Histological effects of melatonin on male rat's alveolar macrophages

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Abstract

Background: Melatonin is a neuro-hormone of the pineal gland. It increases and enhances immunity, whether in animals or human. The mononuclear – phagocyte system; is a single functional immune unit. The pulmonary alveolar macrophages are one of the most important members included within this immune unit.

Objective: This work tried to study the effect of different doses of dietary melatonin on adult rat's pulmonary alveolar macrophages.

Methods: Melatonin was supplied to adult rats, for successive 30 days. Rats were divided into 6 groups. Group I was the control. Group II, III, IV, V and VI were given a daily dose of melatonin as 125, 250, 500, 750 and 1000 µg / kg body weight, respectively. After the last day of treatment, the left lung of the rat was removed under anesthesia for histological study.

Results: The results showed significant beneficial effects on pulmonary alveolar macrophages by normal therapeutic dosages, whereas with further stepping up doses, significant damaging effects were seen.

Conclusion: Dietary melatonin had good effects on the rat's pulmonary alveolar macrophages within therapeutic doses, whereas it had highly damaging changes in overabundance.

Key words: Melatonin, immunity, and alveolar macrophages.

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Introduction

Melatonin is a neuro-hormone of the pineal gland, secreted mostly at night time [(1)]. It increases and enhances both cellular and humoral immunity, whether in animals or human [(2, 3)]. The mononuclear – phagocyte system; is a single functional unit of immunological system, consists of bone marrow precursors (monoblasts and promonocytes), circulating monocytes and tissue macrophages, both free and fixed (histiocytes).

Thus as one member of the immune system; macrophages are affected and activated by melatonin effect [(1, 2, 3)] .The pulmonary alveolar macrophages are one of the most important members included within this immune unit [(4, 5)]. It would be of great interest to study the effect of dietary melatonin on these alveolar macrophages.

Materials and methods

Forty eight Adult male Wister albino rats were used in this work. They were kept in an animal room, with a temperature of 22±2C°, the light - dark cycle was 12:12. Water was offered *ad libitum*. They fed a control diet with free access to food, except for one and half hour prior to melatonin containing meal. Dietary melatonin was provided as a single daily dose, 2 hours prior to sunset.

Animals were divided into 6 groups, each consisting of 8 rats. Group I was the control: rats were provided with the same

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type of drug containing meal, but no drug was added (placebo), though, they were also deprived from food one and half hour prior to the time of treatment as other groups. Group II, III, IV, V and VI were given dietary melatonin as a daily dose of 125, 250, 500, 750 and 1000 µg/kg body weight, in sequence, for 30 successive days. The drug used in this work was N-Acetyl-5-methoxytryptamine (melatonin) tablets, from Nature's Bounty INC, Bohemia NY 11716, and USA. After the last day of treatment, all animals were killed by dissection under effect of diethyl ether. The whole left lung was removed, and processed for paraffin section, using Bouin's solution for fixation and (Haematoxylin & Eosin) for staining. Then 5 serial sections of 5 µm thickness were studied [6].

Histological study was done both as descriptive and morphometric by light microscope. The morphometric data were estimated by using objective micrometer used on a light microscope; by which a distance of 10µm could be calculated, so the average widest diameter of pulmomary alveolar macrophages, as well as the diameter of their nuclei, was estimated. All the values were taken as mean ± SD of 8 rats. The significance of difference between each of treated groups and its control was evaluated by student – t – test [7].

Results

The morphometric results:

In all of the treated groups; there were significant increase ($p<0.05$) both in the average widest diameter of the macrophages and the average diameter of their nuclei (Table1).

The descriptive results:

Unusual types of cells were appeared; among which was the large type of macrophages seen mostly in group II and III. They were large polygonal, with darkly stained cytoplasm containing many vesicles, vacuoles and particles. Their nucleus was pale basophilic and eccentric. They were frequently located near the blood vessels in comparison with the control (Figure1 and 2).

The second type of cells seen was the epithelioid cells; they were large type of macrophages with voluminous pink-stained cytoplasm and pale basophilic, eccentric nucleus. They were viewed mostly in the group IV, V and VI (Figure 3).

Multinucleated giant cells were regarded to represent the third type of unusual cells. They were noticed only in animals received doses of 750 and 1000µg/kg.Those cells were very large, irregular in shape, with pale acidophilic cytoplasm filled with granules, vesicles and debris (Figure 4).

The vascularity of the pulmonary tissues as a whole was positively increased with the stepping up doses of melatonin, till the last dose 1000µg/kg; where areas of hemorrhages appeared (Figure5).

Table1: Average widest pulmonary alveolar Macrophage's diameter and their nuclear diameter in µm, of adult rats; treated with dietary melatonin.

Daily dose of melatonin in µg/kg body wt	***Average alveolar Macrophage's diameter (in µm)***	***Average nuclear diameter of alveolar Macrophage's (in µm)***
Control	16.3±1.6	9.7±1.4
125	19.1±1.8*	11.2±1.8†
250	29.7±2.9**	15.1±2.7**
500	44.3±5.1**	18.9±4.2**
750	91.4±11.4**	(multiple) 21.3±7.1††
1000	168.5±21.6**	(multiple) 24.1±9.1‡

-Data were expressed as mean ± SD.
(* P<0.004; **P<0.0001; † P<0.03; †† P<0.008; ‡ P<0.006).

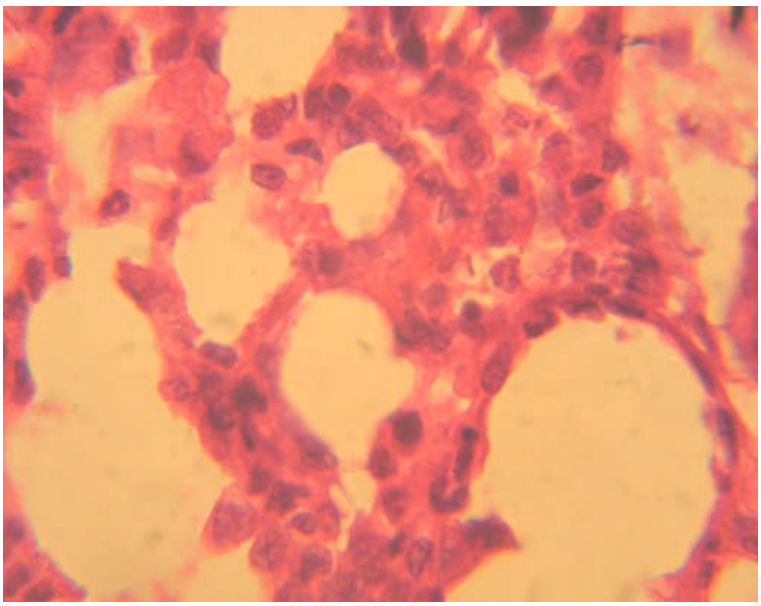

Figure 1: Lung tissue in the control adult rat. H&E, X400

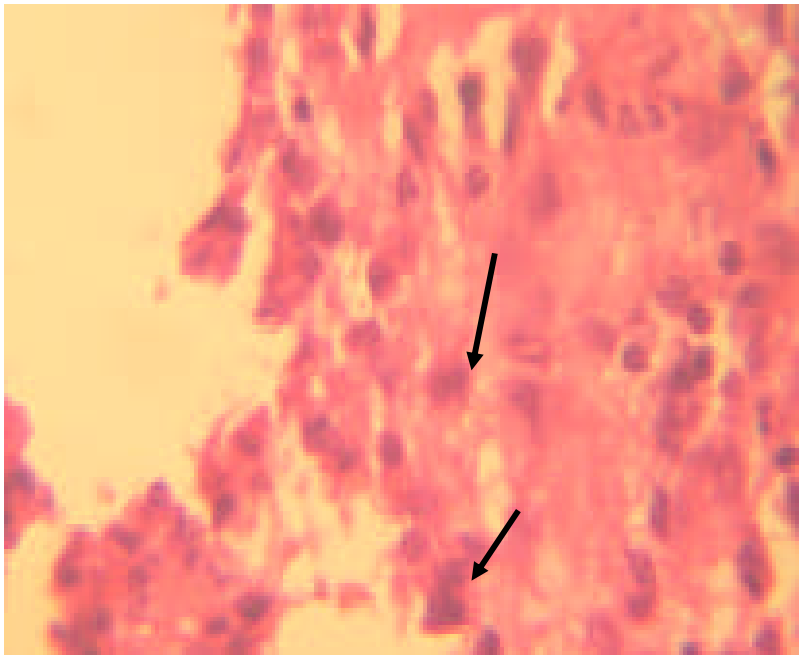

Figure 2: Lung tissue in adult rat treated with 250 µg/kg doses; macrophage (arrows). H&E, X400.

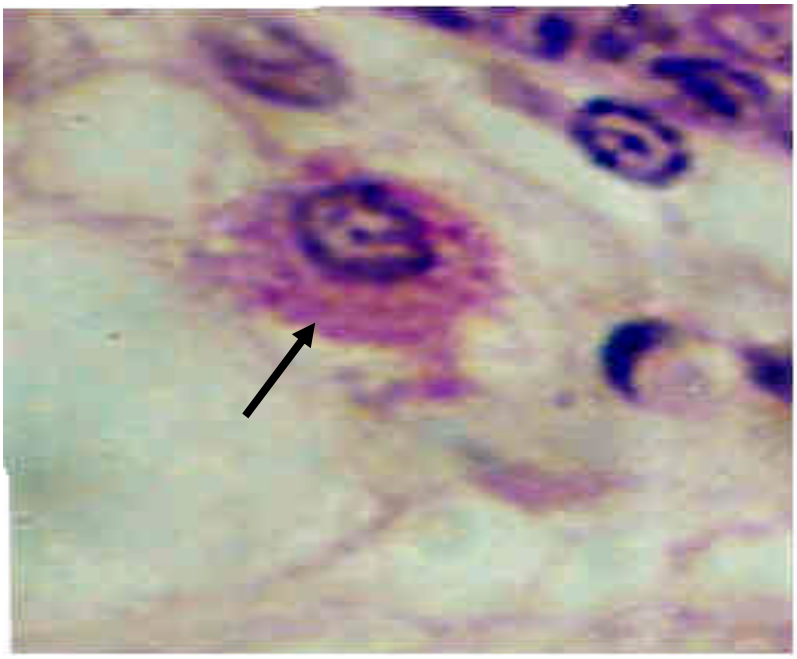

Figure 3: Epithelioid cell (arrow). H&E, X 800.

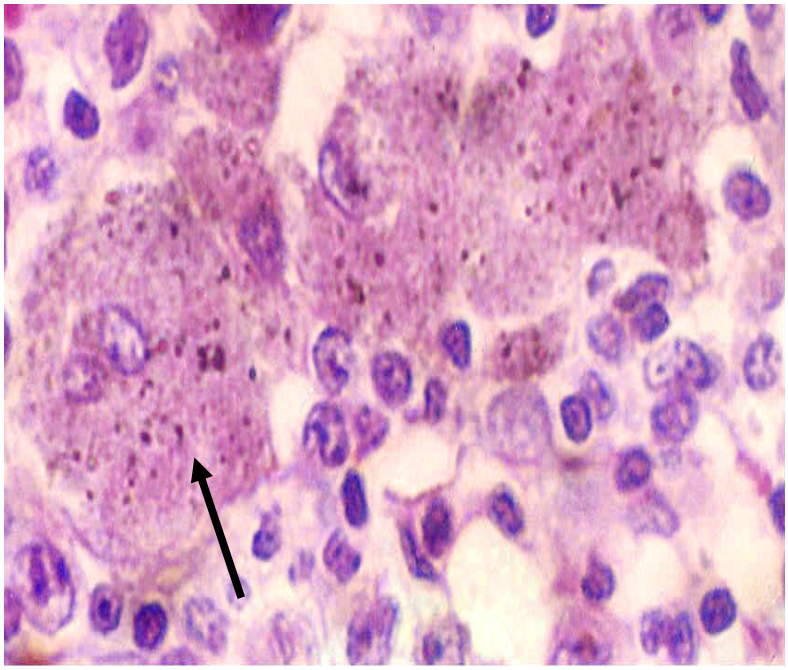

Figure 4: Multinucleated giant cell (arrow).H&E, X450

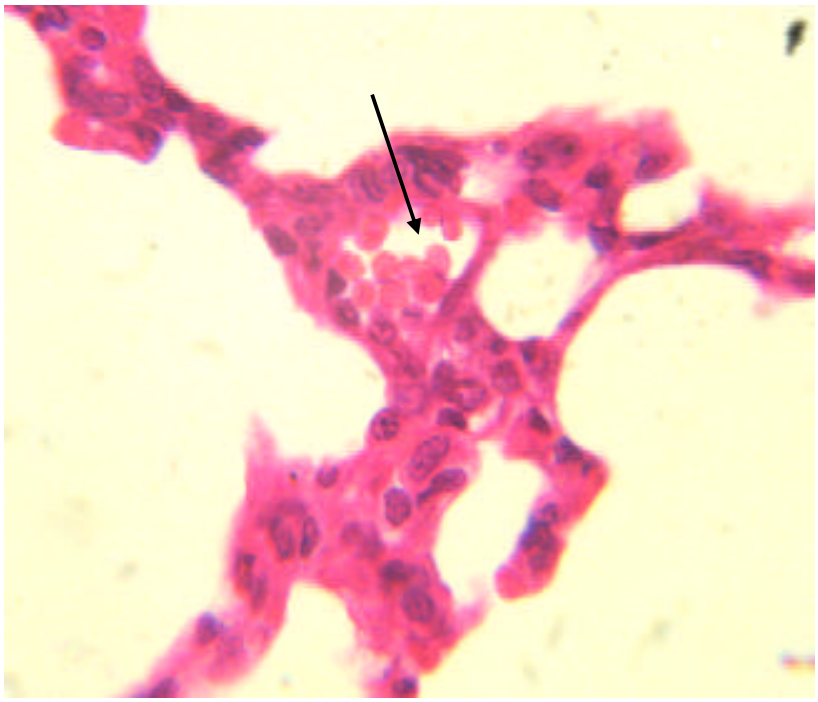

Figure 5: Dilated blood vessel (arrow) in lung tissues in rat given a dose of 500µg/kg. H&E, X400

Discussion

The average widest diameter of pulmomary alveolar macrophages, as well as the diameter of their nuclei was significantly increased by melatonin in the instant work. The explanation for this might be highlighted by the fact that those parameters principally follow the macrophage function status [8, 9]. The physiological condition of the pulmomary alveolar macrophages is determined basically by their histological appearance; so they are considered to be actively functioning whenever their size are larger with paler and larger nuclei, whereas they are said to be insufficient in case they are being smaller with darker relatively smaller nuclei [4, 5, 10]. Hence in the groups treated with 125 and 250µg/kg dose; the unusually large macrophages reflected an actively functioning cells, whereas, the epithelioid cells might represent the over stimulating view, since they are well documented to be seen only in hyper stimulating situations [2, 3, 5, 10].

The other interestingly existed cells in the ongoing study were the multinucleated giant cells; which appeared to highlight a very toxic condition, because those exceptionally rare cells are formed when epithelioid cells coalesce to make huge multinucleated masses termed the foreign body giant cells; that regarded as a characteristic finding consequently seen only in pathological and/or toxic cases. This could be due to the concept that melatonin is a well designed to exert its physiologic action in a dose – dependent manner, being stimulating at normal therapeutic level and harmful at its overabundance [11, 12]. Those findings might indicate the enhancement in the function of macrophages, as a consequence of exogenous melatonin on those cells, affecting them directly through melatonin receptors found in all tissues and cells [13, 14], and/or indirectly through the well known cytokines, namely; granulocyte – macrophage colony stimulating factor (GM – CSF), which is secreted by the macrophages, endothelium and T lymphocytes; activating these cells, and/or through macrophage colony stimulating factor (M- CSF), since the melatonin is the hub director of all types of immunity[2, 3, 15, 16].

The significant effect on average diameter of nuclei in all of treated groups; may lead to the impression that melatonin could affect most of the cell activities, since the nucleus is the archive of the cell [4, 5].

The dilated blood vessels watched in the forgoing study; could be due to the fact, that melatonin has a well known vasodilator action [17]. Those results could be explained by the fact; that melatonin has damaging effects only when it is administered in excess [11, 12].

The results of the instant work went with the concept that melatonin administration within a therapeutic dose might be helpful in the amelioration of the immunity status [18, 19, 20].

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