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Histological effects of melatonin on testes in pinealectomized and normal albino rats

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Abstract
In the present study, the histological effect of melatonin (MEL) on pinealectomized (PINx) and normal rats' testes was investigated. Thirty six adult male rats were used in this study. The rats were divided into six experimental groups as follow: *1st control group*; *2nd sham-operated surgery rats group*; *3rd PINx rats group*, *4th PINx rats+MEL (60 mg / Kg diet) group*; *5th control rats+MEL (60 mg / Kg diet) group*; and *6th control rats+MEL (120 mg / Kg diet) group* and the treatments were continued for six weeks. The result showed mild histological changes in testicular tissue of sham-operated surgery rats, like shrinkage and atrophy of seminiferous tubules (STs) and weakness in spermatogenesis. While, in PINx rats revealed regressive changes in testicular tissue, like sever degeneration, vacuolation and necrosis in germinal epithelium and no spermatogenesis. But as PINx rats were given MEL 60 mg/kg diet, the testicular structure and function were recovered, whereas, in normal rats were given MEL 60 and 120 mg/kg diet, the result show sever vacuolation and sloughing of spermatocytes inside STs lumen and no spermatogenesis at rats+60 MEL and mild edema with well development of germinal epithelium and spermatogenesis at rats+120 MEL. *In conclusion*, the deficiency of MEL level leads to change in histology and physiological activities of rat testes.

Key words: ***melatonin, pinealectomy, rat, testis histology.***

Introduction
Melatonin is a neurohormone manufactured and secreted mainly by the pineal gland. Other tissues and cells are also involved in its synthesis, as evidenced by the fact that plasma melatonin (MEL) level in pinealectomized (PINx) mice decreases significantly, but is not completely nonexistent [1,2].

Several researchers have demonstrated that continuous light exposure (functional pinealectomy) influences the cardiovascular system and abolishes nocturnal rise of blood MEL levels. Functional PINx represents a more physiological model of reduced MEL levels as compared to surgical PINx (removal of pineal gland), which decreases not only the night-time MEL level but also the day-time MEL level [3].

Pinealectomy would leads to atrophy of the vascular wall, which is associated with a decrease in passive distensibility [4]. It has been hypothesized that MEL deprival would impair the distensibility of the cerebral arterioles and that this would impair cerebral blood flow autoregulation [5].

Sirotkin and Schaeffer, (1997) [6], suggested that MEL exerts its antigonadal effects, at least in a part, through the straight decrease of testosterone production. Furthermore, it has been found that PINx induced increased testosterone production in Leydig cells and this elevation of testosterone secretion can be prevented by administration of MEL [7]. In addition, beside to its role as a broad spectrum of free radical scavenger, MEL may also limits the MDA levels [8]. The aim of the present study is to investigate the effects of MEL on the histology of testes in control and PINx rats.

Materials and methods
A. Animals and housing
Thirty six male albino rats, 8-10 weeks of age and about 300-350 gm were used in this study. The rats were inhabited in plastic cages contained wooden chips. They were inhabited under standard conditions, photoperiod at 12:12 light/dark temperature at 22 ± 2 C^{o} [9]. The rats were fed on standard rat chow and tap water *ad libitum*.

B. Experimental Design
This experiment was designed to study the histological effect of two doses of MEL on testes in male albino rats. Animals were allocated randomly into six different experimental groups and were continued for 6 weeks as follows:

1st group: *Control rats*. The rats were given a standard rat chow and tap water *ad libitum*.

2nd group: *Sham-operated surgery rats*. The rats underwent sham-operated surgery and given standard rat chow and tap water *ad libitum*. .

3rd group: *PINx rats*. The rats of this group underwent pinealectomy and given standard rat chow and tap water *ad libitum*.

4th group: *PINx rats + MEL (60 mg / Kg diet)*. The rats of this group underwent pinealectomy and were given standard rat chow supplemented with MEL (60 mg / kg diet).

5th group: *Melatonin (60 mg / Kg diet)*. The rats were given standard rat chow supplemented with MEL (60 mg / kg diet).

6th group: *Melatonin (120 mg / Kg diet)*. The rats were given standard rat chow supplemented with MEL (120 mg / kg diet).

C. Histological analysis
At the end of experiments, the rats were anesthetized with ketamine hydrochloride (50mg/kg), and the testes were collected by post mortem dissection, and then placed in bouin's solution for fixation and processed until preparing paraffin blocks. Five

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micron thick sections were prepared using microtome (microTec Laborgerate GmbH Rudolf-Diesel-Straβe, Walldorf, Germany). Hematoxylin and eosin were used to staining the sections. The specimens were examined under light microscope [10].

Results

The results showed that in sham-operated surgery rats shrink of seminiferous tubules (STs), atrophy in germ epithelium, decreased number of spermatocytes and spermatogenesis were seen, in addition to narrowing of STs, cellular debris appear in the lumen of STs (figures 3, 4), as compared to the control group which revealed well development of germinal epithelium and complete stages of spermatogenesis (figures 1, 2).

Pinealectomized rats showed a high testicular damage, which involved sever cytoplasmic degeneration, sever vacuolation in germinal epithelium, sever necrosis of spermatocytes, narrowing of STs and cellular debris inside the lumen of STs and sloughing spermatocytes inside the lumen of STs (figures 5, 6).

In PINx rats were given 60 mg/kg diet of MEL, showed gradually recovery of structure and function of testes to normal, and in onset activation of germinal epithelium of STs despite found cellular debris in the lumen of few STs (figures 7, 8). Whereas, in normal rats were given 60 mg/kg diet, the STs of most rats completely shrank and sever sloughing spermatocytes were seen in their lumen and spermatogenesis was highly decreased (figures 9, 10). In addition, in normal rats treated with 120 mg/kg diet, the germinal epithelium well developed and spermatogenesis process was increased as compared to PINx rats+60 MEL group, despite mild edema in germinal epithelium (figures 11, 12).

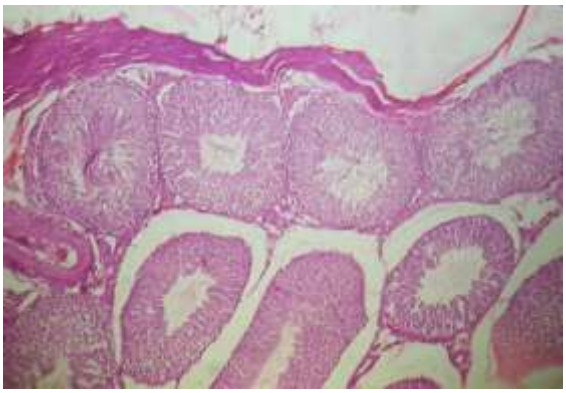

Figure 1: Normal control rat testes show normal structure of STs (H&E X350).

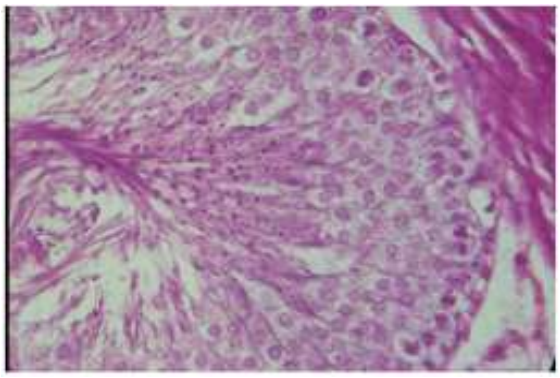

Figure 2: Normal rat testes show well development of germinal epithelium and stages of spermatogenesis (H&E X1000).

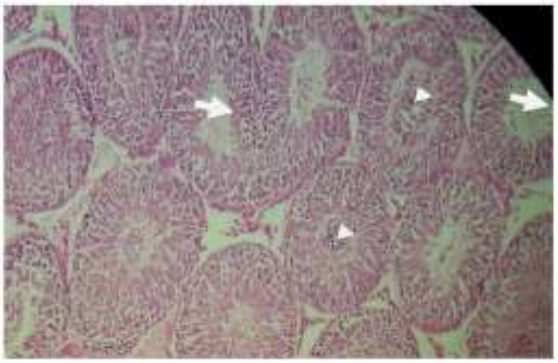

Figure 3: Sham-operated surgery rat testes show cellular debris in STs lumen (head arrow), decrease spermatogenesis (arrow) (H&E X250).

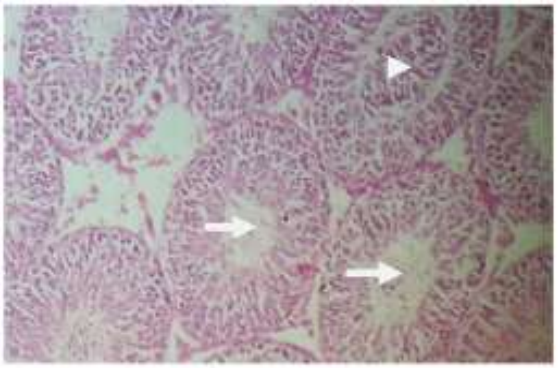

Figure 4: Sham-operated surgery rat testes show cellular debris in STs lumen (head arrow), no spermatogenesis (arrow) (H&E X500).

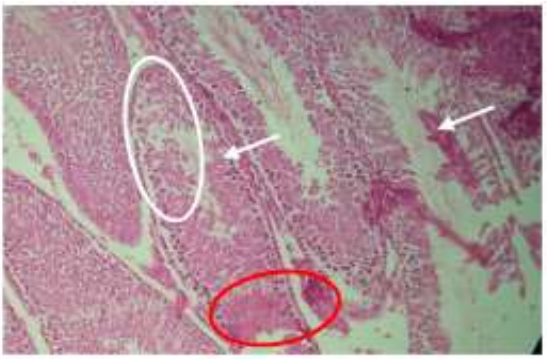

Figure 5: Pinealectomized rat testes show sever vacuolation (white circle) and necrosis in germinal epithelium(red circle), narrowing of STs and no spermatogenesis (arrow) (H&E X350).

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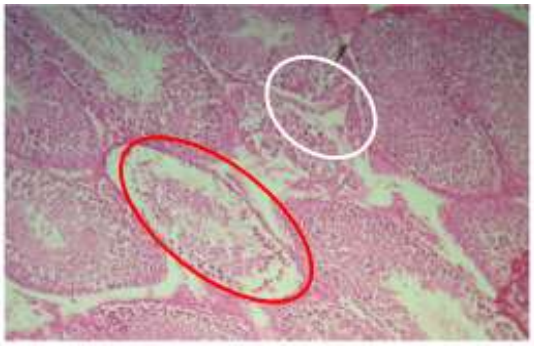

Figure 6: Pinealectomized rat testes show sever vacuolation in germinal epithelium (white circle), sever necrosis and narrowing of STs, spermatocytes slouphing and no spermatogenesis (red circle) (H&E X350).

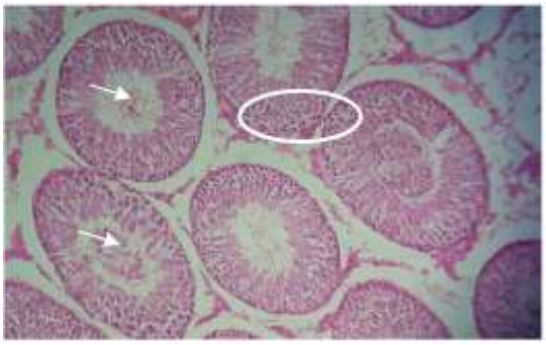

Figure 7: Pinealectomized rat testes were given MEL (60 mg/kg diet) show active spermatogonia, well development of STs (circle), onset stages of spermatogenesis few cellular debris in STs lumen (arrow) (H&E X320).

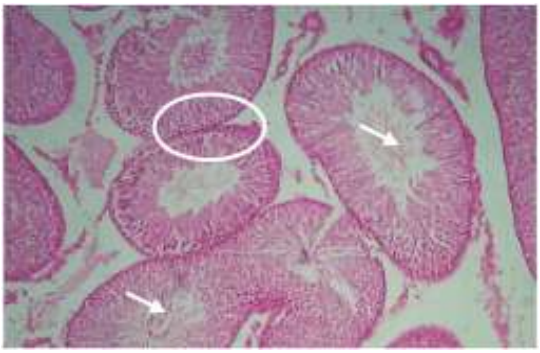

Figure 8: Pinealectomized rat testes were given MEL (60 mg/kg diet) show active spermatogonia, well development of STs (circle), onset stages of spermatogenesis (arrow) (H&E X320).

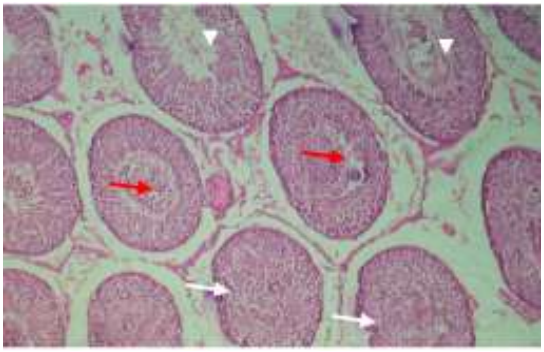

Figure 9: Normal rat testes were given MEL (60 mg/kg diet) show sever shrink most of STs (white arrow), sever sloughing spermatocytes inside STs lumen (red arrow), no spermatogenesis (head arrow) (H&E X320).

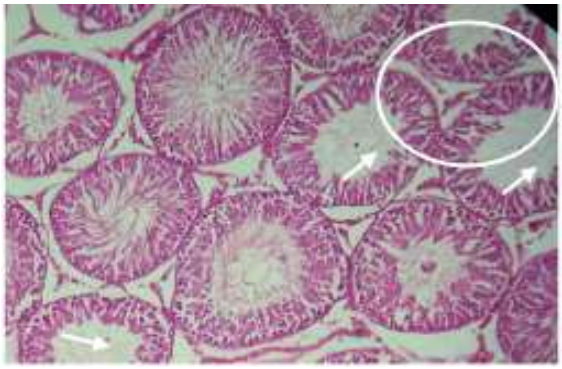

Figure 10: Normal rat testes were given MEL (60 mg/kg diet) show sever vacuolation in germinal epithelia (white circle), sever disturbance of spermatogenesis (arrow) (H&E X360).

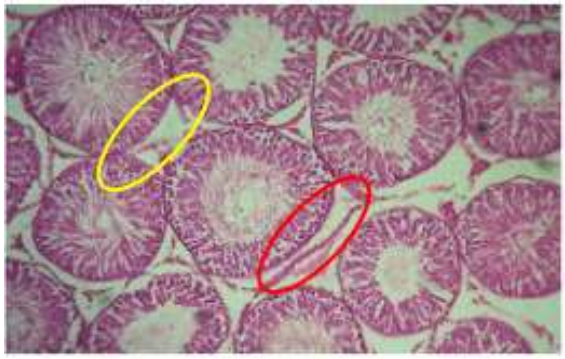

Figure 11: Normal rat testes were given MEL (120 mg/kg diet) show mild edema in germinal epithelia (red circle), well development germinal layer and spermatogenesis (yellow circle) (H&E X320).

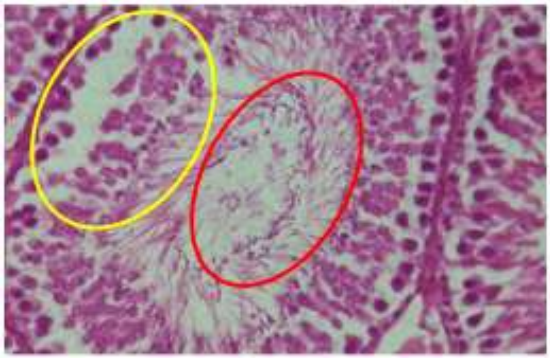

Figure 12: Normal rat testes were given MEL (120 mg/kg diet) show mild edema in germinal epithelia (yellow circle) and well development spermatogenesis (red circle) (H&E X1040).

Discussion

Melatonin is an important one of many hormones implement in the hormonal-replacement therapy, it is antioxidant, which prevents oxidative damaging events to endocrine organs including testis [11,12]. In the current study, histological effects of MEL deficiency and treatment of testicular tissue in normal and PINx rats were study.

It has been recorded that wound cutaneous injury generates reactive oxygen species (ROS) which leads to oxidative stress and damaging of intracellular macromolecules such as protein, lipid and DNA [13,14]. Previously, reported that MEL deficiency

affects the activity and level of cellular mRNA of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and glutathione reductase [15]. Which is confirmed with the recorded data of the present study that in sham-operated surgery and PINx rats, where, MEL oriented to scavenger ROS generated in sham-operated surgery which lift ROS in testis free to damage germinal epithelium and process of spermatogenesis, and MEL deficiency in PINx rats leads to regressive oxidative stress by ROS.

The data of the present study indicated that in sham-operated surgery and *PINx rats leads to regressive histological changes in testicular tissue*. This result is supported by Mahmud and Mahmud, finding that male albino rats were given MEL 60 mg / kg diet and MEL 120 mg / kg, the level of testosterone was significantly decreased in ratio 30% and 60% respectively [16]. Furthermore, Masson-Pevet *et al*., (1987) [17], found that MEL play protective role in gonads, and they showed that exposure of hamster to short photoperiod leads to regression of testicular tissue. In addition, Huang *et al*., (2009) [18], recorded that intraperitoneally injection of MEL 5 mg/kg BW to rats removes oxidative testicular injury induced by 2-bromopropane via ROS scavenger and anti-apoptosis effect. On the other hand, recently, Aslan *et al.,* (2015) [19], found that pretreatment with MEL 25 mg/ kg/ day for 14 days cured the testicular damage by cigar smoke and recover to normal testicular tissue.

The results of the current study showed that administration of 60 mg / kg diet of MEL to PINx rats for six weeks, cured the germinal epithelium and increases the process of spermatogenesis, this may be due to MEL receptors on leydiq, sertoli, epididymis and sperms, which affect directly or indirectly via hypothalamus to control on positive and negative feedback regulation to promote well development of testicular tissue and spermatogenesis.

Eleiwe, (2008) [12], found that MEL at dose 125 µg / kg BW for 14 days didn't induce any change in the rat testis, whereas at dose 500 µg/kg BW induced increasing of testis weight, but at dose 1000 µg / kg BW the testis onset to regression and lost the weight. These results support the data of the current study which revealed of sever shrinkage in most of STs and the spermatogenesis wasn't seen, as in normal rats were given MEL 60 mg/kg diet, but in rats were given 120 mg / kg diet, well development of germinal epithelia, with increasing of spermatogenesis were seen, despite of mild edema in some germinal epithelia. This might be explain that MEL act at optimal concentration through its receptors on leydiq and sertoli cells, in negative and positive regulation and through hypothalamus, it controls testicular growth and function [20,21].

These results are inconsistent with the results of Tuncer *et al*., (2011) [22], they concluded that MEL administration stimulates the effect of implanted testosterone pellets in rats, causing gonadal atrophy of reproductive organ. On the other hand Arendt, (2003) [20], supports the data of the current study that MEL contained diet recovered the normal structure and function of rat testicular tissue.

Melatonin influences directly on leydig cell receptors or indirectly through hypothalamic-hypophysial axis to inhibit the secretion of luteinizing hormone [12] and testosterone [23] for negative feedback [20] and vise versa [21].

Aktas *et al*., (2011) [23] and Power *et al.,* (2003) [24], reported that MEL treatment prevents ischemic injury in testicular tissue, and found that pretreatment with antioxidants can protect testis against ROS injury. Furthermore, MEL is identified to be a free radical scavenger and prevents the peroxidation of membrane lipids [25]. Unlike some other well-known antioxidants, MEL is amphiphilic permitting it to reduce free radical-mediated damage in both lipid and aqueous subcellular compartments. Where, hydrogen peroxide, single oxygen, superoxide anion radicals and peroxyl radical can be scavenged [15]. Furthermore, MEL can reduce non-enzymatic lipid oxidation of rat testicular microsomes and mitochondria [22,26]. Huang *et al*., (2009) [27] and Gwayi and Bernard, (2002) [28], investigated that MEL protects DNA and biological membrane lipids form insult effects of free radicals in reproductive system by decreasing testosterone concentration. Furthermore, Aktas *et al*., (2011) [23], recorded that 100 mg/kg MEL reduce testicular ischemic damage of rats' cellular organelles by entered the nucleus and protect the DNA against oxidative damage. These investigations consistent current results to protect testicular tissue form deleterious effects.

Conclusion

It was clearly demonstrated that MEL plays protective roles in gonads (testes) structure and functions, MEL recovered the histological damage in the testes of PINx rats, and high dose of MEL activates germinal epithelium and increasing spermatogenesis in normal rats.

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التأثيرات النسجية للميلاتونين على خصى الجرذان البيضاء مستأصلة الغدة الصنوبرية والجرذان البيضاء الطبيعية

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الملخص

في الدراسة الحالية، تم التحقق من تأثير الميلاتونين على النسيج الخصوي لكل من الجرذان البيضاء مستأصلة الغدة الصنوبرية والجرذان البيضاء الطبيعية. حيث استخدام ستة وثلاثون ذكر جرذ بالغ. قسمت هذه الجرذان الى ستة مجاميع كما يلي: المجموعة الاولى هي مجموعة السيطرة , والثانية مجموعة الجرذان الشام (معرضة للجراحة فقط), والثالثة مجموعة الجرذان مستأصلة الغدة الصنوبرية PINx, والرابعة مجموعة الجرذان PINx + ميلاتونين 60 ملغم/كغم وجبة, والخامسة مجموعة السيطرة+ ميلاتونين 60 ملغم/كغم وجبة, والسادسة مجموعة السيطرة+ ميلاتونين 120 ملغم/كغم وجبة حيث استمرت المعاملات لمدة ستة اسابيع. اظهرت النتائج تغيرات نسجية معتدلة في النسيج الخصوي لجرذان الشام, كانكماش وضمور النبيبات المنوية وضعف في عملية نشأت النطفة. بينما اظهرت مجموعة الجرذان مستأصلة الغدة الصنوبرية تغيرات تقهقرية في النسيج الخصوي, كتنكس حاد, تفجي ونخر في الظهارة الجرثومية وتوقف عملية نشأت النطفة. لكن عندما اعطت الجرذان مستأصلة الغدة الصنوبرية الميلاتونين وبتركيز 60 ملغم/كغم وجبة, لوحظ استرجاع كل من التركيب النسجي والفعالية الوظيفية للخصى, وعندما تناولت الجرذان الطبيعية الميلاتونين 60 و120 ملغم/كغم وجبة, اظهرت نتائج المعاملة بـ MEL 60 حدوث تفجي حاد وعدم وجود الخلايا النطفية الاولية والثانوية داخل تجويف النبيبات المنوية مع توقف في عملية نشأت النطفة، بينما اظهرت نتائج المعاملة بـ MEL 120 حدوث وذمة متوسطة مع نمو في الظهارة الجرثومية وعملية نشأت النطفة. نستنتج من هذه الدراسة ان لنقص مستويات الميلاتونين يؤدي الى حدوث تغير في التركيب النسجي والفعاليات الوظيفية لخصى الجرذان.