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Melatonin can enhancement p53 concentration and decrease micronucleus production rate in albino mice

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***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 (p53) in serum and micronucleus (MN) in bone marrow stem cells as cytogenetic test .This investigation was conducted at a Research laboratory in the Department of Biology, College of Science, Wasit University and was designed to evaluate the rate of micronucleus (MN) formation as a cytogenetic test and the level of protein p53 as a serological test . Melatonin (5, 10&15) mg/kg was used for seven consecutive days to know their influence on the parameters before mentioned .Results showed a significantly increased in gene expression (protein) of p53 at P≤0.05 for all three doses of melatonin that were used. While Micronucleus production significant decrease at P≤0.05 for the same doses above of melatonin, when all results above were compared with the control group. Because Melatonin can increase the level of tumor suppressor protein p53 concentration in treated mice serum, and decrease MN rate, that will give additional protection to the cells to fight any DNA damage that may cause abnormality in normal cells.

***Keywords*---**P53, Melatonin, Serum, Micronucleus, Albino Mice

1. 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). Melatonin has a wide range of potential uses, particularly in cancer treatment where it inhibits tumor development. All of these effects appear to be achieved by melatonin's regulation of the immune system, redox status of cells, telomerase activity, circadian rhythms, and epigenetic actions. Melatonin also inhibits cancer cell proliferation, modulates cell cycle and induces apoptosis, stimulates cell differentiation, inhibits cell migration and metastasis, and decreases angiogenesis. (Li *et al.*, 2017). Melatonin was used to inhibit cell proliferation and to increase cell death via p53, p27 and pRb phosphorylation (Shen *et al.*, 2016)

P53 is a transcription factor that binds DNA and functions physiologically as a homotetramer (Raj & Attardi, 2017). P5 interacts with other proteins or enzymes directly to mediate actions that are not dependent on transcription. P53 is a transcription factor that binds DNA and functions physiologically as a homotetramer (Kruiswijk *et al.*, 2015). P53 controls hundreds of genes; a conserved core program of about 100 genes is activated, regardless of cell type or response; and these genes work together to support tumor suppression (Andrysik *et al.,* 2017). One of the most sensitive indicators of DNA damage is the micronucleus test. Studies on the genotoxicity of several substances have employed this marker (Fenech, 1993).

In human epidemiological research and animal testing, it is also utilized to evaluate the impact of genetic damage brought on by occupational and environmental conditions (Ishikawa *et al.,* 2000). Acentric fragments (chromosome breaks without centromeres) or entire chromosomes that are unable to move to the spindle poles during mitosis are two examples of cells that exhibit MN during division (Sundararajan & Natarajan2017). The Micronucleus Test (MN Test) is used to determine the genotoxic potential of substances and permits the management of mitosis (Sandoval-Herrera et al., 2021)

2. 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. A room temperature of animal house in the Probation (22-27°C). Standard pelletsand fresh water it was animal food and drink to avoidstressful 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 estimationtumor protein P53 test and micronucleus test.
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 p53/tumor protein (p53/TP53) ELISA Assay
We use the ELISA kit as a method type (Sandwich ELISA). The plate of Elisa kit was provided with specific antibody to p53 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.

Micronucleus (MN) assay :
In this study depending on Schmid procedure to estimate the rate of micronucleus, A sample photograph showing MN is shown in Fig. 1. The micronucleus index was calculated using the following equation Micronucleus Index = $\left(\frac{\text{Number of Micronucle i}}{\text{Total Count (1000)}} \right) \text{x} 100$. (Schmid, 1976).

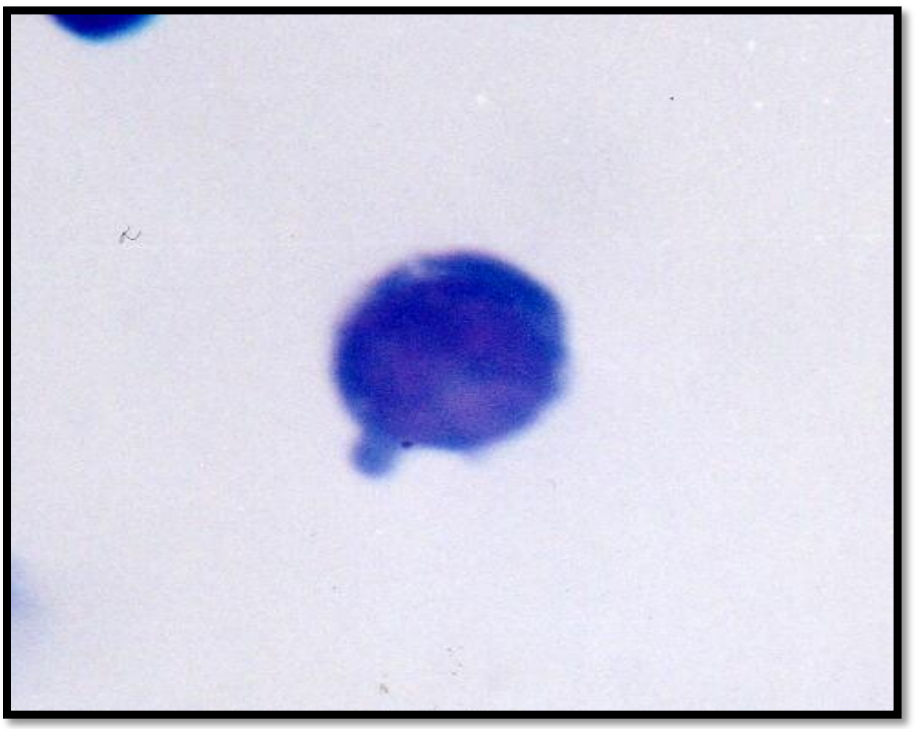

Fig1: Micronucleus in mouse bone marrow below a microscope at (100x)

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 programStatistical

Program for Social Science 23 (SPSS 23)discovery Copyright 2015. The difference is consideredat significant probability value is less than is at p≤0.05.

3. Results and Discussion

1-P53 Test

The P53 concentration in treated mice blood is (53.09, 73.21, and 98.17pg/ml) at (5, 10, and 15 mg/kg of melatonin) sequentially. When compared these results with the control group result

(43.25) showed increasing in P53 concentration with melatonin increasing doses in drinking water. This increase is highly significant as shown in table (1)

Table (1): The P53 concentration (pg/ml) in treated and control mice blood

No. of group	Experimental groups	M+S.E.M	*P value
A	Control	43.25±4.4	0.0001
B	Mice that were given 5 mg/kg of melatonin	53.09±4.4	
C	Mice that were given 10 mg/kg of melatonin	73.21±7.3	
D	Mice that were given 15 mg/kg of melatonin	98.17±9.6	

*p value = (p≤0.05)

The results of table (1) showed melatonin had the ability to increase the levels of p53 sequentially with increasing doses of it in drinking water. This was evident when the control group was compared with the treatment groups and the treatment groups with each other.

When DNA damage or oncogenic signaling occurs, the p53 protein acts as a transcriptional regulator that is stabilized inside the nucleus. P53 protein controls genes involved in cell cycle arrest, cell death, and apoptosis. Senescence and DNA repair to halt tumor proliferation and growth (Gnanapradeepan *et al.*, 2018). Additionally, the p53 protein directly binds to the mitochondria and cytoplasm, where it can control cellular processes in the cytoplasm, such as programmed cell death. Necrosis is one non-canonical function of the p53 protein. (Baumann,2012), and autophagy (Green and Kroemer, 2009). A less well-known role for the protein p53 in iron and lipid-mediated cell death (ferroptosis) occurs in inflammatory programmed cell death (necroptosis) (Wu *et al.,* 2012). The quick intracellular ubiquitination that prevents its accumulation allows the wild type of p53 protein to stay at low levels in the absence of cellular stress Therefore, the value of the control group was almost regarded low (Dornan *et al.,* 2004).

By triggering apoptosis, the melatonin showed a full halt in cell growth and a reduction in the number of cancer cells.Melatonin's anti-tumoral effect has been studied in both in vivo and in vitro carcinogenesis models, and it has been proven in a wide range of endogenous and environmental cancers. According to reports, melatonin inhibits cell division by delaying the G1-S transition, increasing the

proportion of G0/G1 cells and decreasing the number of S cells as a result. This decreases DNA synthesis and lengthens the cell cycle. An overexpression of p53 and p21 and a downregulation of cyclin D1 account for this G0/G1 arrest (Alonso-González *et al.,* 2017).

2- Micronucleus Test

The experiment results in table (2) showed indicated that melatonin had the ability to significantly reduce mean frequencies of MN in mouse bone marrow stem cells. This was observed when melatonin doses (5, 10 and 15) reduced MN number to (2.06, 1.36 and 1.2) respectively when were compare with control group (2.58).

Table (2): The melatonin effect on micronucleus producing mean in bone marrow stem cells

No. of group	Experimental groups	MN Per 5000 cells	M+S.E.M	*p value
A	Control	129	2.58±1.3	0.0001
B	Mice that were given 5 mg/kg of melatonin	103	2.06±0.5	
C	Mice that were given 10 mg/kg of melatonin	68	1.36±0.5	
D	Mice that were given 15 mg/kg of melatonin	60	1.2± 1.1	

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

The melatonin increasing dose does not continue to reduce the mean of MN production in mice bone marrow stem cells. This is cleared in table (3-8) when tested the degree of differences within treatments. Where showed a significant relationship between groups (A with B, C, and D) and (B with C and D) but not significant when comparing groups (C with D).

Micronuclei (MN) are genetic damage markers that indicate somatic inherited and congenital genotoxin-transmitted defects that result in malignant cell transformation (Kirsch-Volders *et al.,* 2011). Micronucleus must be viewed as significant sources of genetic variation rather than merely chromosomal change indicators in the cell in which they are found; Additionally, MN might be an ideal setting for significant genetic rearrangements to occur in proliferating tissues, especially in malignancy (Russo and Degrassi, 2018).

Melatonin can be found in any cellular compartment because of its amphiphilicity (Reiter *et al.,* 2007). In contrast, research indicates that the pineal gland hormone preferentially localizes inside the nucleus and can shield nuclear DNA from oxidative damage by interacting with double-stranded DNA and enhancing its stability (Tan *et al.,*1994). Additionally, melatonin's antioxidant impact involves DNA repair, and the hormone can reverse oxidation brought on by the guanosine (GN) radical (Mahal *et al.,* 1999). If the centromere is missing or damaged, damaged DNA may interfere with the attachment of a chromosome to the spindle because centromeric DNA serves as the binding site for the kinetochore proteins needed to connect the chromosomes to the spindle microtubules. Acentric

(centromere missing) chromosome fragments produced by genotoxic chemical agents are known to result in the formation of micronuclei (Lewis, 2014).

4. Conclusions

The conclusions of this study are:

1. Melatonin can increase the level of tumor suppressor protein p53 concentration in mice serum and that gives additional protection to the cells to fight any DNA damage that may cause a tumor to the normal cells.
2. Melatonin as a powerful antioxidant can reduce the rate of micronucleus formation and give evidence of its effects against the genotoxicity and cytotoxicity on mice bone marrow.

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