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Histological and Physiological Effects of the Drugs Used in the Protocol treatment of COVID-19 on Male Reproductive System in Rats

A thesis

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By

Nawras Jassim Muhammad

B.Sc. Biology (2010)

Supervised by

Assist. Prof. Dr.

Ali Khalaf Ali

2023 A.H

Assist. Prof. Dr.

Mustafa Saddam Chaji

1445 A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿إِنَّا خَلَقْنَا الْإِنْسَانَ مِنْ نُطْفَةٍ أَمْشَاجٍ نَبْتَلِيهِ

فَجَعَلْنَاهُ سَمِيعًا بَصِيرًا﴾

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

سورة الانسان / الآية (2)

Dedication

To the master of humanity, the messenger of mercy dedicated to our master Muhammad and his good and pure family, and his faithful companions

To my country with everything in it.

To my mother, who supported me all the time.

To those who were the reason for my success, my martyr brothers

(Wisam, Hossam, and Essam),

To those who encouraged me to continue my career in science and who endured the hardships of work and study, my husband

To my children, the eyes from which I draw strength and continuity (Maryam, Hassn, and Malak).

To my friends and colleagues and to everyone who stood with me, even with a glimmer of hope, I dedicate the result of my effort.

Nawres

Acknowledgment

Praise be to Allah Almighty, first and foremost, who gave me the blessing of patience, ability, and perseverance to complete the work. I thank God, the Exalted, the Majestic, a great, good, and blessed one who fills the heavens and the earth for what he has honored me with from completing this study, I hope you are satisfied about her. Thank you to the Messenger of Mercy and Humanity, Muhammad Al-Hadi Al-Amin, and his good and pure family. and his faithful companions, who taught us to perform honesty and seek knowledge.

I am pleased to put the last fingerprints on my thesis to express my sincere thanks and gratitude to Assis. Prof. Dr. Ali Khalaf Ali and Assis Prof. Dr. Mustafa Saddam Chaji, who kindly supervised my thesis and provided continuous encouragement to me in all stages of my research work, I wish them all success and continued health.

I also extend my heartfelt thanks to the esteemed Head of the Department of Biology, my distinguished teachers, and the Deanship of the College of Science, especially the esteemed Dean.

I extend my deep thanks and gratitude to my family, especially my mother and my husband. May God reward them with the best reward, and I pray to God to bless them with health, wellness, and healing.

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All thanks and appreciation to my friends, especially Alaa and Israa, for their help and support throughout the study period.

Nawras

Summary

Summary

This study was conducted at the College of Veterinary Medicine, University of Basra from the period 15\10\2022 to 20\3\2023.The study was conducted using 90 male rats and which was divided into six groups, each of group consisting of fifteen rats. The first group, the control group was given saline solution orally (2.5 ml) for 14 days; the second group, the protocol group was given the medication protocol used to treat COVID-19 patients (remdesivir, azithromycin, dexamethasone, heparin, and supplements); the third group received remdesivir (2.5mg/rat); the fourth group received azithromycin (13mg/rat); the fifth group was given dexamethasone (0.15mg/rat); and the sixth group was given heparin (1.5mg/rat) .The treatment was given once daily for fourteen days except for the remdesivir, which was given treatment for five days, as for the supplements, they were given according to the following doses for 14 days orally (zinc 1.2mg/rat , vitamin C 200mg/rat and vitamin D 0.03mg/rat). Whereas the trial lasted five weeks.

At each stage, the weight of the rats was taken before sacrifice of them, five rats from each group were euthanized at the end of the weeks (first, second and fifth), blood samples were collected to obtain a serum for measure hormones (follicle-stimulating hormone FSH, luteinizing hormone LH and testosterone T), the reproductive organs were removed and their weights were taken (testicle, epididymis and seminal vesicle), and sections were prepared to study histological and histomorphometracal changes of seminiferous tubule in the testicle and epididymis and counting the numbers of the cells:(spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, Leydig and Sertoli cells). Semen was collected from the epididymis for the purpose of calculating sperm concentration, sperm motility, and the percentage of dead and abnormal sperm.

The results of this study showed the following:

A significant decrease ($p \leq 0.05$) in the average body weight of the protocol and dexamethasone groups, while significant increased ($p \leq 0.05$) in the weight average of remdesivir group during the second and fifth weeks of the experiment. a significant decrease ($p \leq 0.05$) in the testis weight average in all groups during the second and fifth weeks, while decrease in the epididymis weight in the dexamethasone group during the first second and fifth weeks, and a significant decrease ($p \leq 0.05$) in the epididymis weight of remdesivir group during the second week, a significant decrease ($p \leq 0.05$) in the seminal vesicle weight in all groups during the first and second weeks.

A significant increased ($p \leq 0.05$) in the LH serum level in the first week in the all groups, and increased significantly ($p \leq 0.05$) in the second week in the protocol, remidsvir, dexamethasone and heparin groups, and significant increased ($p \leq 0.05$) in the heparin group during the fifth week compared to the control group. A significant decreases ($p \leq 0.05$) in FSH serum level in the protocol, azithromycin, dexamethasone and heparin groups in the fifth week compared to the control group. The testosterone hormone significant decrease ($p \leq 0.05$) in protocol, remidsvir, azithromycin and dexamethasone groups during the second week from experience compared to the control group.

A significant decrease ($p \leq 0.05$) in the sperm count and motility in all groups during the first, second and fifth weeks. a significant increase ($p \leq 0.05$) in the dead sperm percentage in all group except the heparin group no significant ($p > 0.05$) differences during the first, second and fifth weeks. a significant increase ($p \leq 0.05$) in the abnormal sperm percentage in the protocol, remdesivir, and azithromycin groups in the five weeks periods.

The diameter of the seminiferous tubule a significant decreased ($p \leq 0.05$) in the protocol group and the other groups during the first second

and fifth weeks. While the diameter of the epididymis duct decrease in all groups during the second week. a significant decrease ($p \leq 0.05$) in the number of the (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, Leydig cells, and Sertoli cells) in all groups during the five weeks periods.

The study's findings revealed histopathological changes in the reproductive organs (testis, epididymis and seminal vesicle) which observed in all groups throughout the first, second, and fifth weeks of the experiment. In the protocol group, there is an abnormal form of the seminiferous tubules and a deficiency in the process of spermatogenesis, as well as a degeneration and necrosis of testicular tissue. The epididymis demonstrated sperm loss, an increase in interstitial regions, and an uneven cavity. Furthermore, the basement membrane collapsed, and the morphology of the epididymis cells changed from pseudostratified columnar to simple columnar or cuboidal.

In the remdesivir group, testicular tissue revealed a severe damage, necrosis, and vacuolation in the first and second weeks, and fibrosis with blurred cellular structure in the fifth week. While the epididymis tissue showed a damage of the basement membrane and tubular lumen, which resulted in vacuolation of the epididymis duct epithelial lining and the absence of spermatozoa.

In the azithromycin group, there was deterioration of the germinal epithelium, insufficient spermatogenesis, gaps, and the lumen was filled with detached germ cells, interstitial blood vessel congestion existed. The epididymis anatomy in the azithromycin group showed an abnormal lumen loaded with sperm and epithelial cell hypertrophy. The testis tissue in the dexamethasone group showed seemed noticeably necrotic, and the epididymis tissue displayed an uneven lumen with decreased sperm production. The

presence of gaps within the seminiferous tubule in the heparin group, the first and second weeks exhibited interstitial blood vessel congestion, while the fifth week showed normal structure, sperm proliferation inside the seminiferous tubules, and an epididymis packed with sperm.

The results of the study also showed that the most satisfactory histological changes groups are the protocol group and the remdesivir and were more severe in the second week of the first and fifth weeks.

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List of Abbreviations

Abbreviate	Definition
229E, OC43, NL63, and HKU1	Human Coronavirus
ABP	Androgen-Binding Protein
ACE2	Angiotensin-Converting Enzyme
ARDS	Acute Respiratory Distress Syndrome
ATP	Adenosine triphosphate
AZM	Azithromycin
BTB	blood-testicular barrier
BBB	Blood-Brain Barrier
DEX	Dexamethasone
EBOV	Ebola virus
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
FSH	Follicle –stimulating hormone
GnRH	Gonadotropin releasing hormone
GS-5734	Remidsvir
H&E	Hematoxylin & Eosin
HPG	Hypothalamus pituitary Gonads axis
ICSH	Interstitial cell- stimulating hormone
LH	Luteinizing hormone
LHR	Luteinizing hormone receptors
LMHW	Low Molecular Weight Heparin
LPO	Lipid peroxidation
MDA	Malondialdehyde
MERS-CoV	Middle East respiratory syndrome
NIDDKD	National Institute of Diabetes and Digestive and Kidney Diseases us
RdRp	RNA-dependent RNA polymerase
ROS	Reactive oxygen species
SARS-CoV-2	Serve acute respiratory syndrome-coronavirus 2
SPSS	Social Package of Social Sciences
TMPRSS	Enzyme transmembrane serine protease-2
SARS - COV 1	Serve acute respiratory syndrome-coronavirus 1
TNF α	Tumor Necrosis Factor Alpha
7-DHC	7-Dehydrocholestrol
UVB	Ultraviolet Type B
CASA	Computer-Assisted Semen Analysis

Chapter one

Introduction

1. Introduction

Serve acute respiratory syndrome (SARS-CoV-2) a novel coronavirus, was discovered in patients with the severe pneumonia near the end of 2019 and spread swiftly over the globe. Associated with SARS-CoV-1 is SARS-CoV-2, a member of the beta coronavirus subfamily (Lee *et al.,* 2003;Zhou *et al*., 2020).SARS-CoV-2 infection can damage spermatogenesis, reduce sperm quality in infected people, have other negative consequences for male fertility . It can also cause orchitis through inflammatory or immune reactions (He *et al*., 2021). The angiotensin-converting enzyme ACE2 receptors shared by SARS-CoV-2 and SARS-CoV-1 are found in many human organs (Zhang *et al.,2020).* SARS-CoV-2 infection weakens organs such as the kidney, lung, and testis that mostly express ACE2, as well as having other detrimental effects on the male reproductive system, such as infertility and sexual dysfunction associated with COVID-19, A study examined the impact of COVID-19 on male reproduction from various angles, and a research review revealed the reproductive toxicity of drugs used in the treatment of COVID-19 (Guo *et al*., 2021).

The food and drug administration (FDA) has authorized the use of remdesivir as an antiviral therapy for COVID-19 in hospitalized adult and pediatric patients (COVID-19 Treatment Guidelines., 2021).There is currently no data indicating that remdesivir has any negative effects on the humans; however, there is one study conducted in male mice indicating the reproductive toxicity of remdesivir (Fan ^{B} *et al.,* 2020). Remdesivir's active form works as a nucleoside analog and inhibits coronaviruses like SARS-CoV-2's, RNA-dependent RNA polymerase (RdRp), which stops the virus from replicating (Kokic *et al.,* 2021). Corticosteroids, in particular dexamethasone, has been widely used due to their strong anti-inflammatory

qualities and capacity to treat lung injury in patients with acute respiratory distress syndrome (ARDS) brought on by coronavirus disease 2019 (Tomazini *et al*., 2020).

Dexamethasone a powerful glucocorticoid (Brinks *et al*., 2018), decreases capillary membrane permeability, inhibits neutrophil migration, and improves the stability of lysosomal membranes (Sharma., 2021). Through the hypothalamic-pituitary-gonadal axis (HPGA),corticosteroids may indirectly affect spermatogenesis and oocyte competence (Whirledge and Cidlowski, 2010;Yuan *et al*., 2016). According to the number of prior studies dexamethasone's actions on the anterior pituitary axis and testicles have been associated with the testicular damage, the death of germ cells and Leydig cells, decreased daily sperm production, impaired sperm motility, and decreased testosterone synthesis (Mukherjee *et al.*, *2015;* Brinks *et al*., 2018).

The broad-spectrum antibiotics are frequently given to COVID-19 patients (Beović *et al.*,2020). azithromycin and other macrolide antibiotics have been used to reduce the likelihood of bacterial co-infection in patients with COVID-19 (Metlay and Waterer, 2020). azithromycin is used to treat pneumonia, bronchitis, upper respiratory tract infections and middle ear infections (Zuckerman *et al*., 2009).azithromycin is classified as a macrolide is one of many lipophilic antibacterial drugs that are widely dispersed in bodily fluids and tissues. Due to its immunomodulatory, anti-inflammatory, and antibacterial properties (Retsema *et al.,* 1987). The 50S subunit of the delicate bacterial ribosome is one of the targets of azithromycin's activity, which also inhibits bacterial protein synthesis (Jelić and Antolović,2016;Heidary *et al.,* 2022). azithromycin use impairs male fertility as measured by semen characteristics, testosterone levels, and testicular infection, according to the number of prior studies (Abeer, 2015; El-Sayed *et al*., 2017).

Because severe COVID-19 symptoms include vascular dysfunction and blood clotting, and these clotting issues are linked to the organ failure and higher mortality, unfractionated or low molecular weight heparin has been utilized as an anticoagulant (Kondashevskaya, 2022). At a molecular level, heparin act to blocks viral contact with host cell receptor in one investigation, SARS-CoV-2 was found to interact with cellular heparin sulfate making heparin a potent competitive inhibitor for SARS-CoV-2 receptor (Clausen *et al.,* 2020).

In addition to the antiviral medications and the anti-inflammatory drugs mentioned above, the COVID-19 Treatment Guidelines recommend adjunctive therapies for the prevention or treatment of COVID-19 or its complications. Vitamin and mineral supplements have been used to treat respiratory viral infections for both the treatment and prevention of SARS-CoV-2 infections (COVID-19 Treatment Guidelines, 2021).

The process of spermatogenesis which is a set of events through which the sperm develop in the testis, occurs in the lumen of the seminiferous tubules, which are the components of the testis, and is ongoing in humans starting at puberty and continuing throughout life (Sharma, 2007).Because the process of spermatogenesis takes place in the testis, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone hormone are regarded as indicators of spermatogenesis as well as the activity of the testis (Sofikitis *et al*., 2008).

1.1. Aims of the Study

The study's goal is to evaluate the COVID-19 treatment protocol's medication effects on male reproductive system in rats by study the following:

1-Measurement the body weight and weight of the male reproductive organs.

2-Histological and histomorphometric study of the male reproductive organs (testis, epididymis and seminal vesicle).

3-Estimation levels of some reproductive hormones (FSH, LH and testosterone)

4-Study the sperm parameters (sperm concentration, motility and percentage of dead and abnormal sperm).

Chapter Two

Literature Review

2. Literature Review

2.1.Male Reproductive System

The male reproductive system in mammalian such as rats consists of the testicles, epididymis, carrier vessel, urethra, penis and accessory glands including seminal vesicles, prostate, and cooper glands as shown in figure (2-1). The male reproductive system performs two essential functions: spermatogenesis and the creation of sex hormones in men, which are responsible for puberty and the emergence of masculine traits (Knoblaugh *et al.,* 2021).

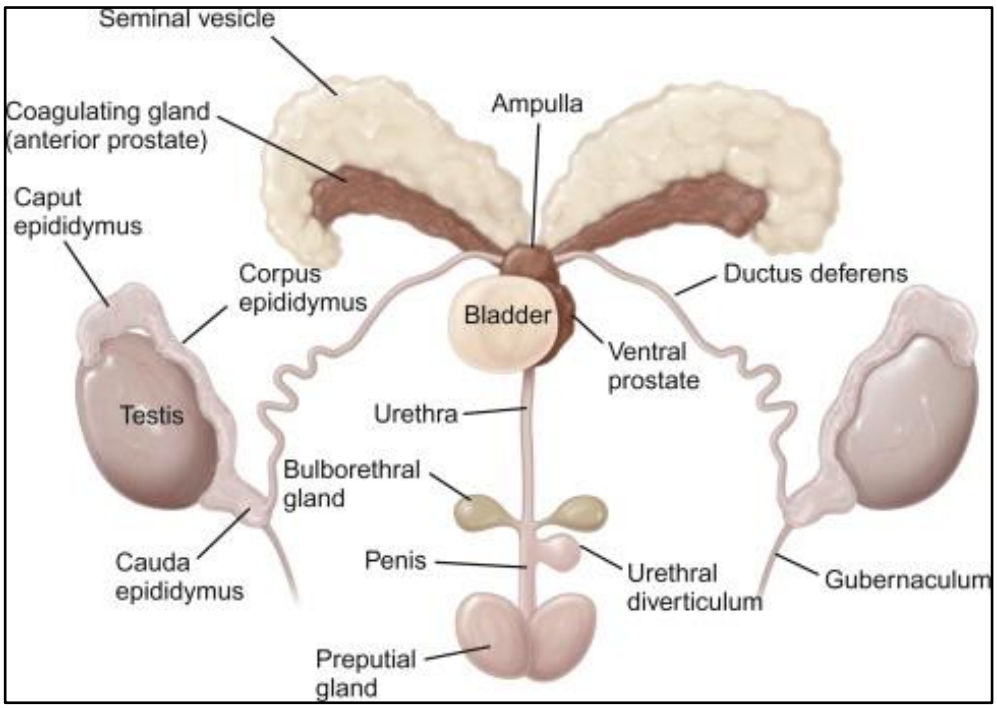


Figure (2-1): Male reproductive system in rats (Treuting *et al* ., 2017).

2.1.1.The Testis

The most important organ in the male reproductive system is the testicle, which contains a pair of testicles. Each testicle is surrounded by a capsule of a thick connective tissue called the tunica albuginea from which barriers extend into the inside of the organ. It divides into pyramidal chambers called testicular lobules, which form convoluted tubes called seminiferous tubules (Mescher, 2018), it's have two main functions, sperm production and steroid synthesis (Carreau *et al*., 2002). The structure of the testicle is formed of a seminiferous tubules that generate the sperm and the interstitial tissue that contains the Leydig cells distributed between the seminiferous that secrete testosterone. Each seminiferous tubule is surrounded by a smooth muscle cells and a basement membrane which lined by Sertoli cells and germ cells (spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids), arranged in layers of varying thickness at different stages of maturity (Creasy *et al*.,2012), as shown in figure (2-2).

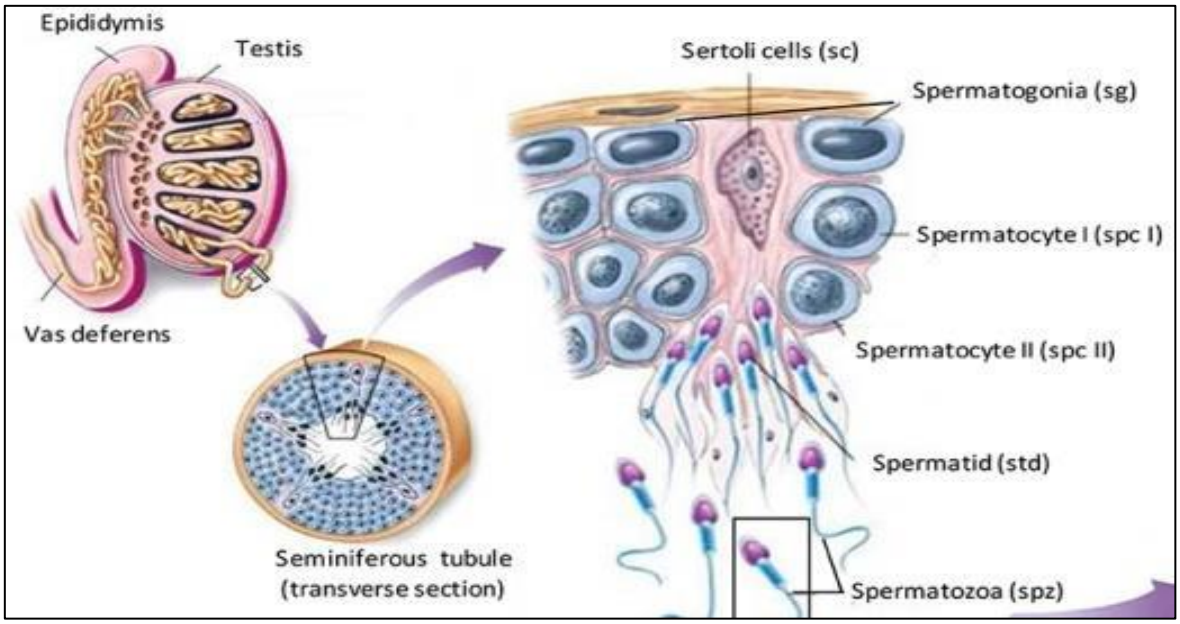


Figure (2-2): Showing the layers of germ cells and the process of spermatogenesis inside the seminiferous tubules (Allais-Bonnet and Pailhoux, 2014).

2.1.2.The Epididymis

It has a single canal and numerous bypasses (Arroteia *et al.*, 2014). A thin coating of smooth muscle fibers encircles it , and between them is a fibrous connective tissue that is made up of a capillaries, cells and fibers (Cornwall , 2009). It consists of two main types of cells, the first type is columnar elongated cells with stereo cilia, which known as principal cells and pyramidal-shaped cells located near the base of the epithelial tissue known as basal cells (Arrotéia *et al*., 2014). The epididymis which is made up of the head, body, and tail regions and is situated on the back of the testicle, in charge of maturing and storing sperm. Because that is where sperm undergo their final maturation, the presence of mature sperm indicates that spermatogenesis is occurring normally. The epididymis tubes are lined with different types of epithelium depending on their located, the head and body are pseudo stratified columnar, whereas the tail has cuboidal to simple columnar epithelium (Treuting *et al*., 2017; Knoblaugh *et al*., 2021). When passing through the epididymis, sperm experience physiological changes (Olaniyan, 2020).

2.1.3.The Seminal Vesicle

Enormous cystic glands on either side of the urinary bladder starting from the dorsal side, they are distinguished by a very bright acidic secretions and a simple or pseudo-stratified columnar epithelium that creates branched mucous folds on their surface (Mescher, 2018;Venditti *et al*., 2019). A wall of three layers surrounds the seminal vesicles, connective tissue with many elastic fibers makes up the outer layer, the intermediate layer made of smooth muscle and the inner layer is made of pseudo-stratified columnar epithelial cells (Pawlina and Ross, 2018).Vesicular fluid, a viscous, white-yellowish fluid with a high fructose, prostaglandin, electrolyte, and fibrinogen content

makes up the majority of the semen (46–80%). Valvin, phosphor choline and vitamin C, Prostaglandin levels in the seminal vesicle are approximately 40 million times greater than those in the blood. When this interacts with cervical mucus, it has been demonstrated to improve sperm motility in the female reproductive canal and to promote smooth muscle contraction in both sexes (Owen and Katz, 2005).

2.2.Spermatogenesis

physiological event that occurs in the seminiferous tubule epithelium to produce sperm (Ni *et al*., 2019). Process which spermatogonial stem cells divided into a spermatocytes during mitosis and tetraploid primary spermatocytes divide during meiosis to form haploid spermatids in the seminiferous tubules. In the final phase of spermatogenesis, spermatids produce spermatozoa (Nishimura and L'Hernault, 2017). High amounts of gonadotropins and testosterone trigger the beginning of spermatogenesis which continues throughout the life with a minor decline as people get older. From the initial stage, mature spermatozoa are produced around 65–70 days (O'Shaughnessy, 2014;Rajender, 2020).

Spermatogenesis is the method through which the testis' seminiferous tubules' germ cells transform into haploid spermatozoa. The process starts with stem cells (a diploid spermatogonium) going through mitosis close to the tubular basement membrane, generating type A and B cells. Type A cells restore the stem cell milieu while type B cells differentiate into diploid intermediate primary spermatocytes, the primary spermatocyte moves to the seminiferous tubules' luminal compartment where it doubles its DNA during meiosis I to produce two haploid secondary spermatocytes, which each undergoes meiosis II to produce two equal haploid spermatids.

Consequently, four spermatids are finally produced by each single spermatocyte. The subsequent step of spermatogenesis involves the functional and morphological transformation of a single spermatid into spermatozoa (sperm) as shown in figure (2-3), (O'Shaughnessy, 2014; Rajender Singh, 2020).

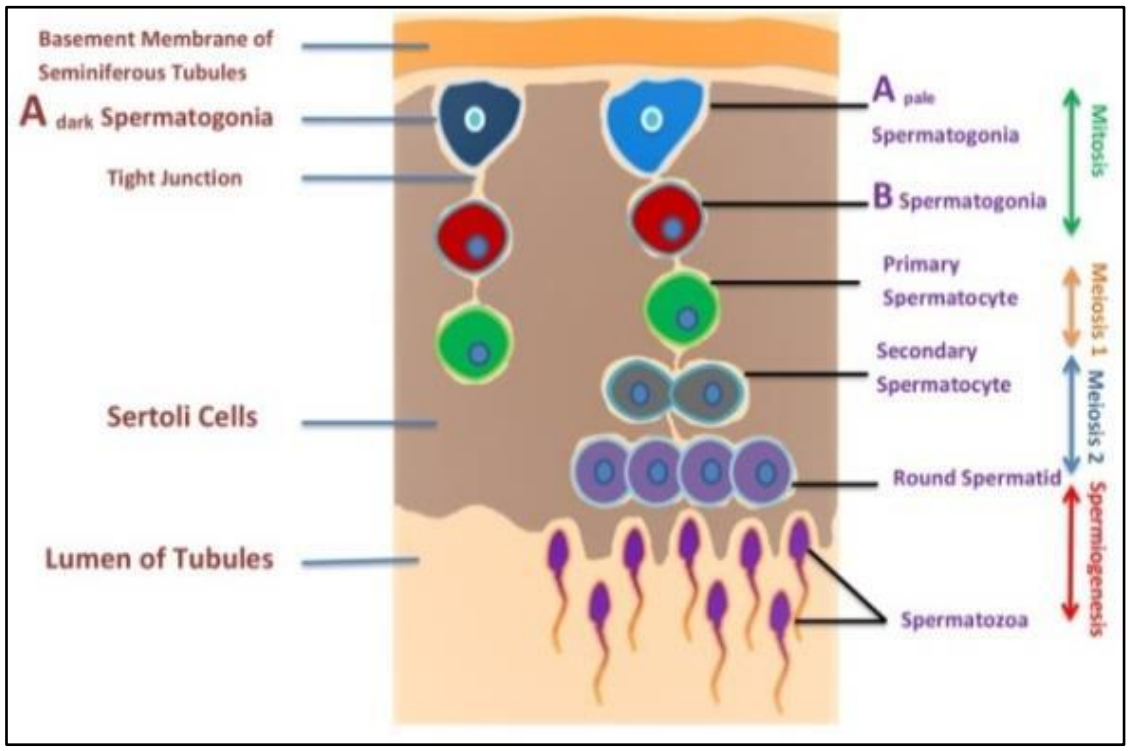


Figure (2-3): The process of spermatogenesis (Ibtisham *et al*., 2017).

2.3. The Hormonal Regulation of Spermatogenesis

The activation of the reproductive system involves three endocrine glands, specifically the hypothalamus gland, which releases Gonadotropin -Releasing Hormone (GnRH), a hormone that releases gonadotropins, pituitary gland and the testicles that create the hormones inhibin and testosterone and the overlap in these third glands' functions, known as the hypothalamus-pituitary-gonadal axis(HPG). It is inactive before puberty for this axis (Kuiri-Hanninen *et al*., 2014).

At puberty, the hypothalamus becomes active and secretes GnRH for less than 10 minutes before being destroyed by pituitary gland cell enzymes. This causes the anterior pituitary gland to respond by secreting FSH and LH (Popa *et al*, 2008;Clasadonate and Prevot, 2018).The Leydig cells, which are situated between the seminiferous tubules, are impacted by the LH hormone as it circulates through the blood to the testicles. Therefore, these cells secrete testosterone which is essential for the development and production of sperm. The blood carries FSH to the seminiferous tubules where it attaches to receptors on Sertoli cells, it causes them to secrete the protein called androgen-binding protein (ABP), which binds the hormone testosterone, increasing its content on the surface of Sertoli cells as a result . Which supports the development and maturation of spermatozoa, the testis' primary function (Allan *et al*., 2010; Lindgren *et al*., 2012).

Negative feedback controls the method through the (HPG)functions. An rise in testosterone levels prevents the production of (GnRH), while when Sertoli cells secrete the inhibin hormone it inhibits the FSH hormone. When the amount of sperm increases, through a negative feedback mechanism, the inhibin hormone goes through the blood to the pituitary gland, where it influences the release of FSH, as shown in figure (2-4),(McLachlan *et al*., 2002;Mcneilly *et al*., 2003).

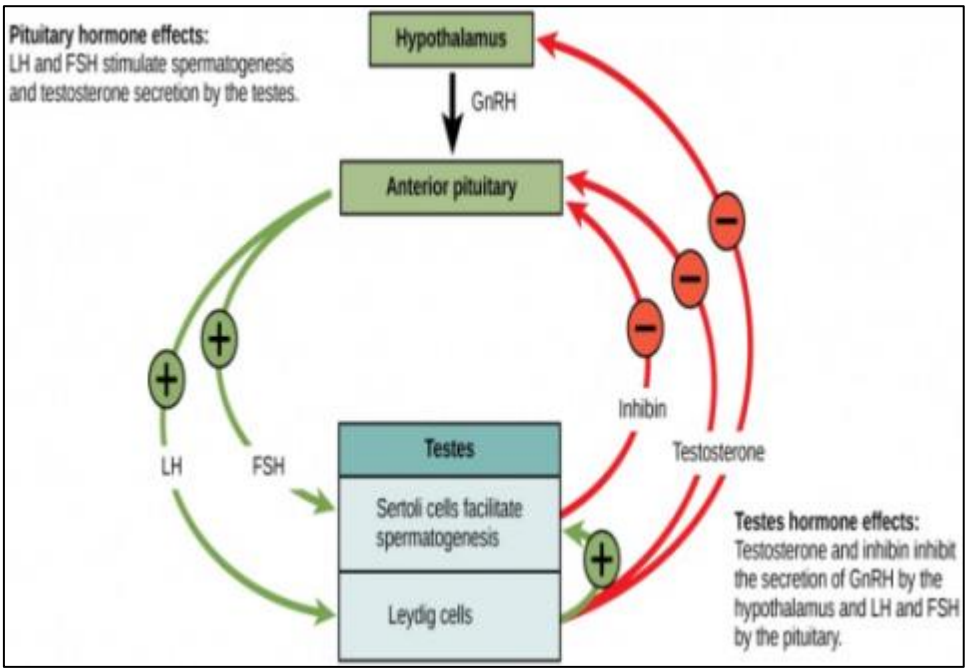


Figure (2-4): Hormones control sperm production in a negative feedback system (Molnar and Gair, 2022).

2.3.1.Follicle Stimulating Hormone (FSH)

It is a glycoprotein consisting of two polypeptide chains, alpha and beta (Santi *et al.*, 2020). The alpha subunit contains 92 amino acids while the beta subunit contains 118 amino acids and the average half-life of the hormone is 3-4 hours (Luboshitzky *et al*.,2005). It is synthesized and secreted by the anterior pituitary gland (Mullen *et al.*,2013). Is essential for sperm production because its plays a crucial role in the differentiation of sperm cells into sperm. Since germ cells lack testosterone receptors this hormone works by binding to the Sertoli cells in the testicles are in charge of feeding germ cells (Hameed *et al*., 2011).

2.3.2.Luteinizing Hormone (LH)

It is a glycoprotein consisting of 85% protein and 15% carbohydrates, (Robert, 2010). Similar in structure to the FSH hormone, as it consists of two subunits alpha and beta (Santi *et al*., 2020). The alpha subunit, as in FSH contains 92 amino acids while the beta subunit is different as it contains 121 amino acids. The biological half-life of LH is shorter than that of FSH which is about 20 minutes (Jiang *et al*., 2012). (LH) is called interstitial cell-stimulating hormone (ICSH), This is because it stimulates the spermatogenesis process by affecting the Leydig cells present in the interstitial cells of the testis to stimulate the secretion of the testosterone hormone (Jimoh *et al*., 2012) .

2.3.3.Testosterone

The primary male hormone responsible for the regulating of sexual differentiation and fertility is testosterone. Additionally, it affects spermatogenesis. It is produced in the interstitial cells distributed in the seminiferous tubules and interacts with receptors in Sertoli cells located in the cytoplasm and nucleus of these cells. It is main purpose is to support spermatogenesis (Nassar and Leslie, 2018). Testosterone is produced by Leydig cells (Gao *et al*., 2018). After receiving a signal from the LH, this pathway is known as the hypothalamic-pituitary-gonadl axis (Hameed *et al*., 2011). It can lead to an imbalance in the HPG can lead to infertility and hypogonadism which leads to failure of this glands in the production of testosterone hormone sufficient for the formation of sperm despite the presence of normal levels of the hormones FSH, LH, and this called the first hypogonadism, which leads to the Loss of testicular function which leads to damage of Leydig or Sertoli cells and thus loss its function (Richard, 2018).

As for the second hypogonadism it results in a disorder of the hypothalamic-pituitary axis a decrease in the GnRH (LH or FSH) leads to a decrease in the testosterone hormone and spermatogenesis (Trevisan *et al.*, 2018), because of the inability of Leydig and Sertoli cells to do their job (Ross and Bhasin, 2016).

2.4. COVID-19:

The word "coronavirus" refers to a few features of the infective form (virion) as observed through an electron microscope such as a strangely shaped bulbous bump (ppeplumeric elevation) which was subsequently discovered to be protein particles connected to the surface of the lipid bilayer membrane (McIntosh., 1974). The family Coronaviridae comprises enclosed viruses with a large single-strand positive-sense RNA genomes (Drosten *et al*., 2003).RNA viruses in the coronavirus family are responsible for the disease in both humans and other animals. They can affect the liver, central nervous system, male reproductive system, and respiratory system of people, dogs, birds, bats, mice, and many other wild species (Chen and Guo, 2016 ; Su *et al*., 2016).

As of 2003 at least seven different types of coronavirus have been linked to the human illness; however, the human coronavirus 229E, OC43, NL63, and HKU1 viruses only induce minor cold symptoms. The final three viruses, which include serious sickness, could be brought on by the virus that caused the SARS pandemic in 2002 and 2003, severe acute respiratory syndrome (SARS COV1) (Ksiazek *et al*., 2003 and Fouchier *et al*., 2003).

A brand-new coronavirus called SARS-CoV-2 was discovered in patients with severe pneumonia at the end of 2019 and has since spread over the world with a steadily increasing number of infection cases.

SARS-CoV-2 and SARS-CoV-1 are also members of the coronavirus subfamily known as the coronaviruses beta (Peiris *et al.*, 2003;Zhou *et al*., 2020).It all began in Wuhan a city of 11 million people in China's Hubei Province (Jingchun *et al*., 2020).China was the first to report this pneumonia with an unknown cause on December 31, 2019 (WHO, 2020).

The World Health Organization (WHO) (2020) named the new coronavirus disease (COVID-19).The virus is also referred to as severe acute respiratory syndrome coronavirus (SARS-CoV-2).Both SARS-CoV-1 and SARS-CoV-2 include angiotensin-converting enzyme (ACE2) receptors, which are present in a variety of human tissues (Zhang *et al.*, 2021).

Prior studies by various research teams demonstrated that SARS-CoV-2 patients had reproductive system insufficiency following infection. previous research has elucidated the molecular underpinnings of viral post infection, clinical characteristics, and detrimental effects on the male reproductive systems of COVID-19 patients (Guo *et al.*, 2021; Rago and Perri, 2023).

2.4.1 Male Reproductive System Infection:

COVID-19 has an impact on several areas of male reproduction, including reproductive system, hormones, gametes, and sexual function. It may cause orchitic or epididymitis, compromising testicular integrity and spermatogenesis. Patients with COVID-19 have lower sperm concentration and motility (Guo *et al* ., 2021). Other conditions such as fever, inflammation, and disruption of the (HPG) may also impair testosterone secretion or sperm production, if these viruses assault the testicles and affect male reproductive function (Ma [A] *et al*., 2021). In addition to the direct relationship between SARS-CoV-2 infection and treatment for COVID-19 includes antiviral medications that have been proven to be associated with oxidant

sensitivity, lower testosterone level, and impaired spermatogenesis (Almasry *et al*., 2017), as shown in figure (2-5).

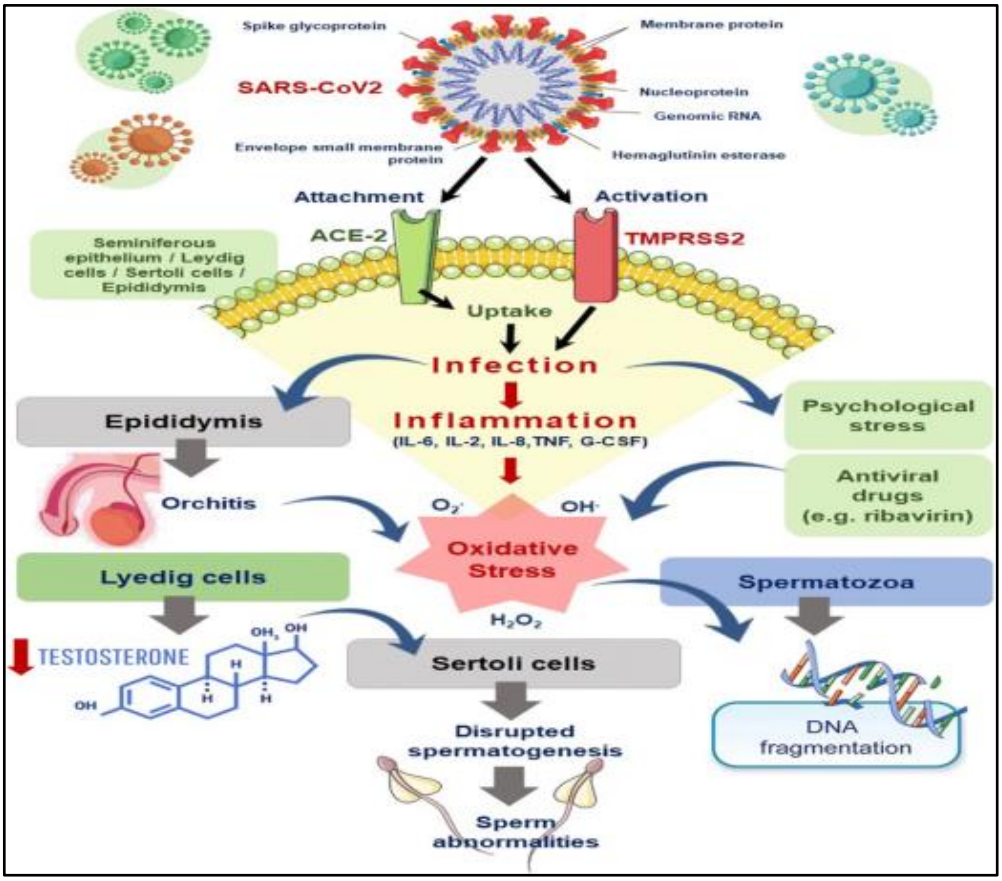


Figure (2-5): The impact of COVID-19 on the male reproductive system in several aspects (Dutta and Sengupta, 2021).

2.4.2.The Effect of Covid-19 on the Testis:-

COVID-19 infection can affect the testicle either directly because SARS-CoV-2 uses ACE2 receptors, which are located in many organs of the body such as the kidneys, heart, intestines, liver and testicle, cells that have a high expression of the enzyme can be a target for the virus (Younis *et al*., 2020).

A previous study showed have shown that there is a high expression rate of ACE2 in the testicular cells especially in cells of the seminal ducts, spermatogonia, Leydig cells and Sertoli cells (Fan [A] *et al*., 2020).Which

indicates that the possibility of infection of the male reproductive system (Sharma *et al*., 2021).

COVID-19 infection penetrates the cells of the body by binding to the ACE2 receptors (Olaniyan *et al*., 2020). It can also penetrate the host cell by the enzyme transmembrane serine protease-2 (TMPRSS), which fuses with the S protein from human coronaviruses through receptors on the membrane and prepares them for viral entry into the cells (Hoffmann *et al*., 2020).In addition to the ACE2, (TMPRSS) is expressed in sperm, Leydig cells, and Sertoli cells. This provides a potential pathway for the virus to enter these cells (Wang and Xu, 2020). It is possible it was concluded that the testicle could be a target to direct damage through binding of SARS-COV-2 to (ACE2) and (TMPRSS2) in tissues testis (Abobaker and Raba, 2021).

Covid-19 affects fertility indirectly by elevating body temperature and inducing inflammation as a result of infection. The resulting fever and the migration of inflammatory cells into cells that disrupt the function of Leydig cells may have an indirect impact on fertility. As a result of heat stress in the testis, oxidative stress and DNA damage occur in the sperms (Albani *et al*., 2019).

According the degraded germ cells sloughed that observed into the lumen of the seminiferous tubules (Ma^{B} *et al*., 2021) Sertoli cell destruction and infiltration of T-lymphocytes, B-lymphocytes, and macrophages in the testicle of the Covid-19 patients, indicating that they have a viral orchitis, which created a malfunction and impaired the spermatogenesis process. Sertoli cells displayed enlargement, vacuolation, and cytoplasmic rarefaction as well as dissociation from tubular basement membranes, and the number of leydig cells was reduced, which is responsible for lower testosterone synthesis (Yang *et al*., 2020).

Testicles of the COVID-19 patients also showed an interstitial edema and moderate lymphocytic inflammation, which are symptoms of orchitis and they also discovered epididymitis (Li *et al*., 2020 and Ma [B] *et al*., 2021). SARS-CoV-2 were observed in blood-testicular barrier (BTB) endothelia, seminiferous tubules and sperm in the epididymis (Yao *et al*., 2021).

2.4.3.The Effect of Covid-19 on Sperm Parameters:-

The (BTB), whose functional integrity may be compromised by a systemic inflammation, allows the virus to enter the semen from the blood. Semen analysis can reveal changes such as decreased sperm count, increased morphological changes, decreased motility, and increased DNA fragmentation.(Rago and Perri, 2023).One of the typical symptoms of COVID-19 patients is febrility (Alimohamadi, 2020).Fever can a significantly affect sperm DNA integrity and semen parameters (Guo *et al.,* 2021).

2.4.4. The Effect of Coved-19 on Male Sex Hormone:-

In the COVID-19 patients, an alternate sex hormone imbalance is demonstrated. SARS-CoV-2 has also been displayed to cross the blood-brain barrier (BBB), and when it infects cells that express ACE2, it encourages neuro inflammation in brain regions including the hypothalamus, disrupting its physiological processes controlling hormone balance and body temperature (Baig *et al*., 2020;Pascual-goni *et al*., 2020). As a result, the higher risk of male infertility found in COVID-19 patients by (HBG) malfunction, which causes aberrant secretion of GnRH, FSH and LH, impacting testosterone creation and spermatogenesis.

Despite the fact that hypogonadism caused by testicular inflammation is becoming more common (Sansone *et al.*, 2021; Dutta and Sengupta, 2021). Some studies have identified (HBG) dysfunction in COVID-19 patients,

particularly in severe cases, as well as elevated circulating levels of gonadotropin activity in men, which could be attributed to the transitory activation of gonadotropin-secreting cells as a result of early inflammatory reactions. (Ma [B] *et al.*, 2021 ; Çayan *et al.*, 2020). Furthermore, damaged Leydig cells during SARS-CoV-2 infection might impair testosterone release, which, through pituitary feedback, may eventually result in an increase in gonadotropin levels (Rastrelli *et al*., 2021).

2.5.The Reproductive Toxicity of Covid-19 Medication:-

In COVID-19 treatment protocols, a variety of medications have been used, including antivirals like remdesivir, antibiotics like azithromycin, corticosteroids, including dexamethasone as an anti-inflammatory, and heparin as an anticoagulant, in addition to dietary supplements like vitamins C and D and minerals like zinc (COVID-19 Treatment Guidelines, 2021;COVID-19 rapid guideline).

Through several previous studies conducted separately, animal models showed that these treatments affect male fertility and spermatogenesis and also affect sex hormones (Abeer, 2015; Hanafy and Khalil , 2015; El-Sayed *et al.*,2017; Sadeghzadeh *et al*., 2019 and Fan[B] *et al*., 2020). A Literature analysis also found a link between the toxicity of the reproductive system and the medications used to treat Covid-19 (Guo *et al*., 2021).

2.6. Remdesivir

Originally known as (GS-5734), this drug shows a broad-spectrum antiviral efficacy against a number of viruses, including respiratory syncytial virus, Nipah virus, Ebola virus (EBOV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-1 (Lo *et al.*, 2017; Agostini *et al.*, 2018). Received an Emergency Use Authorization (EUA) from the US

(FDA), allowing it to be used to treat Covid-19 (SARS-CoV-2 infection) in adult and pediatric patients who were hospitalized with severe illness (Rubin *et al*., 2020). remdesivir interacts with the viral RNA-dependent RNA polymerase (RdRp) which is why remdesivir is effective against SARS-CoV-2. This also explains why remdesivir is effective against zoonotic coronaviruses with a highly divergent RdRp (Sheahan *et al* ., 2017; Brown *et al*., 2019). remdesivir effectively inhibits RdRps from a variety of flaviviruses, including Zika, Dengue, Japanese encephalitis, Tick-borne encephalitis, and West Nile fever (Konkolova *et al*., 2020), with chemical formula $C_{27}H_{35}N_{6O}P_8$ as shown in figure (2-6), (Bakheit *et al.*, 2023).

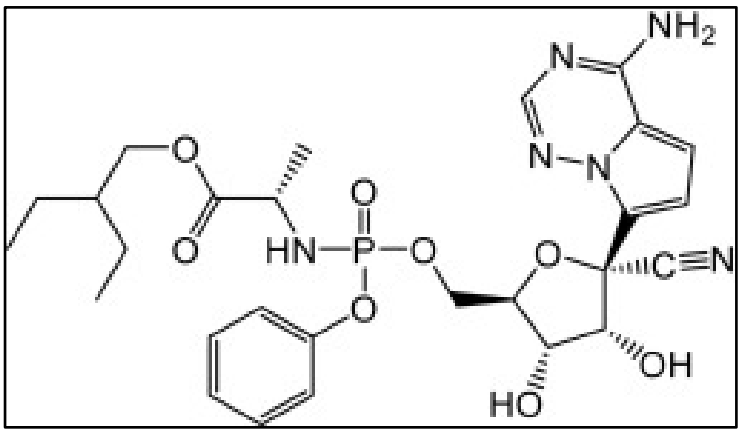


Figure (2-6):chemical structure of remdesivir GS-5734 (Bakheit *et al.*,2023)

2.6.1.Mechanism of Action:-

Remdesivir's active form functions as a nucleoside analog and prevents coronaviruses like SARS-CoV-2 from producing (RdRp). The RdRp incorporates remdesivir into the developing RNA product allowing three more nucleotides to be added before RNA synthesis stalls (Kokic *et al.*, 2021).

2.6.2.Pharmacologically:-

The monophosphate nucleoside analogue GS-441524 can be effectively delivered into cells by remdesivir. The GS-441524 monophosphate quickly

transforms inside the cells into the biologically active nucleoside triphosphate form GS-443902. In order to specifically inhibit viral (RdRp), nucleoside triphosphate GS-443902 functions as an analogue of adenosine triphosphate (ATP) and competes with the natural ATP substrate. The viral (RdRp) incorporates the nucleoside triphosphate GS-443902 into developing RNA chains, delaying RNA chain termination during viral replication, this is the main method of inhibition. remdesivir is a prodrug that, when converted to ATP analogues inside the cell, inhibits viral RNA polymerases (European Medicines Agency, 2020).

2.6.3. Side Effect:-

remdesivir's most frequent side effects are anemia, elevated liver enzymes, a rash on the skin, renal damage and hypotension (Gupta *et al.*, 2020). Additionally, septic shock, hypoalbuminemia, hypokalemia, hypernatremia, pyrexia, hyperglycemia, diarrhea, multiple organ failure, and thrombocytopenia worsened (Badgujar *et al*., 2020). Serious severe cardiac consequences may emerge from pharmaceutical overuse or accumulation (Nabati and Parsaee, 2022). A previous study showed that remdesivir has a negative effect on the male reproductive system such as histopathological effect on testis and epididymis decrease sperm concentration and motility increase abnormal sperm after administration (Fan *et al*., 2020).

2.7. Azithromycin:-

A macrolide is a type of antibiotic like azithromycin (AZM), which marketed as Zithromax. Due to its immunomodulatory, anti-inflammatory, and antibacterial properties, it's chemical formula $C_{38}H_{72}N_2O_{12}$, as shown in the figure (2-7), (Heidary *et al.*, 2022).

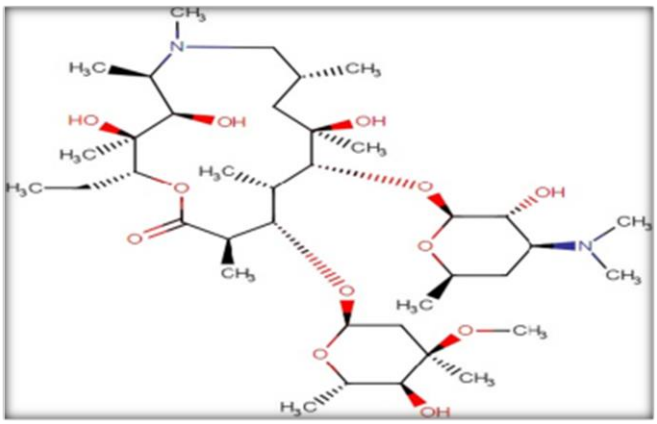


Figure: (2-7) chemical structure of azithromycin (Heidary *et al.*,2022).

2.7.1. Pharmacologically:-

An antibiotic known as a macrolide is azithromycin (Imamura *et al.*, 2005). Because of its dual-base design, numerous cells, including fibroblasts and white blood cells actively absorb AZM (Gladue and Snider,1990). AZM is advantageous for individuals with a variety of inflammatory illnesses of the respiratory tract because it has immunomodulatory, anti-inflammatory, and antibacterial modulatory actions (Albert *et al.*, 2011). In clinical trials, AZM has been used to prevent bacterial infections in individuals with COVID-19 and has been beneficial in doing so (Gautret *et al.*, 2020). Additionally, AZM can modify the functions of the immune system by lowering cytokine production, maintaining the integrity of epithelial cells and preventing lung fibrosis. With AZM, The course of treatment is brief. Adults should take 1500 mg of immediate-release AZM, which is either 500 mg once a day for three days or 500 mg on Day 1 and 250 mg on day 2 through day 5 (Liu *et al.*, 2007).

2.7.2.Mechanism of Action:-

Similar to other macrolide antibiotics, AZM's primary goal is to stop the production of bacterial proteins by targeting on the delicate bacterial

ribosome's 50S component. The decrease in protein synthesis is associated with an increase in the concentration of macrolides (Champney and Burdine, 1998 ; Heidary *et al.*, 2022). AZM binds at a site near peptidyl transferase center on 23S rRNA called nascent peptide exit tunnel (which is approximately 100 Å long and 10–20 Å wide) and partially occludes it (Parnham, 2014 ; Vázquez-Laslop and Mankin, 2018).

2.7.3.Side Effects:-

Hearing loss and cardiovascular arrhythmias are two possible serious side effects. Additionally problematic are interactions with routinely used medications and macrolide resistance (McMullan and Mostaghim., 2015). AZM has a negative impact on male fertility by decrease testosterone hormone secretion, proper spermiograms and the intra-testicular oxidative stress mechanism (Abeer, 2015 ; El-Sayed *et al*., 2017).

2.8.Dexamethasone:-

Glucocorticoid with analgesic and anti-inflammatory effects. It is also recommended for the treatment of postoperative nausea and vomiting, which might have an impact on postoperative development (Ciobotaru *et al.*,2019). Its chemical Formula $C_{22}H_{29}FO$, as shown in the figure (2-8), (Schacke *et al*., 2002).

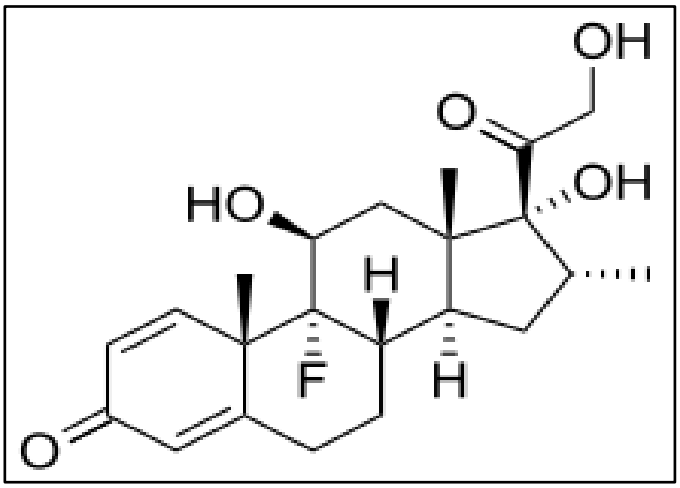


Figure (2-8):chemical structure of dexamethasone (Schacke *et al*., 2002).

In medicine dexamethasone is used for a variety purposes including allergies, cerebral edema, inflammation, and shock. Asthma, atopic dermatitis, contact dermatitis, and medication hypersensitivity (Corssmit and Dekkers, 2019). Dexamethasone has been used successfully as a Cushing syndrome test in endocrinology (Bano *et al.*,2013).Dexamethasone is useful in alleviating chemotherapy-induced nausea and vomiting. It is also used to prevent and cure altitude sickness. It has used in oncology to reduce spinal cord compression caused by metastases (Teachery and Pui, 2019). Dexamethasone is recommended for COVID-19 patients who are critically ill and receiving supplemental oxygen or ventilator support (Agarwal *et al*., 2020).

2.8.1.Mechanism of Action:-

Dexamethasone is a powerful glucocorticoid (Brinks *et al.,* 2018).The body is affected by dexamethasone in a number of different ways. It functions by preventing neutrophil migration and reducing lymphocyte colony proliferation. Additionally, the capillary membrane loses some of its permeability. The stability of lysosomal membranes has been enhanced Prostaglandin, interleukin-1, interleukin-12, interleukin-18, tumor necrosis factor, interferon-gamma, and granulocyte-macrophage colony-stimulating factor are blocked, while serum vitamin A compounds are increased. Dexamethasone has proven to improve pulmonary circulation and increase surfactant levels. Dexamethasone is metabolized through the liver and excreted mainly in the urine. COVID-19 causes an inflammatory state an extreme. Dexamethasone's therapeutic success is therefore probably a result of the extensive anti-inflammatory properties of glucocorticoids (Sharma, 2021).

2.8.2.Administration:-

Dexamethasone comes in a number of formulations. It comes as tablets with strengths ranging from 0.5 mg to 6 mg. An oral solution or an injectable **(**Eckhard *et al*.,2019). Patients with severe COVID-19 should be take 6 mg once daily for ten days (Bhimraj *et al.*, 2020).

2.8.3.Side Effects:-

Even though it is generally well tolerated, dexamethasone has drawbacks as a medication. Sleeplessness is the most common side effect mentioned by patients after taking a medication. Additional typical adverse effects include, weight gain, increased hunger, anorexia in some cases, indigestion, fluid retention, electrolyte imbalances, nausea, vomiting, acne, irritability, and depression. There have been reports of hypokalemia, pulmonary edema, pseudo tumor cerebri, and high intracranial pressure, in addition to adrenal gland suppression (Polderman *et al*., 2018) Long-term steroid therapy causes femoral head osteonecrosis (Wu *et al*., 2019). When given in high doses, it may cause hepatotoxicity (NIDDKD., 2012).Dexamethasone has also been shown to have a negative effect on the spermatogenesis, causing a drop in the secretion of the testosterone hormone, a decrease in germ cells, and a decrease in the motility and concentration of sperm (Sadeghzadeh *et al*., 2019).

2.9.Heparin:-

It is a glycosaminoglycan polysaccharide molecule that is frequently used as an anticoagulant. Heparin has the largest negative density of any known biomolecule. Heparin's major function is anticoagulation, but it also has anti-inflammatory and anti-cancer properties, which are known as pleiotropic effects (Mousavi *et al*., 2015; dit Sollier *et al.*, 2020). LMWH is a

tiny fragment of heparin, a bigger mucopolysaccharide, as shown in figure (2-9), (Mulloy *et al.*, 2016).

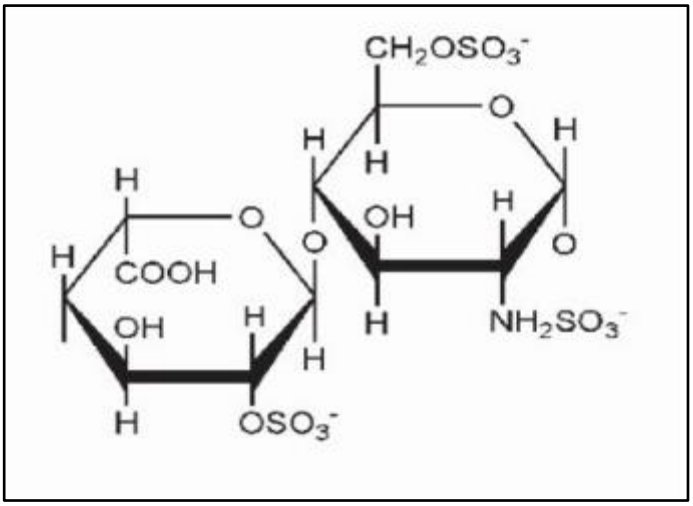


Figure (2-9): chemical structure of Heparin (Gray *et al* .,2008).

2.9.1.Mechanism of Action:-

LMWHs are anticoagulants that work by inhibiting the coagulation cascade's final common pathway (Mulloy *et al*., 2016) . The purpose of the coagulation cascade is to convert blood into a clot, thereby avoiding bleeding. The final common process is the conversion of fibrinogen to fibrin by thrombin activity. Antithrombin III is activated by LMWH, which reduces coagulation. Factor Xa is inhibited by anti-thrombin III. As a result, the final common route is not activated; Xa inactivation means that prothrombin is not converted to thrombin, as a result, fibrinogen is not converted into fibrin for the development of a clot. Heparin works similarly by attaching to and activating antithrombin III. Heparin also has a thrombin binding site, which allows thrombin to interact with anti-thrombin III and heparin, preventing coagulation. Heparin has a faster beginning of anticoagulant activity because it inhibits both Xa and thrombin, whereas LMWH solely inhibits Xa (Mulloy *et al.*, 2016) .

2.9.2.Administration:-

LMWHs are administered subcutaneously (Seaman *et al*., 2014). Because LMWHs have a longer half-life than heparin dosing is more predictable and can be less frequent most often once per day (Mulloy *et al*., 2016).

2.9.3.Side Effect:-

As an anticoagulant, the main risk of LMWH will be bleeding (Mulloy *et al.*,2016). Heparin-induced thrombocytopenia, osteoporosis, spontaneous fractures, and hypersensitivity reactions are some of the less prevalent side effects (Gajic-Veljanoski *et al*., 2016 and Prince & Wenham., 2018).

2.10.Supplements:-

In addition to the antiviral medications and the anti-inflammatory drugs mentioned above, the COVID-19 treatment guidelines recommend adjunctive therapies for the prevention or treatment of COVID-19 or its complications. Vitamin and mineral supplements have been using to treat respiratory viral infections for both the treatment and prevention of SARS-CoV-2 infections (COVID-19 Treatment Guidelines, 2021).

2.10.1.Zinc:-

Zinc and its ionophores are candidates against COVID-19 due to this mineral's immunomodulatory and antiviral properties (Hoang and Han, 2020). The immune system's integrity depends on zinc Its upkeep, growth, and activation of cells throughout innate and adaptive immune responses are crucial (Maggini *et al*., 2010).

2.10.2.Vitamin C:-

Ascorbic acid is a water-soluble nutrient that cannot be produced by humans. Proteins, lipids, and nucleotides are among the macromolecules that are protected from oxidative damage and malfunction by the anti-oxidant activity of vitamin C (Lee and Burckart, 1998). As the COVID-19 infection worsens, cytokine storm increases and vitamin C has been recommended as a preventative measure (Zhou and Xu, 2020). TNF-α and other pro-inflammatory cytokines are known to be decreased by vitamin C, whereas IL-10 and other anti-inflammatory cytokines are increased (Hajishengallis, 2010).

2.10.3.Vitamin D:-

A Fat-Soluble steroid hormone precursor that arises from ultraviolet B (UVB) radiation exposure of 7-dehydrocholesterol (7DHC) in the epidermis of the skin (Gorman *et al*., 2017). Vitamin D's protective effects against viral infection upper respiratory tract infections and blood 25-hydroxyvitamin D levels are inversely correlated, and vitamin D supplementation are known to help lower the incidence and severity of viral illness. Acute respiratory distress syndrome-related mortality in COVID-19 patients could potentially be reduced while vitamin D supplementation's effect on SARS-CoV-2 infection could be reduced proinflammatory cytokines (Zhou *et al.*, 2019).

Chapter Three

Materials and Methods

3. Materials and Methods :-

3.1.Chemicals, Equipment and Tools used:-

3.1.1.Chemicals:-

Table (3-1): Displays the origin and names of the chemicals used in this study

No.	Chemicals name	Country	Company
1	Azthromycin (500mg)	Iraq	Awamedica
2	Canada Balsam	Germany	Roth
3	Chloroform	Switzerland	Sigma
4	Dexamethasone (8mg)	Cyprus	Medochemieltd
5	Distilled water	Iraq	Pioneer
6	Eosin	England	BDH
7	Ethanol (absolute100%)	Spain	Scharlab
8	Formalin	Spain	Scharlan
9	Hematoxylin	England	BDH
10	Heparin	Spain	Pharmex
11	Normal Saline Solution	Germany	Fresenius Kabi
12	Paraffin Wax	Germany	Wollen weber
13	Rat testosterone ELISA Kit	Germany	Abbot
14	Rat (FSH) ELISA Kit	Germany	Abbot
15	Rat (LH) ELISA Kit	Germany	Abbot
16	Remdesivir (100mg)	Egypt	Eva pharma
17	Vitamin C (500MG)	UAE	Natures Aid
18	Vitamin D (5000IU)	UAE	Natures Aid
19	Xylene	India	SDFCL
20	Zinc (50Mg)	UAE	Natures Aid

3.1.2. Equipment:-

Table (3-2): displays the origin and names of the apparatus used in this study :

No	Equipment name	Country	Manufacture Company
1	Digital Camera	Japan	Sony
2	Centrifuge	Germany	Eppendorf
3	Computer-assisted sperm analysis (CASA)	Florida-USA	GENEX
4	Distillation device	Germany	WB2800
5	Electric Oven	Germany	Binder
6	Electrical Balance	Germany	KERM
7	ELISA	Germany	Abbott
8	Hot plate	India	Tglassco
9	Incubator	China	Biocotek
10	Light Microscope	Japan	Olympus
11	Magnetic Stirrer	Korea	Daihan.Lab.tech
12	Oil path	Germany	Memmert
13	Paraffin dispenser	China	premierse
14	Refrigerator	Korea	LG
15	Rotary microtome	Germany	LEITZ
16	Sensitive Balance	Germany	KERM
17	Water bath	Germany	Memmert

3.1.3.Tools:-

Table (3-3): displays the origin and names of the tools used in this study

No	Tools name	country	Company
1	Adapter	USA	Stony Lab
2	Cotton	China	Citioglas
3	Cover slip	USA	Klempa
4	Eppendrof tube	USA	Eppendrof
5	Filter paper	China	Whatman
6	Gloves	China	Broche
7	Micro pipetes	Germany	DRAGON
8	Oral gavage 3ml	China	------
9	Plastic cage	Iran	Kajeen
10	Plastic cups	China	Shanghai Blopak
11	Round flask	Germany	ISOLAB
12	Slides	China	Citioglas
13	Surgical kite	India	Hebson
14	Syringes	China	Citioglas

3.2. Animals used in Experiments:-

This study was conducted at the College of Veterinary Medicine, University of Basra, for the period from 15\10\2022 to 20\3\2023. Ninety male albino rats (weight: 210–230g, age: 5-8 weeks) were procured from University of Basra College of Veterinary Medicine's animal house. Rats were housed and given unrestricted access foods and water in an environment that was controlled (25 ± 2°C, and relative humidity of 50 ± 5%). These rats are pathogen-free. The rats were given two weeks to adjust before being dosed with the medication and placed in plastic cages covered with metal mesh and furnished with sawdust. The mattresses are changed twice weekly while being cleaned and sterilized. The animal food is made up of 50% wheat, 30% fish, 13% greens, 2% salt, sodium chloride (NaCl), and 5% raw fat. For all experimental methods, animals are handled in accordance with institutional norms and are authorized by the local animal ethics committee.

3.3. The Study's Design:-

The experiment, 90 mature male rats participated. The animals were divided into six groups:

Group I:(control): Consisted of 15 male rats that were administrated with normal saline 2.5 ml for 14 days.

Group II:(protocol): Consisted of 15 male rats that were administered the protocol of the drug use to treatment covid- 19 for 14 days .

Based on the surface area technique. The doses were estimated using the human doses and converted in to the animal dose (Nair and Jacob, 2016). Each rat received after diluted once a day :

- Remdesivir intraperitoneal injection (IP), (2.5mg /rat),after diluted 2.5ml.
- Azthromycin orally (13mg /rat),after diluted 1.3 ml.

- Dexamethasone intramuscular injection (IM),(0.15mg/rat),after diluted 0.15ml.
- Heparin subcutaneous injection (SC), (1.5mg/rat),after diluted 1.2ml Supplement orally added to water
- Zink (1.2mg /rat).
- Vitamin C (200mg /rat).
- Vitamin D (0.03mg /rat).

Group III: consisted of 15 male rat that were intraperitoneal injection remdesivir for five day at a dose (2.5mg, after diluted 2.5ml./ rat) once a daily.

Group IV: consisted of 15 male rat that were received azithromycin orally for 14 day at dose (13mg after diluted,1.3ml / rat) once a daily.

Group V: consisted of 15 male rat that were received intra muscular injection dexamethasone for 14 day at dose (0.15mg after diluted 0.15ml / rat) once a daily.

Group VI: consisted of 15 male rat that were received sub cutaneous injection heparin for 14 days at dose (1.5mg after diluted,1.2ml / rat) once a daily.

During the end first, second and fifth weeks, the animals were weighed weekly, and clinical signs and behavior were observed throughout the five weeks Probation. At the end of the first, second, and fifth weeks, five male rats were euthanized for each group. Blood was obtained, the testis, epididymis, and seminal vesicles were excised, and the sperm was extracted to quantify concentration, motility, and the proportion of dead and abnormal sperm as shown in diagram (3-1).

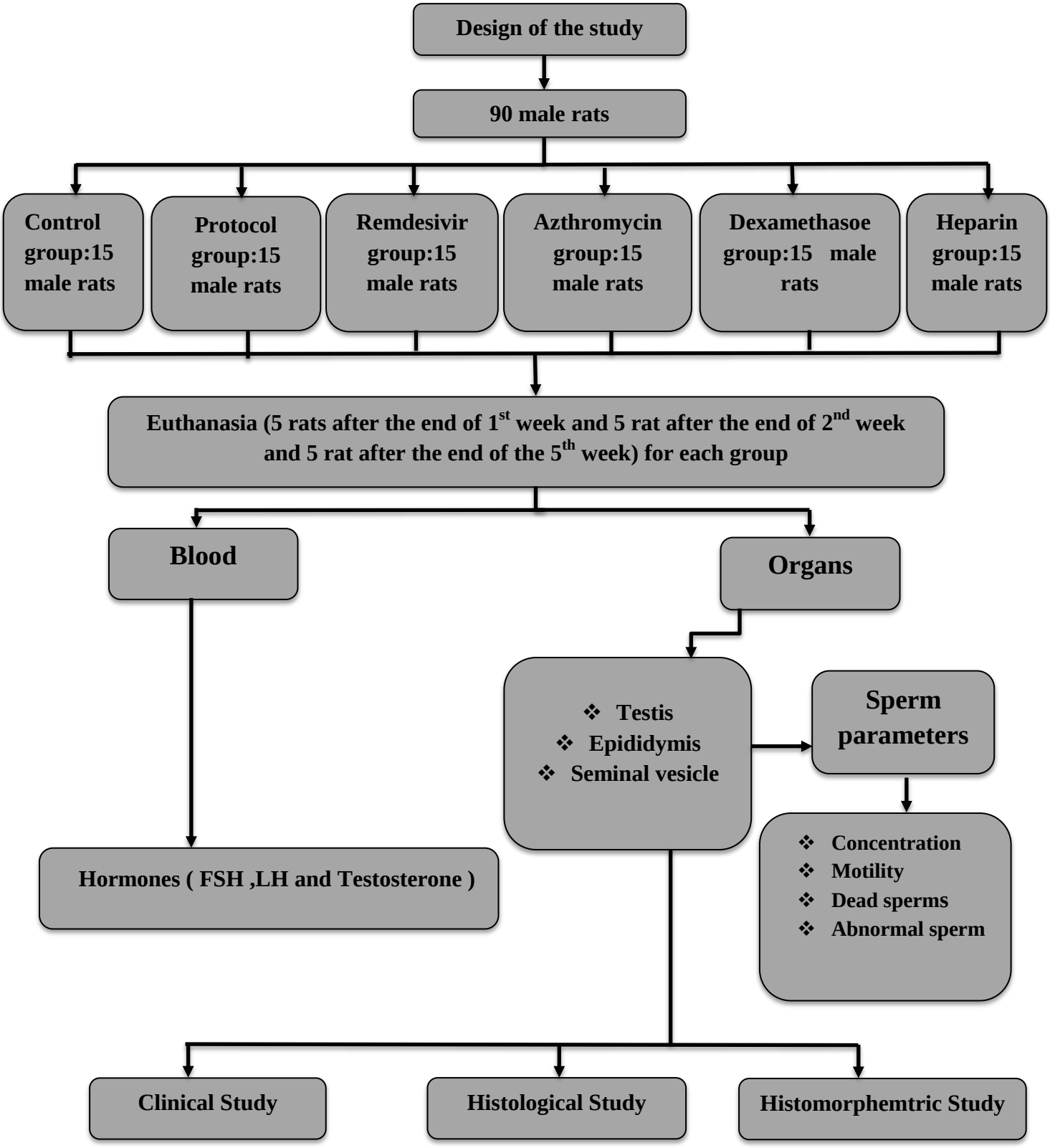


Diagram (3-1): Design of The Study

3.4. Assembly of Blood Samples and Organ Specimens:-

Rats are euthanized by placing them in a closed cage, applying chloroform (CHC13) to cotton, and placing it inside the cage (Blackshaw *et al*.,1988). Blood is extracted from the heart by stabbing it with a syringe (3ml) (Parasuraman *et al*., 2010). Blood samples are collected at the end of the first, second and fifth weeks. The blood is placed in tubes that aid in coagulation in order to collect serum and measure the percentage of hormones (Follicle-stimulating hormone FSH, luteinizing hormone LH and testosterone). The Rat is then placed on the dissection board, and the front and back limbs are fastened before the testis, epididymis, and seminal vesicles are removed and rinsed with normal saline solution (Basim, 2019).

3.5. Sperm Parameters:-

3.5.1.Sperm Motility:-

After being taken, weighed, and placed in a petri dish with 2 ml of saline solution at 37 °C for 5–10 minutes, the epididymis is chopped into small pieces to extract the sperm. An instantaneous sperm sample is obtained, followed by the use of a clean slide, a drop of sperm dilution, and a coverslip. sperm motility was assessed using a computer-assisted semen analysis (CASA). According to (Adamkovicova *et al* .,2016).

3.5.2. Sperm Concentration:-

Computer-Assisted semen analysis was used to assess the sperm concentration in ejaculates (CASA) according (Adamkovicova *et al* .,2016).

3.5.3. Dead Sperm Percentage:-

It Was determined by placing 1-2 drops of the fresh semen sample and 1-2 drops of the pre-warmed eosin-nigrosine on the clean slide, then using the edge of the second slide to mix the semen sample with stain and also to drag the mixture along the surface of the clean slide after smeared was dried. The examination was performed under a light microscope (400x). The background is stained with nigrosine, whereas the dead sperms are stained with eosin, giving the dead sperms a red color while the live sperms appeared color less (Björndahl *et al*.,2003).

3.5.4.Sperm Abnormalities Percentage:-

The slide that counts at least 200 sperm to calculate the proportion of dead sperm is also used to calculate the percentage of abnormal sperm. With a light microscope. The study of sperm anomalies concentrated on identifying head, mid-piece, and tail anomalies (400x) (Björndahl *et al*., 2003).

3. 6. Preparation for Histological Sections:-

The slides were prepared to find out the histological changes in the testicles, epididymis and seminal vesicles of different experimental groups according to the method (Chong *et al*.,2012).

1.Fixation:-

The samples were fixed in 10% buffered formalin solution for 48 hours.

2. Washing:-

After the initial fixation stage, the samples are rinsed under running water for three hours to eliminate any remaining fixative..

3. Dehydration:-

Dehydrating tissue samples in ethanol helped to prevent tissue shrinkage. The fixed tissue was subjected to a succession of ethanol concentrations that rose in strength: 70%, 80%, 90%,100%, 100% (keeping the ethanol concentration constant throughout(tissue in each concentration for two hours).

4. Clearing:-

To make the samples more transparent and to remove the alcohol from the sample, it drank in the xylene solution for half an hour.

5. Infiltration:-

The samples were placed with a mixture of xylene solution and paraffin wax (with a melting point of 56 ° c) and a ratio of 1:1 in an electric furnace at degrees Its temperature is 60°C for 15 minutes In preparation for transferring it to paraffin wax, The samples were transferred in to electric oven at the same temperature, and the process was repeated twice for 30 minutes.

6. Embedding and making blocks:-

The samples prepared in the previous steps were buried with the same type of wax used in the leaching stage, as the molten wax was poured into special molds prepared in advance for this purpose, and the samples were transferred to them and air bubbles were removed using a hot needle around the sample, then the mold was left to solidify

7. Sectioning:-

Sections from paraffin blocks were cut using a rotary microtome with a setting for a 5μm thickness.

8.Sections are attached to slides:-

Tissue fragments were scattered on the surface of a warm water bath (45–48°C) and allowed to float on sterile glass slides.

9.Deparaffinization:-

The parts were cleaned of extra paraffin in the following ways: Sections on slides were treated with xylene in two steps for four minutes after 30 minutes in a 65°C oven, and then processed with ethanol concentrations of 100%, ,90,80,70,.(for two minutes at a time, for each concentration), then finished with a two-minute rinse in water.

Staining Method:-

The samples were stained with hematoxylin and eosin according (Bancroft and Gamble., 2008), As follows.

1- The prepared sections were stained with hematoxylin for 4 minutes, and the sections were washed with tap water for two minutes to get a better blue called bluing.

2- Sections were stained with eosin solution for 10 minutes to describe the cytoplasm.

3-Slides were washed for a full minute using running water.

4- The sections were dried through a series of rising concentrations of ethyl alcohol 70% 90% 80%, for two minutes at a concentration, then at a concentration of 100% for 10 minutes.

5- The slide is transferred to the xylene in two stages for a total of six minutes, each stage lasting three minutes.

6- A little Canada balsam is placed on the slide cover, the slide is covered and placed on a hot plate at 37 ºC .

3.8. Histomorphometrical Study:-

The testis and epididymis were inspected on slides .The testis' seminiferous tubules, epididymis duct and the quantity of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid, Sertoli, and Leydig cells were all measured using light microscope and an ocular micrometer under magnification of (100X.) (Galiher and Kozloff,1964).

3.9.Statistical Analysis:-

Using the SPSS program, the mean and standard deviation of the data were examined for statistical differences below the probability level ($p \leq 0.05$), using a one-way ANOVA (Analyzes Variation) and an LSD test (Nwaigwe and Chrysogonus, 2021).

Chapter Four

Results

4.1.Clinical Study

4.1.1. Body Weights :-

The results showed that there were no significant differences (P>0.05) in the average body weight in all groups during the first week compared to the control group. where the average weight of the control group was (264.00±27.92g), the protocol group (254.40±29.78g), remdesivir (263.60±20.72g), azithromycin (261.00±28.15g), dexamethasone (259.60± 27.39g), heparin (263.80±23.98g).

In the second and fifth weeks, the results showed a significant differences ($p \leq 0.05$) in the average body weights in the protocol remdesivir and dexamethasone groups, while there were no significant differences ($p > 0.05$) in the other groups, the weight of the remidsvir group increased significantly ($p \leq 0.05$), while the weights of the protocol and dexamethasone groups significantly decreased ($p \leq 0.05$) compared to the control group. Whereas, the average weight of the control group was (282.00±18.57g), protocol (247.20±15.12g), remdesivir (287.00±18.57g), azithromycin (278.00±18.23g), dexamethasone (272.80±18.26g), and heparin (281.60±22.41g).

The average weight of the control group in the fifth week was (302.00±9.08g), protocol (263.60±20.72g), remdesivir (309.00±6.51g), azithromycin (299.00±10.84g), dexamethasone (296.80±10.35g), and heparin (297.60±11.01g), table (4-1).

Table (4-1) The changes in the male rat's body weight during the five weeks period.

Group	body weights (g)		
	1st week	**2nd week**	**5th week**
control	264.00 [a] ±27.92	282.00 [a] ±18.57	302.00 [a] ±9.08
protocol	254.40 [a] ±29.78	247.20 [c] ±15.12	263.60 [c] ±20.72
Remidsvir	263.60 [a] ±20.72	287.00 [b] ±18.57	309.00 [b] ±6.51
Azthromycin	261.00 [a] ±28.15	278.00 [a] ±18.23	299.00 [a] ±10.84
Dexamethasone	259.60 [a] ±27.39	272.80 [d] ±18.26	296.80 [d] ±10.35
Heparin	263.80 [a] ±23.98	281.60 [a] ±22.41	297.60 [a] ±11.01

❖ The values represent mean ± SD., different letters represent a significant difference in ($p \leq 0.05$) between groups. Similar letters represent no significant difference.

4.1.2.Testis Weight:-

The result of the study showed that there were no significant differences ($p > 0.05$) in the testis weight in all groups during the first week period compared to the control group, where the average weight of the control group was (1.68±0.52), the protocol group (1.56±0.05g), remdesivir (1.58±0.47g), azithromycin (1.60±0.24g), dexamethasone (1.02±0.17g) and heparin (1.74±0.43g).

The second week there was a significant decrease ($p \leq 0.05$) in the testis weight in all groups compared to the control group and the decrease in the protocol, remdesivir, azithromycin and heparin groups less than the dexamethasone group, the average weight of the control group in the second week was (1.72±0.70g), the protocol group (1.40±0.10g), remdesivir (1.34±0.69g), azithromycin (1.14±0.21g), dexamethasone (0.76±0.05g) and heparin (1.18±0.19g).

In the fifth week a significant decrease ($p \leq 0.05$) in the testis weight in all groups compared to the control group, the average weight of the control group was (1.78± 0.50g), the protocol group (1.48±0.10g), remdesivir (1.50 ±0.24g), azithromycin (1.54±0.29g) , dexamethasone (1.38±0.25g) and heparin (1.58 ±0.25g), table (4-2).

4.1.3.Epididymis Weights:-

The study showed that there were a significant decrease ($p \leq 0.05$) in the epididymis weight in the dexamethasone group and no significant differences ($p > 0.05$) in the other groups during the first-week compared to the control group, where the average weight of the control group was (1.48±0.13g), the protocol group (1.46±0.05g), remdesivir (1.18±0.43g), azithromycin (1.34±0.38g), dexamethasone (0.68 ±0.08g), and heparin (1.44±0.31g).

In the second week, the study showed that there were a significant decrease ($p \leq 0.05$) in the epididymis weight in the dexamethasone and remidsvir groups and no significant differences ($p > 0.05$) in the other groups, the average weight of the control group was (1.50±0.32g), the protocol group (1.46 ± 0.20g), remdesivir (0.74 ± 0.42g), azithromycin (1.18± 0.43 g), dexamethasone (0.54 ±0.05g), and heparin (1.26±0.32g). In the fifth week the study showed that there were significant decrease ($p \leq 0.05$) in the epididymis weight in the dexamethasone group and no significant differences ($p > 0.05$) in the other groups compared to the control group, the average weight of the control group was (1.52±0.34g), the protocol group (1.52± 0.08g), remdesivir (1.44±0.15g), azithromycin (1.50±0.33g), dexamethasone (0.86 ±0.30g), and heparin (1.50±0.32g), table (4-2).

4.1.4. Seminal Vesicle Weights:-

The study showed that there were a significant decrease ($p \leq 0.05$) in the seminal vesicle weight average in all groups during the first and second week period compared to the control group, in the first week the decrease in the dexamethasone group more than other the groups where the average weight of the control group was (0.39±0.08g), the protocol group (0.37±0.15g), remdesivir (0.28±0.08g), azithromycin (0.29±0.02g), dexamethasone (0.26±0.05g), and heparin (0.29±0.22g).

In the second week the decrease in the azithromycin and dexamethasone groups more than other the groups, where the average weight of the control group was (0.40±0.09g), protocol (0.32±0.07g), remdesivir (0.26±0.05g), azithromycin (0.24±0.06g),dexamethasone (0.20±0.00g), and heparin (0.29±0.02g).

In the fifth week, there were no significant differences ($p > 0.05$) in the average weight of seminal vesicle in all groups compared to the control group, the average weight of the control group was (0.41±0.07g), protocol (0.35±0.11g), remdesivir (0.29±0.10g), azithromycin (0.38±0.17g), dexamethasone (0.28±0.08g), and heparin (0.40±0.10g), table (4-2).

Table (4-2) The changes in the testis, epididymis and seminal vesicle weight of male rat over the five weeks.

Groups	weeks	Testis weight (g)	Epididymis weight (g)	Seminal vesicle weight (g)
Control	1st week	1.68[a] ±0.52	1.48[a] ±0.13	0.39[a] ±0.08
	2nd week	1.72[a] ±0.70	1.50[a] ±0.32	0.40[a] ±0.09
	5th week	1.78[a] ±0.50	1.52[a] ±0.34	0.41[a] ±0.07
Protocol	1st week	1.56[a] ±0.05	1.46[a] ±0.05	0.37[b] ±0.15
	2nd week	1.40[b] ±0.10	1.46[a] ±0.20	0.32[b] ±0.07
	5th week	1.48[b] ±0.10	1.52[a] ±0.08	0.35[a] ±0.11
Remdesivir	1st week	1.58[a] ±0.47	1.18[a] ±0.43	0.28[b] ±0.08
	2nd week	1.34[b] ±0.69	0.74[b] ±0.42	0.26[b] ±0.05
	5th week	1.50[b] ±0.24	1.44[a] ±0.15	0.29[a]±0.10
Azithromycin	1st week	1.60[a] ±0.24	1.34[a] ±0.38	0.29[b] ±0.02
	2nd week	1.14[b] ±0.21	1.18[a] ±0.43	0.24[b] ±0.06
	5th week	1.54[b] ±0.29	1.50[a] ±0.33	0.38[a] ± 0.17
Dexamethasone	1st week	1.02[a] ±0.17	0.68[b] ±0.08	0.26[b] ±0.05
	2nd week	0.76[c] ±0.05	0.54[b] ±0.05	0.20[b] ±0.00
	5th week	1.38[b] ±0.25	0.86[b] ±0.30	0.28[a] ±0.08
Heparin	1st week	1.74[a] ±0.43	1.44[a] ±0.31	0.29[b] ±0.22
	2nd week	1.18[b] ±0.19	1.26[a] ±0.32	0.29[b] ±0.02
	5th week	1.58[b] ±0.25	1.50[a] ±0.32	0.40[a] ±0.10

- The values represent mean ± SD., vertically different letters represent a significant difference in ($p \leq 0.05$) between groups. Similar letters represent no significant difference.

4.2. Hormonal Study

4.2.1. Level of Luteinizing Hormone in Serum :-

In the first week, the LH serum level significantly increase ($p \leq 0.05$) in the protocol, remdesivir , azithromycin, dexamethasone, and heparin groups

compared to the control group, where the LH serum level of the control group was (0.004 ± 0.002g), protocol (0.03 ± 0.004 g), remdesivir (0.02 ± 0.01 g), azithromycin (0.03 ± 0.008 g), dexamethasone (0.03 ± 0.01 g), and heparin (0.02 ± 0.01 g). In the second week, the LH serum level significantly increase ($p \leq 0.05$) in the protocol, remdesivir, dexamethasone, and heparin groups while no significant differences ($p > 0.05$) in the azithromycin group compared to the control group, the LH serum level of the control group was (0.004 ±0.002), the protocol group (0.03 ± 0.01 g), remdesivir (0.03 ± 0.01 g), azithromycin (0.00 ± 0.00 g), dexamethasone (0.03 ± 0.01 g), and heparin (0.03 ± 0.004 g).

In the fifth week, there are no significant differences ($p > 0.05$) in LH serum levels in the protocol, remdesivir, azithromycin and dexamethasone groups, while significant increasing ($p \leq 0.05$) in the heparin group compared to the control group, the LH serum level of the control group was (0.004 ± 0.002 g),the protocol group (0.00±0.00g), remdesivir (0.00±0.00g), azithromycin (0.00±0.00g), dexamethasone (0.00 ± 0.00 g) and heparin (0.01±0.008 g), table (4-3).

Table (4-3) The changes in the LH serum levels (mIU/ml) of male rat over the five weeks.

weeks	Groups					
	Control	**Protocol**	**Remdesivir**	**Azithromycin**	**Dexamethasone**	**Heparin**
1st week	0.004^{a} ±0.002	0.03^{b} ±0.004	0.02^{c} ±0.01	0.03^{b} ±0.008	0.03^{b} ±0.01	0.02^{c} ±0.01
2nd week	0.004^{a} ±0.002	0.03^{b} ±0.01	0.03^{b} ±0.01	0.00^{a} ±0.00	0.03^{b} ±0.01	0.03^{b} ±0.004
5th week	0.004^{a} ±0.002	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ±0.00	0.01^{b} ±0.008

❖ The values represent mean ± SD., different letters represent a significant difference in ($p < 0.05$) between groups. Similar letters represent no significant difference.

4.2.2. Level of Follicle Stimulating Hormone in Serum:-

The result showed that there was no significant differences ($p>0.05$) during the first and second weeks compared to the control group, where the FSH serum level in the first week of the control group was (0.01±0.004), the protocol group (0.01±0.004), remdesivir (0.012±0.005), azithromycin (0.002±0.002), dexamethasone (0.008 ± 0.004), and heparin (0.008 ± 0.002).

The FSH serum level of the control group in the second week was (0.01±0.004), the protocol group had (0.00±0.00), remdesivir (0.01±0.006), azithromycin (0.00±0.00), dexamethasone (0.01±0.005), and heparin (0.004±0.002).

In the fifth week, the result showed that there were significant decreases ($p\leq0.05$) in FSH serum level in all groups compared to the control group, except for the remidsvir group, no significant differences ($p>0.05$) were found, where the FSH serum level of the control group was (0.01±0.004), protocol was (0.004±0.002), remdesivir was (0.01±0.00), azithromycin was (0.00±0.00), dexamethasone (0.00±0.00), and heparin (0.004±0.00), table (4-4).

Table (4-4) The changes in the FSH serum levels (mIU/ml) of male rat over the five weeks.

weeks	Groups					
	control	**protocol**	**Remdesivir**	**Azithromycin**	**Dexamethasone**	**Heparin**
1st week	0.01[a] ±0.004	0.01[a] ±0.004	0.01[a] ±0.005	0.002[a] ±0.002	0.008[a] ±0.004	0.008[a] ±0.002
2nd week	0.01[a] ±0.004	0.00[a] ±0.00	0.01[a] ±0.006	0.00[a] ±0.00	0.01[a] ±0.005	0.004[a] ±0.002
5th week	0.01[a] ±0.004	0.004[b] ±0.002	0.01[a] ±0.00	0.00[b] ±0.00	0.00[b] ±0.00	0.004[b] ±0.00

❖ The values represent mean ± SD., horizontally different letters represent a significant difference in ($p\leq 0.05$) between groups. Similar letters represent no significant difference.

4.2.3. Level of Testosterone Hormone in Serum:-

The study showed that there was no significant differences ($p>0.05$) in the testosterone serum level in all groups during the first and fifth weeks period, where the testosterone serum level of the control group the first week was (3.56±0.64), the protocol group (4.00±1.67), remdesivir (2.12±0.72), azithromycin (1.60±0.48), dexamethasone (1.81±1.22), and heparin (2.61±1.46).

In the second week, the result of the study showed that there was a significant decrease ($p\leq0.05$) in the testosterone serum level in all groups, except for the heparin group, no significant differences were found ($p>0.05$) compared to the control group, and the decrease in the dexamethasone groups was less than the protocol, remdesivir , and azithromycin groups, where the testosterone serum level of the control group was (3.58±0.59), the protocol group (0.53±0.28), remdesivir (0.89±0.51), azithromycin (0.60±0.27),dexamethasone (1.84±0.51), and heparin (2.27 ± 0.50).

In the fifth week, the testosterone serum level of the control group was (3.95±0.63), protocol (1.22±0.26), remdesivir (2.37±0.24), azithromycin (2.61±0.44),dexamethasone (1.38±0.22), and heparin (3.91±0.39), table (4-5).

Table (4-5) The changes in the testosterone serum levels (ng/ml) of male rat over the five weeks.

weeks	Groups					
	Control	**Protocol**	**Remdesivir**	**Azithromycin**	**Dexamethasone**	**Heparin**
1st week	3.56[a] ±0.64	4.004[a] ±1.67	2.12[a] ±0.72	1.60[a] ±0.48	1.81[a] ±0.22	2.61[a] ± 0.46
2nd week	3.58[a] ± 0.59	0.53[b] ±0.28	0.89[b] ±0.51	0.60[b] ±0.27	1.84[b] ±0.51	2.27[a] ±0.50
5th week	3.95[a] ±0.63	1.22[a] ±0.26	2.37[a] ±0.24	2.61[a] ± 0.44	1.38[a] ±0.22	3.91[a] ±0.39

- The values represent mean ± SD., horizontally different letters represent a significant difference in ($p\leq0.05$) between groups. Similar letters represent no significant difference.

4.3. Sperm Parameter Study:-

4.3.1. Sperm Concentration :-

The study showed that there was a significant decrease ($p \leq 0.05$) in the concentration of the sperm in all groups during the first, second, and fifth weeks, with the decrease in the protocol, azithromycin, and heparin groups being less than in the remdesivir and dexamethasone groups. where the concentration of sperm in the first week of the control group was (123.84±31.10), protocol (48.06±10.68), remdesivir (26.54±2.69), azithromycin (32.04±1.93), dexamethasone (26.86±5.29), and heparin (50.52±4.47). In the second and fifth weeks, the decrease in the heparin group, less than in the protocol group azithromycin, dexamethasone, and remdesivir groups compared to the control group, where the concentration of sperm in the second week of the control group was (126.42±28.72), protocol (35.02±9.48), remdesivir (21.50±7.94), azithromycin (27.42±3.70), dexamethasone (16.80±3.10), and heparin (68.14±9.45). While the concentration of sperm in the fifth week of the control group was (130.46±26.87), protocol was (35.54±6.56), with remdesivir (30.98±5.52), azithromycin (35.38±4.36), dexamethasone (35.83±3.44), and heparin (76.74±3.33), table (4-6).

4.3.2. Sperm Motility:-

The study showed that there was a significant decrease ($p \leq 0.05$) in the motility of sperm in all groups over five weeks. In the first week, the decreases in the azithromycin, dexamethasone, and heparin groups were less than in the protocol and remdesivir groups, where the motility of sperms in the control group in the first week was (70.72±8.96%), protocol (15.96±2.72%), remdesivir (19.60±1.80%), azithromycin (30.98±6.30%), dexamethasone (33.02±4.04%), and heparin (37.74±4.13%).

In the second week, the decrease in the heparin group was less than the other treated groups, where the motility of sperms in the control group in the second week was (71.68±8.16%), the protocol group (12.68±4.52%), remdesivir (17.88±3.42%), azithromycin (26.14±3.66%), dexamethasone (26.94±4.31%), and heparin (39.26±4.31%).

In the fifth week, the decrease in the heparin and dexamethasone groups was less than in the other treated groups, where the motility of sperms in the control group in the fifth week was (71.94±9.05%), protocol (20.00±2.87%), remdesivir (28.84±4.37%), azithromycin (28.94±3.73%), dexamethasone (36.72±3.99%), and heparin (44.34±5.26%), table (4-6).

4.3.3. Dead Sperms:-

The study showed that there was a significant increase ($p \leq 0.05$) in the percent of dead sperms in all groups except for the heparin group that no significant differences ($p > 0.05$) were found during the first, second, and fifth weeks.

In the first week, the increase in the azithromycin group was less than the protocol , remdesivir and dexamethasone groups compared to the control group, where the percent of dead sperms in the control group was (23.60±4.16%), the protocol group (36.10±3.48%), remdesivir (31.90±4.50%), azithromycin (29.34±1.89%), dexamethasone (35.26±4.12%), and heparin (26.26±3.54%).

The percent of dead sperms in the control group in the second week was (22.28±4.69%), protocol (34.52±3.31%), remdesivir (36.52±5.10%), azithromycin (38.28±2.83%), dexamethasone (31.44±3.04%), and heparin (23.46±1.73%).

The percent of dead sperms in the control group in the fifth week was (22.22±4.69%), protocol (31.94±2.45%), remdesivir (29.42±2.14%), azithromycin (32.62±1.73%), dexamethasone (30.10±2.91%), and heparin (21.12±1.67%), table (4-6).

4.3.4.Abnormal Sperm:-

The study showed that there were a significant increase ($p \leq 0.05$) in the percent of abnormal sperm in the protocol and remdesivir groups, while there were no significant differences ($p > 0.05$) in the azithromycin, dexamethasone and heparin groups during the first, second, and fifth weeks compared to the control group.

In the first week, the percent of abnormal sperm in the control group was (20.36±1.06%), protocol (29.20±5.63%), remdesivir (28.18±1.69%), azithromycin (24.94±3.55%), dexamethasone (21.92±1.20%), and heparin (21.38±1.61%).

In the second week, the percent of abnormal sperm in the control group was (18.76±2.24%), protocol (29.90±4.68%), remdesivir (27.24±2.74%), azithromycin (25.08±3.01%), dexamethasone (19.64±1.82%), and heparin (18.90±3.96%).

In the fifth week, the percent of abnormal sperm in the control group was (19.08±1.37%), protocol (26.00±2.91%), remdesivir (25.28±2.69%), azithromycin (22.54±2.28%), dexamethasone (20.36±1.06%), and heparin (19.09±2.46%), table (4-6).

Table (4-6) The changes in the concentration, motility ,dead of sperm, and abnormal sperm of male rat over the five weeks.

Group	Weeks	Concentration of sperms10^5	Motility of sperms %	Dead of sperms%	Abnormal of sperms%
Control	1st week	123.84^{a} ± 31.10	70.72^{a} ± 8.96	23.60^{a} ± 4.16	20.36^{a} ± 1.06
	2nd week	126.42^{a} ± 28.72	71.68^{a} ± 8.16	22.28^{a} ± 4.69	18.76^{a} ± 2.24
	5th week	130.46^{a} ± 26.87	71.94^{a} ± 9.05	22.22^{a}± 4.69	19.08^{a} ± 1.37
Protocol	1st week	48.06^{b} ± 10.68	15.96^{c} ± 2.72	36.10^{b} ± 3.48	29.20^{b}± 5.63
	2nd week	35.02^{c} ± 9.48	12.68^{d} ± 4.52	34.52^{b} ± 3.31	29.90^{b} ± 4.68
	5th week	35.54^{c} ± 6.56	20.00^{c} ± 2.87	31.94^{b}± 2.45	26.00^{b} ± 2.91
Remdesivir	1st week	26.54^{b}± 2.69	19.60^{c} ± 1.80	31.90^{b} ± 4.50	28.18^{b}± 1.69
	2nd week	21.50^{c} ± 7.94	17.88^{d} ± 3.42	36.52^{b}± 5.10	27.24^{b} ± 2.74
	5th week	30.98^{c} ± 5.52	28.84^{c} ± 4.37	29.42^{b} ± 2.14	25.28^{b} ± 2.69
Azithromycin	1st week	32.04^{b} ± 1.93	30.98^{b} ± 6.30	29.34^{b} ± 1.89	24.94^{a} ± 3.55
	2nd week	27.42^{c} ± 3.70	26.14^{c} ± 3.66	38.28^{b} ± 2.83	25.08^{a}± 3.01
	5th week	35.38^{c} ± 4.36	28.94^{c} ± 3.73	32.62^{b} ± 1.73	22.54^{a}± 2.28
Dexamethasone	1st week	26.86^{b} ± 5.29	33.02^{b} ± 4.04	35.26^{b} ± 4.12	21.92^{a} ± 1.20
	2nd week	16.80^{c} ± 3.10	26.94^{c} ± 4.31	31.44^{b} ± 3.04	19.64^{a} ± 1.82
	5th week	35.38^{c} ± 3.44	36.72^{b} ± 3.99	30.10^{b} ± 2.91	20.36^{a} ± 1.06
Heparin	1st week	50.52^{b} ± 4.47	37.74^{b} ± 4.13	26.26^{a} ± 3.54	21.38^{a} ± 1.61
	2nd week	68.14^{b} ± 19.45	39.26^{b} ±4.31	23.46^{a} ± 1.73	18.90^{a} ± 3.96
	5th week	76.74^{b} ± 3.33	44.34^{b} ± 5.26	21.12^{a} ± 1.67	19.09^{a} ± 2.46

- The values represent mean ± SD., vertically different letters represent a significant difference in ($p \leq 0.05$) between groups. Similar letters represent no significant difference.

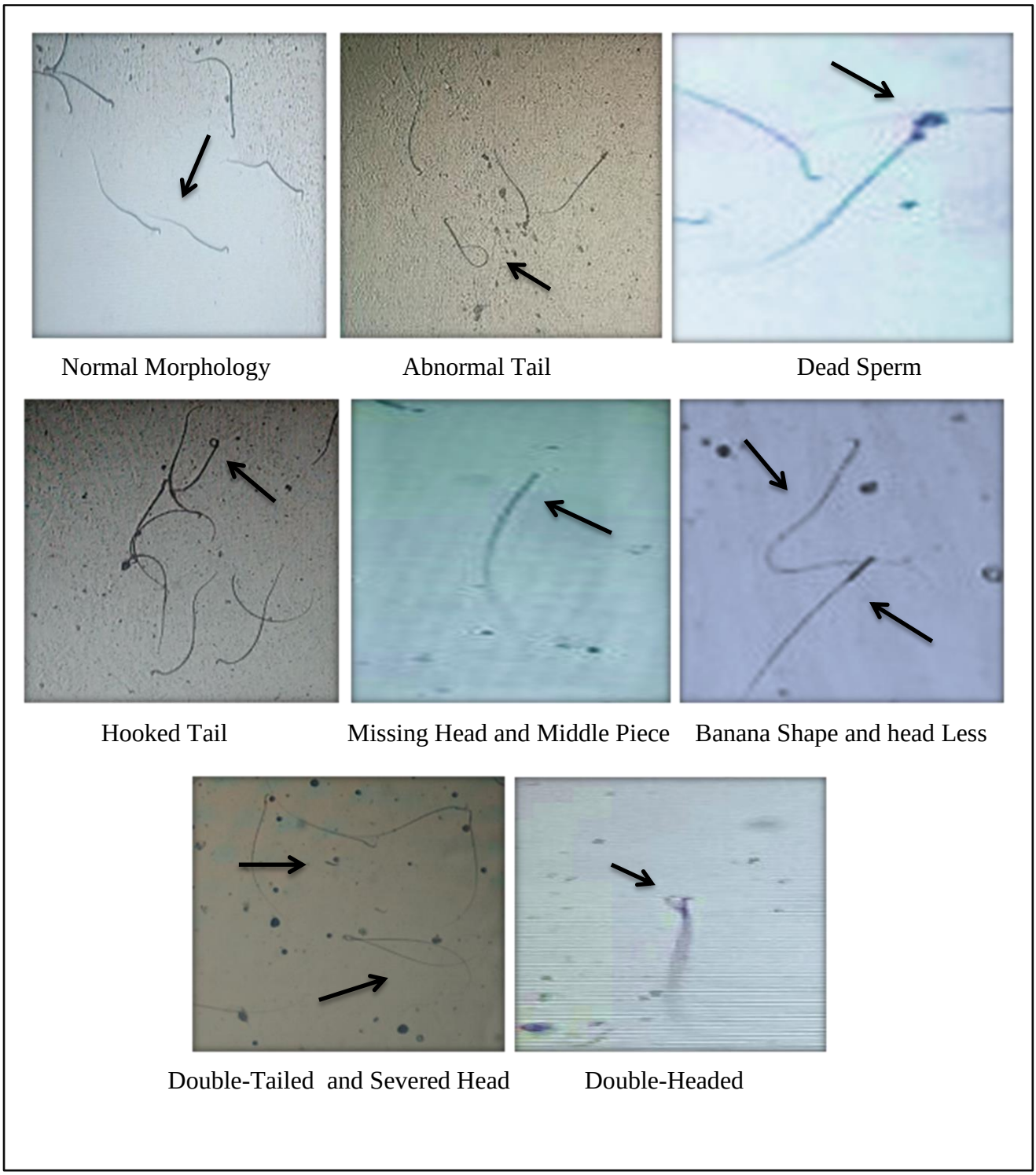


Figure (4-1): Microphotographs Normal Sperm, and Various Sperm Abnormalities , (Nigrosine and Eosin Stain,400x).

4.4.Histological Study

4.4.1.The Testis

The study's findings demonstrated that the control group's testicular tissue had a normal cellular structure in the first week, consisting of seminiferous tubules that contained epithelial germ cells and Sertoli cells arranged with the germ cells in the central layers closely linked to one another, as well as interstitial tissue that was filled with Leydig cells, fibroblasts, and small blood vessels, figure (4-2).

The microscopic image of the testicular tissue in the protocol group of the first week showed an irregular cellular structure, the arrangement of many germ cells and sperm in the late stages was disorganized, and the process of spermatogenesis was disordered and necrotic in several places, figure (4-3).

The group that was given remdesivir showed that the testicular tissue, irregular structure, absence of spermatogenic series, loss of spermatids and severe degenerative changes with vacuolated and necrosis, figure (4-4).

The degradation of the germinal epithelium was seen in the azithromycin group, inadequate spermatogenesis was discovered, gaps were identified, and the lumen was filled with detached germ cells, figure (4-5). In the dexamethasone group, space was found between spermatogonia cells; expansion of the lumen featured irregular and vacuolated seminiferous tubules; and a less compact arrangement of the spermatogenic cells, figure (4-6).

In the heparin group, there were a disorders in the spermatogenesis chain and the presence of spaces within the seminiferous tubule ,figure (4-7).In the second week, testicular tissue from the control group showed that the testes appear typical testicular architecture, the seminiferous tubules was

compact with each other, and spermatogenic layers were present, figure (4-8). In the protocol group, the cells suffering necrotic, with degeneration of epithelial cells and only remnants of the basement membrane and the absence of spermatogenic stages, figure (4-9).

Whereas in the remdesivir group, the cells showed degeneration and the absence of spermatogenic series, and seminiferous tubules showed germ cell disorganization with a necrotic cellular debris, figure (4-10). In the azithromycin group, there was congestion in interstitial blood vessels, poorly differentiated seminiferous tubules, and fewer spermatozoa populations, figure (4-11).

In the dexamethasone group, the testis tissue showed a degeneration of the epithelial cells and appeared markedly necrotic, figure (4-12). In contrast, the heparin group experienced interstitial blood vessel congestion and damaged of the seminiferous tubules, figure (4-13).

In the fifth week, the testis tissue of the control group showed that the tubules lined with seminiferous epithelium with active spermatogenesis, figure (4-14). In the protocol group, the late-stage sperm cells and sperms were greatly reduced or even disappeared, and there was a mild degeneration of the germinal epithelium of some seminiferous tubules, figure (4-15).

In the remdesivir group, the microscopic image showed testicular fibrosis and cell structure disappeared, figure (4-16). Whereas in the azithromycin group, extensive necrosis of spermatogenic cells of seminiferous tubules was observed with the spermatogenesis disorder, figure (4-17).

While in the dexamethasone group, the altered seminiferous tubules showed an irregular shape, disarranged epithelial layers, and a lumen filled with detached germ cells, figure (4-18). In the heparin group showed normal structure and Sperm growth inside the seminiferous tubules, figure (4-19).

The Histopathological changes of testicular tissue during the first and second weeks in the protocol and remdesivir group were more than in other groups. While the remdesivir group was the most affected in the fifth week. Histopathological changes in the testicular tissue appeared in all groups in the second week of the experiment more than in the first and fifth week.

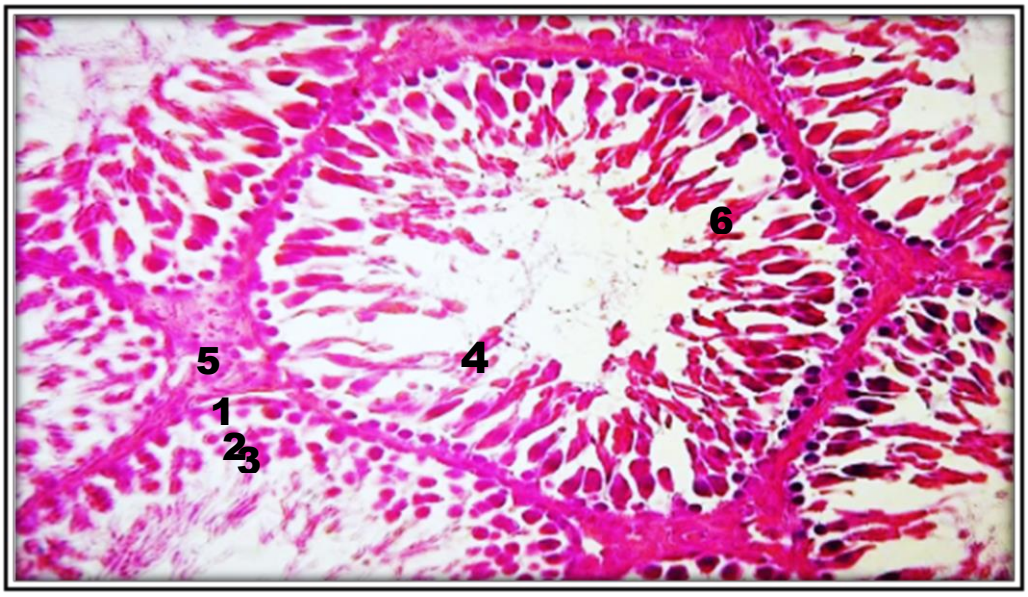


Figure (4-2): tests of the control group in male rats (first week),spermatogonia (1), primary spermatocytes (2), secondary spermatocytes (3), spermatids (4), Leydig cells (5), Sertoli cells (6), (H&E, 400X).

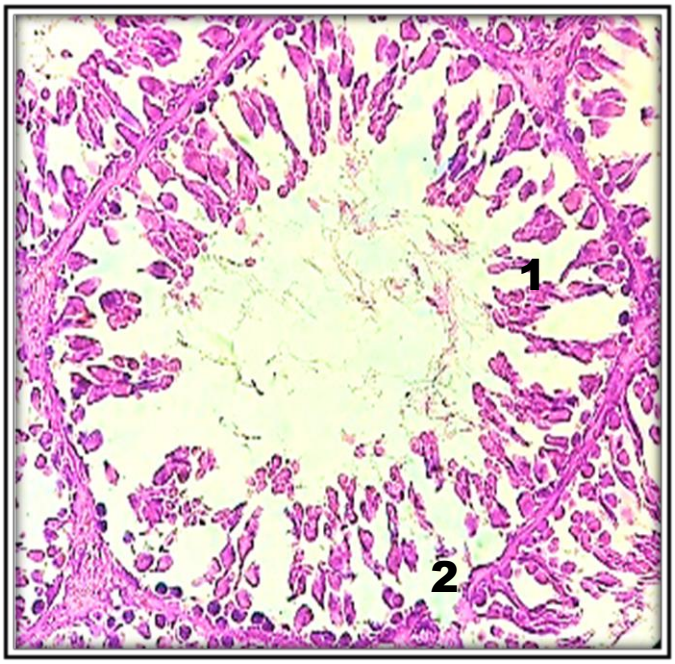


Figure (4-3):testis of protocol group in male rat (first week), organization of the many late-stage sperm cells and sperm disorganized (1), necrosis (2),(H&E.,400X).

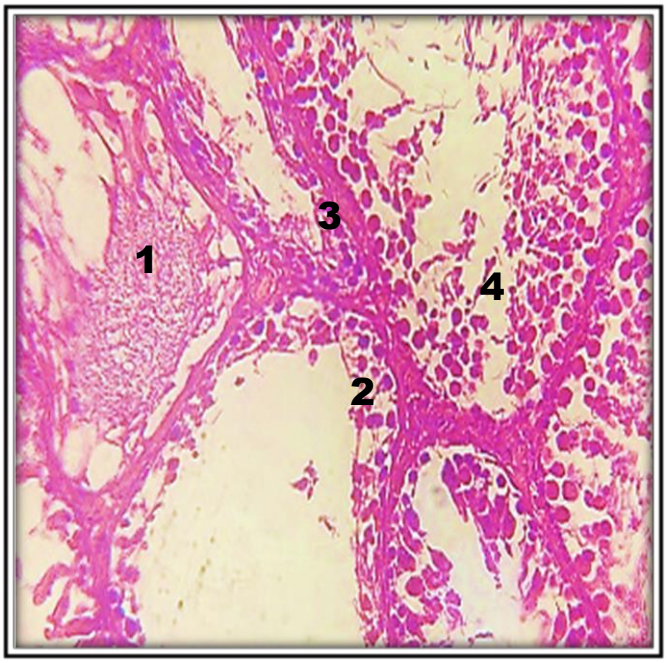


Figure (4-4):testis of remdesivir group in male rat (first week)**,** degeneration (1), absence of spermatogenic stages (2), seminiferous tubules disorganization (3), necrotic cellular debris (4),(H&E.,400X).

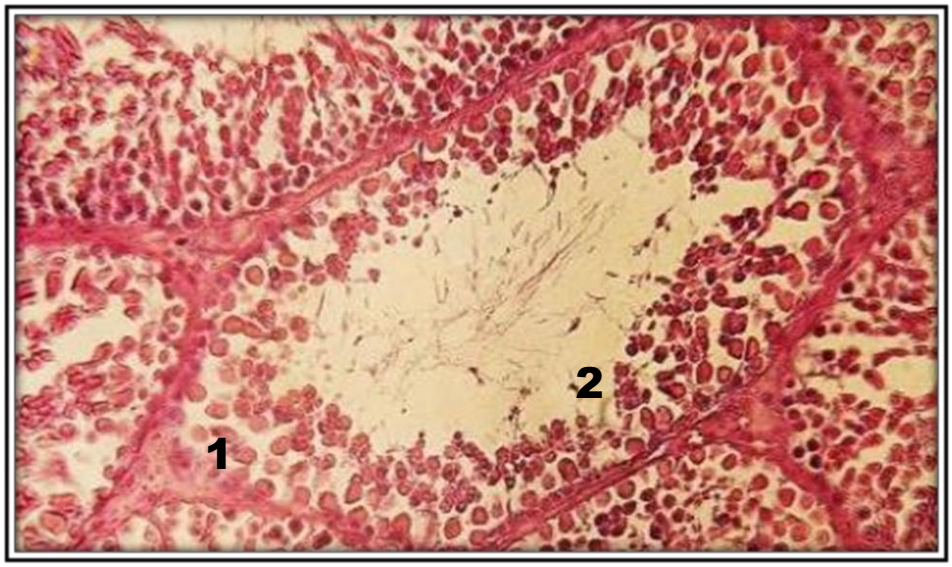


Figure (4-5): Testis of azithromycin group in male rat (first week) , degeneration of germinal epithelium(1), incomplete of spermatogenesis (2),(H&E,400X).

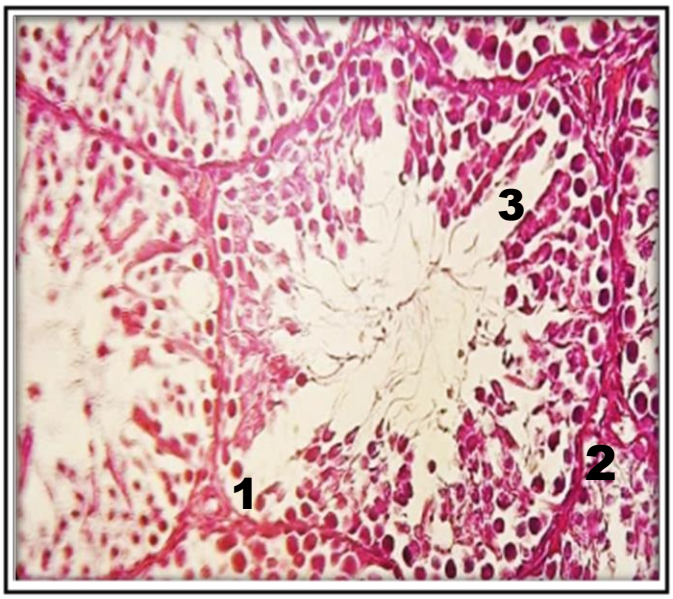


Figure (4-6): Testis of dexamethasone group in male rat (first week), space between spermatogonia cells (1), vacuolated seminiferous tubules (**2**), less compact arrangement of spermatogenic cells (3),(H&E,400X).

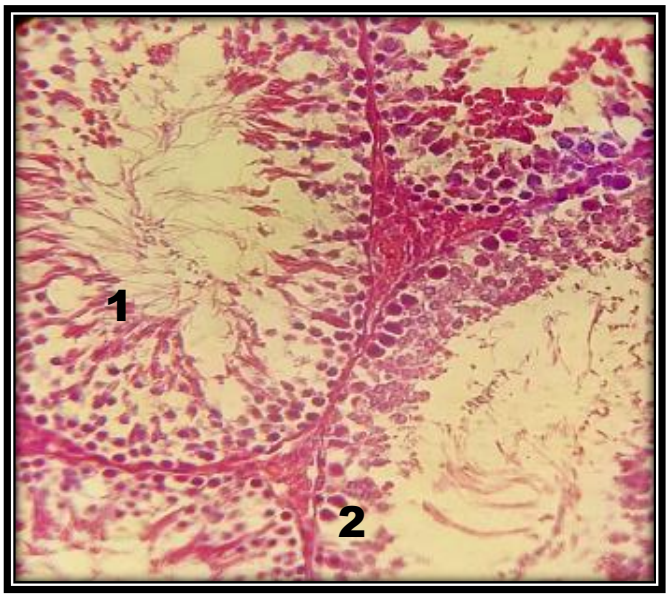


Figure (4-7): Testis of heparin group in male rat (first week), spermatogenesis chain (1), presence of spaces within the seminiferous tubule (2),(H&E,400X).

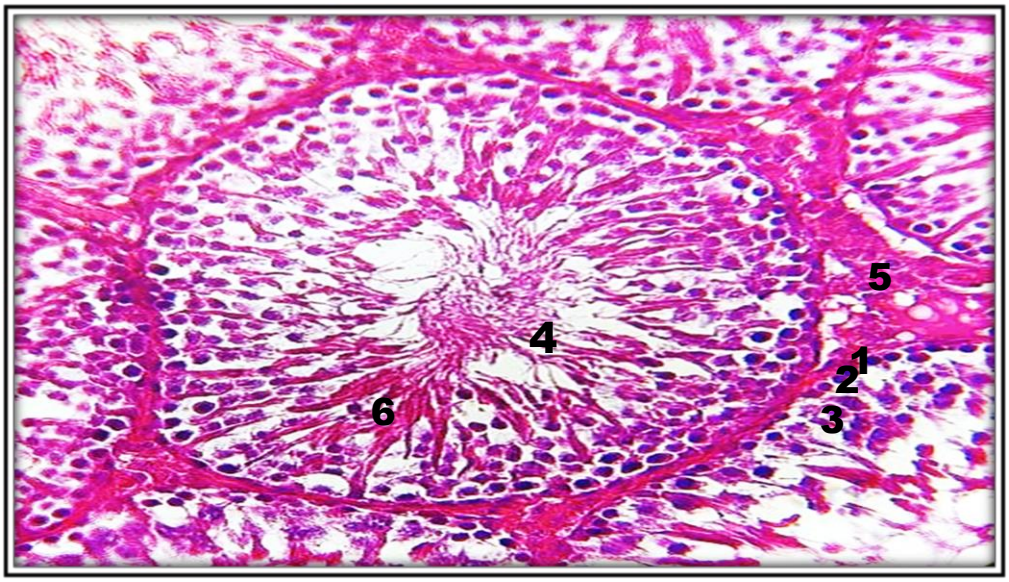


Figure (4-8): Testis of control group in the male rat (second week): spermatogonia (1), primary spermatocyte (2), secondary spermatocyte (3), spermatid (4), Leydig cells (5), Sertoli cells(6), (H&E,400x).

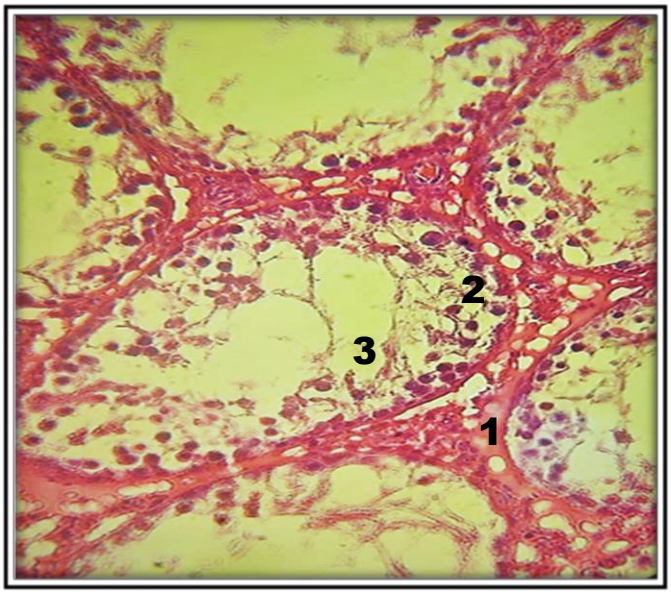


Figure (4-9):testis of protocol group in male rat (second week), necrosis (1), degeneration of epithelial cells remnants of the basement membrane (2), absence of spermatogenic series (3), (H&E,400x).

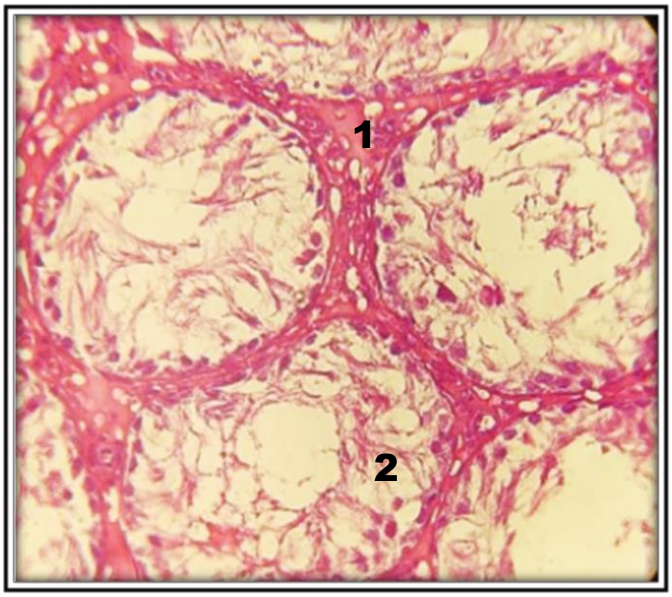


Figure (4-10):testis of remdesivir group in male rat (second week), degeneration (1), absence of spermatogenic series (2), (H&E,400x).

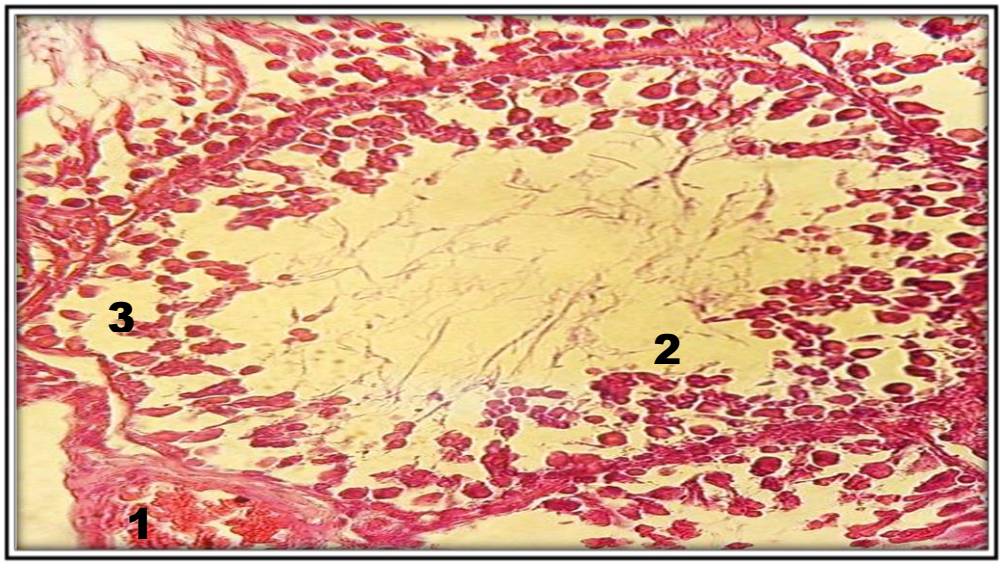


Figure (4-11): Testis of azithromycin group in male rat (second week):, Congestion in Interstitial Blood vessels (1), fewer spermatozoa population (2), mild degeneration of the germinal epithelium (3), (H&E,400x).

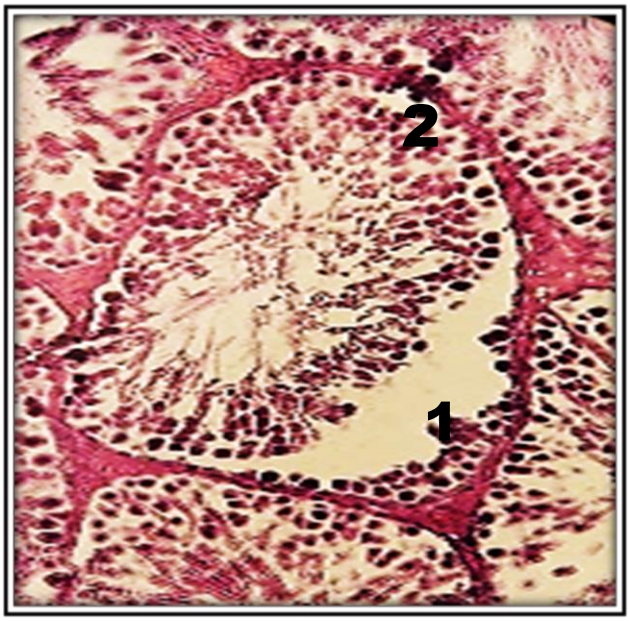


Figure (4-12): Testis of dexamethasone group in male rat (second week), degeneration of epithelial cells(1), necrosis (2), (H&E,400x).

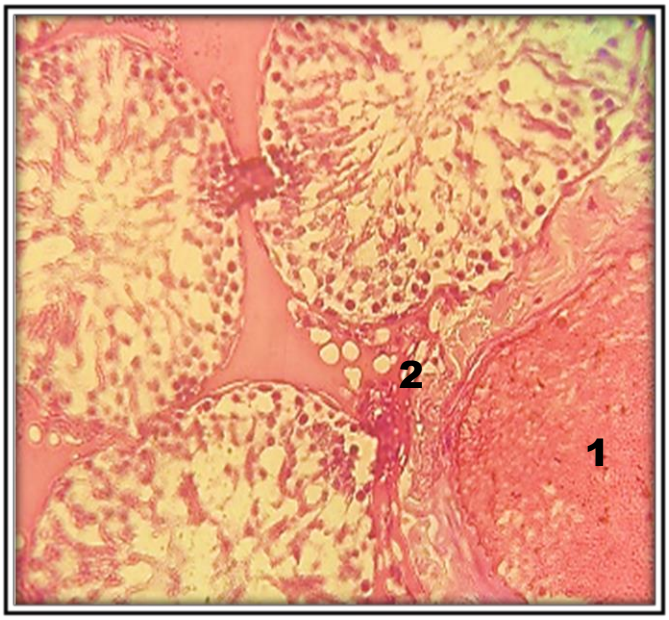


Figure (4-13): Testis of heparin group in male rat (second week): congestion in interstitial blood vessels (1), damaged seminiferous tubule (2), (H&E,400x).

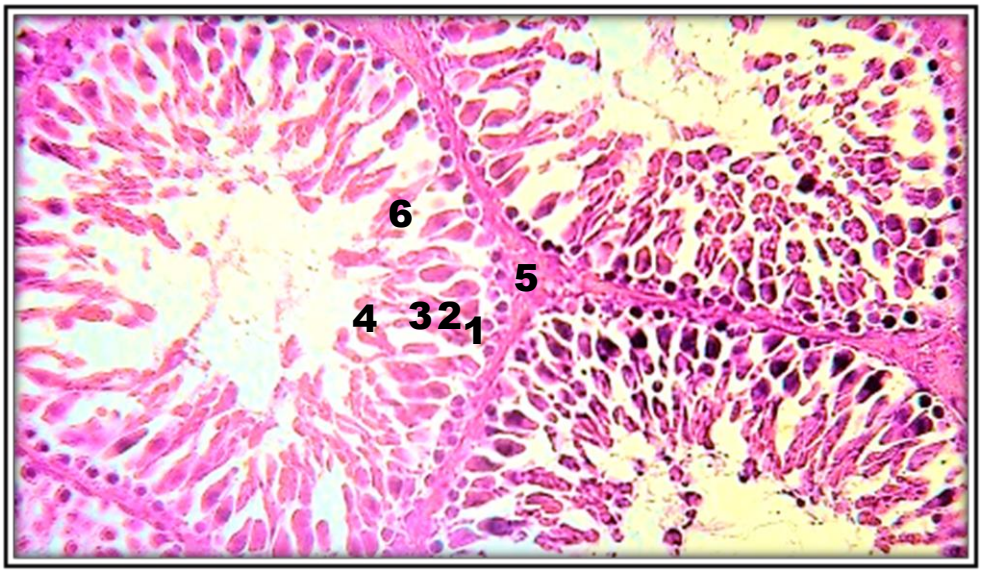


Figure (4-14): Testis of control group in male rat (fifth week) :normal structure, spermatogonia (1), primary spermatocyte (2), secondary spermatocyte (3), spermatid (4), Leydig cells (5), Sertoli cells (6), (H&E,400x).

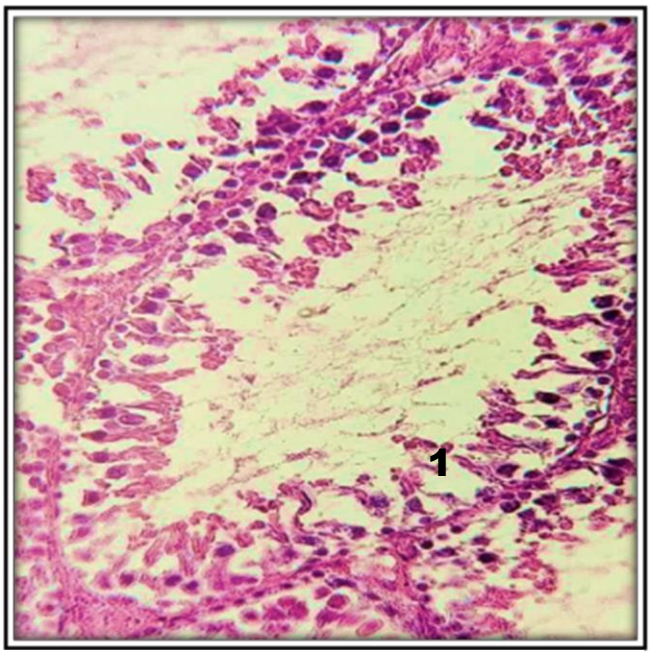


Figure (4-15): Testis of protocol group in male rat (fifth week) degeneration germinal cells (1), (H&E,400X).

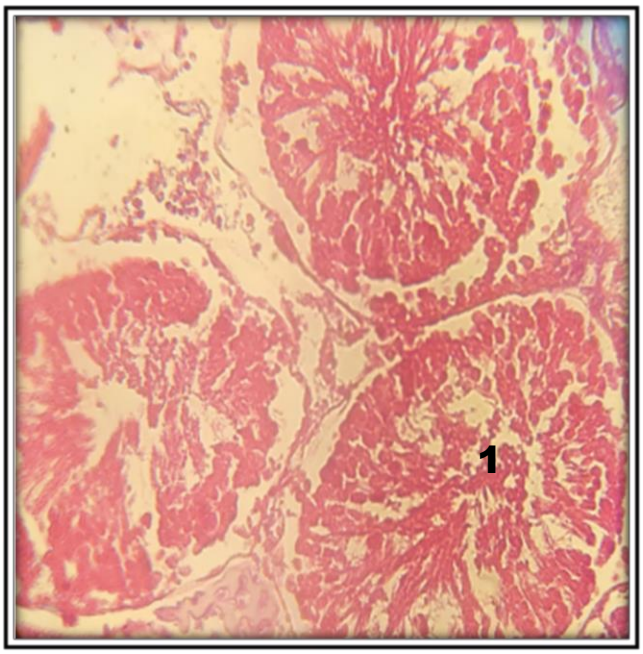


Figure (4-16): Testis of remdesivir group in male rat (fifth week) , fibrosis of testicular tissues and cell structure disappeared(1), (H&E,400X).

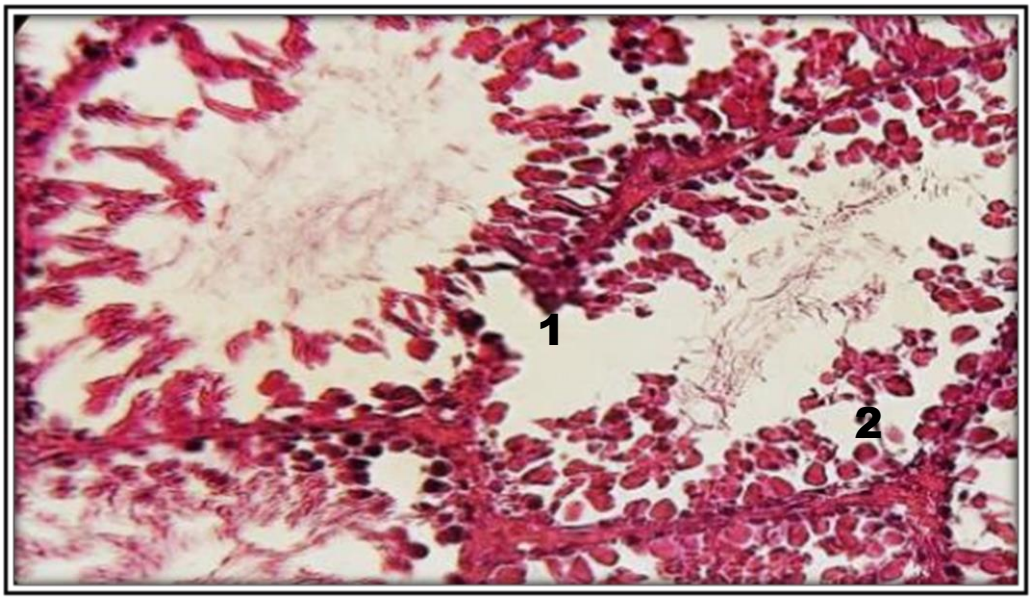


Figure (4-17): Testis of azithromycin group in male rat (fifth week)**:** necrosis (1),irregular spermatogenesis (2), (H&E,400X).

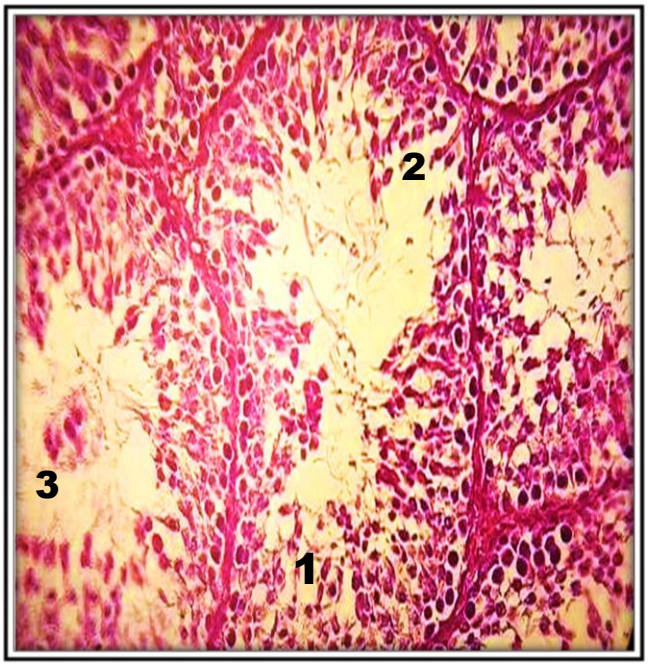


Figure (4-18): Testis of dexamethasone group in male rat (fifth week), irregular shape (1), disarranged epithelial layers (2), lumen filled with detached germ cells (3), (H&E,400X) .

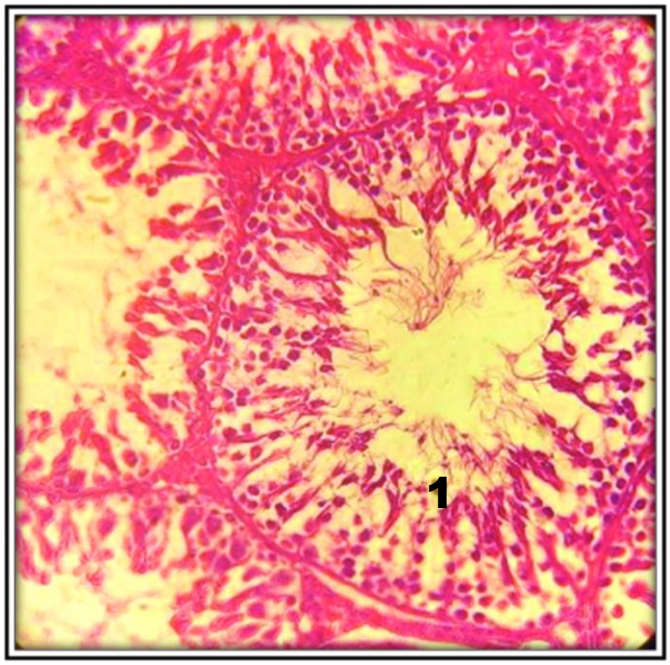


Figure (4-19): Testis of heparin group in male rat (fifth week): normal structure , sperm growth inside the seminiferous tubules (1),(H&E,400X).

4.4.2.The Epididymis

According to the study's findings at the first week, the epididymis of control group has a healthy basement membrane separating the epithelium from the connective tissue and has a blood vessels in the extra tubular area of the interstitial connective tissue. It is lined by a pseudostratified columnar epithelium with long stereocilia and surrounded by a circular layer of smooth muscle and the lumen contains mature sperm, figure (4-20).

the epididymis of protocol group had an irregular lumen and a low spermatozoa density, figure (4-21). But the epididymis in the remidsvir group showed a torn basement membrane and tubular lumen that showed azoospermia, figure (4-22).

In the contrast the epididymis structure in the azithromycin group displayed an irregular lumen filled with the sperm and hypertrophy of epithelial cells, figure (4-23). While the dexamethasone group displayed an irregular lumen with decreased in the sperm production, figure (4-24). The heparin group displayed an irregular lumen filled with sperms and hypertrophy of epithelial cells. figure (4-25).

The epididymis control group's sections in the second week were comparable to those of the control group in the first week, figure (4-26). While the protocol group's epididymis demonstrated hypertrophy in the epithelial cells and an increase in the interstitial space as well as the loss of sperm, figure (4-27).

In the remidsvir group, the microscopic image showed a vacuolation of the epithelial lining of the epididymis duct and the absence of the spermatozoa, figure (4-28). Whereas in the azithromycin group, the epididymis histological structure showed an increase in the interstitial space hypertrophy of epithelial cells and absence of the sperm, figure (4-29).

dexamethasone group's epididymis histological structure demonstrated absence of spermatozoa in some epididymis ducts, figure (4-30). heparin group displayed an interstitial spaces between the ducts of the epididymis and decreased the sperms, figure (4-31).

During the fifth week, the control group showed no change from the first and second weeks, figure (4-32). While the protocol group displayed hypertrophy of epithelial cells, damaged basement membrane, and lumen irregular shape, figure (4-33). The remidsvir group demonstrated epithelial cell hypertrophy, sperm absence, and increased interstitial space, figure (4-34).

azithromycin did not cause any structural changes to be noticed in figure (4-35). dexamethasone caused sperm counts to drop, figure (4-36). heparin did not cause any pathological changes to be noticed, figure (4-37).

The study's findings demonstrated that the regimen and remidsvir group had more severe histological changes in the epididymis tissue than the other groups. In the second week, histopathological changes of epididymis tissue in all groups were more severe than the first and fifth week.

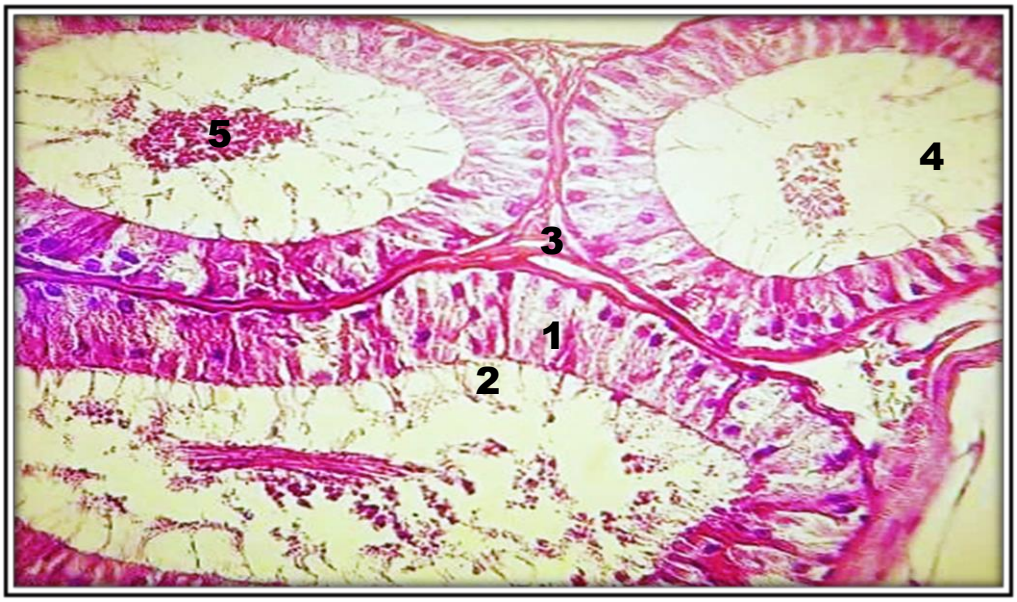


Figure (4-20):Epididymis of control group in male rat (first week): lined by pseudostratified columnar epithelium(1), long stereocilia (2), smooth muscle (3), lumen (4) mature sperms (5), (H&E,400X).

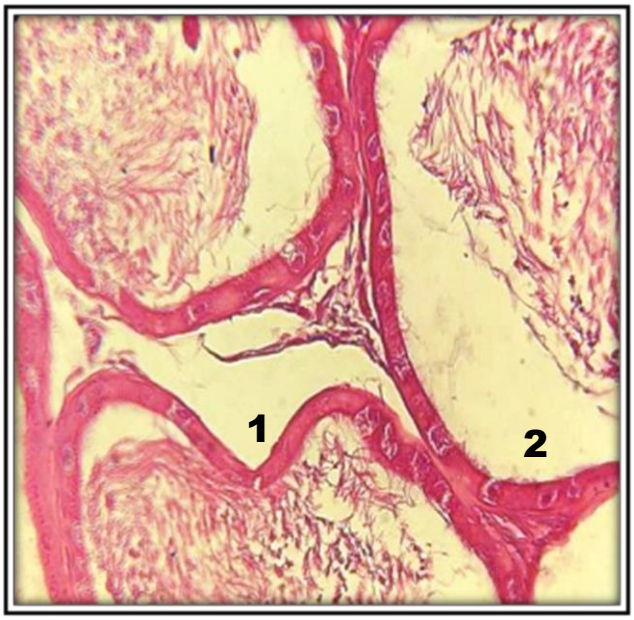


Figure (4-21): Epididymis of protocol group in male rat (first week): large interstitial area (1) low spermatozoa density (2) ,(H&E,400X)

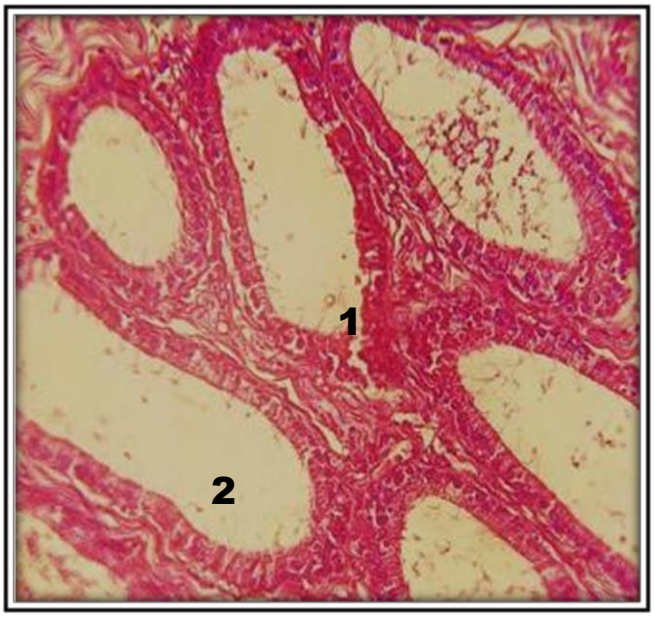


Figure (4-22): Epididymis of remdesivir group in male rat (first week), broken basement membrane (1), lumen revealed azoospermia (2), (H&E,400X).

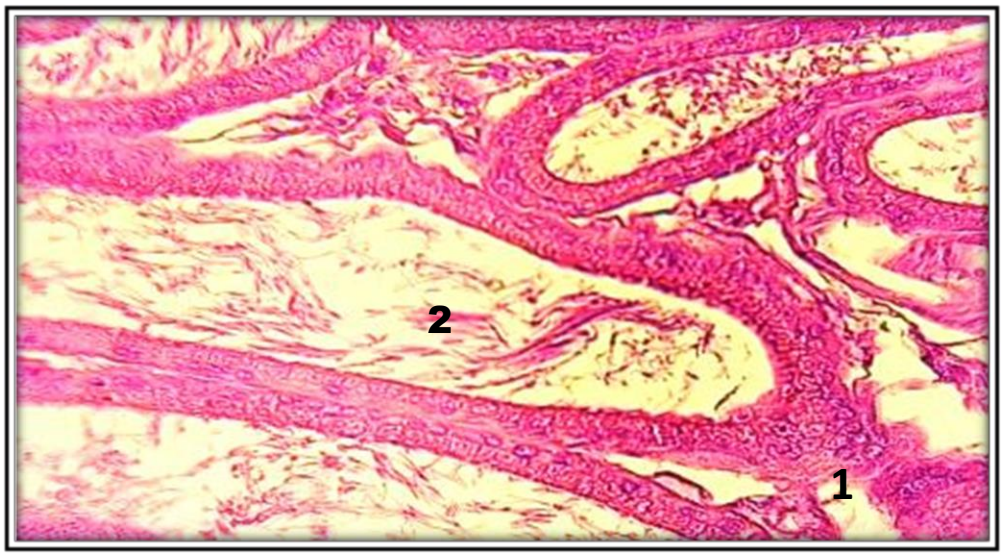


Figure (4-23): Epididymis of azthromycin group in male rat (first week), hypertrophy of epithelial cells (1),lumen irregular shape and filled with sperm(2),(H&E,400X).

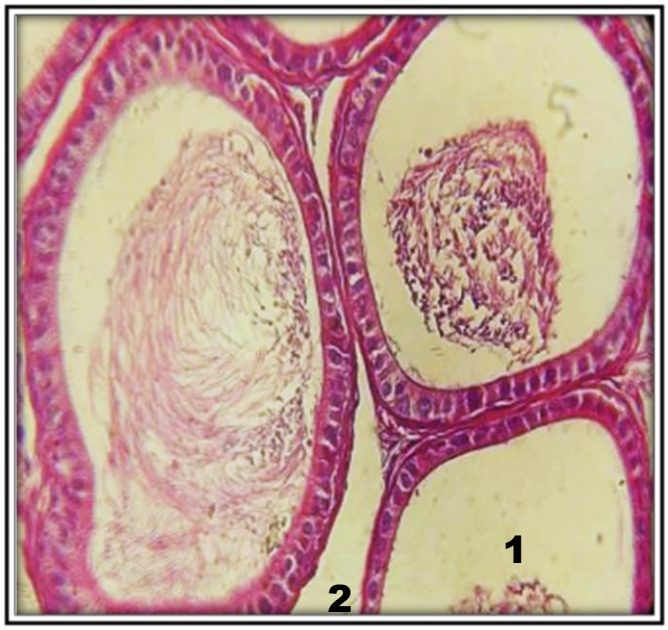


Figure (4-24): Epididymis of dexamethasone group in male rat (first week) decrease the sperms (1), interstitial space (2),(H&E,400X).

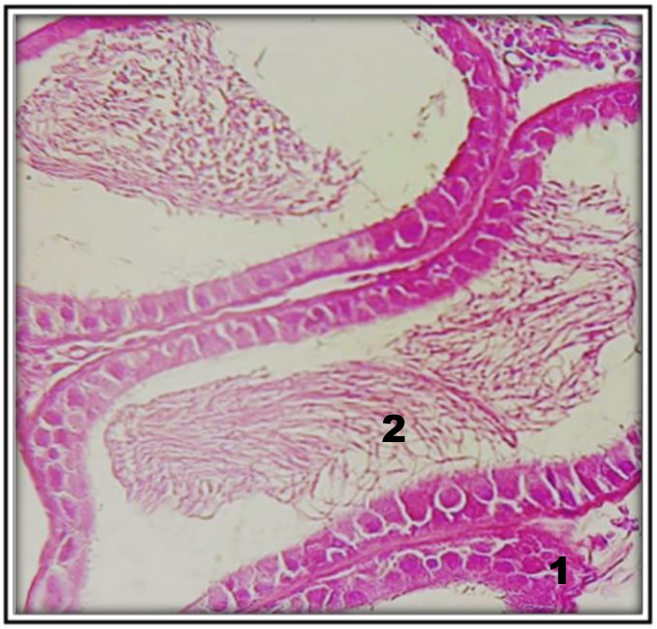


Figure (4-25): Epididymis of heparin group in male rat (first week): hypertrophy of epithelial cells (1),lumen filled with sperm(2),(H&E,400X).

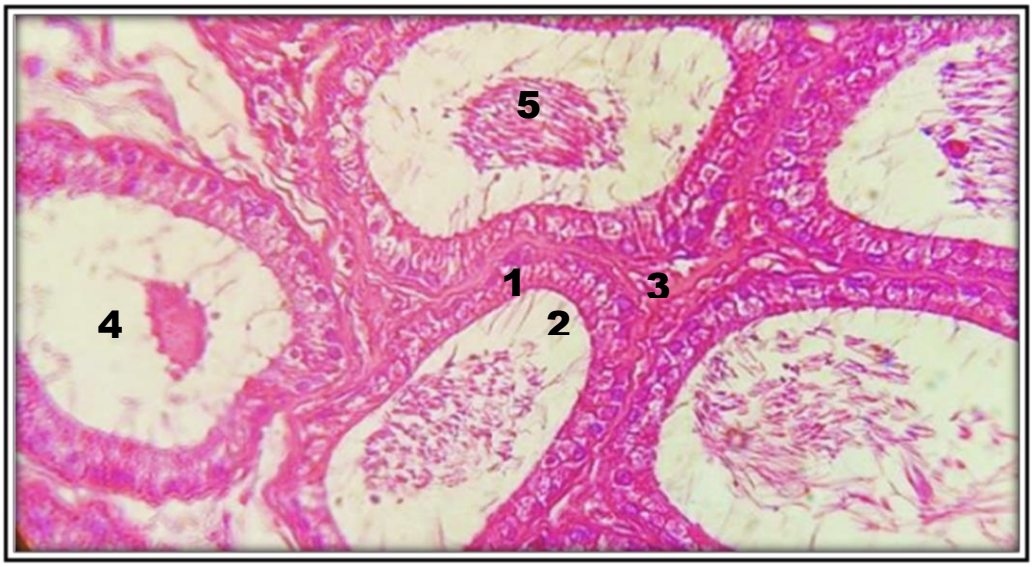


Figure (4-26): Epididymis of control group in male rat (second week): pseudostratified columnar epithelium (1), stereo cilia (2), smooth muscle (3),lumen(4) mature sperms (5), (H&E,400X).

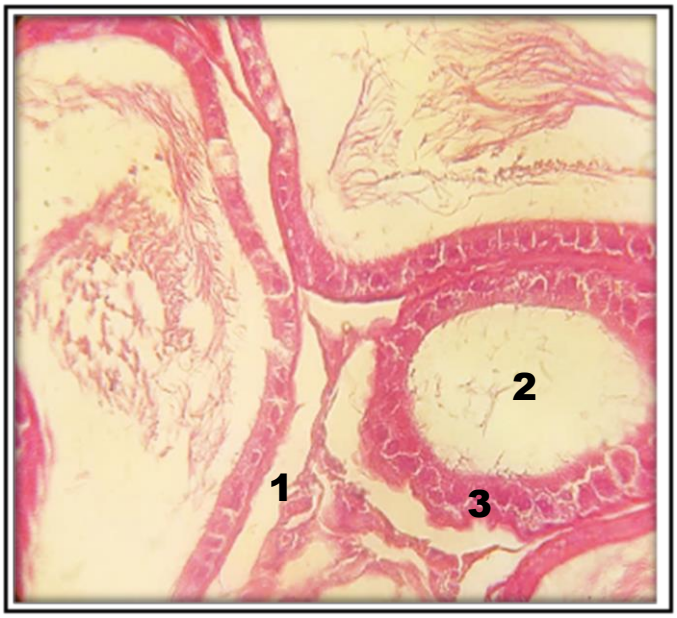


Figure (4-27): Epididymis of protocol group in male rat (second week): increase interstitial space (1),loss the sperm (2), hypertrophy in the epithelial cells (3),(H&E,400X).

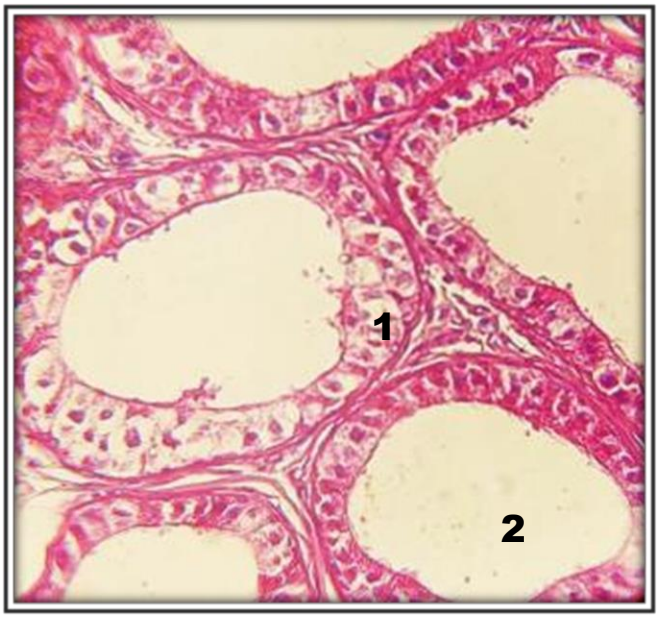


Figure (4-28): Epididymis of remdesivir group in male rat (second week):vacuolation of epithelial (1),absence of spermatozoa (2) ,(H&E,400X).

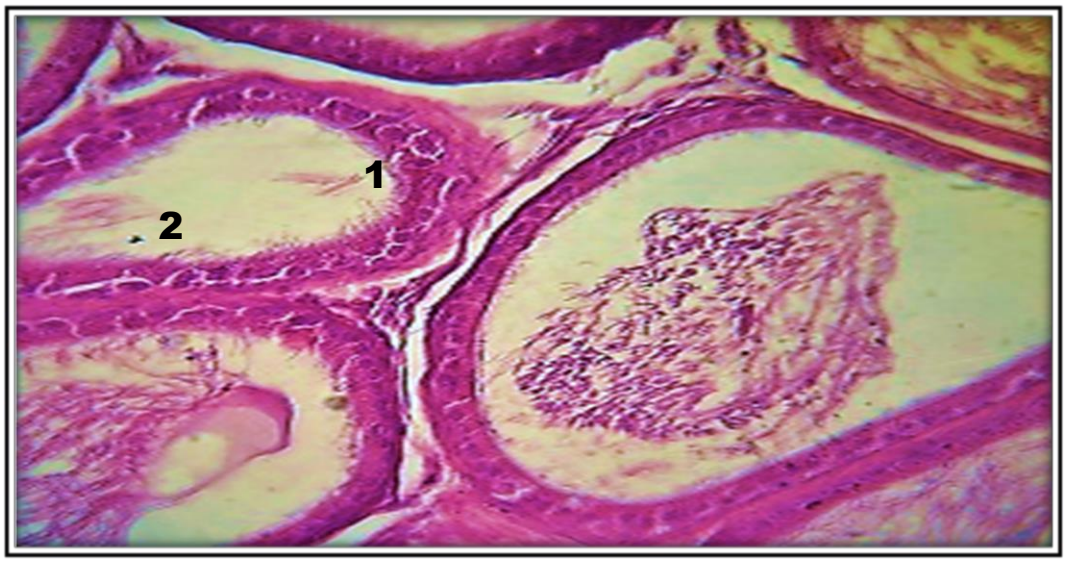


Figure (4-29): Epididymis of azithromycin group in male rat (second week): hypertrophy of epithelial cell (1), absence of sperms (2),(H&E,400X).

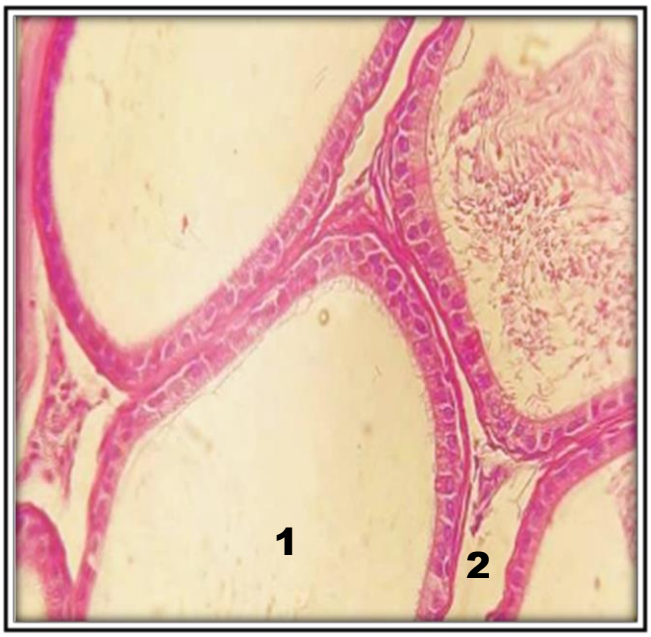


Figure (4-30) : Epididymis of dexamethasone group in male rat (second week) absence of spermatozoa in some epididymis ducts(1),increase interstitial space (2), (H&E,400X).

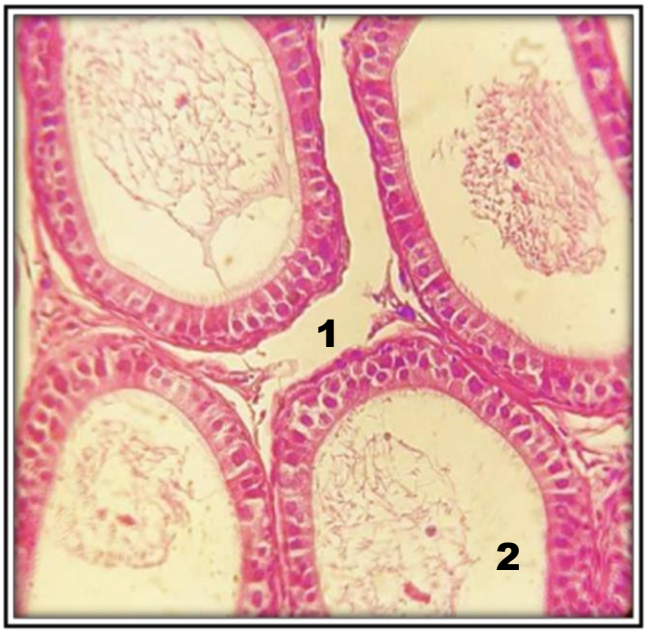
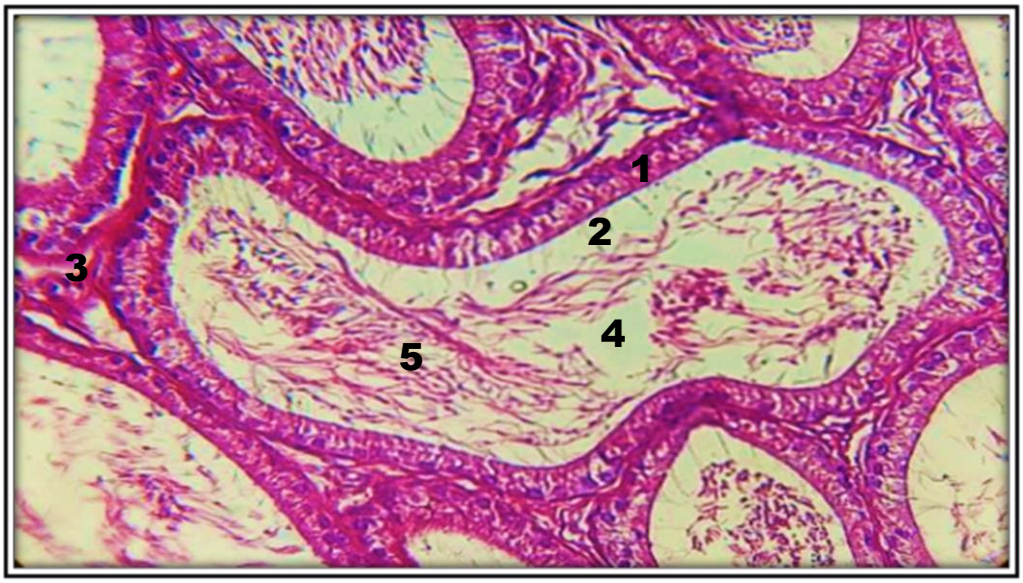


Figure (4-31): Epididymis of heparin group in male rat (second week):Interstitial spaces between the ducts of the epididymis (1), decrease the sperm (2), (H&E,400X).



Figure(4-32): Epididymis of control group in male rat (fifth week): pseudostratified columnar epithelium(1), stereocilia (2), smooth muscle (3), lumen (4), mature sperms (5), (H&E,400X).

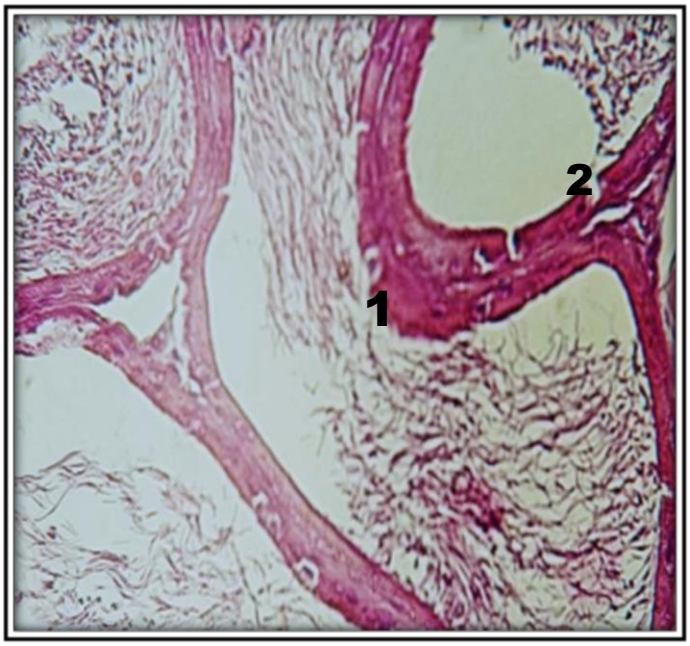


Figure (4-33): Epididymis of protocol group in male rat (fifth week): hypertrophy of epithelial cells (1), broken basement membrane (2),(H&E,400X).

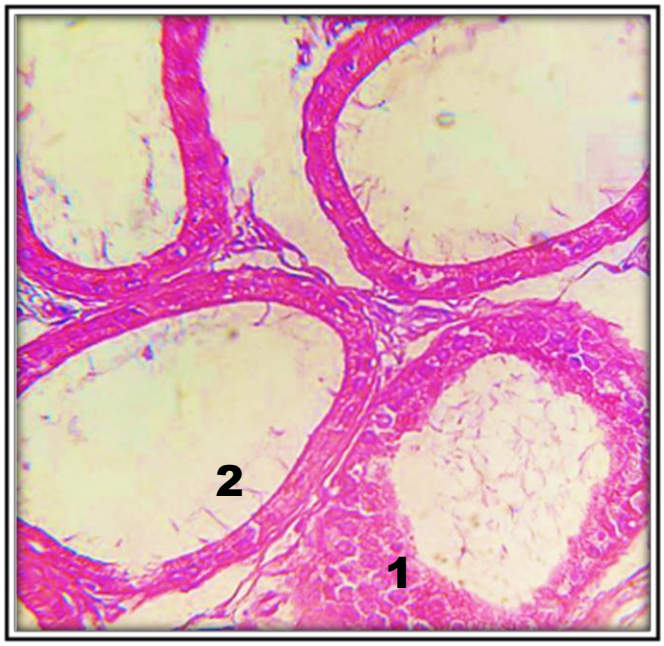


Figure (4-34): Epididymis of remdesivir group in male rat (fifth Week): hypertrophy of epithelial cell (1),absence of sperm (2), (H&E,400X) .

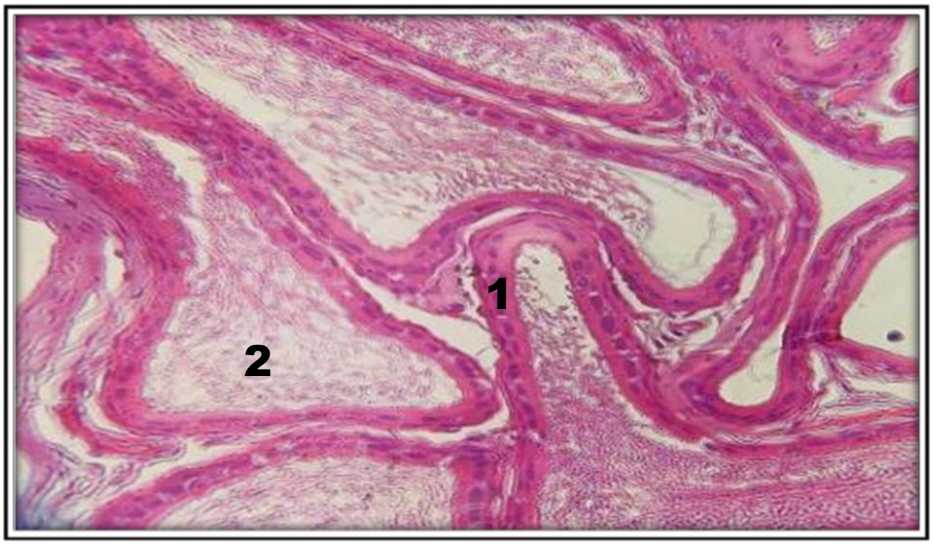


Figure (4-35): Epididymis of azithromycin group in male rat (fifth week): a normal epithelium layer (1), mature sperms (2), (H&E,400x).

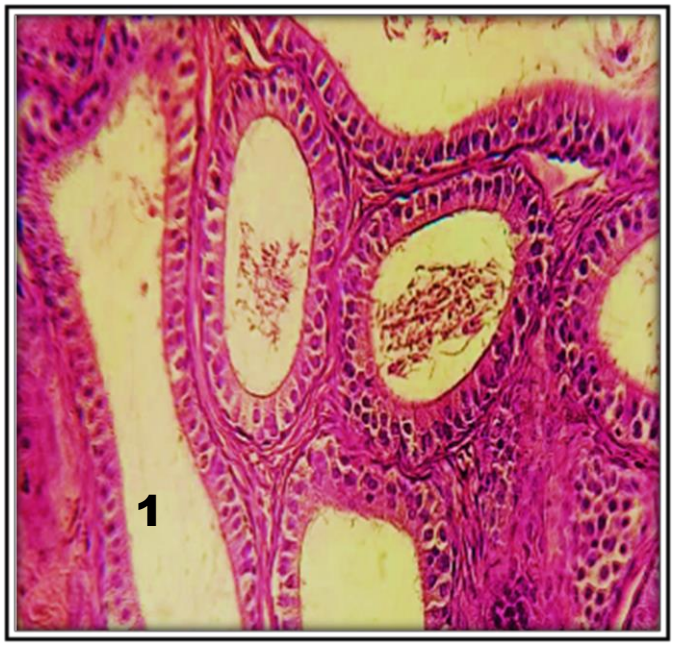


Figure (4-36): Epididymis of dexamethasone group in male rat(fifth week) sperms decrease (1), ,(H&E,400x).

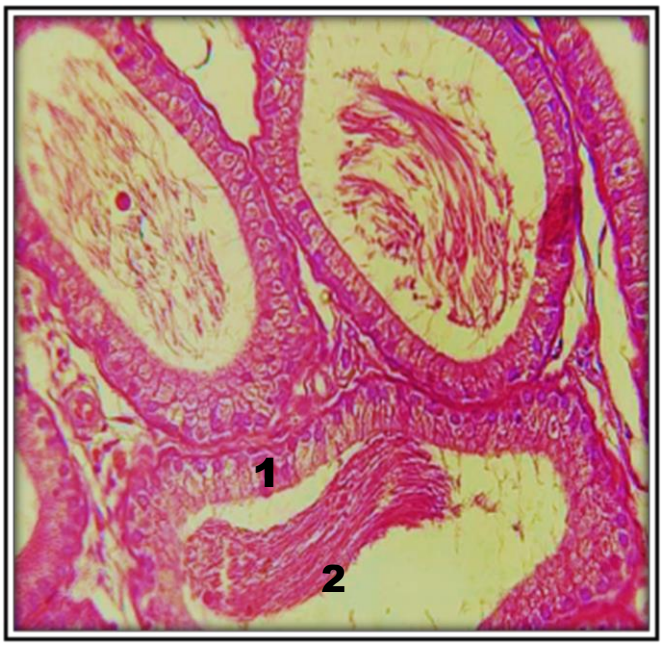


Figure (4-37): Epididymis of heparin group in male rat (fifth week), normal epithelial structure (1),lumen filled with sperms (2),(H&E,400X).

4.4.3.The Seminal Vesicles:-

The transverse section of the seminal vesicle in the control group of the first week exhibited a mucosal folds extending into the lumen, Pseudo-stratified columnar epithelium made up primarily of a single layer of tall columnar primary cells and triangular-shaped basal cells made up the lining epithelium of the seminal vesicle, eosinophilic secretion filled the seminal vesicle's lumen, lamina propria, a thin layer of connective tissue supports the epithelium, Inner circular and outer longitudinal smooth muscle fibers lined the lamina propria, figure (4-38).

The seminal vesical in the protocol group displayed a complex mucosal folds, the lamina propria penetrating the epithelial folds, and a reduction in the eosinophil secretion, figure (4-39). In contrast to the remdesivir group, which displayed numerous mucosal cavities bordered by a simple squamous and simple cuboidal epithelium and reduced eosinophil secretion, figure (4-40).

While the seminal vesical of azithromycin group, the lumen was filled with the mucosal crypts and a little eosinophilic secretion, figure (4-41). So the dexamethasone group, which displayed a complete obliteration of the mucosal folds into the lumen, numerous mucosal cavities bordered with the simple squamous and simple cuboidal epithelium, reduced eosinophil secretion, and focal necrosis of epithelial lining, figure (4-42). The heparin group's seminal vesicle displayed mucosal crypt development and a little eosinophil secretion, figure (4-43).

The seminal vesicle section of the control group in the second week was comparable to that of the control group in the first week, figure (4-44). While in the protocol group, the lamina propria grew and was found crawling within the epithelial folds, and mucosal crypts were filled with a minor

amount of eosinophil secretion, figure (4-45). The remdesivir group demonstrated an increase in the mucosal folding, The lamina properia broke through the epithelial folds with additional crypts, figure (4-46). In the azithromycin group the seminal vesical showed a mucosal folds completely cover the lumen, a large number of mucosal crypts bordered by a simple squamous and simple cuboidal epithelium with a little eosinophilic secretion, epithelial stratification, figure (4-47). Whereas the dexamethasone group displayed modest eosinophil production, a hollow filled with mucosal crypts, and lamina propria infiltrating into the mucosal folds, figure (4-48). In the Heparin group increased mucosal folds and increased crypts, cell stratification, and a reduction in eosinophilic secretion, figure (4-49).

The control group's seminal vesicles in the fifth week, which were similar to those of the control group in the first and second weeks figure (4-50). In contrast to the transversal section of the protocol group's revealed a focal necrosis of the epithelial lining and a decrease in eosinophil secretion, figure (4-51).

In the remdesivir group mucosal crypts and alveoli fill the lumen, which showed a limited eosinophilic secretion figure (4-52). In the azithromycin group, which also showed a minimal eosinophilic secretion, figure (4-53). In the dexamethasone group were observed the stratification of the epithelial cells, the filling of the lumen with mucosal crypts, and the reduction in eosinophilic secretion, figure (4-54). Regarding the Heparin group, folds and eosinophil secretion were visible in the lumen, figure (4-55).

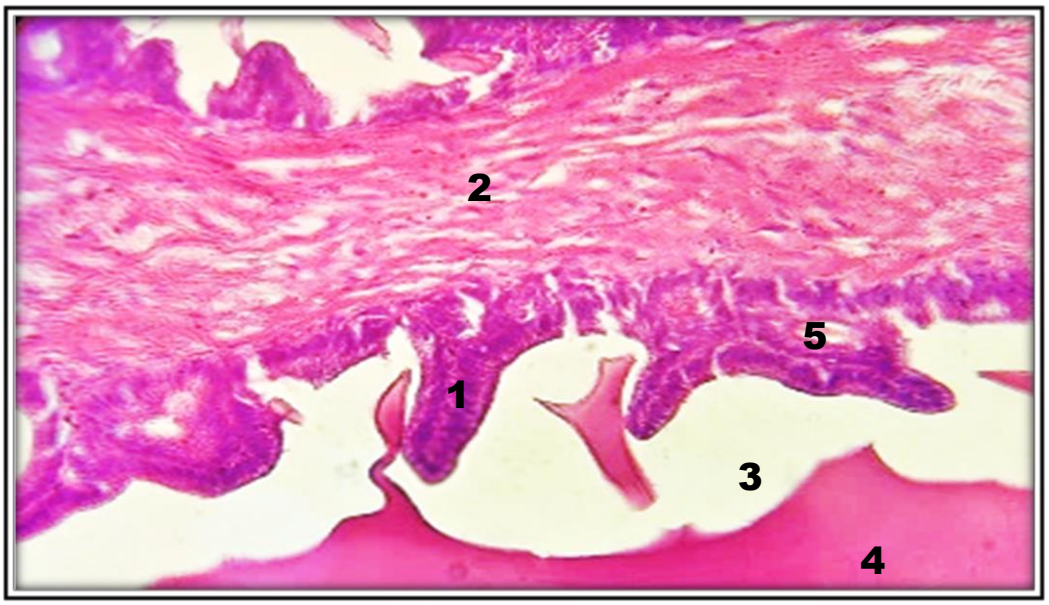


Figure (4-38): Seminal vesical of the control group in male rat (first week): normal pseudostratified columnar epithelium (1), smooth Muscle (2), lumen (3), eosinophilic secretion (4), few mucosal crypts/alveoli (5) ,(H&E,400X).

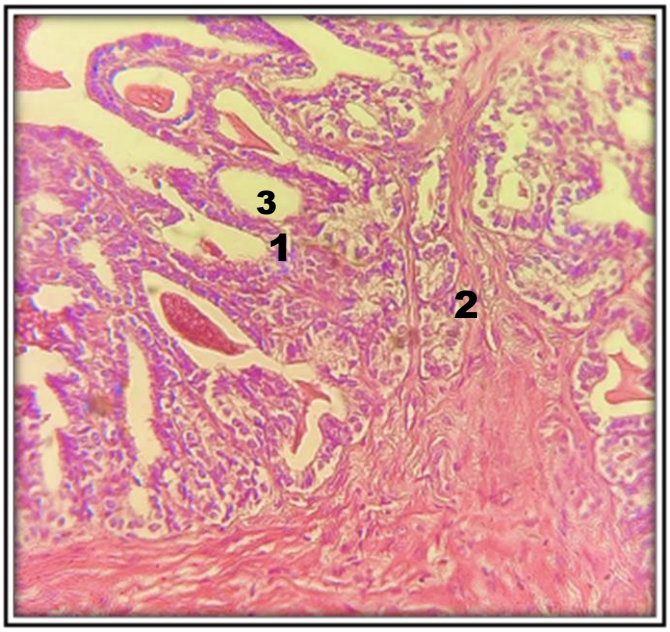


Figure (4-39): Seminal vesical of protocol group in male rat (first week): complex mucosal folds (1), lamina properia penetrating the epithelial folds (2), eosinophilic secretion(3),(H&E,400X).

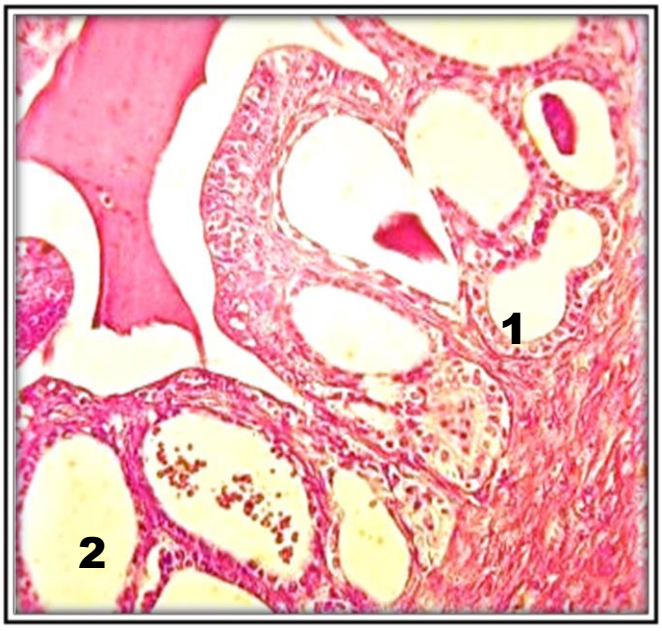


Figure (4-40): Seminal vesical of remdesivir group in male rat (first week): mucosal folds , numerous mucosal cavities bordered with simple squamous or simple cuboidal epithelium (1), reduced eosinophil secretion (2),(H&E,400X).

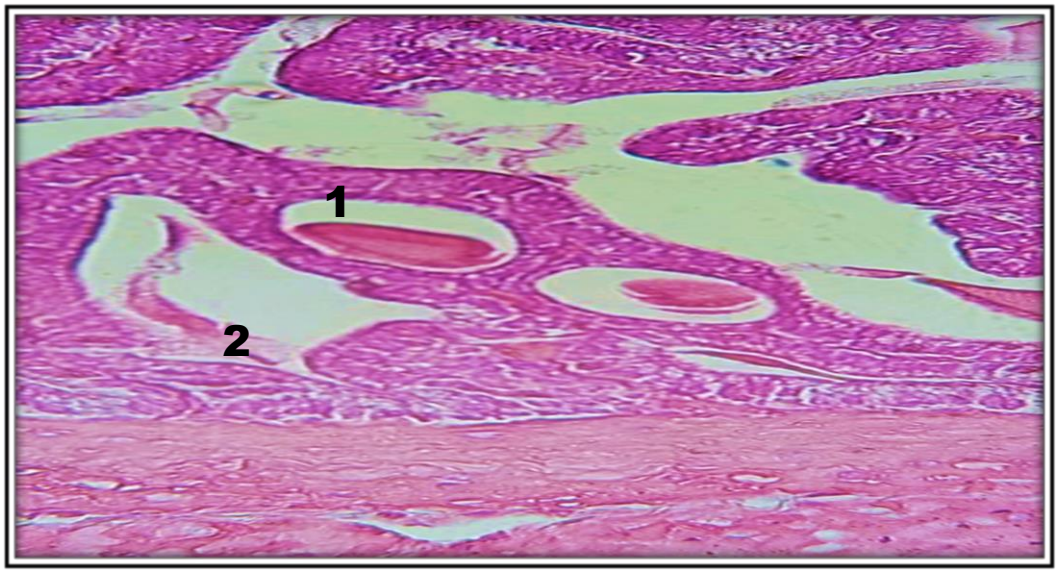


Figure (4-41): Seminal vesical of azithromycin group in male rat (first week): Lumen filled with mucosal crypts (1), eosinophilic secretion (2),(H&E,400X).

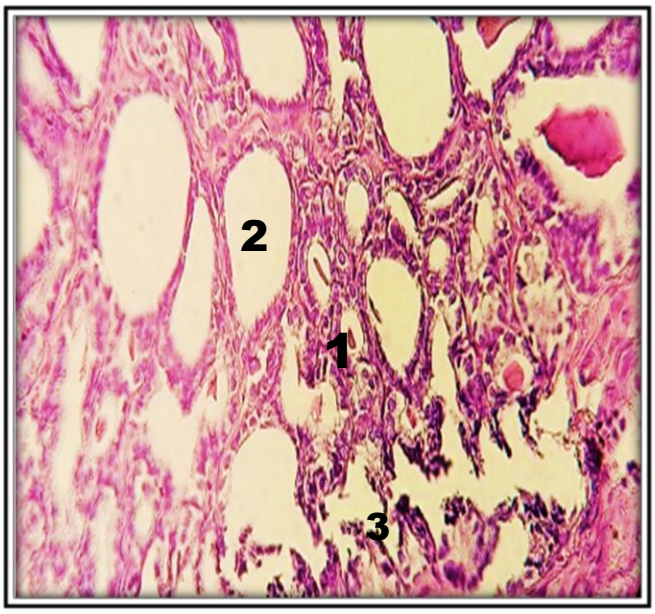


Figure (4-42): Seminal vesicle of dexamethasone group in male rat (first week): complete obliteration of mucosal folds into the lumen (1), eosinophil secretion (2), focal necrosis (3),(H&E,400X).

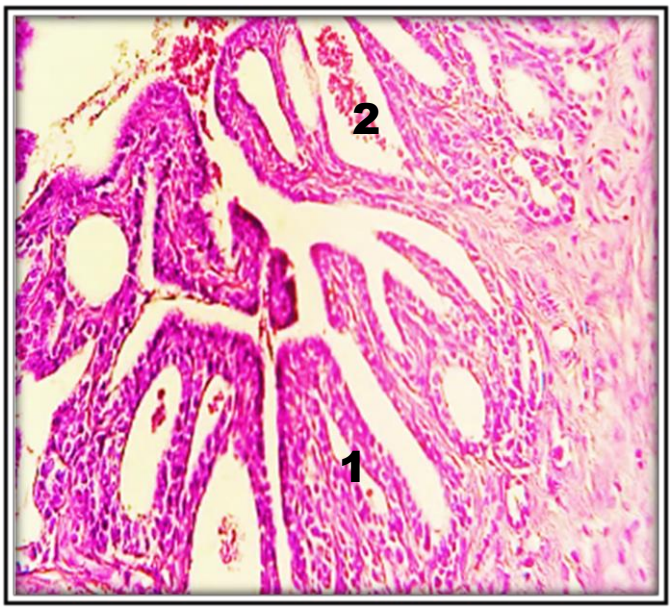


Figure (4-43): Seminal of heparin group in male rat first week, mucosal crypt (1), eosinophilic secretion within the crypts (2), (H&E,400X).

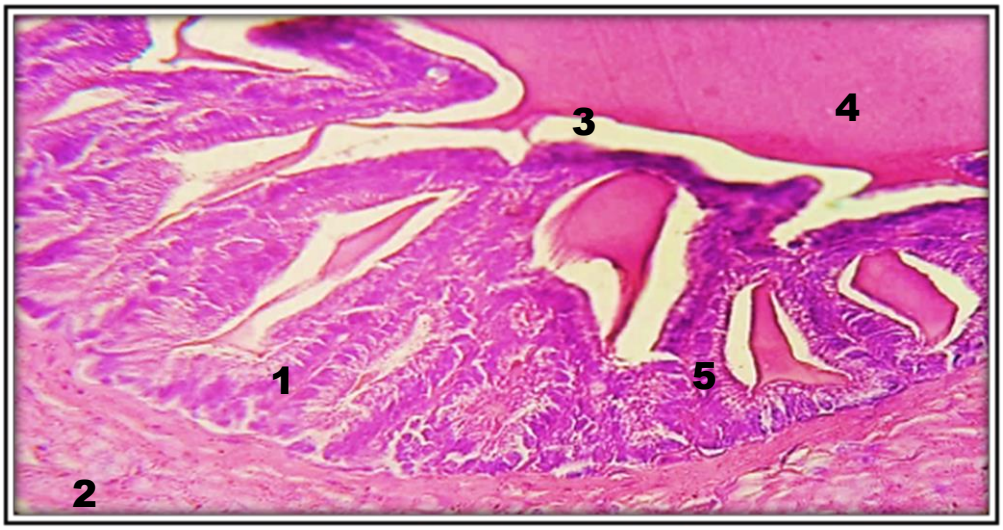


Figure (4-44): Seminal vesical of control group in male rat (second week): pseudostratified columnar epithelium (1), smooth muscle (2), lumen (3) eosinophilic secretion (4), mucosal crypts/alveoli (5),(H&E,400X).

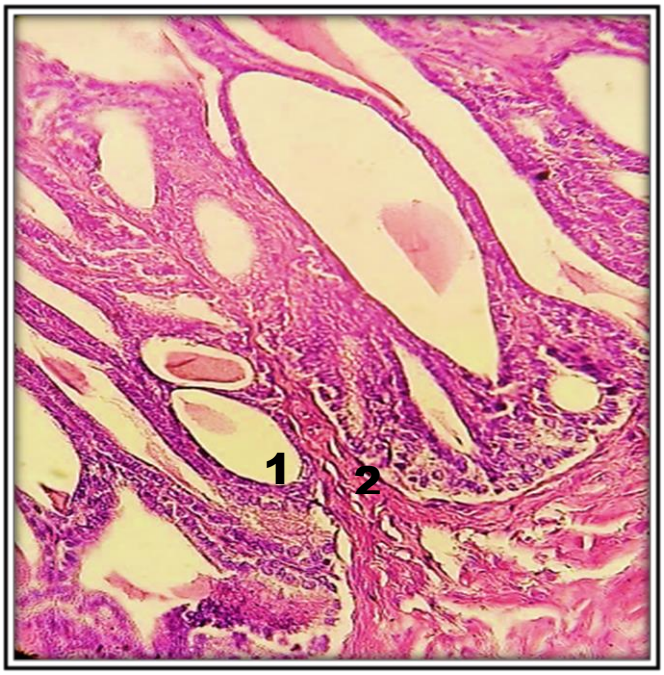


Figure (4-45): Seminal vesical of protocol group in male rat (second week): mucosal crypts (1),the amount of lamina properia increased, and it was crawling within the epithelial folds (2), (H&E,400X).

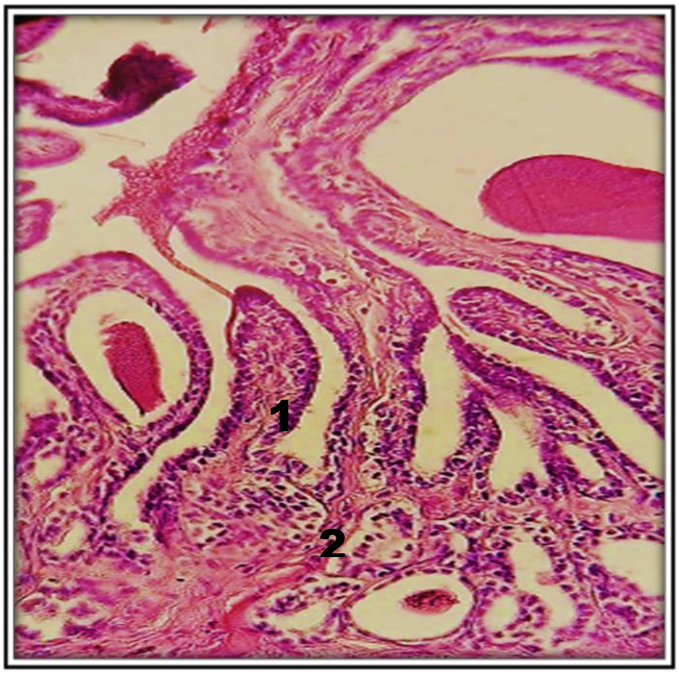


Figure (4-46): Seminal vesical of remdesivir group in male rat in the second week, mucosal folding increased with more crypts (1), lamina properia (2), (H&E,400X).

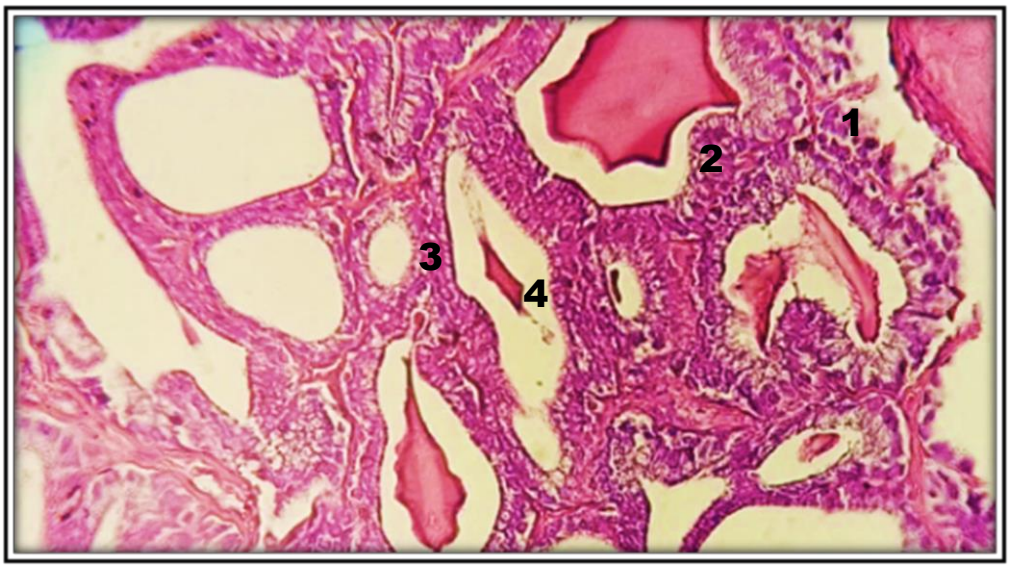
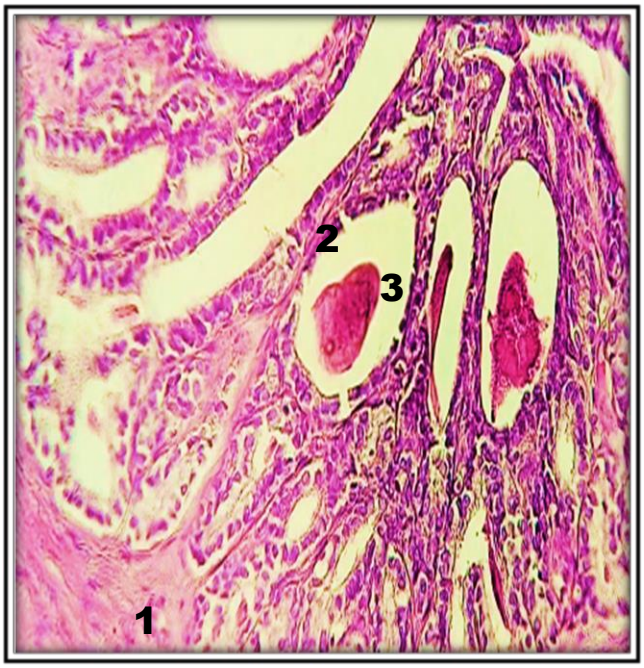


Figure (4-47): Seminal vesical of azithromycin group in male rat (second week): mucosal folds (1), epithelial stratification(2), a large number of mucosal crypt (3),limited eosinophilic secretion (4), (H&E,400X).



Figure(4-48): Seminal vesical of dexamethasone group in male rat (second week): displayed lamina properia penetrating into the mucosal folds (1), mucosal crypts (2), eosinophil secretion(3) ,(H&E,400X).

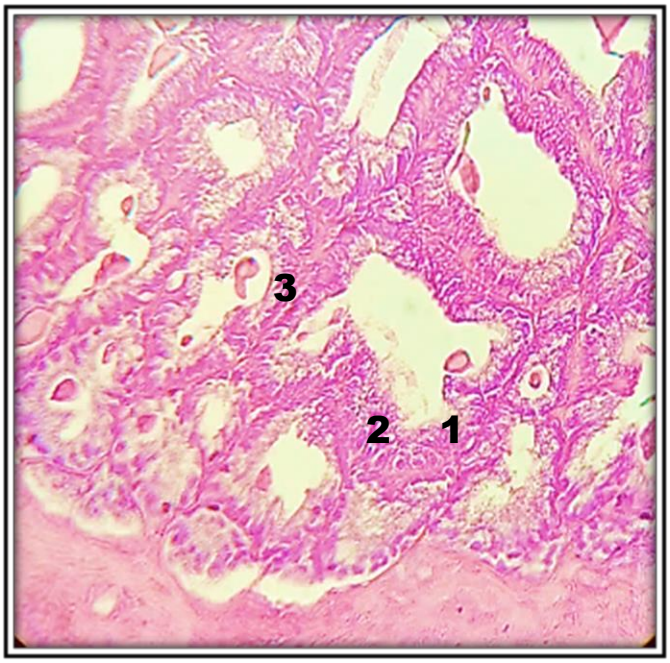


Figure (4-49): Seminal vesical of heparin group in male rat (second week): mucosal folds increased with greater crypts (1), stratification of cells (2), eosinophilic secretion(3), (H&E,400X).

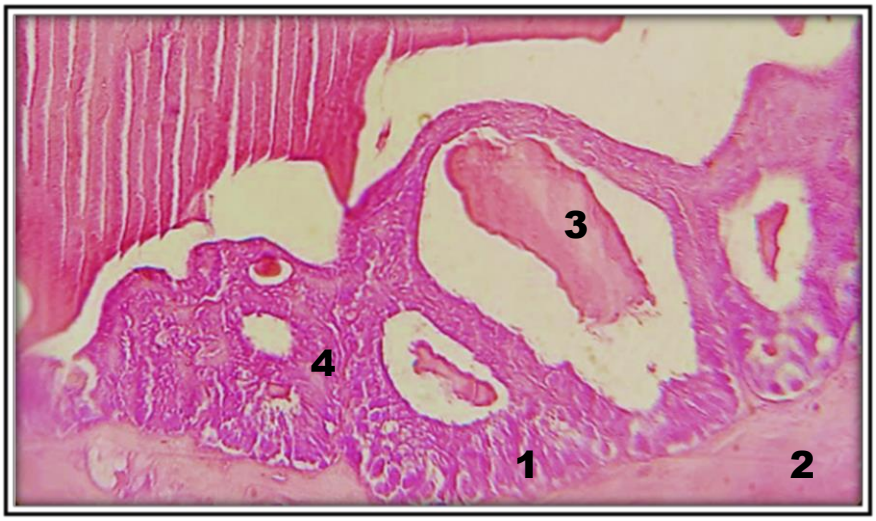


Figure (4-50):seminal vesicle from a male rat in the control group (fifth week): pseudostratified columnar epithelium (1), smooth muscle (2), a eosinophilic fluid (3), mucosal crypts/alveoli (4),(H&E,400X).

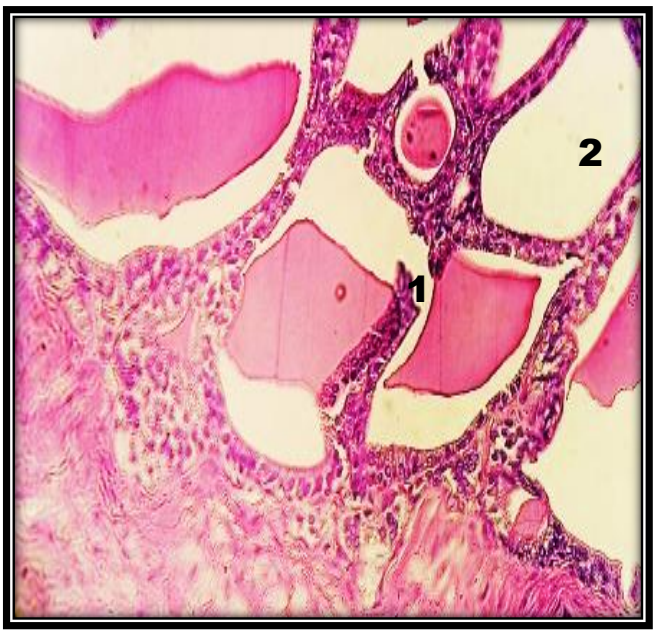


Figure (4-51): Seminal vesicle of protocol group in male rat (fifth week): focal necrosis of epithelial lining (1), eosinophil secretion (2), (H&E,400X).

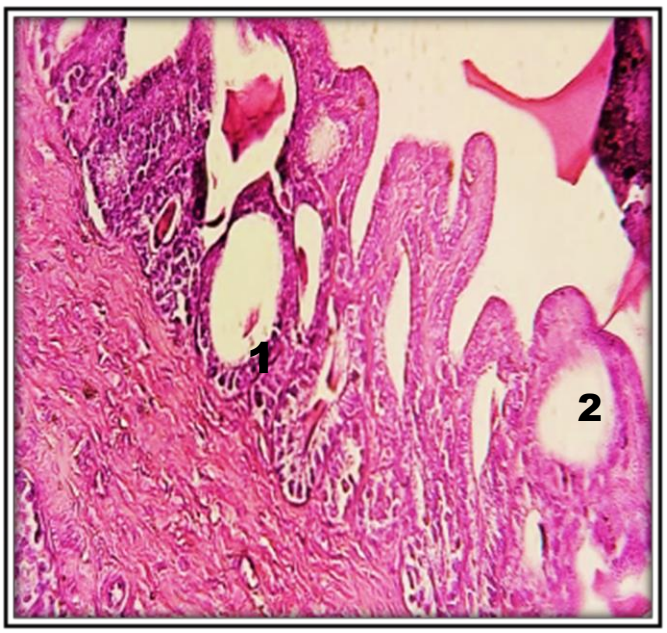


Figure (4-52): Seminal vesicle of remdesivir group in male rat (fifth week): minimal eosinophilic secretion (1), mucosal crypts and alveoli fill the lumen (2), (H&E400X) .

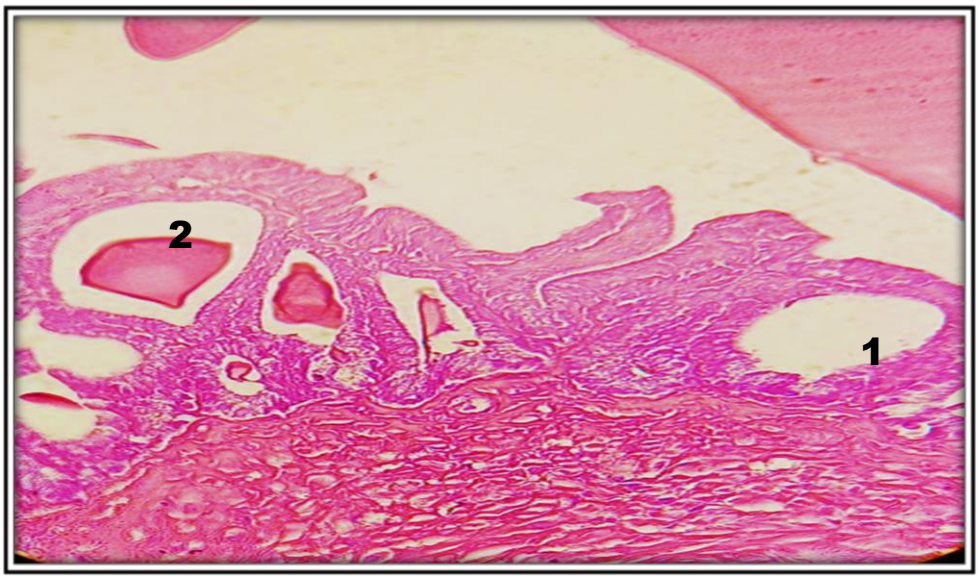


Figure (4-53): Seminal vesical of azithromycin group in male rat (fifth week): eosinophilic secretion (1), mucosal crypts and alveoli fill the lumen (2), (H&E,400X) .

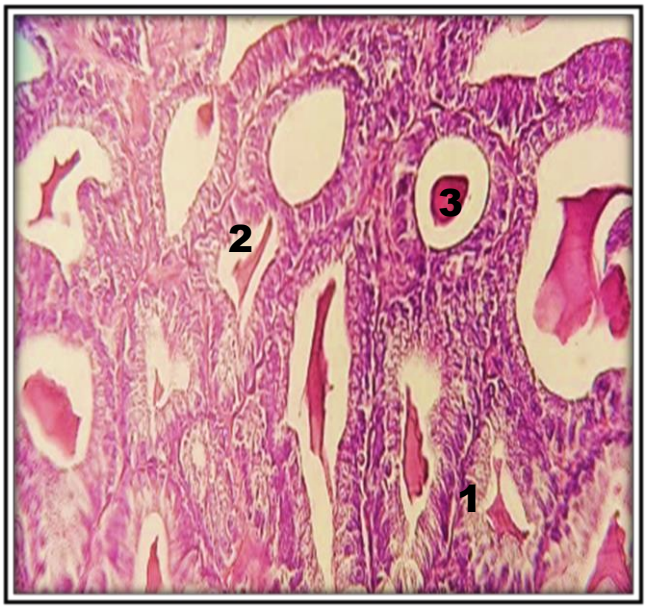


Figure (4-54): Seminal vesicle of dexamethasone group in male rat (fifth week): stratification of epithelial cells (1) , mucosal crypts fill lumen (2), eosinophilic secretion (3), (H&E,400X).

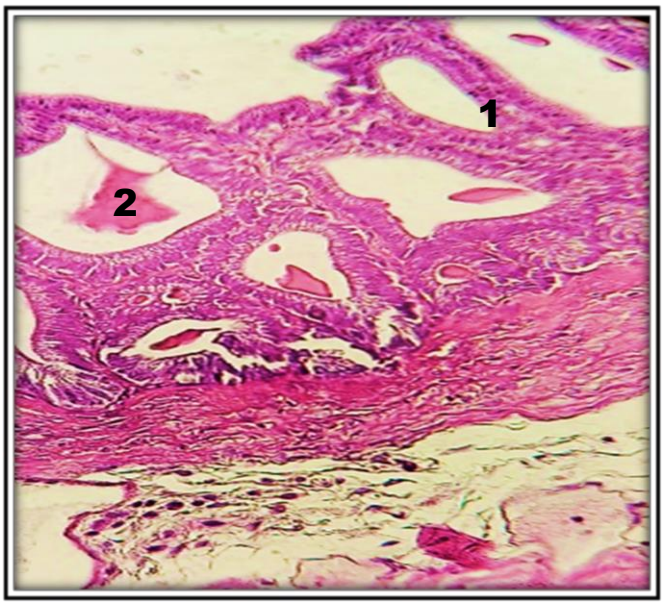


Figure (4-55): Seminal vesicle of heparin group in male rat (fifth week): folds (1), lumen contain eosinophil secretion (2), (H&E,400X).

4.5.Histomorphometric Study:-

4.5.1.Dimeter of Seminiferous Tubules :-

According to the study's findings, there was a significant decrease ($p \leq 0.05$) in the diameter of seminiferous tubules in all groups relative to the control group during the five-week period. In the first week, The diameter of the control group was (280.00±2.24 µm)**;** the diameter of the protocol group was (266.60±5.17µm); remdesivir (219.00±7.48µm); azithromycin (226.00±6.51µm); dexamethasone (227.00±9.87µm); and heparin (246.00±1.40µm) . In the second week, the dimeter of the control group was (283.00±5.70µm), protocol (215.40±6.88µm), remdesivir (218.00±3.93µm), azithromycin (247.00±1.51µm), dexamethasone (217.00±3.27µm), Heparin (254.20±8.86µm). In the fifth week, the control group was (287.40± 5.75µm), protocol (224.00±6.73µm), remdesivir (203.00±4.86µm), azithromycin (248.20± 3.69µm), dexamethasone (235.00± 2.00µm), and Heparin (235.00±9.52µm), table (4-7).

4.5.2.Diameter of Epididymis Duct :-

The study's findings revealed that there were no significant differences ($p > 0.05$) in the diameter of the epididymis duct in all groups in the first week compared with the control group. The diameter of the control group (220.00 ±5.24µm), protocol (203.00±4.66µm), remdesivir (206.00±2.94µm), azithromycin (239.00±7.41µm), dexamethasone (183.00±4.38µm), and heparin (231.00±9.54µm). the diameter of the epididymis duct in the second week in all groups showed a significant decrease ($p \leq 0.05$) compared to the control group, the dimeter of the control group (223.00 ±7.29µm), protocol (195.20±4.09µm), remdesivir (157.80± 6.49µm), azithromycin (198.0±5.81µm), dexamethasone (145.00 ±3.18µm), and heparin (218.0 ±2.54µm). The epididymis duct in all groups in the fifth week showed

no significant differences (p>0.05), the dimeter of the control group (232.00±8.34µm), protocol (228.20±4.51µm), remdesivir (232.0±5.18µm), azthromycin (209.00±7.59µm), dexamethasone (225.00 ±3.69µm), and heparin (249.00± 8.94µm),table (4-8).

Table (4-7) The changes in the diameter of seminiferous tubules of male rats over the fifth weeks

Diameter of seminiferous tubule (µm)			
Group	**1st week**	**2nd week**	**5th week**
Control	280.00^{a} ± 2.24	283.00^{a} ± 5.70	287.40^{a} ± 5.75
Protocol	266.60^{b} ± 5.17	215.40^{c}± 6.88	224.00^{c} ± 6.73
Remdesivir	219.00^{c} ± 7.48	218.00^{c} ± 3.93	203.00^{c} ± 4.86
Azthromycin	226.00^{c} ± 6.51	247.00^{b} ± 1.51	248.20^{b} ± 3.69
Dexamethasone	227.00^{c} ± 9.87	217.00^{c} ± 3.27	235.00^{b} ± 2.00
Heparin	246.00^{b} ± 1.40	254.20^{b} ± 8.86	235.00^{b} ± 9.52

- Values represent mean ± SD, The same letter means no significant between groups while different letters means there was significant (p≤0.05) between groups.

Table (4-8) The changes in the diameter of epididymis duct of male rats over the fifth weeks.

Diameter of Epididymis duct (µm)			
Group	**1st week**	**2nd week**	**5th week**
Control	220.00^{a}± 5.24	223.00^{a} ± 7.29	232.00^{a} ± 8.34
Protocol	203.00^{a} ± 4.66	195.20^{c} ± 4.09	228.20^{a} ± 4.51
Remdesivir	206.00^{a} ± 2.94	157.80^{c} ± 6.49	232.00^{a} ± 5.18
Azthromycin	239.00^{a} ± 7.41	198.00^{c} ± 5.81	209.00^{a} ± 7.59
Dexamethasone	183.00^{a} ± 4.38	145.00^{c} ± 3.18	225.00^{a} ± 3.69
Heparin	231.00^{a} ± 9.54	218.00^{b} ± 2.54	249.00^{a} ± 8.94

- Values represent mean ± SD, The same letter means no significant between groups while different letters means there was significant (p≤0.05) between groups.

4.5.3. Number of Spermatogonia:-

The study showed that there was a significant decrease ($p \leq 0.05$) in the number of spermatogonia cells in all of the groups except the protocol group no significant differences($p > 0.05$) found during the five weeks period compared to the control group, whereas the number of the control group in the first week was (80.8±8.67), protocol (58.00±2.22), remdesivir (66.00±4.35), azithromycin (58.60±12.05), dexamethasone (65.00±21.03) and heparin (58.00±10.77).In the second week, the decrease in the azithromycin and heparin groups was less than the protocol, remdesivir and dexamethasone groups compared to the control group, where the number of the control group was (82.40± 7.92), protocol (43.80±18.51), remdesivir (33.20±18.29), azithromycin (62.20±3.27), dexamethasone (43.60±2.07) and heparin (68.80±23.66). In the fifth week, the number of the control group was (79.40±7.95), protocol (65.00±21.03), remdesivir (43.00±27.47), azithromycin (67.40±2.07), dexamethasone (71.00±2.91), and heparin (69.60 ±23.42), table (4-9).

4. 5.4. Number of Primary Spermatocytes:-

The study showed that there was a significant decrease ($p \leq 0.05$) during the five-weeks period in all groups compared to the control group. In the first week, the number of the control group were (61.40±4.03), protocol (61.20±3.03), remdesivir (41.00±13.28), azithromycin (41.00±12.24), dexamethasone (43.00 ±27.47) and heparin (39.20 ±8.87).In the second week, the number of the control group were (61.60±16.11), protocol (59.00±1.58), remdesivir (41.60±25.28), azithromycin (44.80±3.76), dexamethasone (51.60±2.07) and heparin (54.40 ±5.27).In the fifth weeks, the number of the control group (65.20±5.89), protocol (60.80±2.28), remdesivir (21.00±8.36),

azithromycin (55.00±2.55), dexamethasone (51.60 ±3.43) and heparin (52.00±10.12), table (4-9).

4.5.5. Number of Secondary Spermatocytes:-

The study showed a significant decrease ($p \leq 0.05$) in the number of secondary spermatocyte in all groups during the five-week period, in the first week the number of the control group was (65.60±13.22), protocol (56.00±16.27), remdesivir (36.80±17.25), azithromycin (30.80±5.89), dexamethasone (51.00±23.80) , and heparin (34.40±6.61).In the second week the number of the control group (67.80±15.36), protocol (45.00±11.68), remdesivir (23.80±13.10), azithromycin (31.60±1.51), dexamethasone (39.40±2.40), and heparin (61.00 ±21.96).In the fifth week the number of the control group (69.40±12.77), protocol group (51.00±23.80), remdesivir (14.40±4.15), azithromycin (43.00±2.55) dexamethasone (61.40±2.40) and heparin (61.60 ±23.38), table (4-9).

4. 5. 6. Number of Spermatids:-

The study showed a significant decrease ($p \leq 0.05$)in the number of the spermatid cells in all groups during the five-week period. In the first week the number of the control group (53.40±3.20), protocol (46.80±2.28), remdesivir (36.40±5.03), azithromycin (20.40±1.81), dexamethasone (41.00±1.00) and heparin (37.40±17.55).In the second week, the number of the control group (52.60±2.07), protocol (46.40±16.10), remdesivir (19.20±0.83), azithromycin (17.20 ±3.11), dexamethasone (47.40±2.40) and heparin (46.80±2.28).In the fifth weeks, the number of control group (53.40±2.07), protocol (40.40±3.64), remdesivir (27.20±6.76), azithromycin (35.00±2.55), dexamethasone (41.40±2.40) and heparin (54.20±7.19). table (4-9).

4. 5. 7. Number of Leydig Cells:-

The study showed a significantly decrease ($p \leq 0.05$) in number of Leydig cells in all groups over the course of the five-weeks period as compared to the control group. In the first week, the number of the control group was (91.60±4.82), which was the protocol group (42.20±32.07), remdesivir (45.00±9.61), azithromycin (25.00±12.34), dexamethasone (32.80±2.16),and heparin (30.60±11.05). In the second week, the control group's number was (92.80±4.60), protocol (18.60±12.07), remdesivir (23.20±12.61), azthromycin (28.00±1.87), dexamethasone (23.40±2.40) and heparin (52.40±42.55). In the fifth week, the number of control group was (91.80±5.80), protocol (39.80±20.87), remdesivir (37.60±24.82), azthromycin (34.00±2.55), dexamethasone (65.40±2.40) and heparin (52.40±42.55), table (4-9).

4. 5. 8. Number of Sertoli Cell:-

The study showed a significantly decrease ($p \leq 0.05$) in the number of Sertoli cells in all groups during the five weeks period as compared to the control group. In the first week, the number of the control group was (36.60±11.03) , protocol (24.80±11.69), remdesivir (31.80±7.79), azithromycin (28.00±2.91), dexamethasone (12.60±1.81), and heparin (33.40±5.68). In the second week, the number of control group was (34.20±12.39), protocol (21.20±7.15), remdesivir (21.20±14.41), azithromycin (27.40±2.51), dexamethasone (14.40 ±2.40) and heparin (19.40±6.54) . In the fifth week the number of control group was (28.60±7.73), protocol (19.20±2.38), remdesivir (15.20±9.44), azithromycin (19.00±2.55), dexamethasone (21.80±2.58), and heparin (27.80±9.36), table (4-9).

Table (4-9): The changes in the count of (spermatogonia, primary spermatocyte ,secondary spermatocyte, spermatid, Leydig cell and Sertoli cell) in male rat over the five weeks.

Groups	wk	Spermatogonia	Primary Spermatocyte	Secondary Spermatocyte	Spermatid	Leydig cell	Sertoli cell
Control	1st wk	80.8 ^{a} ± 8.67	61.40^{a} ±4.03	65.60^{a}±13.22	53.40^{a}±3.20	91.60^{a}±4.82	36.60^{a}±11.03
	2nd wk	82.40 ^{a}± 7.92	61.60^{a}±16.11	67.80^{a}±15.36	52.60^{a}±2.07	92.80^{a}±4.60	34.20^{a}±12.39
	5th wk	79.40^{a}± 7.95	65.20^{a}± 5.89	69.40^{a}±12.77	53.40^{a}±2.07	91.80^{a}±5.80	28.60^{a}± 7.73
Protocol	1st wk	58.00^{b}± 2.22	61.20^{a} ± 3.03	56.00^{b}±16.27	46.80^{b}±2.28	42.20^{b}±32.07	24.80^{c}±11.69
	2nd wk	43.80^{b}± 18.51	59.00^{a}± 1.58	45.00^{b}±11.68	46.40^{b}±16.10	18.60^{d}±12.07	21.20^{c}± 7.15
	5th wk	65.00^{b}± 21.03	60.80^{a} ± 2.28	51.00^{b}±23.80	40.40^{b}±3.64	39.80^{c}±20.87	19.20^{c}± 2.38
Remdesivir	1st Wk	66.00^{b} ± 4.35	41.00^{b}±13.28	36.80^{c}±17.25	36.40^{c}±5.03	45.00^{b}±9.61	31.80^{p}± 7.79
	2nd wk	33.20^{b}± 18.29	41.60^{b}±25.28	23.80^{c}±13.10	19.20^{c}± 0.83	23.20^{c}±12.61	21.20^{c}±14.41
	5th wk	43.00^{b}± 27.47	21.00^{b}± 8.36	14.40^{c}± 4.15	27.20^{c}±6.76	37.60^{c}±24.82	15.20^{c}± 9.44
Azthromycin	1st Wk	58.60^{b}± 12.05	41.00^{b}±12.24	30.80^{c}± 5.89	20.40^{c}± 1.81	25.00^{c}±12.34	28.00^{p}± 2.91
	2nd wk	62.20^{b}± 3.27	44.80^{b} ± 3.76	31.60^{c}± 1.51	17.20^{c}± 3.11	28.00^{c}± 1.87	27.40^{b}± 2.51
	5th wk	67.40^{b}± 2.07	55.0^{c}± 2.55	43.00^{b}± 2.55	35.00^{c}± 2.55	34.00^{c}±2.55	19.00^{c}± 2.55
Dexamethasone	1st wk	65.00^{b} ±21.03	43.00^{b}±27.47	51.00^{b}±23.80	41.00^{b}±1.00	32.80^{c}±2.16	12.60^{c}± 1.81
	2nd wk	43.60^{b}± 2.07	51.60^{b}± 2.07	39.40^{b}± 2.40	47.40^{b}±2.40	23.40^{c}±2.40	14.40^{c}± 2.40
	5th wk	71.00^{b}±2.91	51.60^{b}± 3.43	61.40^{b}± 2.40	41.40^{b}±2.40	65.40^{b}±2.40	21.80^{b}± 2.58
Heparin	1st wk	58.00^{b} ± 10.77	39.20^{b} ± 8.87	34.40^{c}± 6.61	37.40^{c}±17.55	30.60^{c}±11.05	33.40^{b}± 5.68
	2nd wk	68.80^{b}± 23.66	54.40^{b}± 5.27	61.00^{b}±21.96	46.80^{b}±2.28	52.40^{b}±42.55	19.40^{c}± 6.54
	5th wk	69.60^{b}± 23.42	52.00^{b}±10.12	61.60^{b}±23.38	52.40^{b}±42.55	75.00^{b}±32.87	27.80^{b}± 9.36

- Values represent mean ± SD, The same letter means no significant between groups while different letters means there was significant ($p \leq 0.05$) between groups.

Chapter Five

Discussion

5.Discussion:-

Medications can affect men's fertility in a variety ways. Drugs may directly and indirectly promote sexual dysfunction and spermatogenesis impairment, as well as change epididymis maturation, by modifying (HPG) axis hormones or by non-hormonal mechanisms (Semet *et al*., 2017).

5.1.The Effect of Mediation used In Protocol Covid-19 on Body Weight:

The study showed a decrease in the average body weight in the protocol and dexamethasone groups, while weight increased in the remdesivir group during the second and fifth weeks of the experiment, table (4-1). May be the cause of weight loss , the direct effect of dexamethasone on carbohydrate metabolism leading to increased glucose utilization that responsible for this drop, these agreement with (Bennett and Brown, 2008).When administered in excess, dexamethasone induces adverse effects such as muscle catabolism (Prelovsek *et al*., 2006).Animals unable to get nutrients due to the effects of drugs on central nervous system, which produce mental stasis, weakness, and myopathy. These results are consistent with (Jahnng *et al*., 2008;Dolatabadi and Zarchii., 2015)

The increase in the body weight in the remdesivir -treated group could be related to the fluid retention in the body, because remdesivir is a nephrotoxic drug that might affect the kidneys, It was also revealed that the rats went hungry after getting remdesivir, and their feeding habits improved. This medicine may effect on the hypothalamic satiety centers, increasing their appetite. It was also discovered that after receiving remdesivir intraperitoneally, the amount of water and food consumed increased and these results consistent with (Hussain *et al*., 2022).Which concluded that after

administering a high dose of remdesivir 150 μg for 10 days. to mice, there is a significant difference in average body weight, which may be attributable to edema. He also mentioned that after administering the mice remdesivir, their eating habits increased, which could be related to the influence of medications on the hypothalamic satiety centers, which stimulates their hunger.

5.2.The Effect of Medication used in Protocol of Covid-19 on Reproductive Organs Weight:-

The result of the study showed decrease in the testis weight average in all groups during the second and fifth week, while decrease in the epididymis weight in the dexamethasone group during the first, second and fifth weeks of the experiment and decrease in the epididymis weight in the remdesivir group during the second week, and decrease in the seminal vesicle weight in all groups during the first and second weeks, table (4-2).

Atrophy and degenerative changes may be the reason for the low weight of the genitals. These findings are consistent with (Abeer, 2015;Elsayed., 2017), a high concentration of the bioactive macrolide antibiotic in the prostate and seminal vesicles, which affects the reproductive histomorphological condition, may also contribute to the weight loss of the accessory sex organs (Schramm *et al*., 1988). These results agree with (Hussain *et al*., 2022) who indicated that the medicine remdesivir effects on the male reproductive organ in mice, these result agreement with study done by (Narayana *et al*., 2005) on rats which revealed that another antiviral agent, ribavirin, acts on the testes and lowers the number of seminiferous tubules, vacuoles at the Sertoli cell.

5.3.The Effect of Medication used in Protocol of Covid-19 on Male Sex Hormones:

The study found that the LH serum level increased in the first and second weeks in all groups except the azithromycin group that no significant differences found, in the heparin group the LH serum levels increased in the fifth week. The FSH hormone decrease during the fifth week in the protocol, azithromycin, dexamethasone and heparin groups. The testosterone hormone decrease in the protocol, remdesivir, azithromycin and dexamethasone groups during the second week of experience, tables (4-3),(4-4) and (4-5).

LH production may have an increased due to the modest negative feedback between testosterone and LH in the pituitary. In the early stages of the hypogonadism, decreased testosterone production may increase the release of LH, which can temporarily sustain testosterone levels.(Shuling *et al*., 2019; Lardone *et al*., 2013). While decrease in hormone levels could have been caused by damage to Leydig's cells, which generate testosterone (Abd- Allah *et al*., 2000;Khaki *et al*., 2009). These outcomes supported the conclusions stated by (Abeer., 2015) who asserted that taking azithromycin dramatically reduced serum testosterone levels. These results agreement with (Sadeghzadeh *et al*., 2019) who indicated dexamethasone reduces serum levels of testosterone. This due to how much transcription of genes encoding testosterone-producing enzymes is significantly reduced as a result (LaVoie and King, 2009).

5.4.The Effect of Medication used in Protocol of Covid-19 on Sperm Parameter:-

The study showed decrease in the sperm concentration and motility in all groups during the first, second and fifth weeks, and increase dead sperm percentage in all groups except the heparin group no significant differences

during the first, second and fifth weeks, and increase in the abnormal sperm percentage in the protocol, remdesivir, and azithromycin groups during the five weeks periods, table (4-6).

Sperm motility may be impacted by a stoppage in ATP production as a result of oxidative stress, (Karbalay and Noorafshan, 2011), these results coincide with (El-Sayed *et al*., 2017; Sadeghzadeh *et al*., 2019 and Fan [B] *et al*., 2020),They discovered that administration azithromycin, dexamethasone, and remidisvir have a detrimental influence on sperm parameters such as motility and concentration. They also discovered that these medications increase the percentage of dead and abnormal sperm. The earlier studies also demonstrated that dexamethasone's primary target is the mitochondria, as the medication affects the regulation of 72% of the genes involved in the mitochondrial respiratory chain, which lowers the ATP levels and slows sperm motility, these result agreed with (Mutsaers and Tofighi, 2012).

The considerable decline in sperm characteristics may be caused by increasing reactive oxygen species(ROS) causing oxidative injury to the testicles (Jeje *et al*., 2017). Lipid peroxidation of the sperm membrane is thought to be the primary mechanism by which ROS cause sperm damage, which may result in the infertility (Agarwal *et al*., 1994).

A key factor in the toxicity of many xenobiotics is oxidative damage, which is indicated by the lipid peroxidation (LPO) marker. Malondialdehyde (MDA) is a stable LPO byproduct that can be used to evaluate cumulative LPO indirectly. Due to the abundance of polyunsaturated fatty acids in the plasma membrane and the low levels of cytoplasmic antioxidants, mammalian spermatozoa are vulnerable to LPO, (Aitken *et al*., 1993).High levels of LPO can reduce sperm motility, most likely by an abrupt loss of intracellular ATP, which causes axonemal harm , decreasing sperm viability and an increase in abnormal morphology with negatively influencing sperm capacitation and

acrosome response (Lenzi *et al*., 1993). As a result, spermatogenesis and testicular steroidogenesis are affected.

Treatment with dexamethasone and azithromycin causes a high level of MDA (El-Sayed *et al*., 2017 and Jeje *et al*., 2020). A higher-than-normal MDA level in the testicles indicates lipid peroxidation. As a result, a rise in MDA levels harms sperm function and causes infertility (Hsieh *et al*., 2006).

5.5.The Histopathological Effect of Medication Used in Protocol Covied-19 :-

The study discovered that over a first, second and fifth weeks of the experiment, the diameter of the seminiferous tubule decreased in male rats in the protocol group and the other groups. while the diameter of the epididymis duct decreased in all groups during the second week, table (4-7 and 4-8). Over the course of the five-weeks study, the number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, Leydig cells, and Sertoli cells decreased in all groups, table (4-9).

Furthermore, histological changes in the reproductive organs (testis, epididymis and seminal vesicle) were observed in all groups throughout the first, second, and fifth weeks of the experiment. In the protocol group, there is abnormal form of the seminiferous tubules and a deficiency in the process of spermatogenesis, as well as degeneration and necrosis of testicular tissue. The epididymis demonstrated sperm loss, an increased in the interstitial regions, and an uneven cavity. Furthermore, the basement membrane collapsed, and the morphology of the epididymis cells changed from pseudostratified columnar to simple columnar or cuboidal.

The changes mentioned above may be could be attributed to the oxidative stress process. Male infertility is thought to be caused by oxidative

stress, which is caused by an imbalance between (ROS) formation and antioxidant system activity (Ross *et al*., 2010), on the other hand suppressing the (HPG) axis can have indirect effects on spermatogenesis (Palomba *et al*., 2014and Leroy *et al*., 2015).

In the remdesivir group, testicular tissue revealed a severe damage, necrosis, and vacuolation in the first and second weeks, and fibrosis with blurred cellular structure in the fifth week. While the epididymis tissue showed damage to the basement membrane and tubular lumen, which resulted in vacuolation of the epididymis duct epithelial lining and the absence of spermatozoa, as well as increased interstitial space.

These results are consistent with (Fan [B] *et al.,* 2020),who indicated remdesivir therapy negatively affects the spermatogenesis of testicular and epididymis tissue significantly, testicular tissue appeared of fibrosis and loss its function, and sperm in the epididymis decreased significantly or disappeared and the effects did not recover within one week of stopping the drug. Study on the nutritional metabolism of drugs have also revealed that while the remdesivir medication persists more in testicular tissue during the initial stages of treatment, it also exhibits a rapid nutritional metabolism in testicular tissues over time (Warren *et al*., 2016).

In the azithromycin group, there was a deterioration of the germinal epithelium, insufficient spermatogenesis, gaps, and the lumen was filled with the detached germ cells. Interstitial blood vessel congestion. The epididymis anatomy in the azithromycin group showed an abnormal lumen loaded with sperm and epithelial cell hypertrophy.

These findings are in line with those of (Abeer, 2015 and El-Sayed *et al*., 2017) who showed that the azithromycin administration resulted in severe histopathologic lesions like vacuolations and degeneration of spermatogonial

cells lining the seminiferous tubules. In a typical situation, the sperm plasma contains antioxidants, which function as inhibitors of (ROS) and shield the sperm from harm (Yadav *et al*., 2006).When azithromycin is administered, antioxidant enzymes (superoxide dismutase, catalase, and glutathione) become less active (Abeer, 2015). When azithromycin is administered, testosterone levels fall (El-Sayed *et al*., 2017) and this might be as a result of a decline in the testosterone-producing Leydig cells (Abd-Allah *et al*., 2000 and Khaki *et al*., 2009).

In the study the testis tissue in the dexamethasone group showed an epithelial cell degradation and seemed noticeably necrotic, changed seminiferous tubules revealed irregular form, and the epididymis tissue displayed an uneven lumen with the decreased sperm production. These results are consistent with a previous study (Sadeghzadeh *et al*., 2019) that indicated dexamethasone administration caused Leydig cells to undergo apoptosis, which lowered the spermatogenesis index as well as the mean number of spermatocytes, spermatids . a cellular redox imbalance and oxidative stress are caused by dexamethasone, which also encourages the generation of free radicals like ROS (Eid *et al*., 2017).

The presence of gaps within the seminiferous tubule was associated with the decrease in the sperm in the heparin group. The first and second weeks exhibited interstitial blood vessel congestion, while the fifth week showed normal structure, sperm proliferation inside the seminiferous tubules, and an epididymis packed with the sperm. perhaps there is no research to date indicating that heparin causes reproductive toxicity .

Chapter Six

Conclusions and Recommendations

6. Conclusion and Recommendation

6.1. Conclusions:-

1. The medications used in the COVID-19 treatment protocol have negative effects on the male reproductive system in the rat. Where it had Histopathological changes on the male reproductive organs (testicle, epididymis, and seminal vesicle), such as severe degeneration of epithelial cells, loss of sperm density, and a decrease in the number of germ cells Leydig and Sertoli cells .
2. The protocol and remdesivir groups saw more severe histological changes than the other groups, and during the second week of the experiment, they had a more detrimental effect than during the first and fifth weeks.
3. In the experiment's fifth week, there were no histopathological alterations found in the heparin group.
4. The medications used in the COVID-19 treatment protocol have a significant change in body weight and reproductive organs weight.
5. The medications used in the COVID-19 treatment protocol have a significant change in balance of male sex hormones
6. The medications used in the COVID-19 treatment protocol have a significant change on the features of semen and thus causes a defect in the process of spermatogenesis.

6.2.Recommendation:-

1. Conducting future studies on the effect of drugs used in the treatment protocol for COVID-19 on the female reproductive system in animals.
2. Conducting more studies to find out the effect of the drugs used in the treatment protocol for Covid-19 on other systems such as urinary, digestive and circulatory.
3. Conducting a study to find the effect of drugs used in the treatment protocol for Covid-19 on hematological parameter and biochemical parameters.
4. Conducting a study of the effect of drugs used in the treatment protocol for Covid-19 on oxidative stress in the testicle.

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Appendixes

Appendix (1):

Dosage calculation method

1-The doses were calculated based on the surface area approach where the dose given to the adult human is taken and divided by the weight of the adult human (60 kg) to extract the human equivalent dose.

2- Extract the animal equivalent dose by multiplying the human equivalent dose by the conversion constant factor for the rat (6.17).

3- Extract Average animal dose by multiplying the animal equivalent dose

In the weight of the rat (250g),divided by 1000.

Human equivalent dose (HED) = $\frac{humen\ dose}{60kg}$

Animal equivalent dose (AED) = HED * convention factor rat (6.17)

Average animal dose = (AED*250/1000) ,(Nair & Jacob., 2016).

Appendix (2):

Measuring the level of interstitial cell stimulating hormone (LH)

LH measurement is a two-step immunological test that uses CMIA technology and flexible screening methods, sometimes known as "Chemiflex," to detect the presence of LH in blood and plasma.

1- The samples is mixed with magnetic small particles that have anti-B LH coatings on them. Anti-B LH-coated tiny particles are attracted to the LH in the sample.

2- Anti-acridinium LH- is added to the reaction mixture after washing.

3- Trigger-pre solutions are added to the reaction mixture following a second wash cycle.

4- Relative units of light (RLUs) are used to quantify the chemical reaction that was created. The RLUs picked up by the optics system are directly proportional to the amount of LH present in the sample.

Appendix (3):

Follicle-Stimulating Hormone (FSH) Measuring Level

FSH measurement is a two-step immunoassay that uses CMIA technology and flexible screening algorithms known as Chemiflex to detect the presence of FSH in serum and plasma.

1- Anti-B FSH-coated magnetic fine particles are mixed with the sample. FSH is attached to tiny particles coated in anti-B FSH in the sample.

2- Anti-acridinium FSH- is then added to the reaction mixture after washing.

3- Trigger-pre solutions are added to the reaction mixture following a second wash cycle.

4- Relative units of light (RLUs) are used to quantify the chemical reaction that was created. The amount of FSH present in the sample and the RLUs identified by I opxics are directly correlated.

Appendix (4):

Testicular Lipid Hormone Testosterone Measurement Level

The determination of serum and plasma testosterone using a one-step immunoassay known as a "testosterone measurement" makes use of CMIA technology and adaptable screening techniques known as "Chemiflex".

1- The appropriate assay diluent and the anti-testosterone (sheep, monoclonal) are mixed with specimen-covered magnetic fine particles. The sample's testosterone binds to the anti-testosterone-coated micro particles.

2- Testosterone is added to the reaction mixture following incubation.

3- Trigger-Pre solutions are added to the reaction mixture following a further wash cycle.

4- Relative units of light (RLUs) are used to measure the chemical reaction that occurs. The RLUs picked up by the Opxics system are inversely correlated with the amount of testosterone present in the sample.

Appendix (5):

Nigrosine Solution (10%) Preparation

Add 50 ml of deionized water after weighing 5 g of nigrosine. Melt with low heat. The liquid is cooled to room temperature before being filtered via filter paper.

Appendix (6):

Neutral formalin preparation (10%)

100 ml of formaldehyde was added were dissolved in 900 ml of distilled water to create neutral formalin (10%).

Appendix (7):

Hematoxylin stain

Hematoxylin solution

Ethanol 100%(100 ml), Glycerin (100 ml), glacial acetic acid(10 ml),Hematoxylin (2g).

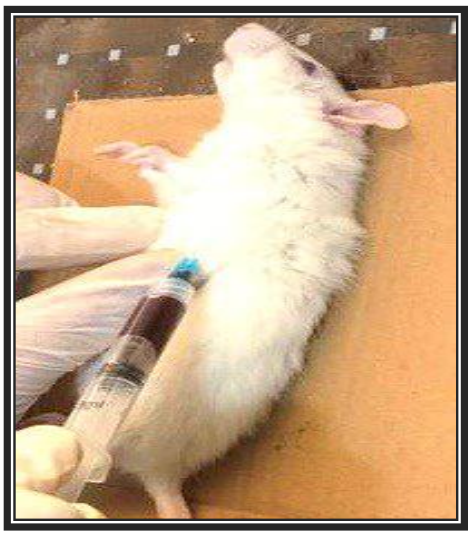
Hematoxylin and ethanol alcohol are combined, followed by the addition of glycerol, glacial acetic acid, and potassium alum. The mixture is then placed in a glass bottle and exposed to sunshine. After briefly opening the vial, it is shut and shook. The tincture must mature for a number of weeks after this process is repeated.

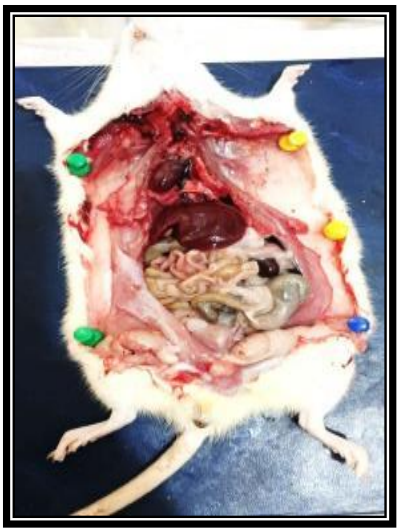
Appendix (8):

Eosin Y stain

Weighs Y (1g),Distilled water (20 ml 95%),Ethanol (80ml),Mix to dissolve and store at room temperature.

Appendix (9) Pictures showing animal dosing, injection, blood samples and anatomy.





الخلاصة

أجريت هذه الدراسة في كلية الطب البيطري جامعة البصرة للمدة من 15\10\2022 إلى20\3\2023 , وتم استخدام (90) ذكر من الجرذان، وتم تقسيمها الى ست مجاميع كل مجموعة مكونة من (15) جرذ، المجموعة الأولى المجموعة (الضابطة) تم تجريعها فمويا بمحلول ملحي (2.5 ml) لمدة 14 يوم.

المجموعة الثانية مجموعة البروتوكول، تم إعطائها بروتوكول الأدوية المستخدم لعلاج مرضى كوفيد-19(الريميدسفير ، الازثرومايسين، الديكساميثازون، الهيبارين، والمكملات الغذائية)، المجموعة الثالثة تم حقنها تحت الصفاق بالريميدسفير (2.5mg/rat)، المجموعة الرابعة جرعت فمويا بالأزثرومايسين (13mg/rat)، المجموعة الخامسة حقنت بالعضلة بالديكساميثازون (0.15mg/rat)، أما المجموعة السادسة حقنت تحت الجلد بالهيبارين (1.5mg/rat). تم استمرار إعطاء العلاج لمدة (14) يومًا بأستثناء مجموعة الريميدسفير التي تم اعطائها العلاج لمدة (5) ايام مرة واحدة يوميا، اما المكملات الغذائية جرعت فمويا حسب الجرع التالية لمدة 14 يوم (الزنك 1.2mg/rat, فيتامين C 200mg/rat وفيتامين D 0.03mg/rat) بينما استمرت التجربة لمدة خمسة أسابيع.

في كل مرحله تم أخذ وزن الجرذان قبل التضحية بها، تم القتل الرحيم لخمسة جرذان من كل مجموعة في نهاية الأسابيع (الأول والثاني والخامس) وتم جمع عينات الدم للحصول على مصل لقياس الهرمونات (الهرمون المنبه للجريب FSH ,الهرمون اللوتيني LH وهرمون التستوستيرونT)، ثم تم استئصال الأعضاء التكاثرية وتم أخذ أوزانها (الخصية، البربخ و الحويصلة المنوية)، تم تحضير المقاطع النسيجية لدراسة التغيرات النسيجية باستخدام صبغات (الهيماتوكسلين والأيوسين) وكذلك دراسة التغيرات في القياسات النسيجية للنبيبات المنوية في الخصية والبربخ وحساب أعداد الخلايا الجرثومية (سليفات النطف وخلايا النطف الأولية وخلايا النطف الثانوية وأرومات النطف وخلايا ليدك وخلايا سرتولي)، وتم جمع السائل المنوي من البربخ لغرض حساب (تركيز الحيوانات المنوية، وحركتها و نسبة الحيوانات المنوية الميته والمشوهة).

أظهرت نتائج هذه الدراسة ما يلي:

انخفاض معنوي ($p\leq0.05$) في متوسط وزن الجسم في مجاميع (البروتوكول و الديكساميثازون)، بينما كان هنالك زيادة معنويه ($p\leq0.05$) في متوسط الوزن لمجموعة الريميدسفير خلال الاسبوعين الثاني والخامس من التجربة.

انخفاض معنوي ($p\leq0.05$) في متوسط وزن الخصية في جميع المجاميع خلال الاسبوعين الثاني والخامس، بينما انخفض وزن البربخ معنويا ($p\leq0.05$) في مجموعة الديكساميثازون خلال الاسبوع الأول والثاني والخامس من التجربة بينما انخفض معنويا ($p\leq0.05$) وزن البربخ في مجموعة الريميدسفير خلال الاسبوع الثاني، كما ولوحظ انخفاض معنوي ($p\leq0.05$) في وزن الحويصلة المنوية في جميع المجاميع خلال الأسبوعين الأول والثاني.

زيادة معنوية ($p\leq0.05$) في مستوى الهرمون المحفز للخلايا الخلالية (LH) في الأسبوع الأول في جميع المجاميع بينما ازداد معنويا($p\leq0.05$) في الاسبوع الثاني في مجاميع (البروتوكول، الريميدسفير، الديكساميثازون والهيبارين). وارتفع معنويا($p\leq0.05$) مستوى(LH) في مجموعة الهيبارين في الاسبوع الخامس بالمقارنة بالمجموعة الضابطة.

انخفاض معنوي ($p\leq0.05$) في مستوى الهرمون المحفز للجريبات (FSH) في مجموعات (البروتوكول، الأزيثروميسين، الديكساميثازون والهيبارين) في الأسبوع الخامس مقارنة بالمجموعة الضابطه. كما وأنخفض هرمون التستوستيرون معنويا ($P\leq0.05$) في مجاميع البروتوكول, الريميدسفير, الازثرومايسين والديكساميثازون خلال الأسبوع الثاني من التجربة.

انخفاض معنوي ($p\leq0.05$) في تركيز الحيوانات المنوية و حركتها في جميع المجاميع خلال الاسبوع الاول والثاني والخامس من التجربة. زيادة معنويه ($P\leq0.05$) في نسبة الحيوانات المنوية الميته في جميع المجاميع بأستثناء مجموعة الهيبارين خلال الاسبوع الاول والثاني والخامس .كما ولوحظ زياده معنويه ($P\leq0.05$) في نسبة النطف المشوهة في مجاميع البروتوكول والريميدسفير والازثرومايسين خلال فترة الاسابيع الخمسة من التجربة.

انخفاض معنوي ($p\leq0.05$) في قطر النبيبات المنوية في جميع المجاميع خلال فترة الاسابيع الخمسة من التجربة. بينما انخفض معنويا ($P\leq0.05$) قطر قناة البربخ في جميع المجاميع خلال الاسبوع الثاني.

انخفاض معنوي ($p\leq0.05$) في اعداد الخلايا (الخلايا الجذعية النطفية, الخلايا النطفية الأولية، الخلايا النطفية الثانوية، النطف، خلايا سرتولي وخلايا ليدك) في جميع المجاميع خلال فترة الأسابيع الخمسة من التجربة.

لوحظت التغيرات النسيجية المرضية في الأعضاء التناسلية (الخصية، البربخ و الحويصلة المنوية) في جميع المجاميع خلال الأسابيع الأول والثاني والخامس من التجربة. في مجموعة البروتوكول، هناك اشكال غير طبيعية من النبيبات المنوية ونقص في عملية تكوين الحيوانات المنوية، وكذلك تنكس ونخر أنسجة الخصية. أظهر البربخ فقدان الحيوانات المنوية، زيادة في المناطق الخلالية ، وتجويف غير متساوي، تضررالغشاء القاعدي ، وتغير خلايا البربخ من عمودي مطبق كاذب إلى عمودي بسيط أو مكعب.

في مجموعة الريميدسفير، كشفت أنسجة الخصية عن تلف شديد ونخر وتفريغ في الأسبوعين الأول والثاني، وتليف مع بنية خلوية غير واضحة في الأسبوع الخامس. بينما أظهر نسيج البربخ تضرر الغشاء القاعدي والتجويف الأنبوبي، مما أدى إلى تفريغ البطانة الظهارية لقناة البربخ وغياب الحيوانات المنوية.

في مجموعة ألازيثرومايسين ، كان هناك تنكس في الظهارة الجرثومية، وعدم انتظام تكوين الحيوانات المنوية، الفجوات، وامتلاء التجويف بالخلايا الجرثومية المنفصلة. كان هناك احتقان في الأوعية الدموية الخلالية. أظهرنسيج البربخ في مجموعة أزيثروميسين تجويفًا غير طبيعي يحتوي على الحيوانات المنوية وتضخم الخلايا الظهارية.

أظهرت أنسجة الخصية في مجموعة الديكساميثازون نخرًا بشكل ملحوظ، وأظهر أنسجة البربخ تجويفًا غير متساوي مع انخفاض إنتاج الحيوانات المنوية. في مجموعة الهيبارين وجود فجوات داخل النبيبات المنوية, أظهر الأسبوعان الأول والثاني احتقان الأوعية الدموية الخلالية، بينما أظهر الأسبوع الخامس بنية طبيعية وتكاثر الحيوانات المنوية.

كما بينت نتائج الدراسة ان اكثر المجاميع تغيرات نسيجية مرضية هي مجموعة البروتوكول والريميدسفير وكانت أكثر شده في الأسبوع الثاني من الاسبوعين الأول والخامس.

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

التأثيرات النسيجية الفسلجية للأدوية المستخدمة في بروتوكول علاج كوفيد-19 على الجهاز التناسلي الذكري في الجرذان

دراسة مقدمة

إلى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

نورس جاسم محمد

بكالوريوس تربية علوم حياة (2010)

بإشراف

أ. م . د. علي خلف علي

أ. م . د. مصطفى صدام خاجي

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