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Decreasing TNFα in serum and increasing mitotic index rate in albino mice treated with melatonin

Marwa Majeed Hameed
Department of Biology, College of Science, University of Wasit, Iraq

Muthana Ibrahim Maleek
Department of Biology, College of Science, University of Wasit, Iraq

***Abstract*---**Melatonin, as an endogenous hormone, is associated with the moderation of the circadian rhythm. Besides controlling the sleep-wake cycle, many physiological functions of melatonin have been identified, such as antioxidant, immune modulating, and anti-inflammatory. The current study was designed to estimate the effect of different doses of melatonin on gene expression of (TNF) in serum and mitotic index (MI) in bone marrow stem cells as cytogenetic test. This study was conducted at Wasit University, College of Science, Department of Biology, Cytogenetic Laboratory, from the period 2/11/2021 to 14/4/2022 using albino mice. The mice were divided into four groups, a control group and three groups divided according to melatonin concentration (5, 10, &15 mg/kg), and were treated for seven consecutive days for every dose . Results showed a significant decrease in gene expression (protein) of TNF α- at P≤0.05 for all three doses of melatonin that were used and mitotic index numbers increased at P≤0.05 for the same doses above of melatonin, when all results above were compared with the control group. Because Melatonin can decrease TNF in treated mice serum, and increase MI rate, that will give additional protection to the cells to fight any DNA damage that may cause abnormality in normal cells.

***Keywords*---**melatonin, TNF α, mitotic index, albino mice.

Introduction

The hormone melatonin, which is mostly secreted by the pineal gland, has been shown to have a variety of effects (Qiu et al., 2019) and controls a number of physiological processes, including neuroendocrine activity, circadian rhythms, and sleep (Zisapel, 2018). The effects of melatonin in the immune system can be either pro- or anti-inflammatory (Hardeland, 2018).Melatonin reduces active

oxygen generation and boosts superoxide dismutase production to shield cells from proinflammatory cytokines (Liu, et al., 2014). TNF- is a newly discovered molecule that functions as the main immune system regulator, a sort of pleiotropic cytokine that may be generated by several cell types and is implicated in a wide range of pathological disorders (Cheng et al., 2020).

TNF is a cytokine with a broad range of physiological functions that regulates inflammation, anti-tumor responses, and homeostasis (Varfolomeev & Vucic, 2018) although b cells, neutrophils, and endothelial cells have all been reported to produce TNF-Alpha, macrophages and t cells are the primary producers of this protein. It is expressed as a type ii transmembrane protein (MbTNF-Alpha), however with higher biological activity; it can be cleaved into its soluble form (s TNF-Alpha), targets include the type I transmembrane receptors TNF receptor I (also known as cd120a) and TNF receptor II for TNF-Alpha (TNFr-ii or cd120b). (Atretkhany et al., 2020) With the exception of erythrocytes, all cell types express TNFr-i, whereas only endothelium and immune cells express TNFr-ii, which can be activated by mbtnf-Alpha. (Salomon et al.,2018) After being stimulated by interleukin-2, t cells proliferate more quickly thanks to the macrophage protein Tnf-Alpha (ii-2). (Vucic & Varfolomeev, 2018). Tnf-Alpha causes b cells to proliferate and differentiate when ii-2 is not present. Tnf-Alpha has anticancer properties, which allow it to kill tumor cells through a variety of methods and attract neutrophils by chemotaxis. Additionally, it can induce osteoblasts to create prostaglandin E2 and collagenase and macrophages to produce acid phosphatase and collagenase. These chemical agents have been linked to bone resorption in the past (salomon et al., 2018).

Mitotic index (MI)

The tissue's cell proliferation can be accurately predicted using the mitotic index. The mitotic index assay is used to analyze proliferating cells and discover the substances that impede mitotic progression, hence lowering the population's MI. It is defined as the proportion of cells that are undergoing mitosis to all of the cells in the body. It is calculated as the total number of cells in the area of view divided by the number of cells with visible chromosomes. The cell cycle can be stopped at this point if you're taking colchicines or one of its derivatives, such as colcemid, leaving the chromosomes in their visible state. Colchicines prevent the chromosomes from being separated by the spindle fibers during anaphase because they prevent the development of microtubules. (Ismeel, 2020) The estimate of the mitotic index is the technique that is most frequently utilized in everyday practice (MI). The number of mitotic figures in a certain location is what is meant by MI (Fitzgibbons et al., 2000).

Materials and Methods

Laboratory animals

In the present study, 40 of Albino Swiss male mice were needed. Mice were purchased from the Animal House in the College of Science, Wasit University, Iraq. The age of mice in the experiments are from (7-8) weeks while the weights of mice was (25±2) gram. Mice were categorized in 7 blocks, each block consisting of

6 mice placed in isolated plastic cages. Aroom temperature of animal house in the Probation (22-27°C). Standard pelletsand fresh water it was animal food and drink to avoid stressful conditions (Maleek et al, 2016). Three different dose of melatonin were used (5, 10, 15) mg/kg (Sumsuzzman, et al. 2021).

Design of the experiment

Each group consists of 10 mice used for estimation TNF α protein and Mitotic Index
Mice were divided into 4 groups
Group A / Control
Group B / Mice were given 5 mg/kg Melatonin
Group C / Mice were given 10 mg /kg Melatonin
Group D / Mice were given 15 mg /kg Melatonin

Blood collection

Blood sample were obtained after the animals had been on melatonin supplement for seven consecutive days by Cardiac puncture method with anesthetic agent (chloroform) by 3 ml syringe because this procedure gives good quality and large volume of blood. Mouse Tumor necrosis factor α (TNF-α) ELIZA Assay. We use the ELISA kit as a method type (Sandwich ELISA). The plate of Elisa kit was provided with specific antibody to TNF-α Protein has been pre-coated the wells. The concentration of p53 Protein can calculate in the samples through comparing the optical density (OD) of the samples to the curve of standards

Mitotic index (MI) assay

The experiments to chromosomes preparation was conducted based on the way of Allen *et al.* (1977)

Mitotic Index (%) $= \left(\frac{\text{Number of Metaphase Cells}}{\text{Total Count}} \right) \text{x}\, 100$. (King *et al.*, 1982; Shubber and Al-Allak, 1986).

Statistical analysis

The values of the parameters are given in terms of mean (M) and standard error (S.E.) and differences between means have been assessed by one-way analysis of variance (ANOVA) using the computer program Statistical Program for Social Science 23 (SPSS 23)discovery Copyright 2015. The difference is consideredat significant probability value is less than is at $p \leq 0.05$

Results and Discussion

TNF Test

The results of this table (1) and figure (1) referred to the ability of melatonin to reduce the concentration of TNF –α (82.19,65.34, and 40.55 pg/ml) in treated

groups when compared with the control group (102.54 pg/ml) this reducing is highly a significant.

Table (1): Show the TNF (pg/ml) serum levels decreased in mice treated groups when getting melatonin

No. of group	Experimental groups	M+S.E.M	*p value
A	Control	102.54±6.9	0.0001
B	Mice that were given 5 mg/kg of melatonin	82.19±3.4	
C	Mice that were given 10 mg/kg of melatonin	65.34±5.1	
D	Mice that were given 15 mg/kg of melatonin	40.55±5.4	

*p value = (p≤0.05)

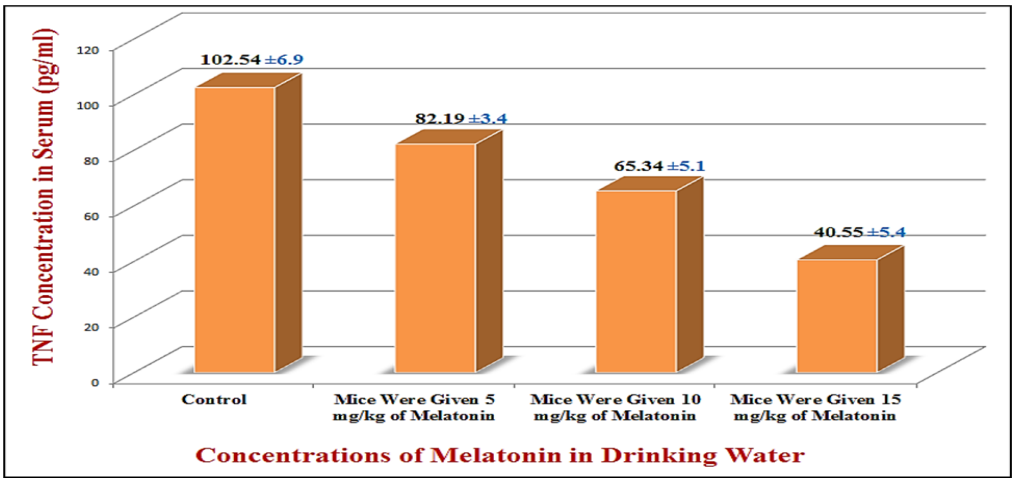

Figure (1): The effects of melatonin on (TNF)-α concentration in blood of control and treated groups.

The results of table (1) showed melatonin had the ability to decrease the concentration of TNF –α sequentially with increasing doses of it in drinking water. This was clear when comparing treatment groups with each other and with the control group. Due to its anti-inflammatory and antioxidant effects, melatonin lowers TNF levels via inhibiting mitogen activation (Ferreira et al., 2014). Signaling via Protein Kinase MAPK Signals from external stimuli are relayed, amplified, and integrated by MAPKs to produce an intracellular reaction (Zhang and Liu, 2015). MAPK signaling pathways control a variety of biological functions. These include the processes of differentiation, proliferation, growth, and death as well as MAPKs' participation in immunological and stress responses (Zhang and Dong, 2005).

Melatonin's anti-inflammatory properties are linked to its suppression of the tumor suppressor TXNIP, which may function in a variety of cancer forms (Chen *et al.*, 2020). Inflammation plays a crucial part in the development of cancer; cancer microenvironments are frequently infiltrated with inflammatory cells. However, this mechanism is not fully understood (Coussens and Werb, 2002).

Melatonin can reduce inflammation by either inhibiting the production of prostaglandins, tumor necrosis factor (TNF), and chemokines, or by preventing the creation of nitric oxide (NO), which causes the release of free radicals. Melatonin can also promote the production of inflammatory mediators such interleukin (IL)-2 and IL-6, interferon (IFN), and IL-1, IL-12, TNF-, and macrophage-colony stimulating factor (M-CSF), particularly in cancer tissues (Vinther and Claesson, 2015).

Cellular insults like ROS, LPS, cytokines (like TNF-), several lncRNAs, and signaling pathways (like MAPKs, hypoxia inducible factor-1, and LOX) activate NFkB signaling, which in turn causes the expression of downstream genes involved in inflammatory responses, such as pro-inflammatory cytokines (like IL1, IL-6, and TNF-), iNOS, adhesion molecules, COX-2, Melatonin's anti-inflammatory properties entail blocking a variety of these inflammatory chemicals, and the end consequence is to reduce inflammation by blocking NF-kB signaling. Melatonin in the regeneration of RA and OA The use of melatonin as a sensitizing agent for chemotherapy and as an adjuvant treatment in a variety of tumor types has been studied in recent decades using both in vitro and in vivo models, Chemotherapy resistance has been linked to circadian disturbance of nocturnal melatonin production by exposure to light at night, raising the possibility that melatonin inhibition may contribute to resistance brought on by chemotherapeutic medicines. (Alonso-González *et al.,* 2020).

Mitotic Index

The mitotic index is a good parameter to evaluate the stem cells' state from where the activity and vilify. It is being done by counting the number of cells in metaphase in a specified whole number of cells. The melatonin showed a significant increase in the mean mitotic index. That is clearly demonstrated in table (2). Because the treated groups with melatonin (5, 10, and 15mg/kg) showed a highly significant increase in mean mitotic index (8.64, 12.90, and 13.1) respectively when compared with the control group (6.22).

Table (2) Effects of different melatonin doses on the mitotic index in bone marrow stem cells

No. of group	Experimental groups	MI Per 5000 cells	M+S.E.M	*p value
A	Control	311	6.22±0.5	0.0001
B	Mice that were given 5 mg/kg of melatonin	432	8.64±0.5	
C	Mice that were given 10 mg/kg of melatonin	645	12.90±0.7	
D	Mice that were given 15 mg/kg of melatonin	655	13.1±1.1	

*p value = ($p \leq 0.05$)

Our bodies have divide millions of cells every day to sustain growth and replenish lost or damaged cells in our tissues. To guarantee that these divisions only take place under perfect growth conditions and with great fidelity, numerous

protections have developed. Aneuploidy is a condition when there are either too many or too few chromosomes in the daughter cells produced during mitosis.With the remarkable exception of trisomy 21 in humans, nearly all aneuploidies that result from errors in meiosis or during early embryonic development are fatal. However, aging and cancer have been connected to mitotic mistakes that result in aneuploidy later in life (Naylor and van Deursen 2016).

With the remarkable exception of trisomy 21 in humans, nearly all aneuploidies that result from errors in meiosis or during early embryonic development are fatal. However, aging and cancer have been connected to mitotic mistakes that result in aneuploidy later in life (Naylor and van Deursen 2016). Aneuploidy occurs in about 70% of solid human tumors, making it a fairly prevalent characteristic of cancer (Duijf; *et al.* 2013). Along with changes in chromosome number, tumor cells frequently exhibit structural changes to their chromosomes, such as translocations, amplifications, and deletions. In addition to being the primary cause of the numerical changes in chromosome numbers seen in cancer, errors in mitosis are now now known to have a role in the production of chromosomal rearrangements (Leibowitz; *et al.*, 2015).

Due to melatonin's antioxidant properties, which promote cell development and raise glutathione levels, it has a protective effect on bone marrow (Rodriguez et al., 2004). Melatonin may potentially operate as a growth factor by causing the release of IL-2 from granulocytes and stimulating bone marrow cells (Beljaards et al., 1993). Besides its well-known antioxidant properties, it might be useful in triggering DNA repair pathways. According to a prior study, melatonin can either activate DNA mending enzymes or the genes that start new protein kinase C-mediated DNA synthesis. (Reiter *et al.*,1998).

Conclusions

The conclusions of this study are:

1. Melatonin has lowing level of TNF- concentration, that due to its anti-inflammatory properties.
2. Melatonin causes an increase in the rate of the mitotic index as a result of its mechanism of action.

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References

Allen, J. W., Shuler, C. F., Mendes, R. W., & Latt, S. A. (1977). A simplified technique for in vivo analysis of sister-chromatid exchanges using 5-bromodeoxyuridine tablets. *Cytogenetic and Genome Research*, *18*(4), 231-237.

Al-Mothafar, N. A., Al-Shahwany, A. W., & Salih, S. J. (2022). Comparing the histological effect of Thymol from multiple sources with blood lipids profile and liver enzymes in Albino Mice. *International Journal of Health Sciences*, *6*(S1), 244–256. https://doi.org/10.53730/ijhs.v6nS1.4770

Alonso-González, C., González, A., Menéndez-Menéndez, J., Martínez-Campa, C., & Cos, S. (2020). Melatonin as a radio-sensitizer in cancer. *Biomedicines*, *8*(8), 247.

Atretkhany, K. S. N., Gogoleva, V. S., Drutskaya, M. S., & Nedospasov, S. A. (2020). Distinct modes of TNF signaling through its two receptors in health and disease. *Journal of leukocyte biology*, *107*(6), 893-905.

Beljaards, R. C., Kaudewitz, P., Berti, E., Gianotti, R., Neumann, C., Rosso, R., ... & Willemze, R. (1993). Primary cutaneous CD30-positive large cell lymphoma: definition of a new type of cutaneous lymphoma with a favorable prognosis. A European Multicenter Study of 47 patients. *Cancer*, *71*(6), 2097-2104

Bromodeoxyuridine tablets with improved depot effect for analysis in vivo of sister-chromatid exchanges in bone-marrow and spermatogonial cells. *Mutation Research/Environmental Mutagenesis and Related Subjects*, *97*(2), 117-129.

Chen, Y., Ning, J., Cao, W., Wang, S., Du, T., Jiang, J., ... & Zhang, B. (2020). Research progress of TXNIP as a tumor suppressor gene participating in the metabolic reprogramming and oxidative stress of cancer cells in various cancers. *Frontiers in oncology*, *10*, 568574.

Chen, Y., Ning, J., Cao, W., Wang, S., Du, T., Jiang, J., ... & Zhang, B. (2020). Research progress of TXNIP as a tumor suppressor gene participating in the metabolic reprogramming and oxidative stress of cancer cells in various cancers. *Frontiers in oncology*, *10*, 568574.

Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. *Nature*, *420*(6917), 860-867.

Duijf, P. H., Schultz, N., & Benezra, R. (2013). Cancer cells preferentially lose small chromosomes. *International journal of cancer*, *132*(10), 2316-2326.

Ferreira, G. M., Martinez, M., Camargo, I. C. C., Domeniconi, R. F., Martinez, F. E., & Chuffa, L. G. A. (2014). Melatonin attenuates Her-2, p38 MAPK, p-AKT, and mTOR levels in ovarian carcinoma of ethanol-preferring rats. *Journal of Cancer*, *5*(9), 728.

Fitzgibbons, P. L., Page, D. L., Weaver, D., Thor, A. D., Allred, D. C., Clark, G. M., ... & Schnitt, S. J. (2000). Prognostic factors in breast cancer: College of American Pathologists consensus statement 1999. *Archives of pathology & laboratory medicine*, *124*(7), 966-978.

Gamez, M. R., Perez, A. V., Falcones, V. A. M., & Bazurto, J. J. B. (2019). The geoportal as strategy for sustainable development. *International Journal of Physical Sciences and Engineering*, *3*(1), 10–21. https://doi.org/10.29332/ijpse.v3n1.239

Hardeland, R. (2018). Melatonin and inflammation—Story of a double-edged blade. *Journal of pineal research*, *65*(4), e12525

Ismeel, A. O. (2011). *Cytogenetic and cytotoxic studies on the effect of phytoinvestigated active compounds of Hyoscyamus niger (in vivo and ex vivo)* (Doctoral dissertation, Ph. D. Thesis in Philosophy of Sciencein Biotechnology, University of Al-Nahrain, Iraq).

King, M. T., Wild, D., Gocke, E., & Eckhardt, K. (1982).

Leibowitz, M. L., Zhang, C. Z., & Pellman, D. (2015). Chromothripsis: a new mechanism for rapid karyotype evolution. *Annual review of genetics*, *49*, 183-211.

Liu, X., Xu, Y., Chen, S., Tan, Z., Xiong, K., Li, Y., & Gong, Y. (2014). Rescue of proinflammatory cytokine-inhibited chondrogenesis by the antiarthritic effect of melatonin in synovium mesenchymal stem cells via suppression of reactive oxygen species and matrix metalloproteinases. *Free Radical Biology and Medicine*, *68*, 234-246.

Maleek M I, Faraj S A and Alzubaidi D A (2016) Cytogenetic Effects of Ranitidine on Stem Cell L-Asparaginase Treated Mice. Int. J.

Naylor, R. M., & Van Deursen, J. M. (2016). Aneuploidy in cancer and aging. *Annual review of genetics*, *50*, 45.

Qiu, X., Wang, X., Qiu, J., Zhu, Y., Liang, T., Gao, B., ... & Huang, D. (2019). Melatonin rescued reactive oxygen species-impaired osteogenesis of human bone marrow mesenchymal stem cells in the presence of tumor necrosis factor-alpha. *Stem cells international*, *2019*.

Reiter, R. J., Mayo, J. C., Tan, D. X., Sainz, R. M., Alatorre-Jimenez, M., & Qin, L. (2016). Melatonin as an antioxidant: under promises but over delivers. *Journal of pineal research*, *61*(3), 253-278.

Rodrigues, M. A., Beaton-Green, L. A., Wilkins, R. C., & Fenech, M. F. (2018). The potential for complete automated scoring of the cytokinesis block micronucleus cytome assay using imaging flow cytometry. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *836*, 53-64.

Salomon, B. L., Leclerc, M., Tosello, J., Ronin, E., Piaggio, E., & Cohen, J. L. (2018). Tumor necrosis factor α and regulatory T cells in oncoimmunology. *Frontiers in immunology*, *9*, 444.

Sci. & Engineering Investigations 5, 45-51

Shubber, E. K., & Al-Allak, B. M. A. (1986). Spontaneous chromosomal aberrations and SCEs in human lymphocytes. Effects of culture conditions. *Nucleus*, *29*, 92-8.

Sumsuzzman, D. M., Khan, Z. A., Choi, J., & Hong, Y. (2021). Differential role of melatonin in healthy brain aging: A systematic review and meta-analysis of the SAMP8 model. *Aging (Albany NY)*, *13*(7), 9373.

Varfolomeev, E., & Vucic, D. (2018). Intracellular regulation of TNF activity in health and disease. Cytokine, 101, 26-32.

Vinther, A. G., & Claesson, M. H. (2015). The influence of melatonin on immune system and cancer. *Int. J. Cancer Clin. Res*, *2*(4).

Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202

Zhang, C. Z., Spektor, A., Cornils, H., Francis, J. M., Jackson, E. K., Liu, S., ... & Pellman, D. (2015). Chromothripsis from DNA damage in micronuclei. *Nature*, *522*(7555), 179-184.

Zhang, Y. L., & Dong, C. (2005). MAP kinases in immune responses. *Cell Mol Immunol*, *2*(1), 20-27.

Zhang, Y. L., & Dong, C. (2005). MAP kinases in immune responses. *Cell Mol Immunol*, *2*(1), 20-27.

Zisapel, N. (2018). New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *British journal of pharmacology*, *175*(16), 3190-3199.