

## Effect of melatonin on histology of the epididymidis of adult rat.

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### **Abstract**

**Background:** The spermatozoa are provided with the needed capacity for normal motility, in the epididymidis, so the function of epididymidis is very important for the normal fertility. Melatonin is the basic neuro-hormone of the pineal gland, regulates the sexual and reproductive activities in all mammals including man.

**Objective:** To study the effect of different doses of dietary melatonin on the histology of adult rat's epididymidis.

**Methods:** Melatonin was supplied to adult Wister albino rats with their diet, 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 125, 250, 500, 750 and 1000 µg / kg body weight, respectively. After the last day of treatment, animals were killed under effect of anesthesia; epididymidis was removed, fixed in Bouin's solution and processed routinely for histological study.

**Results:** The results showed significant positive effects on epididymidis, since it increased the epididymal wall thickness, epididymal, as well as spermatozoal clump within epididymal tubules, with normal therapeutic dosages, whereas significant damaging effects were seen with raising dosages.

**Conclusion:** Dietary melatonin has clear positive effects on the rat's epididymidis within therapeutic doses, since it increased the epididymal wall thickness, epididymal, as well as spermatozoal clump within epididymal tubules, whereas it had highly damaging changes in surplus doses.

**Keywords:** Epididymidis, melatonin, and infertility.

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### **Introduction**

The epididymidis is the site of accumulation, storage and physiological maturation of spermatozoa; hence spermatozoa get their capacity for normal motility<sup>(1, 2)</sup>, so the function of this part of male genital system is very vital for the normal fertility<sup>(3, 4)</sup>.

Melatonin is the basic neuro-hormone of the pineal gland<sup>(5, 6)</sup>.

This hormone evidently plays an important regulatory role in the sexual and reproductive activities in all mammals including man<sup>(7, 8, 9)</sup>, hence, it would be of great interest to study the relationship between melatonin and epididymidis structural and so functional status. Histological morphometric study could be estimated by using Zeiss Integrating Micrometer – disk Turret I of 25 point system, (which measures the relative surface area by counting the points superimposed through a disk put on the microscopic eye piece during slide examination, so the number of these points positively related with the relative measurement of the surface area), the total points falling on each epididymidis wall, lumen, and

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spermatozoa clump within the lumen, could give the idea about the structural and hence the functional status of epididymidis.

#### **Materials and methods**

Adult male Wister albino rats, 48 in number, were used in this work. They were kept in an animal room, with a temperature of  $22 \pm 2^\circ\text{C}$ , 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 sundown. Animals were divided into 6 groups, each consisting of 8 rats. Group I was the control: rats were provided with the same 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  $\mu\text{g/kg}$  body weight, in sequence, for 30 successive days. After the last day of treatment, all animals were killed by dissection under effect of diethyl ether. The whole epididymidis was removed, separated from the surrounding connective tissues under a dissecting microscope, weighed by an electric sensitive balance. Fixed in Bouin's solution, embedded in paraffin, and processed routinely for histological study. Then 5 serial sections of 5  $\mu\text{m}$  thickness from the mid- part (body) of the left organ were stained with Haematoxylin & Eosin and selected for study<sup>(10, 11)</sup>. epididymidis was removed, under a dissecting microscope, weighed by an electric sensitive balance.

Histological study was done both as descriptive and morphometric by a light microscope. The morphometric data were estimated by using Zeiss Integrating Micrometer – disk Turret I of 25 point system, (which measures the relative surface area by counting the points superimposed through a disk put on the microscopic eye piece during slide examination, so the number of these points positively related with the relative measurement of the surface area), the total points falling on each epididymidis wall, lumen, and spermatozoa clump within the lumen, were calculated. From each section 5 fields were taken randomly examined at 150X magnification. All the values were taken as mean  $\pm$  SD of 8 rats. The significance of difference between each of treated groups and its control was evaluated by student – t – test<sup>(12)</sup>.

#### **Results**

Descriptive and morphometric studies for all groups were done, as follows:

Epididymidis weight was unaffected significantly in all groups (Table 1).

Morphometric results:

(1) The number of points overlying the epididymal epithelial wall, was raised till the dose of 500 $\mu\text{g/kg}$ , then it was significantly decreased at the dose of 750 $\mu\text{g/kg}$ , and a great decrease was clear at group received 1000  $\mu\text{g/kg}$  (Table2).

(2) The number of points superimposed on the lumen of the epididymidis, followed an opposite manner to that of the wall (Table2).

(3) The number of points superimposed on the spermatozoa clump within lumen of the epididymidis, followed an opposite manner to that of the wall (Table2).

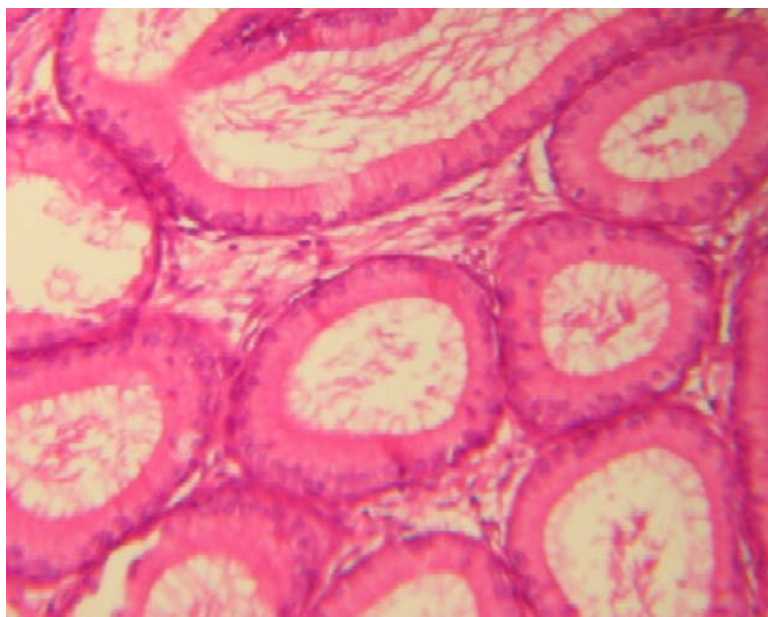
The descriptive histological result:

Cells of the epididymal epithelial wall, in the groups treated with 125, and 250µg/kg were almost thicker than that of the control group (Figure 1); so each epididymal duct was bound by a single layer of specialized epithelium which rest on a thick basement membrane and enclosed a lumen filled with clumps of spermatozoa which are more abundant than that of the control. The epididymal duct had tall columnar epithelium bearing numerous very long microvilli, and basal nuclei (Figure 2). In the group treated with 250µg/kg, apoptotic and pyknotic cells seen frequently.

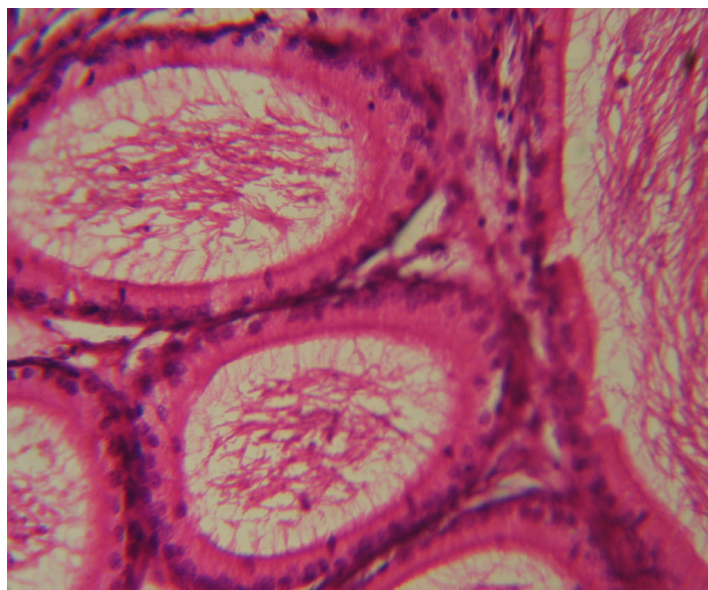
In groups received 500 µg/kg dose, cells of epididymal duct were seen commonly tall columnar

epithelium, having basal nuclei which were appeared as more crowded at the periphery of the duct, with unusually long micovilli, lined narrow lumina which were noticed to have less population of spermatozoa (Figure 3).

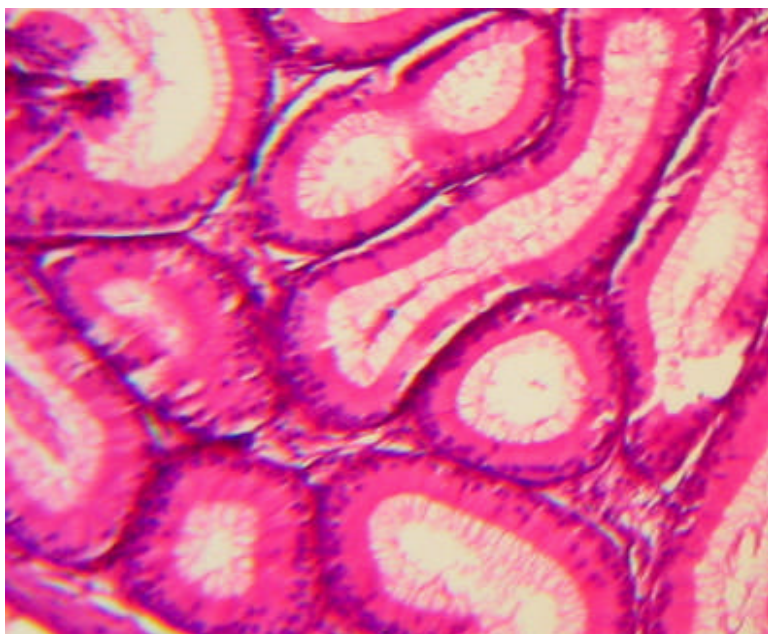
The group treated with 1000 µg / kg dose: The epididymal duct was viewed with thickened basement membrane, some areas showed fibrosis & necrotic changes. There was abundance in the number of spermatozoa , epithelial cells were looked more or less regressed in their height, their nuclei were viewed as less abundant at periphery of the duct, the micrivili also seemed to be shorter, and the lumina were appeared larger (Figure 4).



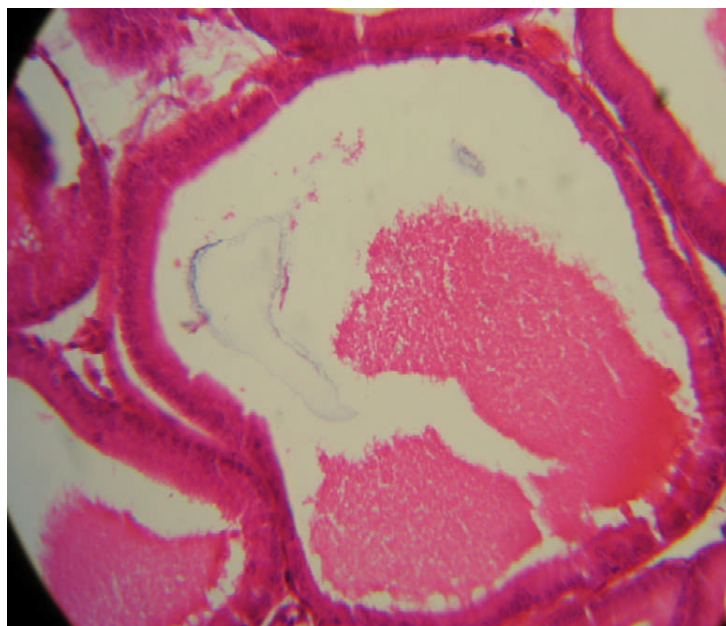
**Figure 1: Epididymidis in control adult rat, X 200, H&E.**



**Figure 2: Epididymidis of adult rat, treated with 250 µg/kg dose, epididymal wall thickness increased, lumen dilated and spermatozoal Clumps became abundant than that of control, X 200, H&E.**



**Figure 3: Epididymidis of adult rat treated with 500 µg/kg dose, epididymal wall thickness increased, lumen became smaller and spermatozoal clumps diminished than that of control, X 200, H&E.**



**Figure 4: Epididymidis of adult rat treated with 1000 µg/kg dose, epididymal wall thickness decreased, lumen dilated hugely and spermatozoal clumps became much abundant than that of control, X 200, H & E.**

**Table 1: The Effect of Melatonin on Epididymal Weight of Adult Male Rats.**

| <i>Daily dose of melatonin<br/>in µg/kg body weight</i> | <i>Epididymal weight at<br/>autopsy<br/>(in mg) of adult rats</i> |
|---------------------------------------------------------|-------------------------------------------------------------------|
| Control                                                 | 4.3±64.3                                                          |
| 125                                                     | 4.9±28.4 NS                                                       |
| 250                                                     | 5.1±23.2 NS                                                       |
| 500                                                     | 5.9±26.3 NS                                                       |
| 750                                                     | 6.6±31.1 NS                                                       |
| 1000                                                    | 9.2±61.3 NS                                                       |

-Results were expressed in mean± SD of 8 rats.

-The difference of each dose group was statistically insignificant when compared with its control:

(\* P>0.05; NS= not significant).

**Table 2: Number of Points Overlying the Wall and Lumen, as well as the Spermatozoal Clump, in Epididymidis of Adult Rats Treated with Dietary Melatonin (in unit area of 0.0025mm<sup>2</sup>).**

| <i>Daily dose of melatonin in µg/kg body wt</i> | <i>Points on epididymal wall</i> | <i>Points on epididymal lumen</i> | <i>Points on epididymal Spermatozoal clump</i> |
|-------------------------------------------------|----------------------------------|-----------------------------------|------------------------------------------------|
| Control                                         | 16.17±1.93                       | 31.96±1.34                        | 7.33±2.15                                      |
| 125                                             | 17.16±0.09<br>NS                 | 41.41±1.21*                       | 10.15±0.11‡                                    |
| 250                                             | 17.98±1.01†                      | 40.35±1.02*                       | 11.06±1.02*                                    |
| 500                                             | 29.87±1.62*                      | 10.26±1.31*                       | 5.09±1.42**                                    |
| 750                                             | 21.26±1.07<br>NS                 | 19.24±1.28*                       | 6.92±1.91NS                                    |
| 1000                                            | 11.13±1.05*                      | 51.92±1.97*                       | 24.43±2.16*                                    |

-Data were expressed as mean ± SD of 8 rats.

-When any dose-group was compared with its control, the difference was statistically significant:

(\* P<0.00001; \*\* P<0.004 † P<0.008; ‡ P<0.02; NS= non significant).

### **Discussion**

The epididymal weight was significantly unaffected by melatonin in the instant work (Table 1). The explanation for this might be highlighted by the fact that epididymal weight principally dose not follow its function status <sup>(13)</sup>. Changes in epididymal wall thickness, lumen diameters, and spermatozoal clumps showed a clear positive effect of melatonin on those parameters (Table 2); i.e., they were steadily increased with the increase in amount of doses up to the level of 500 µg/kg dose, then after decreased with 750 µg/kg dose and they were noticed to increased again at 1000 µg/kg dose. This could be due to the concept that melatonin is well designed to exert its physiologic

action in dose – dependent manner, being stimulating at normal therapeutic level and harmful at higher doses <sup>(14, 15)</sup>.

The epididymal tubule wall was significantly thicker with more frequent existence of nuclei observed in those groups treated with 125 , 250 and 500 µg/kg dose, and much less in group of 750 µg/kg ,then regressed at 1000 µg/kg dose, these findings might indicate the increase in number of epididymal epithelial cells, which could be the consequence of exogenous melatonin on the those cells, and affecting their function directly through melatonin receptors found in all tissues and cells <sup>(16)</sup>, or indirectly through the pituitary gland affecting its secretion of FSH there by promotes other sexual

hormones secretion<sup>(17)</sup>. Nevertheless, direct and/or indirect role, also there could be probably an induction of Sertoli cells to secrete increasing amount of androgen binding protein (Abp), which binds testosterone and hydroxytestosterone produced outside the genital ducts, high concentration of these hormones are required within the genital epithelium and lumen for normal function<sup>(18, 19)</sup>. The epididymal wall thickness was decreased with 750 µg/kg doses and a great regression noticed at 1000 µg/kg dose. This could be due to the concept that melatonin is stimulating at normal level and harmful at higher doses<sup>(14, 15)</sup>.

The suggestion for those findings could be through suppression of hormone inhibin, which is secreted by Sertoli cells normally, inhibiting the secretion of FSH by the pituitary under control of hypothalamus and therefore plays an important feedback role in controlling the suppression of inhibin, which could be the cause of that regression consequently<sup>(4, 13)</sup>.

The number of points overlying the spermatozoa clump within the duct was increasing incrementally in the groups treated with the dose of 125 and 250 µg/kg, then at the dose of 500 µg/kg, it was adversely proportionate with those points on the wall & lumen of the tubules, this may be due to the effect of melatonin either directly on the main cells of spermatogenic lineage, through melatonin receptors proposed to be present in all body tissues and cells<sup>(16)</sup>, and / or indirectly by melatonin effect on hypothalamic-hypophyseal axis suppresses the secretion of FSH, hence decreases cells of spermatogenic lineage activity and number<sup>(20)</sup>. The other proposed explanation could be through over stimulation of these

Leydig cells by melatonin inducing over secretion of androgen; which acts by its negative feedback mechanism on hypothalamus leading to suppression of FSH secretion also<sup>(1, 4)</sup>.

The increase in frequency of apoptotic and pyknotic cells seen in groups treated with 250 µg/kg, might be caused by the effect of melatonin on Sertoli cells to control the large number of spermatogenic cells competing for survival in a so called programmed cell death (apoptosis) which is very different from that which occurs as a direct result of deleterious events to the cells, termed necrosis<sup>(1, 4, 13)</sup>.

In the group treated with 1000 µg/kg dose, some areas showed fibrosis & necrotic changes, this picture offers the extent of the highly damaging effect exerted by that dose of melatonin, since in any damaging events to any given tissue, a similar histological view will be sighted<sup>(1, 13, 17, 21)</sup>. Those results could be explained by the fact that melatonin has damaging effects only when it is administered in high doses<sup>(14, 15)</sup>. The thickening of the basement membrane could be resulted from the increase in production of fibrocollagenous tissues, since melatonin hormone has special effect on fibroblasts<sup>(22)</sup>, which are the active collagen – secreting cells and the basic forming cells of the connective tissues<sup>(1, 4, 23)</sup>. The increase in spermatozoa clump might be the consequence of decrease in motility of the spermatozoa so accumulated inside the widened lumen<sup>(13, 17)</sup>.

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