viability. Melatonin, primarily produced by the pineal gland, regulates various endocrine processes in the body (Cipolla-Neto and Amaral, 2018). At physiological concentrations (0.01 to 10 nM), it influences intracellular cyclic nucleotides, calcium levels, protein kinase C subtypes, steroid hormone receptor localization, and G protein signaling proteins (Nikolaev *et al.,* 2021; Cook, 2010). At higher concentrations (1 to 100 μM), melatonin affects receptor expression, and endogenous production, and may impact various disorders including circadian rhythm sleep disorders, Alzheimer's, Parkinson's, glaucoma, depression, and cancers (Dubocovich, 1988; Al-Wasidi *et al.,* 2020). Recent research emphasizes the dual physiological and pharmacological effects of melatonin, with outcomes contingent on its concentration (Zawilska *et al.,* 2009).

There is great interest in melatonin neuroprotective and antioxidant properties, as well as its impact on cell viability (Wang *et al.,* 2019B). At physiological concentrations, melatonin may mitigate oxidative stress and protect cells from damage, whereas, at pharmacological levels, it could significantly influence cell growth and survival, often explored in studies on neuroprotection or cellular resilience (Corpas *et al.,* 2018; Wang *et al.,* 2019B). However, the specific outcomes vary depending on experimental conditions and study context, given the complexity of melatonin's effects (Niska *et al.,* 2015).

The variability in melatonin dosage across existing literature highlights a significant gap in our understanding of its optimal concentration for therapeutic effects in cell cultures, particularly in models of neuronal damage and protection. Studies have employed a wide range of melatonin concentrations, from as low as 100 nM to as high as 1 mM, to investigate its protective effects against oxidative stress and other forms of cellular injury (Herrera *et al*., 2007; García-Santos *et al*., 2006). For instance, melatonin concentrations have varied dramatically across different studies, with some using low doses (200 nM) to demonstrate reduction in cell death during serum deprivation (Di Sario *et al*., 2017), while others have used much higher concentrations, such as 500 μM, to protect against serum deprivation (Cesarini *et al*., 2018), (1-10 μM) MEL by (Kwon *et al*., 2010) or even up to 1 mM in lipid peroxidation assays (García-Santos *et al*., 2006; Walters-Laporte *et al*., 1998).

Moreover, the concentration-dependent effects of melatonin have also been noted in studies assessing neuroprotective properties against beta-amyloid-induced neurodegeneration, with ranges spanning from 1 μM to 500 μM (Gao *et al*., 2023). Melatonin was also investigated at both higher (10^{-5} M) and low (10^{-9} M) concentrations on microtubule polymerization and cytoskeletons (Huerto Delgadillo *et al*., 1994). This wide variation in dosages underscores the lack of consensus and the need for a systematic investigation to establish a standardized, effective concentration that maximizes melatonin's therapeutic benefits while minimizing potential cytotoxic effects.

This study aims to refine the understanding of melatonin's optimal dosage by analyzing its effects across a controlled range of concentrations, evaluating both efficacy and safety in cell viability, morphological changes, and genetic expression responses. It also aims to determine the melatonin doses that have the greatest impact on the HT22 cell line over spans of 24-48-72 h. By determining the ideal concentration, this research hopes to provide a foundational guideline that can be used to enhance the design and outcome of future therapeutic strategies involving melatonin in various biomedical applications.

2. Materials and Methods

2.1 Reagents

Melatonin, dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), 100 U/ml penicillin, 10 mg/ml streptomycin, L-glutamine 200 mM (29.2 mg/ml), trypsin-ethylene diamine tetra acetic acid (EDTA), and dulbecco's phosphate-buffered saline (PBS), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT). Chemical reagents were purchased from the biological industries of Israel Beit Haemek Ltd. and Gibco BRL.

2.2 MTT assay protocol

Three replicates of the HT22 hippocampal neural cell line were seeded in a 96-well plate. The complete media was aspirated after 24 h, and cells were washed with PBS. Melatonin was applied at various concentrations (0.1 to 5 mM), and the 96-well plate was incubated for 24-48-72 h. MTT solution was added, and the well plates were incubated for 2 h. Then the DMSO was added, and the plate was covered with folio and incubated or left at room temperature for 10 min. The results were analyzed at 570 nm absorbance using an ELISA microplate reader at 570 nm to measure cell samples (Uðuz and Nazýroðlu, 2012; Phelan and May, 2015).

2.3 Statistical analysis

Statistical analysis was performed using SPSS 21.0 (IBM Corporation, Armonk, NY, USA) software, Microsoft excel 365 and graphpad prism version 9.5.1 The data were presented as mean % and ± SD. One-way ANOVA test and tukey's multiple comparisons test were used in this study to compare between the results of the groups.

3. Results

This study assessed the effects of melatonin on cell viability over three distinct periods: 24, 48, and 72 h increasing concentrations of melatonin ranging from 0.1 mM to 5 mM. At 24 h, a dose-dependent decrease in cell viability was observed. Viability remained relatively high at low doses but began to decline markedly at higher concentrations. For instance, cell viability was 84.64% at 0.1 mM and dropped to 55.45% at 0.75 mM. At doses higher than 1 mM, the viability sharply decreased, reaching as low as 4.95% at 4 mM. This indicates a significant cytotoxic effect of melatonin at higher concentrations within the first 24 h. The half-maximal inhibitory concentration (IC_{50}) for melatonin at 24 h was calculated to be 0.83 mM. This value represents the concentration at which melatonin reduces the cell viability by 50% relative to the control within the first day of treatment.

Table 1: Illustrates the mean number of cells with viability and the significant degrees of the various Mel concentrations after 24 h of cell treatment.

Melatonin doses (mM)	Mean cell viability% (SD)	*p* value Cont. vs. Mel.
0.1	84.64% (SD ± 4.21)	0.6528
0.25	76.17% (SD ± 25.64)	0.1297
0.5	59.75% (SD ± 6.05)	0.0012*
0.75	55.45% (SD ± 13.97)	0.0003*
0.9	61.8% (SD ± 7.12)	0.0022*
1	57.75% (SD ± 3.3)	0.0007*
2	6.91% (SD ± 1.89)	<0.0001*
3	5.56% (SD ± 1.52)	<0.0001*
4	4.95% (SD ± 1.31)	<0.0001*
5	5.82% (SD ± 0.79)	<0.0001*

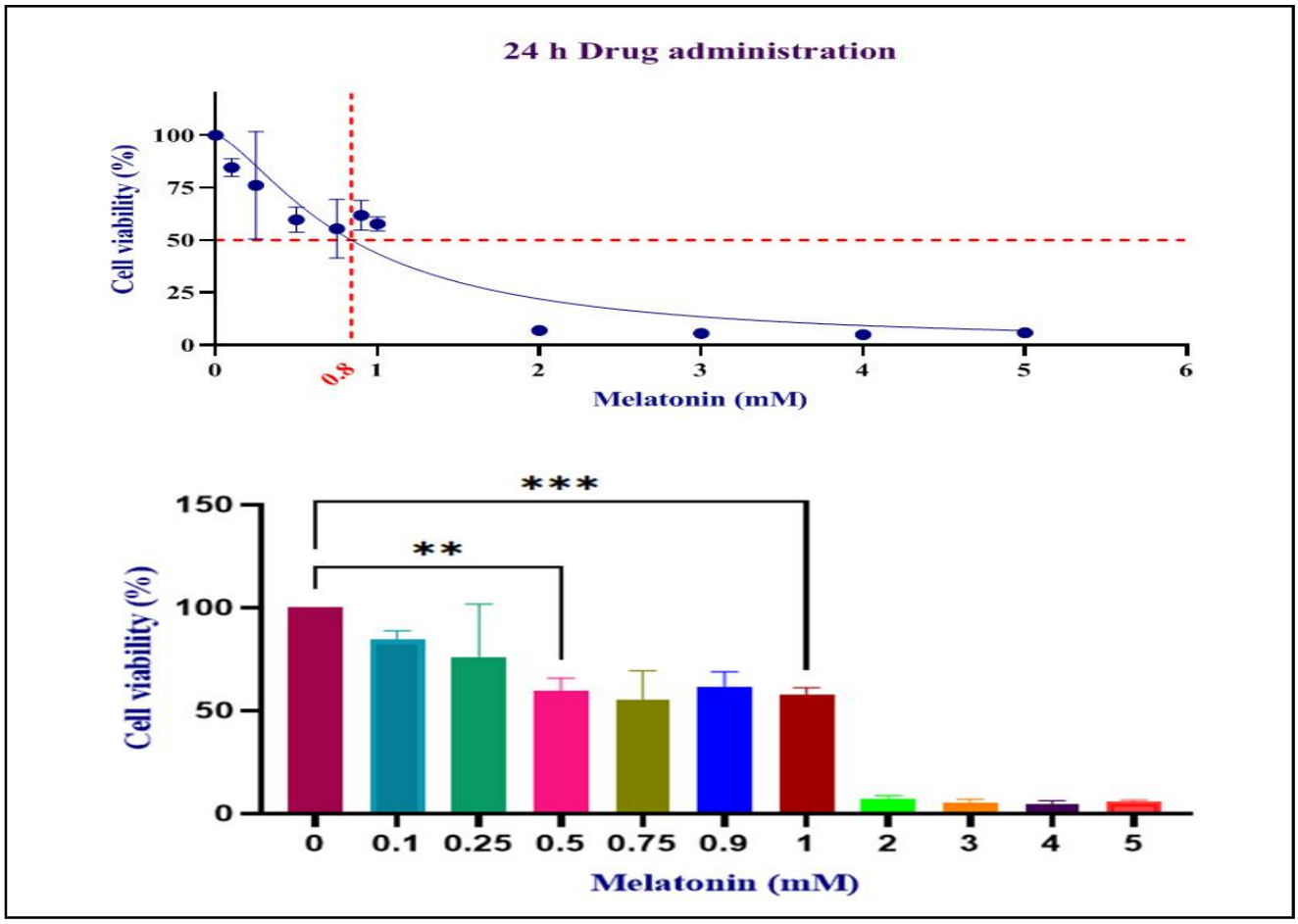


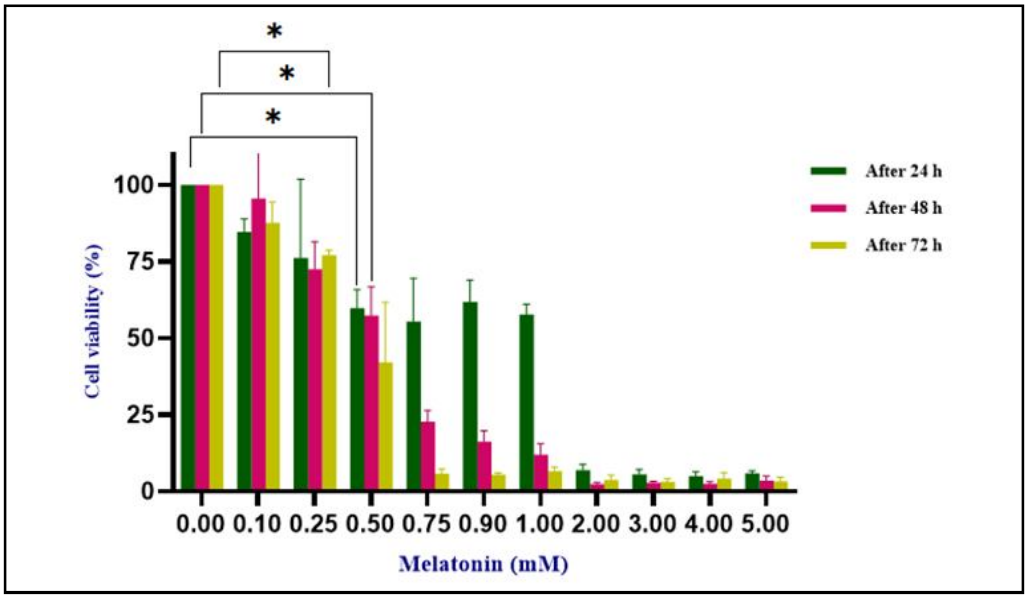
Figure 1: A. Shows the cells started to lose viability in the low doses and the death rate increases with the increase in the Mel. doses at the concentration of 0.5 mM, the cells viability was around 59.75, and at the concentrations of 0.8 mM nearly half of cells died. B. Compared to the control, no significant difference was seen at Mel doses under 0.5 mM. However, the significance was present starting form 0.5 mM. 1 mM showed very high significance when compared to the Cont. group.

The analysis focused on how cell viability changed over the three time intervals assessed (24, 48, and 72 h) at varying doses of melatonin. At 0.1 mM, cell viability was relatively high at 84.64% at 24 h and slightly increased to 95.52% at 48 h before decreasing to 87.57% at 72 h. This fluctuation suggests an adaptive response to melatonin exposure, followed by a decline. At 0.25 mM, there was a gradual decrease from 76.17% at 24 h to 72.42% at 48 h, followed by a slight increase to 77.04% at 72 h. This pattern indicates a possible acclimatization to the melatonin exposure over time. At 0.5 mM, viability started at 59.75% at 24 h, decreased slightly to 57.33% at 48 h, and more significantly to 42.04% at 72 h, demonstrating a continuous decline with extended exposure.

Table 2: Illustrates the mean number of cells with viability of the various Mel concentrations after 24, 48, and 72 h of cell treatment

Melatonin (M)	After 24 h % (SD)	*p* value Cont. vs. Mel	After 48 h % (SD)	*p* value Cont. vs. Mel	After 72 h% (SD)	*p* value Cont. vs. Mel
Control	100% (SD ± 0)	-	100% (SD ± 0)	-	100% (SD± 0)	-
0.1	84.64% (SD ± 4.21)	.076	95.52% (SD ±17.51)	.997	87.57% (SD ± 6.81)	.259
0.25	76.17% (SD ± 25.64)	.632	72.42% (SD ± 8.93)	.103	77.04% (SD ± 1.57)	.005*
0.5	59.75% (SD ± 6.05)	.024*	57.33% (SD ± 9.33)	.049*	42.04% (SD ± 19.62)	.112
0.75	55.45% (SD ± 13.97)	.097	22.67% (SD ± 3.66)	.002*	5.76% (SD ± 1.41)	<.001*
0.9	61.8% (SD ± 7.12)	.036*	16.16% (SD ± 3.56)	.002*	5.43% (SD ± 0.55)	<.001*
1	57.75% (SD± 3.3)	.006*	12.01% (SD ± 3.52)	.002*	6.57% (SD ± 1.34)	<.001*
2	6.91% (SD ± 1.89)	<.001*	2.36% (SD ± 0.56)	<.001*	3.74% (SD ± 1.49)	<.001*
3	5.56% (SD ± 1.51)	<.001*	2.83% (SD ± 0.44)	<.001*	3.07% (SD ± 1.06)	<.001*
4	4.95% (SD ± 1.31)	<.001*	2.45% (SD ± 0.69)	<.001*	4.13% (SD ± 1.98)	<.001*
5	5.82% (SD ± 0.79)	<.001*	3.4% (SD ± 1.61)	<.001*	3.21% (SD ± 1.39)	<.001*

At higher concentrations, the reduction was noted: At 0.75 mM, the viability decreased from 55.45% at 24 h, to 22.67% at 48 h, and to 5.76% at 72 h. This indicates a significant cytotoxic effect as both dose and exposure time increased. Concentrations of 0.9 mM and 1 mM followed similar trends, with initial viabilities of 61.8% and 57.75% at 24 h, respectively, dropping dramatically by 72 h to 5.43% and 6.57%. These levels suggest that the cytotoxic threshold for these concentrations lies between the 24 and 48 h marks. At the highest tested concentrations (2 mM, 3 mM, 4 mM, and 5 mM), cell viability was already below 10% at 24 h and continued to decline, reaching minimal levels by 72 h (ranging between 3.07% to 4.13%).



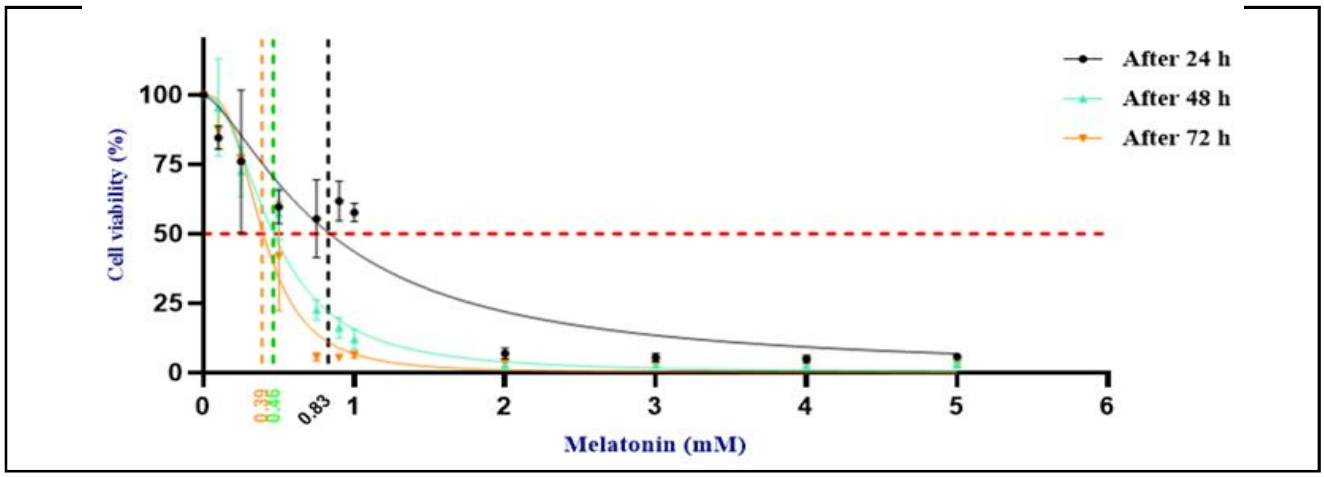


Figure 2: A. Neuroprotective effects of Mel on HT22 cell viability for 24, 48, and 72 h. The values were given as the mean ± standard deviation (n=4). 24 h cell viability was compared to 48 and 72 h. cell viability. B. Comparison of different concentrations of the protective melatonin substance at various periods (24-48-72 h), the IC_{50} was 0.83,0.46, and 0.39 for 24, 48, and 72 h, respectively.

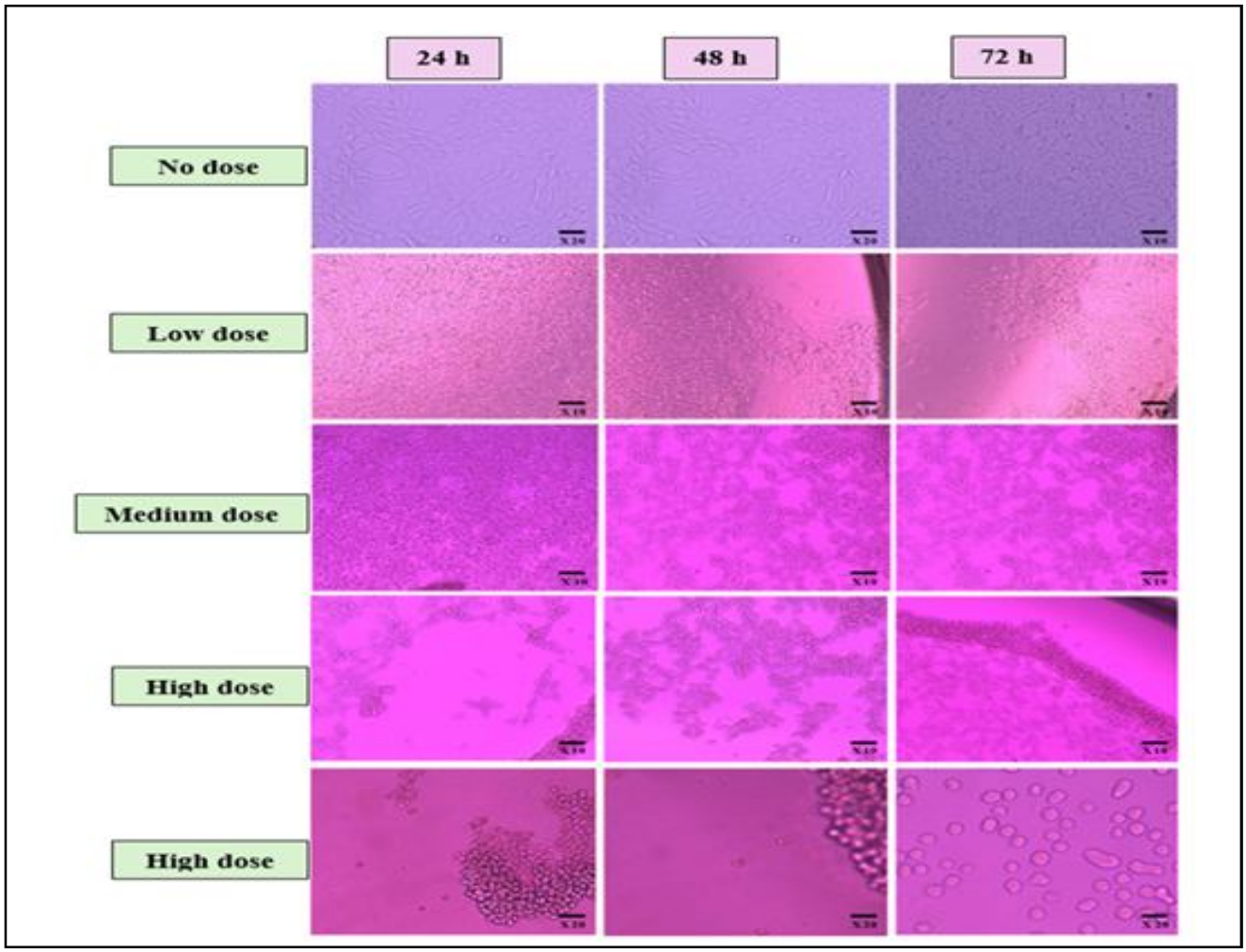


Figure 3: HT22 hippocampal neural cell line in culture cell well-plate taken from the control group and Mel at different doses from 0.1 mM to 5 mM and durations 24, 48, and 72 h. The image was captured by an inverted microscope at 4X to 10X magnification.

Histological analysis under the microscope revealed notable dose-dependent and time-dependent alterations in cellular morphology. At low concentrations cells retained normal morphology through 24 h, with increasing concentration, mild effects were noted and there was an increase in cytoplasmic vacuolization, indicating a stress response. By 48 h, there were few signs of stress such as shrinkage and chromatin condensation. however, with increasing concentrations, the cells showed more apoptotic features. At 0.5 mM, signs of apoptosis were evident even at 24 h, with increased nuclear fragmentation and cytoplasmic vacuolization. By 72 h, a significant number of cells displayed apoptotic features, nuclear fragmentation and loss of membrane integrity.

At 0.75 to 1 mM, marked morphological changes were visible from 24 h onward, with a significant increase in apoptotic bodies and cell shrinkage. By 72 h, the majority of cells exhibited a rapid onset of cytotoxic effects with severe apoptotic changes including extensive cytoplasmic blebbing and nuclear condensation.

At very high concentrations, drastic changes were observed from the outset. By 24 h, most cells had undergone necrosis, as evidenced by loss of plasma membrane integrity and leakage of cellular contents. By 48 and 72 h, the remaining cells were largely necrotic or in late stages of apoptosis, with very few intact cells observed.

4. Discussion

This study aimed to delineate the optimal dosage of melatonin for maintaining cell viability in the HT22 hippocampal neuronal cell line, considering the discrepancies in melatonin dosages reported in existing literature (García-Santos *et al*., 2006; Herrera *et al*., 2007; Cesarini *et al*., 2018; Di Sario *et al*., 2017; Gao *et al*., 2023). The variability observed in past studies reflects a broad spectrum of experimental conditions and outcomes, which underscores the need for establishing a standardized approach to melatonin usage in neuroprotective research.

Previous research has highlighted a wide range of melatonin concentrations used to assess its protective and therapeutic effects. For instance, Herrera *et al*. (2007) and García-Santos *et al*. (2006) employed melatonin dosages varying from 100 nM to 1 mM to explore its antioxidant properties in lipid peroxidation assays (García-Santos *et al*., 2006; Herrera *et al*., 2007). Similarly, other studies have utilized doses ranging from 200 nM to 500 µM to investigate melatonin's protective effects against cellular stress and serum deprivation (Cesarini *et al*., 2018; Di Sario *et al*., 2017). This significant variance in dosages has led to inconsistent findings, particularly in terms of cytotoxicity and neuroprotection, thus complicating the development of a consensus regarding effective therapeutic concentrations.

The present study analyzed the effects of melatonin across a controlled range of concentrations (0.1 mM to 5 mM) over periods of 24, 48, and 72 h. Our findings indicate a dose-dependent decrease in cell viability, particularly at higher concentrations. For instance, cell viability significantly dropped at concentrations exceeding 0.5 mM, with the most profound decreases observed at concentrations of 1 mM and higher. This dose-dependent cytotoxicity aligns with the observations made by Gao *et al*. (2023), who noted similar trends in beta-amyloid-induced neurodegeneration models (Gao *et al*., 2023). The optimal concentration for neuroprotection without inducing cytotoxic effects appears to lie below the higher thresholds used in some previous studies. For example, concentrations as high as 500 µM, which were employed by Cesarini *et al*. (2018), may exceed the therapeutic window, as suggested by the increased cytotoxicity observed at these levels in our study (Cesarini *et al*., 2018). Our findings suggest that lower concentrations, potentially in the range of 0.1 mM to 0.25 mM, may offer a balance between promoting cell viability and minimizing harmful effects, thereby supporting the use of melatonin in neuroprotective therapies.

This study underscores the importance of establishing a clear, evidence-based understanding of melatonin's dose-dependent effects on cell viability. By determining the IC_{50} values across various time points and meticulously documenting the cellular responses to different dosages, we provide a foundational guideline that can be employed to refine experimental designs in neurobiology and pharmacology. Future research should focus on corroborating these findings across different cell lines and in vivo models to validate the generalizability of the optimal dosage range for melatonin.

5. Conclusion

In conclusion, the variability in melatonin dosage documented in the literature emphasizes the critical need for standardized, dose-escalation studies like the present one. Our findings contribute to narrowing the gap in understanding the precise, effective dosages of melatonin that balance neuroprotection with minimal cytotoxicity, paving the way for its therapeutic application in neurodegenerative disease models.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Al-Wasidi, A. S.; Naglah, A. M.; Refat, M.;El-Megharbel, S. M.; Kalmouch, A. and Moustafa, G. O. M. (2020). Synthesis, spectroscopic characterization and antimicrobial studies of Mn (II), Co (II), Ni (II), Cr (III) and Fe (III) melatonin drug complexes. Egyptian J. Chem., **63**(4):1469-1481.

Casañas-Sánchez, V.; Pérez, J. A.; Quinto-Alemany, D. and Díaz, M. (2016). Sub-toxic ethanol exposure modulates gene expression and enzyme activity of antioxidant systems to provide neuroprotection in hippocampal HT22 cells. Front. Physiol., **7**:204269.

Cesarini, E.; Cerioni, L.; Canonico, B.; Di Sario, G.; Guidarelli, A.; Lattanzi, D.; Savelli, D.; Guescini, M.; Nasoni, M. G. and Bigini, N. (2018). Melatonin protects hippocampal HT22 cells from the effects of serum deprivation specifically targeting mitochondria. PloS One, **13**(8):e0203001.

Cipolla-Neto, J. and Amaral, F. G. D. (2018). Melatonin as a hormone: New physiological and clinical insights. Endo. Rev., **39**(6):990-1028.

Cook, J. S. (2010). Neurovascular responses to melatonin in humans.

Corpas, R.; Griñán Ferré, C.; Palomera Ávalos, V.; Porquet, D.; García de Frutos, P.; Franciscato Cozzolino, S. M.;Rodríguez Farré, E.; Pallàs, M.;Sanfeliu, C. and Cardoso, B. R. (2018). Melatonin induces mechanisms of brain resilience against neurodegeneration. J. Pineal Res., **65**(4):e12515.

Di Sario, G.; Cesarini, E.; Canonico, B.; Gabrielli, S.; Zamai, L.; Papa, S. and Luchetti, F. (2017). Role of melatonin in HT22 cells challenged with serum deprivation. Italian J. Anat. Embryol., **121**(1):156.

Dludla, P. V.; Jack, B.; Viraragavan, A.; Pheiffer, C.; Johnson, R.; Louw, J. and Muller, C. J. (2018). A dose-dependent effect of dimethyl sulfoxide on lipid content, cell viability and oxidative stress in 3T3-L1 adipocytes. Toxicol. Rep., **5**:1014-1020.

Fernández-Acosta, R.; Hassannia, B.; Caroen, J.; Wiernicki, B.; Alvarez-Alminaque, D.; Verstraeten, B.; Van der Eycken, J.; Vandenabeele, P.; Vanden Berghe, T. and Pardo-Andreu, G. L. (2023). Molecular mechanisms of nemorosone-induced ferroptosis in cancer cells. Cells, **12**(5):735.

Fukui, M.; Song, J.-H.; Choi, J.; Choi, H. J. and Zhu, B. T. (2009). Mechanism of glutamate-induced neurotoxicity in HT22 mouse hippocampal cells. Euro. J. Pharmacol., **617**(1-3):1-11.

Gao, X.; Sun, H.; Hao, S.; Sun, H. and Ge, J. (2023). Melatonin protects HT-22 cells against palmitic acid-induced glucolipid metabolic dysfunction and cell injuries: Involved in the regulation of synaptic plasticity and circadian rhythms. Biochem. Pharmacol., **217**:115846.

García-Santos, G.; Antolín, I.; Herrera, F.; Martín, V.; Rodriguez-Blanco, J.; Del Pilar Carrera, M. and Rodriguez, C. (2006). Melatonin induces apoptosis in human neuroblastoma cancer cells. J. Pineal Res., **41**(2):130-135.

He, M.; Liu, J.; Cheng, S.; Xing, Y. and Suo, W. Z. (2013). Differentiation renders susceptibility to excitotoxicity in HT22 neurons. Neural. Regen. Res., **8**(14):1297-1306.

Herrera, F.;Martin, V.;García-Santos, G.; Rodriguez-Blanco, J.;Antolín, I. and Rodriguez, C. (2007). Melatonin prevents glutamate-induced oxytosis in the HT22 mouse hippocampal cell line through an antioxidant effect specifically targeting mitochondria. J. Neurochem., **100**(3):736-746.

Huerto Delgadillo, L.; Antón Tay, F. and Benitez King, G. (1994). Effects of melatonin on microtubule assembly depend on hormone concentration: Role of melatonin as a calmodulin antagonist. J. Pineal Res., **17**(2):55-62.

Karatop, E. U.; Cimenci, C. E. and Aksu, A. M. (2022). Colorimetric cytotoxicity assays. Intech. Open, 17:111-121.

Kwon, K. J.; Kim, H.-J.; Shin, C. Y. and Han, S.-H. (2010). Melatonin potentiates the neuroprotective properties of resveratrol against beta-amyloid-induced neurodegeneration by modulating AMP-activated protein kinase pathways. J. Clin. Neurol., **6**(3):127.

Nikolaev, G.; Robeva, R. and Konakchieva, R. (2021). Membrane melatonin receptors activated cell signaling in physiology and disease. Int. J. Mol. Sci., **23**(1):471.

Niska, K.; Santos-Martinez, M. J.; Radomski, M. W. and Inkielewicz-Stepniak, I. (2015). CuO nanoparticles induce apoptosis by impairing the antioxidant defence and detoxification systems in the mouse hippocampal HT22 cell line: protective effect of crocetin. Toxicol. *in vitro*, **29**(4):663-671.

Park, H. J.; Kwak, M. and Baek, S. H. (2020). Neuroprotective effects of *Dendropanax morbifera* leaves on glutamate-induced oxidative cell death in HT22 mouse hippocampal neuronal cells. J. Ethnopharmacol., **251**:112518.

Phelan, K. and May, K. M. (2015). Basic techniques in mammalian cell tissue culture. Current Protocols in Cell Biology, **66**(1):1-22.

Shekh, K.; Ansari, R. A.; Omidi, Y. and Shakil, S. A. (2023). Molecular impacts of advanced nanomaterials at genomic and epigenomic levels. Impact of Engineered Nanomaterials in Genomics and Epigenomics, pp:5-39.

Uðuz, A. C. and Nazýroðlu, M. (2012). Effects of selenium on calcium signaling and apoptosis in rat dorsal root ganglion neurons induced by oxidative stress. Neurochem. Res., **37**(8):1631-1638.

Walters-Laporte, E.; Furman, C.; Fouquet, S.; Martin-Nizard, F.; Lestavel, S.; Gozzo, A.; Lesieur, D.; Fruchart, J.;Duriez, P. and Teissier, E. (1998). A high concentration of melatonin inhibits in vitro LDL peroxidation but not oxidized LDL toxicity toward cultured endothelial cells. J. Cardiovas. Pharmacol., **32**(4): 582-592.

Wang, C.; Cai, X.; Hu, W.; Li, Z.; Kong, F.; Chen, X. and Wang, D. (2019). Investigation of the neuroprotective effects of crocin *via* antioxidant activities in HT22 cells and in mice with Alzheimer's disease. Int. J. Mol. Med., **43**(2): 956-966.

Wang, D. d.; Jin, M. f.; Zhao, D. J. and Ni, H. (2019). Reduction of mitophagy-related oxidative stress and preservation of mitochondria function using melatonin therapy in an HT22 hippocampal neuronal cell model of glutamate-induced excitotoxicity. Front. Endocrinol., **10**:550.

Zatulovskiy, E.; Zhang, S.; Berenson, D. F.; Topacio, B. R. and Skotheim, J. M. (2020). Cell growth dilutes the cell cycle inhibitor Rb to trigger cell division. Sci., **369**(6502):466-471.

Zawilska, J. B.;Skene, D. J. and Arendt, J. (2009). Physiology and pharmacology of melatonin in relation to biological rhythms. Pharmacol. Rep., **61**(3):383-410.

Zhang, H. G; Wang, F. J.; Wang, Y.; Zhao, Z. X. and Qiao, P. F. (2021). lncRNA GAS5 inhibits malignant progression by regulating macroautophagy and forms a negative feedback regulatory loop with the miR 34a/mTOR/SIRT1 pathway in colorectal cancer. Oncol. Rep., **45**(1):202-216.

Zhang, M.; Zheng, H.; Zhang, X.; Tian, X.; Xu, S.; Liu, Y.; Jiang, S.; Liu, X.; Shi, R. and Gong, K. (2018). Involvement of nerve growth factor in mouse hippocampal neuronal cell line (HT22) differentiation and underlying role of DNA methyltransferases. J. Toxicol. Environ. Health, Part A, **81**(21):1116-1122.

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