

Cellular Response of Thymic Macrophages to Exogenous Specific Pineal Hormone

Nibras Hatim Khamees, Khalida Ibrahim Noel, Samia Abbas Eleiwe

Department of Human Anatomy, College of Medicine, Mustansiriyah University, Baghdad, Iraq

Abstract

Background: Thymus gland is an essential component of the immune system which contains an array of immune cells, most notably, lymphocytes and macrophages. The circadian rhythm of human is under the influence of melatonin hormone which in turn may modulate the body immune response through its effect on certain cellular aspects. Macrophages are specialized cells of the connective tissue that play a pivotal role in the immune system. **Objective:** Thymic macrophages are studied here as a type of connective tissue cell to see how they respond to exogenous melatonin as an indicator of immune response. **Materials and Methods:** Melatonin was administered orally to adult male rats for 30 days. There were six distinct groups of animals. The first group served as a control, while the remaining five received melatonin at increasing doses from 125 µg/kg to 250 µg/kg to 500 µg/kg to 750 µg/kg to 1000 µg/kg daily. Anesthesia was used to remove the right thymic lobe at the end of the experiment. **Results:** Descriptive results showed the prevalence of unusual types of macrophages, such as very large macrophages, epithelioid cells and multinuclear giant cells, in varying proportions across all treated groups in comparison to the control group. Morphometric results demonstrated a noteworthy rise in the mean diameter of both macrophages and their nuclei across all treatment groups with varying statistical significance. **Conclusion:** Using dietary melatonin within the daily recommended doses can boost the immune system, whereas high doses could have detrimental effect.

Keywords: Exogenous, immunity, macrophages, pineal, thymus

INTRODUCTION

The pineal gland's primary neurohormone, melatonin, is secreted primarily at night and plays a significant part in the maintenance of the body's circadian rhythm, among other tasks. Over-the-counter sleep aids like tablets and syrup containing exogenous melatonin are very popular today. Because of this, there is a greater potential for abuse and overdosing.^[1-3] It is widely known that melatonin hormone has a significant effect on immunity, in that it contributes to the body defense system against various microorganisms that could negatively impact the functions of the different body tissues and attenuate their natural response.^[4-6] The thymus is the immune system's center, where T-lymphocytes develop and released to participate in a variety of immune reactions.^[7]

Connective tissue contains phagocytes called macrophages; these cells can be found in a wide variety of tissues and

organs throughout the body, including the thymus. These cells may be of the free or fixed (histocyte) variety.^[8]

It is well known that macrophages play a crucial role in the immune system of the body,^[9-11] and that their activity can be influenced by melatonin.^[12,13] Determining how different dosages of melatonin affect thymic phagocytes (macrophages) is, therefore, of great importance.

MATERIALS AND METHODS

Thirty male adults Wistar albino rats were kept in the animal house at a controlled environment in which the

Address for correspondence: Dr. Nibras Hatim Khamees,
Department of Human Anatomy, College of Medicine, Mustansiriyah
University, P.O. Box 14132, Baghdad, Iraq.
E-mail: nh.khamees@uomustansiriyah.edu.iq

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temperature was maintained at around $22 \pm 2^\circ\text{C}$ with a 12:12h dark to light cycle. The animals were treated according to the general guidelines of laboratory animals (Iraqi general health law, experimental protocol section, 1981) and according to the protocol of the laboratory animal center of the University of Baghdad, Iraq.

The animals had a free access to water and diet, on need. The only exception is that feeding was withhold 2h before the administration of oral melatonin, which was added to the animal diet once daily, two hours before sunset.^[12,14]

Rats were assigned into six experimental groups (five animals in each group), including one control group and five treatment groups. Animals in the control group (Group 1) were fed melatonin-free regular diet (placebo) and were fasted for 2h before the administration of melatonin, just like the other groups.

For 30 consecutive days, dietary melatonin was administered to the treatment groups (groups 2, 3, 4, 5, and 6) at doses of 125, 250, 500, 750, and 1000 $\mu\text{g/kg}$ body weight, respectively.

This study utilized the use of commercially available melatonin tablets (manufactured by Nature's Bounty Company, Bohemia, NY, USA). The active compound in the tablets was n-acetyl 5-methoxy tryptamine.

After the last day of melatonin supplementation, control and treated animals were anaesthetized with diethyl ether inhalation and then dissected. During dissection, the right lobe of the thymus was removed in all animal groups, kept in 4% formalin, and then subjected to histological processing according to the standard protocols. For subsequent histological examination, five serial sections of 5 μm thickness were obtained for each rat. The sections were stained with hematoxylin and eosin.^[2,5,15]

The histological examination was descriptive and morphometric.

The prepared slides were examined with compound light microscope to which an objective micrometer (a specialized lens with ruler) was inserted in place of ocular lens. Then, a distance of 10 μm could be estimated during the morphometric investigation and used to measure the size of macrophages and their nuclei. The size of thymic macrophages was determined using their average diameter. Also, the average diameter of macrophages nuclei was estimated to record any variation in their size.^[5,9]

Statistical analysis was performed using GraphPad Prism (version 9, Dotmatics, Boston, MA, USA). For each set of data, the average was calculated with standard deviation from five biological samples. Then, the significance of variance was measured by applying one way ANOVA (analysis of variance) test in order to compare between different experimental groups included in the study.^[16]

RESULTS

Our results were divided into two sections, descriptive study, and morphometric study.

Descriptive study

Unusual types of macrophages were observed in varying proportions across all treated groups in comparison to the control group, as indicated below.

Macrophages of considerable size were initially detected in experimental groups 2 and 3. The polygonal macrophages exhibited a heavily pigmented cytoplasm, which was packed with a multitude of particles, vacuoles, and vesicles. The observed cells exhibited nuclei that were eccentric and displayed a significant size with a pale, basophilic appearance. The prevalence of these macrophage varieties was observed to be highest in the vicinity of the blood vessels, as depicted in Figure 1.

Epithelioid cells were observed as the second type of macrophages with a pinkish stain, large size, and abundant cytoplasm. Additionally, the nucleus was basophilic, pale, and predominantly eccentric, as seen in Figure 2. The majority of these cells were detected in experimental groups 4, 5, and 6.

Multinucleated giant cells were the other kind of macrophage. They observed only in the group of animals administered 750 and 1000 $\mu\text{g/kg}$. These cells had an irregular morphology and an abundance of acidophilic, pale cytoplasm. Their cytoplasm was filled with fragments, inclusions, granules, and vacuoles [Figure 3].

Morphometric study

The morphometric findings indicate a noteworthy rise in the mean diameter of both macrophages and their nuclei across all treatment groups ($P < 0.05$) (refer to Figures 4 and 5). The study found that there was a proportional

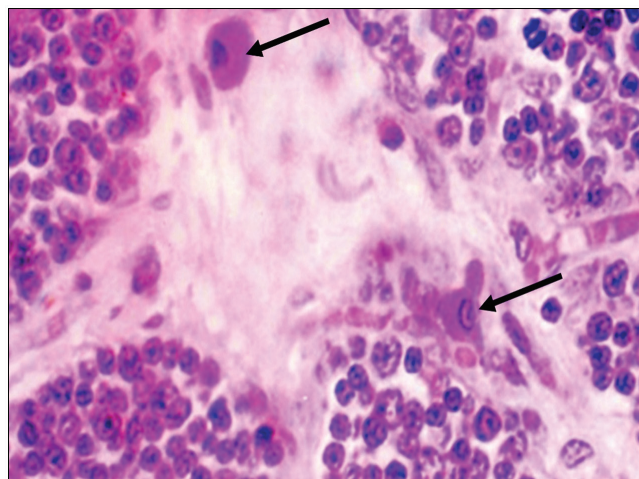


Figure 1: Histological section of thymus gland showing the presence of large macrophages (arrows). H&E. stain. 100 X

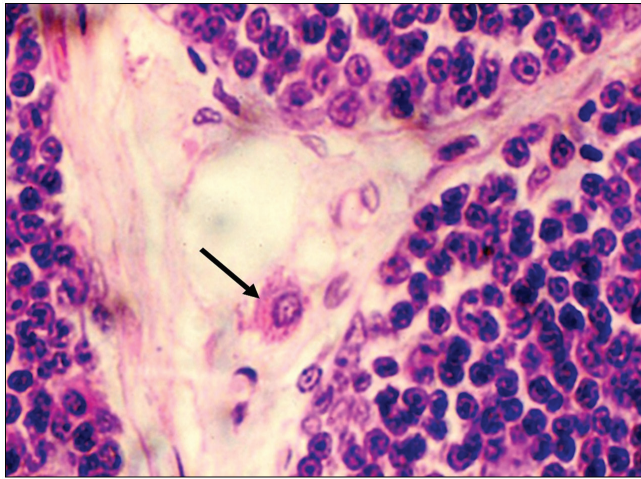


Figure 2: Histological section of thymus gland showing epithelioid cell (arrow). H&E. stain. 100 X

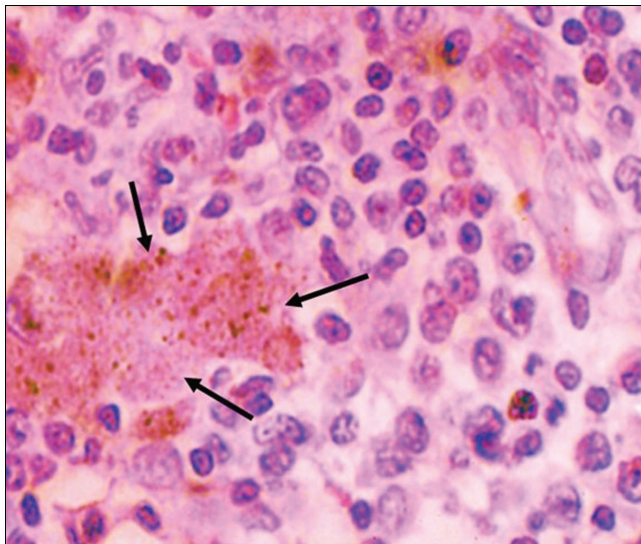


Figure 3: Histological section of thymus gland demonstrating the presence of Multinucleated giant cell (arrows). The cell has an amorphous form and an abundance of pale, acidophilic cytoplasm. H&E. stain. 100 X

increase in the average diameter of thymic macrophages with an increase in the dose of melatonin. The average diameter of thymic macrophages was statistically higher in group 4 ($P < 0.001$) and groups 5 and 6 ($P < 0.0001$) as compared to the control group [Figure 4]. In addition, the average diameter of thymic macrophages nuclei was statistically higher in group 4 ($P < 0.05$), group 5 ($P < 0.01$) and group 6 ($P < 0.001$) as compared to the control group [Figure 5].

DISCUSSION

The histological appearance of macrophages is commonly used as an indicator of their physiological condition. Therefore, they are considered to be active and over

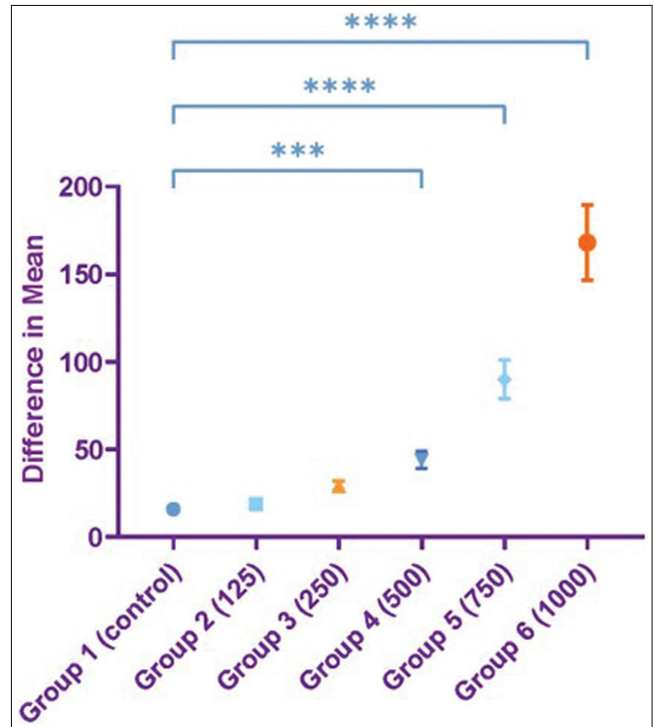


Figure 4: morphometric measurement of the average of the widest diameter (μm) of thymic macrophage cells in adult rats that were administered different doses of exogenous melatonin through their diet. Data were expressed as mean $\pm$ SD. [*** $P < 0.001$; **** $P < 0.0001$]

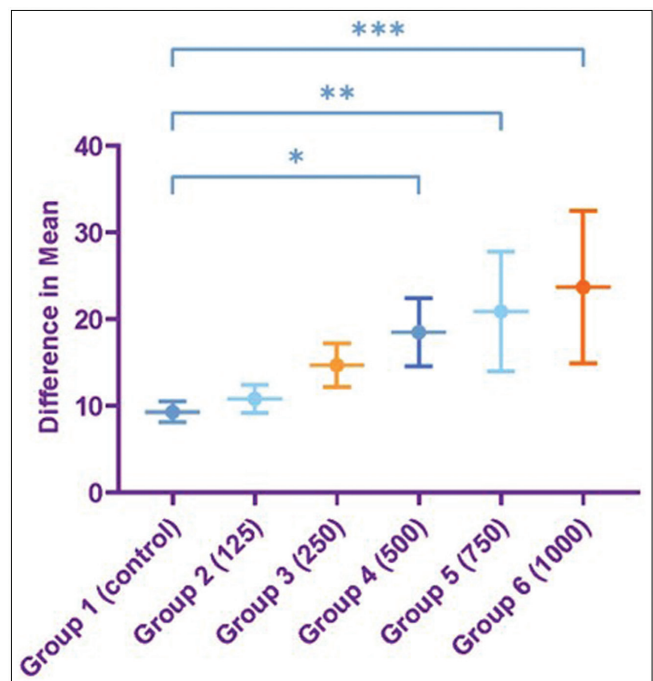


Figure 5: morphometric measurement of the average diameter (μm) of nuclei of thymic macrophages in adult rats that were administered different doses of exogenous melatonin through their diet. Data were expressed as mean $\pm$ SD. [* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$]

functioning whenever they have larger size with pale large nuclei and are considered to have low activity and less operating whenever their size is relatively smaller with somewhat darker nuclei.^[5,9,17] We attribute the significant increase in cell and nucleus diameter in our study to the activity of these cells. As the phagocytic activity of the cells increase, in part of their immune function, the size of cell increases with more cytoplasm with accumulation of phagocytic vacuoles. On the same line, increased cell activity is associated with more protein synthesis and thus larger nucleus size.^[18,19]

The study revealed that there was a proportional increase in the size of macrophages with increased dose of melatonin and this could be attributed to the stimulatory effect of melatonin on macrophages. However, the emergence of epithelioid cells was only observed in instances of over-functioning and hyperstimulation, such as severe immune response and serious inflammations, as reported in previous studies.^[2,20-22]

The third form of cell is the multinucleated giant cell, which is rarely observed outside of pathological settings but can represent a toxic situation and lead to serious diseases.^[2,9] Foreign body giant cells (a huge, multi-nucleated tissue mass with an indistinct cellular membrane.) are formed when a collection of epithelioid cells fuse in the presence of large foreign body to phagocytose it and protect the body from any harmful effect. The toxic and pathogenic effects of this cell type have been studied extensively. Researchers concluded that melatonin hormone activation was responsible for the peculiar behavior (granulation) of all three types of macrophages examined. It is well-documented that the physiological effects of this hormone vary with dosage. At the right dose (the therapeutic dose), it can activate the cell's natural reaction, but at higher doses, it is harmful.^[14,23,24]

These results could be explained by a direct or indirect action of melatonin on macrophages. Melatonin receptors, which are naturally present in every cell and tissue of the body, are responsible for the direct action. However, granulocyte-macrophage colony-stimulating factor (GM-CSF) has an indirect effect because of its direct effect on other essential growth factors. The macrophage-derived, endothelial-derived, and T-lymphocyte-secreted growth factor GM-CSF either augments the impact of macrophage colony stimulating factor (M-CSF) or directly activates macrophages. Melatonin is the primary director of all types of immunity, according to the available literature.^[12,13,20,21]

Different types of macrophages were found to have nuclei that were considerably larger in diameter than those of the control group. This data demonstrate that melatonin can influence nuclear activity. This will have far-reaching consequences for cellular function.^[5,25-27]

Our findings indicate that the body's reaction to exogenous melatonin treatment varies with dose. In this case, low doses within the therapeutic range could be associated with improved body's resistance to illness as suggested by increase in size of macrophage and their nuclei without the appearance of unusual cells. Overdosing of the hormone, however, could be associated with unfavorable effects on immunity, according to our study and this finding is in accordance with previous studies.^[28-32]

Limitations

We did not study the effect of different doses of melatonin on macrophage receptors, as this will add more value to the current research, due to limited resources.

CONCLUSION

Building on the results of this study we can conclude that the usage of dietary melatonin within the recommended daily allowance can have an immune boosting effect. On the other hand, high doses of dietary melatonin will affect the immune system in detrimental way. This message is important to be highlighted as over the counter melatonin supplement are widely used by elderly people as sleep aid which increase the possibility of misuse and overdosing.

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Conflicts of interest

There are no conflicts of interest.

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