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 (5 months 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, T3 and T4 hormones levels. The melatonin treated group showed significant decrease in urea level with highly significant decrease in creatinine, T3 and T4 hormones. The testosterone treated group showed highly significant increase in urea, T3 and T4 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 T3 with a significant increase in T4.

The histological changes induced by aging and anti-aging hormones included intertubular haemorrhage, oedematous 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 T3 &T4 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). Dona 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 for 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 1 ml/kg 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 (T4) was carried out by using solid phase enzyme-immunoassay. Measurement of serum triiodothyronine (T3) 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.

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

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

* = Significant  ** = Highly Significant
Effects of Aging…..

**Serum triiodothyronine (T₃) and thyroxine (T₄) hormones level**

Both the triiodothyronine T₃ and thyroxine (T₄) hormones levels showed response to aging and the anti-aging hormones. **Table (2)** showed that T₃ & T₄ levels exhibited highly significant (P<0.01) increase after treatment with testosterone (group 4) and melatonin plus testosterone (group 5), whereas, both hormones (T₃ & T₄) 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 (T₃&T₄) (ng/dl) of male albino rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Group (1) C</th>
<th>Group (2) Ag</th>
<th>Group (3) M</th>
<th>Group (4) T</th>
<th>Group (5) M+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum T₃ (mg/dl)</td>
<td>Mean±S.E.</td>
<td>91±1.52</td>
<td>60.23±2.25**</td>
<td>79.10±1.15**</td>
<td>194.87±1.58**</td>
<td>160.03±2.21**</td>
</tr>
<tr>
<td>% of change from adult group</td>
<td>-33.81</td>
<td>-13.10</td>
<td>+114.14</td>
<td>+75.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum T₃ (mg/dl)</td>
<td>% of change from untreated aged group</td>
<td>+31.33</td>
<td>+223.54</td>
<td>+165.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum T₄ (mg/dl)</td>
<td>Mean±S.E.</td>
<td>5.31±0.26</td>
<td>2.68±0.19**</td>
<td>2.91±0.23**</td>
<td>13.57±1.88**</td>
<td>8.17±1.07*</td>
</tr>
<tr>
<td>% of change from adult group</td>
<td>-49.53</td>
<td>-45.20</td>
<td>+155.56</td>
<td>+53.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum T₄ (mg/dl)</td>
<td>% of change from untreated aged group</td>
<td>+8.6</td>
<td>+406.34</td>
<td>+204.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = 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 depriv (Fig. 3). Area of haemorrage and oedema were detected in the interstitial tissue and the hypoplasia of the interstitial tissue is also detected (Figs. 3&4).

**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 significantly 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 13 months 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 $T_3$ 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 $T_3$, $T_4$ and TSH concentration. On the other hand, also Vaughan et al. (1983) reported a depression in $T_4$ values after melatonin injection with no changes in $T_3$ 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 T3/T4 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 interstitial 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 gonadotropin–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 antagonadal 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 haemorrhage 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 diffusible 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;X320)

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;X825)

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;X200)

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;X400)
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; X400)

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

(Hx-E; X400)

Fig (7): Photomicrograph of a section of the testis of testosterone treated-aged rat, showing haemorrhagic (*) and oedematous areas (O) between the seminiferous 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; X200)

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; X400)
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;X200)

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;X200)

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Effects of Aging…..

تأثير الشيخوخة والهرمونات المضادة للشيخوخة على وظائف الكليّة والغدة الدرقية وأنسجتها الخصية في ذكور الجرذان البيضاء

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تهدف الدراسة الحالية لدراسة تأثير الشيخوخة والهرمونات المضادة للشيخوخة على الكليّة والغدة الدرقية من الناحية الفسيولوجية وأنسجتها الخصية لذكور الجرذان البيضاء.

وقد استخدم في هذه الدراسة خمسة وثلاثين من ذكور الجرذ البيضاء، قسمت إلى خمس مجموعات.

المجموعة الأولى: تأثير الشيخوخة على الكلي والغدة الدرقية من الناحية الفسيولوجية، وتأثر بزيادة في نسبة الحرارة والبصريات والإجهاد البصريات في الكلي والغدة الدرقية.

المجموعة الثانية: تأثير هرمون الميلاتونين على الكلي والغدة الدرقية من الناحية الفسيولوجية، وتأثر بزيادة في نسبة الحرارة والبصريات والإجهاد البصريات في الكلي والغدة الدرقية.

المجموعة الثالثة: تأثير هرمون التستوستيرون على الكلي والغدة الدرقية من الناحية الفسيولوجية، وتأثر بزيادة في نسبة الحرارة والبصريات والإجهاد البصريات في الكلي والغدة الدرقية.

المجموعة الرابعة: تأثير هرمون الميلاتونين والتستوستيرون على الكلي والغدة الدرقية من الناحية الفسيولوجية، وتأثر بزيادة في نسبة الحرارة والبصريات والإجهاد البصريات في الكلي والغدة الدرقية.

وقد أظهرت المجموعات المسنة تغيرات واضحة في نسب يجب التوثيق في مستوى البصريات وزيادة في نسبة الحرارة والبصريات والإجهاد البصريات في الكلي والغدة الدرقية.

ومن نتائج هذه الدراسة أن الشيخوخة والهرمونات المضادة للشيخوخة لها أضرار على كل من الناحية الفسيولوجية للكلي والغدة الدرقية وأيضاً على التركيب النسيجي في الجرذان البيضاء. وكذلك أظهرت هذه الدراسة تحسن في مستوى هرموني T3 و T4 وقد أظهرت نتائج هذه الدراسة تغيرات واضحة في نسب يجب التوثيق في مستوى البصريات وزيادة في نسبة الحرارة والبصريات والإجهاد البصريات في الكلي والغدة الدرقية.