

Histopathological effects of exogenous melatonin on connective tissues of thymus gland in male rats

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Abstract

Background: The neurohormone namely melatonin is secreted by the pineal body in brain. It could reach all the bodily tissues and cells; affecting their function, depending on its biological level. Thymus gland is well known to be the main central immunity director, and its wellness is rather proportional to melatonin level.

Aims: This work was done to study the histopathological effect of exogenous melatonin on thymic connective tissue bulk.

Materials and Methods: Dietary melatonin was supplied to adult rats, for successive 30 days. Rats were divided 6 groups. Group 1 was the control, group I was the control. Group II, III, IV, V and VI were supplied with a daily measured quantity of melatonin as 125, 250, 500, and 1000 µm/kg body wt, respectively. After the last day of treatment all animals were killed then the left thymic lobe was removed under anesthesia for histopathological study.

Results: No noteworthy effect of melatonin was seen on the thymic connective tissue on its normal doses, whilst it had significant effect on the connective tissue bulk with its large doses.

Conclusions: The treatment of dietary melatonin had no important effects on the rat thymic connected tissues, on its little doses, but it has considerable effects when administrated in large doses.

Keywords: Connective tissue, melatonin, and thymus

INTRODUCTION

The neuro-hormone explicitly melatonin is secreted by the pineal gland, with a climax at night.^[1-3] The main central immune system organ;^[4] namely thymus gland is well documented to be functioning under melatonin control.^[5-7] Thymus gland is consisted of lymphatic as well as connective and fatty tissues.^[8] The connective and fatty tissue bulk, within thymus, increases with age and with any deleterious event. This increase in the bulk of those tissues is on the regard of lymphatic tissues, since they proportionate adversely.^[9-11]

MATERIALS AND METHODS

Adult male Wister albino rats were used in the instant work. They were 24 in number. They were put on the temperature of about 22±2, with light-dark cycle of about 12:12. They were provided with water ad libitum; and fed a controlled diet freely, except for two hours prior to melatonin supplement. Dietary melatonin was provided as a single daily dose mixing with the food, two hours prior to sunset.^[12, 13] Animals were divided into 6 groups, each consisting of four rats. Group I was the

control: rats were fed the similar type of melatonin containing meal but with nothing added (placebo), however, they were also prevented from food for 2 hours before the time of melatonin supplement, just like the other groups.

Group II, III, IV, V and VI were given dietary melatonin as a daily dose of 125, 250, 500, 750, and 1000µm/kg body weight respectively for 30 successive days. The melatonin used in this study was n- acetyl 5- methoxytryptamine (melatonin) tablets, from NATURES BOUNTY INC, bohemia NY 11716 and USA. After the last day of melatonin supplement all animals were dissected under effect of anesthesia diethyl ether at the same day.

The left lobe of the thymus was removed and investigate to study the histopathological changes might see in the connective tissue constituent within thymus gland, in each group of animals using (haematoxylin and eosin) for staining and paraffin for embedding. For each rat 6 serial sections of 4 µm thickness were examined from the middle part of the lobe.^[6, 14]

Histopathological study was done, using light microscope. Morphometric as well as descriptive studies were done.

The morphometric analysis was estimated by using Zeiss Integrating Micrometer–disk Turret I of 25 point system, used on a light microscope, the total points falling on surface area occupied by the connective tissue were estimated. From each section 5 fields were taken randomly examined at 150X magnification.

Fibroblasts and fibrocytes cells were studied done by using objective micrometer used a light microscope by which a distance of 10 µm could be estimated, hence, the average cell diameter of the thymic fibrocytes and fibroblast as well as the diameter of their nuclei were calculated.^[6, 9]

All the values were taken as mean ± SD of 4 rats. Whenever a difference was present between each of the treated groups and its control, the significance of that difference was evaluated by student t – test.^[15]

RESULT

The descriptive results

Microscopically examination of all treated groups, in comparison with the control group (Fig. 1), illustrated the increase in the connective tissue bulkiness appeared clearly in group IV and V (Fig. 2), while in group VI; the whole organ was appeared consisted of connective and

fatty tissues, with some scattered patchy small areas of loose lymphatic tissues (Fig. 3).

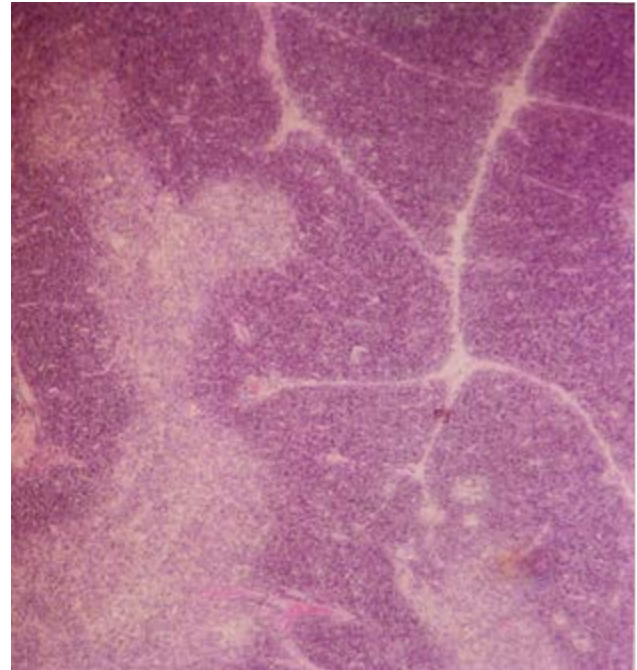


Figure 1. Thymus tissue in 8 weeks old male rat (control), H&E stain, X 40.

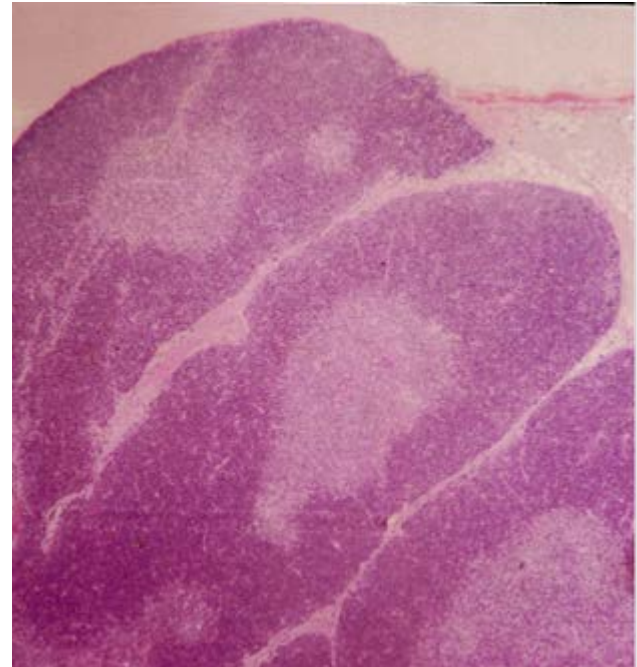


Figure 2.Thymus tissue in 8wk male rat of group IV, thickened septa (arrow), H&E stain, X 40.

Those connective tissues seemed to be hyper vascular than usual, since numerous, dilated blood vessels seen within those connective tissues (Fig. 4).

Fibrocytes cells were the dominant type of connective tissue cells in the control group I. these cells were small cells having spindle shape with few cytoplasmic processes, small dark elongated nucleus, acidophilic cytoplasm (Fig. 5). In group II, III, IV, V, and IV the fibroblasts were the foremost; they were larger than fibrocytes, having abundant basophilic cytoplasm, irregular processes, large vesicular pale nucleus (Fig. 6).

Morphometric results

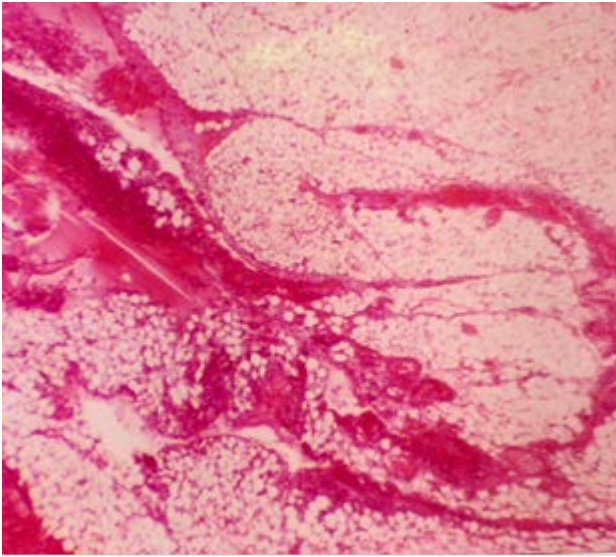


Figure 1. Thymus tissue in 8 wk old male rat of group VI, thickened septa (arrow), H&E stain, X40.

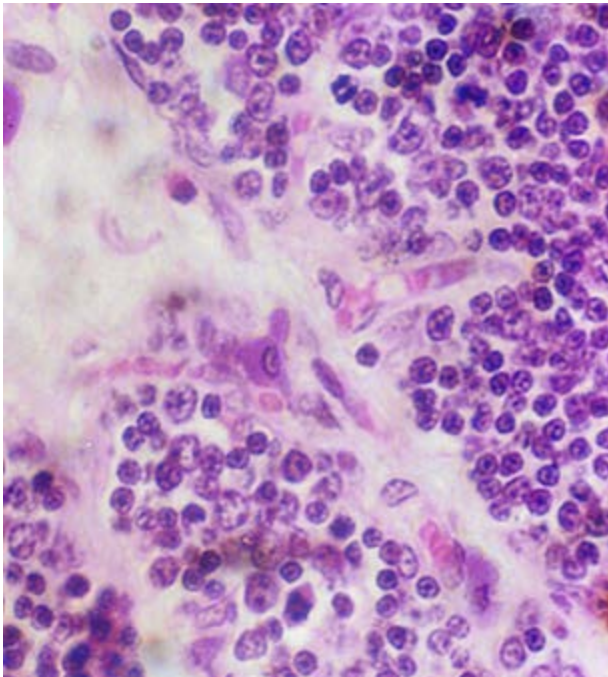


Figure 4. Thymus tissue in 8 wk male rat of group IV, blood vessels (arrows), H & E stain, X 400.

In all melatonin - treated groups of rats; there was significant increment of surface area occupied by the connective tissue septae (table 1).

The average widest cell diameter of thymic fibroblasts and fibrocytes as well as their nuclear diameter in μm , were increased in all treated groups.

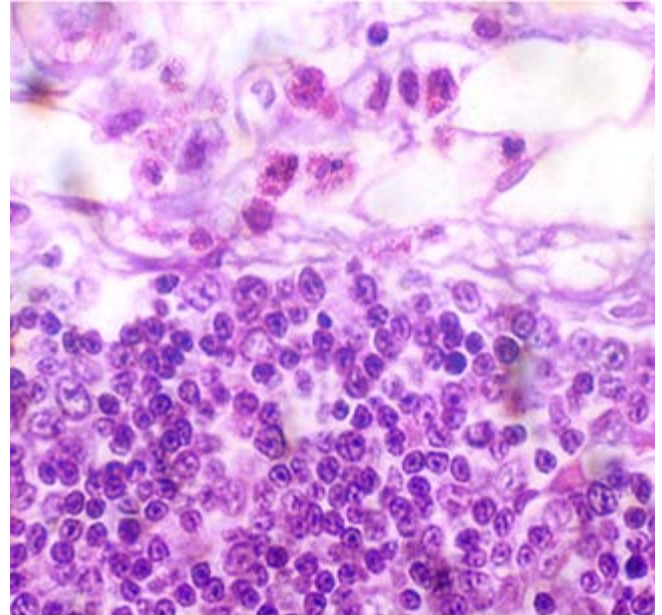


Figure 5. Thymus tissue in 8 wk male rat of group V, Fibrocytes (arrows), H & E stain, X 400.

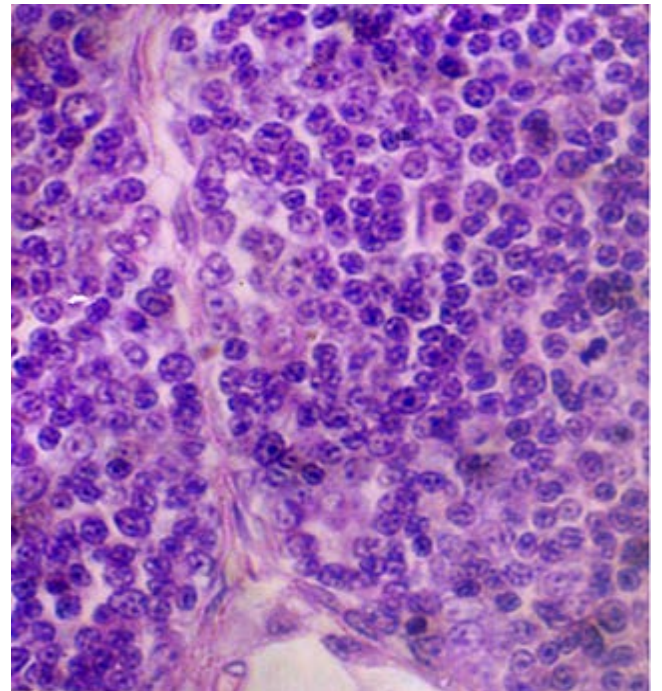


Figure 6. Thymus tissue in 8 wk male rat of group IV, Fibrocytes (arrows), H & E stain, X 400.

Though, the increase was positively related to the dose of melatonin, accept for the last group VI; where these cell and nuclear dimensions was all regressed (table 2).

Table 1. The average diameter of fibroblasts and fibrocytes and their average nuclear diameter in μm .

Daily dose of melatonin in $\mu\text{g/kg}$ body wt	Fibroblast diameter in μm	Fibroblast Nuclear Diameter in μm	Fibrocytes diameter in μm	Fibrocytes Nuclear diameter in μm
Control	13.4 $\pm$ 1.9	5.52 $\pm$ 0.17	10.3 $\pm$ 0.9	3.13 $\pm$ 0.13
125	14.5 $\pm$ 1.7*	6.12 $\pm$ 1.21*	10.7 $\pm$ 1.9NS	3.14 $\pm$ 0.24NS
250	15.1 $\pm$ 1.9*	6.54 $\pm$ 0.13**	10.9 $\pm$ 1.8*	3.20 $\pm$ 0.11*
500	15.7 $\pm$ 2.1*	6.11 $\pm$ 0.17*	11.4 $\pm$ 1.9	3.22 $\pm$ 0.18*
750	16.4 $\pm$ 2.0*	7.05 $\pm$ 1.13*	11.8 $\pm$ 2.1*	3.25 $\pm$ 1.12*
1000	17.2 $\pm$ 2.4**	7.43 $\pm$ 0.42**	12.1 $\pm$ 2.5*	3.26 $\pm$ 0.41*

- Data were expressed as mean $\pm$ SD of 4 rats.

When each treated group was compared with the control, the significance was as follows:

- (* P<0.03;** P<0.001; NS: not significant).

Table 2. Number of points overlying the connective tissue septae of thymus in adult rat treated with dietary melatonin (in a unit area of 0.0025 mm^2).

Daily dose of melatonin in $\mu\text{g/kg}$ body wt	Average septal connective tissue thickness in μm
Control	4.11 $\pm$ 0.18
125	4.76 $\pm$ 0.23 NS
250	5.16 $\pm$ 0.12*
500	5.22 $\pm$ 1.18**
750	7.53 $\pm$ 0.13* *
1000	4.91 $\pm$ 0.51***

-Data were expressed as mean $\pm$ SD of 4 rats.

-When any dose-group was compared with its control, the difference was statistically significant (* P<0.05, ** P<0.03, ***P < 0.001, NS=Not significant).

DISCUSSION

The reason for choosing rats in the instant work, to study the connective tissue response, within the central immune organ; namely thymus gland, was planned because rats immune system is the most similar one to that of human, than that of other animals even mouse.^[15] This ongoing study was attended for evaluation of melatonin effect on size of the connective tissue; because, regulation of its amount is very important for the thymic function welfare.^[2,17] The connective tissue bulk is adversely proportionate to the lymphatic tissue bulk within the thymus.^[2,18] The body normally start to raise vastness of the connective tissues and fatty tissues against favor of lymphatic tissues containing within thymus gland, soon after puberty, otherwise, many abnormal immune

problems might be initiated, which could be fatal, such as autoimmune diseases .^[2, 17, 18]

Fibrocytes cells were the dominant type of connective tissue cells in the control group. They were identified from their appearance; looking relatively smaller cells having spindle shape with few cytoplasmic processes, small dark elongated nucleus, acidophilic cytoplasm, and these are the quiescent cells. On other hand; the type of connective tissue cell seen in the treated groups, was the fibroblasts; they were identified by the followings: they were larger than fibrocytes, having abundant basophilic cytoplasm, irregular processes, and large vesicular pale nucleus.^[1, 2, 6]

The cause of the significant increase both in cell diameter and nucleus diameter could be due to the increase in the

activity of these cells,^[2, 6, 17, 18] and it is well known that the physiological condition of these cells are determined by their biological appearance, thus, they are regarded as active and over functioning whenever they are larger with pale large nuclei, whilst they are considered to be non-dynamic and biologically non active whenever they have relative smaller size with somewhat dark small nucleus.^[14, 19, 20, 21]

These findings could be described as; the melatonin might affect the fibrocytes and the fibroblasts directly through the melatonin receptors find normally in all body cells and tissue and/or indirectly through the well-known effect of melatonin as the main director of all types of immunity.^[12, 23, 24, 25]

The significant effect on average diameter of nuclei of fibrocytes and fibroblasts, may give the view that melatonin could affect most of the cell activities, since the nucleus is well known to be the hub director of the cell.^[6, 26, 27, 28]

This stimulation effect of melatonin could be the main cause of the increase in the connective tissues thickness.

These results in the instant study could give the impression that the hormone melatonin might be helpful and has beneficial effect on body immunity in its small, therapeutic and normal dosage, while it has a clear harmful and adverse effect on immunity in its over dosage.^[29-32]

The dilated blood capillaries seen in this study, is due to the well-known vasodilatation action of melatonin.^[33]

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