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COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF ANATOMY

PROJECT PAPER ON THE EFFECT OF MELATONIN IN THE TESTES AND OVARIES OF ANIMAL MODELS

A PROJECT PAPER SUBMITED TO ADDIS ABABA UNIVERSITY, SCHOOL OF MEDICINE, GRADUATE STDUYS, DEPARTMENT OF ANATOMY INPARTIAL FULFILLMENT OF MASTERS OF SCIENCE DEGREE IN HUMAN ANATOMY

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DEDICATION

To my father Ato Assefa Beyene, for nursing me with affection, unreserved assistance and for his dedicated encouragement in my academic carrier. However, he is unlucky to share with me the success I have been achieving in academic endeavors. I pray to God to rest his soul in peace.

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Above all, I would like to thank the Almighty God who blessed my life in all movements I have been making. I thank Him for the wonderful change, peace, joy and love brought to my life and for the helpful people He has been providing me throughout my study as well as this project.

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ABBREVIATION

SCN - Suprachiasmatic nucleus

HE - Haematoxylin-Eosin

ROS- Reactive oxygen species

Rpm -Revolutions per minute

EDTA - Ethylene diamine tetra acetic acid

ANOVA - Analysis of variance

SPSS - Statistical package for social science

SE- Standard error

GnRH- Gondotropin–releasing hormone

LH -Luteinizing hormone

FSH-Follicle-stimulating hormone

ELISA -Enzyme linked immunosorbent assay

IP-Intraperitonium

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SUMMARY

Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone synthesized and secreted primarily by the pineal gland during the dark hours at night. Other tissues and cells are also involved in its synthesis in the retina, gastrointestinal tract, lymphocytes and the skin. There has been increasing evidence that extrinsic doses of melatonin cause certain pharmaceutical, biochemical and physiological effects on the mammalian genital organs (such as in the testes and ovaries). 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). This project paper is designed to review and discuss scientific research articles regarding the effect of melatonin on the testes and ovaries of animal models. For these purpose different animal models, different time of exposure and different doses of melatonin administration was employed. As a result, the experimental animals treated with melatonin displayed inhibited spermatogenesis, tubular degeneration and necrosis, obstruction in tubular lumen and lymphocytic infiltration. In addition, melatonin administration caused marked reductions in absolute and relative testicular weight, size, serum testosterone concentration, atretic follicles and sperm of experimental animals. Though there was no difference in ovarian volume and relative ovarian weights, a differential count of various follicles has revealed significantly higher number of primordial, primary, secondary and antral follicles in melatonin treated rats. In the 90-day-old ovary, there was almost double the number of corpora lutea between control and melatonin programmed rats. Moreover, the histological effect of the testicular and ovarian damage increased with increased dosage of melatonin administration and with increased days after exposure to different doses of melatonin.

Keywords: Antral follicles, Atretic follicles, Corpora lutea, Melatonin, Ovaries, spermatogenesis, Testes.

1. INTRODUCTION

1.1. General Descriptions

In the past few decades, many studies regarding the biochemistry and physiology of a hormone called melatonin (*N*-acetyl-5-methoxytryptamine) were conducted. This hormone is secreted during the dark hours at night by the pineal gland (epiphysis cerebri) and is responsible for the regulation of a variety of important central and peripheral actions related to circadian rhythms and reproduction (Tamura *et al*., 2012). In recent years, many studies have been focusing on the role of melatonin in the process of reproduction system. Studies have shown that melatonin reduces oxidative stress and contributes to oocyte maturation, embryo development, and luteinization of granulosa cells. Clinical studies have demonstrated that melatonin treatment for infertile women increases intra-follicular melatonin concentrations, reduces intra-follicular oxidative damage, and increases the chances of pregnancy (Lampiao *et al*., 2013). Although melatonin is primarily synthesized and secreted by the pineal gland, it has been reported that it is also formed in tiny amounts by other organs such as the retina, gastrointestinal tract, lymphocytes, and the skin (Anderson *et al*., 1993; Bubenik, 2002 and Carrillo-Vico *et al*., 2004 cited by Lampiao *et al*., 2013).

The pineal gland is a small roughly spherical gland 6-10 mm in diameter, and lies in the midline of the brain, just below the posterior end of the corpus callosum. It represents an evagination of the posterior part of the roof of the third ventricle. It is connected to the brain by a short stalk containing nerve fibres, some of which communicate with the hippocampus. The pineal gland synthesises the hormone melatonin which acts as an endocrine transducer, inducing rhythmical changes in the endocrine activity of the hypothalamus, pituitary, ovaries and testes in response to changes in light received by the retina. Melatonin production by the pineal is induced by darkness and inhibited by light, probably through sympathetic nerves transmitting messages from the eye through the suprachiasmatic nucleus, central sympathetic pathways and the superior cervical ganglion. Postulated effects of melatonin in man include an influence on the onset of puberty and body biorhythms. In other animals it plays a role in the timing of seasonal reproductive cycles and, in reptiles and other lower vertebrates is responsible for changing skin colour through its action on melanophores, pigmented cells analogous to melanocytes in mammals (Young *et al*., 2007).

The pineal consists of two main cell types: pinealocytes (pineal chief cells) and neuroglial cells. Pinealocytes are highly modified neurones arranged in clusters and cords surrounded by a rich network of fenestrated capillaries. Pinealocytes have round nuclei with prominent nucleoli and granular cytoplasm, and many highly branched processes, some of which terminate near or upon blood vessels. The cytoplasmic granules of pinealocytes contain melatonin and its precursor, serotonin. The neuroglial cells, which are similar to the astrocytes of the rest of the CNS, are dispersed between the clusters of pinealocytes and in association with capillaries. A characteristic feature of the ageing pineal is the presence of basophilic extracellular bodies (pineal sand) consisting of concentric layers of calcium and magnesium phosphate in an organic matrix. The calcified pineal can be seen on X-rays of the skull and its position can be a useful guide to pathological conditions causing the midline to be displaced to one side (Young *et al*., 2007).

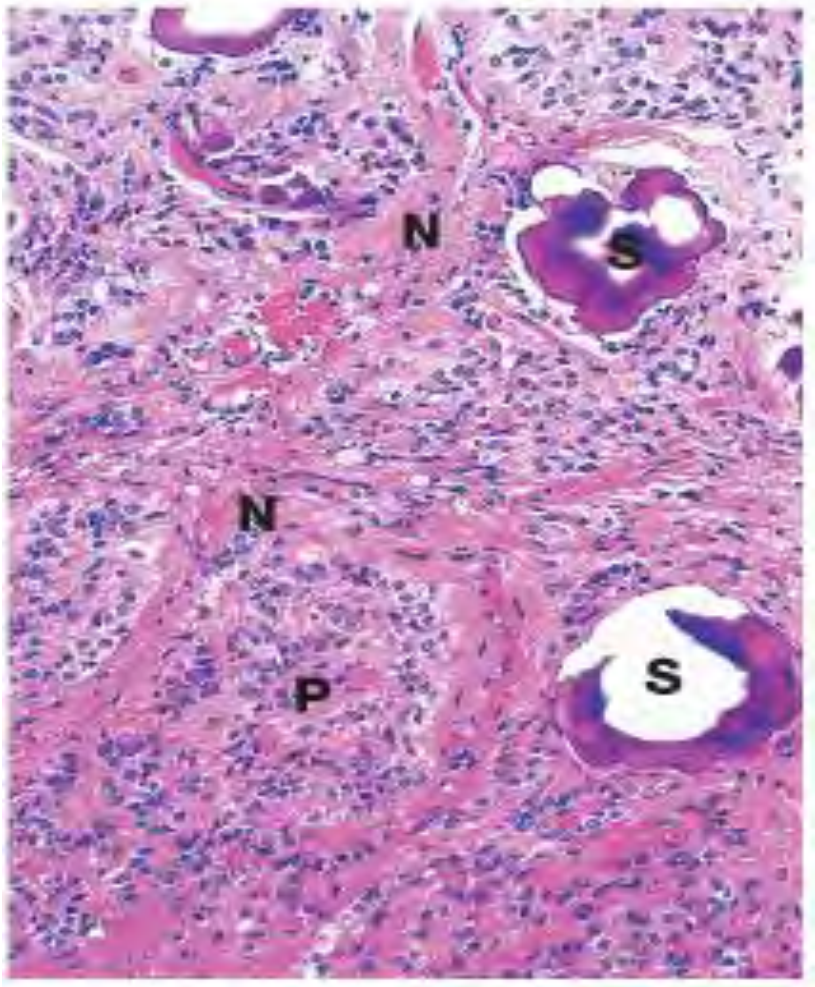

Figure 1: Histology of pineal gland consists of two main cell types: pinealocytes (P) and neuroglial cells (N) and presence of basophilic extracellular bodies: pineal sand (S), (Adopted from Young *et al*., 2007).

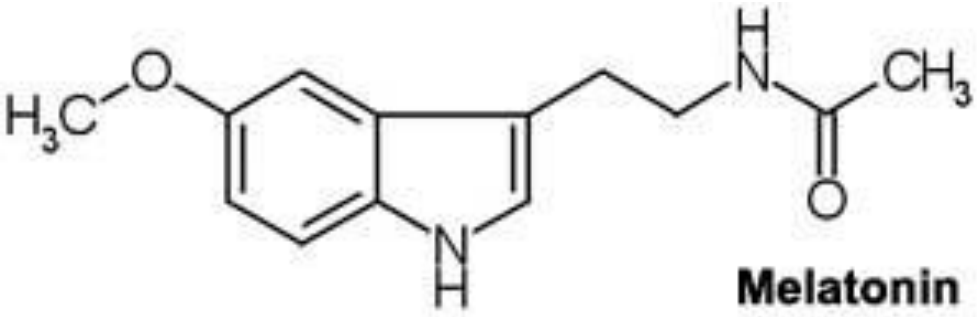

Figure 2: Chemical Structure of Melatonin, (Adopted from Lampiao *et al*., 2013).

1.2. Synthesis and regulation of melatonin

The production of melatonin by the pineal gland exhibits a circadian rhythm with low level of production during day time and high levels during the night (Brzezinski, 1997; Pang *et al*., 1998). During the process of melatonin synthesis, tryptophan is hydroxylated to 5-hydroxy-tryptophan and subsequently into serotonin. Serotonin is acetylated to form N-acetylserotonin and then converted into melatonin. The suprachiasmatic nucleus, which is the major circadian oscillator that receives light input from the retina through the retino-hypothalamic tract, is the one that regulates the circadian melatonin production. When melatonin is formed in the pineal gland, it is not stored there, but released immediately into the blood or into the cerebrospinal fluid. It is metabolized mainly in the liver (Berson *et al*., 2002).

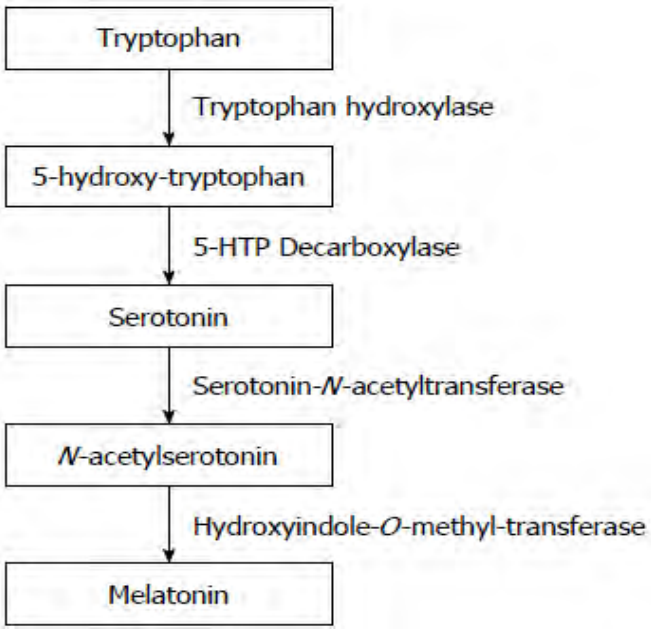

Figure 3: Biosynthesis of melatonin. 5-HTP: 5-hydroxy-tryptophan, (Adopted from Lampiao *et al*., 2013).

1.3. Histological features of testes

Each human testis (testicle) is surrounded by a capsule of dense connective tissue (tunica albuginea) which is thickened on the posterior side of the testes to form the mediastinum testis, from which fibrous septa penetrate the organ and divide it into 250 pyramidal compartments or testicular lobules and each lobule is occupied by one to four seminiferous tubules that is surrounded by interstitial loose connective tissue rich in blood and lymphatic vessels, nerves, and endocrine interstitial cells (Leydig cells) which secrete testosterone. Sperms are produced in the seminiferous tubules at a rate of about 2×10^8 per day in the adult male. Each testicle has 250–1000 seminiferous tubules in its lobules, with each tubule measuring about 150–250μm in diameter and 30–70 cm in length. The combined length of the tubules of one testis is about 250 m. Each tubule is a convoluted loop linked via a short, narrower segment, the straight tubule, to the rete testis, a labyrinth of epithelium-lined channels embedded in the mediastinum testis. Ten to twenty efferent ductules connect the rete testis to the head of the epididymis. Each seminiferous tubule is lined with a complex specialized stratified epithelium, germinal or seminiferous epithelium. The seminiferous epithelium consists of two types of cells: nondividing supporting or sustentacular cells (Sertoli cells) and proliferative cells of the spermatogenic lineage. The cells of the spermatogenic lineage comprise four to eight concentric cell layers and their function is to produce the cells that become sperm. The part of sperm production that includes cell division through mitosis and meiosis (spermatogenesis) (Mescher, 2010).

The rat testes are located in the scrotum (freely retractable into the abdominal cavity) and consist of convoluted seminiferous tubules enclosed by the tunica albuginea, a fibrous capsule of connective tissue. The seminiferous tubules are lined by seminiferous epithelium. At the base of this epithelium are, at regular intervals, the Sertoli (sustentacular) cells, which have large oval nuclei. The seminiferous epithelium further contains cells undergoing spermatogenesis; the least mature cells (spermatogonias) are located at the base of the epithelium, more matured cells (spermatogonias, spermatocytes, round spermatids, elongated spermatids) move towards the lumen, and the most mature cells, the spermatozoa, are released at the lumen. In the interstitial space between the seminiferous tubules are Leydig (interstitial) cells, which have abundant, eosinophilic cytoplasm, and blood vessels (Prata *et al.*, 2004).

The testes develop retroperitoneally in the dorsal wall of the embryonic abdominal cavity. They are moved during fetal development and eventually are suspended within the two sides of the scrotum at the ends of the spermatic cords. Because of the migration from the abdominal cavity, each testis carries with it a serous sac (the tunica vaginalis), derived from the peritoneum. The tunic consists of an outer parietal layer lining the scrotum and an inner visceral layer, covering the tunica albuginea on the anterior and lateral sides of the testes (Mescher, 2010).

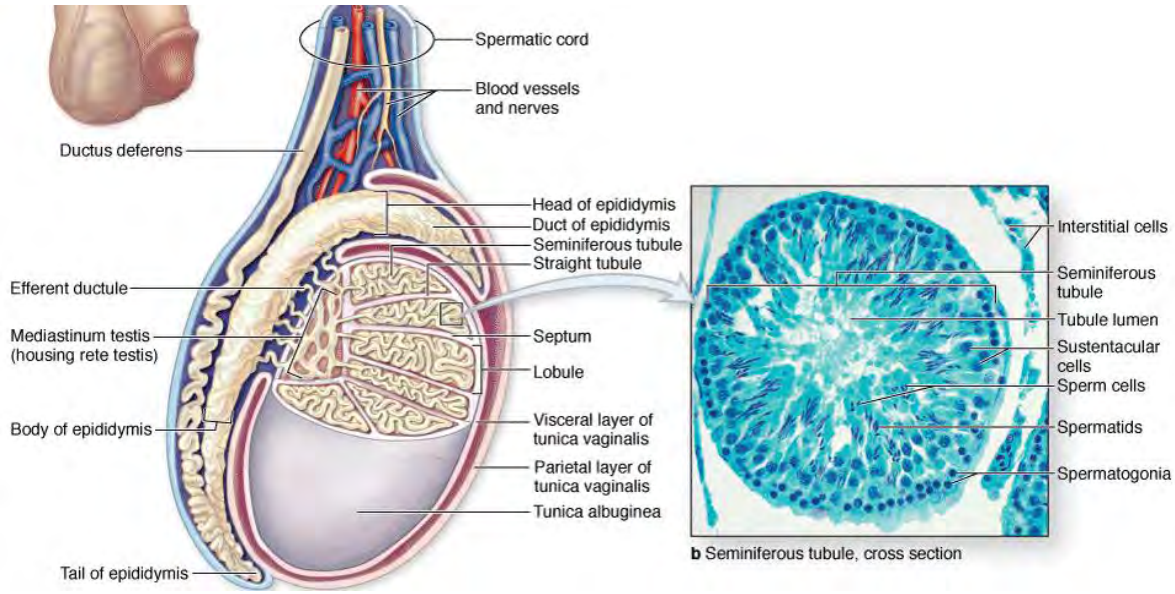

Figure 4: The histology of human testis is shown. (a): The diagram shows a partially cut-away sagittal section. (b): The micrograph shows a cross section of one seminiferous tubule. The micrograph shows seminiferous tubules surrounded by connective tissue, containing many large rounded or polygonal interstitial cells secreting androgens. Inside the tubule it is a unique seminiferous epithelium composed of columnar supporting cells called Sertoli cells, which usually have oval nuclei and distinct nucleoli, and germ cells of the spermatogenic lineage. Prominent among the latter are spermatogonia, diploid cells always located near the basement membrane, and primary spermatocytes, (Adopted from Mescher, 2010).

1.4. Histological features of ovaries

The human ovaries are almond-shaped body's approximately 3 cm long, 1.5 cm wide and 1 cm thick. Each ovary is covered by a simple cuboidal epithelium, the germinal epithelium, continuous with the mesothelium and overlying a layer of dense connective tissue capsule, the tunica albuginea, like that of the testis and responsible for the whitish color of the ovary. Most of the ovary consists of the cortex, a region filled with a highly cellular connective tissue stroma and many ovarian follicles, which in the adult ovary vary greatly in size. The most internal part of the ovary is the medulla, which contains loose connective tissue and blood vessels entering the organ through the hilum from mesenteries suspending the ovary (Mescher, 2010).

The ovaries of rats are enclosed by a clear membrane, the ovarian bursa, and consist of a poorly defined central medulla and outer cortex covered by the germinal epithelium, a single layer of cuboidal to columnar cells. The medulla comprises vascular, dense fibrous connective tissue stroma, while the ovarian cortex consists of primordial and maturing follicles and corpora lutea separated by loose fibrous stroma and groups of ovarian interstitial cells. Primordial follicles contain oocytes, clear round cells, surrounded by a single layer of flat cells. The majority of primordial follicles eventually undergo atresia, but each estrus several primordial follicles mature to larger follicles (primary unilaminar follicle, secondary multilaminar follicle) and the preovulatory Graafian follicles. In the maturing follicles, many layers of small basophilic granulosa cells have replaced the single layer of flat cells, a vascular theca layer has formed from stromal cells, and the oocyte is surrounded by the acellular zona pellucida and the cumulus oophorus, a cluster of follicle cells. The maturing follicles cause the germinal epithelium to bulge, which ruptures at ovulation, releasing the oocyte with its cluster of cells from the Graafian follicle. Next, the granulosa and theca cells change the follicle to a corpus luteum, which consists of lutein cells, large cells with a clear eosinophilic cytoplasm and a large nucleus, organized in cords around sinusoids (Prata *et al*., 2004).

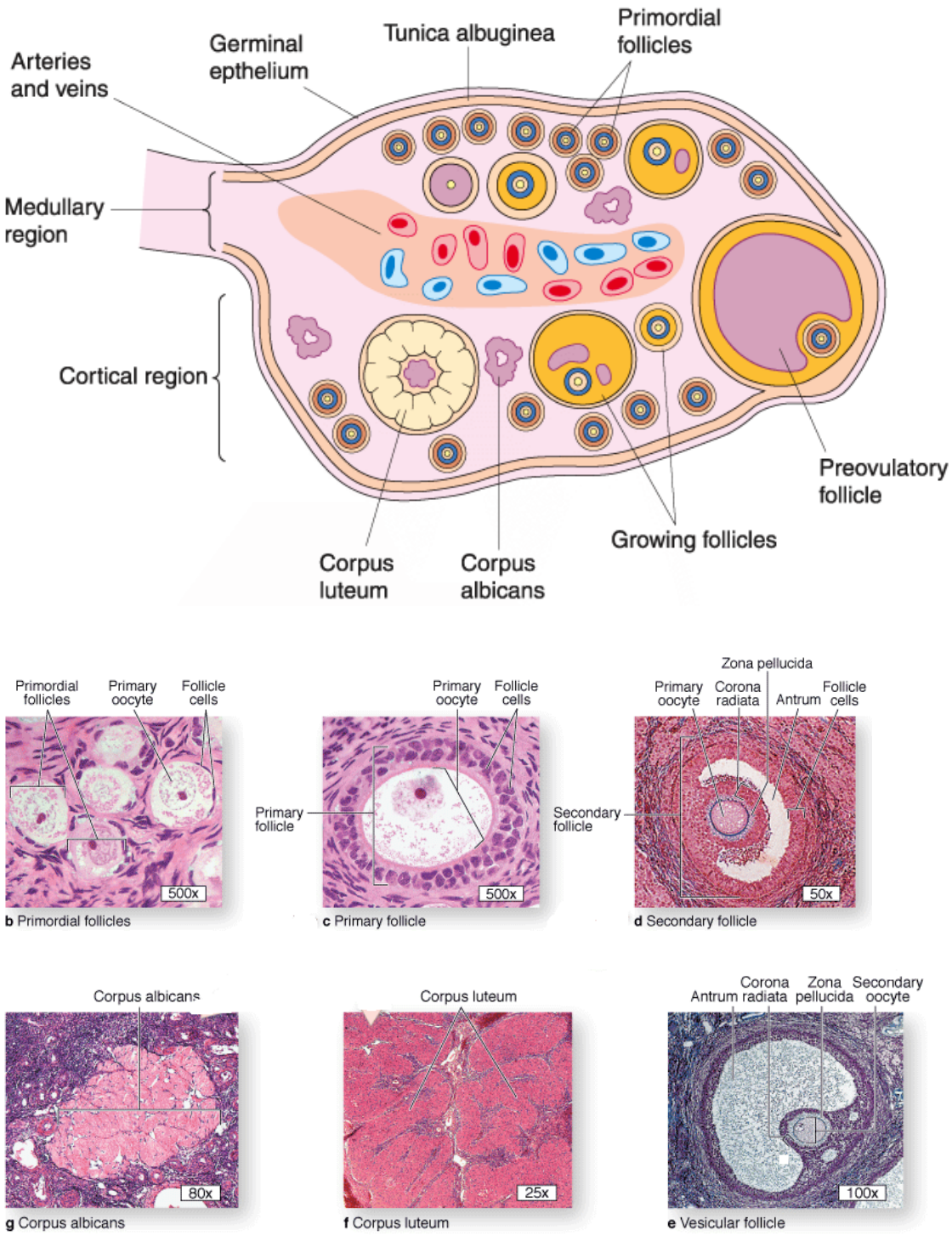

Figure 5: A human diagram of a sectioned ovary (a), shows the different stages of follicle maturation, ovulation, and corpus luteum formation and degeneration. The histological sections identify primordial follicles (b), a primary follicle (c), a secondary follicle (d), and a large vesicular follicle (e). After ovulation, the portion of the follicle left behind forms the corpus luteum (f), which then degenerates into the corpus albicans, (Adopted from Junqueira and Carneiro, 2005 and Mescher, 2010).

2-OBJECTIVES OF THE PROJECT

2.1. General objective

To review and discuss literature concerning the effect of melatonin on the testes and ovaries of animal models.

2.2. Specific objectives

1- To review and discuss literature concerning the effect of two different doses of melatonin on spermatogenic cells of adult rat *rattus norvegicus*, at the light and electron microscopic levels.

2- To review and discuss literature concerning melatonin and its effect on the histology of the testis of male albino rats.

3- -To review and discuss literature concerning the administration of different doses of melatonin and its effect on testosterone in albino rats.

4- To review and discuss literature concerning the neonatal melatonin treatment and its effect on adult ovarian and body weight in rats.

5- To review and discuss literature concerning the administration of neonatal melatonin and its effect on adult follicle type of female rats.

6- To review and discuss literature concerning the synthesis and regulation of melatonin, deliberates the mechanism of action and its effect on testicular and ova function.

3. REVIEW OF PUBLISHED RESEARCH ARTICLES ON THE EFFECT OF MELATONIN ON TESTES AND OVARIES OF ANIMAL MODELS

To demonstrate the effects of two different doses of melatonin on the spermatogenic cells of rat testes (a light and electron microscopic study), Rashed–Mourad et *al*. (2010) conducted a research by utilizing thirty adult male albino rats (weighing 170±15 g). All rats were healthy and kept in cleaned and good aerated animal cages at ~ 25±2 ºC, relative humidity of 45-50% and 12 hours light-cycle and they were fed with standard pellet diet and provided water *ad libitum*. 5 mg and10 mg of melatonin available commercially as capsule, tablet, cream or lozenges from Weider, USA, was dissolved in 1.0 ml ethanol and mixed with 99 ml physiological saline at 10 mg/100 ml. This solution was given orally through a stomach tube to the adult male rats in two different single doses, 0.05 mg/kg and 0.1 mg/kg body weight. These doses represent half and full therapeutic doses for the rats. Since the studied animals are known to secrete the endogenous melatonin during the night, the exogenous melatonin was given to them at early night. The rats were assigned into two experimental groups (group I and group II).

Group I (control group), included 6 rats that received only once an equivalent amount of oral physiological saline-ethanol (99:1, v/v). Group II (treated group), involved 24 rats and was used for treatment with melatonin. It was subdivided into four subgroups with 6 rats on each subgroup. Subgroup A (received a single dose of 0.05 mg/kg body weight of melatonin) and were killed after 48 hours. Subgroup B (received a single dose of 0.05 mg/kg body weight of melatonin) and were killed after 10 days. Subgroup C **(**received a single higher dose of 0.1 mg/kg body weight of melatonin) and were killed after 48 hours. Subgroup D (received a single higher dose of 0.1 mg/kg body weight of melatonin) and were killed after 10 days.

For light microscopic examination, the testes of the control and treated rats were dissected out through an abdominal incision and fixed immediately in aquous Bouin's solution, dehydrated in ascending grades of ethyl alcohol, cleared in chloroform and were then embedded in paraplast for histological examinations. Five µm-thick sections were cut using American Optical microtome (AO-821), stained with haematoxylin and eosin and were examined and photographed.

For electron microscopic examination, the testes were decapsulated and the exposed seminiferous tubules were cut into small pieces and rapidly fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffered for 4 hours at 4 ºC. They were washed in the same buffer, post-fixed in 1% cacodylate buffered-osmium tetroxide for 2 hours, dehydrated in ascending grades of ethyl alcohol (70%, 95% and 100%), cleared in propyleneoxide and embedded in epoxy resin. Ultrathin sections were cut with an ultramicrotome (Hitachi Sci. Inst.,Mountain View, CA), stained with uranyl acetate and lead citrate and then examined with a Hitachi (H500H, Hitachi Sci. Inst.) electron microscope.

The light microscopic investigation in group I suggested that the normal morphology of the seminiferous tubules, including all stages of the spermatogenic cycle (the different possible characteristic associations of developing germ cells), two types of spermatogonia (type A and type B), were observed. Primary spermatocytes were markedly larger in size than spermatogonia with large spherical nuclei with darkly stained chromatin. The rapidly dividing secondary spermatocytes were infrequently seen. Different steps of spermatids appeared throughout the different stages of the spermatogenic cycle. Sertoli cells, located on the basement membrane, had characteristic oval, indented nuclei with prominent nucleoli as shown in figure 6. Similarly, the gross appearance of all rats after melatonin administration (group II) appeared in a good health, had normal body weight and size and exhibited a normal behavior. However, on dissection, especially the rats which received higher dose of melatonin (0.1 mg/kg b.wt.) and killed after ten days (subgroup D), their testes were lesser in weight and smaller in size than those of the control rats. Besides few normal tubules, most of the seminiferous tubules exhibited abnormal structures regarding the nature of the testicular damage.

Forty-eight hours after melatonin administration of 0.05 mg/kg (in the sections of testes of the subgroup A), all the seminiferous tubules showed no marked changes in their histology as compared to the control groups except that the late spermatids were missed from these tubules, normal spermatogenesis, well-preserved Sertoli cells and well-delineated boundary tissue were observed. By the 10^{th} day after melatonin administration of 0.05 mg/kg (in subgroup B), the seminiferous tubules were slightly damaged, exfoliation of few primary spermatocytes (showed some elongation in their shape) and early spermatids into the lumina of the tubules was seen as indicated in figures 7.

Forty-eight hours after melatonin administration of 0.1 mg/kg b.wt (in subgroup C) the rat testes showed, a reduction in the diameter of their damaged seminiferous tubules and looked intact with a normal spermatogenic progression and inhibition of spermatogenesis were recognized. In rats killed ten days after melatonin administration 0.1 mg/kg b.wt (subgroup D), in addition to massively lost of primary spermatocytes and early spermatids from most of the seminiferous tubule, many seminiferous tubules were severely damaged and some others were partially damaged, many tubules had only Sertoli cells and spermatogonia because Sertoli cells and spermatogonia were more resistant to melatonin treatment than the other spermatogenic cells of the seminiferous tubules (they possessed a thickened boundary tissue) and many tubular vacuoles of different sizes were observed as shown in figures 8.

Electron microscopic examination of the seminiferous tubules of control group (group I) rats revealed that their epithelia were composed of two types of cells (Sertoli cells and Spermatogenic cells). Sertoli cells membranes possessed complex infoldings and their extensive cytoplasm appeared to ramify throughout the whole seminiferous epithelium enclosing all spermatogenic cells and spermatogenic cells. These cells had basally located, oval, indented nuclei with prominent nucleoli. Their cytoplasm contained a moderate number of mitochondria, many lipid droplets and inclusion products. Spermatogenic cells were seen in various stages of maturation. Spermatogonia were located in the basal compartment of the seminiferous tubules with type-A (had flattened oval nuclei) whereas type-B spermatogonia (had round nuclei and occupied the basal compartment little distant from the boundary tissue) were recognized. Primary and secondary spermatocytes were also observed.

For electron microscopic examination of the treated group (group II) testes of rats received 0.05 or 0.1 mg/kg b.wt. of melatonin and killed after 48 hours, in subgroups A and C, respectively showed the normal characteristics of the spermatogenic cycle at stage VII, few small vacuoles within the head cap and nearby region of cytoplasm of some early spermatids and the mitochondria showed normal arrangement as one layer under the cell membrane whereas the electron microscopic examination of testicular tubules of rats received 0.05 mg/kg b.wt. of melatonin and killed after 10 days (subgroup B) proved the formation of elongated primary spermatocytes that had elongated nuclei and some vacuoles in their granular cytoplasm.

These cells had a dilated endoplasmic reticulum, finely granular cytoplasm and many structurless mitochondria that were seen randomly distributed throughout the cytoplasm. Ultrastructural study of testes of rats received 0.1 mg/kg b.wt. of melatonin and killed after 10 days showed marked infoldings of the boundary tissue beneath the spermatogonia. The mitochondria of these spermatogonia showed a prominent disintegration of their cristae. In addition, some early spermatids were degenerated and abnormally located at or near the margin (basal compartment) of the seminiferous tubules close to the damaged boundary tissue. The mitochondria of these cells were completely damaged and showed a marked disintegration of their cristae.

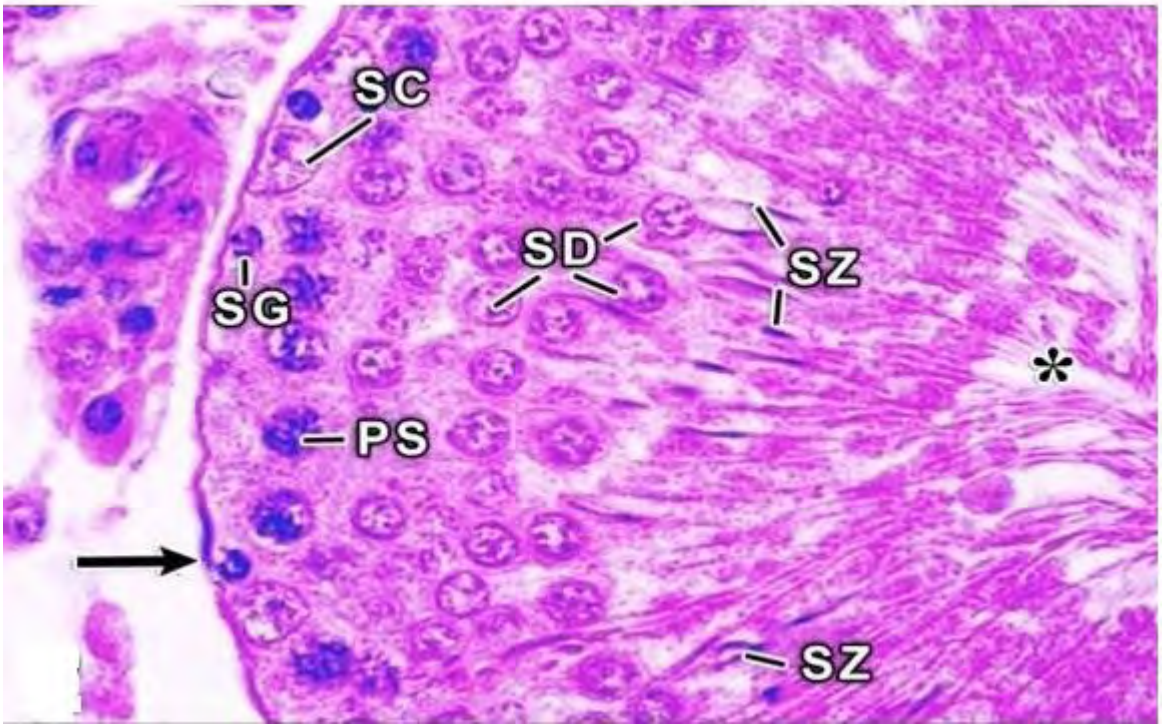

Figure 6 : Photomicrograph of a section of the testes of a control rat, showing successive stages of spermatogenesis which include spermatogonia (SG), primary spermatocytes (PS), different stages of spermatids (SD) and spermatozoa (SZ) surrounding a central lumen (*). Notice Sertoli cells (SC) are attached by their bases to the basement membrane (arrow), (Adopted from Radwan *et al*., 2011).

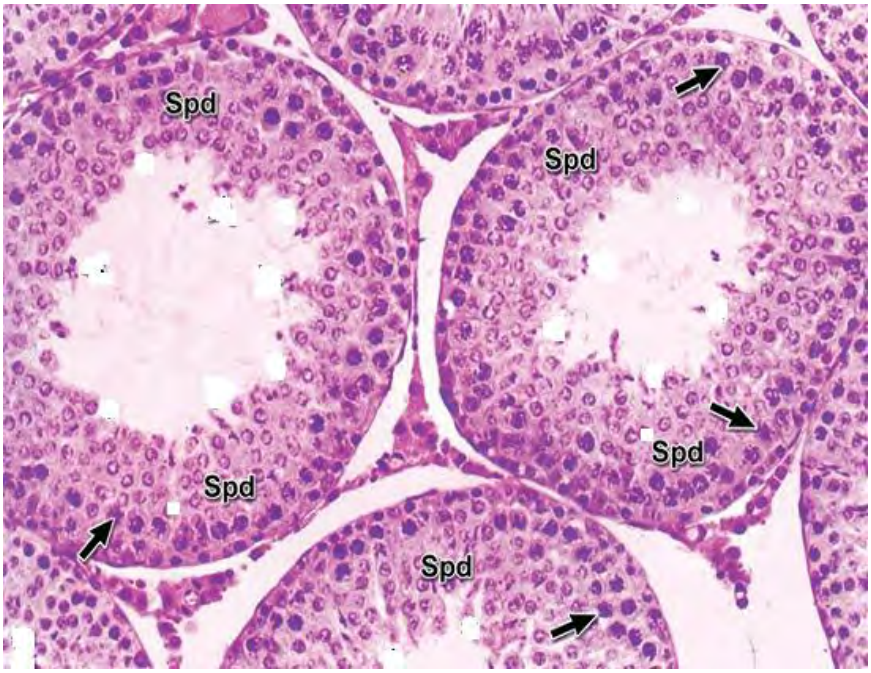

Figure 7: A photomicrograph of a cross-section from the testes of a rat received 0.05 mg/kg b.wt of melatonin and were killed after 48 hours showing well preserved primary spermatocytes (arrows) and early spermatids (Spd), (Adopted from Rashed–Mourad *et al*., 2010).

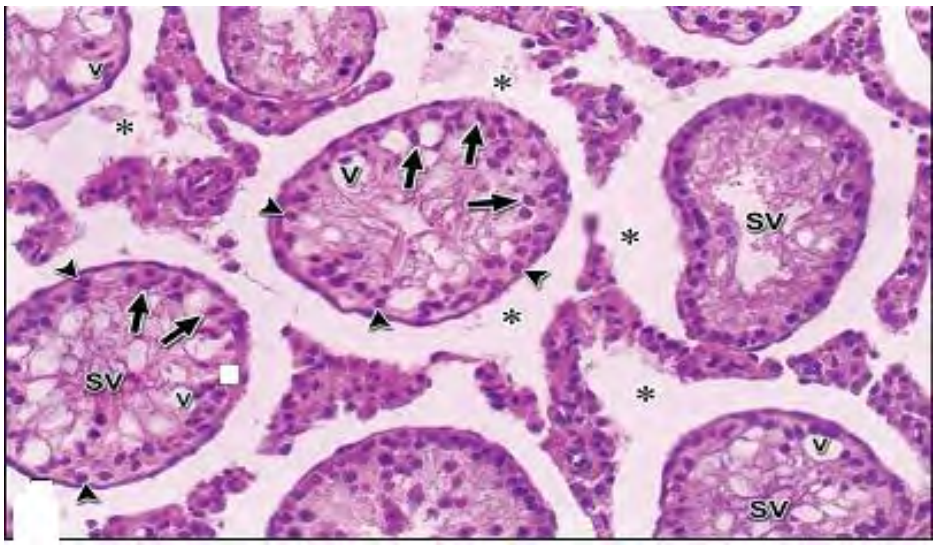

Figure 8: Photomicrographs of a cross-section from the testes of a rat received 0.1 mg/kg b.wt of melatonin and were killed after 10 days showing diminution of seminiferous tubules diameter and the wide spaces around them are shown (asterisks). Many vacuoles among the spermatogenic cells (V) are evident. Note the severely damaged tubules (SV), Sertoli cell nuclei (arrows) and spermatogonia (arrowheads), (Adopted from Rashed–Mourad *et al*., 2010).

The histological effect of melatonin on rat testes was evaluated by Tuncer et al. (2011). The research was performed on twenty adult Sprague-Dawley male rats. The experimental animals were housed in stainless steel cages that were washed and cleaned daily and maintained at 21±1 °C in a 12-hour light/dark cycle. All rats were given a standard diet and water *ad libitum* throughout the study period by dividing them into two groups of 10 animals each and treated as:group1 (normal controls receiving a standard diet and not subjected to any procedure), group 2 (rats receiving I.P. injections of saline containing 3 mg/kg melatonin) during one month. The injections were administered daily between 9 AM and 10 AM. 40-mg melatonin (Sigma M-5250) were suspended in 3 ml ethanol and 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. 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 histological changes in the testis tissues of melatonin treated groups were seen to reveal inhibition of the tubular degeneration, necrosis and obstruction in tubule lumens or lymphocytic infiltration (were contain 5.00 median value) whereas the microscopic examination of tissues from general control groups did not reveal inhibition of tubular degeneration and necrosis, obstruction in tubule lumens or lymphocytic infiltration (were contain1.00 median value) as indicated in table 1. The histological difference in the testis tissues of melatonin treated groups were 4.00 median values. The spermatogenesis scoring of the control group were 7.00 seen to contain a sufficient amount of cells as compared to the rats administered with melatonin reached a score 6.50 and displaying inhibited spermatogenetic activity as shown in table 2. The reductions in scoring spermatogenesis activity of the treated groups were 0.5 ($p<0.001$).

Table 1- Histological changes in the testes tissues of study groups.

Groups	Tubular degeneration and necrosis (Median value)	Obstruction in tubule lumens and lymphocytic infiltration (Median value)
General control (n=10)	1.000^{a}	1.000^{a}
Melatonin administrated (n=10)	5.000^{b}	5.000^{b}

*Means with different superscripted letters (1.000^{a} and 5.000^{b}) in the same column are representing the statistically significant.

*Means with the same superscripted letters (1.000^{a}) and ($5.000^{b)}$ in the same column has no statistically significant **($p<0.001$).**

Table 2- Spermatogenesis scoring of study groups.

Groups	Spermatogenetic activity
General control (n=10)	7.00±0.00
Melatonin administrated (n=10	6.50±0.50

Sarbast *et al*. (2013) conducted research on the effect of two doses of melatonin treatment on the testosterone of male albino rats. Thirty seven adult male albino rats aged between 9 and11 weeks and weighing (300-350gm) were used for the study. Animals were housed in plastic cages bedded with wooden chips, under standard laboratory conditions, 12:12 light/dark photoperiod at 22 ± 2 ºC. The animals were fed on standard rat chow and tap water *ad libitum*. The rats were assigned randomly into three groups and were continued for 6 weeks: Group 1(Control rats), given a standard rat chow and tap water *ad libitum*. Group 2, given standard rat chow supplemented of 60 mg / kg diet of melatonin. Group 3, given standard rat chow supplemented with melatonin 120 mg / kg diet of melatonin. At the end of each experiment, the rats were anesthetized with ketamine hydrochloride (50 mg/kg). Blood samples were taken by cardiac puncture into chilled tubes with ethylene diamine tetra acetic acid (EDTA) (4.5mm) as anticoagulant and centrifuged at 3000 rpm at 0°C for 15 minutes; then sera were stored at -80°C (Sony, Ultra low, Japan).The serum testosterone concentrations were determined by using enzyme linked immunosorbent assay (ELISA) kit. All data were expressed as means + standard error (S.E) and statistical analysis was carried out using available statistical software (statistical package for social science (SPSS) version 11.5). P values <0.05 were considered significant.

The serum testosterone concentration of the control groups were 2.829 (ng/ml) whereas the oral administration of melatonin for 60 mg/kg and 120 mg/kg diet were 1.952 and 1.171(ng/ml) respectively as shown in table 3.The reduction for the serum testosterone concentration among the melatonin treated groups of 60 mg/kg were 0.877 (ng/ml whereas, the reduction difference for the serum testosterone concentration among the melatonin treated groups of 120 mg/kg diet were 1.658 as compared to control groups respectively.

Table 3- Effect of melatonin on serum testosterone in male albino rats.

Groups	Serum testosterone (ng/ml) *
Control	2.829 ± 0.408 ^{c}
Melatonin (60mg/kg diet)	1.952 ± 0.208 ^{b}
Melatonin (120mg/kg diet)	1.171 ± 0.124 ^{a}

Means with different superscripted letters (**a, b** and **c**) indicated statistical differences (***=P<0.05**)

Still another study by Radwan *et al.* (2011) determined the histopathologic changes in male albino rats by utilizing fourteen rats (aged 5 and18 month) used in these trials were divided into two groups of 7 animals each (control group) were received 1 ml/kg b.w. corn oil intramuscular injection and (experimental group) were received 1ml of a daily dose of melatonin (2mg/kg body weight orally) 2 hour before lights out through a period of two weeks. 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. By the end of the two weeks, the animals (both control and treated 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.

The testes of the control rat were surrounded by a dense fibrous tissue capsule. Thus, the thin fibrous septa divide the testis into lobules; each lobule contains several seminiferous tubules which are surrounded by the interstitial tissue as shown in figure 9. The spermatogenic lineage of the control groups were composed of spermatogonia, primary and secondary spermatocytes, spermatides and mature spermatozoa that occupy the center of tubule (figure 9). However, the testes of melatonin treated group 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 (figure 10). The basement membranes of some seminiferous tubules were detached and congestion of some intertubular blood vessels was also detected and hypoplasia of interstitial tissue was also observed as shown in figure 10.

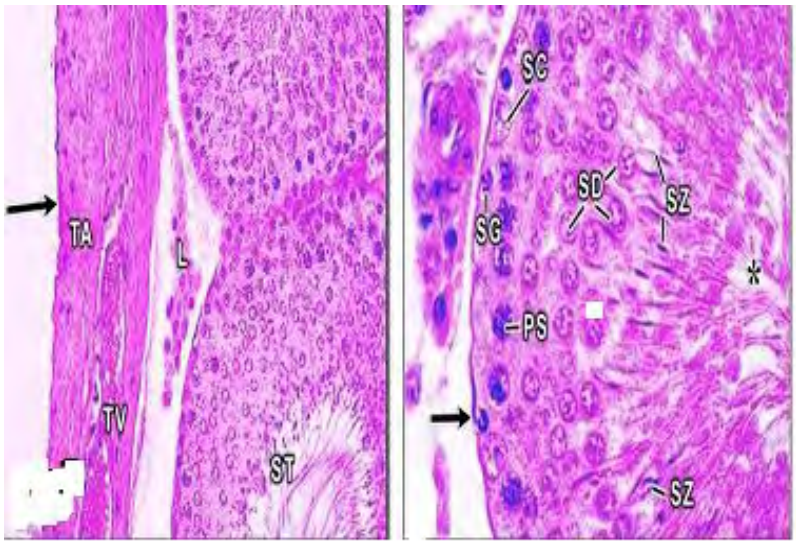

Figure 9: Photomicrograph of a section of the testes of a control rat, showing connective tissue capsule (arrow) which is formed of tunica albuginea (TA), successive stages of spermatogenesis which include spermatogonia (SG), primary spermatocytes (PS), different stages of spermatids (SD) and spermatozoa (SZ) surrounding a central lumen (*). Notice Sertoli cells (SC) are attached by their bases to the basement membrane and tunica vascuolosa (TV), the seminiferous tubules (ST) and interstitial tissue (L), (Adopted from Radwan *et al.*, 2011).

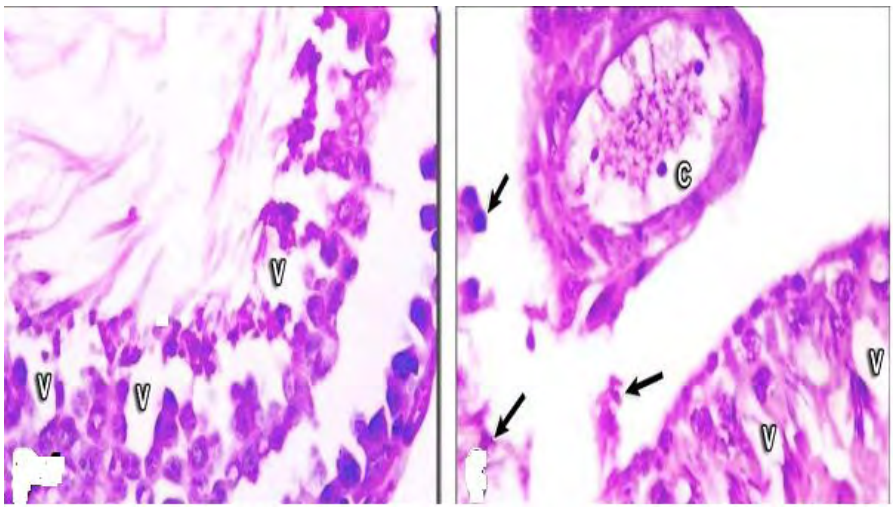

Figure 10: Photomicrograph of a section of the testis of melatonin treated rats showing disorganization of the germ cells of some tubules, degeneration of some of primary spermatocytes and presence of large vacuoles (V) among spermatogenic cells and congestion (C) of interstitial blood vessel, hypoplasia of the interstial tissue (arrows). Notice presence of large vacuoles (V) between spermatogenic cells, (Adopted from Radwan *et al.*, 2011).

An effect of neonatal melatonin treatment on adult ovarian functions in rat was investigated by Ramachandran and Thakkar (2011). The study was performed on 30 neonates' albino Wistar rats under a constant temperature range of 21±2°C. Melatonin (Sigma Co, USA) for administration was prepared by dissolving weighed amount in 1.0 ml of ethanol and diluted with 99 ml saline at10 mg/100 ml. The experimental setup consisted of two groups of study. Group I (Female rats maintained until day 90 served as controls). This consisted of two subgroups of 10 animals each as control rats (maintained as such) and injected i.p with vehicle (99 ml saline) in the evening at 16:30 hours. Group II (consisting 10 neonates female rat) were injected i.p with 40 µg melatonin/animal/day from day 0 to day 21 postpartum at 16:30 hours.

After treatment for 21 days, the animals were sacrificed on days 22, 45 and 90. The animals were sacrificed and blood was collected by brachial venipuncture in Eppendorff tubes. After centrifugation at 4000 revolutions per minute (rpm), separated serum was stored at -4^0C. The viscera was cut open and the ovaries were excised, blotted free of tissue fluids and weighed accurately in a Mettler balance. Relative weight was calculated and expressed as percentage of body weight. The fixed ovaries (Bouin's fluid) were processed for paraffin sectioning. Paraffin sections of 3µm thickness were cut on a microtome and stained with Haematoxylin-Eosin (HE). For morphometry and enumeration of ovarian follicles, homologous cross sections of entire ovary showing better area of vision were chosen. The section area was calculated by integrating the area inside the traced perimeter and volume calculated by multiplying by the section thickness. The section volume was multiplied by 10 (to count for the number of sections skipped) to give the '10-section' volume, all of 10 section volumes were summed to obtain an estimate of the total ovarian volume in mm3 and a count of different types of follicles was also made. The data were analyzed by student's test and analysis of variance (ANOVA) wherever applicable, at 95% confidence limit.

The body weight of the control groups injected with vehicle (0.9% saline) in the evening at 16:30 hours for 22, 45, 90 days were 51.33, 136.67 and 226.167g respectively whereas for the melatonin treated groups were 46.67, 129.33 and 194.83g. Thus, the difference in body weight reduction in melatonin treated groups compared to control groups were 4.66, 7.34 and 31.337g respectively.

The relative ovarian weight in melatonin treated group were 0.038, 0.040 and 0.038 g/100g for 22, 45, 90 days as compared to control groups 0.046, 0.045 and 0.034 respectively as indicated in table 4. The body weight of melatonin treated animals was significantly less at all ages of the study. However, there was no significant difference in relative ovarian weights. The total follicular count (primordial) in ovary of control group within 22, 45 and 90 days were 821, 426 and 300 whereas the total number of primordial follicle in ovary of melatonin treated group were 891, 446 and 373 respectively as shown in table 5. The total increment of primordial follicle in melatonin treated group was 70, 20 and 73 respectively. The total number of primary follicle in ovary of control group was 531, 162 and 168 whereas in melatonin treated group were 391, 229 and 217. The number of primary follicle in melatonin treated group in day 22 was decreased by140 whereas the number of primary follicle in melatonin treated group in day 45 and 90 was increased by 67 and 49 respectively.

The of secondary follicles in ovary of control group were 350, 245 and 96 whereas the number of secondary follicles in ovary of melatonin treated group were 452, 277 and 108 number respectively as indicated in table 4. The number of secondary follicles was increased by 102, 32 and 12 in day 22, 45 and 90 melatonin treated group as compared with control group. The number of antral follicles in ovary of melatonin treated group was 183, 166 and 96 whereas in the control group they were 181, 142 and 72. Thus, the number of antral follicle in ovary of melatonin treated groups in day 22 were increased by 2 however, the difference between the age of 45 and 90 were 24 as compared with control groups. In the 90-day-old ovary, there was almost double the number of corpora lutea in melatonin treated rats (72) as compared to controls (48) by 24. The number of atretic follicles in ovary of melatonin treated group was 5, 9 and 13 as compared to control groups 10, 29 and 36 respectively as shown table 5. The total reduction of atretic follicle in ovary of melatonin treated group was 5, 20 and 23 as compared to the control groups. Thus, melatonin treated group in the 22, 45 and 90- day-old ovary had no difference in ovarian volume (0.45, 0.88 and 1.29 mm^3) as compared to control rats (0.58, 0.68 and 1.31 mm^3) as shown in table 5. Though there was no difference in ovarian volume, a differential count of various follicles has revealed significantly higher number of primordial, primary, secondary and antral follicles in melatonin treated rats. In the 90-day-old ovary, there was almost double the number of corpora lutea in melatonin treated rats as compared to controls.

A count of atretic follicles has also shown significantly lesser number in melatonin treated rats. The ovary of melatonin treated animals showed greater population of follicles at all ages of study compared to corresponding controls as indicated in figure 11 and 12.

Table 4: Chronological alterations in body weight (g) and relative ovarian weight (g/100g) in control and melatonin treated rats.

Groups	Body weight (g)			Relative ovarian weight (g/100g)		
	Age in days			Age in days		
	22	45	90	22	45	90
Control	51.33±1.83	136.67±4.176	226.167±9.55	0.046±0.0006	0.045±0.005	0.034±0.0025
Melatonin	46.67±0.83	129.33±2.917	194.83±6.66[a]	0.038±0.0023[b]	0.040±0.009	0.038±0.0023

Values are expressed as Mean± SEM of ten observations with **a= p <0.05**, **b= p<0.005.**

Table 5: Differential total follicular count in ovary of control and melatonin treated female rats.

Treatment	Age in days	Number of Follicle Type						Ovarian Volume (mm^3)
		Primordial	Primary	Seconday	Antral	CL	Atretic	
Control	22	821±48	531±25	350±20	181±04	-	10±0.8	0.58
	45	426±17	162±11	245±10	142±08	-	29±02	0.68
	90	300±13	168±08	96±04	72±05	48±5	36±03	1.31
Melatonin	22	891±42[b]	391±1[b]	452±12[b]	183±0[a]	-	05±0.4[c]	0.45[a]
	45	446±15	229±1[b]	277±28	166±11	-	09±0.1[c]	0.88[b]
	90	373±15[b]	217±1[b]	108±04	96±04[b]	72±6[b]	13±01[c]	1.29

Values are expressed as Mean±SEM of ten observations as **a = p <0.05, b = p<0.005, c= p<0.0005**.

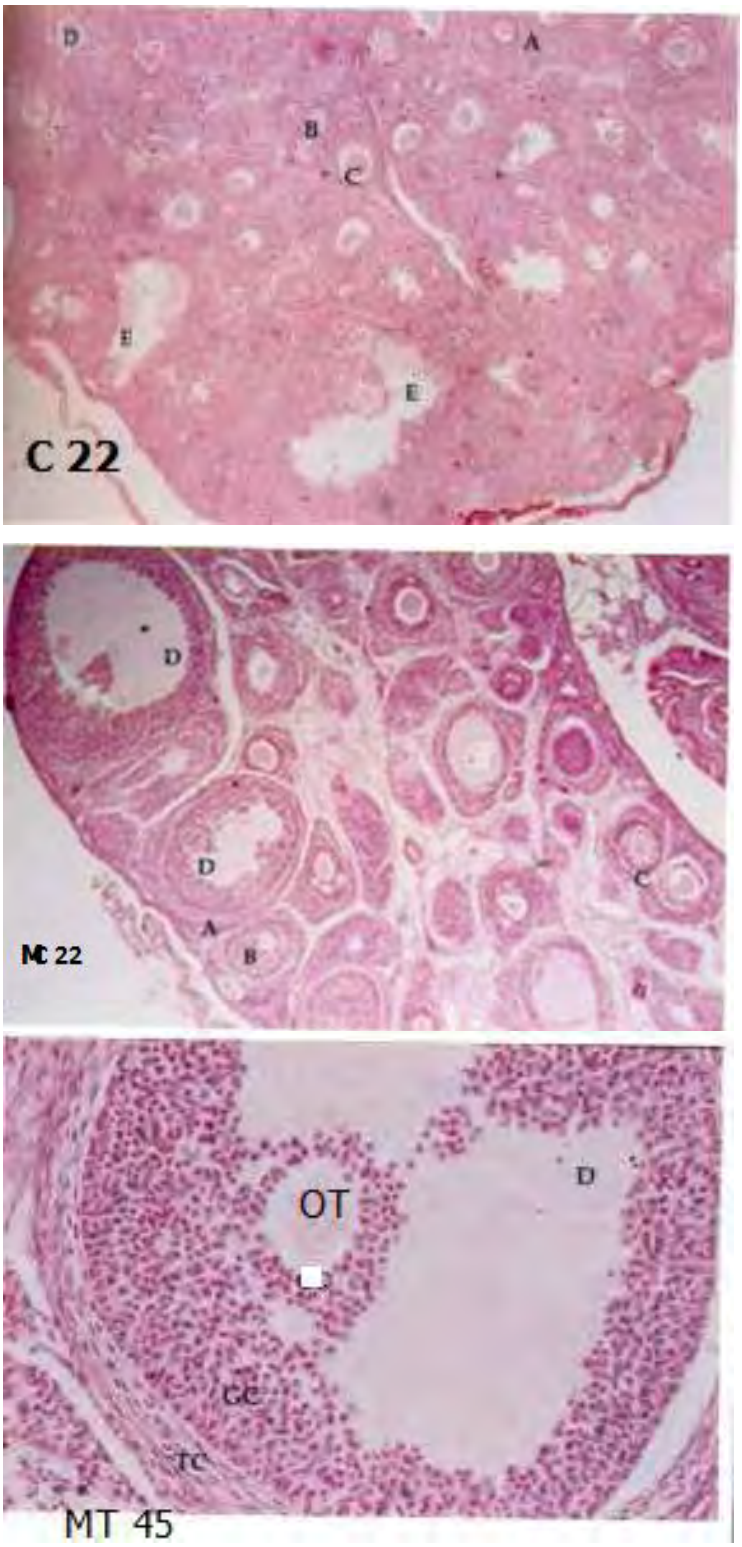

Figure 11: Photomicrograph of sections of ovaries of 22 and 45 day old of control (C) and melatonin (MT) treated rats showing more number of pre antral follicles and fewer atretic follicles compared to treated groups. A-Primordial follicle; B- Primary follicle; C- Secondary follicle; D- Antral follicle; E- Atretic follicle; GC Granulosa cells; TC- Thecal cells; OT- Oocyte, (Adopted from Ramachandran and Thakkar , 2011).

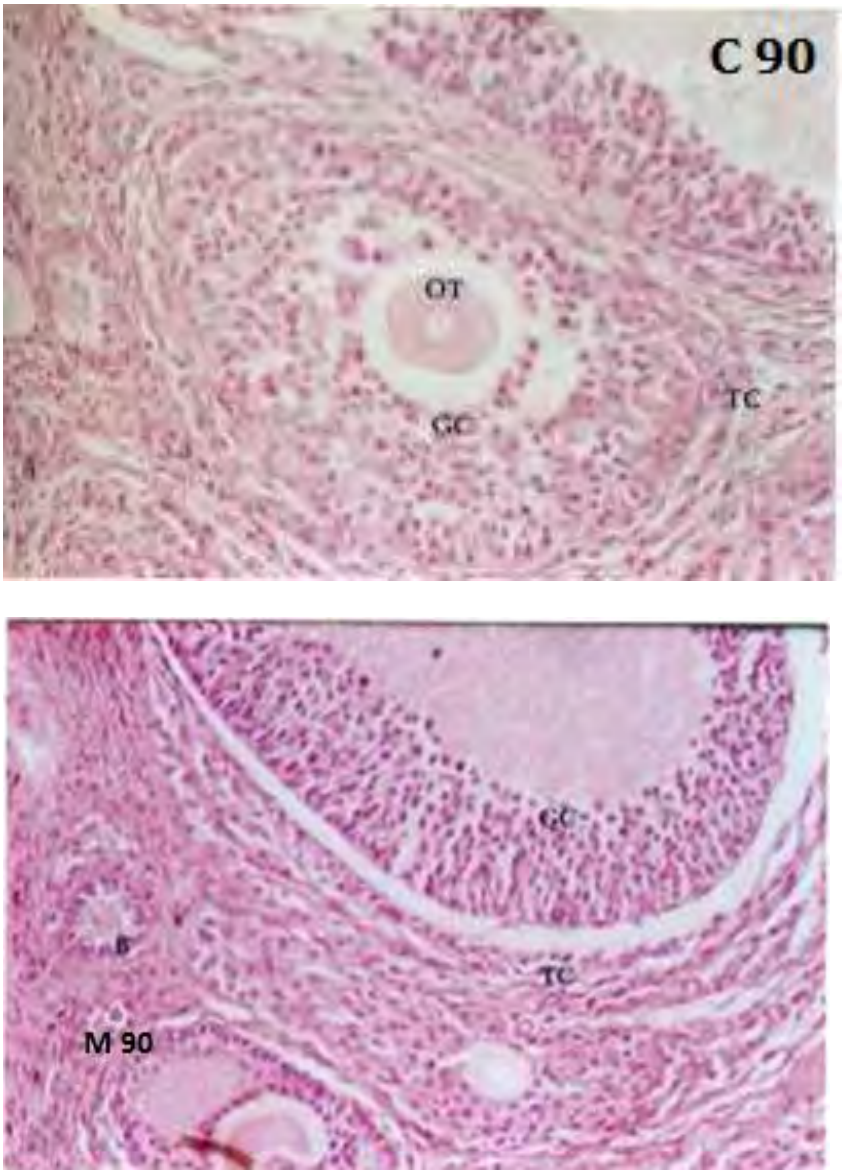

Figure 12: Photomicrograph of sections of ovaries of 90 day old control (C) and melatonin (M) treated rats showing more number of secondary and tertiary follicles in melatonin treated rats, GC Granulosa cells; TC- Thecal cells; OT- Oocyte, (Adopted from Ramachandran and Thakkar , 2011).

4. DISCUSSION

Several studies were undertaken on animal models to investigate the adverse effects of administration of melatonin on testes and ovaries of rats in different doses and times of exposure in the presence of control group as well.

One of the effects of melatonin treatment was conducted by Radwan *et al*. (2011), the testes of melatonin treated group (received two mg/kg body weight of melatonin orally) two hours before lights out through a period of two weeks revealed histopathological alterations in both the seminiferous tubules and interstitial tissue. Radwan *et al*. (2011) and coworkers also added that large vacuoles appeared between the spermatogenic cells and some nuclei of spermatogonia exhibited signs of pyknosis, degeneration of some primary spermatocytes and hypoplasia of interstitial tissue. The basement membranes of some seminiferous tubules were detached and congestion of some intertubular blood vessels were also detected as compared to the control groups. In support to the findings of Radwan *et al.* (2011) and Tuncer *et al.* (2011) administration of three mg/kg melatonin for one month displayed inhibition of tubular degeneration, necrosis, obstruction in tubule lumens and lymphocytic infiltration with the median value of 5.00 and inhibited spermatogenetic activity whereas the microscopic examination of tissues from the control group did not reveal any of these symptoms. The control rats were also seen to contain a sufficient amount of cells (score 7), while the rats administered with melatonin reached a score 6.5.

Treatment of six mg/kg /day melatonin for twenty-eight days by Leena-Patil and Balaraman (2009) brought to a close the ideas of Radwan *et al.* (2011) and Tuncer *et al*. (2011), by suggesting the final body weight (228.3 g) of melatonin treated groups were increased by 0.8g as compared to the control groups (227.5g) whereas the absolute (1.95 g) and relative (0.87per bw,%)) testes weight of the melatonin treated groups was decreased by 0.05g and 0.02% as compared with that of the control groups (2g and 0.85. per bw,%) respectively. Thus, the number of sperm ($14.55x10^6$/mg epididymis) and testosterone (0.78 ng/ml) in treated group were decreased by 1.15 $x10^6$/mg in epididymis and 0.02 ng/ml as compared to the control groups (15.7 $x10^6$/mg epididymis and 0.80 ng/ml).

In line with Leena-Patil and Balaraman (2009); Radwan *et al.* (2011) and Tuncer *et al.* (2011), the effect of melatonin on the reduction of the testicular weight and size of rats was indicated by Rashed-Mourad *et al.* (2010); in which the rats treated with 0.05 mg/kg b.wt dose of melatonin and killed after 48 hours, all the seminiferous tubules showed no marked changes in their histology: normal spermatogenesis, well-preserved Sertoli cells and well-delineated boundary tissue in the sections of testes as compared to the control rats. However, the late spermatids were missed from the seminiferous tubules.

By the 10 day after melatonin administration of 0.05 mg/kg the seminiferous tubules were slightly damaged, exfoliation of few primary spermatocytes and early spermatids were seen. Likewise, 48 hours after melatonin administration of 0.1 mg/kg b.wt of the rat testes showed a moderate reduction in the diameter of their damaged seminiferous tubules and inhibition of spermatogenesis. So, when the rats treated with the higher dose of melatonin (0.1 mg/kg b.wt.) and were killed after 10 days, it was observed that many seminiferous tubules were severely damaged, many tubules had only Sertoli cells and spermatogonia, primary spermatocytes and early spermatids were massively lost from most of the seminiferous tubules and many tubular vacuoles of different sizes were observed. So, the histological alterations in testis have a strong link with increased dose of melatonin and exposure duration periods.

In support to the findings of Leena Patil and Balaraman (2009); Rashed-Mourad *et al.* (2010); Radwan *et al.* (2011) and Tuncer *et al.* (2011), there were dose and time dependent histological effects by examined two doses of melatonin treatment (60 mg/kg and 120 mg/kg diet) on the testosterone of male albino rats and reported a significant decrease in serum testosterone concentration by 1.952 and 1.171 ng/ml (in 60 mg/kg and 120 mg/kg diet) respectively as compared to control groups (2.829 ng/ml). The reduction of serum testosterone concentration among the melatonin treated groups of 60 mg/kg were 0.877 ng/ml whereas, the reduction difference for the serum testosterone concentration among the melatonin treated groups of 120 mg/kg diet was 1.658 as compared to control groups (2.829 ng/ml) respectively (Sarbast *et al.*,2013).

Malpaux *et al*. (1999) also showed the presence of a negative relationship between sperm production and melatonin secretion in male rats and reported the nocturnal secretion of melatonin regulates the pulsatile release of GnRH from hypothalamus. This was supported by Sirotkin and Schaeffer (1997) who reported that change in GnRH release in turn affects LH secretion and leads to decrease of sperm production and attributed to antigonadal effects of melatonin, at least in part, that exerts through the direct decrease of testosterone production. These results are in agreement with Noorafshan *et al*. (2005) and Leena-Patil and Balaraman (2009) who showed that prolonged and uncontrolled use of melatonin causes various histological and morphological abnormalities in the testis, including reduction of testicular volume and seminiferous tubule length.

Since the Leydig cells are responsible for the production of testosterone and epididymis is important for the maturation and storage of spermatozoa before they are ejaculated into the female reproductive tract in mice and rats, it was reported that administration of melatonin has an inhibitory effect on Leydig cells and epididymal physiology (Shiu *et al*., 1997 and Persengiev and Kehajova, 2003). Moreover, long term administration of melatonin to healthy men is associated with decreased semen quality, sperm concentration and motility as well as testosterone levels. In another study in which melatonin levels were measured in fertile and infertile men, it was found that serum and seminal melatonin levels in infertile men were significantly reduced as compared to the levels in the fertile men. This demonstrated that melatonin may be involved in the modulation of the reproductive neuroendocrine axis in male fertility. With regard to sperm morphology, melatonin interferes in sperm differentiation producing anomalies in sperm tails in treated rats which alter fertility significantly (Luboshitzky *et al.,* 2002; Awad *et al*., 2006; Ortiz *et al*., 2011 and Sarabia *et al.,* 2011).

Ramachandran and Thakkar (2011) evaluated the toxicity of neonatal melatonin administration with 40 μg melatonin/animal/day exposed from day 0 to day 21 postpartum at 16:30 hours. From this finding, neonatal melatonin administration has favorable influence on the adult ovarian functions marked by significantly increased number of primordial follicle, primary follicle, secondary follicle, antral follicle in 22, 45 and 90-day-old ovaries as compared to the control groups. In the 90-day-old ovary, there was increased the number of corpora lutea in melatonin treated rats. However, the body weight of melatonin treated animals was significantly decreased at all ages of study (in the 22, 45 and 90) day-old ovaries respectively. Likewise, the numbers of atretic follicles in ovaries of melatonin treated group were reduced. Thus, melatonin treated group in the 22, 45 and 90- day-old ovary had no difference in ovarian volume and relative ovarian weights as compared to control rats. Since there is significant increase in primordial, primary, secondary and antral follicles, neonatal melatonin seems to have a favorable influence on the survival of follicles on a long term basis (Ramachandran and Thakkar, 2011).

On the other hand, melatonin improves the quality of oocytes by preventing degeneration as well as by preventing intrafollicular lipid peroxidation in the human ovaries. It was suggested that, melatonin regulates ovarian functions by way of progesterone production, LH receptor expression as well as GnRH and GnRH receptor gene expression through melatonin receptors in the human granulosa and thecal cells (Woo *et al*., 2001 and Takasaki *et al*., 2003).

Rönnberg *et al*. (1990) and Nakamura *et al*. (2003) have shown that high levels of melatonin are found in human preovulatory follicular fluid at concentrations which are much higher than those in serum. It has been reported that the follicular fluid melatonin levels depend on the follicular growth (the larger the follicle the higher the melatonin concentration). The ability of melatonin to promote embryo development in different species has correspondingly been reported. When oocytes are incubated in medium with melatonin supplementation during *in vitro* maturation, they have lower levels of ROS than control (without melatonin treatment) oocytes. When mouse embryos were cultured in medium containing melatonin, increased blastocyst development rates were observed. This suggests that melatonin is involved in embryo development (Jahnke *et al*., 1999 and Ishizuka *et al*., 2000).

Since melatonin secretion has an inhibitory influence on the hypothalamic secretion of GnRH in humans. It is therefore speculated that before puberty, melatonin concentrations are too high thus inhibiting the hypothalamic activation. But prior to puberty, the levels of melatonin decline below the threshold value thus forming the trigger signals of GnRH from the hypothalamus which leads to the onset of pubertal changes. Therefore, it is the decline of melatonin levels that trigger puberty and high nocturnal melatonin secretion in children delays puberty (Buchanan and Yellon, 1991). Moreover, the effect of melatonin administration during fetal stage has an altered neonatal hormonal status more particularly with reference to LH and prolactin and a sexual difference marked by elevated LH levels until the prepubertal period in female offsprings born to melatonin treated mothers and, decreased LH level in male offsprings. Both pinealectomy of the mother or melatonin treatment could affect fetal development and influence the postnatal ontogeny of the hormones involved in the neuroendocrine reproductive axis in developing rats (Diaz *et al*., 1995 cited by Lampiao *et al*., 2013).

Furthermore, maternal melatonin treatment or pinealectomy during gestation has also indicated the requirement of maternal melatonin for normal somatic growth and postnatal development of the reproductive organs of the offsprings (Diaz *et al*., 1999). In support to the findings of Diaz *et al*. (1995); Diaz *et al*. (1999) and Ramachandran and Thakkar (2011), administration of fetal neonatal melatonin had no significant difference in ovarian growth. However, administration of neonatal melatonin cause hormonal disturbances known to influence the adult phenotype and physiology by inducing plasticity changes at critical phases of development, gained recognition as hormonal programming and altered germ cell kinetics and hormonal axes (Lee *et al*., 2001 and Dufty *et al*., 2002).

5. CONCLUSION

In recent years, many studies have been focusing on the role of melatonin in the process of reproductive system. Since spermatogenesis is a long, complex and finely tuned process, the developing sperm cell is sensitive to endogenous or exogenous stresses. Although, the germ cells are more resistant to adverse conditions including ionizing radiation, exposure to reproductive cytotoxic agents (administration of extrinsic doses of melatonin) also known to affect testicular function; morphology and spermatogenesis and damage testicular cells or germ cells at different stages of differentiation, leading to a temporary or permanent impairment of fertility in males.. Its effect increased as dose of melatonin and time of exposure increased. After treatment of rats with extrinsic doses of melatonin revealed inhibited spermatogenesis activity, tubular degeneration, necrosis, obstruction of tubular lumen and lymphocytic infiltration. Administrations of excess melatonin caused marked reductions in absolute and relative testicular weight as well as their size of rats. So, when the rats treated with the higher dose of melatonin they revealed histopathological alterations in both the seminiferous tubules and interstitial tissues with decreased semen quality, sperm concentration, level of testosterone and exhibited sign of pyknosis in some nuclei of spermatogonia, degeneration of primary spermatocytes and hypoplasia of interstitial tissue, many seminiferous tubules were severely damaged, many tubules had only Sertoli cells and spermatogonia. The prolonged and uncontrolled use of melatonin causes various histological and morphological abnormalities in the testes, including reduction of seminiferous tubule length, primary spermatocytes and early spermatids were massively lost from most of the seminiferous tubules. Since, the histological alterations in testes have a strong link with increased dose of melatonin and exposure duration periods, the basement membranes of seminiferous tubules were detached and congestion of intertubular blood vessels was also detected.

Administration of extrinsic doses of melatonin also caused histological effect in ovaries of animal models. Though there was no difference in ovarian volume and relative adult ovarian weights between control and melatonin programmed rats, there was increased density of follicles with significantly higher number of primordial follicle, primary follicle, secondary follicle, antral follicles and corpora lutea. The number of atretic follicles and body weight of melatonin treated rats were significantly decreased.

Since there is increased number of follicles except atretic follicles, neonatal melatonin have a favorable influence on the survival of follicles on a long term basis and could affect fetal development and influence the postnatal ontogeny of the hormones involved in the neuroendocrine reproductive axis in developing rats. Likewise, administration of neonatal melatonin caused hormonal disturbances known to influence the adult phenotype and physiology by inducing plasticity changes at critical phases of development. However, studies have shown that administration of neonatal melatonin contributes to oocyte maturation, embryo development, and luteinization of granulosa cells. Clinical analyzes have also demonstrated that melatonin treatment for infertile women increases intra-follicular melatonin concentrations, reduces intra-follicular oxidative damage and increases the chances of pregnancy.

6. RECOMMENDATIONS

In this project paper of the histological effects of melatonin on testes and ovaries of animal models, the following important concerns are recommended:

Despite the effect of melatonin on testes and ovaries of the animal models, more investigations are required to elucidate the mechanism of melatonin in another system.

The excess administration of melatonin inhibits production of sex hormones. However, further investigation needs to be performed in order to understand the mechanism underlying the effects on gonadothropin releasing hormone secretion on sperm production.

More carefully designed studies are needed to offer clear histopathological effects of melatonin on the testes and ovaries of animal models with less dosage, graded exposure duration and appropriate animal models in consideration.

Further study is needed to find genetic and genomic markers that could identify individual's susceptibility to melatonin side effects and before direct using of them.

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