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EXPERIMENTAL STUDY

Histological effects of zinc and melatonin on rat testes

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Abstract: *Background:* The objective of this study is to examine the histological effects of zinc and melatonin, alone or in combination, on rat testes.
Methods: For this purpose four study groups of ten Sprague-Dawley male rats were formed and treated for one month as follows: (1) Controls; (2) Rats injected with 3 mg/kg/day zinc, as zinc sulfate; (3) Rats injected with 3 mg/kg/day melatonin and (4) Rats injected with 3 mg/kg/day each zinc and melatonin.
Results: After four weeks the rats treated with melatonin showed inhibited spermatogenesis, testicular tubular degeneration and necrosis, obstruction of tubular lumen and lymphocytic infiltration. The two zinc-treated groups showed no histological differences to controls but the melatonin-only group showed inhibited spermatogenetic activity, tubular degeneration and necrosis, as well as obstruction of tubule lumens and lymphocytic infiltration.
Conclusion: The obtained results suggest that 4-week treatment with melatonin leads to histological and physiological impairments of testis and that zinc supplementation might offset these damaging effects *(Tab. 2, Fig. 4, Ref. 12)*. Full Text in free PDF *www.bmj.sk.*
Key words: zinc, melatonin, testes, rat.

Melatonin is a neurohormone synthesized and secreted primarily by the pineal gland. Other tissues and cells are also involved in its synthesis, as evidenced by the fact that plasma melatonin level in pinealectomized mice decreases significantly, but is not completely nonexistent (1, 2). The better-known effects of melatonin are those associated with reproductive physiology. It has an inhibitive effect on hypothalamus-hypophysis-gonads system. Furthermore, it increases the secretion of opioid peptides, which in turn decrease the secretion of gonadotropin-releasing hormone (GnRH) (3).

Melatonin increases the intestinal absorption of zinc (1). That pinealectomized rats had significantly reduced plasma zinc levels, parallel to the decreased levels of plasma melatonin, is a noteworthy indicator of the correlation between melatonin and zinc (4).

Zinc plays a key role in the reproductive system (5). It is the only metal found in almost all classes of enzymes (6). High concentrations of zinc found in the testes and accessory sex glands show that it plays a crucial role in the reproductive system (7). Zinc deficiency in rats results in atrophy of seminiferous tubules and disruption of spermatogenesis (8). Zinc is also involved in functions that are important for sperm physiology. It is known to provide sperm membrane integrity, to increase sperm motility and to regulate the spiral movements of the sperm tail (9).

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Evaluating the histopathological effects of zinc on the reproductive system together with those of melatonin on rat testes is expected to be a positive contribution to the current knowledge on the subject.

Methods

Study groups

The experiments were conducted at the Experimental Medicine Application and Research Center of Selcuk University (SUDAM), which provided the forty adult Sprague-Dawley male rats that were used in the study. The study protocol was approved by the local Ethics Committee.

The experimental animals were housed in stainless steel cages that were washed and cleaned daily and maintained at 21±1 °C in a 12-h light/dark cycle. The rats were fed a standard diet (Aytekin's Grain Co. (Konya, Turkey) and allowed to drink tap water *ad libitum*.

Four study groups of ten rats each were formed and treated as follows during one month:

Group 1. Normal controls receiving a standard diet and not subjected to any procedure.

Group 2. Rats that received I.P. doses of 3 mg/kg zinc (as zinc sulfate, in saline).

Group 3. Rats receiving I.P. injections of saline containing 3 mg/kg melatonin.

Group 4. The rats were injected I.P. with saline containing 3 mg/kg each zinc (as zinc sulfate) and melatonin.

The injections were administered daily between 9 AM and 10 AM.

Tab. 1. Histological changes in the testis tissues of study groups.

Groups	Tubular degeneration and necrosis (Median value)	Obstruction in tubule lumens and lymphocytic infiltration (Median value)
1 General control (n=10)	1.000^{B}	1.000^{B}
2 Zinc administrated (n=10)	1.000^{B}	1.000^{B}
3 Melatonin administrated (n=10)	5.000^{A}	5.000^{A}
4 Zinc+Melatonin administrated (n=10)	1.000^{B}	1.000^{B}
P	0.05	0.05

*Means with different superscripted letters in the same column have statistical significance ($p<0.001$)

Tab. 2. Spermatogenesis scoring of study groups.

Groups	Spermatogenetic activity
1 General control (n=10)	7.00±0.00
2 Zinc administrated (n=10)	7.00±0.00
3 Melatonin administrated (n=10)	6.50±0.50
4 Zinc+Melatonin administrated (n=10)	3.50±0.25
P	0.01

*Means with different superscripted letters in the same column are statistically significant ($p<0.001$)

Preparation of the solutions

Zinc sulfate was dissolved in doubly distilled water and aliquots were taken and diluted with saline so that the dose administered intraperitoneally would be 3 mg/kg.

40-mg melatonin (Sigma M-5250) were suspended in 3 ml ethanol. The suspension was stored in a freezer until required. For injection 0.1 ml of the stock solution were dissolved in 0.9 ml saline to a concentration of 3 mg/kg.

Histological methods

After the rats were sacrificed by decapitation under anesthesia, the testes were removed and 5 µm cross sections were taken from each sample. The cross sections were stained with hematoxylin-eosin and examined under a microscope for evaluation of the spermatogenic activity of 20 seminiferous tubules in five different fields in each cross section. The findings of the examination were assessed according to modified Johnson criteria: 1 Azoospermia; 2 Presence of Sertoli cells; 3 Spermatogonia; 4 Presence of spermatocytes; 5 Presence of spermatids; 6 Presence of spermatozoa and 7 Normal spermatogenesis (sufficient amount of cells).

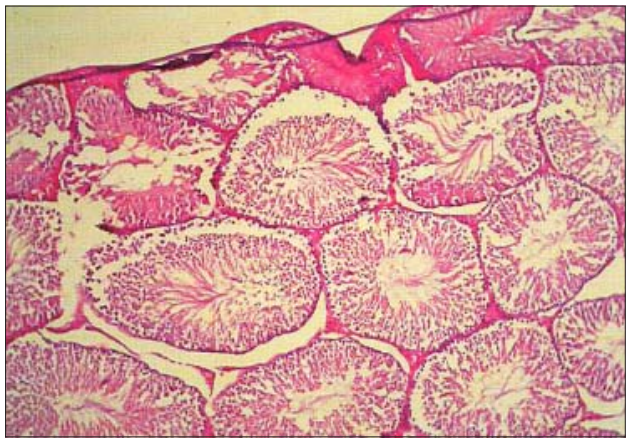

Fig. 1. Normal Cells and Spermatogenesis.

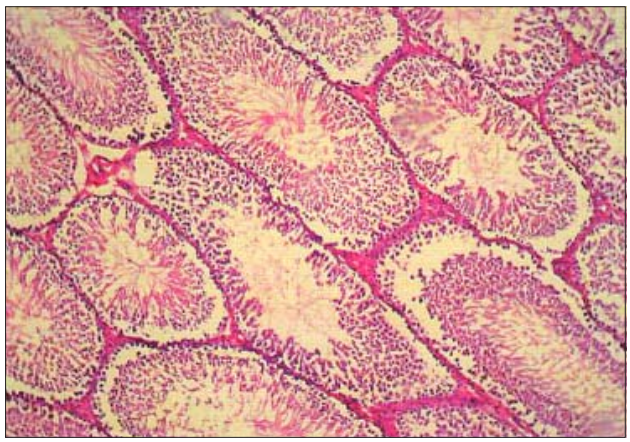

Fig. 2. Normal Germinal Epithelium, Unobstructed Tubule Lumens.

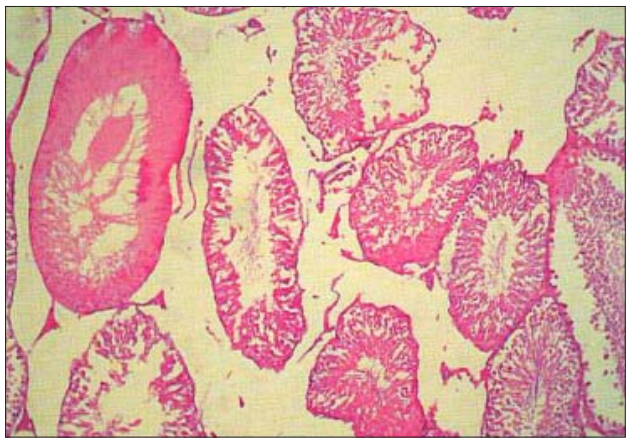

Fig. 3. Tubular Degeneration, Edema, Obstructed Seminiferous Tubules.

Treatment of the data

Statistical evaluation of results was conducted through computer software. The data did not meet the prerequisites of variance analysis, so they were subjected to square root conversion. The values obtained after conversion were subjected to variance

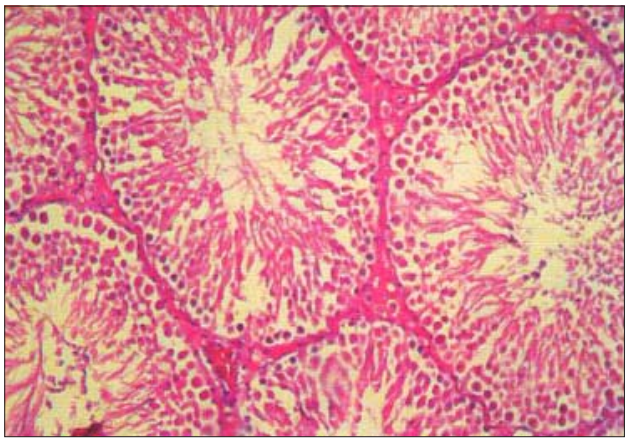

Fig. 4. Almost Normal Appearance.

analysis. The Duncan test was used to establish differences between groups.

Results

The microscopic examination of tissues from groups 1, 2 and 4 did not reveal inhibition of spermatogenetic activity, tubular degeneration and necrosis, obstruction in tubule lumens or lymphocytic infiltration.

The controls and zinc-supplemented rats (groups 1 and 2) were seen to contain a sufficient amount of cells (score 7), while the rats administered with zinc and melatonin reached a score 6. The melatonin-only group had scores 3 or 4, displaying inhibited spermatogenetic activity (Tab. 1), tubular degeneration and necrosis, obstruction in tubule lumens and lymphocytic infiltration (Tab. 2, Figs 1–4).

Discussion

Melatonin is a neurohormone secreted from the pineal gland that has a circadian rhythm. Consequently, animals used in experimental studies are subjected to controlled short or long photoperiods. Vaughan et al implanted 15 mm Silastic testosterone pellets in rats subjected to short and long photoperiods and found that the decrease in testicular weight in the rats subjected to a short photoperiod was less than that in rats subjected to a longer photoperiod (10). In a subsequent study the authors injected melatonin to rats that were implanted testosterone pellets and subjected them to longer photoperiods, finding that melatonin enhanced the effects of testosterone causing gonadal atrophy in reproductive organs (11). Jarrige et al reported that there was no change of testicular dihydrotestosterone content despite absolute atrophy, but a decrease in the plasma dihydrotestosterone levels in the rats born to pinealectomized mothers (12).

In agreement with these reports, in the present study we observed that testes of rats given melatonin experienced histological changes and inhibited spermatogenetic activity when compared to the other groups. When zinc is given in combination with melatonin the differences observed were not significant in comparison with normal controls or rats treated with zinc alone. These results suggest that zinc might have a protective effect against the testicular damage caused by melatonin.

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