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Effects of Aging and Anti-Aging Hormones on The Kidney, The Thyroid Functions and The Histology of The Testis of Male Albino Rats

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Abstract

Introduction
The present study was carried out to evaluate the effect of aging and anti-aging hormones on the kidney, the thyroid and the testis of aged male albino rats from the physiological and histological points of view.

Material & Methods
Thirty five male rats were used in the present study. They were allocated into five groups. The first group (5months old) served as control group and the other remaining groups are (18 months old). The second group 1 ml/kg b.w. corn oil intramuscular injection through a period of two weeks .The third group received 2mg/kg b.w. of melatonin hormone orally daily for two weeks. The fourth group received 0.57 mg/kg b.w. of testosterone hormone via intramuscular injection through two weeks. The fifth group received the same dose of both hormones (Melatonin & Testosterone) for two weeks. Some biochemical parameters of the kidney, the thyroid and histological structure of the testis were examined.

Results
The untreated aged group showed insignificant change in urea level with highly significant decrease in creatinine, T_3 and T_4 hormones levels. The melatonin treated group showed significant decrease in urea level with highly significant decrease in creatinine, T_3 and T_4 hormones. The testosterone treated group showed highly significant increase in urea, T_3 and T_4 hormones and highly significant decrease in creatinine level. Whereas, fifth group showed significant decrease in urea accompanied with a highly significant decrease in creatinine and highly significant increase in T_3 with a significant increase in T_4.
The histological changes induced by aging and anti-aging hormones included intertubular haemorrhage, odematous areas present between the seminiferous tubules. The interstitial tissue was degenerated. The degenerated seminiferous tubules revealed maturation arrest in late-stage spermatides.

Conclusion
In conclusion, aging and anti-aging hormones administration into adult male rats exerts a clear effect on the kidney and the thyroid functions and on the testicular structure. On the other hand, amelioration in T_3 &T_4 serum level was found in anti-aging treated rats compared with untreated aged rats.

Keywords: Aging; Anti-aging Hormones; Melatonin; Testosterone; Biochemical Parameters; Testis; Histology; Male albino rats.

Introduction

Aging is a universal biological phenomenon but our understanding of why and how the human being age remains limited. It refers to a progressive loss of physiological functions, decline in fertility, decreased ability to respond to a wide range of stresses, increased risk of age–associated diseases and disorders, and more likelihood of mortality.

Age-related declines in albumin during normal aging have been documented in human studies **(Rall *et al.*, 1995)**.

Donda and Lemarchand-Béraud (1989) reported low serum T4 and T3 with normal serum TSH in aged male rats, and related this to an increased pituitary T3 generation from T4.

Hajjar *et al.* (1997) found that no significant difference in the initial blood tests in 45 elderly hypogonadal men receiving testosterone (200 mg testosterone enanthate or cypionate i.m. every 2 weeks). At 2 year follow-up, a decrease in the urea nitrogen to creatinine ratio was not statistically significant.

O'Connor and Persiger (1996) have determined a relationship between melatonin and thyroid metabolism. In fact, in pineal gland-removed rats, application of one dose of melatonin was reported to affect thyroid activity at different times of the following day and through the night. Similarly, injection of melatonin in the evening to rats and mouse is reported to affect thyroid hormone synthesis during a 10-day period **(Selmaoui *et al*., 1997).**

Hussein *et al*. (2006) in their study on the effect of melatonin against x-ray-induced early and acute testis damage of albino rats, they reported ultrastructural features of apoptosis (condensation of the nuclei, vacuolization of the cytoplasm, increased cytoplasmic density, and apoptotic bodies) in irradiated testes, which were absent when the irradiated animals were pretreated with melatonin.

According to **Sun *et al*. (2009)** they found that aged mice tend to show reduced fertility and the seminiferous tubules in the mice degenerate with age. The authors added that some seminiferous tubules lost mainly spermatogonia, but retain other germ cells, suggesting that the exhaustion of spermatogonial cells leads to loss of all germ cells in the seminiferous tubules.

Material & Methods

The experimental animals

Thirty five of male albino rats (*Rattus norvegicus)* were used in this investigation. Rats were obtained from Schistosoma Biological Supply Program (SBSP) Theodor Bilharz Research Institute,they were allocated into five groups, each group was contained seven rats. Rats in the first group are aged 5 months (control group), while the other four groups aged about 18 months. At the beginning of the experiment, each two rats were placed in a metal cage, and kept under normal laboratory conditions during the whole period of experimentation and were fed on a standard diet. Food and water were available *ad libitum*.

The synthetic hormones (anti-aging hormones) used in the present investigation are **melatonin** and **testosterone** (**Cidotestone**).

Dosage, periods and rout of administration

Rats were allocated into five groups of 7 individual each, as follows:

Group (1): control rats, aged 5 months (C) were received 1 ml/kg b.w. corn oil intramuscular injection through a period of two weeks.

Group (2): untreated aged-rats, 18 months (Ag) were received 1 ml/kg b.w. corn oil intramuscular injection through a period of two weeks.

Group (3): treated aged-rats (M) were received 1ml of a daily dose of melatonin (2mg/kg body weight orally) 2 hour before lights out according to **Demas *et al.*, 2004**,daily for two weeks.

Group (4): treated aged-rats (T) were injected with *1ml* of testosterone (0.57 mg/kg. body weight) intramuscularly through two weeks.

Group (5): treated rats aged (M+T) were received the same doses of both hormones together (2mg/kg. of melatonin orally and 0.57 mg/kg. i.m. of testosterone).

By the end of the two weeks, the animals (both control and treated-aged groups) were sacrificed by decapitation. Individual blood sample was collected for biochemical analysis, then the rats were dissected immediately and small pieces of testes were immediately fixed in aqueous Bouin's solution for 24 hours. They were dehydrated in alcohol, cleared in terpineol and embedded in paraffin wax. Sections of 5µm thickness were stained with hematoxylin and eosin **(Bancroft and Gamble, 2002)**.

Biochemical Methods

-Serum content of urea was estimated according to urease-colorimetric method described by **Patton and Crouch (1977)**.

-Serum creatinine was determined according to the method described by **Young *et al.* (2001)**.

-Determination of thyroxin (T_4) was carried out by using solid phase enzyme-immunoassay.Measurement of serum tri-iodothyronine (T_3) concentration was done by using **Enzyme-Immunoassay** kit purchased from (Boehringer Manheim West Germany). The methods were carried out according to **Wood (1980)**.

-The obtained results were statistically analyzed by using the student T-Test according to the method of **Snedecor and Cochran (1980)**.

Results

Biochemical studies

Effect of aging and anti-aging hormones on biochemical parameters.

Serum content of urea and creatinine

The data represented in **table (1)** display the effect of aging and anti-aging hormones on serum urea level of male albino rats. Untreated aged rats (group 2) showed non significant change in the seum urea level compared with the control group. In groups 3&5 that treated with melatonin and testosterone respectively, the urea level showed a significant ($P<0.05$) decrease. While in testosterone treated group, the serum urea level recorded highly significant increase ($p<0.01$). The same table revealed the effect of aging and anti-aging hormones on serum creatinine level of male albino rats. The serum creatinine level of all groups (2, 3, 4 and 5) were decreased markedly (P<o.o1).It was found also in melatonin with testosterone treated group (5) amelioration in serum creatinine level when compared with untreated aged group.

Table (1): Effect of aging and anti-aging hormones on serum urea and serum creatinine concentration (mg/dl) of male albino rats.

Parameters \ Groups		Group (1) C	Group (2) Ag	Group (3) M	Group (4) T	Group (5) M+T
Serum urea (mg/dl)	**Mean± S.E.**	31.57±2.099	30.29±0.97	23.71±1.02*	46.86±3.12**	24.14±0.98*
	% of change from adult group		-3.10	-24.9	+48.43	-23.54
Serum urea (mg/dl)	**% of change from untreated aged group**			-21.72	+54.70	-20.30
Serum creatinine (mg/dl)	**Mean± S.E.**	1.03±0.07	0.65±0.04**	0.65±0.05**	0.54±0.02**	0.67±0.03**
	% of change from untreated aged group		-36.89	-36.89	-47.58	-34.95
Serum creatinine (mg/dl)	**% of change from untreated aged group**			0	-16.92	+3.1

* = Significant ** = Highly Significant

Serum triiodothyronine (T_3) and thyroxine (T_4) hormones level

Both the triiodothyronine T_3 and thyroxine (T_4) hormones levels showed response to aging and the anti-aging hormones. **Table (2)** showed that T_3 &T_4 levels exhibited highly significant (P<0.01) increase after treatment with testosterone (group 4) and melatonin plus testosterone (group 5).whereas, both hormones (T_3 & T_4) revealed highly significant (P<0.01) decrease in aged rats (group 2) and melatonin treated rats (group 3) compared with control group (group 1).But, the melatonin treated group showed to some extent improvement in T3&T4 serum level than untreated aged group (group 4).

Table (2): Effect of aging and anti-aging hormones on thyroid hormones level (T_3&T_4) (ng/dl) of male albino rats.

Groups / Parameters		Group(1) C	Group (2) Ag	Group (3) M	Group (4) T	Group (5) M+T
Serum T_3 (mg/dl)	**Mean±S.E.**	91±1.52	60.23±2.25**	79.10±1.15**	194.87±1.58**	160.03±2.21**
	% of change from adult group		-33.81	-13.10	+114.14	+75.90
Serum T_3 (mg/dl)	**% of change from untreated aged group**			+31.33	+223.54	+165.70
Serum T_4 (mg/dl)	**Mean±S.E.**	5.31±0.26	2.68±0.19**	2.91±0.23**	13.57±1.88**	8.17±1.07*
	% of change from adult group		-49.53	-45.20	+155.56	+53.86
Serum T_4 (mg/dl)	**% of change from untreated aged group**			+8.6	+406.34	+204.90

* = Significant ** = Highly Significant

Histological studies

Testis of the control adult rat.

The testis of the control rat is surrounded by a dense fibrous tissue capsule, i.e., the tunica albuginea. Thin fibrous septa divide the testis into lobules; each lobule contains several seminiferous tubules which are surrounded by the interstitial tissue (Fig. 1). Each tubule is lined with germ cells in various stages of spermatogenesis, with sertoli cells in between. The spermatogenic lineage is composed of spermatogonia, primary and secondary spermatocytes, spermatides and mature spermatozoa that occupy the center of tubule (Fig. 2).Sertoli cells are found between the spermatogonia and rest on the basal lamina; these cells nourish the developing spermatozoa. In the interstitial support Leydig cells are shown. They occur singly or in clump and are embedded in the rich plexus of blood and lymph capillaries, which surrounded the seminiferous tubules (Fig. 2).

Testis of untreated-aged rats.

Histological examinations of the testes of untreated aged rats (group 2) showed several changes in some seminiferous tubules and interstitial tissue. The basement membranes of some seminiferous tubules were detached. Large vacuoles were observed between the spermatogonic cells (Fig.4). The lumina of some seminiferous tubules were sloughed with cellular depris (Fig. 3).Area of haemorrage and oedema were detected in the interstial tissue and the hypoplasia of the interststial tissue is also detected (Figs. 3&4).

Testis of melatonin treated-aged rats.

The testes of rat treated with melatonin revealed histopathological alterations in both the seminiferous tubules and interstitial tissue. Large vacuoles appeared between the spermatogenic cells and some nuclei of spermatogonia exhibited signs of pyknosis. Degeneration of some primary spermatocytes was also observed (Fig. 5&6). The basement membranes of some seminiferous tubules were detached and congestion of some intertubular blood vessels were also detected, hypoplasia of interstitial tissue were also observed (Fig. 6).

Testis of testosterone treated-aged rats.

The histopathological examination of the testes of the rats treated with testosterone revealed severe pathological changes in both the seminiferous tubules and interstitial tissue of the testes of this group. Detachment of the basement membrane of some seminiferous tubules was observed. Large vacuoles were observed in some seminiferous tubules among spermatogonic cells and some spermatogenic cells showed pyknotic nuclei (Figs. 7&8).The lesions in the intertubular spaces appeared in the form of haemorrage and oedema (Fig.7). Leydig cells have undergone degeneration (Fig. 8).

Testis of melatonin & testosterone treated-aged rats.

After two weeks of treatment with both hormones (group 5) the testes of the rats of this group revealed an advanced degree of injury indicated by atrophy and disorganization of germinal epithelium (Fig.9). The basement membrane of some seminiferous tubules were detached. Large vacuoles were observed between spermatogonic cells (Figs.9&10).The nuclei of both spermatogonia and primary spermatocytes exhibited signs of pyknosis.Some tubules showed spermatogenic arrest (Fig.10).The seminiferous tubules showed intertubular oedema. Interstitial haemorrage was also detected (Fig.9). hypoplasia of Leydig cells was also observed in figures 9 & 10.

Discussion

Biochemical studies

Kidney function

The determination of urea is the most widely used test for the evaluation of kidney function. The test is frequently used in conjunction with the determination of creatinine for the differential diagnosis of prerenal hyperuremia, renal hyperuremia, chronic nephritis and postrenal hyperuremia.This study revealed that, the untreated aged group showed insignificant change in urea concentration. Whereas; the creatinine concentration showed highly signifcantly decreased.

Lowseth *et al.* (1990) who distinguished age-related changes concluded that serum creatinine decreased with age. Whereas, **Musch *et al. (*2006**) confirmed that in humans, an age-related increase in plasma urea levels and no correlation between plasma creatinine and age.

It is widely known that glomerular filtration decreases with age, but this is not associated with an increase in plasma creatinine, as a result of a concomitant age-related decrease in muscle mass and creatinine production (**Choudhury *et al.*, 2005**).

Significant decrease in urea concentration and a highly significant decrease in creatinine concentration in melatonin treated group were observed in the present study.

While, **Ogeturk *et al.* (2004**) stated that melatonin treatment did not cause significantly change in serum urea, total protein, and albumin levels. **Kaplan *et al.* (2009)** showed that N-acetylcysteine (NAC) prevented and ameliorated kidney damage induced by Cadmium. Melatonin achieves this by its direct antioxidant effect and by increasing the antioxidant enzyme activities without changing the kidney tissue Cadmium level.

In testosterone treated group, there were highly significant increase in urea concentration and highly significant decrease in creatinine concentration.Whereas, group treated with testosterone and melatonin revealed significant decrease in serum urea concentration level and highly significant decrease in serum creatinine concentration level compared with the control group.

According to **Ali and Ahmed (2006)** who used rats model of chronic renal failure (CRF) revealed that, there is depressed growth; significant increases in the plasma concentrations of creatinine, urea, indoxyl sulphate and anemia. All these signs were significantly and partially reversed by estradiol and testosterone therapy equally in female and male rats, respectively.

In a previous study involving male Wistar rats, the glomular filtration rate (GFR) began diminishing at 16 months (**Tanaka *et al.*, 1995**), two months of testosterone replacement at 13months old accelerated a reduction of the GFR.

Thyroid function

Results obtained in the present study revealed a highly significant decrease in T_3 & T_4 hormones level in untreated aged rats and melatonin treated group compared with control group. Whereas, in testosterone treated rats and melatonin plus testosterone treated group there were a highly marked increase in T_3 & T_4 level.

Results of the present investigation seems to be in agreement with **Pipes *et al.*(1963)** who reported that in adult animals of several species, thyroid activity appears to decrease with increasing age. The response, as in human beings, may be homeostatic. The data of this survey suggest that the functionality of the thyroid reduces as the age of Sprague-Dawley rats increases. Several observers, however, have noted a decline in total T3 in subjects over 60 **(Jeske and Thorner, 1977).**

Generally, the decrease in thyroid hormones could be attributed to one or more of the following reasons;deficient iodide trapping,structural changes in follicular cells or inhibition of enzymes necessary for synthesis of thyroid hormones.

A study of **Vriend *et al.* (1982)** reported that injection of melatonin reduced plasma T3, T4 and TSH concentration. On the other hand, also **Vaughan *et al.* (1983)** reported a depression in T4 values after melatonin injection with no changes in T3 and TSH values.

Also, **Ianas *et al*. (2007)** reported that the melatonin treatment induced an opposite circadian variation of serum T3, T4 and pineal 5'-D activity suggesting an interaction between the light/dark cycle, 5'-D activity and responsiveness to melatonin.

But, according to **Gordon *et al.* (1980)** melatonin increased the thyroid gland size relative to body weight and increased the total T4 content and T_4:T_3 ratio in the thyroid gland.

This is attributed to the counter-antithyroid effect of melatonin on thyroid hormone secretion. Since Pinealectomy revealed the stimulatory effect on thyroid growth processes, while melatonin treatment reversed the effect of the surgery **Wajs and Lewiski (1992).**

Similar to these findings, **Bisschop *et al.* (2006)** who found that oral estrogen administration increased thyroid hormone-binding globulin (TBG) concentrations, whereas testosterone decreased (TBG) concentrations.Testosterone administration increased T3/T4 ratios, indicating increased 50-deiodinase activity.

Histological studies

The present study showed that untreated aged rats and anti-aging hormones treated groups have some histopathological changes in the seminiferous tubules and interstial tissue of the testis. The lumina of the seminiferous tubules were obliterated and filled with remnant of ruptured cells and residual bodies. Residual bodies are thought to be cytoplasm shed by developing spermatids **(Russell, 1979)** and normally become phagocytosed by Sertoli cells. Several investigators have suggested that Sertoli cells resorb these residual bodies cast off by spermatides **(Dietert, 1966 and Nicander, 1967).** The abnormal presence of such residual bodies suggests that Sertoli cells capacity to ingest them may be affected **(Somkuti, 1987).**

The present results are in agreement with **Takano and Abe (1987)** who reported that age-related changes in the testis were studied histologically in dd-mice from 2 months to 2 years of age. After 6 months of age, vacuoles appeared first singly and later became clustered in the seminiferous epithelium. With the appearance of the vacuoles, the epithelium started to release spermatids and spermatocytes into the lumen.

Also, in harmony with the present results, **Malpaux *et al*. (1999)** found a negative relationship between sperm production and melatonin secretion in male rats. The study reported that the nocturnal secretion of melatonin regulates the pulsatile release of gondotropin–releasing hormone (GnRH) from hypothalamus. Change in GnRH release in turn affects luteinizing hormone secretion and leads to decrease of sperm production.This may be attributed to antigonadal effects of melatonin, at least in part, that exerts through the direct decrease of testosterone production **(Sirotkin and Schaeffer, 1997).**

Also, the present study showed that the testes of rats treated with anti-aging hormones exhibited histopathological changes which included vacuoles among spermatogenic cells and the congestion of some intertubular blood vessels, hypoplasia of interstitial tissue, area of haemorrage and oedema in the interstitial tissue.These lesions may be attributed to accumulation of blood in the vessels causing increase of the blood pressure in blood capillaries **(Gomaa, 2000).**

These results are in agreement with, **Lombardo *et al*., 2005** they found that the adverse effects of nandrolone, 19-nortestosterone (a synthetic androgenic-anabolic steroid) promoting muscle growth. Prolonged and uncontrolled use of nandrolone cause various histological and morphological abnormalities in the testis, including reduction of testicular volume and seminiferous tubule length **(Noorafshan *et al*., 2005),** germ and Sertoli cells' sloughing **(Takahashi *et al*., 2004)**, and severe depletion of Leydig cells in the interstitial compartment **(Nagata *et al*., 1999)**. Also, it is well recognized that a long term use of nandrolone frequently results in male infertility, as a predominant side effect.

Kim *et al*. (2002) reported that the number of Leydig and connective tissue cells per testis was unchanged with aging.

Leydig cells exhibit hyperplasia, particularly around the atrophied seminiferous tubules in the testes of aged men **(Honore, 1978)** and in experimentally damaged testes (**Sato *et al*., 1981)**. **Aoki and Fawcett (1978)** believed that the atrophied seminiferous tubules radiate some diffusable agents which influence Leydig cells to proliferate.

Sloughing and exfoliation observed in the present investigation may be correlated to loss of contact between Sertoli cells and germ cells; this separation is rapidly followed by exfoliation of the germ cells into the lumina of the tubules and their subsequent loss **(Haschek and Rousseaux, 1991).**

The maturation arrest observed in the present investigation was explained by **El-Zayat (1988)** who correlated this arrest to the testosterone inhibition which caused stopping of spermatogenesis.

Balasubramanian *et al*. (1980) explained the congestion of the blood vessels as being due to the inhibition of prostaglandins synthesis, since these compounds are known to be involved in regulation of testicular blood flow. Also, **Singwi and Lall (1980)** suggested that such congestion was due to the assumption that increased breakage of blood capillaries leads to further augmentation of interstitial oedema and consequent to disorganization effect on Leydig cells in the interstitial tissue of the testes.

In conclusion, it could be stated that anti-aging hormones induced disturbance in many biochemical parameters and have deleterious impacts on the testes of treated rats. So,this research needs further study.

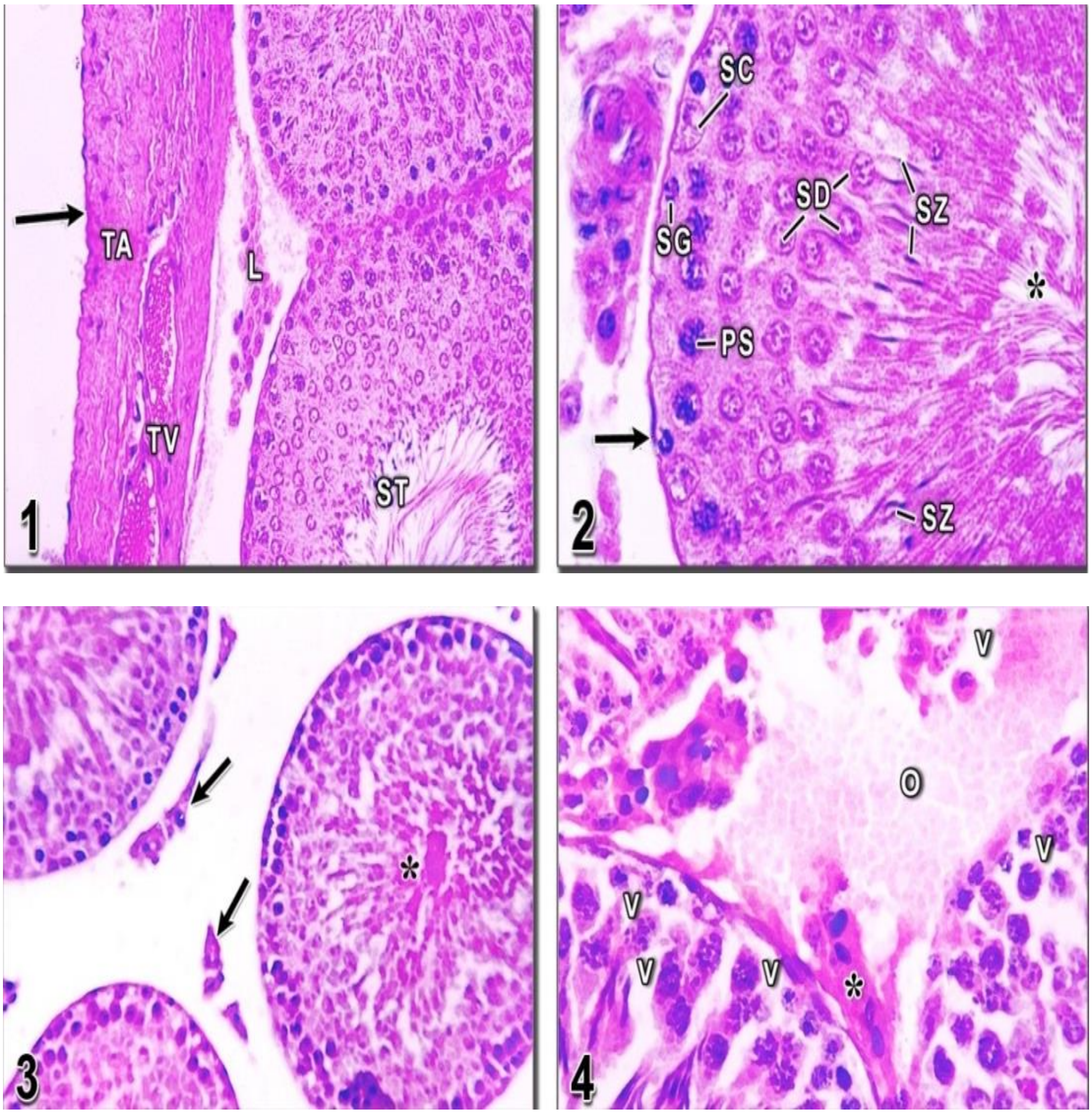


Fig (1): Photomicrograph of a section of the testis of a control rat, showing connective tissue capsule (arrow) which is formed of tunica albuginea (TA) and tunica vascuolosa (TV). The seminiferous tubules (ST) and interstitial tissue (L) are also illustrated.

(Hx-E;×320)

Fig (2): Photomicrograph of a section of the testis of a control rat, showing successive stages of spermatogenesis which include spermatogonia (SG), primary spermatocytes (PS), different stages of spermatids (SD) and spermatozoa (SZ) surrounding a central lumen (*). Notice Sertoli cells (SC) are attached by their bases to the basement membrane (arrow).

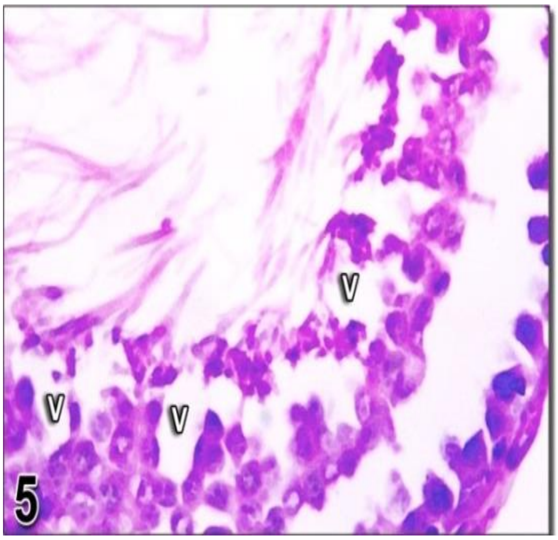
(Hx-E;×825)

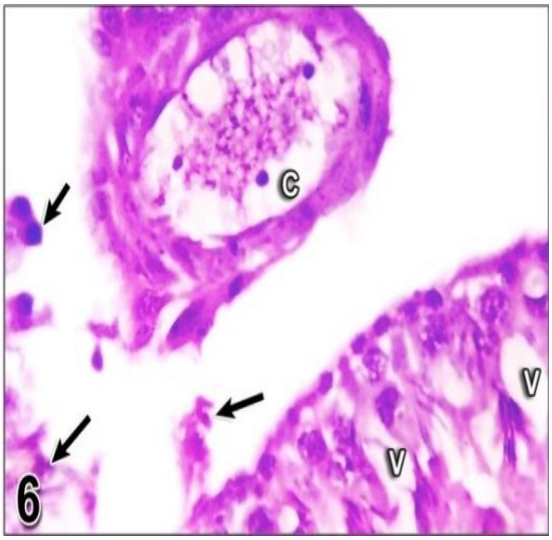
Fig (3): Photomicrograph of a section of the testis of untreated-aged rat, showing sloughing of some seminiferous tubules (*), pyknotic nuclei of the spermatogonia and hypoplasia of interstitial tissue (arrows) is also observed.

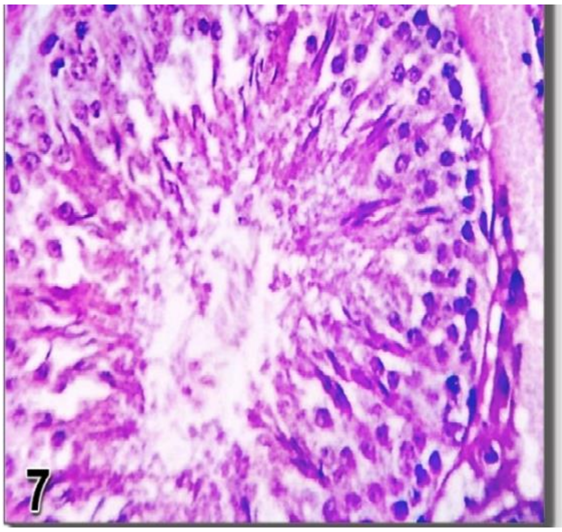
(Hx-E;×200)

Fig (4): Photomicrograph of a section of the testis of untreated-aged rat, showing some seminiferous tubules with several vacuoles (V) among the spermatogenic cells. Notice that presence of haemorrhagic (*) and oedematous (O) areas between the seminiferous tubules.

(Hx-E;×400)







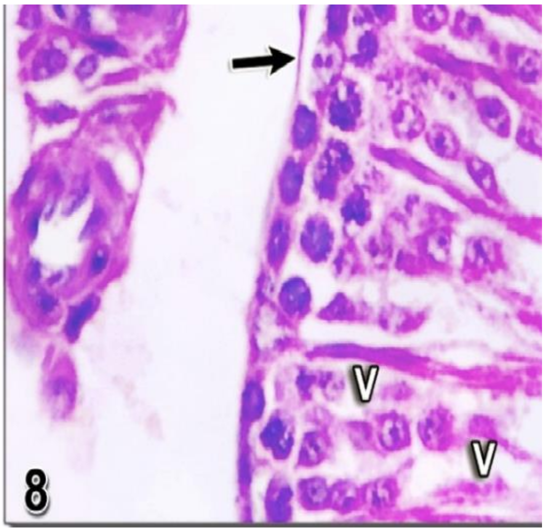


Fig (5): Photomicrograph of a section of the testis of melatonin treated-aged rat, showing disorganization of the germ cells of some tubules, degeneration of some of primary spermatocytes and presence of large vacuoles (V) among spermatogenic cells.

(Hx-E;×400)

Fig (6): Photomicrograph of a section of the testis of melatonin treated-aged rat, showing congestion (C) of interstitial blood vessel, hypoplasia of the interstial tissue (arrows). Notice presence of large vacuoles (V) between spermatogenic cells.

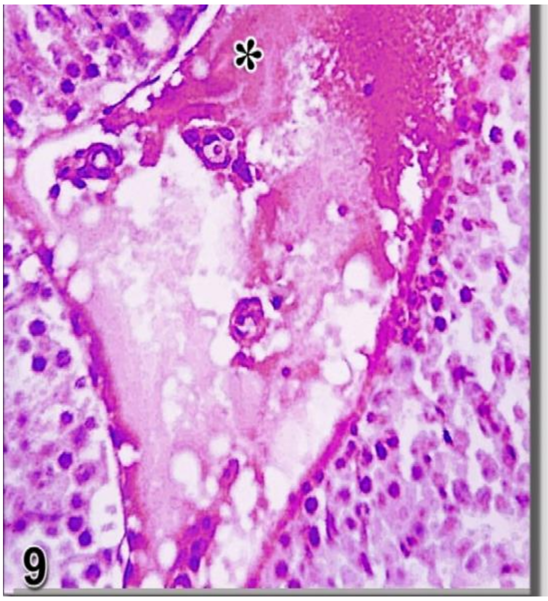
(Hx-E;×400)

Fig (7): Photomicrograph of a section of the testis of testosterone treated-aged rat, showing haemorrhagic (*) and oedematous areas (O) between the seminiferrous tubules. Notice that most of Leydig cells have undergone degeneration and nuclear pyknosis of spermatogenesis. Detached basement membrane of some tubules (arrow), presence of vacuoles (V) among spermatogenic cells and the lumina of seminiferous tubules are sloughed.

(Hx-E;×200)

Fig (8): Photomicrograph of a section of the testis of testosterone treated-aged rat, showing detachment of the basement membrane of some seminiferous tubules (arrow), presence of some vacuoles (V) among the spermatogenic cells and hypoplasia of the interstitial tissue (head arrows).

(Hx-E;×400)



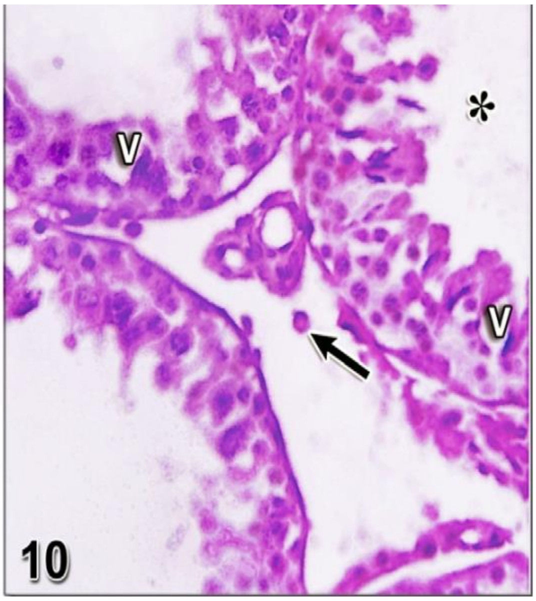


Fig (9): Photomicrograph of a section of the testis of melatonin and testosterone treated-aged rat, showing haemorrhagic (*) and oedematous (O) areas between the seminiferous tubules. Notice that most of Leydig cells have undergone degeneration and nuclear pyknosis of spermatogonia is also observed in this figure.

(Hx-E;×200)

Fig (10): Photomicrograph of a section of the testis of melatonin and testosterone treated-aged rat, showing maturation arrest of seminiferous tubules (*) and presence of large vacuoles (V) between the spermatogenic cells. Notice hypoplasia of the interstitial tissue (arrow).

(Hx-E;×200)

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تـأثير الشـيخوخة والهرمونـات المضـادة للشـيخوخة علـى وظـائف الكليـة و الغـدة الدرقية وأنسجة الخصية فى ذكور الجرذان البيضاء

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تهدف الدراسة الحالية لدراسة تأثير الشيخوخة والهرمونات المضادة للشيخوخة على الكلى والغدة الدرقية من الناحية الفسيولوجية وأنسجة الخصية لذكور الجرزان البيضاء.
وقد استخدم فى هذه الدراسة خمسة وثلاثون من ذكور الجرذ الأبيض قسمت الى خمس مجموعات- المجموعة الأولى اعتبرت هى المجموعة الضابطة (الذين تتراوح أعمارهم خمسة أشهر) وباقى الجموعات تتراوح اعمارهم ثمانية عشر شهرا.أعطيت المجموعة الثانية 1 مل زيت ذرة عن طريق الحقن العضلى خلال فترة أسبوعين وأعطيت المجموعة الثالثة هرمون الميلاتونين عن طريق الفم (2 مجم/كجم يوميا لمدة أسبوعين). أعطيت المجموعة الرابعة 0.57 مجم/كجم من هرمون التستوستيرون عن طريق الحقن العضلى خلال أسبوعين. المجموعة الخامسة أعطيت نفس الجرعة من الهرمونين خلال الاسبوعين.تم فحص بعض المعايير البيوكيميائية لكل من الكلى والغدة الدرقية والتركيب النسيجى للخصية.

أظهرت المجموعة المسنة غير المعالجة تغير ضئيل فى مستوى اليوريا مع نقص ذو دلالة احصائية فى مستوى الكرياتينين وكل من هرمونى T_3 ، T_4.وأظهرت المجموعة المعالجة بالميلاتونين نقص ذو دلالة احصائية فى مستوى اليوريا ومستوى الكرياتينين، وهرمونى T_3 ، T_4.وأظهرت المجموعة المعالجة بالتستوستيرون زيادة ذو دلالة احصائية عالية فى اليوريا، وهرمونى T_3 ، T_4 ونقص ذو دلالة احصائية فى مستوى الكرياتينين. فى حين أظهرت المجموعة المعالجة بالميلاتونين والتستوستيرون نقص ذو دلالة احصائية فى اليوريا و الكرياتينين مع زيادة ذو دلالة احصائية فى هرمونى T_3 ، T_4.
وقد أظهرت نتائج هذه الدراسة تغيرات واضحة فى نسيج خصى الحيوانات المعالجة, تضمنت مظاهر التهدم والتحلل فى الأنيبيبات المنوية ومنها فى حدوث خلل فى ترتيب وتنظيم الطلائية الجرثومية,مع النقص الواضح فى سمك طبقاتها مع ظهور بعض مظاهر التحلل ودرجات مختلفة من التهدم لخلايا الطلائية الجرثومية (أمهات المنى والخلايا المنوية). فضلا عن ظهور فجوات كبيرة فى الطلائية الجرثومية وعدم اكتمال تكوين الحيوانات المنوية.أما فى النسيج البينى فقد لوحظ حدوث نزف دموى وارتشاح مع احتقان بعض الأوعية الدموية.

ونستنتج من هذه الدراسة أن الشيخوخة والهرمونات المضادة للشيخوخة لها أضرار على كل من الناحية الفسيولوجية للكلى والغدة الدرقية وأيضا على التركيب النسيجى للخصى فى الجرذان البيضاء.وكذلك أظهرت هذه الدراسة تحسن فى مستوى هرمونى T_3 & T_4 فى المجموعات المعالجة بالهرمونات المضادة للشيخوخة عند مقارنتها بالمجموعة المسنة الغير معالجة.