Histological modulation of adult rat's testis in response to anti-aging factor

Samia A. Eleiwe
MBChB, MSc

Abstract:

Background: Melatonin overtops other hormones in the studies of hormonal – replacement therapy nowadays. It is an anti-aging and antioxidant, prevents the oxidative damaging events to endocrine organs including testis.

Objective: The on going study tried to document that the fertility of testes must be increased by melatonin supply within the therapeutic doses.

Methods: Melatonin was supplied to adult male albino rats, for successive 14 days. Rats were divided into 6 groups. Group I was the control. Group II, III, IV, V and VI were given melatonin as 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. Left testis was studied to evaluate the fertility status.

Result: The results showed significant increase in the reproductive morphology with the therapeutic doses, while highly degenerative changes seen with larger doses.

Conclusion: Melatonin increases the reproductive morphology of rat's testes within therapeutic doses, whereas it induces vast damages with large doses.

Key Words: Testis, melatonin, antiaging, and hormonal replacement therapy

Introduction:

The endocrine system is a "pace marker" of male aging. Though not as dramatic as in women, men also show declining hormone concentrations with aging. Hence, hormonal replacement therapy has a potential role, in aging men. The goal of hormonal replacement would be the improvement of body well being, virility, muscle strength and quality of life in men, thereby reducing mortality and morbidity [1].

Melatonin overtops other hormones in the studies of hormonal – replacement therapy nowadays, since it is a potent antioxidant agent, prevents the oxidative damaging events to endocrines including testes, which is an endocrine as well as exocrine organ[2,3]

Materials & Methods:

Adult male Wister albino rats, 48 in number, were used. Their average body weight at the beginning of the experiment was 355 gram. They were kept in an environmentally controlled animal room. They fed a controlled diet with a free access to food, except for one and half hour prior to the melatonin meal. Dietary melatonin was provided as a single daily dose, 2 hours prior to sundown. Water was offered *ad libitum*.

Animals were divided into 6 groups, each consisting of 8 rats. Group I was the control: rats were provided with the same type of meal, but here no drug was added (placebo), though, they were also deprived from food one and half hour prior to the time of treatment, like other groups. Group II, III, IV, V and VI were given melatonin as a daily dose of 125, 250, 500, 750 and 1000 µg / kg body weight, respectively, for successive 14 days .After the last day of treatment, animals were killed under effect of diethyl ether. Left testis was removed, weighed and used for paraffin section using Bouin's solution for fixation and (Haematoxylin & Eosin) for staining. Form each testis, 5 serial sections of 3 µm thickness from the mid part of the organ, were studied [4].

The morphometry was estimated by using Zeiss Integrating Micrometer–disk Turret I of 25 point system, used on a light microscope, the total points falling on each seminiferous tubule wall as well as lumen, were calculated. Also the Points overlying the interstitial spaces were estimated. From each section 5 fields were taken randomly examined at 150X magnification. The significance of difference was evaluated by student – t – test [5].

Result:

Testis weight was increased incrementally with the increase in melatonin dose up to the dose of 500 µg / kg, then, it began to decrease, with a clear regression at the dose of 1000 µg /kg (table 1).

Morphometric results (table 2):

1-The number of points overlying the wall of seminiferous tubule, was significantly increased steadily, with the increase in doses of melatonin, till the dose of 500µg / kg dose, then the number started to decrease (but still more than the control) , the great regression was clear at group received 1000 µg / kg dose.

2-The number of points superimposed on the lumen of the seminiferous tubule, followed the same manner as that of the wall.

3-The number of points on the interstitial spaces within the same given surface area, ran in an opposite way than that of the serminiferous tubules wall. Hence it had a frequency proportionate adversely with melatonin doses, and the maximum value was noticed at 1000 µg/kg dose.

Table1: the effect of melatonin on testis weight of adult male rats.

Daily dose of melatonin in µg/kg body weight	Testis weight at autopsy (in µg) of adult rats
Control	1517.4±61.3
125	1538.6±48.4NS
250	1587.4±53.2*
500	1612.6±56.3**
750	1578.7±47.3*
1000	1192.3±61.1**

-Results were expressed in mean± SD of 8 rats.
-The difference of each dose group was statistically significant when compared with its control (* P<0.05; ** P<0.001; NS not significant).

Table2: Number of points overlying the seminiferous tubule wall and lumen, as well as the interstitial space, in testis of adult rats treated with dietary melatonin (in unit area of 0.0025mm²).

Daily dose of melatonin in µg/kg body wt	Points on seminiferous tubule wall	Points on seminiferous tubule lumen	Points on interstitial space
Control	14.18±1.94	3.86±1.29	7.53±2.13
125	14.91±1.61 NS	4.41±1.60 NS	7.16±2.04NS
250	15.26±1.51*	4.35±1.27NS	6.76±1.01*
500	15.67±1.62*	5.21±1.31*	5.09±1.98*
750	15.27±1.72*	4.24±1.28*	6.92±1.91*
1000	12.11±1.63*	2.12±1.33*	8.43±2.12*

-Data were expressed as mean ± SD of 8 rats.
-When any dose-group was compared with its control, the difference was statistically significant (* P<0.05); NS= non significant.

The descriptive histological result: Cells of spermatogenic lineage, were more abundant in the groups treated with 125, 250, 500 and 750µg/kg especially; spermatogonia (both dark and pale type), also primary spermatocytes, spermatid and spermatozoa (both developing and mature ones) were increased (Fig.1, 2&3).

In groups received 750 µg/kg dose, multinucleated spermatogenic cells were seen. Groups treated with 500 and 750 µg/kg all showed an increasing frequency of apoptotic and pyknotic cells. Mitotic figures in spermotogenic cells were demonstrated clearly in groups treated with 125, 250 and 500 µg/kg and rarely in those rats treated with 750 µg /kg.

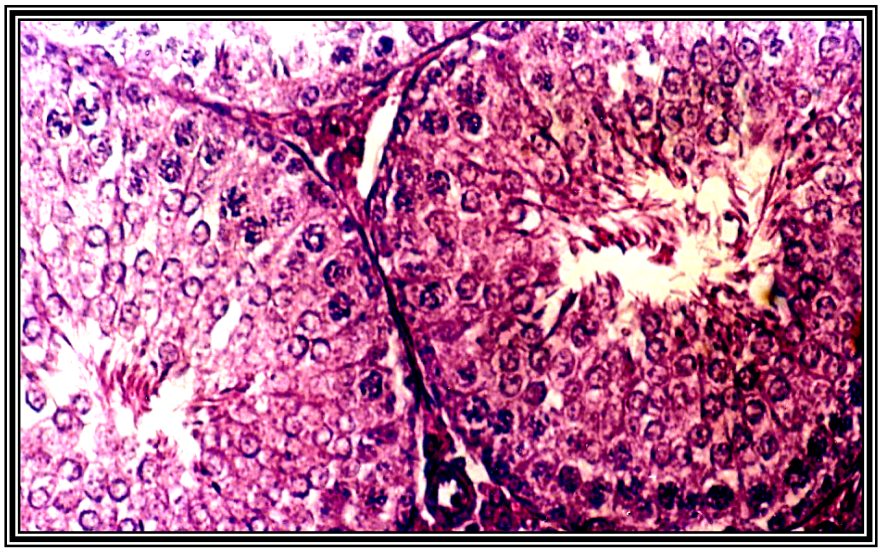

Figure 1: Photomicrograph of testicular tissues in control of male rat H&E stain X100

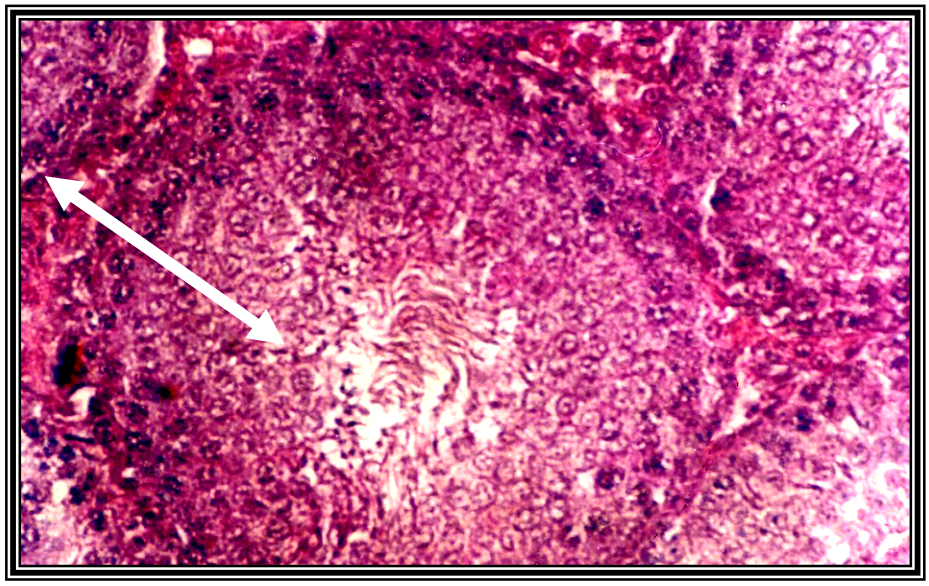

Figure 2: Photomicrograph of testicular tissues in male rat treated With 250µg/kg melatonin, in creased wall thickness (double head arrow). H&E stain X100

The group treated with 1000 µg / kg dose: The seminiferous tubules were viewed with thickened basement membrane consisted of hyalinized connective tissue. There is concomitant increase in the interstitial stoma Leydig cells less frequent than in other previous treated groups but they appeared to be more prominent. Most of seminiferous tubules showed Sertoli cells mainly, though, a few of them still retaining some cells of spermatogenic lineage (Fig.4).

Some areas showed severe destruction of the testicular architecture represented by only necrotic tissues that heavily infiltrated with lymphocytes, plasma cells, fibroblasts, and large types of macrophages as well as epithelioid cells. Though a few recognized seminiferous tubules remained which lacked the spermatogenic cells, the lumen of which was obliterated completely by Sertoli cells. The wide interstitial spaces were almost completely fibrosed with a few cells of Leydig's (Fig.5). In all groups

except group VI; Sertoli cells were not clearly identified, hence it was so difficult to be studied.

Blood vessels dilatation was obvious in all treated groups and the degree of dilatation proportionate positively with the amount of melatonin used.

Leydig cells were identified by their polygonal shape; the nucleus is central, vesicular, round and one or two nucleoli. The cytoplasm is granular, pale eosinophilic with intracytoplasmic, eosinophilic, elongated, rectangular or rhomboid masses.

Some of Leydig's cells showed yellow - brown pigmented dots in their cytoplasm. Whereas the epithelioid cells, were identified by their voluminous, pink – stained cytoplasm, with their pale eccentric nucleus. The fibroblasts were designed with abundant irregularly branched basophilic cytoplasm, with ovoid central pale nucleus with distinct nucleoli.

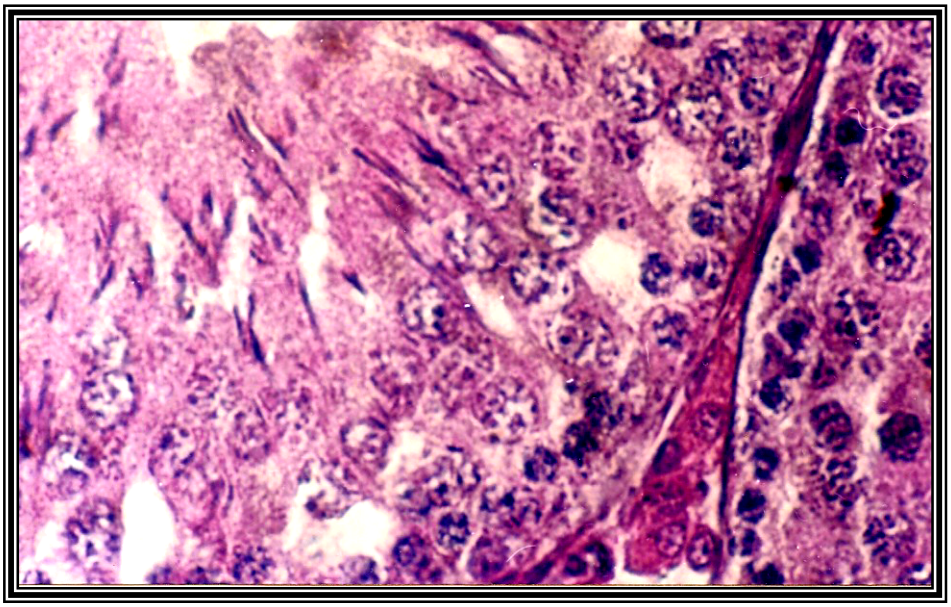

Figure 3: Photomicrograph of testicular tissues in male rat treated with 250µg/kg melatonin, (pale spermatogonia: short arrow, dark spermatogonia: long arrow). H&E stain X 400

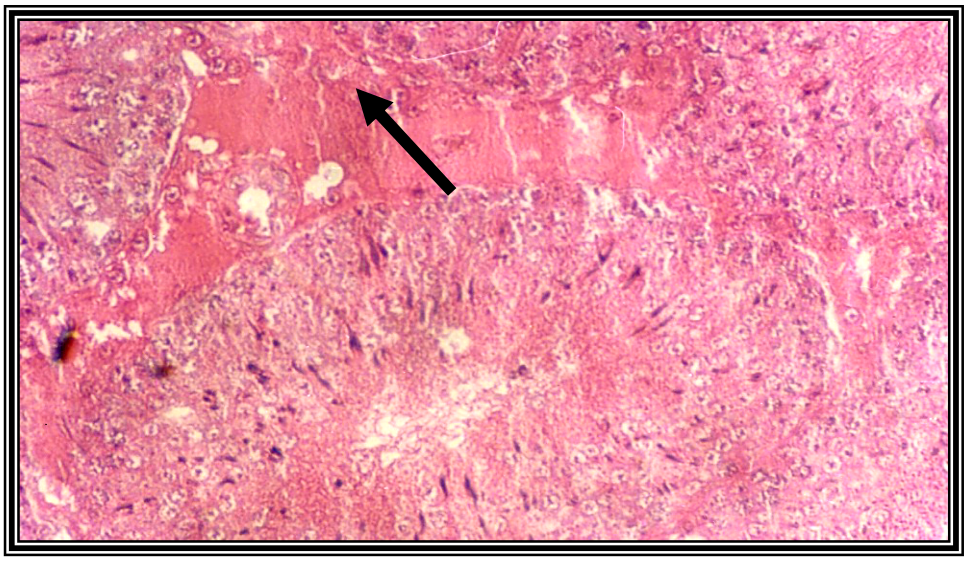

Figure 4: Photomicrograph of testicular tissues in male rat treated with1000µg/kg melatonin, increased interstitial surface area (arrow), H&E stain 100

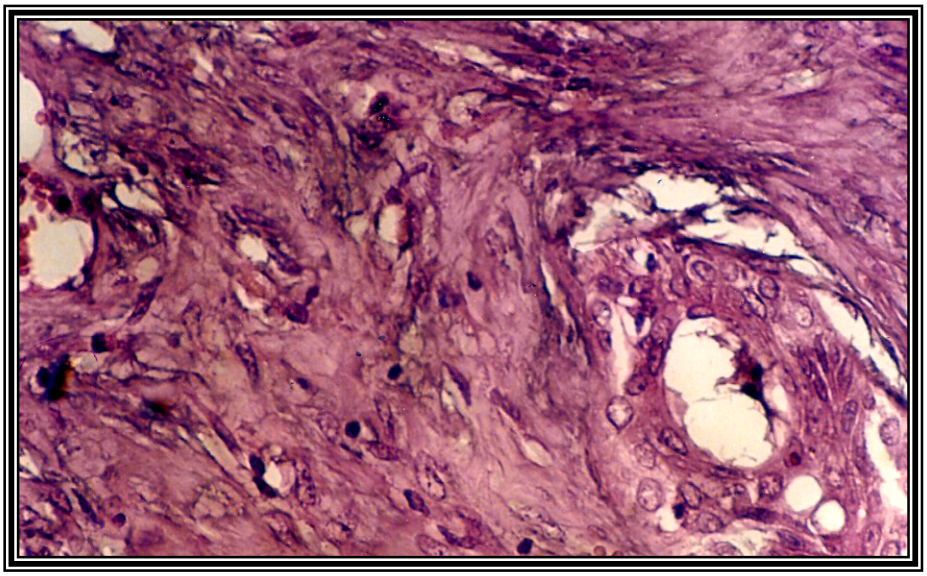

Figure5: Photomicrograph of the testicular tissue in male rat treated with 1000µg/kg melatonin; seminiferous tubule (arrow), H&E, X100

Discussion:-

The testicular weight was significantly affected by melatonin in the instant work (Tab.1). The explanation for this might be highlighted by the fact that melatonin hormone, controls the gonadal axis (1&2). Other possibility is that melatonin controls the gonads directly through its receptors which are realized to be present in all bodily tissues and cells [6]. Hence, melatonin influenced the weight since it is a well known that testicular weight principally follows its function status [7]. Testicular weight as well as seminiferous tubules wall thickness and lumen showed a clear positive effect of melatonin on these parameters (Tab.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 a great regression noticed 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 [8].

The seminiferous tubule wall was significantly thicker with more frequent existence of mitotic figures observed in those groups treated with 125 , 250 and 500 µg/kg dose, and much less in group of 750 µg/kg , these findings might indicate the increase in number of spermatogenic cell lineage , which could be the consequence of exogenous melatonin on the Sertoli cells of the seminiferous tubules affecting their secretion directly through melatonin receptors found in all tissues and cells[5], or indirect through the pituitary gland affecting its secretion of FSH there by promotes spermatogenesis[3]. Nevertheless, direct and/or indirect role, there is probably an induction of Sertoli cells to secret increasing amount of androgen binding protein (ABP), which binds testosterone and hydroxytesteron produced out side the seminiferous tubule; high concentration of these hormones are required within the germinal epithelium and tubule lumen for normal function[3&6]. The seminiferous tubule wall thickness was significantly 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 [7].

The other suggestion for these finding could be through suppression of hormone inhibin, which is secreted by Sertoil cells normally, inhibiting the secretion of FSH by the pituitary under control of hypothalamus and therefore plays an important feed back role in controlling the suppression of inhibin, which could be the cause of increasing the rate of spermatogenesis consequently [2&3].

The number overlying the interstitial space was proportionate adversely 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 interstitium termed the Leyding cells, through melatonin receptors proposed to be present in all body tissues and cells[6], and / or indirectly by melatonin effect on hypothalamic-hypophysial axis suppresses the secretion of LH which acts upon the Leydig cells affecting their activity and number[3].

The other proposal explanation could be through over stimulation of these Leydig cells by melatonin inducing over secretion of androgen; which acts by its negative feed back mechanism on hypothalamus leading to suppression of LH secretion also[2&3] .

Multinucleated spermatogenic cells were seen in 750 µg/kg dose-group, since those cells are seen only in the degenerative and toxic status of the testis [9].

The increase in frequency of apoptotic and pyknotic cells seen in groups treated with 250, 500 and 750 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 [4].

In the group treated with 1000 µg/kg dose, some areas showed severe fibrosis & necrotic changes with very few retained spermatic tubules, this picture offers the extent of the highly damaging effect exerted by that dose of melatonin, since in any damaging events to the testis similar histological view will be sighted [7].

With exception to group VI, Sertoli cells were not identified in all other groups, because it is a well known that Sertoli cells are not distinguished by routine histological paraffin section stained with hemataxylin & Eosin[10], therefore they were not studied in this work. Dilatation of blood vessels was clearly anticipated in all treated groups, proportionate positively with the increasing in the doses of melatonin, might be resulted by the vasodilatation action of melatonin [11].

Melatonin administration might be helpful in the prevention, or, at least, in the delay of damaging stress events on testicular tissues [12].

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Lecturer in Department of Anatomy, Histology and Embryology
College of medicine- Al- Mustansirya University