REPUBLIC OF TURKEY
ACIBADEM MEHMET ALİ AYDINLAR UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

THE EFFECT OF MELATONIN ON BLOOD TESTIS BARRIER INTEGRITY TO RATS FED WITH HIGH FAT DIET: MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATION

NİLSU ATAY

MASTER THESIS

DEPARTMENT of MEDICAL BIOTECHNOLOGY

SUPERVISOR
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THESIS APPROVAL

Department:	Medical Biotechnology.
Program:	Medical Biotechnology Master Program.
Thesis Title:	The Effect of Melatonin on Blood-Testis Barrier Integrity to Rats Fed with High Fat Diet: Morphological and Biochemical Investigation.
Student's name and Surname:	Nilsu Atay.
Date of Defance:	21/05/2019

This is to certify that Ihave examined this copy of master thesis. I have found that she/he prepared after fulfilling requirements specifed in the associated legislalations before the final examining committee whose signatures are below.

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STATEMENT

I declare that this thesis study is my own work. I have no unethical behavior in all stages from the planning of the thesis to the writing. I have obtained all the information in this thesis within the academic and ethical rules. I refer to all the information and interpretations that have not been obtained by this thesis. These references have been included in the part of references. There is no violation of patent and copyright rights during the study and writing of this thesis.

Nilsu ATAY

ACKNOWLEDGMENT

I would like to express my special thanks to my thesis supervisor, Prof. Dr. Serap ARBAK. I deeply appreciate her great efforts for my thesis. Working with Prof. Dr. Serap ARBAK has provided a great contribution to my academic perspective.

I would like to thank Assist. Prof. Deniz YÜCEL for her support.

I would like to send my gratitude to Dr. Merve AÇIKEL ELMAS for her enormous contribution to assist me during my experimental studies and tissue processing.

I would like to thank Prof. Dr. Fikriye URAS and Assoc. Dr. Özlem BİNGÖL ÖZAKPINAR for biochemical analysis.

I am grateful for my family and my fiancée Sercan TOSUN for their supports during my thesis.

This study was supported by Acıbadem Mehmet Ali Aydınlar University ABAPKO. (2017/01/11)

March, 2019

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LIST OF SYMBOLS/ ABBREVIATIONS

ABP: Androgen-binding protein

AFMK: Acetyl-N-formyl-5-methoxykynurenamine

ANOVA: Analysis of variance

CRP: C-Reactive protein

cAMP: Cyclic adenosine monophosphate

DAB: 3, 3-diaminobenzidine

DTNB: 5,5'-dithiobis 2-nitrobenzoic acid

EDTA: Ethylenediamine tetraacetic acid

ELISA: Enzyme-linked immunosorbent assay

FSH: Follicle stimulating hormone

GSH: Glutathione

HPG: Hypothalamus-Pineal-gonadal axis

IL-6: Interleukin-6

JAM: Junctional adhesion molecule

LH: Luteinizing hormone

MDA: Malondialdehyde

PPT1: Palmitoyl-protein thioesterase 1

PBS: Phosphate buffered saline

ROS: Reactive oxygen level

SOD: Sodium dismutase

TG: Triglyceride

TNF-α: Tumor necrosis factor-alpha

TUNEL: Terminal deoxynucleotidyl transferase dUTP Nick End Labeling

ZO-1: Zonula occludens-1

ZO-2: Zonula occludens-2

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SUMMARY

Effect of exogenously administered melatonin in high-fat diet has been investigated on the basis of morphology and oxidative stress markers of testis tissue by morphological and biochemical methods. Thirty-two Wistar albino male rats were fed with standard (STD group; a diet containing 6% fat) or high-fat diet (HFD group; a diet containing 45% fat) for 14 weeks. Melatonin (25μg/mL) was added to the drinking water of half of the experimental animals constituting the STD+MEL and HFD+MEL groups. Animals were weighed regularly during the experiment and all data were analyzed statistically. At the end of the 14th week, testis tissues were removed under deep anesthesia from all groups and processed for light microscopical and biochemical analysis. Following hematoxylin-eosin (H&E) and periodic acid-Schiff (PAS) stainings, seminiferous tubules were scored as normal, regressive, degenerative and atrophic. Immunohistochemistry for ZO-1 and TUNEL were also applied to the tissue samples. Testis tissues were examined biochemically by using malondialdehyde, glutathione, IL-6 and TNF-α and SOD criteria. In addition, testosterone, glucose, triglyceride, FSH, LH, and leptin levels were determined in blood serum samples. Based on the histopathological scoring, regular seminiferous tubule morphology was observed in STD and STD+MEL groups. Increased number of regressive and degenerative tubules, impairment of basement membrane, and significant cell debris in the lumen of the seminiferous tubule were among the important morphological findings HFD group. Seminiferous tubules showed normal tubular morphology in HFD+MEL group. While ZO-1 immunoreactivity was higher in STD, STD+MEL and HFD+MEL groups, HFD group showed a weak ZO-1 immunoreactivity. TUNEL positive cell number was less in STD, STD+MEL, HFD+MEL compared to HFD group. In HFD group, a prominent increase in glucose, triglyceride, MDA, leptin levels and a significant decrease in GSH, FSH, LH and testosterone levels including SOD activity, were observed. In HFD+MEL group, all these histological and biochemical findings seemed to be ameliorated compared to the HFD group. In conclusion, this study shows that obesity deteriorated seminiferous tubule structure by causing oxidant effect, and melatonin was ameliorated testis degeneration by reducing reactive oxygen species.

Keywords: Blood-testis barrier, Melatonin, Microscopy, Obesity, Testis.

ÖZET

Yüksek Yağlı Diyet ile Beslenen Sıçanların Kan Testis Bariyer Bütünlüğü Üzerine Melatoninin Etkisi: Morfolojik ve Biyokimyasal Çalışma.

Yüksek yağlı diyetle beslenmiş sıçanlarda ekzojen olarak verilen melatoninin testis dokusunun morfolojisi ve oksidatif stres belirteçleri üzerine etkisi bu çalışmada morfolojik ve biyokimyasal yöntemler eşliğinde incelenmiştir. 32 adet Wistar albino cinsi erkek sıçan 14 hafta boyunca standart (STD grubu; %6 yağ içeren yem) veya yüksek yağlı yem (YYD grubu; %45 yağ içeren yem) ile beslendi. STD ve YYD gruplarını oluşturan deney hayvanlarının yarısının içme sularına melatonin (25µg/mL) eklendi (STD+MEL ve YYD+MEL grupları). On dört haftanın bitiminde derin anestezi altında tüm gruplardan alınan testis dokuları ışık mikroskopisi ve biyokimyasal analizlere yönelik olarak hazırlandı. Hematoksilen-eozin (H&E) ve Periyodik asit- Schiff (PAS) boyamalarını takiben histopatolojik skorlama sonrası seminifer tübüller normal, regresif, dejeneratif ve atrofik olarak değerlendirildi. Doku örneklerine ayrıca ZO-1 ve TUNEL immünhistokimyası uygulandı. Testis dokuları biyokimyasal olarak malondialdehid, glutatyon, IL-6 ve TNF-α ve SOD kriterleri bazında incelenirken, serumda testosteron, glukoz, trigliserid, FSH, LH ve leptin seviyeleri belirlendi. Hayvan ağırlıkları deney süresince düzenli olarak tartılarak tüm veriler istatistiksel olarak analiz edildi. Histopatolojik skorlama bazında STD ve STD+MEL gruplarında normal seminifer tübül morfolojisi gözlenirken, YYD grubunda regresif ve dejeneratif tübül sayısında artış, bazal membranda bozulma ve seminifer tübül lümeninde çok fazla döküntü hücresi izlendi. YYD+MEL grubunda ise normale yakın seminifer tübül morfolojisi gözlendi. ZO-1 immünreaktivitesi STD ve STD+MEL grubunda yüksek pozitif olarak izlenirken, YYD grubunda immünreaksiyon azalmıştı. ZO-1 immünreaktivitesi YYD+MEL grubunda orta şiddette izlendi. STD, STD+MEL ve YYD+MEL gruplarında TUNEL pozitif hücre sayısı YYD grubuna kıyasla daha azdı. YYD grubunda, glukoz, trigliserid, MDA ve leptin seviyesinde artış görülürken, GSH seviyesinde ve SOD aktivitesinde, FSH, LH ve testosteron seviyelerinde ise azalma görüldü. YYD+MEL grubunda ise YYD grubuna kıyasla tüm bu histolojik ve biyokimyasal bulguların düzeldiği gözlendi. Sonuç olarak, obezitenin oksidan bir etki oluşturarak, seminifer

tübül yapısını bozduğu, melatoninin ise obezite sonucu oluşan hasarı, oluşan reaktif oksijen türevlerini azaltma yolu ile düzelttiği sonucuna varılmıştır.
Anahtar Kelimeler: Kan testis bariyeri, Melatonin, Mikroskopi, Obezite, Testis.

1. INTRODUCTION

Obesity is one of the most common health problems involving both genders and all age ranges in developing countries (1). Obesity can be defined as an excessive accumulation of fat in adipose tissue in the body. According to the World Health Organization, excessive weight and obesity are one of the main risk factors in cancer, the pathogenesis of many chronic diseases such as Type II diabetes, hypertension, cardiovascular diseases and reproductive disorders such as infertility (2). Although enormous efforts have increased to reduce the epidemic of obesity, such as healthy diets, physical activity, and pharmacological treatment, obesity is not only seen in the adult population but also in children (3). For these reasons; medical complications from obesity is a significant. In recent years, the mechanisms underlying the development of obesity have been extensively investigated. These mechanisms include behavioral such as calorie-rich diets, low physical activity, and sleep deprivation, as well as intrinsic factors such as impaired fat tissue regulation, chronic inflammation, and oxidative stress. Sleep deprivation is another problem thought to include the pathogenesis of obesity in the industrialized world (4). Epidemic studies have revealed the relationships between chronic sleep deprivation and long-term weight gain (5, 6, 7).

Circadian disorders affecting the endocrine function of fat tissue are associated with obesity. Leptin and adiponectin are adipokines producing from fat tissue associated with energy balance and have strong relationships with glucose and lipid metabolism. Circadian rhythm, which is impaired in obesity, also causes deterioration in the synthesis and secretions of these hormones. Hyperleptinemia results in leptin resistance and hypoadiponectinemia is associated with the pathophysiology of obesity-related diseases. Melatonin deficiency is one of the consequences of sleep deprivation and has been shown to be associated with obesity.

Melatonin is a pineal secretory product that acts as an antioxidant and anti-inflammatory agent, involved in numerous events such as the regulation of internal biological clock and energy metabolism. There are many publications supporting the

beneficial effects of melatonin supplementation on obesity and obesity-related complications (8).

Circadian rhythms (9, 10) form basic biological processes. These rhythms are synchronized with the dark and light cycle; the periodic organization of biochemical; molecular and physiological processes and genetically encoded molecular precursors (9, 10). The number of publications showing the regulation of metabolism and energy balance with the circadian clock system is increasing (6,11, 12, 13, 14). Circadian rhythm deteriorates due to lifestyle changes, increased awakening time, use of artificial light, television and the use of computer monitors. The impairment of the secretion pattern of melatonin, which is a hormone involved in the regulation of the circadian clock in mammals, is one of these consequences.

Melatonin deprivation and obesity have been associated, shown in several studies (11, 12, 13). In addition, recent studies have shown promising results in the role and complications of melatonin in the prevention of obesity (15, 16, 17). The antioxidant structure of melatonin is stimulating the immune system, activating brown adipose tissue, and metabolic rate and chronobiological agent regulating the energy balance in the organism (11, 13, 18, 19, 20, 21).

Fat tissue, which serves as the main reserve of long-term energy, is very important for ensuring energy balance. Fat tissue constitutes 10 to 15% of body weight in men and over 50% in obese men (22). The fat tissue does not consist only of adipocytes. Connective tissue matrix, nerve tissue, and macrophages constitute 50% of the total cell content of this tissue (23, 24). Brown adipose tissue is found not only in children but also in adults (25). Because of its high metabolic activity, it plays a role in thermogenesis and participates in energy expenditure (24, 26, 27). The main role of white adipose tissue is lipid storage, also is a very important endocrine organ.

In obesity, many mechanisms are involved in the pathogenesis of white adipose tissue. Initially, the immune cell composition of white adipose tissue changes

because of hypertrophy and hyperplasia of adipocytes. This situation creates a modification in the adipokine secretion profile because of the dysfunction of white adipose tissue (28, 29, 30). Secondly, hypoxia and necrosis of adipocyte are observed in obesity with decreasing white fat tissue capacity to expand to the capillaries surrounding the adipocytes (31). These causes also leads to the development of some metabolic complications such as insulin resistance and dyslipidemia. Modifications in adipose tissue are associated with low-level inflammation and increased oxidative stress (32, 33). Oxidative stress is observed with increased reactive oxygen species (ROS) level. It is seen that ROS increases in obese individuals (34). Adipokines, including adiponectin and leptin, affect energy use and production. Modifications to their secretion are thought to play a major role in the pathophysiology of obesity and metabolic dysfunction such as obesity-induced metabolic syndrome, Type II diabetes and atherosclerosis (35, 36). Adipokines also influence the synthesis of inflammatory mediators in the development of chronic low-level inflammation in obesity (37). It is known that obesity changes in cellular and molecular levels, protein structure and increases oxidative stress and apoptosis (38). Lipid peroxidation and oxidative modification of protein molecules are the most important oxidative damage mechanisms seen in tissues (39).

The aim of this study is to investigate the effects of administration of melatonin on testicular tissue of high fat diet-induced obese rats, by morphologic immunohistochemical and biochemical methods.

2. BACKGROUND INFORMATION

2.1. Histology of Testis

The testis is part of the male reproductive system. Testis has responsibility for the formation of male gametes (spermatogenesis) and synthesis and secretion of testosterone (steroidogenesis) (40).

Testis, paired and ovoid shaped structure organs, are suspended in the scrotum where outside of the body (Figure 1). During development of testis, primordium germ cells migrate to the genital ridges and differentiate into testis via induction of the testis-determining factor. Testis develops retroperitoneally on the posterior wall of the abdominal cavity. During descending of testis, they carry some part of peritoneum which constitutes tunica vaginalis. Tunica vaginalis, lining the anterolateral region of each testis permits mobility of testis in the scrotum. Tunica albuginea consists of dense irregular connective tissue capsule that covers each testis. A loose connective tissue, tunica vasculosa that is rich in blood vessels is found under the tunica albuginea. Tunica albuginea becomes thicker in the posterior side of the testis to form mediastinum of testis. Connective tissue septa from the mediastinum divide each testis into nearly 250 pyramidal compartments, called as lobuli testis. Each lobule consists of highly convoluted and blindly ending seminiferous tubules and intertubular connective tissue containing Leydig (interstitial) cells. End of seminiferous tubule is opened (connect with) to the short and straight tubules known as tubuli recti (straight tubule). These tubules lined with simple cuboidal epithelium, connect the seminiferous tubules to rete testis (Figure 2). Rete testis, lined with simple cuboidal or low columnar epithelium, is a network system within the mediastinum testis. Spermatozoa in the seminiferous tubules first enter the rete testis via tubuli recti, then pass through 10 to 20 ductuli efferentes (efferent tubules, efferent ductules) which are connected with epididymis. Both the testis and the epididymis are hanged by the spermatic cord containing vas deferens, spermatic artery, venous and lymphatic plexuses in the scrotal sac (41).

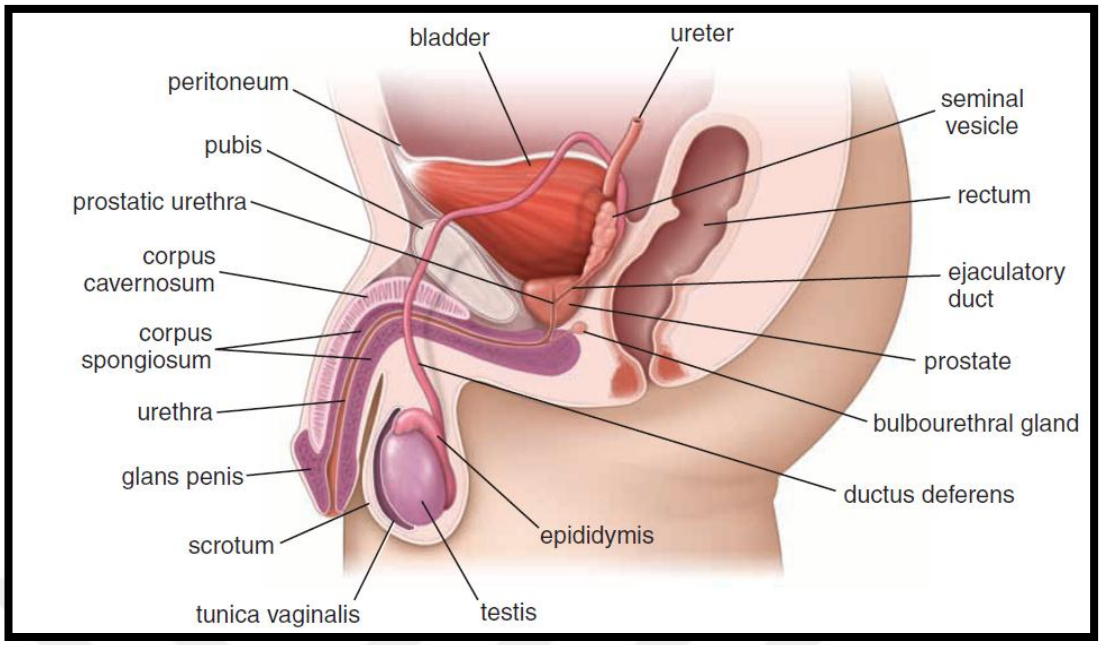


Figure 1. Male reproductive system (Pawlina W, Ross MH. Histology a Text and Atlas with Correlated Cell and Molecular Biology. 8th edition. China: Wolters Kluwer Health, 2016).

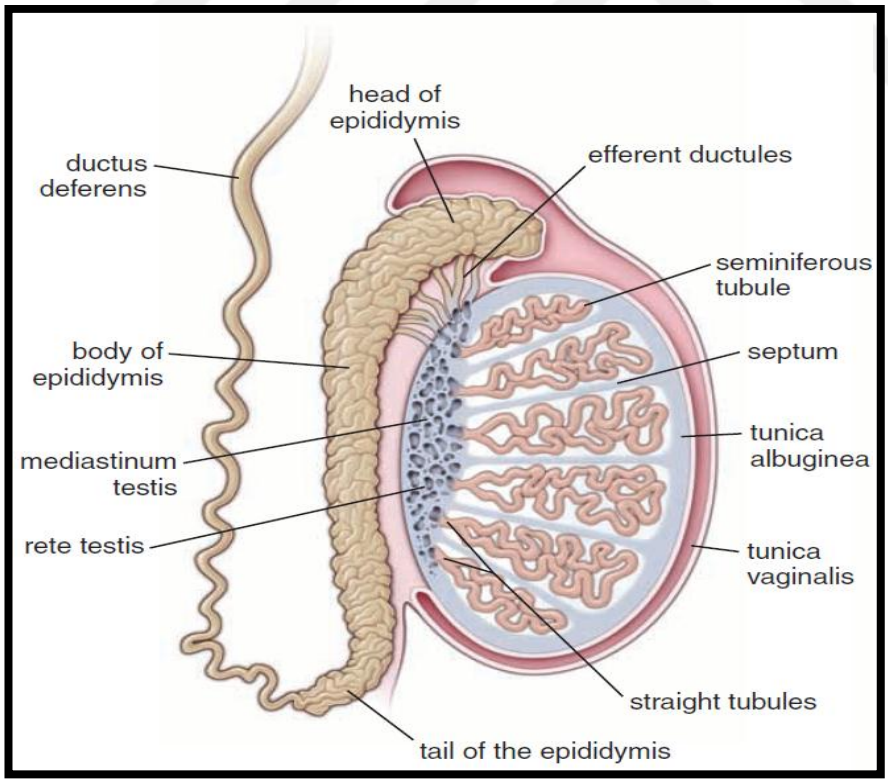


Figure 2. Structure of testis, epididymis and its components (Pawlina W, Ross MH. Histology a Text and Atlas with Correlated Cell and Molecular Biology. 8th edition. China: Wolters Kluwer Health, 2016).

2.1.1 Seminiferous tubules

Highly convoluted seminiferous tubules are approximately 30-70 cm long, 150-250 μm in diameter. Between the seminiferous tubules loose connective tissue, known as tunica (lamina) propria or peritubular tissue is found. Blood vessels, macrophages, and Leydig cells are found in lamina propria. Leydig cells are responsible for the production of testosterone. Each seminiferous tubule lies on the basal lamina. Smooth muscle-like myoid cells are found in the surroundings of the basal lamina of seminiferous tubules. They permit some contractions which help to the movement of spermatozoa. Seminiferous tubules constitute thick, specialized epithelium, called as germinal (seminiferous) epithelium which consists of two different kinds of cells: Sertoli cell as a support cell and spermatogenic cell (male immature germ cell) at different stages of spermatogenesis (Figure 3).

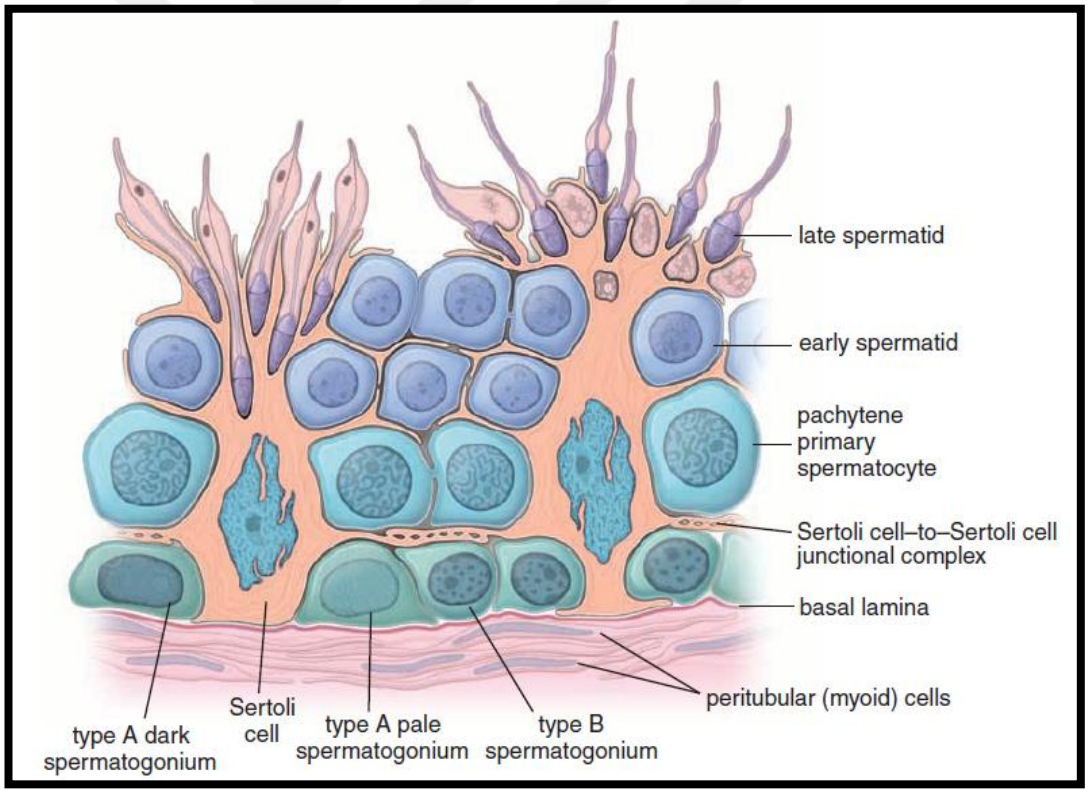


Figure 3. Part of germinal epithelium of seminiferous tubule (Pawlina W, Ross MH. Histology a Text and Atlas with Correlated Cell and Molecular Biology. 8th edition. China: Wolters Kluwer Health, 2016).

2.1.1.1 Sertoli cells

Sertoli cells are known as supporting or sustentacular cells and do not replicate themselves after puberty. Sertoli cells are columnar cells and have irregular wide apical and lateral processes. These processes surround the adjacent spermatogenic cells and fill the spaces between Sertoli and spermatogenic cells. They lie on basal lamina. Sertoli cells have an apparent, ovoid nucleus with an oversized (big), centrally located nucleolus. The cytoplasm of Sertoli cells has inclusion referred as crystalloids of Charcot-Böttcher and its composition and function are unknown. When Sertoli cells are examined at ultrastructural level by electron microscope, they contain a large amount of smooth endoplasmic reticulum, some rough endoplasmic reticulum, well-developed Golgi complex, abundant mitochondria, and lysosomes. They constitute plentiful cytoskeletal elements that give support structurally for spermatogenic cells. Adjacent Sertoli cells form Sertoli-Sertoli cell junction that is typical tight junctions (zonulae occludentes). This junction divides the seminiferous epithelium into two compartments: the basal compartment and adluminal compartment (Figure 4). The division of the seminiferous epithelium creates the blood-testis barrier in the tubule. The basal compartment is extended between basal lamina and occluding junctions, in contact with blood and lymph circulation. Dark Type A dark spermatogonia lie in this compartment. The adluminal compartment is located between tight junctions and lumen of seminiferous tubule, isolated from blood and lymph. Thus, spermatogenic cells are dependent on the Sertoli cell for their nourishment. Developing spermatogenic cells are found in adluminal compartment. Adluminal compartment protects the developing gametes from immune system cells and harmful chemicals. Sertoli cells have many functions in the seminiferous tubules:

- Blood-testis barrier is created by tight junctions between adjacent Sertoli cells
- Residual body (the remnant of cytoplasm during spermiogenesis) is phagocytosed.
- Developing spermatogenic cells are supported physically and nutritionally.
- They are responsible for synthesis and release of androgen-binding protein which increases testosterone hormone concentration in the tubule.

- ❖ Antimüllerian hormone which prevents the formation of müllerian duct is synthesized and secreted by Sertoli cells during embryogenesis.
- ❖ Testicular transferrin is synthesized and released for maturation of spermatogenic cells.
- ❖ Inhibin hormone which suppresses secretion of follicle- stimulating hormone is synthesized and released.

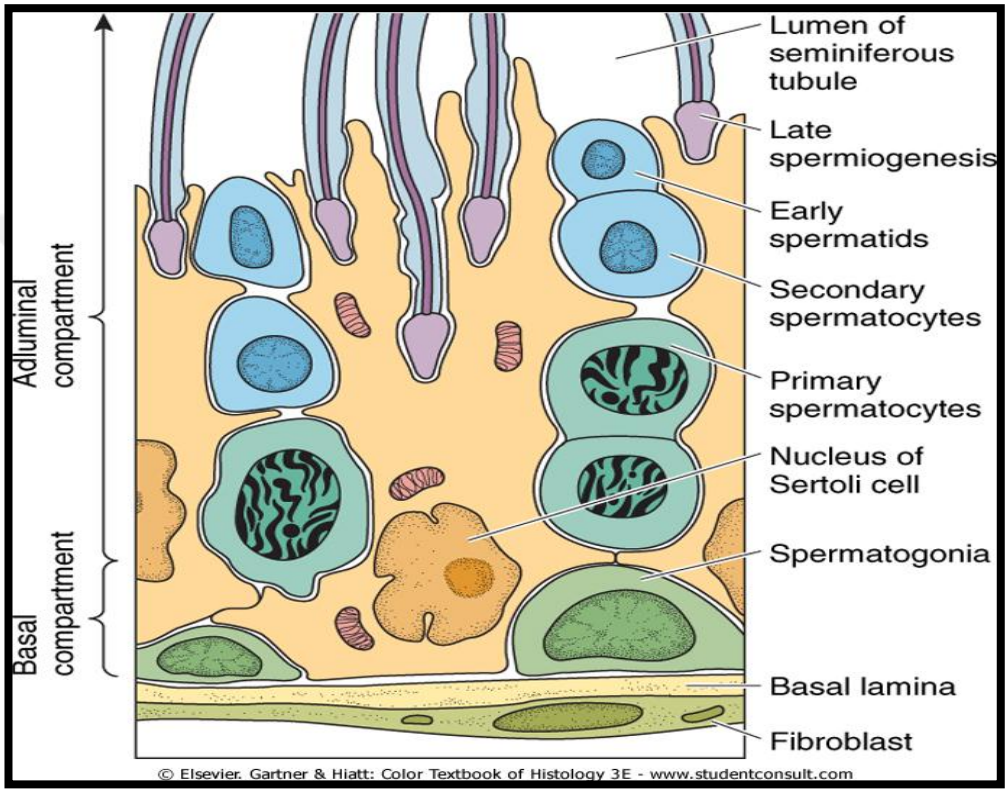


Figure 4. Basal and adluminal compartments of seminiferous epithelium (Gartner L, Hiatt J. Color Textbook of Histology 3rd edition. Saunders, 2006).

2.1.1.2 Spermatogenic cells

Spermatogenic cells are differentiated into mature sperm through a certain process called spermatogenesis. During development of testis, spermatogenic cells were constituted from primordial germ cells coming from yolk sac. Spermatogenic cells are located between adjacent Sertoli cells at various stages of maturation. Immature spermatogenic cells, called type A dark spermatogonia, are stayed on the basal lamina, whereas the mature cells formed by meiosis, called spermatids, are found on the apical part of the Sertoli cells. Spermatids form the boundary of the lumen of seminiferous tubules.

Spermatogenesis is the process of maturation of sperm and consists of three phases: spermatogonial phase (spermatocytogenesis), spermatocyte phase (meiosis) and spermiogenesis.

In the spermatogonial phase, the spermatogonia undergo mitosis to form daughter cells and primary spermatocytes. These cells are diploid cell (2n) and lie in the basal compartment of seminiferous tubule. Human spermatogonia are classified as three types according to the appearance of nuclei in routine histological preparations.

Dark type A spermatogonia are 12 μm in diameter and have ovoid-shaped nuclei. These cells are darker due to heterochromatin in their nuclei. These are stem cells. They undergo mitotic division to form daughter cells as reserve cells and give rise to another type of spermatogonia called pale type A spermatogonia. Pale type A spermatogonia resemble dark type A spermatogonia; the only difference is their nuclei containing euchromatin. In routine preparation, their nuclei are paly stained. They undergo mitosis to proliferate by induction of testosterone hormone. They produce pale type A spermatogonia and type B spermatogonia. Type B spermatogonia are similar to pale type A spermatogonia, with round nuclei presenting condensed chromatin. They give rise to primary spermatocytes by mitotic division.

In every step of spermatogonial phase, dark type A spermatogonia are divided into two pale type A spermatogonia. Pale type cells are connected by cytoplasmic bridges. These connections are present every mitosis and meiosis steps of spermatogenesis. The cytoplasmic bridges remain until the end of sperm maturation and are necessary for the synchronous development of each clone from type A dark spermatogonia. After the formation of primary spermatocyte, they undergo meiotic division.

In the spermatocyte phase (meiosis), primary spermatocytes undergo meiosis to reduce amount of DNA and chromosome number to form spermatids. Primary spermatocytes are formed by mitotic division of type B spermatogonium. They replicate their DNA amount before the start of meiosis. Thus, each primary spermatocyte contains normal chromosomes number (2n) and double DNA amount (4d), because each chromosome consists of two sister chromatids. In meiosis I, the chromosomes number and the DNA amount are halved. At the end of the meiosis I, secondary spermatocytes are formed and contain n chromosome number and 2d DNA amount. Secondary spermatocytes enter meiosis II without replication of DNA. Newly formed cells are called spermatids. Spermatids contain 22 autosomal and one Y or one X chromosome. They are located near lumen of seminiferous tubule and still have a connection with Sertoli cells.

In spermiogenesis phase, spermatids change their morphologies. Thus, small and round spermatids change the organization of their organelles, form the flagellum, and have a decreased volume of cytoplasm. Spermiogenesis consist of four phases; Golgi phase, cap phase, acrosomal phase, maturation phase.

In the Golgi phase, hydrolytic enzymes are produced on the rough endoplasmic reticulum, altered in the Golgi apparatus. They pass to the trans-Golgi network to package as small, vesicles containing preacrosomal granules. These vesicles come together to form an acrosomal vesicle. Acrosomal vesicle grows its content and binds to nuclear envelope to form the anterior pole of developing spermatozoa. Centrioles of the spermatozoa migrate to the posterior pole to establish axoneme of sperm.

In the cap phase, acrosomal vesicle enlarges and partially covers the nucleus of developing sperm. This vesicle becomes acrosome also called an acrosomal cap.

In the acrosomal phase, spermatids with changing morphology are more similar to mature sperm. They elongate to become flattened and have condensed nucleus. Their head is embedded in the Sertoli cells whereas their flagellum extends into

lumen of seminiferous tubule. Cylindrical microtubules become organized to form a cylindrical structure, manchette which maintains the elongation of the spermatid.

Maturation phase is the last phase that spermatid transforms into the mature sperm. Spermatids discard their cytoplasm around the flagella and Sertoli cells phagocytose remnant of cytoplasm. Thus, cytoplasmic bridges are disrupted. Spermatozoa become free and are released into the lumen of seminiferous tubule.

Spermatids are no longer interconnected and detached from Sertoli cells. This process is called spermiation. The number of sperms in the ejaculate is associated with the rate of spermiation in the testis. Newly released sperms which are inactive, gain motility in the epididymis. Spermatogenesis in humans takes approximately 74 days.

2.1.1.3 Leydig cells

Leydig cells (interstitial cells) are big, polygonal shaped (15 μm), eosinophilic cells with centrally located nuclei, have a characteristic feature in cytoplasm, called ascrystal of Reinke. These cells synthesize and secrete steroid hormone, testosterone, and therefore contain plenty of smooth endoplasmic reticulum, well- developed Golgi complex and mitochondria with tubulovesicular cristae. Testosterone secretion is crucial for embryonic development, normal development of gonads in male fetus and sexual maturation; therefore Leydig cells synthesize testosterone throughout early development of embryo for sexual differentitation. In puberty, testosterone secreted from Leydig cells is responsible for sperm production, and development of secondary sex characters. In adulthood, secretion of testosterone is necessary to ensure the continuity of spermatogenesis (40).

2.1.2. Blood-testis barrier and tight junctions

During the spermatogenesis, newly formed cells have a different number of chromosome and express different cell surface receptor thus, these cells are considered foreign by immune system cells. They would attack them; therefore, the blood-testis barrier eliminates any interaction between developing sperms and the

immune system and prevents immunoglobulin to pass through the inner part of seminiferous epithelium. The blood-testis barrier thus protects the seminiferous epithelium from an autoimmune reaction. The presence of this barrier between the blood and the inner part of the seminiferous epithelium limits the passage of very certain substances. Capillary in testis is fenestrated and allows free passage of large molecules.

Adjacent Sertoli cells are connected with each other by tight junctions that compromise the blood-testis barrier. This barrier protects the germ cells from immune system and harmful agents. The tight junctions in the seminiferous epithelium are formed by tight junction proteins between two Sertoli cells located close to the basal lamina of the seminiferous epithelium. These tight junctions divide the seminiferous epithelium into two parts as basal compartment and adluminal compartment. The basal compartment contains spermatogonium and preleptotene spermatocytes while the adluminal compartment contains other germ cells (41). The tight junction site in the testis consists of a series of peripheral membrane proteins and three different tight junction integral membrane proteins. These are; zonula occludens varieties, zonula occludens-1 (ZO-1), zonula occludens-2 (ZO-2), occludin, claudin, as tight binding proteins. The tightest junction on the surface is the zonula occludens. It shows folding and curling to form a network-like structure. This structure is important for passage of spermatogenic cells and molecules. The occludin located on the blood-testis barrier plays an important role in the regulation of this barrier. Occludin is also important for localization of ZO-1 tight junction protein.

2.1.3. Histophysiology of testis

Heat is very important in the regulation of spermatogenesis. Spermatogenesis is heat- dependent event that occurs at 34 °C and controlled by several mechanisms. A rich venous plexus (pampiniform plexus) surrounds each testicular artery, providing a countercurrent flow that is important in maintaining testicular temperature lower than normal body temperature.

The most important effect on spermatogenesis is endocrine factors. Spermatogenesis depends on the effects of follicle stimulating hormone (FSH) and luteinizing hormone (LH) on cells in the testis. LH acts on Leydig cells, stimulating the production of testosterone, which is necessary for the normal development of spermatogenic series cells. LH binding to LH receptor on the Leydig cell activates adenylate cyclase to produce cyclic adenosine monophosphate (cAMP). Then, cAMP induces protein kinases in Leydig cells. Protein kinase activates cholesterol esterase that cleaves cholesterol from lipid droplets.

Also, cholesterol desmolase activated by LH transforms free cholesterol into pregnenolone. Formed pregnenolone passes through the smooth endoplasmic reticulum and mitochondria till testosterone is formed and is secreted by Leydig cells. However, this is not enough to start and maintain spermatogenesis. Sertoli cells are induced by FSH, gonadotropin hormone, and synthesize and release androgen-binding protein (ABP) that binds testosterone hormone and keep testosterone inside the seminiferous tubules. The environment of seminiferous tubules is suitable for spermatogenesis (42).

2.2. Obesity

Obesity is an energy metabolism disorder caused by excessive white adipose tissue in the body, which can cause mental and physical problems. According to the WHO, obesity which is a worldwide problem is characterized by increased body fat mass compared to lean body mass and obesity is defined as a metabolic disorder caused by higher energy consumed by the nutrients than the energy consumed by the body (2). It is resulting from behavioral, environmental and heritable factors. It is also caused by disrupted chronobiology, circadian misalignment, increased oxidative stress, reduced antioxidant status, high calorie consumption, and insufficient physical activity. Obesity can be treated with healthy diets, physical activity, and pharmacological treatment (3). The risk of cardiovascular disease, type 2 diabetes, hypertension, cancer, and reproductive disorders are increasing in obese people with the deterioration of adipose tissue (hypertrophy, and hyperplasia)

2.2.1 Effect of obesity on male infertility

Obesity is an increasing problem in the world. Obesity is negative effect with male fertility (43). The number of sperms in obese male has decreased in the last 10 years all over the world, and this is one of the proofs of an increase in male infertility as the body mass index increases in the population (44). Obesity not only changes the sperm quality but also changes the structure of seminiferous tubules, spermatogenic germ cells and this effects the spermatogenesis (45). Obesity alters hormone profile in male especially decreasing testosterone and LH levels (46). Conversion of testosterone into estrogen is increased in obese male by aromatase activity which is responsible for production of estrogen. Depend on this, while testosterone hormone level decreases, estrogen level increases in obese people. Increasing estrogen level negatively affects spermatogenesis. (47). White adipose tissue as an endocrine organ secretes adipose-derived hormones and peptides such as leptin. Leptin is a peptide which is produced in white adipose tissue. Leptin has several responsibilities such as regulation of energy expenditure, hematopoiesis, angiogenesis, puberty and reproduction (48). Leptin secretion increases with increasing fat tissue in the body, (49, 50). It causes a significant reduction in the production of testosterone from Leydig cells. Thus, elevated levels of leptin in obese men change the Hypothalamus-Pineal-gonadal (HPG) axis to reduce testosterone production (47).

2.3. Melatonin

The ancient molecule, melatonin (5-methoxy-N-acetyltryptamine, IUPAC name), is found by Aron B. Lerner in 1958 while he was doing an experiment on frog. He thought that this might be used for skin disorders. Melatonin is synthesized in almost all life forms such as bacteria, protozoa, plants, invertebrates, and vertebrates (51). In humans, it is mostly produced and secreted at night in pineal gland (52). Also, it is synthesized in the gastrointestinal tract, skin, lymphocytes (51), epithelial hair follicles, retina, salivary glands, platelets, and developing brain (53). Melatonin has three distinct receptors which are called as MT1, MT2, and MT3. MT1 and MT2 are found in mammals, MT3 are found in amphibian and birds (54). These receptors are G protein-coupled membrane proteins which are present in the cell membrane as dimers and heterodimers. They are expressed in the central nervous

system, retina, immune cells, prostate, ovary, testis, mammary gland, skin, and adipocytes (55).

Melatonin which is the lipophilic molecule is a derivative of tryptophan (54). Melatonin synthesis is activated during dark and inhibited by light. Synthesis of melatonin starts at 8 pm, peaks between 12 pm- 4 am, decreases at day time. Melatonin synthesis is negatively correlated with age (56).

Melatonin is produced by pinealocytes which give a response to photoperiod information collected from retinohypothalamic pathways. Then, the suprachiasmatic nucleus takes the information from light and forwards the impulses to pineal gland to induce secretion of melatonin (53). When stimulation occurs, tryptophan is converted to 5-hydroxytryptophan via hydroxylation. Serotonin is formed from it by an aromatic L-amino acid decarboxylase enzyme. Then, serotonin is acetylated by serotonin N-acetyltransferase. Finally, biosynthesis of melatonin occurs with the help of hydroxy indole-O-methyl transferase (57).

Melatonin is a very effective hormone and has many roles in our body. It is a scavenger of reactive oxygen species (ROS) as an antioxidant (58). Melatonin synthesis creates the circadian rhythm which changes in a body's biological event during 24-hour period; any hormone, enzyme or a certain physiological function in the body with natural increases and decreases during the day.

Melatonin has many other physiological functions such as regulation of immune response, thermoregulation, blood pressure regulation, oncostatic and anti-aging (59, 60). In addition, recent studies have shown that melatonin reduces plasma total cholesterol and triglyceride levels associated with obesity and cardiovascular diseases (61, 59).

2.3.1. Melatonin and obesity

It has been shown that melatonin takes role in metabolic regulation (19, 21) and prevents the obesity by reducing fat accumulation, affecting both energy intake and

energy expenditure, body mass index (62, 63). Melatonin is also involved in energy metabolism 11, 18). Melatonin deficiency due to sleep reduction in aging or night-shift workers was reported to induce insulin resistance IR and glucose intolerance and cause obesity by metabolic circadian rhythm impairment (64). A study has shown that people with sleep problems are more likely to develop obesity. Melatonin production deficiency develops insomnia in individual. On the other hand, sleep problem may cause develops inadequate melatonin secretion (65). When melatonin production decreases, the biological rhythm is affected. Consequently, obesity occurs as a result of insulin resistance, glucose intolerance, sleep disturbance, and metabolic imbalances.

3. MATERIALS AND METHODS

Before the experimental study, ethical approval was obtained from Acıbadem Mehmet Ali Aydınlar University Animal Experiments Local Ethics Committee (05.01.2017- ACU-HADYEK 2017/5). In this study, 32 Wistar albino male rats (200-250 g) provided from Acıbadem Mehmet Ali Aydınlar University Laboratory Animal Application and Research Center were used. All animals were kept on a 12 h light/dark cycle with food available ad libitum, at average room temperature of 21±2 °C. For 11 weeks, they were fed with a high-fat diet (HFD) containing 45% fat and standard diet (66). Melatonin solution (25 μg/mL) was administered to rats via drinking water. The stock solution of melatonin was prepared with 50% ethanol and the final ethanol concentration in drinking water was 0.015% (v/v%). Fresh melatonin solution to be administered to the animals was prepared daily from the stock solution. Water containers were monitored daily. Water containers were also wrapped in foil to protect them from light. Each rat received 90-95% of the daily water requirement of approximately 20 mL during the night period (66). For this reason, 45 mL of water for control groups (STD and HFD) and 45 mL of water containing approximately 500 μg melatonin for the experimental group (STD+MEL and HFD+MEL) were given per day (2 animals/cage). Before starting the experiment, the rats were weighed and randomly divided into 4 groups. The weights of the rats were determined on a weekly basis. The groups and design of the study were as follows:

1. Group: STD (Control) (n=8): Rats were fed with standard chow (STD) for 14 weeks.

2. Group: High Fat Diet (HFD) (n=8): Rats were fed with chow containing high fat (45%, w/w%) for 14 weeks.

3. Group: HFD+Melatonin (n=8): Rats were fed with chow containing high fat for 14 weeks and were given melatonin (25 μg/mL) in drinking water for 14 weeks.

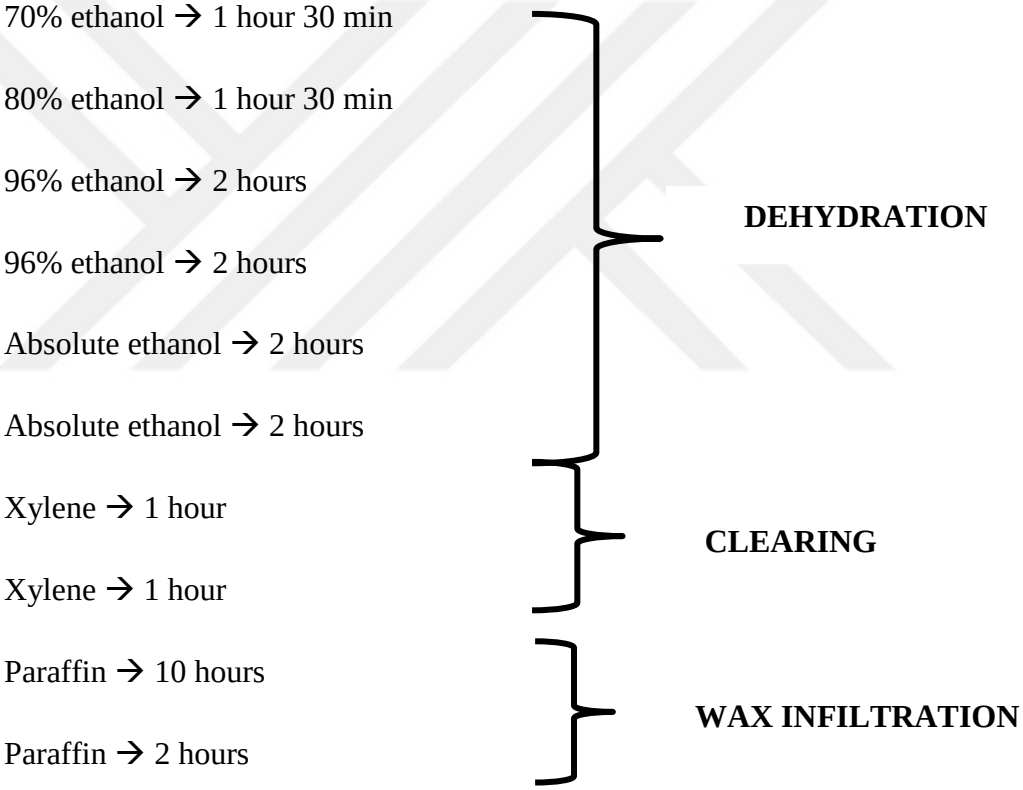
4. Group: STD+Melatonin (n=8): Rats were fed with standard chow (STD) for 14 weeks and were given melatonin (25 μg/mL) in drinking water for 14 weeks.

All rats were sacrificed under deep anesthesia with ketamine (0.9 cc/kg)/ xylazine (0.7 cc/kg). Testis tissues taken from rats were processed for histological, immunohistochemical and biochemical methods. Blood samples were collected by cardiac puncture for biochemical analysis.

3.1. Methods

3.1.1. Tissue processing

Testis tissues were fixed with neutral buffered 10% formalin solution. Fixed testis tissues were kept in tap water for overnight. Then, they were placed in the tissue processor. Tissue processing steps were as follows:



After tissue processing, testis tissues were embedded in paraffin. Tissue sections of 5 μm were obtained following sectioning of paraffin blocks by rotary microtome.

3.1.2. Hematoxylin and eosin staining

Testis tissue sections were deparaffinized with xylene for 40 minutes. Then, they were hydrated with descending series of ethanol (100%, 90%, and 70%) for 10 minutes each. Sections were stained with Mayer's hematoxylin for 5 minutes. They

were immersed in running tap water for 5 minutes and rinsed with distilled water. They were kept in 70% ethanol for 1 minute. Sections were stained with eosin solution for 2 minutes. Following dehydration by ascending ethanol series (90%, 100%) for 1 minute each, testis tissue sections were cleared with xylene 2 times for 1 minute. Then, the sections were mounted with Entellan.

Histopathological Scoring:

The degree of histopathological damage in the sections was evaluated, and the scoring was performed by counting 100 tubules with light microscopy. The histopathological score evaluation of the seminiferous tubules in all experimental groups was performed at x400 magnification from 5 randomly selected areas (67). The scoring was done according to following criteria;

Normal: Normal spermatogenesis and normal intercellular junction.

Regressive: Seminiferous tubules contain one or more defects, the intercellular junction is normal, but the cellular organization is loose, and disrupted (pyknotic nucleus, granular eosinophilic cytoplasm, karyolysis).

Degenerative: Small number of germ cells and Sertoli cells with irregular location and tight junction.

Atrophic: There are only Sertoli cells or a few germ cells. Sertoli cells or germ cells are without tight junctions.

3.1.3. Periodic acid- Schiff reaction

Testis tissue sections were deparaffinized in the xylene for 40 minutes. Then, they were hydrated through the descending ethanol series (100%, 90%, and 70%) for 10 minutes each. They were rinsed with distilled water. The periodic acid solution was applied to the sections for 20 minutes. Tissue sections were washed in running tap water for 5 minutes and rinsed with distilled water. Following application of Schiff reagent to the sections for 20 minutes, sections were kept in sulfurous acid solution for 12 minutes. Tissue sections then were washed with running tap water for 5 minutes and rinsed with distilled water again. They were stained with Mayer's hematoxylin for counterstaining. They were immersed in running tap water for 5

minutes and rinsed with distilled water. They were dehydrated through the ascending ethanol series (70%, 90%, and 100%) for 3 minutes each. Testis tissue sections were cleared with xylene 2 times for 2 minutes. All the sections were mounted with Entellan®.

3.1.4. ZO-1 immunohistochemistry

Five μm thick sections were kept at 37 ° C for overnight. They were placed in xylene for 30 minutes and were hydrated for 10 minutes in absolute alcohol and 96% alcohol. They were placed in 3% hydrogen peroxide solution prepared with methanol for 20 minutes for endogenous enzyme blockage. All sections were rinsed with distilled water before antigen recovery. Sections in EDTA (ethylenediamine tetraacetic acid) buffer was exposed to the 200W microwave oven for 20 minutes. Chilled sections at room temperature were washed with phosphate buffered saline (PBS) 3 times for 5 minutes and then left in blocking solution for 10 minutes. Rabbit anti-ZO-1(1:100) antibody was applied to the sections. Following an overnight incubation at 4 °C, all sections were washed with PBS 3 times for 5 minutes. The biotinylated secondary antibody was applied to the sections for 10 minutes then washed with PBS 3 times for 5 minutes. Streptoavidin peroxidase was applied to the sections for 10 minutes. After washing with PBS 3 times for 5 minutes, AEC chromogen (20 μL AEC chromogen + 1 mL AEC substrate buffer) was applied to the sections for 15 minutes. After washing with distilled water, the sections were mounted with Keiser glycerin gel. In the seminiferous tubules, the degree of ZO-1 immunoreactivity was semiquantitatively evaluated as none (-), very low (+), moderate (++) and intense (+++).

3.1.5. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method

The TUNEL method was performed according to the description by the manufacturer (ApopTag Plus, In Situ Apoptosis Detection Kit, S7101, Millipore). Sections of 5 μm of thickness mounted on positively charged slides were deparaffinized with xylene three times for 5 minutes. They were hydrated through the descending ethanol series (100% ethanol for 2x5 min, 95% ethanol for 3 min,

70% ethanol for 3 min) and then they were washed with PBS for 5 minutes. For protein digestion, Proteinase K (20 µL/mL) was applied on the testis tissue sections for 15 minutes at room temperature. Sections were washed with distilled water two times for 2 minutes, incubated with 3% H_2O_2 prepared in PBS to mask endogenous peroxidase activity for 5 minutes at room temperature and then rinsed with PBS two times for 5 minutes. Then, 15 µL equilibration buffer was applied to the sections. They were incubated for 30 minutes at room temperature. Then sections were incubated with 15 µL terminal deoxynucleotidyl transferase (Tdt) enzyme in a humidified chamber at 37 °C for 1 hour. They were put in a Coplin jar containing stop/wash buffer, agitated for 15 seconds, and incubated for 10 minutes at room temperature. Sections were rinsed with PBS three times for 5 minutes, and then 13 µL anti-digoxigenin peroxidase was applied on each section and incubated in a humidified chamber at room temperature for 30 minutes. Sections were rinsed with PBS four times for 2 minutes. 15 µL of 3, 3-diaminobenzidine (DAB) substrate solution was applied to each section. After 5 minutes, color development was monitored under the microscope. Sections were washed with dH_2O three times for 1 minute then incubated in distilled water for 5 minutes at room temperature. Sections were stained with Mayer's Hematoxylin and dehydrated with 96% alcohol two times for 2 minutes. All sections were mounted with entellan. TUNEL positive cells were counted by examining 20 randomly selected seminiferous tubules. The apoptotic index was calculated by the ratio of the number of seminiferous tubules containing 3 or more TUNEL positive cells to the total number of seminiferous tubules.

3.2. Biochemical Analysis

3.2.1. Biochemical analysis in serum samples

Blood samples taken from the heart before decapitation were taken to dry vacuum tubes without anticoagulant agents. After 30 minutes at room temperature, it was centrifuged at 4000 rpm for 15 minutes and the separated serum samples were stored at -80 °C until the study was performed. The analyses were carried out in Marmara University, Faculty of Pharmacy, Department of Biochemistry.

3.2.1.1. Measurement of glucose, triglyceride, and leptin

Determination of glucose level: Serum samples were dissolved at room temperature after removal from the freezer. Glucose concentration in serum was determined by spectrophotometer (BioTek) using a commercial kit (Biolabo, Glucose GOD-PAP). The tubes prepared as shown in Table 1 were mixed with vortex and left at room temperature for 20 minutes. Then, the tubes were read at 550 nm.

Table 1. Sample preparation to determine glucose concentration in serum.

	Sample	**Standard**	**Blank**
Reactive	1 mL	1 mL	1mL
Sample	10 µL	-	-
Standard	-	10 µL	
Distilled water			10 µL

Serum glucose concentration was calculated with the following formula and the results were given in mg/dL.

$$\text{Glucose amount} = \frac{\text{Absorbance (Sample)}}{\text{Absorbance(Standard)}} \text{x Standard concentration (100)}$$

Determination of triglyceride (TG) levels: Triglyceride concentration was determined using a commercial kit (Biolabo, GPO-PAP) using a spectrophotometer (BioTek). The tubes prepared as shown in Table 2 were mixed with vortex and kept at room temperature for 10 minutes. Then, the absorbances of samples were determined at 500 nm against the blank.

Table 2. Sample preparation to determine serum triglyceride concentration.

	Sample	**Standard**	**Blank**
Reactive	1 mL	1 mL	1 mL
Sample	10 µL	-	-
Standard	-	10 µL	-
Distilled water	-	-	10 µL

$$\text{TG amount} = \frac{\text{Absorbance (Sample)}}{\text{Absorbance(Standard)}} \text{x Standard concentration (200)}$$

Determination of leptin level: Serum leptin concentration was measured using the commercial rat leptin ELISA kit (Bioassay Technology Laboratory). The leptin concentration in the samples was determined using standard leptin graphs generated following the procedures in accordance with the kit procedure and the results were given in ng/mL.

3.2.1.2. Measurement of FSH, LH, testosterone concentration

Serum FSH, LH and testosterone concentrations were measured using the commercial rat FSH ELISA kit, the rat LH ELISA kit, and the rat testosterone ELISA kit (Bioassay Technology Laboratory). The FSH, LH and testosterone concentrations in the samples were determined using standard graphs generated following the procedures in accordance with the kit procedure. Results were given in mIU/L for FSH and LH; for testosterone, nmol/L.

3.2.2. Biochemical measurements in testis tissue samples

Left testicular tissue removed immediately after decapitation was washed with PBS buffer and stored in Eppendorf tubes at -80 °C. On the day of biochemical measurements, tissue samples were homogenized in cold 150 mM KCl solution and tissue homogenate were prepared.

3.2.2.1. Measurement of malondialdehyde (MDA) level

The method developed by Ohkawa et al. (68) was used to determine the levels of MDA levels indicating lipid peroxidation. The principle of the method is based on the measurement of the color intensity of the pink colored complex formed by MDA with thiobarbituric acid at a wavelength of 532 nm.

<u>Reagents used in analysis:</u>

8.1 % sodium dodecyl sulfate

20 % acetic acid

0.8 % 2-thiobarbituric acid

2 mmol/L 1,1', 3,3' tetraethoxypropane

The prepared solutions were added to the test tubes as indicated in Table 3, then vortexed well to mix the solutions. The tubes were kept in boiling water (at least 95

°C) for 1 hour, cooled in running tap water and centrifuged at 5000 rpm for 10 minutes. The absorbance of pink supernatants was read at 532 nm and MDA levels in nmol/g tissue were found by using the standard graph prepared with 1.1', 3.3' tetraethoxypropane.

Table 3. Solutions used to determine MDA level.

	Sample	**Blank**
Homogenate	100 µL	-
8.1% SDS	200 µL	200 µL
20% acetic acid	1500 µL	1500 µL
0.8% TBA	1500 µL	1500 µL
Distilled water	700 µL	800 µL

3.2.2.2. Measurement of glutathione (GSH) level

Glutathione (GSH) acts as an antioxidant molecule in the structural and functional preservation of cell, tissue and organ systems. GSH analysis in testis tissues was performed according to the Beutler method. The principle of the method is that the GSH in the analysis tube reacts with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) to give a yellowish color and the light intensity of this color is read by the spectrophotometer at 410 nm.

Tissue homogenates were centrifuged at 5000 rpm at +4 °C for 20 minutes. The 10% trichloroic acid supernatant was added into supernatant, stirred and centrifuged again at 3000 rpm at 4 °C for 20 minutes to allow the proteins to precipitate. Light colored supernatant samples were used for GSH analysis.

<u>Reagents used in analysis:</u>

10 % trichloroacetic acid

1 % trisodium citrate

0.4 % 5.5'-DTNB

0.3 M disodium hydrogen phosphate

The test tubes were prepared according to Table 4, and the tubes were vortexed to ensure good mixing of the solutions. The absorbance of the colored samples which were kept at room temperature for 5 minutes was read at 410 nm with a

spectrophotometer and GSH levels in μmol/g tissue were calculated by using the glutathione standard graph.

Table 4. Solutions used to determine the GSH level.

	Sample	**Blank**
10% Homogenate	500μL	-
Na_2HPO_4 (0.3M)	4 mL	4 mL
DTNB	500μL	500μL
Distilled water	-	500μL

3.2.2.3. Measurement of sodium dismutase (SOD) level

Wells were labeled as sample, blank 1, blank 2, and blank 3. Twenty μl of sample solution was added each sample and blank 2 well and 20 μl ddH2O (double distilled water) was added to each blank 1 and blank 3 well. WST Working Solution (200 μl) was added to each well, and mixed. Then, 20 μl of Dilution Buffer was added to each blank 2 and blank 3 wells. After that, 20 μl of Enzyme Working Solution was added to each sample and blank 1 well, and then mixed thoroughly. Plate was incubated at 37 °C for 20 min. The absorbance was read at 450 nm by using a microplate reader. The SOD activity (inhibition rate %) was calculated the following equation:

Inhibition rate (%) = [(A blank 1-A blank 3) - (A sample-A blank 2)] / (A blank 1- A blank 3)

3.2.2.4. Determination of inflammation levels

Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels were measured to determine the inflammation in testis tissues. Measurements were done using the commercially available A Bioassay Technology Laboratory Rat TNF-α ELISA kit and Rat IL-6 ELISA kit. TNF-α and IL-6 concentrations in serum samples were determined using standard graphs following the procedures performed in accordance with the kit procedure. The results were given in pg/mL.

3.3. Statistical Analysis

Statistical analysis was performed using Graph-Pad Prism 6.0 (GraphPad Software, San Diego, CA, USA). One-Way or Two-Way ANOVA method was used for statistical analysis and Tukey test was used for comparative analysis. $P < 0.05$ was considered significant.

4. RESULTS

4.1. Food Intake

The food intake was 22.58±1.35 g in the STD group, 19.87±2.01 g in the STD+MEL group, 16.40±3.19 g in the HFD group and 14.14±2.22 g in the HFD+MEL group (Figure 5). The food intake of the HFD group during the experiment was significantly less than that of the STD and STD+MEL groups.

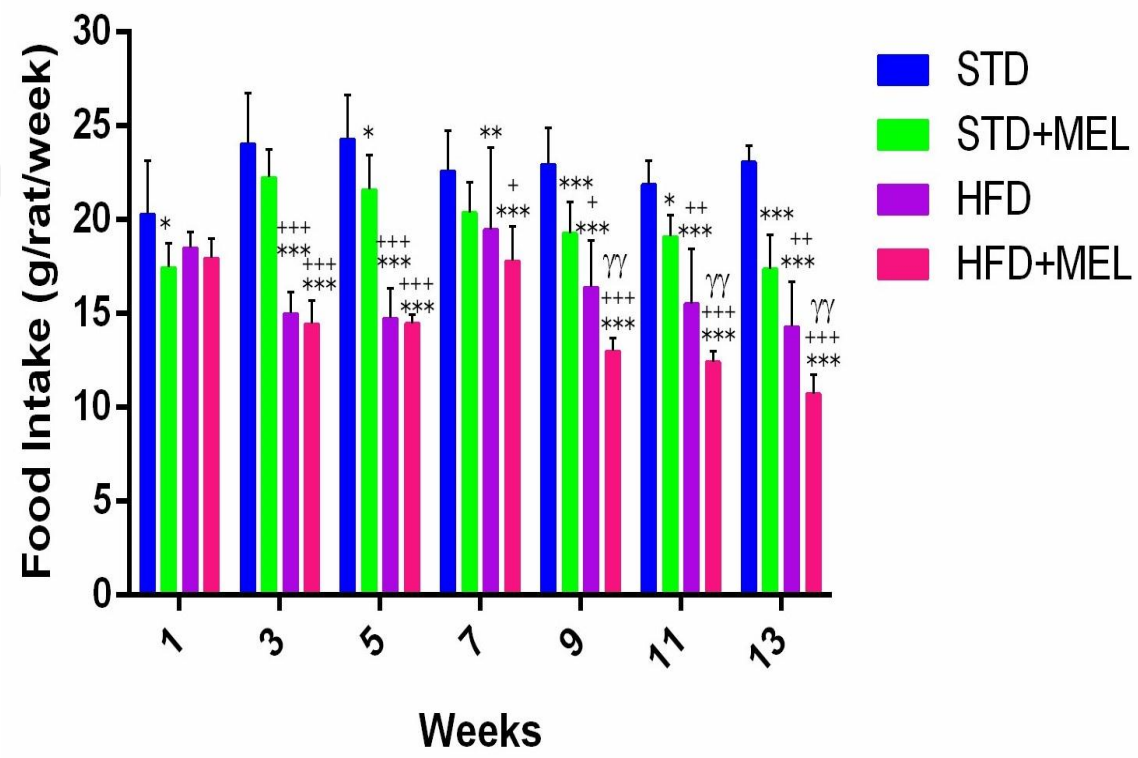


Figure 5. Weekly food consumption of rats. P* <0.05, P** <0.01 and P*** <0.001 compared to STD group. P^{+} <0.05, P^{++} <0.01 and P^{+++} <0.001 compared to STD+MEL group. $P^{\gamma\gamma}$ <0.01 compared to HFD group.

4.2. Weights of Rats

Average weight of rat weight was 305.7±7.78 g in STD group, 303.2±9.18 g in STD+MEL group, 318.9±12.17 g in HFD group and 301.5±10.3 g in HFD+MEL group. A significant increase in rat weight was observed in HFD ($P < 0.05$) and A significant decrease in rat weight was observed in HFD+MEL ($P < 0.01$) groups compared to the HFD group (Figure 6).

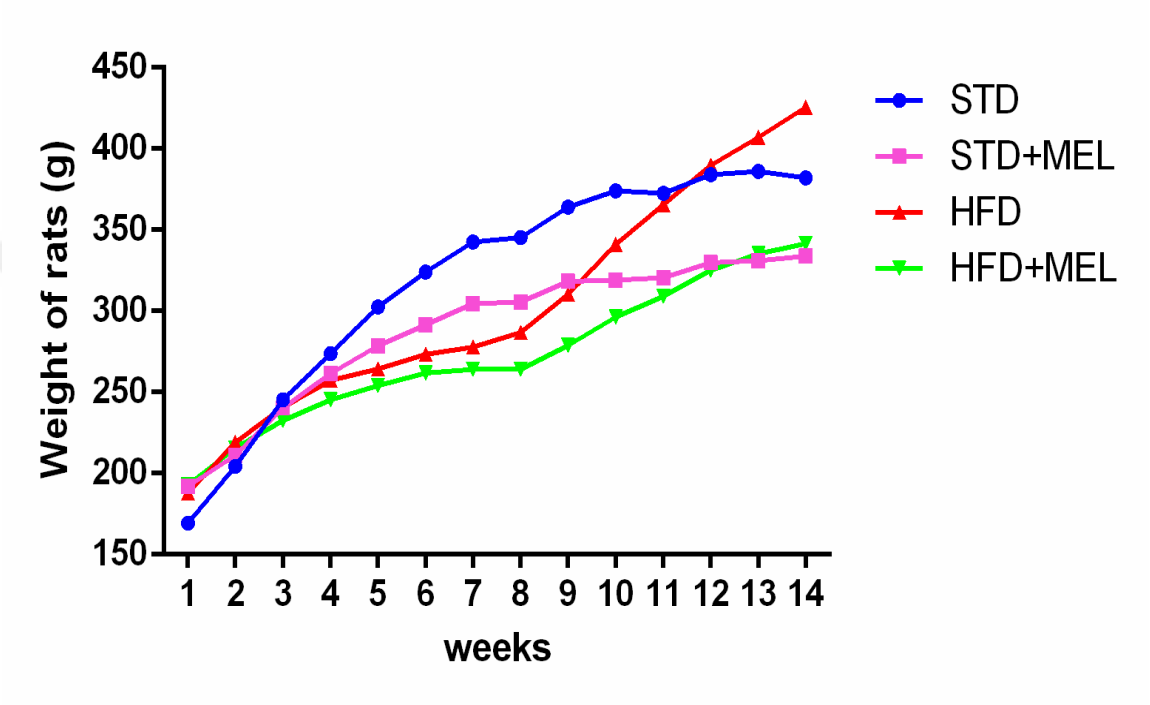


Figure 6. Weekly changes in body weights of rats in experimental groups.

4.3. Weight of Testis

The testis weight was 1.863±0.05 g in the STD group, 1.85±0.04 g in STD+MEL group, 1.703±0.05 g in HFD group and 1.87±0.07 g in HFD+MEL group. A significant decrease (P <0.05) was observed in the HFD group compared to the STD group, whereas the testis weight of HFD+MEL group was significantly increased (P <0.05) compared to the HFD group (Figure 7).

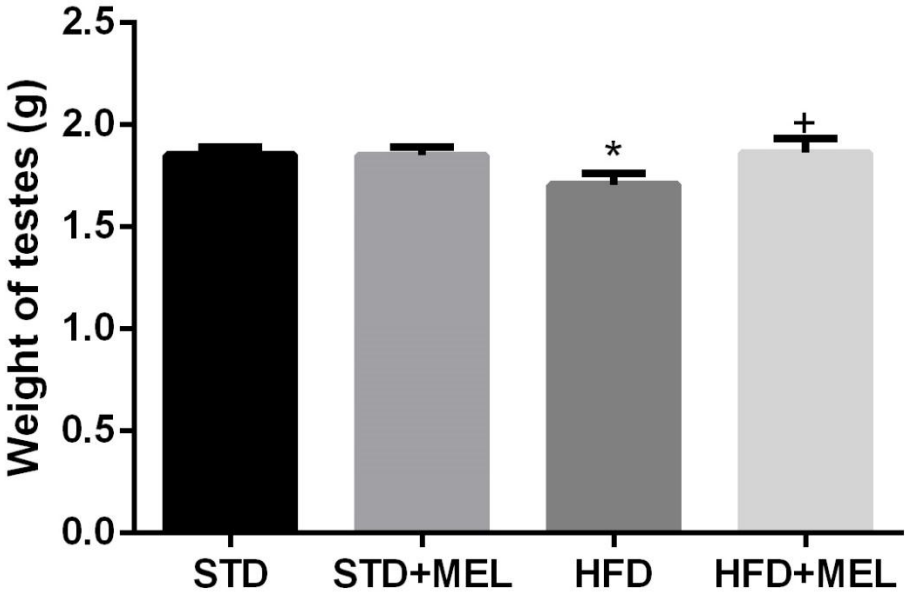


Figure 7. Testis weight of rats for the experimental groups. P* <0.05 compared to STD group, P^{+} <0.05 compared to HFD.

4.4. Morphological Results and Histopathologic Scoring

In the light microscopy examination, the normal morphological structure was observed in the seminiferous tubules of STD group. Spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids were observed in the seminiferous tubules from the basal part to the lumen. Sertoli cells were observed in normal morphology (Figure 8A). In STD+MEL group, testicular morphology similar to the STD with scanty regressive seminiferous tubules were noticed. HFD group presented regressive, degenerative and atrophic seminiferous tubules in the germinal epithelium. Cell debris in the lumen of seminiferous tubules was observed in the sections of that group (Figure 9A). Tissue sections from HFD+MEL group depicted a normal seminiferous tubular morphology (Figure 9B).

In order to monitor the changes in the basal membrane, paraffin sections were stained with PAS. PAS-stained paraffin sections indicated normal morphology of the basal membrane with an intense PAS positivity in STD and STD+MEL groups (Figure 10A and B) groups. In the HFD group, seminiferous tubules presented modulated contours of the basal membranes with decreased PAS positivity (Figure 11A). A few seminiferous tubules in the HFD+MEL (Figure 11B) group show irregularity with a moderate PAS positivity in the basement membrane. In HFD+MEL group, basal membrane with regular morphology had an increased PAS positivity (Figure 11B) compared to HFD group.

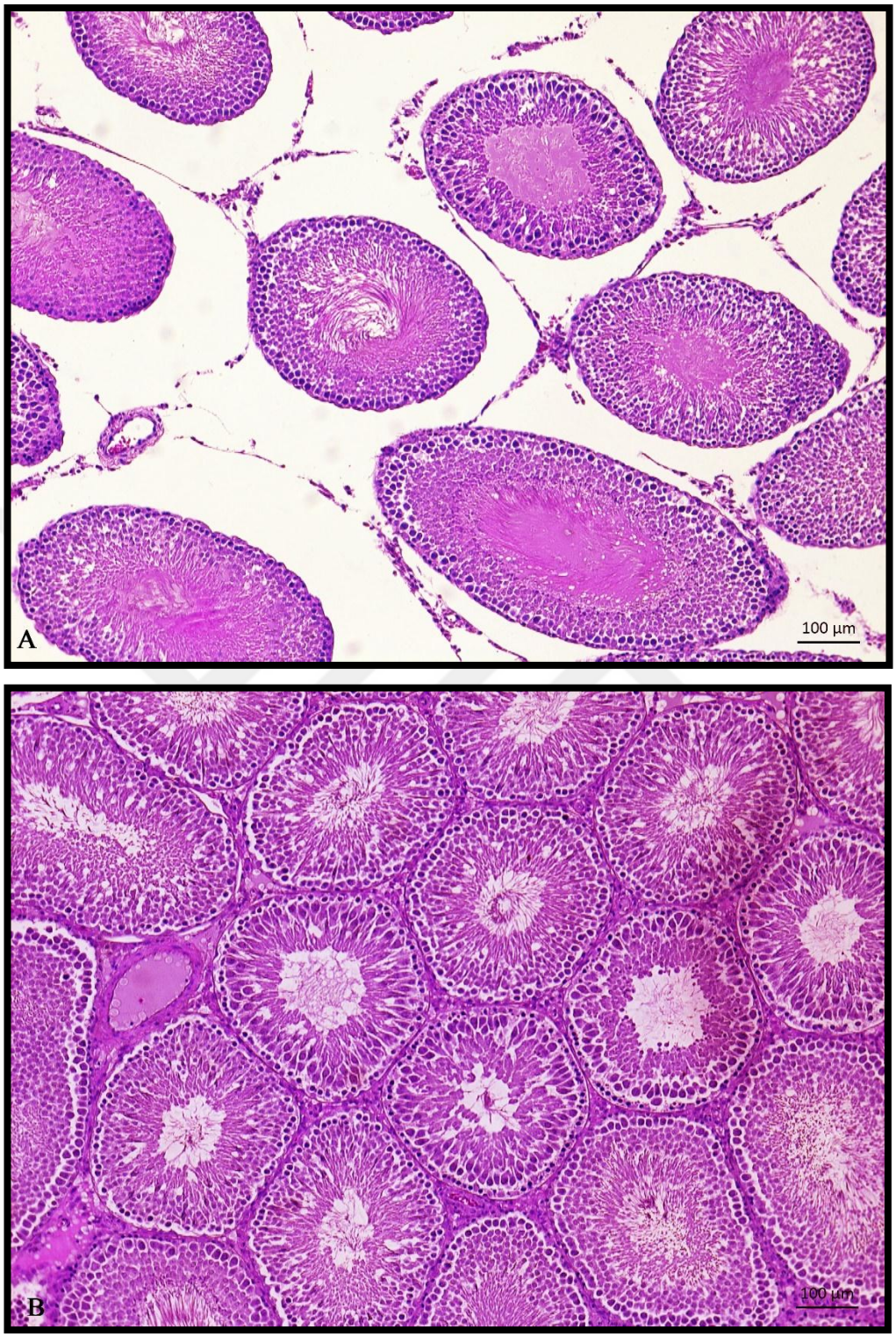


Figure 8. STD (A) and STD+MEL (B) groups show the regular structure of spermatogonium, primary spermatocytes, secondary spermatocytes, spermatids, spermatozoa and seminiferous tubule contours in the germinal epithelium. H&E staining.

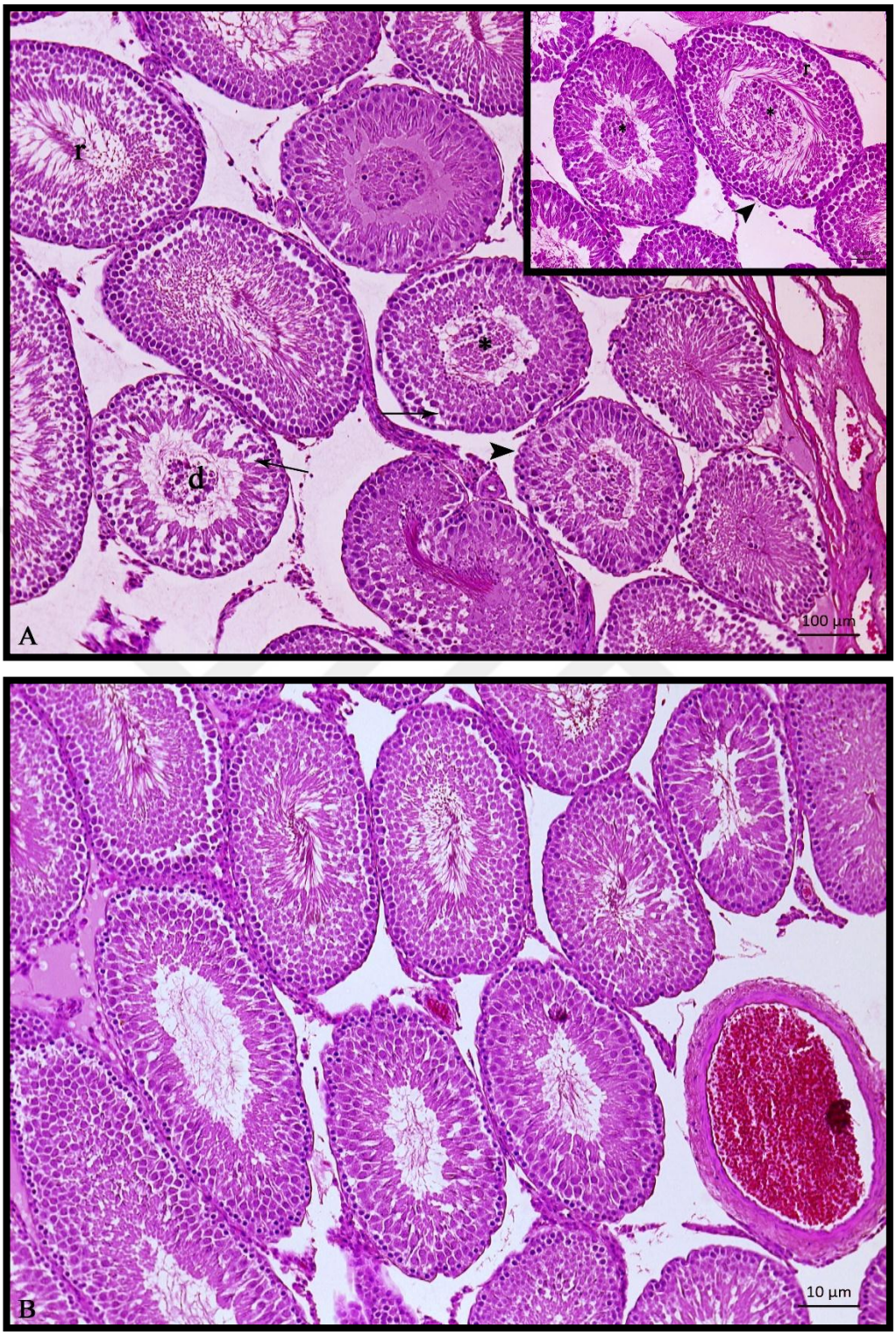


Figure 9. In the HFD (A) group: Basal membrane of seminiferous tubules with an irregular contour (►), many cellular debris (*) in the lumen and separations in the cell-to-cell junction (→) on the germinal epithelium of degenerative (d) seminiferous tubules are noticed. In the HFD+MEL (B) group, regular morphology of the germinal epithelium of the seminiferous tubules is seen. H&E staining.

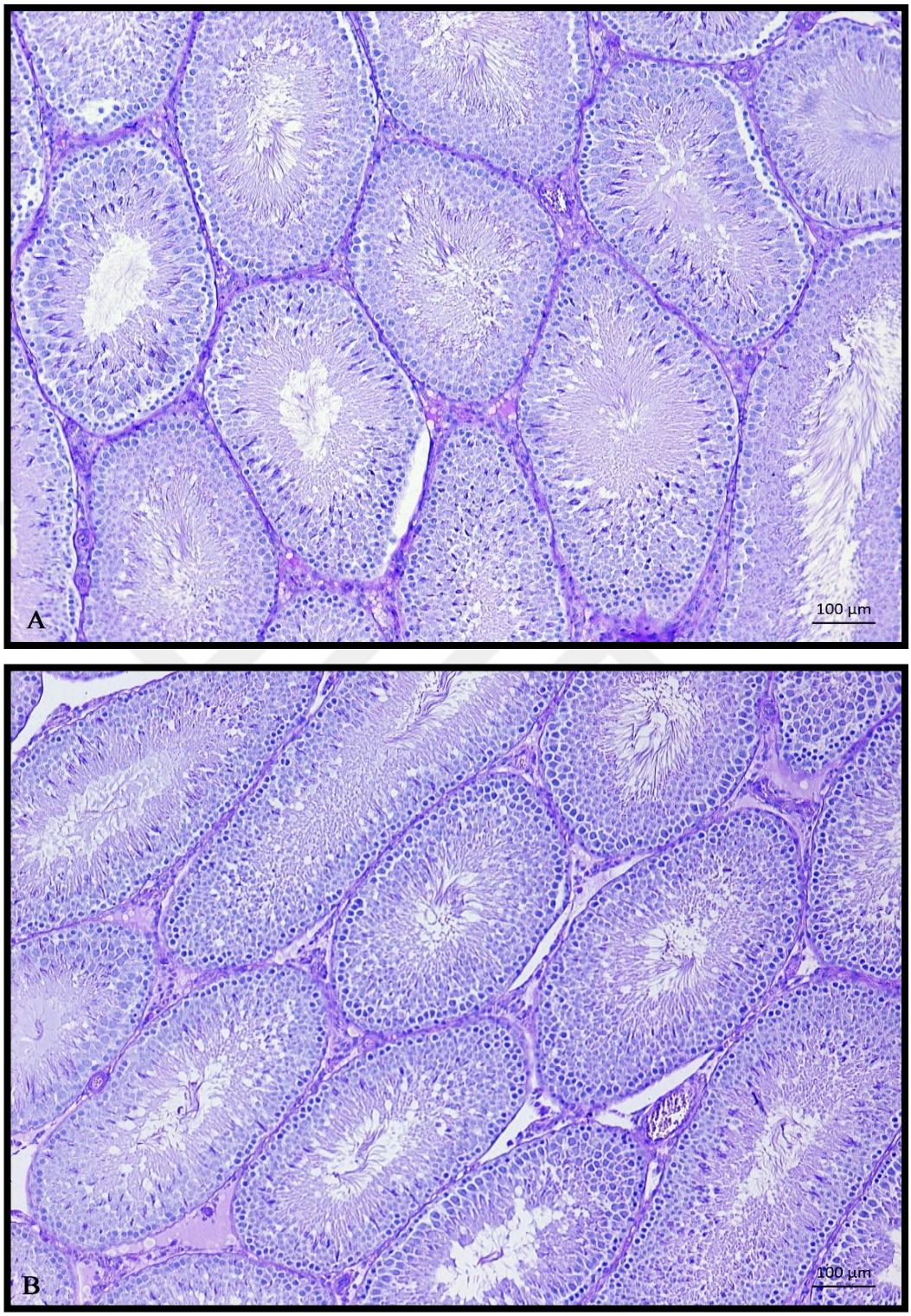


Figure 10. STD (A) and STD+MEL (B) groups show a deep intensity of PAS positivity in the basement membranes of seminiferous tubule PAS reaction.

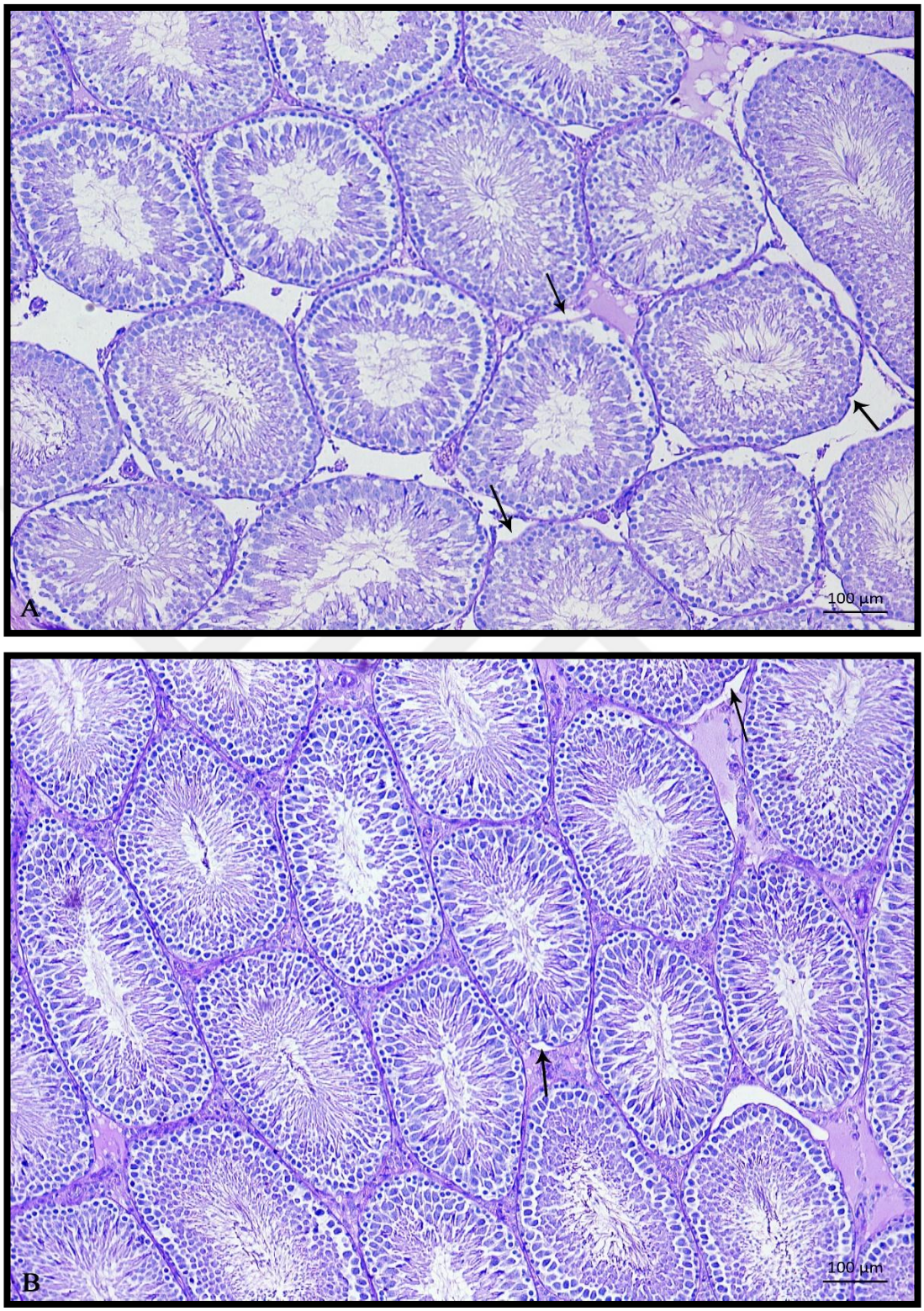


Figure 11. In the HFD (A) group, irregular basal membrane (→) of the seminiferous tubule shows decreased PAS positivity; a few seminiferous tubules in the HFD+MEL (B) group show irregularity (→) with a moderate PAS positivity in the basement membrane. PAS reaction.

The number of normal tubules was 17.67±2.07 in STD group, in STD+MEL group 15.83±1.60, in HFD group 2.17±1.47 and in HFD+MEL group 15±0.89. The number of regressive tubules was 1±0.89 in STD group, 2.34±1.63 in STD+MEL group, 9.17±2.04 in the HFD group and 2.33±0.52 in HFD+MEL group. The number of degenerative tubules was 0.83±0.98 in STD group, 0.84±0.75 in STD+MEL 7.5±2.51 in HFD group and 1.66 ± 0.52 in HFD+MEL group. The number of atrophic tubules was 0.5±0.84 in STD group, 0.66±0.51 in STD+MEL group, 1.17±1.17 in HFD group and 0.67±0.82 in HFD+MEL group. HFD group showed a significant decrease in the number of normal tubules (P <0.001) compared to the control group, and a significant increase in the number of regressive and degenerative tubules (P <0.001). HFD+MEL group showed a significant increase in the number of normal tubules (P <0.001) compared to the HFD group, and a significant decrease in the number of regressive and degenerative tubules (P <0.001) (Figure 12).

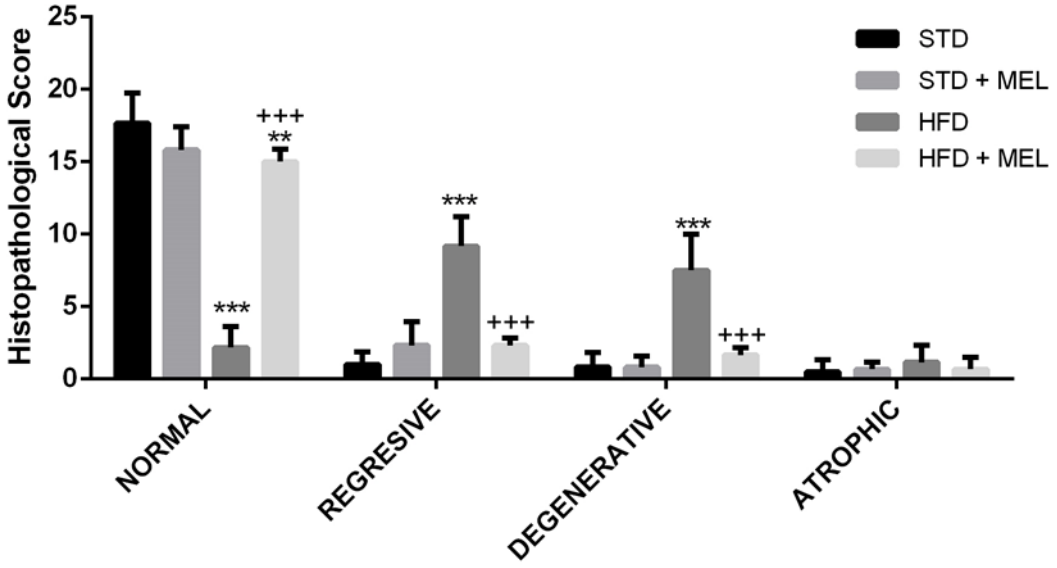


Figure 12. Histopathological scoring of seminiferous tubules in experimental groups: distribution of normal, regressive, degenerative and atrophic tubules among experimental groups. P** < 0.01, P*** <0.001 compared to the STD, P^{+++} <0.001 compared to the HFD group.

4.5. ZO-1 Immunohistochemistry

In STD and STD+MEL groups, intense ZO-1 immunoreactivity was observed between adjacent Sertoli cells (Figure 13A and 13 B). In HFD group, the density of ZO-1 immunoreactivity was very low (Figure 14A). HFD+MEL group showed moderate intensity of ZO-1 immunoreactivity (Figure 14B).

Table 5. Semiquantitative evaluation of ZO-1 immunopositivity in seminiferous tubules.

	STD	**STD+MEL**	**HFD**	**HFD+MEL**
ZO-1	+++	+++	+	++
-: none, +: very low, ++: moderate and +++: intense				

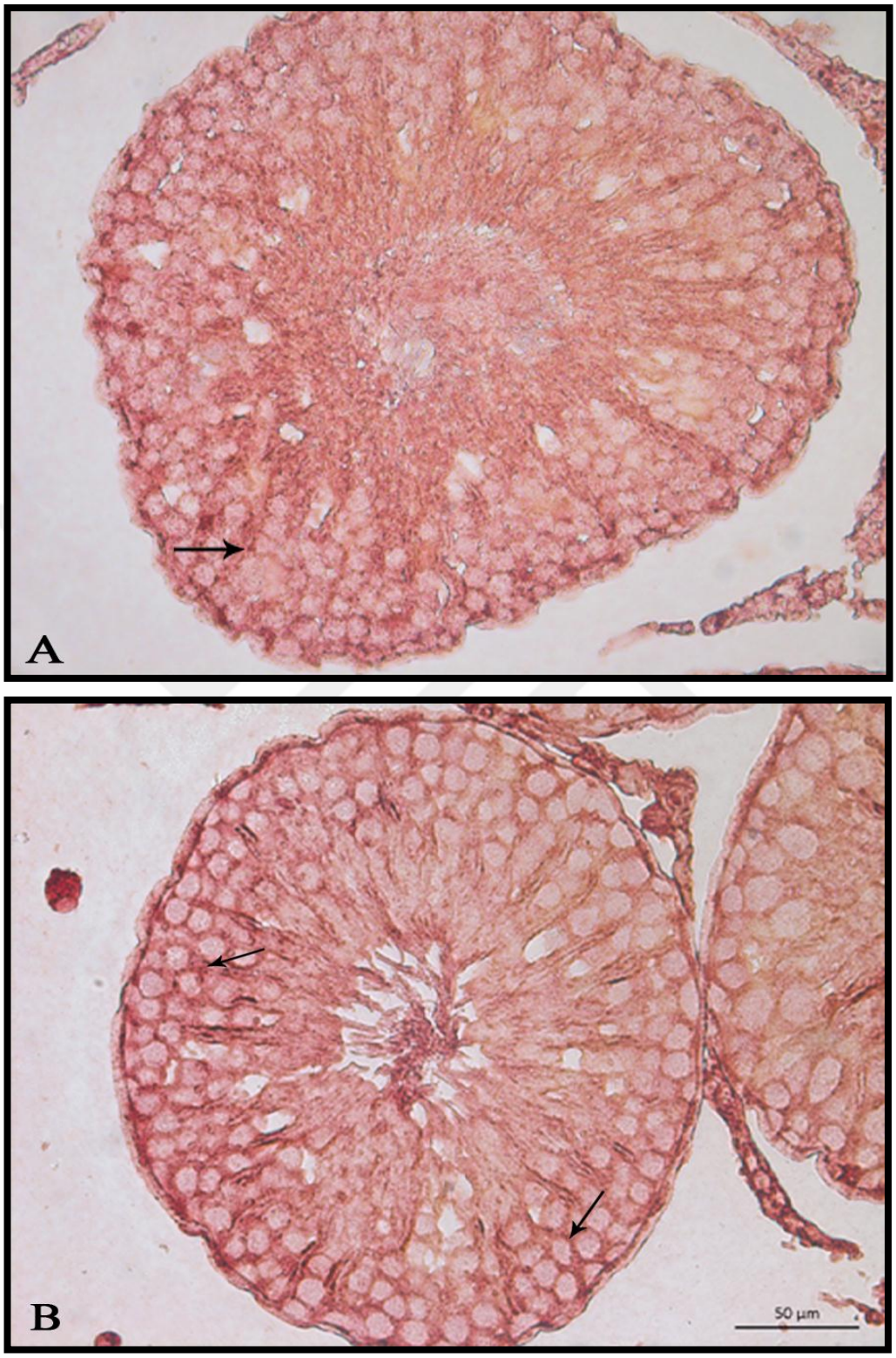


Figure 13. STD (A) and STD+MEL (B) groups indicate strong ZO-1 immunoreactivity (→) in the basolateral regions of Sertoli cells. ZO-1 immunohistochemistry.

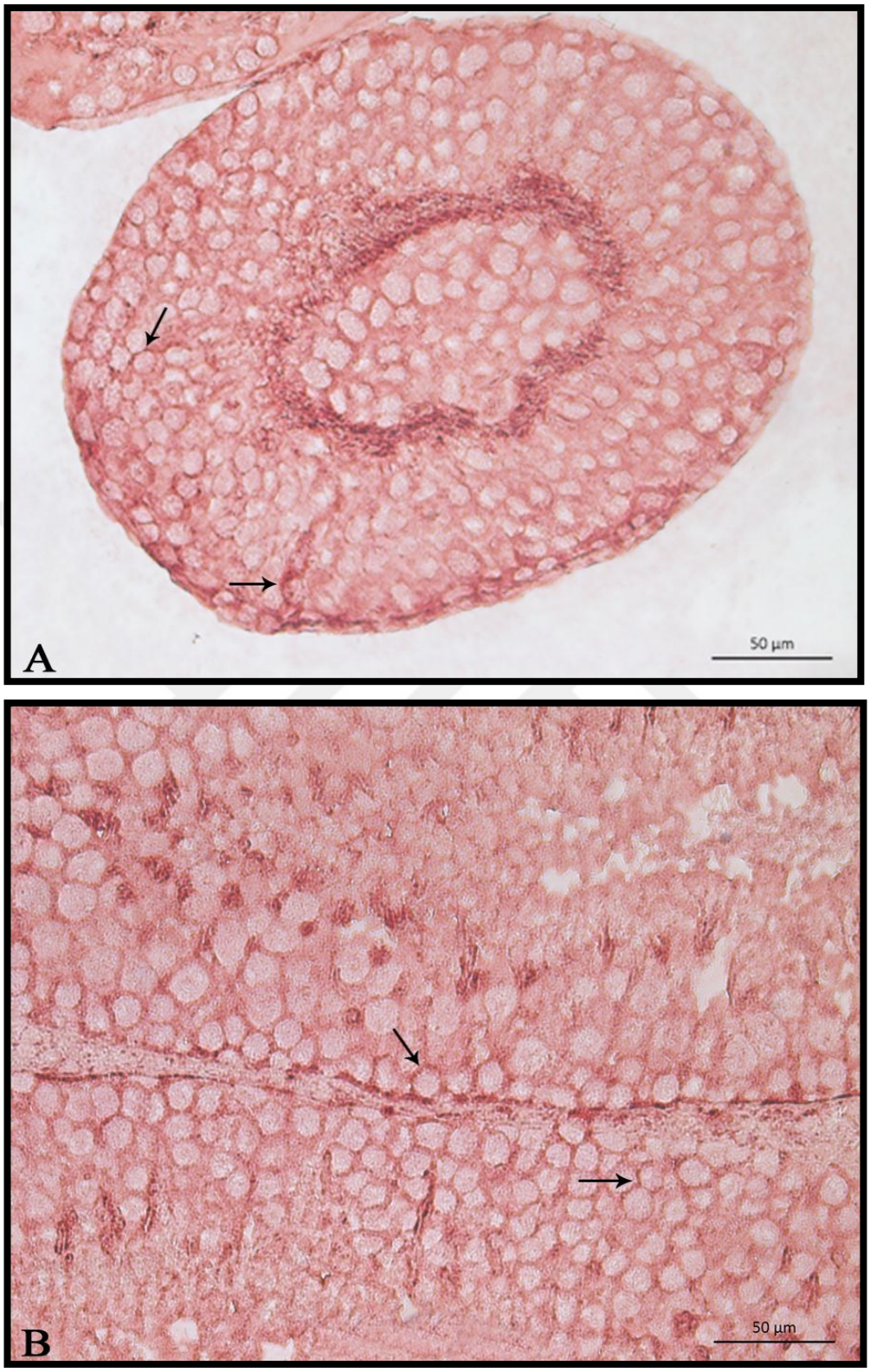


Figure 14. Weak immunoreactivity of ZO-1 (→) in the HFD (A) group; In HFD+MEL (B) group, mild ZO-1 immunoreactivity (→) are seen in the basal regions of Sertoli cells.

4.6. TUNEL and Apoptotic Cell Index

TUNEL positive cells were detected with dark brown color. A small number of TUNEL positive cells were found in the germinal epithelial cells of the seminiferous tubules of STD and STD+MEL groups (Figures 16A and B). The number of seminiferous tubules containing 3 or more TUNEL positive cells was apparently increased in HFD group compared to STD group (Figure 17A). In HFD+MEL group, seminiferous tubules containing 3 or more TUNEL positive cells were scanty (Figure 17B).

Apoptotic index was found to be 1.67±0.52 in STD group, 4.0±1.26 in STD+ MEL group, 18.83±0.75 in HFD group and 2.0±0.63 in HFD+MEL group. Compared to STD group, apoptotic index was significantly higher in HFD group ($P < 0.001$). A significant decrease ($P < 0.01$) was observed in HFD+MEL group compared to the HFD group (Figure 15).

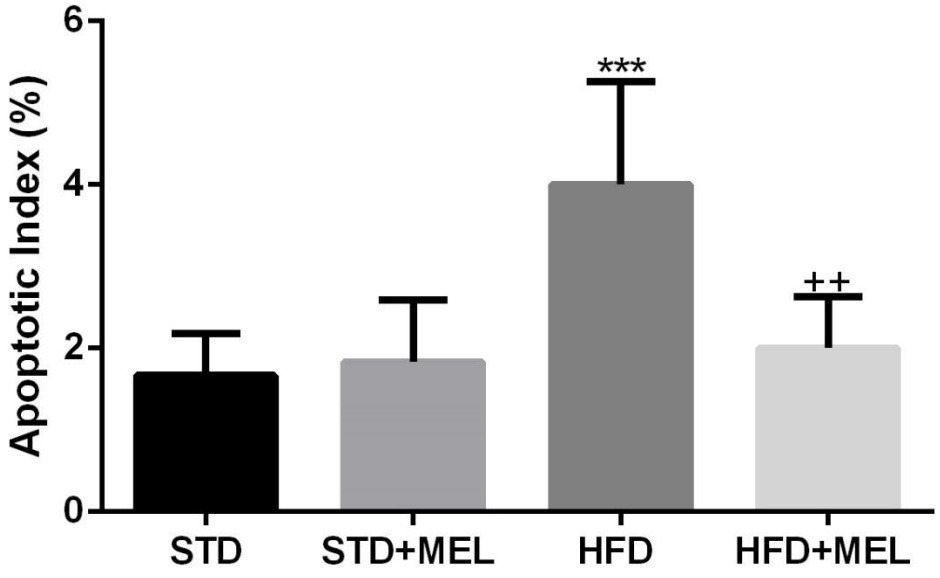


Figure 15. The graph shows the apoptotic index of all experimental groups. P*** <0.001 compared to STD group. P^{++} <0.01 compared to HFD.

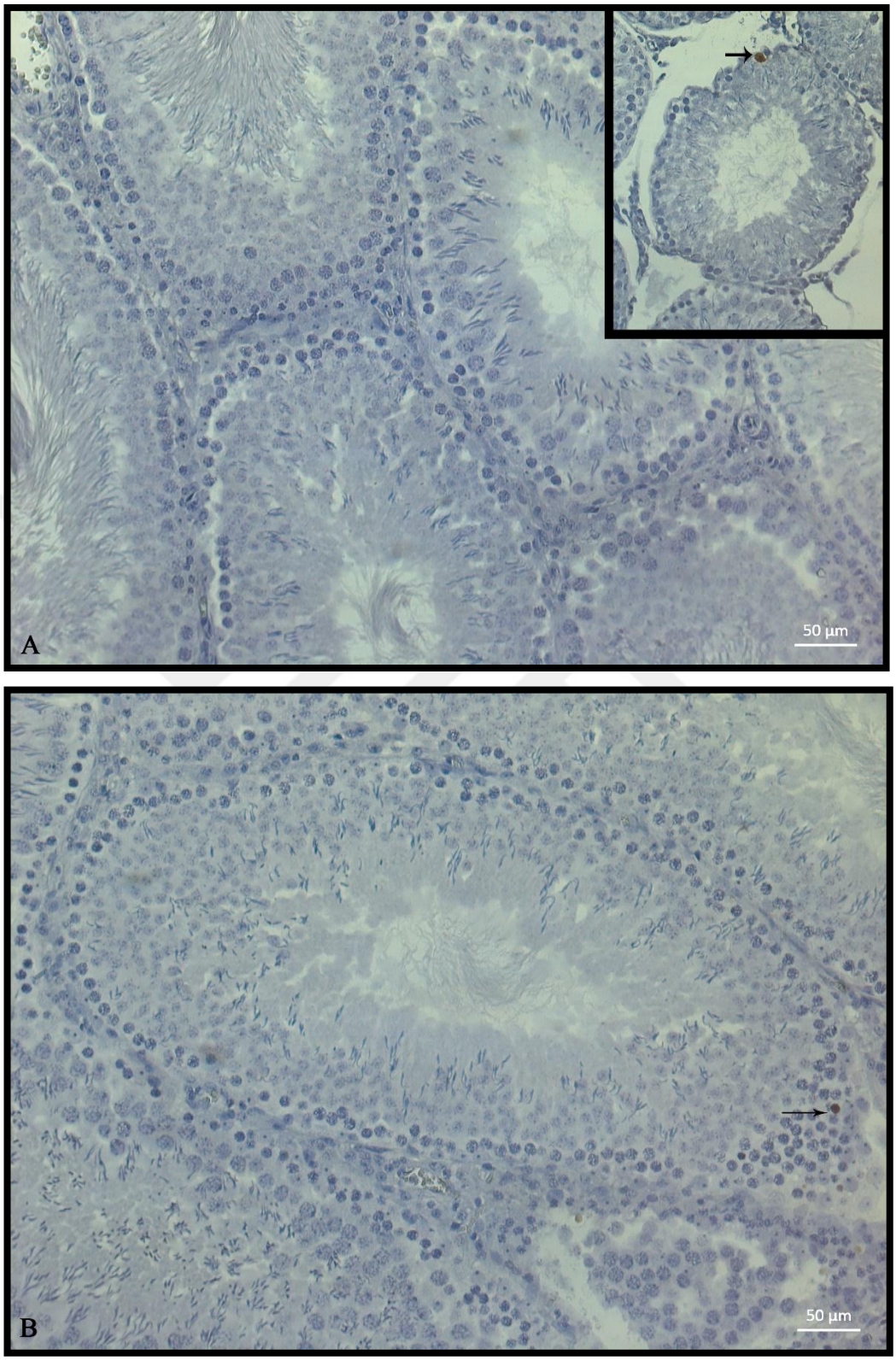


Figure 16. TUNEL positive cell (→) in STD (A) and STD+MEL (B) groups. TUNEL staining.

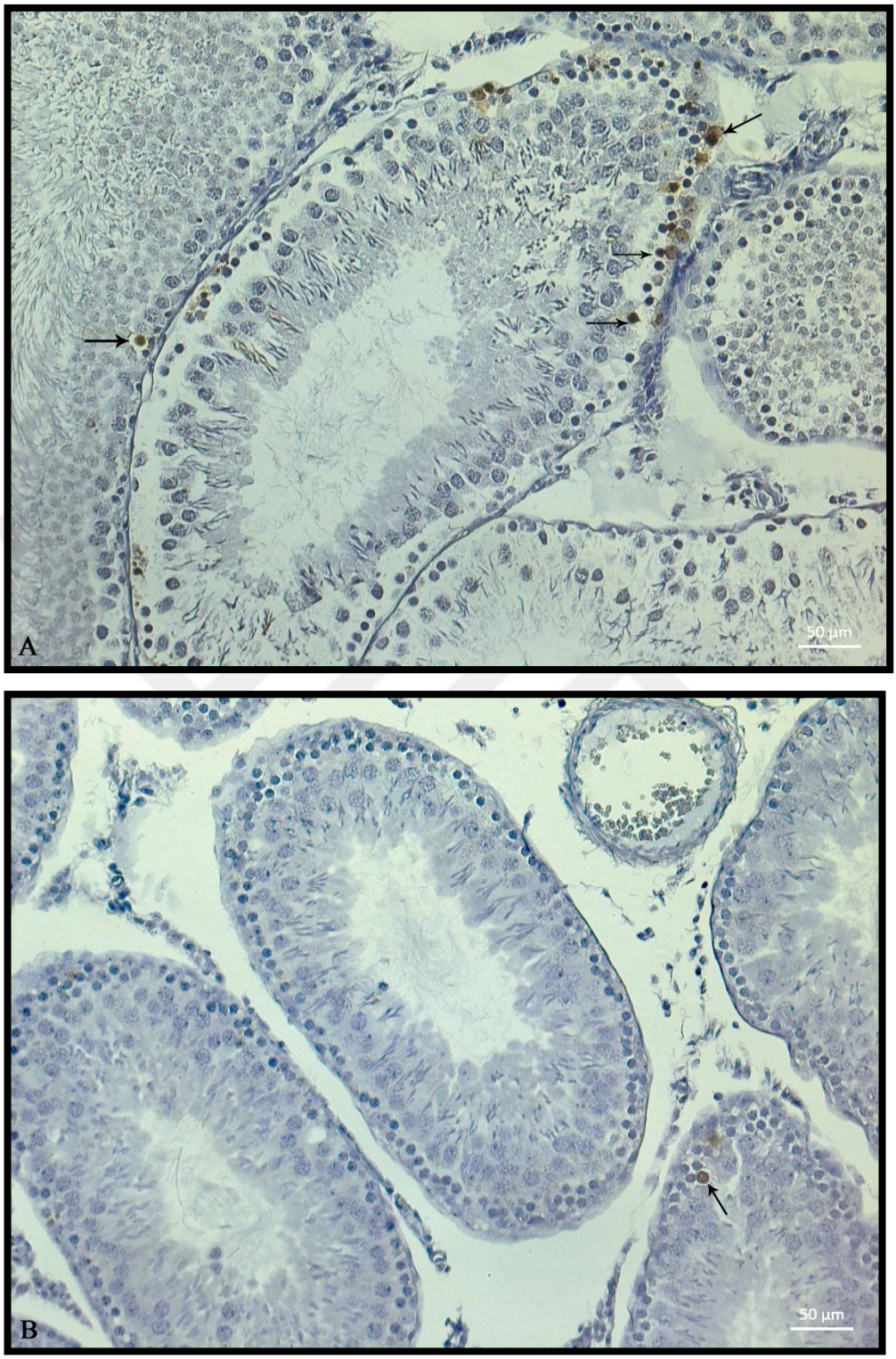


Figure 17. Numerous TUNEL positive cells (→) in seminiferous tubule of the HFD (A) group; In the HFD+MEL (B) group, TUNEL positive cells (→) are few.

4.7. Biochemical Results

4.7.1. Levels of glucose, triglycerides and leptin

According to the results of the analysis, glucose concentration of HFD, STD+MEL and HFD+MEL groups were determined respectively as 263.9±31.59 245.60±22.95 and 248±33,18 mg/dL, depicting a respective significant increase compared to STD group (183.7±42.76 mg/dL) ($P < 0.01$ and $P < 0.01$). In HFD+MEL group, there was a statistically nonsignificant ($P < 0.05$), decrease in glucose concentration (248±33.18 mg/dL) compared to HFD group (Figure 18).

According to the results of serum triglyceride analysis, the serum concentration of HFD group (146.9±40.35 mg/dL) was increased significantly compared to the other experimental groups. This increase was significant compared to STD group (91,81±6,5 mg/dL); STD+MEL (56.70±24.05 mg/dL) and HFD+MEL (83.14±16.13 mg/dL), as $P < 0.001$ and $P < 0.01$ (Figure18).

Leptin hormone level of HFD group (2.19±0.5 ng/mL) increased significantly compared to STD group (1.22±0.24 ng/mL) ($P < 0.001$). In addition, leptin levels of STD+MEL (1.35±0.45 ng/mL) and HFD+MEL (0.92±0.29 ng/mL) groups were found to decrease significantly compared to HFD group ($P < 0.01$ and $P < 0.001$, respectively) (Figure18).

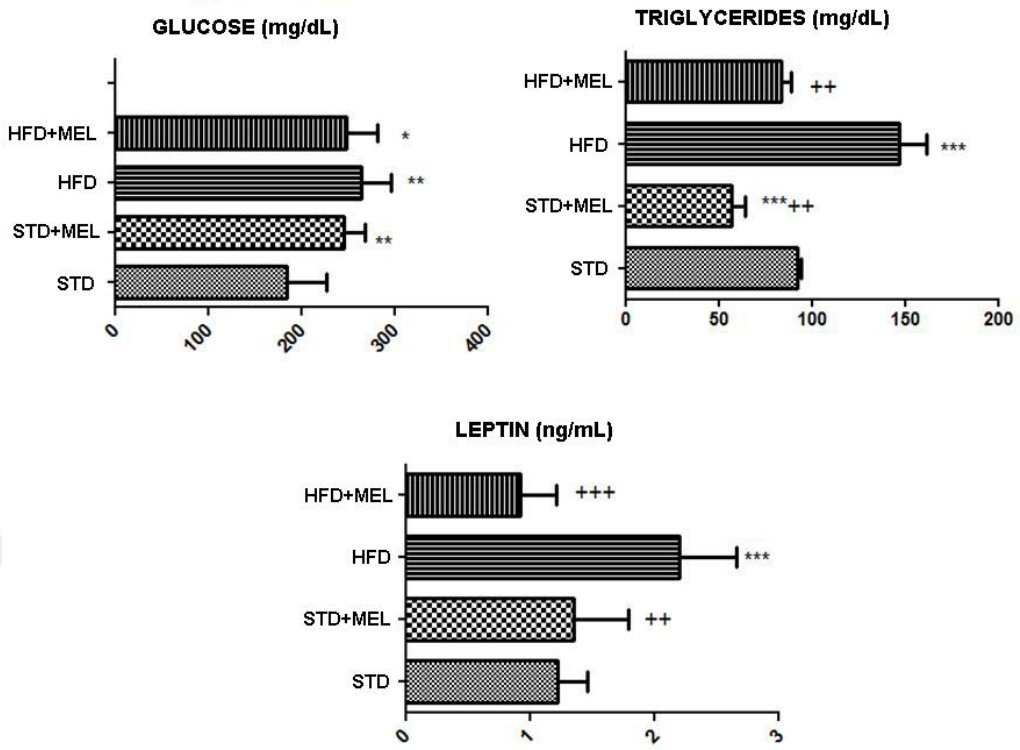


Figure 18. Glucose, triglycerides, leptin levels of all experimental groups. P* <0.05, P** <0.01 and P** <0.001 compared to STD group; P^{++} <0.01, P^{+++} <0.001 compared to HFD group.

4.7.2. Levels of FSH, LH and testosterone hormone

Based on hormone analysis in serum samples; LH level was significantly decreased in HFD group (1.19±0.67 mIU/L) compared to STD group (2.40 ± 0.8 mIU/L) (P<0.01). LH levels of STD+MEL (2.87±0.35 mIU/L) and HFD+MEL (2.49±0.65 mIU/L) groups were significantly increased compared to HFD group (P < 0.001 and P<0.01, respectively) (Figure19).

In HFD group (1.98±0.41 mIU/L), there was a statistically significant decrease of FSH level compared to STD group (2.99±0.72 mIU/L) (P< 0.01). FSH level of STD+MEL (2,68±0,56 mIU/L) was significantly higher compared to HFD group (P <0.05). FSH level of HFD+MEL group (2.14±0.36 mIU/L) was slightly higher compared to HFD group but this increase was not statistically significant (Figure19).

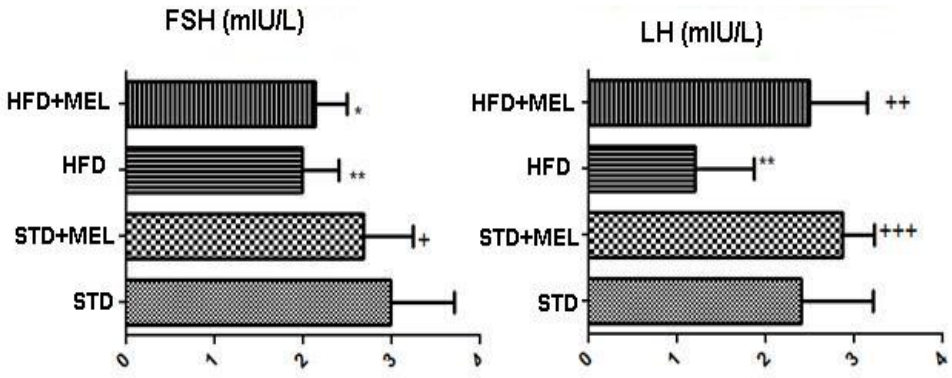


Figure 19. FSH and LH levels of all experimental groups. P* <0.05 and P** <0.01 compared to the STD group, P^{++} <0.01 and P^{+++} <0.001 compared to the HFD group.

Serum testosterone level was found to be similar to FSH and LH. In HFD group (15.57±2.16 nmol/L), testosterone level was significantly decreased compared to STD group (18.83±1.04 nmol/L) (P <0.01). STD+MEL (19.95±2.16 nmol/L) and HFD+MEL (19.53±1.11 nmol/L) groups showed a significant increase in testosterone level compared to HFD group (P <0.001 and P <0.01, respectively) (Figure20).

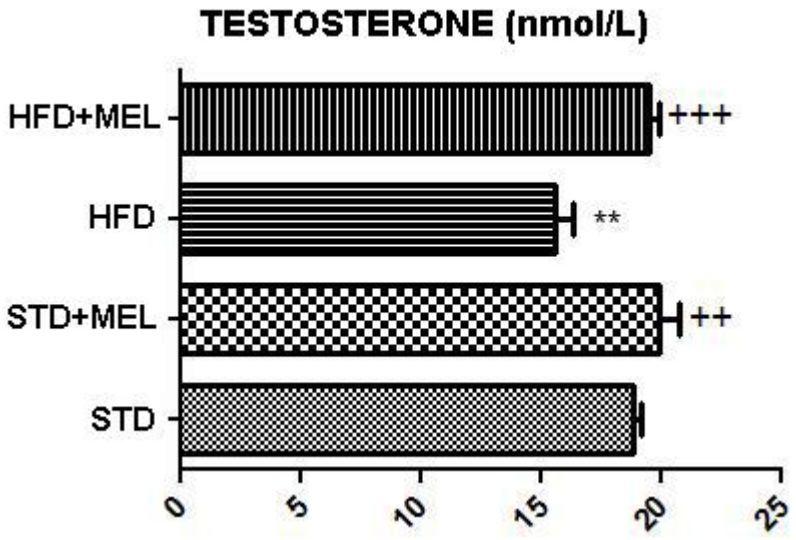


Figure 20. Testosterone level of all experimental groups. P** <0.01 compared to STD group, P^{++} <0.01, P^{+++} <0.001 compared to HFD group.

4.7.3. MDA levels

MDA level of the HFD (27.02±7.09 nmol/g tissue) increased significantly compared to STD and STD+MEL groups (18.87±1.85 nmol/g tissue and 19,36±3.7 nmol/g tissue; respectively) (P <0.01 and P <0.05; respectively). However, in the HFD+MEL group (25.32±6.03 nmol/g tissue), there was a statistically insignificant decrease in MDA level compared to HFD group(P> 0.05) (Figure21).

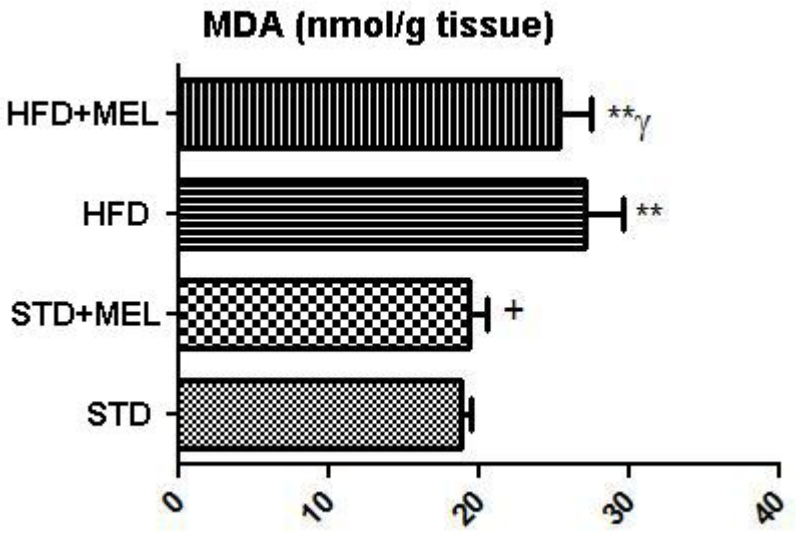


Figure 21. MDA level of all experimental groups. P** <0.01 compared to STD group, P^{+} <0.05 compared to the HFD and P^{γ} <0.05 compared to the STD+MEL group.

4.7.4. GSH levels

In the HFD group (18.60±2.76 µmol/g tissue), the testicular GSH level was significantly decreased compared to STD group (21.31±1.35 µmol/g tissue) (P <0.05). GSH level was found 23.40±5.76 µmol/g tissue in STD+MEL. In HFD+MEL group (23.66±5.9 µmol/g tissue), GSH level was significantly increased compared to HFD group (P <0.05).

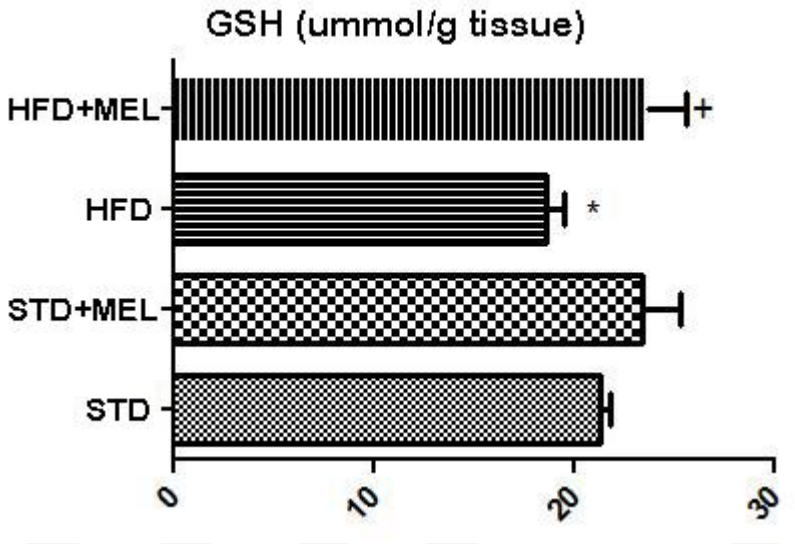


Figure 22. GSH level of all experimental groups. P* <0.05 compared to STD group, P^{+} <0.05 compared to HFD group.

4.7.5. SOD activity levels

The SOD activity of the HFD group (77.0±10.26 %) was found to be statistically lower than that of STD (112.4±11.68 %) and STD+MEL (112.77±10.54 %) groups (P <0.001). SOD activity level of HFD+MEL group (96.74±12.89 %) was also significantly lower than STD and STD+MEL groups (P <0.05) (Figure 23).

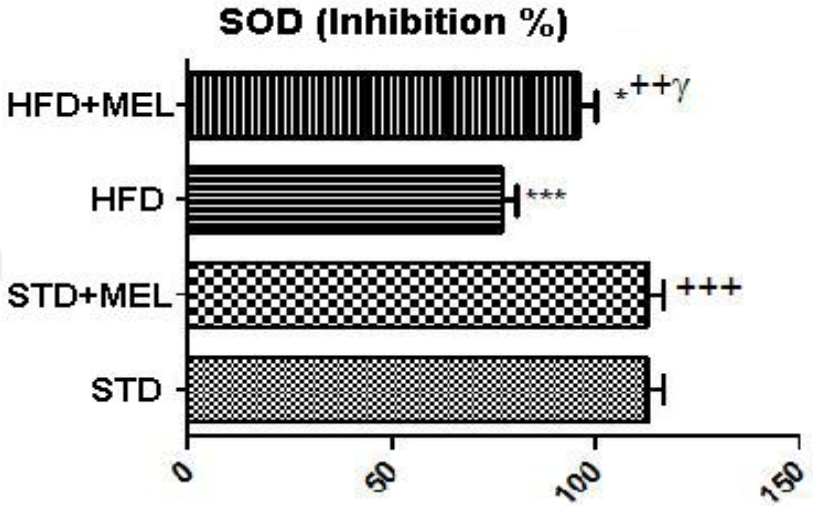


Figure 23. SOD inhibition level of all experimental groups. P*** <0.001 compared to STD group; P^{++} <0.01, P^{+++} <0.001 compared to HFD group and P^{γ} <0.05 compared to STD+MEL group.

4.7.6. TNF-α and IL-6 levels

TNF-α level of HFD group (253.3±41.05 pg/mL) was significantly increased compared to STD group (126±29.5 pg/mL) group ($P < 0.01$). TNF-α levels were significantly decreased in STD+MEL (132.9±69.65 pg/mL) and HFD+MEL (159±33.64 pg/mL) groups compared to HFD group. It was statistically significant ($P < 0.01$ and $P < 0.001$ respectively,) (Figure 24).

Tissue IL-6 level was also measured to determine the degree of inflammation. IL-6 level was measured in HFD group as 8.85±0.85 pg/L, in STD as 5.73±1.6 pg/L and STD+MEL as 6.78±0.58 pg/L ($P < 0.01$ and $P < 0.001$, respectively). On the other hand, IL-6 level of HFD+MEL group (4.74±1.30 pg/L) decreased significantly ($P < 0.01$) compared to HFD group (Figure 25).

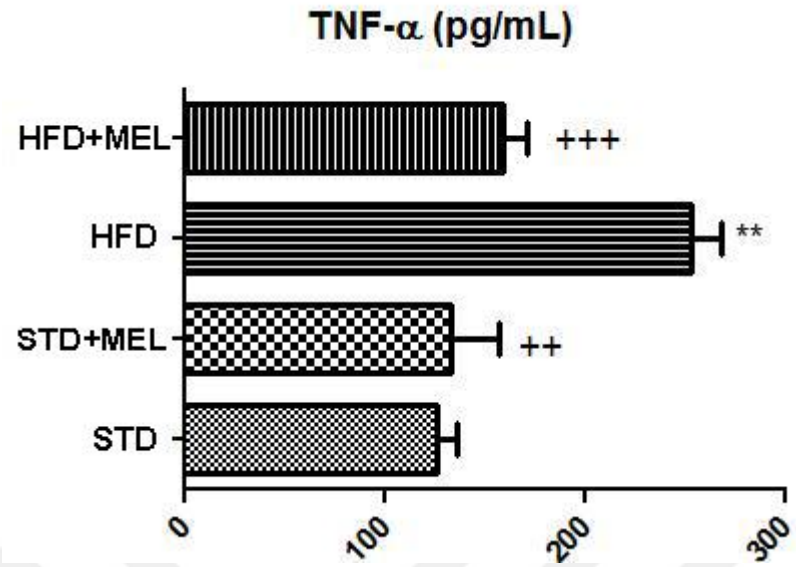


Figure 24. TNF-α level of experimental groups. P* <0.01 compared to STD group, P^{++} <0.01, P^{+++} <0.001 compared to the HFD group.

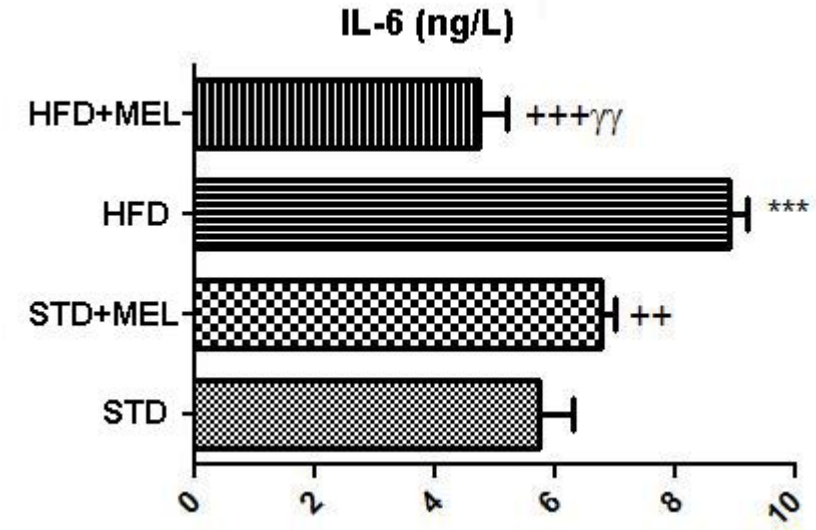


Figure 25. IL-6 level of experimental groups. P*** <0.001 compared to STD, P^{++} <0.01, P^{+++} <0.001 compared to HFD and, $P^{\gamma\gamma}$ <0.01 compared to STD+MEL group.

5. DISCUSSION

Obesity is known to be negatively correlated with male fertility. In this current study, melatonin is intended to improve testicular tissue damage of obese rats induced by a high-fat diet. The presence of regressive, degenerative and atrophic seminiferous tubules in the testis of high fat diet-induced obese rats (HFD group) suggested a high degree of tissue damage. TUNEL (apoptotic cell markers) positive cells were significantly increased in the obese rats. It was observed that ZO-1 protein which is one of the tight junction proteins in the blood-testis barrier was depleted. In addition, a prominent increase in serum glucose and triglyceride levels; a decrease in FSH, LH and testosterone levels was monitored. A parallel increase in leptin and MDA levels was contrasted with a decrease in GSH and SOD levels. Inflammation markers, IL-6, and TNF-α levels were increased in testicular tissue. It was found that melatonin administration was improved all these disturbed histological and biochemical parameters in the testes of high fat diet-induced obese rats.

As a general health problem in the world, obesity is an energy metabolism disorder that is defined as excessive fat accumulation in the body. According to WHO, the prevalence of obesity is increasing day by day and approximately 2 billion people are obese or overweight (2). This increase is not only seen in the adults but also children obesity is increasing (2). Increased fat tissue increases the level of inflammation and oxidative stress in the body because of changes in the functions of fat tissue (69). Obesity can develop several diseases such as Type 2 diabetes, cardiovascular diseases, hypertension, endocrine disorders, respiratory disorders, immunodeficiency, various types of cancer, psychological problems and infertility (70, 71). Sedentary lifestyle reduces daily physical activity, and eating lifestyle which is over-consumption of foods containing high calories triggers an increase in body fat mass (72). Obesity can be a preventable disorder by exercise, bariatric surgery, or pharmacological method (73).

Melatonin is a pharmacological strategy for controlling obesity and weight gain. Several studies have shown that melatonin reduces inflammation in obese mice and

rats (74, 75, 20). Although the amount of feed consumed in rats with fed high-diet is low, a prominent weight gain is observed (76). Melatonin decreases body weight in obese rats (77, 74). In this study, although the food intake was lower in rats fed with high-fat diet compared to fed with standard rat chow, it was observed that weight gain was higher in HFD group because of higher calorie diet. During the experimental period, the weight of rats fed with a high-fat diet was higher than the HFD+MEL group. A statistically significant decrease was observed between the STD group and STD+MEL group.

Increased serum glucose and triglyceride levels observed in rats fed a high-fat diet were significantly decreased with melatonin treatment (66). Melatonin regulates glucose metabolism via regulating the expression of GLUT4 which take a role in the insulin-signaling pathway (11). In our current study, glucose and triglyceride levels were higher in obese rats and melatonin improved these parameters.

Melatonin is a strong antioxidant. It binds to the cell and is easily introduced into the cell due to its lipophilic properties (78). Melatonin has two different antioxidant effects (79). First, it acts directly by connecting to its own receptor in the cell wall. It detoxifies the free radicals (HO^-, H_2O_2, O_2, HOCl, NO, $ONOO^-$) that can lead to oxidative stress and thus can prevent their harmful effects on biomolecules (80, 81). The antioxidant property of melatonin is due to the pyrrole ring in its structure. In the presence of O_2, enzymatic degradation of the pyrrole ring by indoleamine 2,3-dioxygenase or nonenzymatic degradation with hemin results in the formation of acetyl-N-formyl-5-methoxykynurenamine (AFMK) with high reactivity. It was determined that melatonin also produced AFMK in the presence of H_2O_2 and this metabolite performed radical scavenging activity (82). Secondly, melatonin acts on the cell indirectly through the antioxidant enzymes (glutathione, superoxide dismutase) (83). It has been shown that melatonin at both pharmacological and physiological levels increases gene expression or activities of some antioxidant enzymes such as SOD, GSH, glucose-6-phosphate dehydrogenase, and g-glutamylcysteine synthetase and thus suppresses the oxidative stress (84). Oxidative stress, occurring by the accumulation of antioxidants in the cell, is associated with

obesity-related type 2 diabetes (insulin resistance) (85, 86). Increased fatty tissue results in increased free radicals by activation of NADPH oxidase. Increased free radicals in the fat tissue negatively affects glucose uptake into the cell and cause insulin resistance (87). Oxidative damage is increased through reduction of SOD activity and GSH levels. Increased levels of MDA, resulting in cell membrane damage is associated with oxidative stress (88). Erdeve et al. found that MDA levels were significantly higher in obese children compared to the control group (89). In a parallel study, MDA level was found higher in obese patients than in the non-obese group (90). Results of our present study supported these mentioned literature data. In HFD group, GSH level and SOD activity were decreased while an increase in MDA level was detected. In the melatonin-treated HFD group, the deteriorated oxidant/ antioxidant balance was apparently improved.

Leptin is a 16 kDa hormone and regulates food intake and weight gain (91), bone formation (92), inflammation and reproduction in the body. This regulation is made by receptors found in various tissues such as hypothalamus, testis, pancreas, sperm tail (93). Serum leptin levels are higher in obese individuals than those with normal weight (94). Increased leptin level with obesity has a negative effect on male fertility (95). In high fat fed rats, leptin levels increased and sperm motility decreased as weight gain increased (96). In a study based on leptin-deficient mice, the testes of animals were atrophied along with disturbed morphological and biochemical parameters. Fewer spermatozoa have been found in the seminiferous tubules with atrophic Leydig cells (97). According to another study, leptin treatment resulted in decreased fertilization potential (98). Leptin regulates the reproduction by affecting hypothalamus-pituitary-testis axis directly or indirectly (99). Leptin can act directly on testicular steroidogenesis. Increased leptin level adversely affects testosterone production in Leydig cells (100). Leptin is released by circadian rhythm and release times fit into the release time of the melatonin. Increased fat tissue and impaired chronobiology with obesity cause an increase in leptin levels (8). As a parallel finding, serum leptin level was increased in the HFD group and melatonin was found to reverse this increase by lowering the leptin levels.

In our study, decreased levels of testosterone, FSH and LH hormone levels in HFD group were increased by melatonin administration. Obesity disrupts the balance of adipose-derived hormones and adipokines. With increased aromatase activity testosterone is converted to estrogen and then level of testosterone decreases. The decrease in the level of Testosterone, a vital hormone for spermatogenesis, adversely affects spermatogenesis (101). A given decrease in the level of LH causes a decrease in the level of testosterone. According to a study, FSH, LH, and testosterone hormone levels were decreased in obese men (102). Obesity causes deterioration of the hypothalamus-pituitary-testis axis. This deterioration causes a decrease in the level of sex hormones (103).

Adipocytes are responsible for secretion of free fatty acid, as well as secretion of tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) which trigger the development of insulin resistance. Obesity is associated with inflammation originating from adipose tissue. Inflammatory cytokines such as TNF-α and IL-6, which are secreted excessively by adipose tissue in individuals with a high amount of fat, trigger systemic inflammation (104). Increased inflammatory mediators such as C-Reactive Protein (CRP) and IL-6 with obesity led to the characterization of obesity as a mild inflammatory disease. Interleukin-6 is one of the most important cytokines involved in inflammatory activity (105, 106). Interleukin-1 is secreted by the action of TNF- α and has synergistic effects with these cytokines. Interleukin-6 production and circulating levels increase in parallel with obesity and at least 1/3 of circulating IL-6 is secreted by adipose tissue. Abdominal obesity and insulin resistance increase IL-6 and CRP levels (107). IL-6 was found to be correlated with BMI, waist-hip ratio, and insulin resistance (108). IL-6 and TNF-α modulating the release of vascular nitric oxide and superoxide can mediate obesity-related diseases or disorders such as arteriosclerosis, and insulin resistance (109). A positive correlation between IL-6 and BMI in morbid obesity, especially in males, is related to the enlargement of abdominal fat mass (110). Inflammatory cytokines are responsible for insulin resistance and an increase in plasma free fatty acid. In obese patients, there is an increase in plasma levels of IL-6, TNF-α and TNF-α receptors secreted from adipose tissue (111). There is an increase in circulating levels of IL-6, TNF-α and

their messenger RNA (mRNA) levels in obese (112). According to a study in obese mice (ob/ob), TNF-α level of the melatonin-treated group for 8 weeks was shown to be significantly lower than those of the obese group (74). According to a clinical study in obese women, melatonin-treated group (6 mg/day) was found to be significantly decreased in terms of inflammation marker TNF-α and IL-6 levels compared to obese female group (113). In our study, we observed parallel results as TNF-α and IL-6 levels were significantly increased in rats fed with a high-fat diet (HFD). On the other hand, TNF-α and IL-6 levels of the melatonin administered group (HFD+MEL) were significantly decreased compared to the HFD group.

Obesity has a negative effect on testicular morphology as well as disrupting the function of sex hormones. Spermatogenesis is a temperature-dependent event and occurs at about 35 degrees. The testicle temperature increases with increasing amount of fat in the scrotum. Thus, impairment of spermatogenesis occurs with increasing temperature. Obesity increases adipose in the connective tissue between the seminiferous tubules and causes the shrinkage of the Leydig cell (secretion of testosterone hormone decreases) and decreases the diameter of the seminiferous tubules. Obesity causes a reduction in spermatogenic cells, degradation of germinal epithelium, a decrease in cell proliferation and an increase in apoptotic cells (114, 115). In rats fed with high fat, an increase in the number of regressive, degenerative and atrophic tubules was observed. Germinal cell lining is disrupted (116, 117). In addition, there was a decrease in testicular weight due to atrophy (116, 118). In the present study, our results suggest that regressive and degenerative tubules increased statistically in the testis of high fat fed rats. However, the number of regressive and degenerative tubules was decreased in HFD+MEL group.

Age, alcohol, smoking, high testicular temperature, oxidative stress, obesity, radiation, and infection may cause DNA fragmentation in all germinal epithelial cells of the testis. The resulting DNA fragments lead to apoptosis (programmed cell death). Apoptotic cells could be determined by TUNEL assay. In radiation-induced testicular damage, TUNEL-positive cells were decreased by the antioxidant and anti-inflammatory effect of the polydatin-treated testis. Polydatin also inhibited Bax and

caspase 3 proteins associated with apoptosis (119). In testis injury induced by methotrexate (a chemotherapy agent), decreased TUNEL positive cells and inflammation had observed in melatonin-administered rats (120). Melatonin administered as a protective agent for treatment of radioiodine (intraperitoneal 12 mg/kg/day melatonin, 2 days before oral radioiodine administration), had a protective effect on the sperm taken from epididymis and testis. In the melatonin+radioiodine group, sperm DNA fragmentation, FSH level, and apoptotic cell count have decreased compared to the radioiodine group (121). The protective effect of melatonin on the testis tissue has been investigated in streptozotocin-induced diabetic rats. Intravenously induced melatonin (10 mg/kg for 5 days) significantly decreased TUNEL positive cells compared to the diabetic group (122). In our study, apoptotic cell index increased in rats fed with HFD, prominently deceased in melatonin-treated group.

Occludin, claudin-3, -5 and -11, ZO-1, -2 and -3 and Junctional Adhesion Molecule (JAM) - A and -B proteins form the blood-testis barrier (BTB). BTB protects newly formed spermatocytes from harmful chemicals (116). Therefore, damage to BTB directly affects spermatogenesis and causes infertility. Factors such as temperature, radiation, leptin, and obesity cause a decrease in the expression of one of the tight binding proteins, ZO-1, and impair the integrity of the BTB. ZO-1 and occludin expressions were reported to be decreased in Sertoli cell line supplemented with high fat (123). Another study claimed that exogenous leptin had negative effects on sperm parameters with increased apoptosis in germinal cell and suppressed steroidogenesis in testis. Exogenous leptin has been described to affect BTB integrity *in vivo*; as a possible result of decreased intensity of tight junction proteins (120). In our present study, leptin level, as well as the immunoreactivity of ZO-1 was decreased in rats fed high-fat diet. Melatonin reversed these deteriorations and decreased the level of leptin with an obvious increase in the immunoreactivity of ZO-1.

Studies have been shown that melatonin has a positive effect to prevent organ damages on various organs such as kidney, urinary bladder and liver by reducing

oxidative stress (124, 125, 126, 127). Melatonin has been shown to have positive significant impacts on male fertility in many human and animal studies. Melatonin reduces morphological damage, oxidative stress parameters (GSH, MDA, SOD), DNA fragmentation and apoptosis in various testis injuries (39, 128, 129, 130, 131). In another study, FSH and testosterone levels were detected to be increased in melatonin-treated rats (132). Administration of melatonin to nicotine-treated mice improved the sperm motility and morphology all together with increased levels of testosterone and LH (133). Expression of blood-testis barrier protein ZO-1 has been observed to be increased in melatonin-treated mice (128). In the present study, the improvement of testicular morphology through administration of melatonin has been reported. Prominent decrease both in apoptotic index, and inflammation, and restored hormone levels are among the important data to support the beneficial effect of melatonin. Melatonin had also a well-defined positive effect on oxidative stress parameters.

To conclude, administered melatonin to rats treated with high-fat diet could possibly contribute to the prevention of testicular damage by regulating the formation of reactive oxygen species and then by regulating the HPT axis, reducing apoptosis and maintaining the integrity of the blood-testicle barrier.

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7. ETHICAL APPROVAL

ACIBADEM ÜNİVERSİTESİ HAYVAN DENEYLERİ YEREL ETİK KURULU KARAR FORMU

BAŞVURU TARİHİ: 05.01.2017	**KARAR TARİHİ:** 05.01.2017
BAŞVURU SAYISI: 2017/5	**KARAR SAYISI:** 2017/5

Acıbadem Üniversitesi Tıp Fakültesi Histoloji ve Embriyoloji Anabilim Dalı'ndan Prof. Dr. Serap ARBAK'ın yürütücüsü olduğu **"Yüksek Yağlı Diyet ile Beslenen Sıçanların Kan Testis Bariyer Bütünlüğü Üzerine Melatoninin Etkisi: Morfolojik ve Biyokimyasal Çalışma"** isimli proje başvurusu Acıbadem Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun "05.01.2017" tarih ve "31" sayılı toplantısında görüşülmüş ve etik açıdan

☒ **Uygun** ☐ **Düzeltilmesi Gerekir** ☐ **Koşullu Olarak Uygun** ☐ **Uygun Değil**

olarak değerlendirilmiştir.

Kurul Üyesi	İmza	Karara Katılıyorum	Karara Katılmıyorum
Prof. Dr. Güldal Süyen (Başkan)		(X)	( )
Prof. Dr. Serap Arbak (Başkan Vekili)		( )	( )
Prof. Dr. İsmail Hakkı Ulus		(V)	( )
Prof. Dr. Alp Bayramoğlu		( )	( )
Prof. Dr. Yeşim Işıl Ülman		(X)	( )
Yrd. Doç. Dr. Devrim Öz Arslan		(X)	( )
Yrd. Doç. Dr. Figen Demir		( )	( )
Sabiha Turgut Genç		( )	( )
Vet. Hek. Samed Özer (Sekreter)		(X)	( )

8. RESUME

Personal Information

Name	Nilsu	**Surname**	Atay
Place of Birth	Konak/Izmir	**Date of Birth**	09.04.1991
Nationality	Turkish	**Phone**	0535 915 90 93
e-mail	nilsuatay@gmail.com		

Educational Information

	Name of Instution	**Year**
Master	Acıbadem Mehmet Ali Aydınlar University	**2019**
University	Yeditepe University-Gecetic and Bioengineering	**2016-February**
High school	Gaziemir Nevvar-Salih İşgören Anatolian High School	**2009**

Job/Internship Experience

Intern	Acıbadem Mehmet Ali Aydınlar University-Histology and Embryology department	2016
Intern	Ege University-Endustrial Microbioloy	2014
Intern	Yeditepe University-Microbiology	2012

Foreign Languages	Reading Comprehension	Talking	Writing
English	Good	Good	Good

Foreign language grade									
KPDS	ÜDS	ELTS	TOEFL IBT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE	Yökdil
									83.75

	Quantitative	Equally-weigthed
ALES Score	63,83	58,86

Computer Skills

Microsoft Office	Very good
Photoshop	Very good

Member of Association

Turkish Society for Electron Microscopy

European Microscopy Society

International Federation of Societies for Histochemistry and Cytochemistry

Papers Presented in National Scientific Meetings

Serap Arbak, Meltem Kolgazi, Edanur Arsoy, Gülşen Öztosun, Müge Yalçın, Simge Oral, Neslişah Z Ünan, Sümeyye Çilingir, Merve Açıkel Elmas, **Nilsu Atay**, "Sıçanlarda etanol ile oluşturulan deneysel ülser modelinde p-kumarik asitin iyileştirici etkisinin mikroskopik ve biyokimyasal düzeyde incelenmesi", 14. Ulusal Histoloji Kongresi, s185, 2018, 10-13 Mayıs, Antalya, **2018**.

Papers presented in international scientific meetings and published in the proceedings.

Meltem Kolgazi, Edanur Arsoy, Gulsen Oztosun, Muge Yalcin, Simge Oral, Neslisah Z. Unan, Sumeyye Cilingir, Merve Açıkel Elmas, **Nilsu Atay**, Serap Arbak, "Healing effect of p-coumaric acid on ethanol induced gastric damage", s240, 13th Multinational Congress on Microscopy, Rovinj, Hırvatistan 2017.

Merve Açıkel Elmas, **Nilsu Atay**, Özlem Bingöl Özakpınar, Meltem Kolgazi, Göksel Şener, Feriha Ercan, Serap Arbak "Putative effect of exercise on liver in high-fat diet-fed rats", s248, 13th Multinational Congress on Microscopy, Rovinj, Hırvatistan 2017.