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Comparative Histological, Histochemical and Physiological Study on Effect of (Amitriptyline, Escitalopram) on Some Organs of Reproductive System in Adult Male Mice (*Mus musculus*)

A thesis

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By

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﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

صَدَقَ اللهُ الْعَلِيُّ الْعَظِيمُ

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Supervisor's Certificate

I certify that this thesis entitled "Comparative Histological, Histochemical and Physiological Study on Effect of (Amitriptyline, Escitalopram) on Some Organs of Reproductive System in Adult Male Mice (*Mus musculus*)

Submitted by (Israa Abdulameer Naeem) has been prepared under my supervision at the College of Science, University of Misan; as partial fulfillment of the requirements for the Degree of Master in Biology.

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Recommendation of Head of Biology Department

Because of the available recommendations; I forward this thesis to debate by the examining committee.

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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……. those who stayed up nights and drowned me with their kindness and tenderness and taught me honesty, patience, perseveranc, giving and loyalty my mother and father

To ……. my support in my life my brothers and sisters for everyone who taught me

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

To ……. my husband is more wonderful than the embodied of love with all its meanings, so the bond and giving gave me a lot in pictures of patience, hope and love. I will not say thank you, but I will always live thanks to you

To……. my children, the eyes from which I draw strength and continuity, the sweetest of my life (Wissam, Youssef) and to the newcomer who was patient with my uncle (Kinan)

To ……. my friends (Zainab, Hawraa and Zahraa) who accompanied me and encouraged my steps when the days overwhelmed me. You have my love and gratitude

ISRAA

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I am pleased as I put the last fingerprints of my thesis to express my heartfelt thanks and gratitude to my esteemed Assis. Prof. Dr. Ali Khalaf Ali, who kindly supervised my thesis and suggested the subject of the thesis, and his continued encouragement to me in all stages of the research work, I wish him all success and continued health, and may God reward him on my behalf.

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Summary

Summary

In the experiment, (90) male mice were used and divided into three groups, each group consisting (of 30) mice, the first group (control) administration of normal saline, the second group administration Amitriptyline, and the third group administration Escitalopram. The dose for each group was (0.42µl/day) for six weeks twice a day. During the study period , the weights were measured at the end of the week (second, fourth, sixth) , euthanasia and blood collection for CBC test , and hormones examination (luteinizing hormone(LH), follicle- stimulating hormone (FSH), testosterone), organ weight (testis, epididymis, seminal vesicles), semen collection from the epididymis for sperm examination (concentration, motility ,dead) , determined the tissue changes of the organs (testis, epididymis, seminal vesicles) due to the drug by using two types of staining, hematoxylin- eosin ,periodic acid – Schiff's reagent (PAS) and histomorphometric study (the diameter of the seminiferous tubes, the diameter of the epididymis duct, numbers of cells: spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid).

The results of the study showed the in Amitriptyline group, a significant decrease ($p<0.05$) in body weight, while in the Escitalopram group there was an increase in weight ($p<0.05$) compared to the control group, and there was a significant decrease ($p<0.05$) in the weight of testis, epididymis and seminal vesicles for each Amitriptyline and Escitalopram groups in fourth week. significant changes were observed in blood parameters , a significant decrease ($p<0.05$) in the level of FSH hormones in the Amitriptyline groupin second , fourth and six weeks and no significant changes in the level of FSH in the Escitalopram group , significant decrease in the level of LH hormone and the decrease in the Amitriptyline group was more than the decrease in the Escitalopram group in the second and fourth weeks , while in the sixth week

there were no changes in the level of LH in the Escitalopram group , while the decrease continued in the Amitriptyline group, significant decrease in the testosterone level in the Amitriptyline group was more than that in the Escitalopram group over six weeks, Significant decrease in sperm concentration and motility in the Amitriptyline group more than the decrease in the Escitalopram group and significant increase in the number of dead sperm in both groups.

There is no significant change in the diameter of the seminiferous tubules and the diameter of the epididymis in both groups over six weeks, as well as a significant decrease in the number of cells (spermatogonia, primary spermatocyte, secondary spermatocyte, and spermatid) in both group.

The results of the study showed histological changes in the testis of mice in the Amitriptyline group are present space between spermatogonia cells, present space between the layer of the spermatogonia and primary spermatocyte cells, the proliferation of sertoli cells, decrease of primary spermatocyte and lumen of seminiferous tubules is wider and decrease in spermatid.

As for the Escitalopram group , it showed decrease in spermatogonia cells, present space between the layer of the spermatogonia and primary spermatocyte cells, and layers of cells(spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid) irregular in arrangement , absence of spermatid layer, lumen wider, , absence of lumen , change in the size of cells , absence lumen and the interaction of the basement membrane with PAS in the second and fourth week was moderate for both Amitriptyline and Escitalopram , but in the sixth week the interaction was strong for both groups compared to the control group, where the interaction of the control group was weak.

There were histological changes in the epididymis in both group, the changes in Amitriptyline group are presence of gap between the epithelium cells, and the absence of sperm in the lumen, the lumen became narrow and irregular, hypertrophy of epithelial cells. While the changes in the Escitalopram group are hypertrophy of epithelial cells, decrease sperm in the lumen , presence of circular immature sperm ,the epithelial cell layer cells changed from the pseudostratifid columnar to simple columnar , the cell shape changed from the columnar to the cuboidal shape and the interaction of the basement membrane with PAS PAS in the second week was moderate for both Amitriptyline and Escitalopram , but in the fourth and sixth week the interaction was strong for both groups compared to the control group, where the interaction of the control group was moderate.

The results of the study showed histological changes in the seminal vesicles in both groups, the changes in Amitriptyline group were an increase in the number of folds, stratification of epithelial cells, epithelium metaplasia, lumen narrow and a decrease in eosinophil secretion. The changes in the Escitalopram group were an increase in the number of folds, narrowing of the lumen of seminal vesicles , decrease in eosinophil secretion , stratification of epithelial cells and the interaction of the basement membrane with PAS in the second week was moderate for Amitriptyline and strong for Escitalopram , but in the fourth week the interaction was weak for both groups compared to the control group, and in the sixth week was strong for both groups compared to the control group, where the interaction of the control group was strong.

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List of Abbreviations

Abbreviate	Definition
PLT	Blood platelet
BDNF	Brain – derived Neurotrophic factor
CBC	Count blood cell
CYP2C19	Cytochrome 2C19
CYP2D6	Cytochrome 2D6
CYP3A4	Cytochrome 3A4
FGF2	Fibroblast growth factors
FSH	Follicle –stimulating hormone
FDA	Food and Drug Administration
FAA	formalin acetic acid alcohol
GnRH	Gonadotropin releasing hormone
H&E	Haematoxylin & Eosin
HCT	Hematocrit
HGB	Hemoglobin concentration
H1	Histamine receptor
HPG	Hypothalamus pituitary Gonads
ICSH	Interstitial cell- stimulating hormone
L	liter
LH	Luteinizing hormone
LHR	Luteinizing hormone receptors
MDD	major depressive disorder
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MPV	mean platelet volume

ML	milliliter
MAOIs	Monoamine oxidase inhibitors
M1	Musccarinic receptor
NERT	Norepinephrine transport
NF-KB	Nuclear factor kappa
PAS	Periodic acid Schiff
PCT	platelet crit
PDW	platelet distribution width
QT	QT interval
ROS	Reactive oxygen species
RBC	Red blood cell count
RDW	red blood cell distribution width
NaCl	salt sodium chloride
SSRIs	Selective serotonin re-uptake inhibitors
SNRIs	Serotonin norepinephrine re-uptake inhibitors
SERT	Serotonin transport
SPSS	Social Package of Social Sciences
CHCI3	Trichlormethane
TCAs	Tricyclic antidepressant
WBC	White blood cell count

Introduction

1- Introduction

Antidepressants: this class of drugs is commonly used in some countries and is used to treat depression and can be used to treat anxiety disorders (Beeder and Samplaski, 2020).

There are several types of antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), serotonin norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressant (TCAs), and other antidepressants medications, and each type has its mechanism of action and is slightly different from the other types, and therefore takes several ways in its effect on sperm. (Pratt *et al*., 2017).

Amitriptyline is a sedative tricyclic antidepressant frequently used in neuropsychiatry for depressive personality disorder (Millan, 2002; Khurshid *et al*., 2017), it is intended for the treatment of various neuropathies, migraine prophylaxis, and fibromyalgia (Kia and Choy, 2017; Tousson *et al*., 2020), it act inhibiting the re- uptake of serotonin and norepinephrine, thus it intensifies the neurotransmitters in the synapses (Mika *et al*., 2013).

Escitalopram is an antidepressant of the SSRIs class (Erdemir *et al*., 2014). This type act according to the mechanism of stimulating post-synaptic receptors by blocking the re-uptake of serotonin in neurons (pre-synaptic), thus increasing the concentration in the (synaptic space). (Koyuncu *et al*., 2012).

Male reproductive health is important to public health because the viability and quality of semen are directly or indirectly affected by public health (Salonia *et al*., 2009). Antidepressants are medicines used to treat depression, and anxiety-related illnesses (Novio *et al*., 2011), there are many different studies on the negative effect of antidepressants on the male reproductive system (Drobnis and Nangia, 2017; Bandegi *et al*., 2018).

The reason for low male fertility is the use of some drugs for long or short periods, as the use of drugs for short periods causes temporary infertility, while the use of drugs for long periods brutally affects fertility (DA silv junior *et al*., 2014).

The process of spermatogenesis is continuous from puberty and throughout life in humans and takes place in the lumen of the seminiferous tubes, which it the components of the testis, the process of spermatogenesis is a series of events through which sperm develop in the testis. (Sharma, 2007).

Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), and Testosterone hormone are considered signs of spermatogenesis as well as the activity of the testis in males because the process of spermatogenesis takes place in the seminiferous tubules, this process includes stimulating the Leydig cells to secrete the testosterone hormone, and this is stimulated by the (LH) hormone secreted by the pituitary gland and stimulating the Sertoli cells by (FSH) hormone secreted by the pituitary gland as well. Through the count and movement of sperm, the disturbances that occur in the process of spermatogenesis are known (Sofikitis *et al*., 2008).

All antidepressants have reported sexual dysfunctions, and clinicians generally mention them: problems maintaining an erection, loss of sexual desire, delayed ejaculation, and decreased sexual arousal (Higgins *et al*., 2010).

The data about antidepressants, which leads to a negative effect on the reproductive system are limited and focus mostly on general observations associated with semen parameters and other markers of male fertility (Beeder and Samplaski, 2020).

Laboratory studies have shown that antidepressants lead to a decrease in the number of sperm cells, changing the morphological appearance and affecting movement (Kumar *et al*., 2006; Koyuncu *et al*., 2012).

1-1- Aims of the study

Since the Antidepressant drug is commonly used in some countries, its negative effect on the male reproductive system, hence the study was designed to achieve the following objective:

1. Illastrate the histological, histochemical, histomorphometric changes in (Testis, Epididymis, Seminal vesicle) of experimental animals post Amitriptyline and Escitalopram administration.
2. Determined biochemical parameter like (CBC), also measurement testosterone, FSH and LH hormones in serum of experimental animals post Amitriptyline and Escitalopram administration.
3. Sperm analysis (concentration, motility and viability) post Amitriptyline and Escitalopram administration.

Chapter Two

Literature Review

2-1- Histological structures of the male reproductive system

2-1-1- Testis in mice

The function of the testis in mice is to produce and release sperm, as well as produce and release of hormones, an example of these hormones is the androgenic steroid testosterone. In humans and mice, the location of the testis is inside the scrotum. (Suede *et al*., 2020).

Seminiferous tubules and Leydig cells are the structural components of the testis, there are two types of cells in seminiferous tubules of rodents:

1. Sertoli cells: were found resting on the basement membrane.
2. Spermatogenic cells: arranged in several layers (spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, and finally spermatozoa). (Tousson *et al*., 2018).

When using antidepressant drugs causes histological changes in the tissue of the testis, including changes the decrease in the count of the spermatogenic cells, hemorrhage, and congestion of the blood vessels, necrosis, and dissociation of the interstitial tissue between seminiferous tubules (Madlool *et al*., 2019).

2-1-2- Epididymis in mice

On the posterior side of the testis is the epididymis, at the upper pole is the head and at the lower pole is the tail in mice, the primary function of the epididymis is the collection, maturation, and storage of the mature sperm. In humans and mice, the epididymis consists of three parts: the head, the body, and the tail. (Treuting *et al*., 2017).

In rodents, the pseudostratified columnar epithelium lining the epididymis is composed of triangular cells at the base, long columnar cells, and migrating lymphocytes.The nuclei are elongated and at different levels and the lumen is filled with sperms. The loose connective tissue that contains small blood vessels, fibroblasts, abundant ground substance, and collagen fiber, it is an interstitial tissue component of the epididymis. Antidepressant drugs adversely affect the epididymis tissue, leading to changes that include, an increase in the height of the columnar epididymis epithelium, appearance of intracytoplasmic vacuolation, and absence of spermatozoa in the lumen and the stereocilia showed a decrease. (Aggarwal *et al*., 2013).

2-1-3- seminal vesicles in mice

The seminal vesicles are bilateral, large cystic glands dorsal to the bladder, and the intercourse plug consists of the collected secretions from the bulbourethral glands, seminal vesicle, and prostate. (Treuting *et al*., 2017).

They are cystic organs and contain many lateral out pocketing that form an irregularly shaped lumen and are branched. The wall consists of an outer layer, a middle layer, and inner layer. The outer layer is connective tissue rich in elastic fibers and the middle layers consists of smooth muscle. The inner epithelium is pseudo stratified columnar consisting of round cells at the base between large cuboidal or low columnar cells and the mucous membrane forms primary, secondary and tertiary folds. The testosterone hormone has a role in the seminal vesicles as it maintains of the height of the mucosal epithelium as well as affects smooth muscle function. (Pawlina and Ross, 2018)

Antidepressant drugs cause a reduction in the lumen of seminal vesicle due to proliferation of mucosal folds, decrease in eosinophilic secretion, stratification of epithelial cells, decrease in the height of epithelial cells, and increased in the amount of connective tissue. (Aggarwal *et al*., 2014).

2-2- Spermatogenic cells

Spermatogonia are undifferentiated stem cells that support daily sperm production , and self-renewal, leading to the formation of large numbers of sperms, they are diploid and undergo differentiation and division to form spermatocyte, which enters the meiosis process , recombination takes place during the meiotic prophase length, the process of random distribution of the parental chromosomes into the haploid daughter, which cells is called spermatid .In the last stage , spermatids develop from round cells to highly specialized cells and released into the lumen of the seminiferous tubules called immature spermatozoa (Kubota and Brinster, 2018). As in figure (2-1).

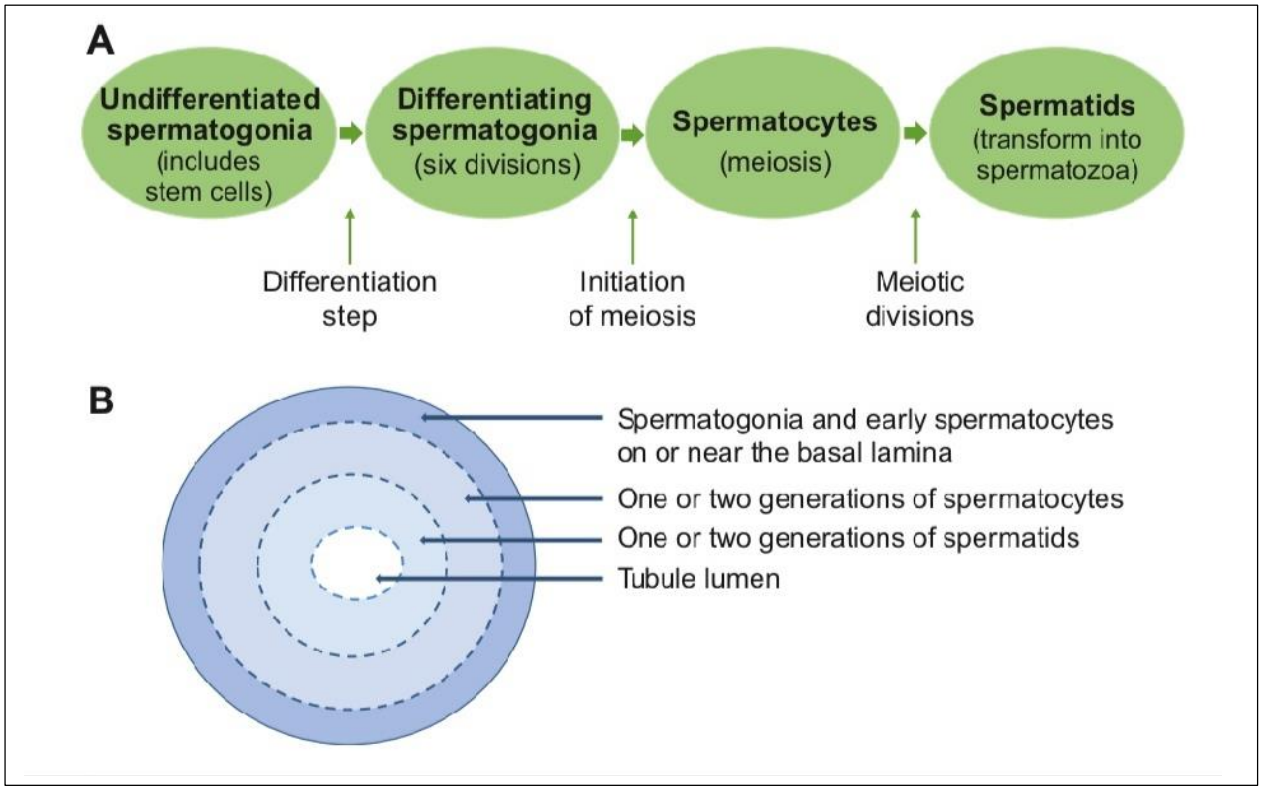

Figure (2-1): spermatogenic cells, **A:** shows the basic cell types, events and the most important steps for spermatogenesis. **B:** shows the position of the spermatogenic cell types in the seminiferous tubules. (De Rooij, 2017).

2-2-1 Spermatogenesis

The process of sperm development in the seminiferous tubule epithelium, it's a physiological process called spermatogenesis (Ni and Yang, 2019). Which starts with division of spermatogonial cell for spermatocytes and then the production of haploid spermatids in the seminiferous tubules, spermatids produce spermatozoa in the final stage of the spermatogenesis process. (Nishimura and L` Hernault, 2017).

High levels of gonadotropins and testosterone cause the sperm to start forming and it lasts for life, in old age it decreases. To produce mature spermatozoa, requires 65-70 days from the first stage (O` Shaughnessy, 2014; Singh *et al*., 2019).

Stem cells are the cells from which the process of spermatogenesis begins and are adjacent to the basement membrane of the tube and undergo division and produce two types of cells: A and B. Diploid intermediate primary spermatocytes arise from differentiation of type B cells, while type A cell renovate the stem cell milieu, the primary spermatocytes replicate their DNA, which undergoing meiosis I after moving to the luminal part of the seminiferous tubules in the testis to produce two haploid secondary spermatocytes. Through the meiosis of each haploid secondary spermatocyte, two equal haploid spermatids are formed, and then sperms are formed after the individual spermatozoa are transformed functionally and morphologically during the process of spermiogenesis (Singh, 2019; O`Shaughnessy, 2014).

2-2-2- The factors affecting spermatogenesis

Neurotransmitters are secreted from the neuroendocrine, testosterone is secreted from Leydig cell, as well as growth factors are secreted into the intertubular space. Leydig cells, blood vessels, the seminiferous tubules lamina properia, and Sertoli cells receive hormones, transmitters, and growth factors, which they play several roles including keeping nutrition for Sertoli cells and per-tubular tissue cells as well as transferring sperm by controlling the peristaltic movement of the seminiferous tubules by contraction myofibroblasts, and also the control of blood flow in the intertubular microvasculature .(Silber, 2018).

The control of spermatogenesis in the testis is through additional testicular stimuli given by the hypothalamus, gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus, and the secretion of this hormone leads to stimulating the pituitary gland to secret luteinizing hormone (LH), as result of this stimulation, testosterone is released from Leydig cell. The process of spermatogenesis is throughout the seminiferous tubules of the testis is under the control of this hormone and provides feedback to the pituitary gland on the secretory activity of Leydig cell. The pituitary gland secretes the follicle-stimulating hormone (FSH), which stimulates the Sertoli cells and is required for the maturation of germ cells (Ramaswamy and Weinbauer, 2014).

The process of spermatogenesis in the seminiferous tubules is maintained by factors as in the figure. (2-2).

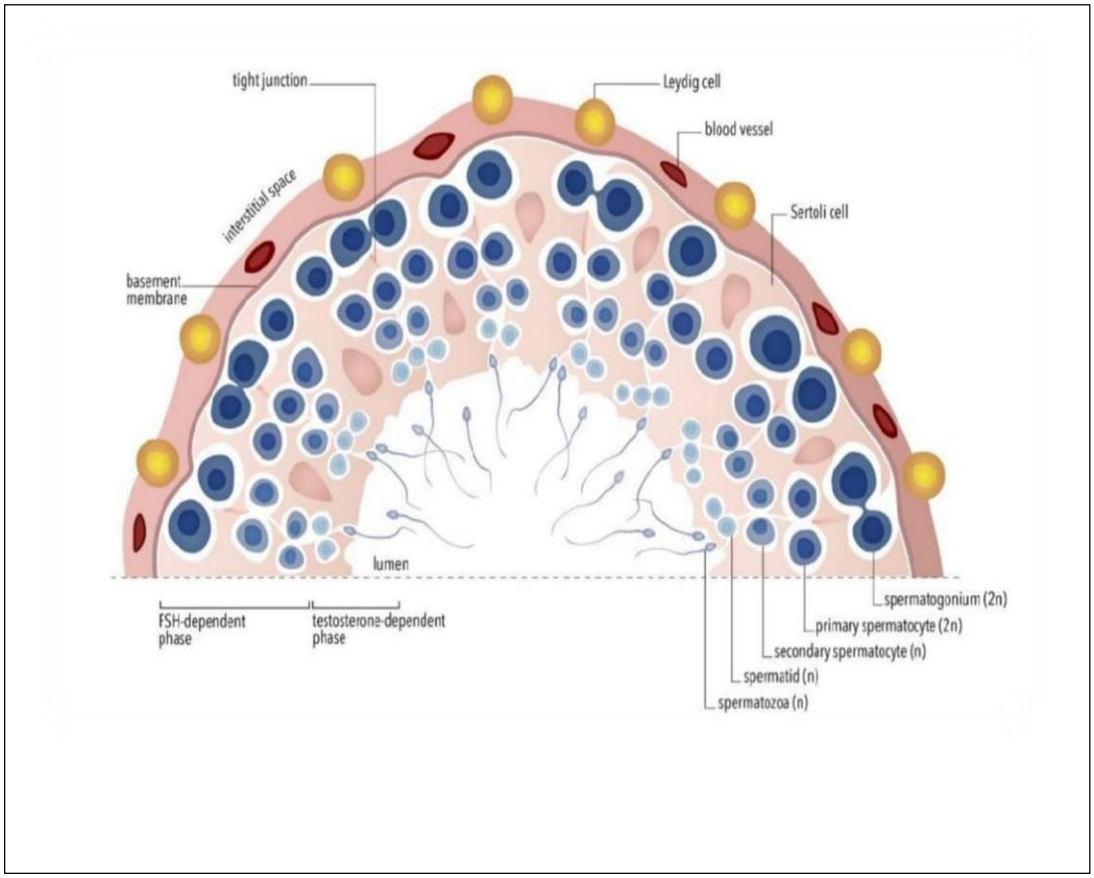

Figure (2-2): shows the process spermatogenesis until formation spermatozoa of a seminiferous tubules from the basement membrane to the lumen under the control of the follicle – stimulating hormone (FSH) and by testosterone hormone under luteinizing hormone (LH) Stimulus (Santi *et al*., 2020)

2-3- Hormones of the male reproductive system

2-3-1- Luteinizing hormone

Luteinizing hormone (LH), also called interstitial cell-stimulating hormone (ICSH) is a heterodimeric glycoprotein that consists of a well-preserved alpha chain and a discrete beta subunit that give biological specificity to hormone-receptor interaction in target tissues.

LH is produced by the anterior pituitary gland, LH is linked to a receptor, which is a receptor that linked and activates LH (Gheorghiu, 2019). Luteinizing hormone receptors (LHR) enhance the differentiation of Leydig cells and produce Testosterone, so it is demanded for the development of the male sex and spermatogenesis, LHR is expressed by Leydig cells in the testis in addition to ovarian granulosa and thecal cells. LHR enhances folliculogenesis, ovulation, and progesterone excretion in women (Latronico and Arnhold, 2013; Gheorghiu, 2019). Antidepressant drugs cause a reduction in the level of LH (Saleem *et al*., 2020).

2-3-2-Follicle-stimulating hormone (FSH)

FSH is a glycoprotein that participates in mammalian reproduction and development, FSH prepares the reproductive system for fertilization, implantation, pregnancy, control folliculogenesis, and the oocyte is chosen. (Messinis *et al*., 2014).

In the male, it mediates testis development and spermatogenesis (Huhtaniemi, 2015). This hormone pulsatile- organized by the hypothalamic gonadotropin-releasing hormone (GnRH) is secreted by the pituitary's gonadotropin cell. (Stamatiades and Kaiser, 2018). FSH contains an alpha subunit that is like other thyrotropin and gonadotropin, add to the beta-subunit. (Casarini and Crepieux, 2019). FSH supports spermatocyte meiosis, but testosterone becomes more involved as sperm matures, the action of androgens is essential for the perfecting meiosis, spermatogenesis, and fertility (Chang *et al.*, 2004; De Gendt *et al*., 2004). FSH levels decrease when taking an antidepressant. (Erdemir *et al*., 2014).

2-3-3-Testosterone hormone

Testosterone is secreted by the testis and to a lesser extent by the adrenal glands, which is the main male sex hormone. The function of this hormone is the maturation of the male sexual organ, responsible for secondary sexual characteristics. Testosterone creates by Leydig cells in the testis. When testosterone feedback to the hypothalamus and pituitary, a negative feedback loop is full, although, testosterone has a role in sex drive. Also, testosterone is demanded has evolved sperm production. Testosterone has an anabolic characteristic, which involves maturation, strength, and increased muscle mass, and bone thickness. In the first place, testosterone is associated with sex hormone-binding globulin (SHBG), and albumin in blood circulation, with little quantities, circulating freely or attached with corticosteroid-binding globulin. With age, testosterone decrease, and its decline is linked to increased arteriosclerosis and cardiovascular risk (Kloner *et al*., 2016; Trost and Mulhall, 2016). Several studies have shown that the use of antidepressants causes a decrease in the level of testosterone hormone. (Madlool *et al*., 2019).

2-4- causes and risk factors that affect sperm parameters

In the first place, disease of the reproductive system is diagnosed by semen analysis which includes: sperm movement, morphology, concentration, and total number. The risk factors affecting sperms parameters as shown in table (2-1). (Tuttelmann *et al*., 2018).

Table (2-1): explains some risk factors affecting sperm parameters (Agarwal *et al*., 2021)

Unknown reason	Nutritional factors Overweight Alcohol Smoking Parental aging Psychological stress Environmental exposure to toxins
Congenital factors	Genetic endocrinopathy Congenital obstruction Mild androgen insensitivity syndrome Kallmann syndrome Cryptorchidism Congenital absence of vas deference
Acquired factors	Varicocele Testicular torsion Testicular trauma Germ cell tumors Urogenital tract obstruction External factors (heat, radiation, medications) Systemic diseases (liver cirrhosis, renal failure)

2-5-The effect of drugs on sperm parameter

Cytotoxic drugs are not only drugs that affect negatively, but other types of drugs effect on (sperm concentration, sperm motility, sperm vitality) and with different mechanisms, which include hormonal and non-hormonal mechanisms, causing sexual dysfunction, impaired spermatogenesis, and negatively affecting epididymis maturation. The process of spermatogenesis is affected by drug treatment by changing the germs cells and Sertoli cells, and this change cause a defect in the secretory function of the testis. When the level of testosterone decreases, it affects the production of sperm, and any molecule that causes a change in the tissue of the testis or the function of the epididymis affects. Some of these drugs :(antidepressants, anti-infective agents and anti-inflammatories). (Semet *et al*., 2017).

2-6- Antidepressants

Antidepressants are medications that alter the chemical disturbances of neurotransmitters in the brain, thus helping to decrease symptoms of depressive disorders and that chemical imbalances lead to changes in mood and behavior, Neurons in the brain communicate through neurotransmitters, the vesicles in neurons contain the neurotransmitters by which neurons communicate with each other, Neurotransmitters such as serotonin, dopamine and noradrenaline or norepinephrine are released from the outer end of one nerve and received from the other. The phenomenon is called reuptake; antidepressants focus a specific neurotransmitter around the nerves in the brain by blocking the re- uptake of neurotransmitters as shown in Table (2-2). (Wessling and Ramsberg, 2008).

Table (2-2): Shows the most commonly used antidepressant and their mechanism of action (Wessling and Ramsberg, 2008).

No	Name of antidepressant	Mechanism of action
1	Moclobemide	MAO inhibitor
2	Escitalopram	Serotonin Reuptake inhibitors
3	Sertraline	Serotonin Reuptake inhibitors
4	Citalopram	Serotonin Reuptake inhibitors
5	Paroxetine	Serotonin Reuptake inhibitors
6	Fluoxetine	Serotonin Reuptake inhibitors
7	Maprotiline	Serotonin-norepinephrine reuptake inhibitors
8	Nortriptyline	Serotonin-norepinephrine reuptake inhibitors
9	Amitriptyline	Serotonin-norepinephrine reuptake inhibitors
10	Clomipramine	Serotonin-norepinephrine reuptake inhibitors

2-6-1 Antidepressant and their classification

Antidepressants are divided into five classes: (Goodman, 1996).

1. Serotonin- norepinephrine reuptake inhibitors (SNRI).
2. Selective serotonin- reuptake inhibitors (SSRIs).
3. Tricyclic antidepressants (TCAs).
4. Monoamine oxidase inhibitors (MAOIs).
5. Other antidepressant medications**.**

1- Serotonin norepinephrine reuptake inhibitors (SNRIs)

Some of the currently available drugs that belong to this category are Venlafaxine, Desvenlafaxine, and Duloxetine (Shah, 2015), studies have shown that each of these drugs is as effective as SSRIs in general (Gartlehner *et al*., 2008), as well as demonstrated through data collection that they have modest

clinical benefits for patients with more severe depression or patients who have not responded to treatment (Bauer *et al*., 2009), however, other studies have shown that both have similar efficacy (Cipriani *et al*., 2005).

2- Selective serotonin reuptake inhibitors (SSRIs)

SSRIs are the number one medications that treat depression and anxiety disorders, including Escitalopram, Citalopram, Paroxetine, Fluvoxamine, Fluoxetine, and Sertraline. SSRIs act by inhibiting serotonin reuptake, which significantly affects sexual function including erectile dysfunction, alteration of circulating hormones, and increased premature ejaculation (Montejo *et al*., 2001). Study have shown that 25-73% of people treated with SSRIs experienced higher sexual dysfunction than another antidepressant (Higgins *et al*., 2010).

3- Tricyclic antidepressants (TCAs)

The most common types of this class are Imipramine, Protriptyline, Amitriptyline, Nortriptyline, Desipramine, Doxepin, and Trimipramine, it can be compared with other types of antidepressants for the treatment of depressive disorder in severe cases (Anderson, 2000). This class has good efficacy, but it is not used much today because it has potentially more serious side effects. Tricyclic may mainly be effective in certain population groups, such as hospital patients (Barbui and Hotopf, 2001).

The mechanism of action of this class is by blocking the reuptake of serotonin and norepinephrine. (Saleem *et al*., 2020).

4- Monoamine oxidase inhibitors (MAOIs)

This class includes antidepressant is Ocarboxazid, Selegiline, Tranylcypromine, and Phenelzine. Patients who fail to use first-line treatment for depression are treated with this class of antidepressants, male and female patients taking MAOIs have 40% sexual dysfunction (Higgins *et al*., 2010). The mechanism of action of this class is by blocking the reuptake of serotonin and norepinephrine. (Khushboo and Sharma, 2017).

5- Other antidepressant medications

It includes this class of drugs Trazodone, Nefazodone, Mirtazapine, and Bupropion, it differs in its pharmacological action and composition from other classes, bupropion, and its mechanism of action is unknown, but believes it is a norepinephrine and dopamine reuptake inhibitor (Fava *et al*., 2005). But is not known to affect sexual functions, it has been shown that patients with major depression and anxiety were treated better with SSRIs than with bupropion (Papakostas *et al*., 2008).

Trazodone is used as an antidepressant to a lesser extent than as a hypnotic (Shah, 2015).

Mirtazapine has similar efficacy to that of a class of antidepressant SSRIs (Papakostas *et al*., 2008) and works through the serotonergic and noradrenergic mechanisms. (Artigas *et al*., 2002).

Nefazodone, its efficacy is similar to SSRIs and it is structurally similar to trazodone but differs in its chemical properties (Papakostas and Fava, 2007).

2-6-2-The effect of antidepressants on sperm parameters and sexual function

The antidepressant (selective serotonin reuptake inhibitors (SSRIs), tricyclic (TACs) class) inhibit catecholamine re-uptake, and these classes of antidepressants cause hyperprolactinemia, which suppresses the hypothalamic-pituitary axis. (Schlosser *et al*., 2007).

Antidepressants affect the count and motility of sperm as well as negatively affect the shape of sperm. It can also alter sperm quality through its effect on sperm transport, and antidepressants cause an increase in DNA fragmentation. (Paul *et al*., 2012).

SSRIs act by inhibiting the reuptake of serotonin, a substance that affects male sexual behavior, causing loss of libido and delayed ejaculation. (Althof *et al*., 2010).

As for as tricyclic antidepressants(TCAs), cause delayed or absent ejaculation and erectile dysfunction, while monoamine oxidase inhibitors (MAOIs) antidepressants cause the least erectile dysfunction because this factor is less linked to blood prolactin, and other types do not cause sexual disorders such as bupropion and mirtazapine, which belongs to a class of other antidepressant medications. (Taylor *et al*., 2013).

2-6-3 -A side effects of antidepressants on the reproductive system

Studies have reported that antidepressants negatively affect or lead to defects in organelle separation and spindle formation, other studies have shown that the effect of most antidepressants when treatment is used for long periods and causes various modifications to vital processes, including reproductive system processes, the effect of antidepressants on the cell can be summarized:

1. Oxidation and reduction and increase production.
2. Impaired mitochondrial function.
3. Disruption of enzymatic and non-enzymatic cell protection mechanisms.
4. Defects of spindle apparatus assembly during cell division.
5. Defects in organelle distribution during cell division.
6. Changes in calnexin protein (Solek *et al*., 2021).

2-7- Amitriptyline

Amitriptyline belongs to the class of tricyclic antidepressants and the basic structure of this class is a central structure consisting of three rings with the side chain side by side and it is action is by inhibiting the uptake of neurotransmitters (serotonin, norepinephrine) (Gillman, 2007). And its chemical formula is $C_{20}H_{23}N$ according to Food and Drug Administration (FDA) .As shown in the figure (2-3).

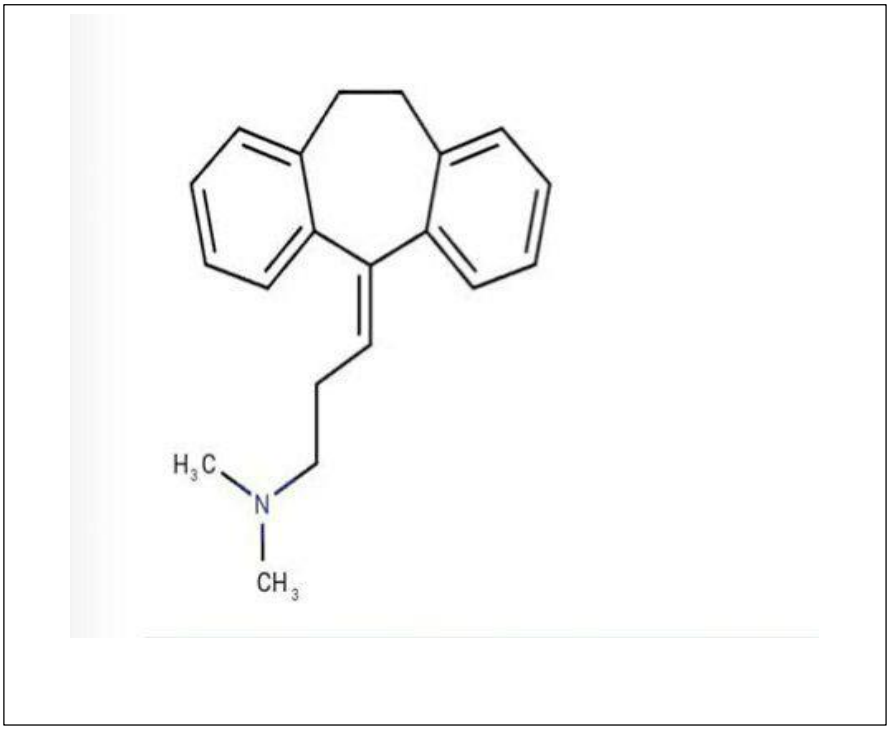

Figure (2-3): describes the chemical structure of amitriptyline (FDA)

2-7-1- Indications

In the treatment of adults with major depressive disorder (MDD) (Dopheide, 2006). amitriptyline, an FDA approved drug, is used as a treatment for anxiety, post-traumatic stress disorder, diabetic neuropathy, fibromyalgia, insomnia, irritable bowel syndrome, migraine prevention and interstitial cystitis (bladder pain syndrome) (Radley *et al*., 2006).

2-7-2- Mechanism of action

At the presynaptic terminals, Amitriptyline increases neurotransmission serotonin and norepinophrine by inhibiting the neurotransmitter (serotonin transport protein SERT, norepinephrine transport NET), if used as a chronic treatment, it leads to changes in monoamine neurotransmission and these changes are long-lasting (Hamon and Blier, 2013), Amitriptyline is a tertiary amine and has a strong affinity for histamine (H1) and muscarinic (M1) receptors (Gillman, 2007).

Compared to other TCAs, it has anticholinergic properties as in another antidepressant, within 2 to 4 weeks the therapeutic effect begins, as the levels of brain-derived neurotrophic factor (BDNF), it is increased with the use of Amitriptyline chronically and the symptoms associated with MDD improve, BDNF has a major role in participating in the formation of neurons and maintaining their survival. (Levy *et al*., 2018).

2-7-3-Administration

Amitriptyline comes in several forms. The most commonly used form is the oral form. Amitriptyline is taken at bedtime at a dose of 25 mg/day. It can lower the dose by about 10- 20 mg/day. The dose can be modified at bedtime. The patient continues to take amitriptyline for three months to treat depression.

Amitriptyline is not used for depression in children according to FDA, so it is taken in a small dose of about 10mg/day for children and the elderly (Thour, and Marwaha, 2022).

Within 2-12 hours of administration, a peak concentration occurs if amitriptyline is given by intramuscular and intravenous administration (Deisenhammer *et al*.,2000), Amitriptyline is taken at night because it leads to sedation (Leucht *et al*.,2012) and from 10-28 hours the half-life of Amitriptyline is mainly metabolized by (CYP3A4) and (CYP2C19) turning it into nortriptyline (Venkatakrishnan *et al*., 2001).

2-7-4- side-effects

Side effects of Amitriptyline include cardiac signs and symptoms (tachycardia, conduction abnormalities, hypotension and prolongation (QT), neurological signs (sedation and coma), and anticholinergic symptoms (dilated pupils, urinary retention and dry mouth). (Ramasubbu *et al*., 2016).

Among the other symptoms are tingling in the arms and legs, skin rash, unexpected weight increase or decrease, swelling of the face and tongue, confusion and constipation or diarrhea. (Khushboo and Sharma, 2017).

2-8- Escitalopram

Selective serotonin reuptake inhibitors, and is common to treat depression. (Sanchez *et al*., 2014). Escitalopram is oxalate, a fine salt of white to yellow crystalline color that is completely soluble in ethanol and normal saline, moderately soluble in water, and its chemical formula is $C_{20}H_{22}FN_{2O}$+ C (Martindale, 2009). As shown in the figure (2-4).

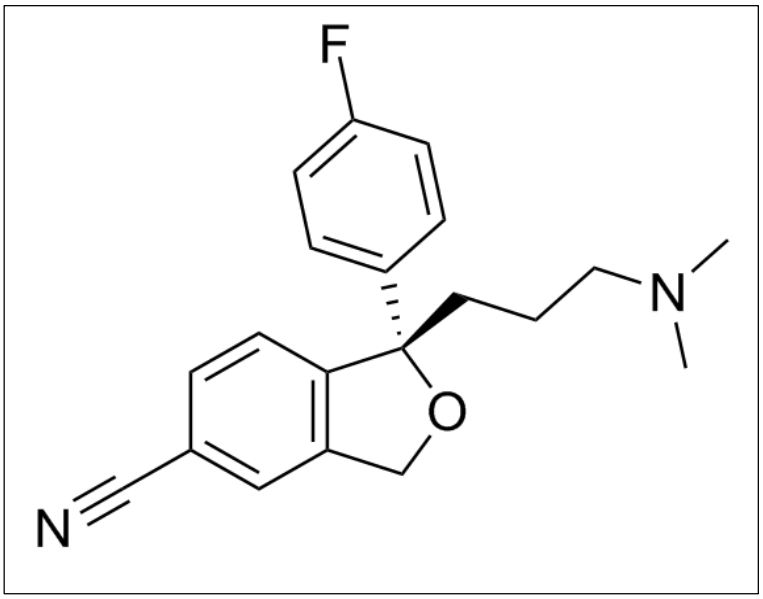

Figure (2-4): describes the chemical structure of Escitalopram (Martindale, 2009).

2-8-1- Indications

Escitalopram is used to treat the vasomotor symptoms of amenorrhea, panic disorder, premenstrual dysphoric disorder, obsessive-compulsive disorder, and post-traumatic stress disorder. SSRIs are commonly used in the treatment of anxiety, depression, and other related disorders (Baldwin *et al*., 2016). The most selective of SSRIs is Escitalopram, which is a serotonin reuptake inhibitor (Sanchez *et al*., 2014).

2-8-2--Mechanism of action

In presynaptic neurons there is the serotonin transporter, a sodium-dependent serotonin transporter protein (SERT), Escitalopram performs its action by binding to this protein, in the synaptic cleft, the amount of serotonin is increased by blocking the reuptake of serotonin and this is this when Escitalopram binds to SERT (Kasper *et al*., 2009).

From the synaptic cleft to the presynaptic neuron, the action of SERT is by re-uptake of serotonin. In the synaptic cleft, the amount of serotonin increases when

SSRIs bind to SERT, because this connection prevents the uptake of serotonin, and thus this activity strengthens the effect of serotonin in the central nervous system (Berger *et al*., 2009). The main serotonin receptors are (5-HT4, 5-HT7, 5-HT6, 5-HT1A, and 5-HT1B) (Yohn *et al*., 2017).

2-8-3--Administration

Escitalopram is available 5mg or 10mg or 20mg. orally, it is taken once a day before or after food, initially, Escitalopram 10mg is taken and the dose is increased depending on symptoms after a week (Rao, 2007). The highest recommended of Escitalopram is 20 mg/ day (Bruggeman, and O'Day, 2021).

If the treatment is switched from Escitalopram to another kind SSRIs, the dose should be reduced for 4 weeks, 27-32 the half-life of Escitalopram (Keks *et al*., 2016). Escitalopram is converted to dimethyl Escitalopram by cytochrome enzymes, that is by three enzymes (CYP3A4, CYP2C19, CYP2D6) according the following proportions, respectively (35%, 37%, 28%). (Cooke and Waring, 2013).

2-8-4- side effects

Escitalopram is characterized as compared antidepressant of the old tricks and is less toxic (Dodd *et al*., 2011), and common side effects are impotence (delayed ejaculation in males, decreased sexual desire) insomnia, nausea and fatigue, Escitalopram causes a decrease in sodium in the blood especially in the elderly. The decrease in sodium results in loss of appetite, headache and vomiting, and decrease may lead to coma (Kirpekar and Joshi, 2005).

Escitalopram causes QT prolongation by a mechanism that is not well understood but may be dose dependent (Cooke and Waring, 2013), Escitalopram also causes an increase in the amount of serotonin in the central and peripheral

nervous system called serotonin syndrome. This syndrome occurs in persons taking high doses of SSRIs or person taking more than one type of serotonin drugs (Scotton *et al*., 2019), among the other symptoms are Dry mouth, diarrhea, constipation, sexual dysfunction, tremor, dizziness, nausea, somnolence, anxiety, sweating, anorexia and agitation. (Khushboo and Sharma, 2017).

Chapter Three
Materials and Methods

3- Materials and Methods

3-1- Chemicals, Apparatus and Instruments used

3-1-1-chemicals

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

No.	Name chemicals	Country	Company
1	Amitriptyline 10 mg	Accord	UK
2	Canada Balsam	Germany	Roth
3	Charcoal Activated	England	BDH
4	Chloroform	Switzerland	Sigma
5	Distilled water	Iraq	Pioneer
6	Escitalopram 10 mg	Accord	UK
7	Ethanol (absolute100%)	England	BDH
8	Eosin	England	BDH
9	Formalin	England	BDH
10	Fuchsin Basic	Denmark	Dakocytomation
11	Glacial acetic acid	England	BDH
12	Glycerin	USA	RPI
13	Hematoxylin	England	BDH
14	Hydrochloric acid	Switzerland	Sigma
15	M Mice testosterone ELISA Kit	Germany	Abbot
16	Mice(FSH) ELISA Kit	Germany	Abbot
17	Mice(LH) ELISA Kit	Germany	Abbot
18	Normal Saline Solution	Germany	Fresenius Kabi
19	Paraffin Wax	Germany	Merck
20	Periodic acid	Denmark	Dakocytomation
21	Potassium alum	India	Hi media
22	Sodium phosphate, Monobasic, monohydrate	India	Hi media
23	Sodium phosphate, Dibasic, anhydrous	England	BDH
24	Xylene	England	BDH

3-1-2- Instruments

Table (3-2): the table shows the origin and names of the instruments used in this study

No	Name instruments	Country	Manufacture Company
1	Adapter	New York	StonyLab
2	Cotton	China	Citioglas
3	Cover slip	USA	Klempa
4	EDTA tube	China	Vacuette
5	Eppendrof Tube 1.5 ml	USA	Eppendrof
6	Filter paper	China	Whatman
7	Glove	China	Broche
8	Knife of Microtome	Korea	LG
9	Micro pipetes	Germany	DRAGON
10	Oral gavage	China	
11	Plastic cage	Iran	Kajeen
12	Plastic cups	China	Shanghai Blopak
13	Round flask	Germany	ISOLAB
14	Slide	China	Citioglas
15	Surgical kite	India	Hebson
16	Syringe	China	Citioglas

3-1-3- Apparatus

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

No	Name Apparatus	Country	Manufacture Company
1	Automated Hematological Analyzer	China	Mindray
2	Camera	Japan	Sony
3	Centerifuge	Germany	Eppendorf
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	Microtome	Germany	Leitz
13	Paraffin dispenser	China	Premiere
14	Refrigerator	Korea	LG
15	Sensitive Balance	Germany	Kerm
16	Water bath	Germany	Tafesa Hannover

3-2- Experimental Animals

The age range of male mice BABL/c used in the experiment 8-12 weeks with an average weight of 28gm, the mice were selected from the animal house of the Faculty of Science/ university of Misan /Department of Biology. These mice are free of pathogens. the mice were left for 2 weeks to adapt before the start of the experiment and dosed with antidepressants after placing them in plastic cages covered with metal mesh and furnished with sawdust, and the mattresses are changed 2 times a week while continuing to clean and sterilize the cages the study included 90 male mice, 5 in each cage with a 12/12 h light/ dark cycle with food and tap water, The animal food consists of 50% wheat, 30% fish, greens 13%, salt sodium chloride (NaCl) 2% and raw fat 5%. Animals are handled according to institutional guidelines and approved by the local animal ethics committee for all experimental procedures. (Al-Attar and Jihad, 2013).

3-3-Design of the Study

The number of animals in this experiment was 90 adult male mice. The animals were divided into three groups as follows:

Group I (control): This group consisted of 30 male mice that were administered normal saline for six weeks.

Group II: This group consisted of 30 male mice that were administered Amitriptyline for six weeks at a dose (0.42µl/day) twice daily.

Group III: This group consisted of 30 male mice that were administered Escitalopram for six weeks at a dose (0.42µl/day) twice daily (Bruggeman, and O'Day, 2021).

During the six weeks, the animals were weighed weekly and clinical signs and behavior were observed throughout the six -weeks. At the end of the second, fourth, and sixth week, male mice were euthanized ten for each group. Blood samples were taken, testis, epididymis and seminal vesicles were removed, and Sperm were withdrawn to calculate concentration, motility and vitality as shown in the diagram (3-1).

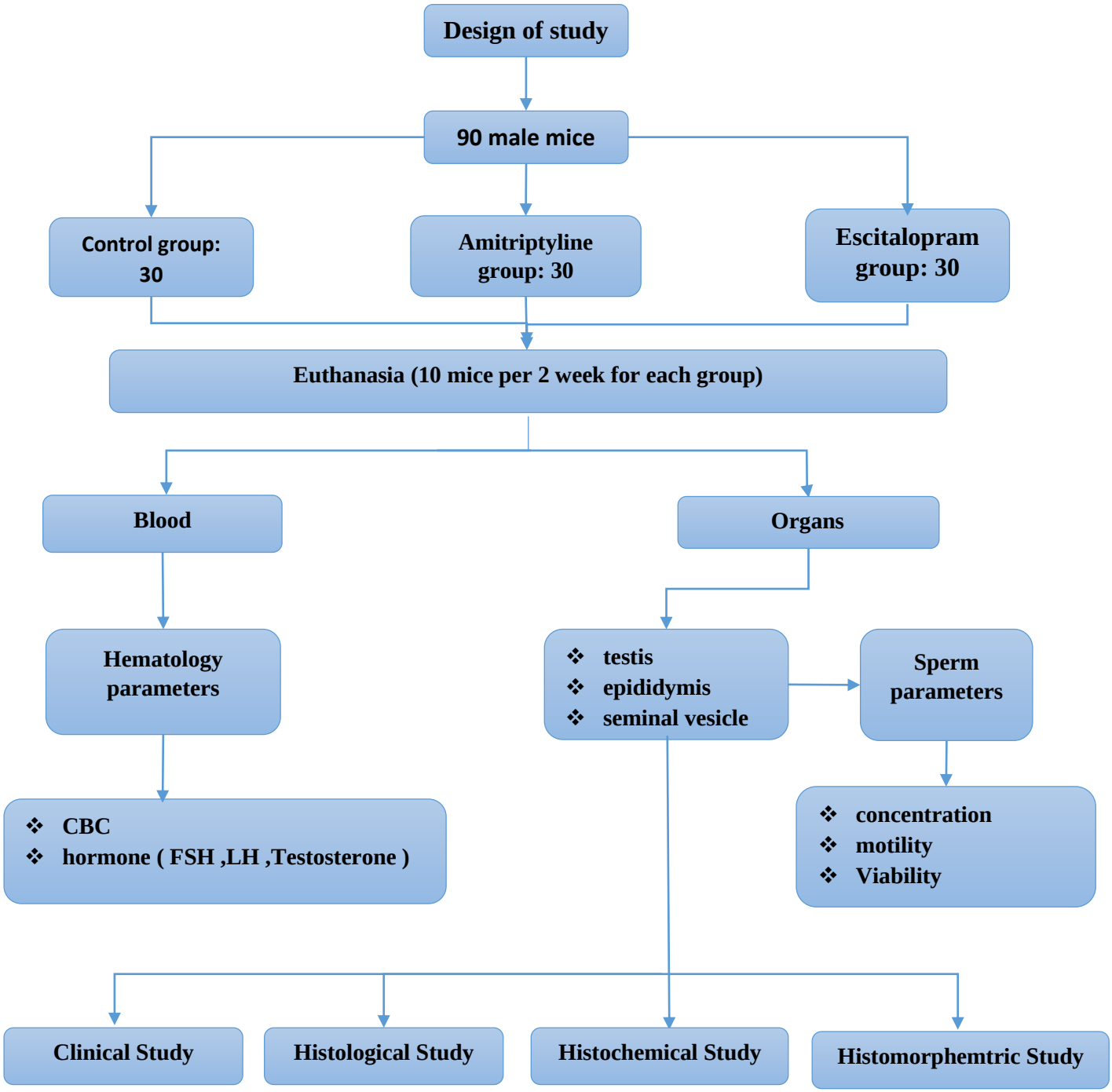

Diagram (3-1): Design of study

3-4- Collection of Blood Samples and organ specimens

Euthanasia of mice is done by placing the mice in a closed cage placing chloroform (CHC13) on cotton and placing it inside the cage (Blackshaw *et al*., 1988).

Blood is taken from the heart about (1ml) (Parasuraman *et al*., 2010). Blood samples are taken every two weeks, preferably from the ventricle and slowly so that the heart does not collapse, using a syringe (3ml), and then the blood is divided into two parts, the first part is placed in tubes containing an anticoagulant, the blood parameters measured using automated hematological analyzer, and this measurement includes, White blood cell count (WBC), neutrophils (GRAN), lymphocytes (LYM), monocytes (MONO).

Red blood cell count(RBC), blood platelet (PLT), platelet distribution width (PDW), Hemoglobin concentration (HGB), mean corpuscular volume(MCV), mean platelet volume(MPV), Hematocrit(HCT), mean corpuscular hemoglobin(MCH), platelet crit (PCT), mean corpuscular hemoglobin concentration(MCHC) and red blood cell distribution width(RDW). (Zainab, 2021).

The second part of blood, it is placed in tubes that help coagulation to obtain the serum so that the proportion of hormones is measured, and these hormones include, luteninizing hormone (LH) (appendix1), Follicle-stimulating hormone (FSH) (appendix2), and Testosterone (appendix 3). Then, the mice are placed on the dissection board and the front and rear limbs are fixed, the testis, epididymis and seminal vesicles are removed and then washed with normal saline solution. (Basim, 2019).

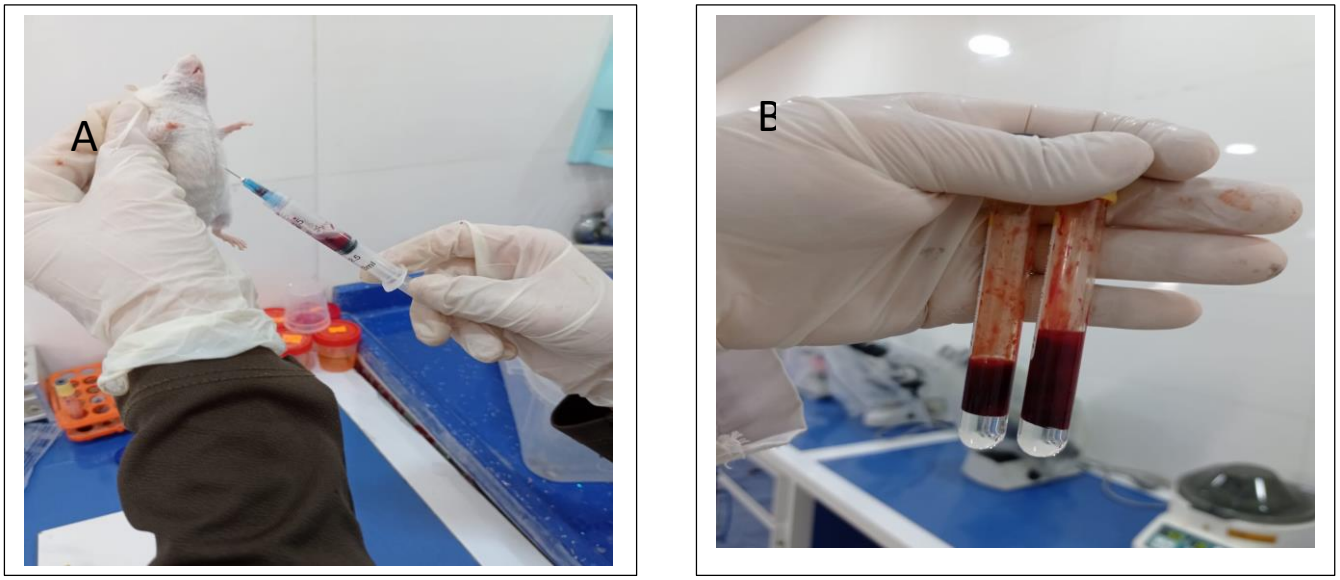

Figure (3-1): The study samples, **(A):** Euthanasia, **(B):** Blood, **(C):** Serum

3-5- sperm parameters

3-5-1- Sperm motility

The epididymis is taken and weighed and then cut into small pieces to extract the sperm after being placed in a petri dish containing 2 ml of saline solution 37c and left for 5-10 minutes. Semen is taken immediately and then a clean slide is taken and a drop of sperm suspension is placed on it and covered with a coverslip. The sperm is evaluated manually according to the instruction of seed et al (seed *et al*., 1996). And then the percentage of motility is calculated.

3-5-2- Sperm concentration

To calculate the sperm concentration (Luthfi, 2015), the contents of the epididymis are extracted in 2 ml normal saline were diluted to 10 ml NS and left for a while until the sperm disperse in the solution. Then drops are taken and placed in the chamber. To obtain the concentration of sperm in one chamber, the number of sperm in 5 squares is multiplied by 5, the number is multiplied by 100000.

The concentration of sperms = the number of sperm in 5 large squares *5*100000

3-5-3- Sperm viability

“Eosin- Nigrosin one-step staining technique"(appendix 4) was used to determine the viability (Bjorndahl *et al*., 2003). To assess viability, in this method is taken (40 µl) from the semen and mixed with drop of eosin and nigrosin, Prepared with normal saline. After a few minutes, a clean slide is taken and a drop of the eosin and semen mixture is placed on it and covered with a glass coverslip. The examination with a high-resolution 100 bright field objective at a

magnification of 1,000 under oil immersion and at least 200 sperms is examined. The sperms that appear in white (unstained) are alive, while the sperms colored in red or pink are dead (Bjorndahl, 2003; Kamijima *et al*., 2004).

3-6- histological section preparation

Tissue slides were prepared according to the instructions of Luna (1968) according to the following steps:

1. fixation:

By placing the study samples for 48 hours in a neutral formalin solution of 10 %. (appendix 5)

2. Washing

After the previous fixation stage, the samples are washed for 3 hours with running tap water to remove the fixative residues from the samples.

3. Dehydration

Using ethyl alcohol with different concentrations, the water is removed according to the following table:

Solution	**Time**
70% ethyl alcohol	2 h
80% ethyl alcohol	1h
96% ethyl alcohol	2 h
96% ethyl alcohol	2h
96% ethyl alcohol	2h
100% ethyl alcohol	7 h
100% ethyl alcohol	2 h

4. Clearing

Samples are placed in xylene in two-stage, each stage for 2 hours.

5. Infiltration and Embedding

A solution of xylene and paraffin wax is prepared and the samples are placed in this solution for 1 hour at 60 c degrees in an electric oven. Then samples are placed in paraffin wax for 4 hours and distributed in two-stage after that the samples are placed in wax molds.

6. Trimming and Sectioning

The wax molds containing the samples that were prepared previously were trimmed with a sharp scalpel and then, longitudinally or transversely, they are cut with a thickness of 5 microns and a slide is taken and a drop of egg white is placed on it. The tape obtained from cutting is transferred to a water bath, and then loaded onto the slide containing egg white, and this slide is transferred to a hot plate 37 c to fix the sample tape on the slide.

7. Staining

Installed according to the instruction (Luna, 1968).

1. Wax removal: the slide containing the samples is placed in the xylene solution for 30 minutes into the stage to remove the wax.
2. Hydration: the slide is placed in ethyl alcohol at a decreasing concentration (100%, 96%, and 70%) for 5 minutes for each stage.
3. Place the slide in distilled water for 3 minutes.
4. Transfer the slide to hematoxylin stain (appendix 6) for 12-15minutes, then wish it with tap water for 8 minutes.

5. The slide is placed in the eosin stain (appendix7) for 5 minutes, then wished with tap water for 3 minutes.
6. The slide was transferred to an ethyl alcohol solution with an increasing concentration for 5 seconds for each stage, then transferred to xylene for 15 minutes into the stage.
7. Canada balsam put on a cover slip, cover the slide and transfer to a hot plate 37 ºC.

3-7- Histochemical staining

According to the instructions (Bancroft *et al*., 1996) the samples were stained with periodic acid Schiff (PAS) (appendix8) as follows:

1. The slide containing the sample is placed in the xylene to remove wax in two-stage, each stage 5 minutes.
2. The slide containing the sample is placed in ethanol alcohol in decreasing concentrations (100%, 96% and 70%) for each stage for 3 minutes.
3. The slide containing the sample is placed for 5 minutes in distilled water.
4. The slide containing the sample is placed for 5 minutes in periodic acid solution.
5. The slide containing the sample is placed is washed for 5 minutes in tap water, then transferred to distilled water for 2 minutes.
6. The slide containing the sample is placed for30 minutes in Schiff's reagent then it is placed for 2 minutes in distilled water.
7. For 10 minutes' slide is washed with tap water.
8. The slide is placed in ethyl alcohol for 5 seconds with a concentration of 70%, 5 seconds with a concentration of 96%, and 5 seconds with a concentration of 100%.
9. The slide is transferred to the xylene for 6 minutes in two-stage, each stage is 3 minutes.

10. Put Canada balsam on the coverslip, cover the sample with it and put it on a hot plate at 37 c.

Then, at the University of Misan/College of science / Animal tissue laboratory, tissue samples were examined under a light microscope with magnification powers (40X), and the photo was taken using a camera.

3-8- Histomorphometric

Slides of the testis and epididymis were examined. To calculate the diameter of seminiferous tubules of the testis, diameter of the epididymis duct, and count of (spermatogonia, primary spermatocyte, secondary spermatocyte and spermatid), an optical microscope was used with (an ocular micrometer). (Galigher and Kozloff, 1964).

3-9- Statistical Analysis

The mean and standard deviation of the data were analyzed by SPSS software using one-way ANOVA (Analyses variation) followed by an LSD test for the statistical differences, statistical significance was set at ($p>0.05$). (Griffith, 2007).

Chapter Four

Result

4-1- Clinical study

4-1-1- Body weights

The results showed that there are no significant differences(P>0.05)) in the average weights between the groups during the period of the second and fourth week compared to the control group, where the average weight of the control group in the second week was (32.88±3.55), Amitriptyline (29.50±5.54) and Escitalopram (35.40-±4.33). While in the fourth week, the average weight of the control group was (34.22±3.36), Amitriptyline (29.00±4.71), and Escitalopram (35.84±4.84)

The sixth-week period showed significant differences (P<0.05) in the average weights, where the average weight of the control group was (35.66±2.84), while the weights of the Amitriptyline group decreased and the average weight was (28.26±4.57), as for the weights of the Escitalopram group increased, and the average weight (39.66±2.59) according to the table (4-1).

Table (4-1): The changes in the body weight of male mice over the six weeks:

Group	body weights (gram)		
	2^{nd} week	**4^{th} week**	**6^{th} week**
Control	32.88 [a] ± 3.55	34.22 [a] ± 3.36	35.66 [a] ±2.84
Amitriptyline	29.50 [a] ± 5.54	29.00 [a] ± 4.71	28.26 [c] ±4.57
Escitalopram	35.40 [a] ± 4.33	35.84 [a] ± 4.84	39.66[b] ± 2.59

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

4-1-2- Testis weights

The result of the study showed that there were no significant differences($p>0.05$) in the testis weight in each of the Amitriptyline and Escitalopram groups during the second week period compared to the control group, where the average weight of the control group was (0.14±0.04), Amitriptyline (0.12±0.01**)** and Escitalopram (0.16±0.04**).**

While in the fourth week period, the testis weight showed a significant decrease($p<0.05$) in the Amitriptyline and Escitalopram group, and the decrease in the Amitriptyline group was less than the decrease in the Escitalopram group compared to the control group, where the average weight of the control group was (0.14±0.02), Amitriptyline (0.062±0.03) and Escitalopram (0.060±0.04).

In the sixth week period also, no significant differences($p>0.05$) in the testis weight in each of the Amitriptyline and Escitalopram groups compared to the control group, where the average weight of the control group was (0.14±0.05), Amitriptyline (0.14±0.02) and Escitalopram (0.15±0.03) according to the table (4-2).

4-1-3- Epididymis weights

The result of the study showed that there were no significant differences($p>0.05$) in the epididymis weight in each of the Amitriptyline and Escitalopram groups during the second week period compared to the control group, where the average weight of the control group was (0.15±0.008), Amitriptyline (0.14±0.02) and Escitalopram (0.28±0.34).

While in the fourth week period, the epididymis weight showed a significant decrease($p<0.05$) in the Amitriptyline and Escitalopram groups, and the decrease in the Amitriptyline group was less than the decrease in the Escitalopram group

compared to the control group, where the average weight of the control group was (0.15±0.02), Amitriptyline (0.10±0.008) and Escitalopram (0.07±0.01).

In the sixth week period also, no significant differences ($p>0.05$) in the epididymis weight in the Amitriptyline group while the Escitalopram group showed that there was a significant decrease ($p<0.05$) compared to the control group, where the average weight of the control group was (0.14±0.03), Amitriptyline (0.14±0.01) while the Escitalopram group (0.07±0.02) according to the table (4-2).

4-1-4- Seminal vesicle weights

The result of the study showed that there were significant differences ($p<0.05$) in the seminal vesicle weight in the Amitriptyline group, while the Escitalopram group showed that there was no significant decrease ($p>0.05$) during the second week period compared to the control group, where the average weight of the control group was (0.18±0.01), Amitriptyline (0.07±0.05) and Escitalopram (0.19±0.04).

While in the fourth week period, the seminal vesicles weight showed a significant decrease ($p<0.05$) in the Amitriptyline and Escitalopram group, and the decrease in the Amitriptyline group was less than the decrease in the Escitalopram group compared to the control group, where the average weight of the control group was (0.18±0.02), Amitriptyline (0.08±0.02) and Escitalopram (0.07±0.01).

In the sixth week period also, no significant differences ($p>0.05$) in the seminal vesicle weight in each of the Amitriptyline and Escitalopram groups compared to the control group, where the average weight of the control group

was (0.19±0.02), Amitriptyline (0.18±0.05) and Escitalopram (0.11±0.11) according to the table (4-2).

Table (4-2): The changes in the testis, epididymis seminal vesicle weight of male mice over the six weeks:

Group	Week	Testis weight (gram	Epididymis Weight (gram)	Seminal weight (gram)
Control	**2^{nd} week**	0.14 [a] ± 0.04	0.15 [a] ± 0.008	0.18 [a] ± 0.01
	4^{th} week	0.14 [a] ± 0.02	0.15 [a] ± 0.02	0.18 [a] ± 0.02
	6^{th} week	0.14 [a] ± 0.05	0.14 [a] ± 0.03	0.19 [a] ± 0.02
Amitriptyline	**2^{nd} week**	0.12 [a] ± 0.01	0.14 [a] ± 0.02	0.07 [b] ± 0.05
	4^{th} week	0.062 [b] ± 0.03	0.10 [b] ± 0.008	0.08 [b] ± 0.02
	6^{th} week	0.14 [a] ± 0.02	0.14 [a] ± 0.01	0.18 [a] ± 0.05
Escitalopram	**2^{nd} week**	0.16 [a] ± 0.04	0.28 [a] ± 0.34	0.19 [a] ± 0.04
	4^{th} week	0.060 [c] ± 0.04	0.07 [c] ± 0.01	0.07 [c] ± 0.01
	6^{th} week	0.15 [a] ± 0.03	0.07 [b] ± 0.02	0.11 [a] ± 0.11

*The values represent mean ± SD.*Different small letters represent a significant difference in ($p< 0.05$) between groups. *Similar small letters represent no significant difference.

4-2- Hematological study

The result of the study in the second week showed that there was a significant increase ($p<0.05$) in the (WBC, LYM , MONO) in each of the Amitriptyline and Escitalopram groups, and the increase in the Escitalopram group was less than the increase in the Amitriptyline group compared to the control group, while (GRAN) no significant difference , but (RBC, HGB, HCT, RDW, PLT, MPV, PDW) showed that there was no significant difference ($p>0.05$), while (MCV) only a significant decrease in Escitalopram was observed, (MCH) a significant decrease in Amitriptyline was observed less than that of Escitalopram,(MCHC) Only a significant decrease in Amitriptyline was observed, and (PCT) a significant increase in Escitalopram was observed.

While in the fourth week period, the (WBC, MONO)showed a significant increase($p<0.05$) in the Amitriptyline and Escitalopram groups, and the increase in the Escitalopram group was less than the increase in the Amitriptyline group compared to the control group , while (LYM, RBC, PCT, PDW) showed a significant increase($p<0.05$) in the Amitriptyline and Escitalopram group and the increase in the Amitriptyline group was less than the increase in the Escitalopram group compared to the control group, while (GRAN) showed a significant increase($p<0.05$) in the Amitriptyline group only, (HGB, HCT, MPV) only a significant increase in Escitalopram was observed, but (MCV, RDW) showed that there was no significant difference ($p>0.05$),(MCHC, MCH)) a significant decrease in Escitalopram was observed less than that of Amitriptyline, and (PLT) a a significant decrease in Escitalopram was observed less than that of Amitriptyline.

In the sixth week period, a significant increase ($p>0.05$) showed in the (WBC, MCV, RDW, MPV, PCT, LYM, GRAN) in the Escitalopram group compared to the control group , but (MONO) a significant increase ($p>0.05$) showed in

Amitriptyline less than Escitalopram group, while (RBC) only a significant increase in Amitriptyline was observed, but (HGB, HCT, MCH, MCHC) showed that there was no significant difference ($p>0.05$), (PLT) a decrease in Escitalopram was observed less than that of Amitriptyline, and (PDW) a significant increase in the Amitriptyline group was less than the increase in the Escitalopram was observed. Table (4-3).

Table (4-3): The changes in the hematological parameter of male mice over the six weeks:

Hematological Parameters	2nd week			4th week			6th week		
	Control	Amtript-yline	Escitalo-pram	control	Amtript-yline	Escitalo-pram	control	Amtrip-tyline	Escital-opram
WBC ($x10^9$/L)	5.0 a ±0.72	10.2 c ± 2.85	9.3 b ± 2.19	4.8 a ±0.69	10.0 c ± 0.70	9.6 b ±2.56	4.8 a ±0.72	7.2 a± 3.08	11.2 b ±0.60
LYM ($x10^9$/L)	3.0 a ±0.77	6.2 c ± 1.72	6.0 b ± 1.95	2.6 a ±0.30	4.8 b ± 0.92	7.0 c ±2.00	2.8 a ±0.61	4.8 a ± 3.13	8.0 b ± 0.94
MONO ($x10^9$/L)	0.7 a ±0.12	2.7 c ± 1.11	1.8 b ±0.36	0.7 a ±0.10	1.66 c ± 0.67	1.64 b ±0.45	0.6 a ±0.14	0.9 b ± 0.29	1.1 c ± 0.15
GRAN ($x10^9$/L)	1.2 a ±0.31	1.1 a ± 0.04	1.3 a ±0.41	1.2 a ±0.30	3.5 b ± 0.87	0.9 a ± 0.15	1.2 a ±0.29	1.3 a ± 0.21	2.0 b ± 0.48
RBC ($x10^{12}$/L)	6.2 a ± 0.54	7.1 a ± 0.87	6.8 a ± 0.94	6.2 a ± 0.57	8.4 b ± 0.28	9.0 c ± 0.52	6.1 a ± 0.50	7.4 b ± 0.38	6.5 a ± 1.38
HGB (g/dL)	11.7 a ± 1.45	12.0 a ± 0.86	11.8 a ± 0.25	11.5 a ± 1.24	11.5 a ± 0.49	12.7 b ± 0.57	11.8 a ± 1.37	12.2 a ± 0.62	11.7 a ± 1.41
HCT (%)	33.5a ±5.68	35.0 a ± 3.13	27.8 a ±6.03	32.3a ±3.84	35.1 a ±2.52	38.0 b ±1.36	32.7 a ±4.00	32.8 a ± 2.66	36.2 a ± 3.67
MCV (fL)	45.1a ±1.87	43.8 a ±0.39	40.9 b ±2.68	43.2 a ±2.52	43.1a ±1.74	43.2 a ±0.45	42.5 a ±2.22	46.6 a ±2.40	47.5 b ±4.01
MCH (pg)	16.2 a ±0.90	15.0 b ±0.23	14.6 c ±0.54	16.0 a ±0.74	12.9 c ±0.68	14.1 b ±0.28	15.9 a ±0.30	15.6 a ±0.43	16.0 a ±0.79
MCHC (g/dL)	36.6 a ±0.35	35.3 b ±0.34	35.6 a ±1.32	36.4 a ±0.46	30.4 c ±0.45	33.1 b ±0.25	34.9 a ±2.70	35.1 a ±2.77	32.6 a ±1.05
RDW %	24.1 a ±4.96	20.9 a ±0.55	24.9 a ±0.36	24.0 a ±4.39	25.2 a ±2.69	26.1 a ±2.00	23.5 a ±4.00	26.7 a ±2.03	30.7 b ±5.37
PLT ($x10^9$/L)	518 a ± 128	425 a ± 57	632 a ± 63	469 a ± 125	199 c ± 33	281 b ± 58	422 a ± 140	146 c ± 37	255 b ± 100
MPV (fL)	5.8 a ±0.74	6.3 a ±0.70	5.6 a ±0.75	5.4 a ±0.60	6.2 a ±0.44	7.9 b ±0.71	5.7a ±0.68	6.4 a ±0.35	7.9 b ±1.45
PCT (mL/ L)	0.25 a ± 0.06	0.24 a ± 0.04	0.34 b ± 0.06	0.25 a ± 0.02	1.23 b ± 0.11	2.2 c ± 0.53	0.24 a ± 0.03	0.69 a ± 0.26	2.0 b ± 1.26
PDW %	10.0 a ± 1.02	10.0 a ± 0.96	9.2 a ± 2.87	9.9 a ± 1.03	15.1 b ± 0.27	15.6 c ± 0.60	10.1 a ± 1.18	15.8 b ± 0.51	16.1 c ± 0.64

*The values represent mean ± SD. *Different small letters represent a significant difference in ($p< 0.05$) between groups. *Similar small letters represent no significant difference.

4-3- Hormonal study

4-3-1- LH serum levels

The result of the study showed that there was a significant decrease ($p<0.05$) in the LH serum level in each of the Amitriptyline and Escitalopram groups during the second and fourth week period, and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group , where the LH serum level of the control group in the second week was (0.01±0.00), Amitriptyline (0.002±0.00)and Escitalopram (0.004±0.00), while in the fourth week, the LH serum level of the control group was (0.01±0.00), Amitriptyline (0.002±0.00) and Escitalopram(0.003 ±0.00**).**

In the sixth week period, showed that there was a significant decrease ($p<0.05$) in the LH serum level in the Amitriptyline group compared to the control group, where the LH serum level of the control group was (0.01±0.00), Amitriptyline (0.001±0.00) while the Escitalopram group no significant differences ($p>0.05$) in the LH serum level compared to the control group, where the LH serum level of the Escitalopram group was (0.04±0.06). According to the table (4-4).

4-3-2- FSH serum level

The result of the study showed that there was a significant decrease ($p<0.05$) in the FSH serum level in the Amitriptyline group over six weeks, while the Escitalopram group did not have significant differences ($p>0.05$) over six weeks compared to the control group , where the FSH serum level of the control group in the second week was (0.01±0.00), Amitriptyline (0.001±0.00)and Escitalopram (0.01±0.00), while in the fourth week, the FSH serum level of the control group was (0.01±0.00), Amitriptyline (0.001±0.00) and Escitalopram (0.01±0.00),and in the sixth week, the FSH serum level of the control group was

(0.01±0.00), Amitriptyline (0.001±0.00**)** and Escitalopram(0.03±0.06**).** According to table (4-4).

4-3-3- Testosterone serum level

The result of the study showed that there was a significant decrease ($p<0.05$) in the Testosterone serum level in each of the Amitriptyline and Escitalopram groups during the second, fourth and sixth week period, and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group , where the Testosterone serum level of the control group in the second week was(9.48±0.85), Amitriptyline (0.41±0.06)and Escitalopram (2.21±**0.05**), while in the fourth week, the Testosterone serum level of the control group was (9.48±0.93), Amitriptyline (0.12±0.07) and Escitalopram (2.56±2.23),and in the sixth week ,the Testosterone serum level the of the control group was (9.48±), Amitriptyline (0.74±0.15**)** and Escitalopram (3.54±1.48**)**. According to the table (4-4).

Table (4-4): The changes in the LH, FSH and Testosterone serum levels of male mice over the six weeks:

Group	Week	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (ng/ml)
Control	**2^{nd} week**	0.01 [a] ± 0.00	0.01 [a] ± 0.00	9.48 [a] ±0.85
	4^{th} week	0.01 [a] ± 0.00	0.01 [a] ± 0.00	9.48 [a] ±0.93
	6^{th} week	0.01 [a] ± 0.00	0.01 [a] ± 0.00	9.48 [a] ±0.83
Amitriptyline	**2^{nd} week**	0.002 [c] ± 0.00	0.001 [b] ± 0.00	0.41[c] ± 0.06
	4^{th} week	0.002 [c] ± 0.00	0.001 [b] ± 0.00	0.12 [c] ±0.07
	6^{th} week	0.001 [b] ± 0.00	0.001 [b] ± 0.00	0.74 [c] ±0.15
Escitalopram	**2^{nd} week**	0.004 [b] ± 0.0	0.01[a] ± 0.00	2.21[b] ± 0.05
	4^{th} week	0.003 [b] ± 0.0	0.01[a] ± 0.00	2.56 [b] ± 2.23
	6^{th} week	0.04 [a] ± 0.0	0.03[a] ± 0.00	3.54 [b] ±1.48

*The values represent mean ± SD. *Different small letters represent significant differences in ($p< 0.05$) between groups. *Similar small letters represent no significant difference

4-4- Sperm parameter study

4-4-1- Sperm concentration

The result of the study showed that there was a significant decrease ($p<0.05$) in the concentration of sperms in each of the Amitriptyline and Escitalopram groups during the second, fourth and six weeks period, and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group, where the concentration of sperms in the second week of the control group was (290.0±56.01), Amitriptyline (165.00±18.37) and Escitalopram (197.00±35.10). While the concentration of sperms of the control group in the fourth week was (282.00±22.80), Amitriptyline (158.00±25.88) and Escitalopram (161.80±44, 40), and in the sixth week, the concentration of sperms of the control group was (288.40±57.53**),** Amitriptyline (157.00±80.28), Escitalopram (158.00±39.46). according to the table (4-5).

4-4-2- Sperm motility

The result of the study showed that there was a significant decrease ($p<0.05$) in the motility of sperms in each of the Amitriptyline and Escitalopram groups over six weeks, and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group , where the motility of sperms of the control group in the second week was (81.80±4.49), Amitriptyline (45.80±4.49)and Escitalopram (63.00±10.70), while in the fourth week, the motility of sperms of the control group was (80.00±7.31), Amitriptyline (41.00±7.03) and Escitalopram (61.40±13.84),and in the sixth week, the motility of sperms of the control group was(82.20±5.40), Amitriptyline (29.60±8.44**)** and Escitalopram **(**54.00 ±6.55). According to table (4-5).

4-4-3- Sperm dead

The result of the study showed that there was a significant increase ($p<0.05$) in the count of dead sperms in each of the Amitriptyline and Escitalopram groups during the second week period, and the increase in the Amitriptyline group was less than the increase in the Escitalopram group compared to the control group, where the count of dead sperms of the control group was (18.20±4.49), Amitriptyline (34.20±11.45) and Escitalopram (35.60±4.03).

While in the fourth week period, the count of dead sperms showed a significant increase ($p<0.05$) in the Amitriptyline and Escitalopram groups, and the increase in the Escitalopram group was less than the increase in the Amitriptyline group compared to the control group, where the count of dead sperms of the control group was (19.80±4.76), Amitriptyline (38.60±8.73) and Escitalopram (33.80±7.15).

In the sixth week period, no significant differences ($p>0.05$) in the count of dead sperms in the Amitriptyline group compared to the control group, where the count of dead sperms of the control group was (18.80±2.16), Amitriptyline (25.80±8.04), while the Escitalopram group showed that there was significant increase ($p<0.05$) in the count of dead sperms compared to the control group, where a count of dead sperms of the Escitalopram group was (33.80±13.16). According to the table (4-5).

Table (4-5): The changes in the concentration, Motility and Dead of sperms of male mice over the six weeks:

Group	Week	Concentration of sperms10^5	Motility of sperms %	Dead of sperms%
Control	**2^{nd} week**	290.0 [a] ± 56.01	81.80 [a] ± 4.49	18.20 [a] ± 4.4
	4^{th} week	282.00 [a] ±22.80	80.00 [a] ± 7.31	19.80 [a] ±4.76
	6^{th} week	288.40 [a] ±57.53	82.20 [a] ± 5.40	18.80 [a] ±2.16
Amitriptyline	**2^{nd} week**	165.00[c] ±18.37	45.80 [c] ± 4.49	34.20[b] ±11.5
	4^{th} week	158.00 [c] ±25.88	41.00 [c] ± 7.03	38.60 [c] ±8.73
	6^{th} week	157.00 [c] ±80.28	29.60 [c] ± 8.44	25.80 [a] ±8.04
Escitalopram	**2^{nd} week**	197.00[b] ±35.10	63.00[b] ±10.70	35.6[c] ±4.03
	4^{th} week	161.80 [b] ±44.40	61.40 [b] ±13.84	33.80 [b] ± 7.15
	6^{th} week	158.00 [b] ±39.46	54.00 [b] ± 6.55	33.80[b] ±13.6

*The values represent mean ± SD. *Different small letters represent a significant difference in ($p < 0.05$) between groups. *Similar small letters represent no significant difference

4-5- Histomorphometric study

4-5-1- Diameter of seminiferous tubules

The results of the study showed that there are no significant differences (p>.0.05) in the diameter of seminiferous tubules in both groups Amitriptyline and Escitalopram compared to the control group over six weeks. In the second week, the diameter of the control group was (1.73±0.11), Amitriptyline (1.75±0.14), Escitalopram(1.77±0.21) , while in the fourth week ,the diameter of the control group was(1.73±0.11), Amitriptyline(1.75±0.26), Escitalopram(1.73±0.08) , as for in the sixth week, the diameter of the control group was (1.73±0.11), Amitriptyline(1.74±0.20), Escitalopram(1.68±0.24).according to the table (4-6).

4-5-2- Diameter of Epididymis duct

The results of the study showed that there are no significant differences (p>.0.05) in the diameter of the epididymis duct in both groups Amitriptyline and Escitalopram compared to the control group over six weeks. In the second week, the diameter of the control group was (1.33±0.29), Amitriptyline (1.29±0.09), Escitalopram (1.25±0.08), while in the fourth week, the diameter of the control group was (1.33±0.29), Amitriptyline (1.28±0.05), Escitalopram (1.25±0.15), as for in the sixth week, the diameter of the control group was (1.33±0.29), Amitriptyline (1.25±0.15), Escitalopram (1.26±0.09). according to the table (4-6).

Table (4-6): The changes in the diameter of seminiferous tubules and epididymis duct of male mice over the six weeks:

Diameter of seminiferous tubule (µm)				Diameter of Epididymis duct(µm)		
Group	**2^{nd} week**	**4^{th} week**	**6^{th} week**	**2^{nd} week**	**4^{th} week**	**6^{th} week**
Control	1.73 [a] ± 0.11	1.73 [a] ± 0.11	1.73 [a] ± 0.11	1.33 [a] ± 0.29	1.33 [a] ± 0.29	1.33 [a] ± 0.29
Amitriptyline	1.75 [a] ± 0.14	1.75 [a] ± 0.26	1.74 [a] ± 0.20	1.29 [a] ± 0.09	1.28 [a] ± 0.05	1.25 [a] ± 0.15
Escitalopram	1.77 [a] ± 0.21	1.73 [a] ± 0.08	1.68 [a] ± 0.24	1.25 [a] ± 0.08	1.25 [a] ± 0.15	1.26 [a] ± 0.09

*The values represent mean ± SD. *Similar small letters represent no significant difference in ($p< 0.05$) between groups

4-5-3- Count of spermatogonia

The result of the study showed that there were no significant differences($p>0.05$) in the count of spermatogonia cells in each of the Amitriptyline and Escitalopram groups during the second week period compared to the control group, where the count of the control group was (61.2±6.53), Amitriptyline (64.6±3.78) and Escitalopram (54.8±5.93).

While in the fourth week period, the count of spermatogonia cells showed a significant decrease ($p<0.05$) in the Amitriptyline and Escitalopram group, and the decrease in the Amitriptyline group was less than the decrease in the Escitalopram group compared to the control group, where the count of the control group was (59.2±5.93), Amitriptyline (26.8±2.28) and Escitalopram (24.2±1.64).

In the sixth week period also, no significant differences($p>0.05$) were shown in the count of spermatogonia cells in each of the Amitriptyline and Escitalopram groups compared to the control group, where the count of the control group was (58.4±5.36), Amitriptyline (61.0±2.91) and Escitalopram (57.8±3.11) according to the table (4-7).

4-5-4- Count of primary spermatocytes

The result of the study showed that there was a significant decrease ($p<0.05$) in the count of primary spermatocyte cells in each of the Amitriptyline and Escitalopram groups during the second week period, and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group, where the count of the control group was (57.40±8.84), Amitriptyline (37.2±4.81) and Escitalopram (40.6±3.84).

While in the fourth week period, the number of primary spermatocyte cells showed a significant decrease ($p<0.05$) in the Amitriptyline and Escitalopram groups, and the decrease in the Amitriptyline group was less than the decrease in the Escitalopram group compared to the control group, where the count of the control group was (55.6±10.04), Amitriptyline (28.2±3.11) and Escitalopram (22.0±4.30).

In the sixth week period also, no significant differences($p>0.05$) were shown in the count of primary spermatocyte cells in each of the Amitriptyline and Escitalopram groups compared to the control group, where the count of the control group was (57.2±9.68), Amitriptyline (48.4±10.28) and Escitalopram (49.2±10.54). According to the table (4-7).

4-5-5- Count of secondary spermatocytes

The result of the study showed that there was a significant decrease ($p<0.05$) in the count of secondary spermatocyte cells in each of the Amitriptyline and Escitalopram group during the second and fourth week period and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group ,in the second week, the count of the control group was (59.0±7.87), Amitriptyline (24.6±5.03)and Escitalopram (31.4±2.88), while in the fourth week, the count of the control group was (59.4±5.89), Amitriptyline (29.2±7.36)and Escitalopram (30.4±5.68)

In the sixth week period, no significant differences ($p>0.05$) were shown in the count of secondary spermatocyte cells for the Amitriptyline group, while the Escitalopram group showed that there was a significant decrease ($p<0.05$) compared to the control group, where the count of the control group was (56.6±7.73), Amitriptyline (51.0±1.58) and Escitalopram (49.8±1.92). According to the table (4-7).

4-5-6- Count of spermatids

The result of the study showed that there was a significant decrease ($p<0.05$) in the count of spermatid cells in each of the Amitriptyline and Escitalopram groups during the second and fourth week period, and the decrease in the Escitalopram group was less than the decrease in the Amitriptyline group compared to the control group, in the second week, the count of the control group was (55.4±5.55), Amitriptyline (26.0±3.74)and Escitalopram (29.4±6.02), while in the fourth week, the count of the control group was (56.2±6.61), Amitriptyline (23.0±2.00)and Escitalopram (24.4±3.05)

In the sixth week period, no significant differences ($p>0.05$) were shown in the count of spermatid cells for Amitriptyline and Escitalopram group compared to

the control group, where the count of the control group was (56.2±6.05), Amitriptyline (57.0±9.05) and Escitalopram (54.6±4.33). According to the table (4-7).

Table (4-7): The changes in the count of (spermatogonia, primary spermatocyte, secondary spermatocyte and spermatid) in male mice over the six weeks:

Count of					
Group	**Week**	**Spermatogonia**	**Primary Spermatocyte**	**Secondary Spermatocyte**	**Spermatid**
Control	**2^{nd} week**	61.2 ^{a} ± 6.53	57.40 ^{a} ± 8.84	59.0 ^{a} ± 7.87	55.4 ^{a} ± 5.55
	4^{th} week	59.2 $^{\mathbf{a}}$ ± 5.93	55.6 ^{a} ± 10.04	59.4 ^{a} ± 5.89	56.2 ^{a} ± 6.61
	6^{th} week	58.4 ^{a} ± 5.36	57.2 ^{a} ± 9.68	56.6 ^{a} ± 7.73	56.2 ^{a} ± 6.05
Amitriptyline	**2^{nd} week**	64.6 ^{a} ± 3.78	37.2 ^{c} ± 4.81	29.2 ^{c} ± 7.36	26.0 ^{c} ± 3.74
	4^{th} week	26.8 ^{b} ± 2.28	28.2 $^{\mathbf{b}}$ ± 3.11	24.6 ^{c} ± 5.03	23.0 $^{\mathbf{c}}$ ± 2.00
	6^{th} week	61.0 ^{a} ± 2.91	48.4 $^{\mathbf{a}}$ ± 10.28	51.0 $^{\mathbf{a}}$ ± 1.58	57.0 $^{\mathbf{a}}$ ± 9.05
Escitalopram	**2^{nd} week**	54.8 ^{a} ± 5.93	40.6 ^{b} ± 3.84	31.4 ^{b} ± 2.88	29.4 ^{b} ± 6.02
	4^{th} week	24.2 ^{c} ± 1.64	22.0 ^{c} ± 4.30	30.4 ^{b} ± 5.68	24.4 ^{b} ± 3.05
	6^{th} week	57.8 $^{\mathbf{a}}$ ± 3.11	49.2 $^{\mathbf{a}}$ ± 10.54	49.8 $^{\mathbf{b}}$ ± 1.92	54.6 ^{a} ± 4.33

*The values represent mean ± SD. *Different small letters represent a significant difference in ($p< 0.05$) between groups. *Similar small letters represent no significant difference

4-6- Histological study

4-6-1-The testis

Results of the study in the second week showed that the histological structure of the testis consists of seminiferous tubules, it has shown regularity of tissue sections of the control group and the presence of all stages of spermatogenesis (spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa) as well as the presence Sertoli cells and Leydig cells. Figure (4-1).

While, the testis sections for the Amitriptyline group showed spaces between spermatogonia cells, clear spaces between the spermatogonia layer and primary spermatocyte layer. Figure (4-2), and the Escitalopram group showed spaces between the spermatogonia layer and the primary spermatocyte layer, but they are not clear and spaces between spermatogonia cells in most of the seminiferous tubules, which leads to a decrease in spermatogenesis. Figure (4-3).

As for the fourth week, the testis sections for the control group were similar to the control group in the second week. Figure (4-4), while the Amitriptyline group showed proliferation of Sertoli cells, decrease of primary spermatocyte and separation of spermatogonia layer from primary spermatocyte layer. Figure (4-5), and the Escitalopram group showed irregular layers of cells (spermatogonia, primary spermatocyte and secondary spermatocyte), an absence of a spermatid layer and a lumen wider. Figure (4-6).

While in the sixth week the control group no change compared to the second and fourth weeks. Figure (4-7), for the Amitriptyline group showed a decrease in spermatid and lumen wider and absence of leydig cells. Figure (4-8), and the Escitalopram group showed a change in the size of the cells, irregular cell layers (spermatogonia, primary spermatocyte and secondary spermatocyte), absence lumen and absence of leydig cells. Figure (4-9).

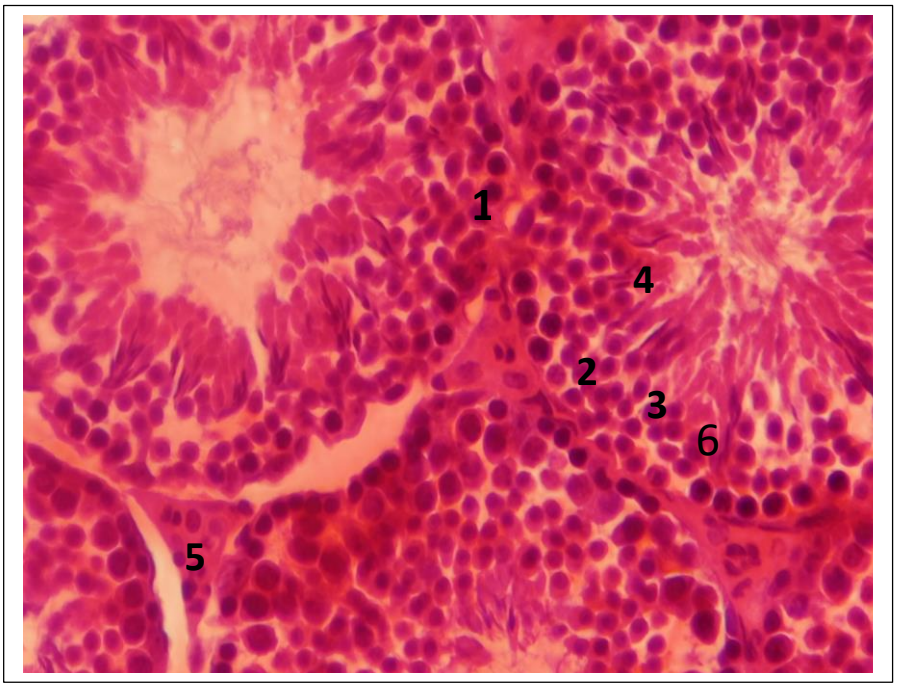

Figure (4-1): Testis of control male mice two week showing the normal structure, 1 spermatogonia,, 2 primary spermatocyte, 3 secondary spermatocyte, 4 spermatid , 5 Leydig cells, 6 sertoli cells ,(H&E, 400x).

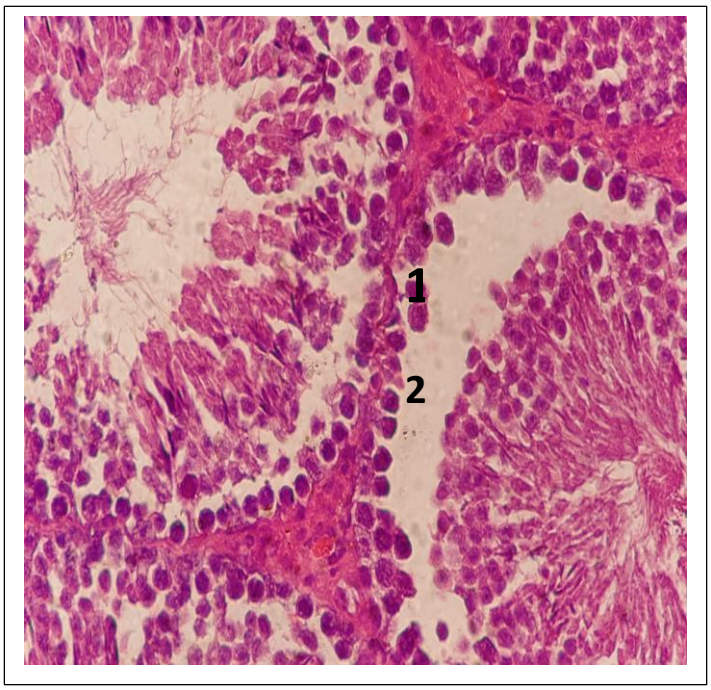

Figure (4-2): Testis of Amitriptyline male mice two week showing, 1 spaces between spermatogonia cells ,2 spaces between spermatogonia and primary spermatocyte, (H&E,400x).

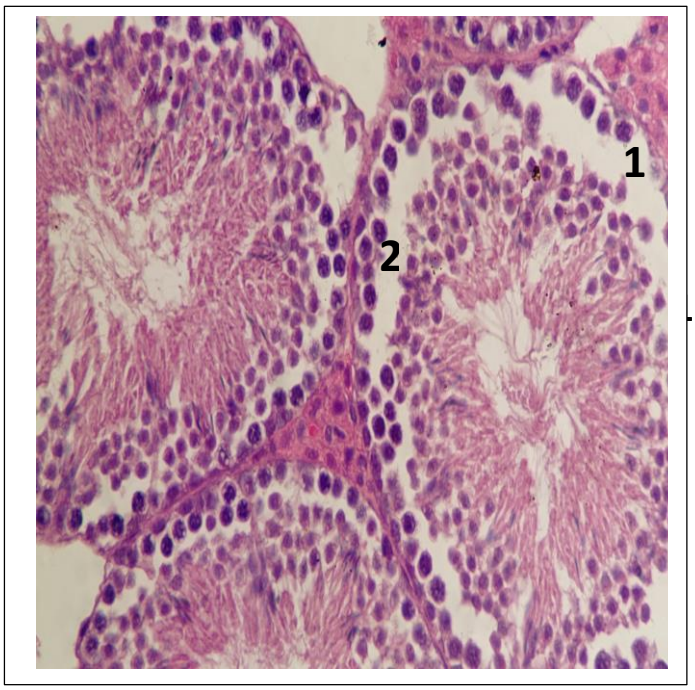

Figure (4-3): Testis of Escitalopram male mice two week showing, 1 decrease spermatogonia, 2 spaces between spermatogonia and primary spermatocyte, (H&E, 400x).

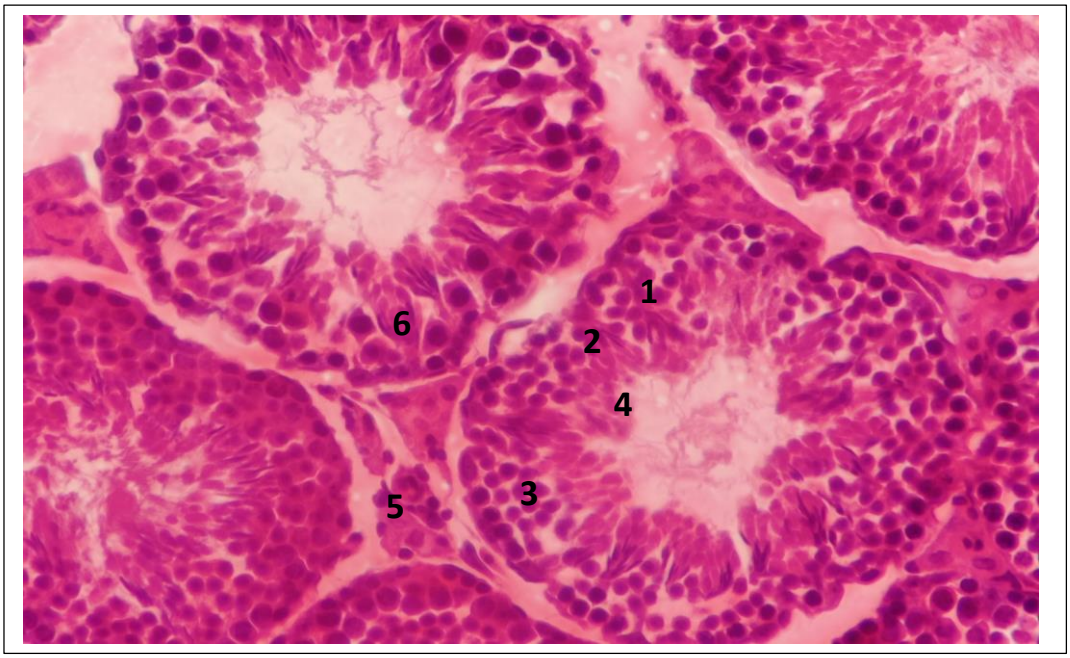

Figure (4-4): Testis of control male mice four week showing the normal structure, 1 spermatogonia, 2 primary spermatocyte, 3 secondary spermatocyte, 4 spermatid, 5 Leydig cells, 6 sertoli cells ,(H&E, 400x).

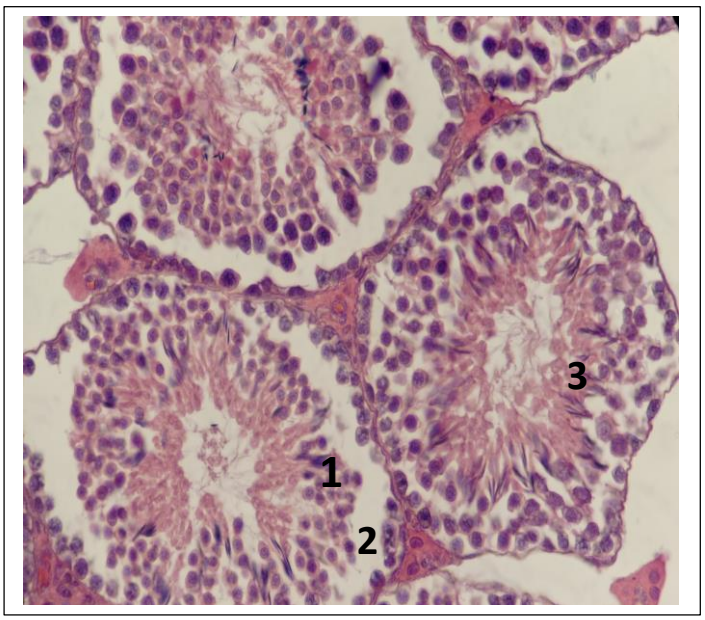

Figure (4-5): Testis of Amitriptyline male mice four week showing, 1 decrease of primary spermatocyte, 2 spaces between spermatogonia layer and primary spermatocyte layer ,3 proliferation sertoli cells, (H&E, 400x).

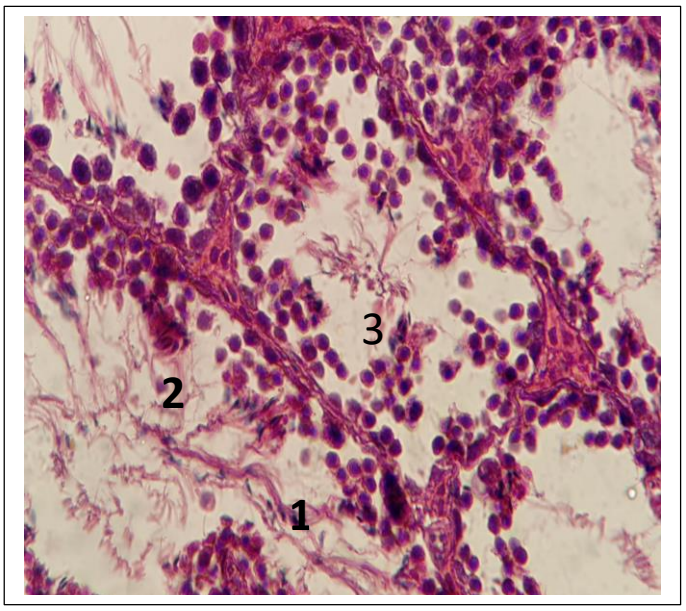

Figure (4-6): Testis of Escitalopram male mice four week showing, 1 absence spermatid layer, 2 lumens wider, 3 irregulars layers' cells, (H&E, 400x).

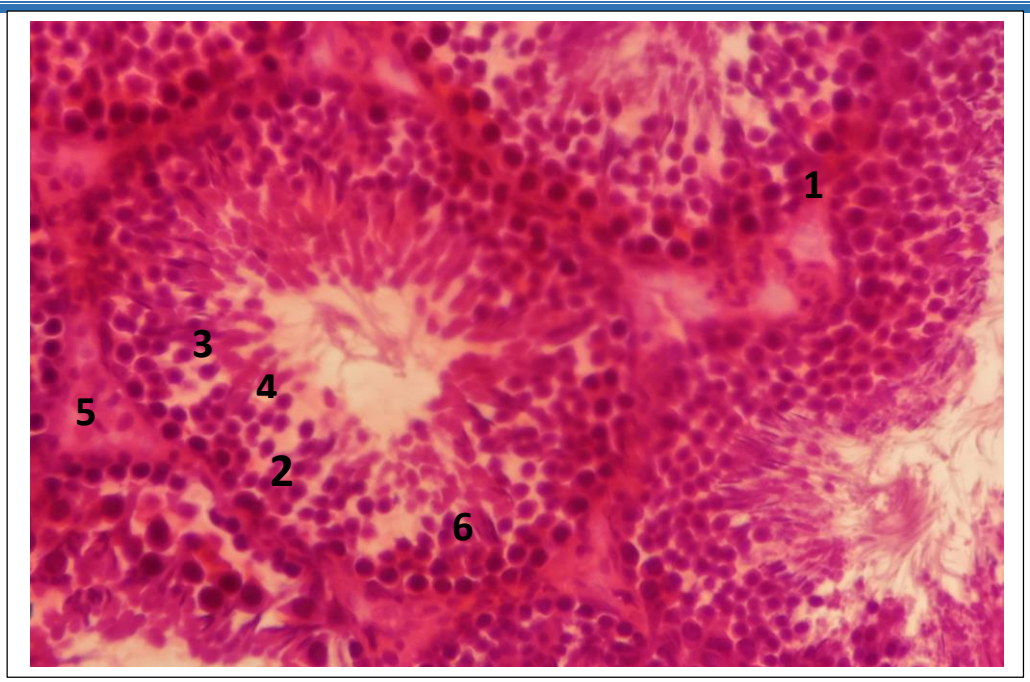

Figure (4-7): Testis of control male mice six week showing the normal structure, 1 spermatogonia, 2 primary spermatocytes, 3 secondary spermatocytes, 4 spermatids, 5 Leydig cells, 6 sertoli cells, (H&E, 400x).

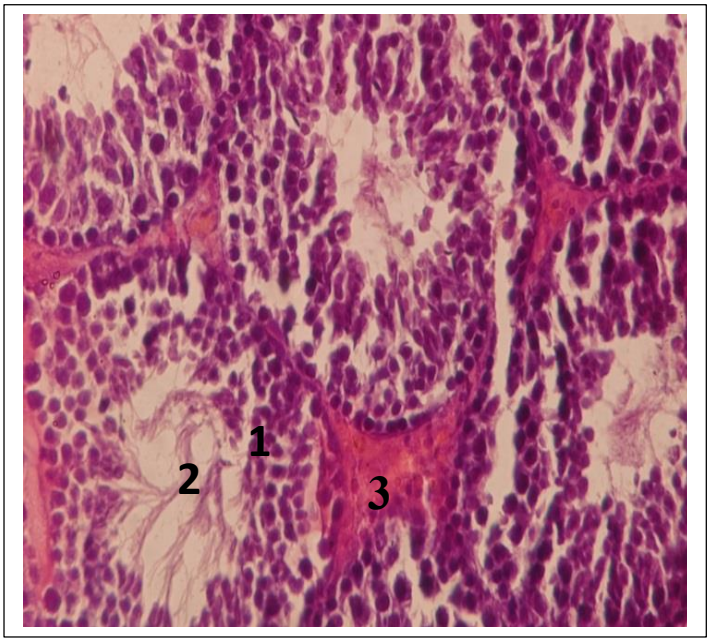

Figure (4-8): Testis of Amitriptyline male mice six week showing, 1 decrease spermatid, 2 lumens wider, 3 absences of leydig cells, (H&E, 400x)

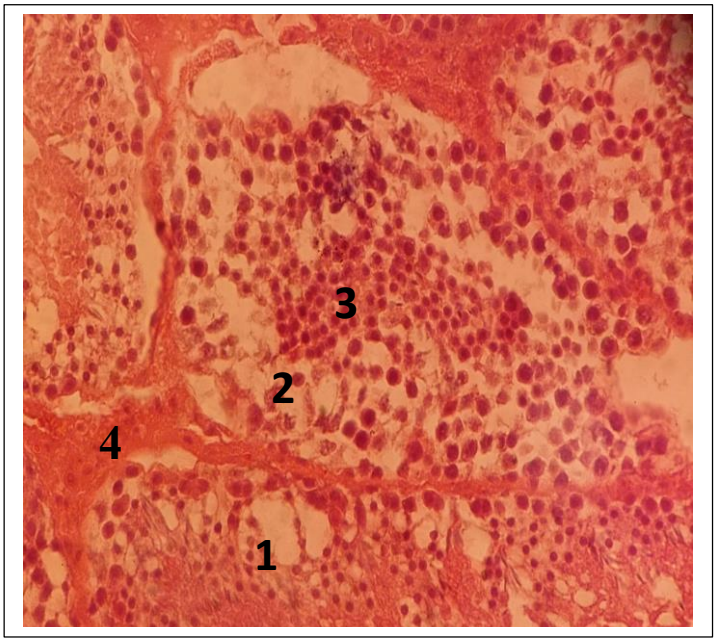

Figure (4-9): Testis of Escitalopram male mice six week showing,1 change the size of the cells, 2 irregular cell layers, 3 absence lumen, 4 absences of leydig cells, (H&E, 400x)

4-6-2-The epididymis

The result of the study in the second week showed that the histological structure of Epididymis in the control group is lined with pseudostratified columnar epithelium, this epithelium stereociliated and lumen filled with mature sperms figure(4-10), while The epididymis section for the Amitriptyline group showed normal tissue of the epididymis duct and the presence of many sperms in the lumen of the epididymis figure (4-11), and Escitalopram group showed hypertrophy of epithelial cells and the lumen contains small numbers of sperm. Figure (4-12).

As for the fourth week, the epididymis sections for the control group were similar to the control group in the second week. Figure (4-13), while the Amitriptyline group showed a normal epithelium layer, lumen filled with mature sperms and the presence of a gap between the cells of the epithelium. Figure (4-14), and the Escitalopram group, it showed, hypertrophy of epithelial cells, the presence of rounded immature sperms and absence in sperm. Figure (4-15).

While in the sixth week, the control group no change compared to the second and fourth weeks. Figure (4-16). The Amitriptyline group showed hypertrophy of epithelial cells, presence rounded immature sperms in the lumen, and the lumen appears narrow and irregular. Figure (4-17), and the Escitalopram group, showed the epithelial cell layer changed from pseudostratified columnar type to the simple columnar type, the cell shape changed from the columnar to the cuboidal shape and the lumen contained mature sperms. Figure (4-18).

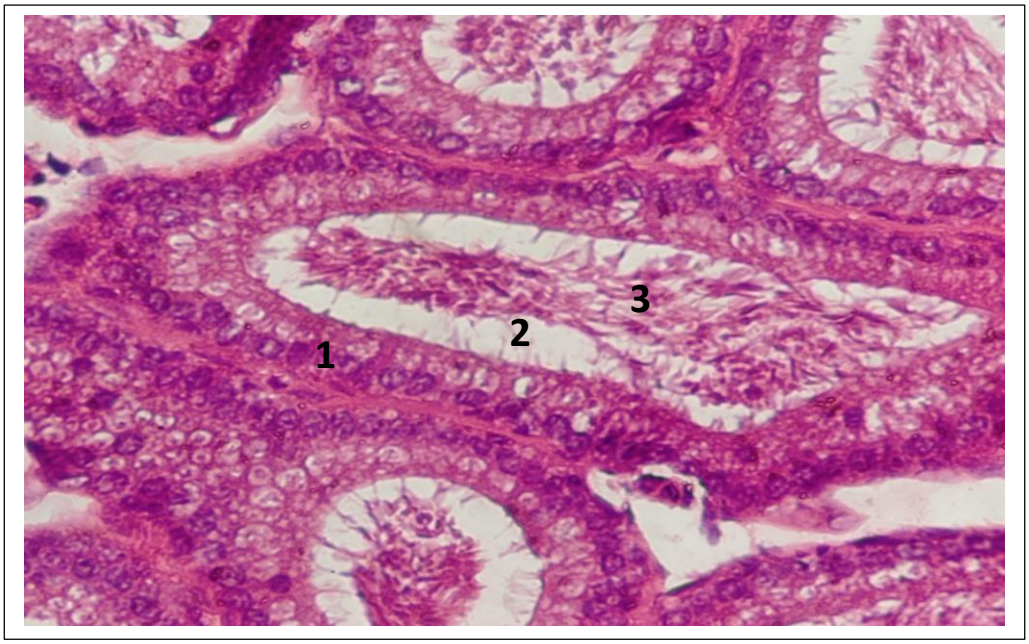

Figure (4-10): epididymis of control male mice two week showing, 1 normal pseudostratified columnar epithelium, 2 lumens, 3 mature sperms, (H&E, 400 X).

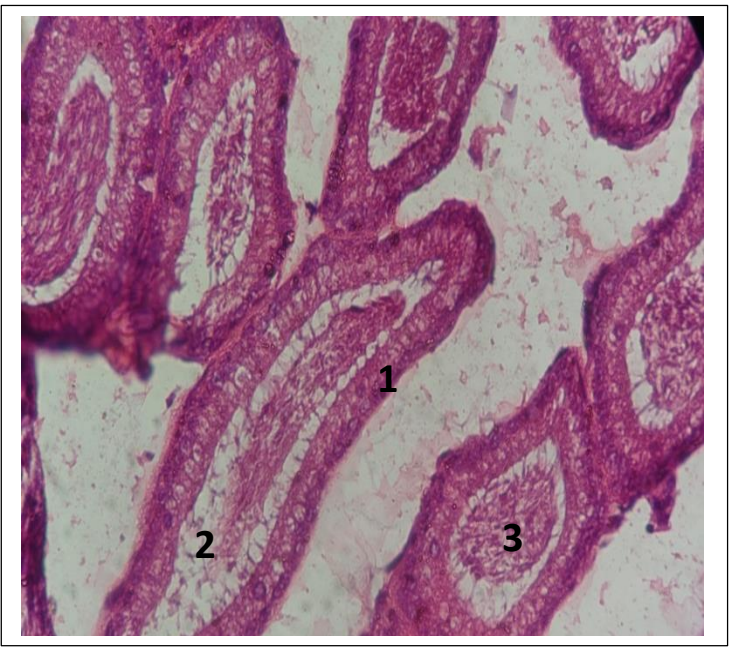

Figure (4-11): epididymis of Amitriptyline male mice two week showing, 1 normal pseudostratified columnar epithelium, 2 lumen filled with mature sperms, 3 mature sperms, (H&E, 400 X).

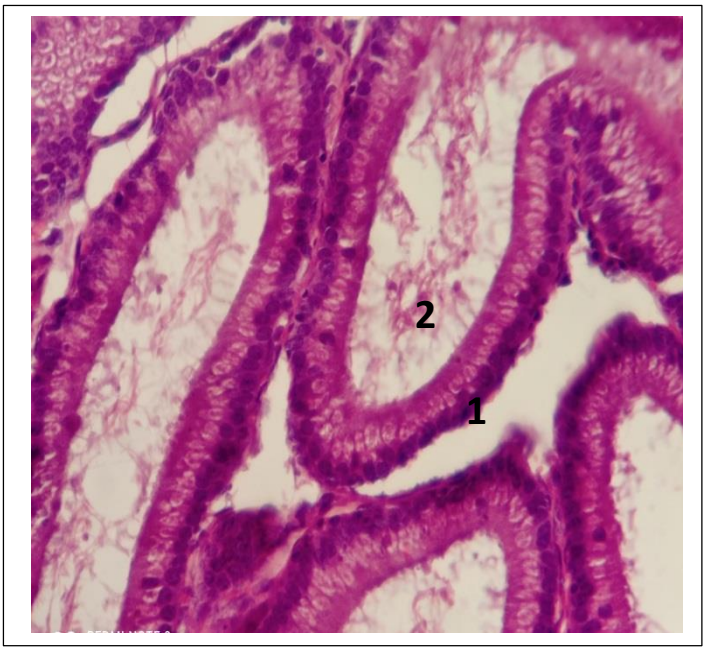

Figure (4-12): epididymis of Escitalopram male mice two week showing, 1 hypertrophy of epithelial cells, 2 decrease of sperms, (H&E, 400 X).

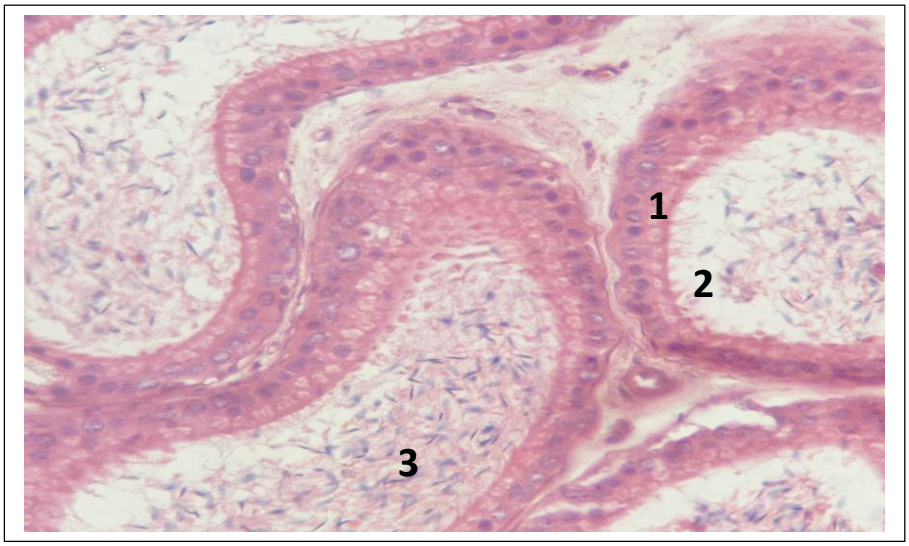

Figure (4-13): epididymis of control male mice four week showing, 1 normal pseudostratified columnar epithelium, 2 lumen filled with mature sperms, 3 mature sperms, (H&E, 400 X).

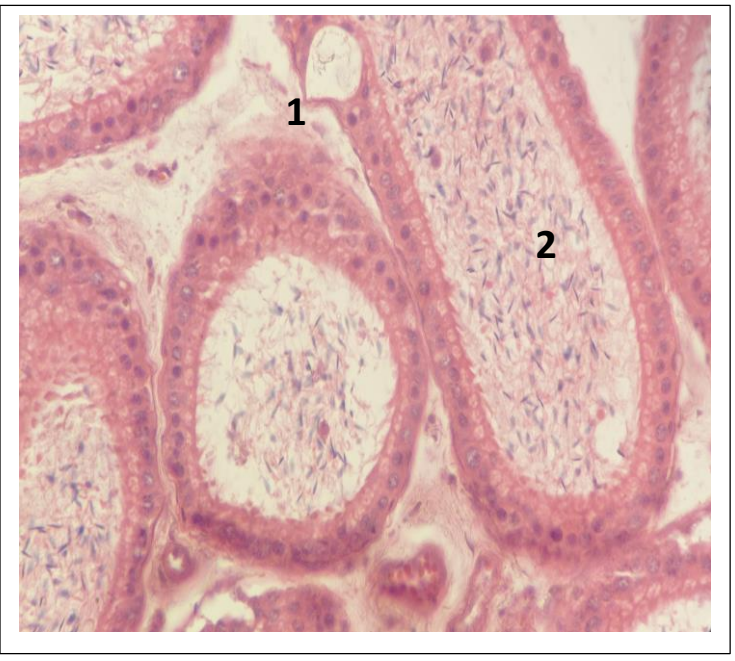

Figure (4-14): epididymis of Amitriptyline male mice four week showing, 1 gap between the cells of the epithelium, 2 lumen filled with mature sperms, (H&E, 400 X).

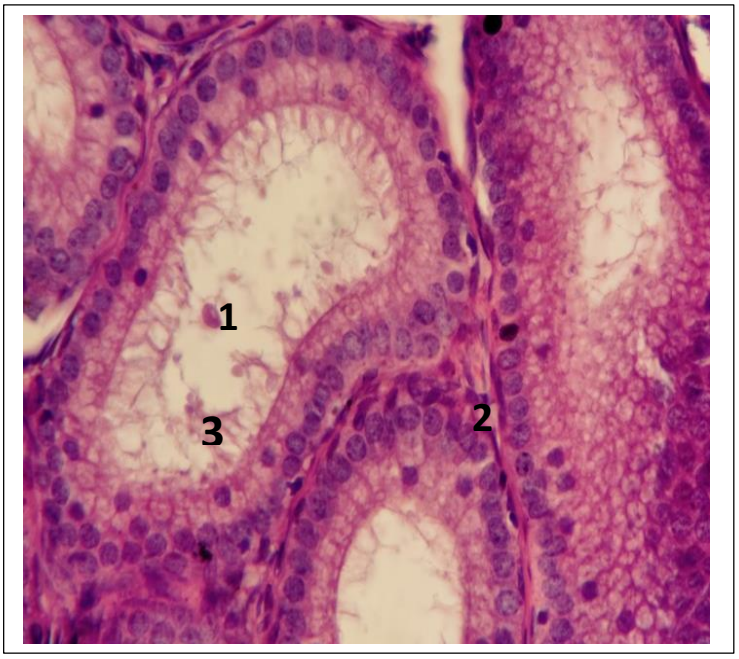

Figure (4-15): epididymis of Escitalopram male mice four week showing, 1 presence of rounded immature sperm, 2 hypertrophy of epithelial cells, 3absence of sperms, (H&E, 400 X).

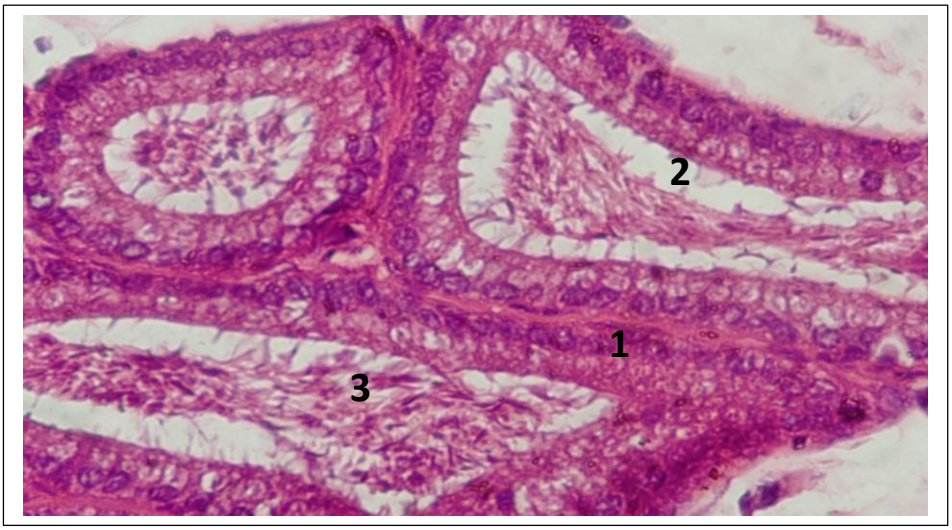

Figure (4-16): epdidymis of control male mice six week showing, 1 normal pseudostratified columnar epithelium, 2 lumens, 3 mature sperms, (H&E, 400 X).

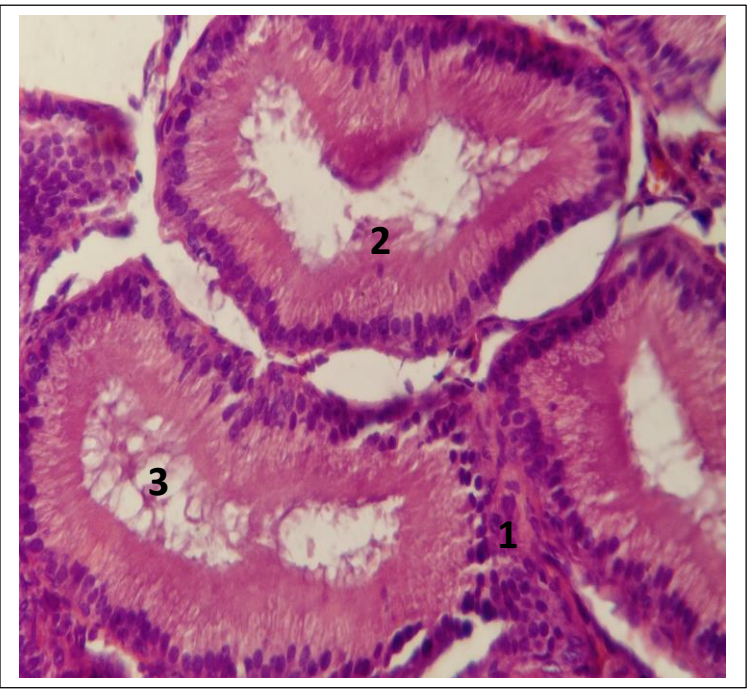

Figure (4-17): epididymis of Amitriptyline male mice six week showing, 1 hypertrophy of epithelial cells, 2 lumen appears narrow and irregular, 3 presences of rounded immature sperm, (H&E, 400

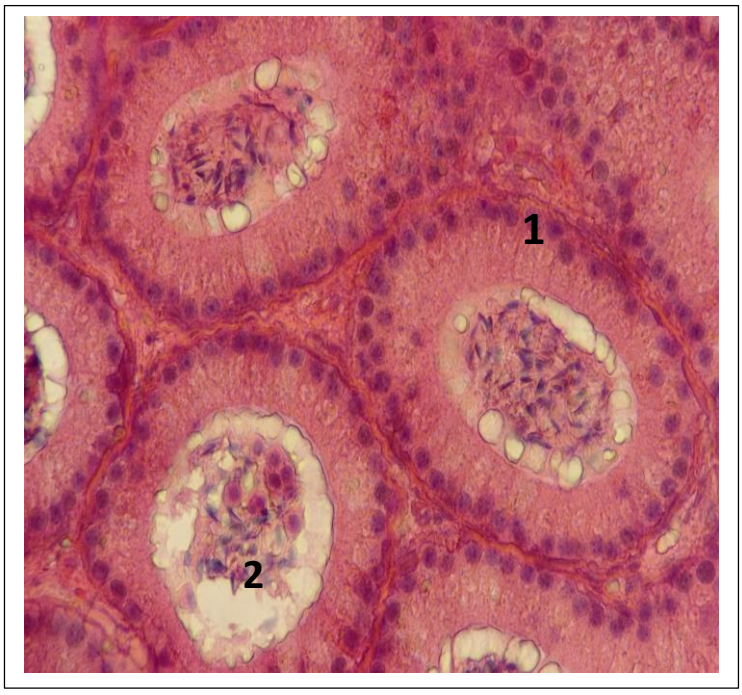

Figure (4-18): epididymis of Escitalopram male mice six week showing, 1 simple layer, 2 lumen contained mature sperms, (H&E, 400 X).

4-6-3- The seminal vesicles

The result of the study in the second week showed that the histological structure of the seminal vesicles in the control group is lined with pseudostratified columnar epithelium is composed of basal cells and tall columnar principal cells and the presence of mucous folds/edges extending into the lumen. The lumen of the seminal vesicles is filled with eosinophilic secretion**,** and the presence of smooth muscle. Figure (4-19), while the seminal vesicle sections for the Amitriptyline group showed an increase in the number of folds, lumen filled with eosinophilic secretion**.** Figure (4-20), and the Escitalopram group showed a narrow lumen due to an increased number of folds and the lumen contains few eosinophilic secretions. Figure (4-21).

As for the fourth week, the seminal vesicle sections for the control group were similar to the control group in the second week. Figure (4-22), while the Amitriptyline group showed epithelial metaplasia from columnar to cuboidal, increased number of folds and narrow lumen. Figure (4-23), and the Escitalopram group showed stratification of epithelial cells and filled with eosinophilic secretion. Figure (4-24).

While in the sixth week, the control group no change compared to the second and fourth weeks. Figure (4-25), for the Amitriptyline group showed epithelial metaplasia from columnar to cuboidal and the lumen contains few eosinophilic secretions. Figure (4-26), and the Escitalopram group showed a significantly increase in the number of folds and the lumen almost disappears. Figure (4-27).

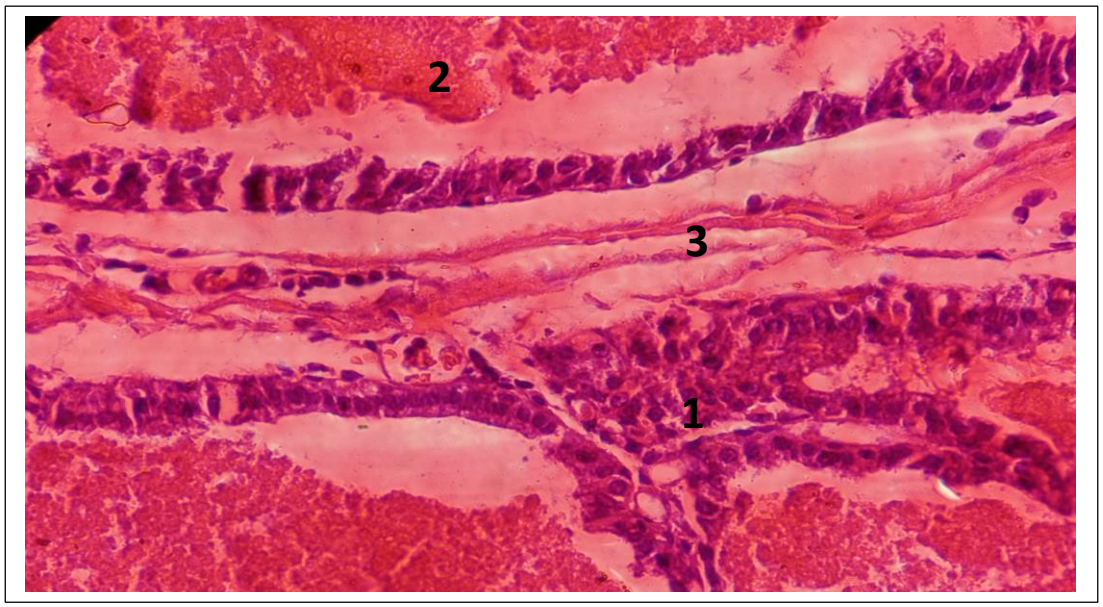

Figure (4-19): seminal vesicle of control male mice two week showing, 1 normal pseudostratified columnar epithelium, and 2 lumen filled with eosinphilic secretion, 3 smooth muscle, (H&E, 400 X).

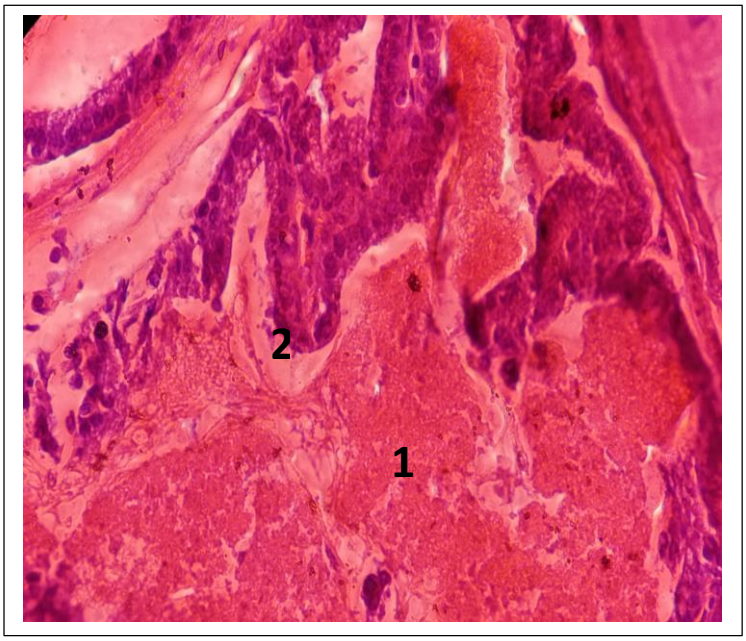

Figure (4-20): seminal vesicle of Amitriptyline male mice two weeks showing, 1 eosinophilic secretion**, 2** folds, (H&E, 400 X).

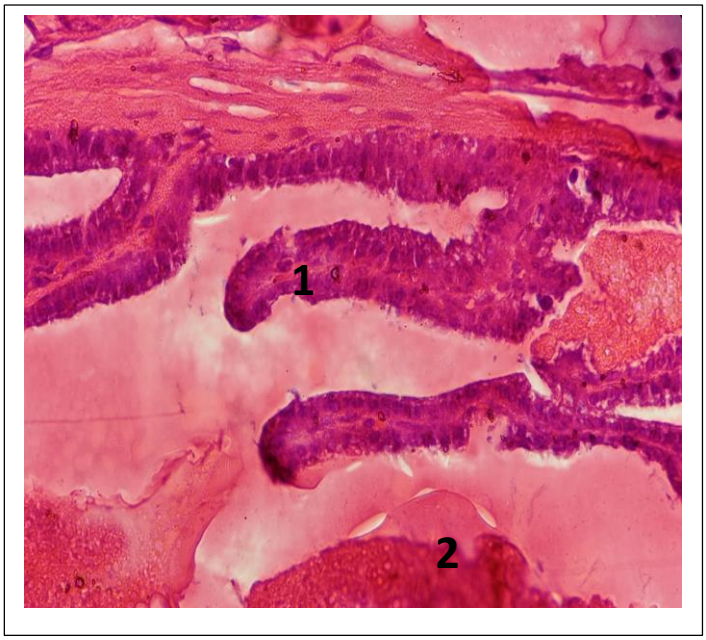

Figure (4-21): seminal vesicle of Escitalopram male mice two week showing, 1 folds, 2 lumen contains few eosinphilic secretion, (H&E, 400 X).

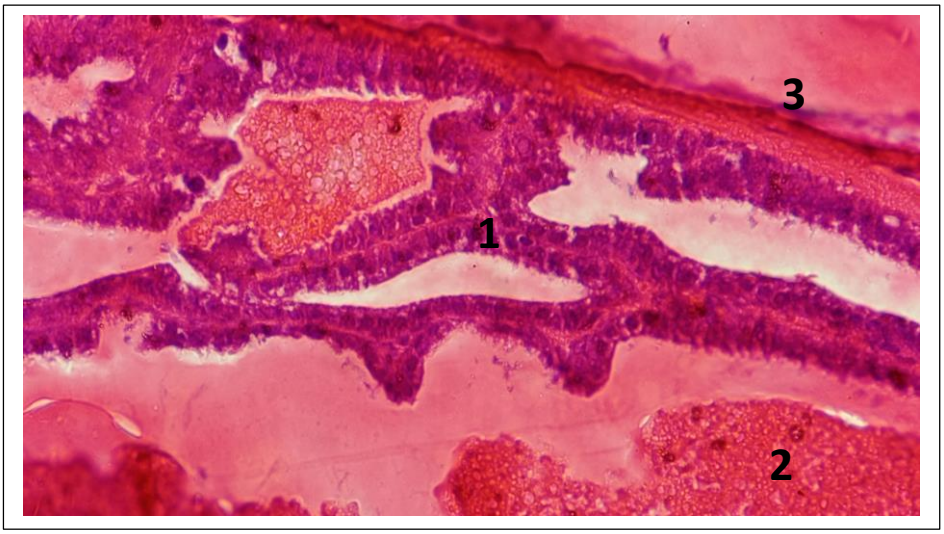

Figure (4-22): seminal vesicle of control male mice four week showing, 1 normal pseudostratified columnar epithelium, 2 lumen filled with eosinphilic secretion, 3 smooth muscle, (H&E, 400 X).

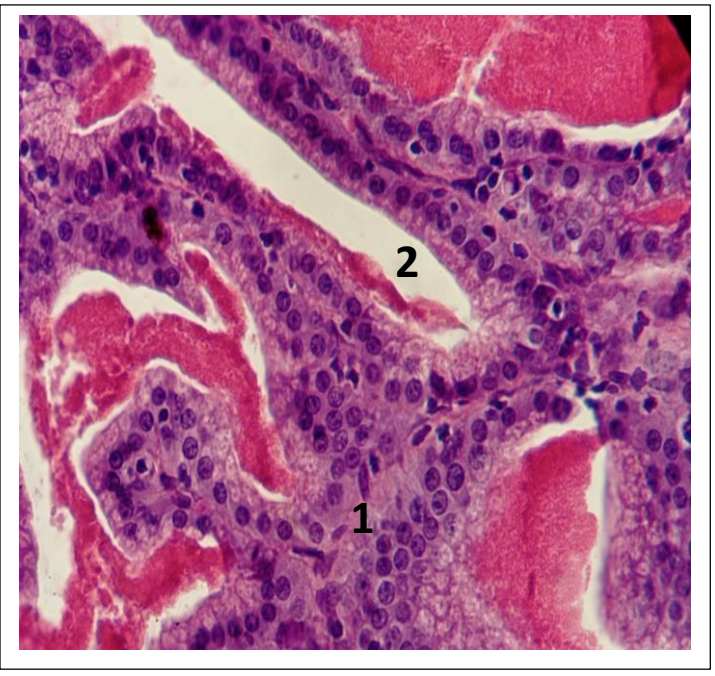

Figure (4-23): seminal vesicle of Amitriptyline male mice four week showing, 1, epithelial metaplasia, 2 lumen narrow, (H&E, 400 X).

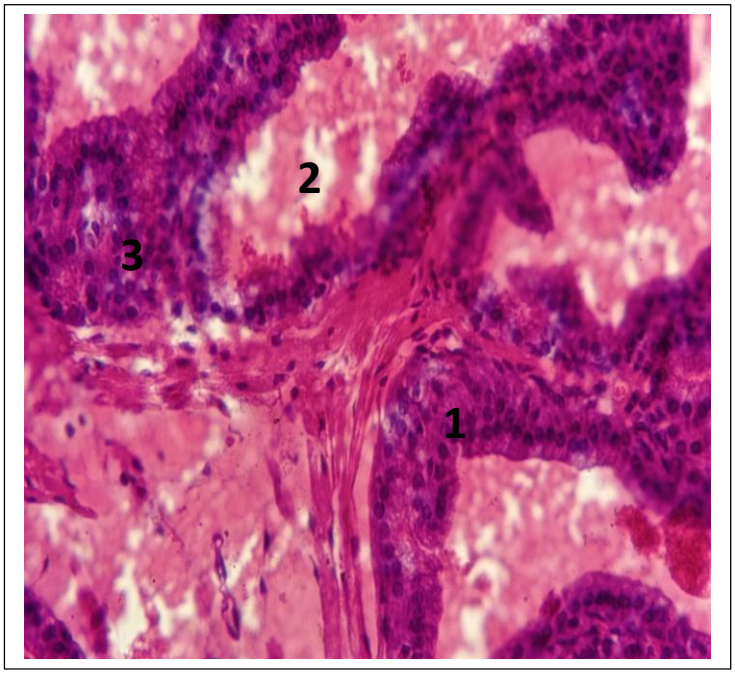

Figure (4-24): seminal vesicle of Escitalopram male mice four week showing, 1 stratification of epithelial cells, 2 lumen filled with eosinphilic secretion, 3 folds, (H&E, 400 X).

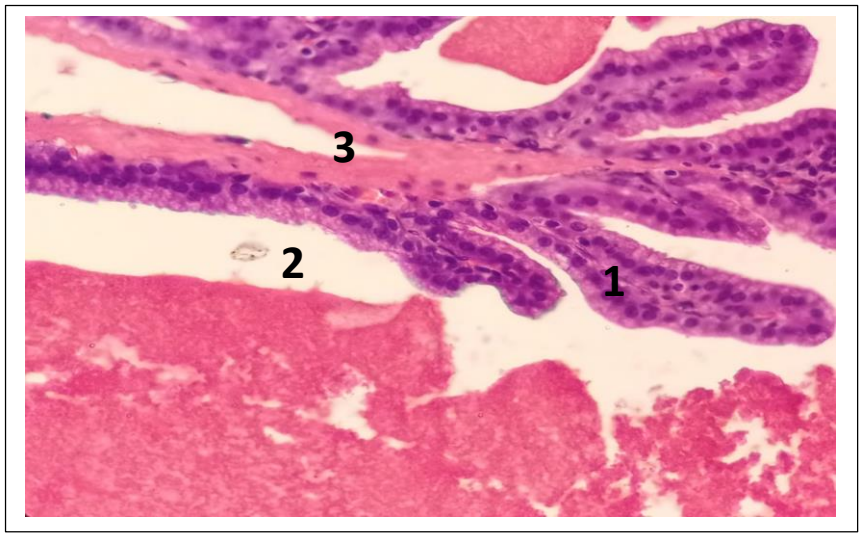

Figure (4-25): seminal vesicle of control male mice six week showing, 1 normal pseudostratified columnar epithelium, 2 lumen filled with eosinphilic secretion, 3 smooth muscle, (H&E, 400 X).

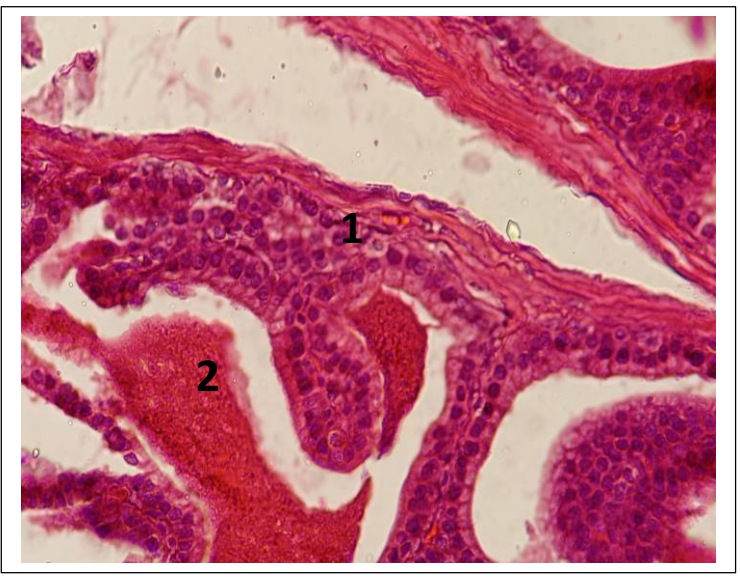

Figure (4-26): seminal vesicle of Amitriptyline male mice six week showing, 1 epithelial metaplasia, 2 lumen contains few eosinphilic secretion, (H&E, 400 X).

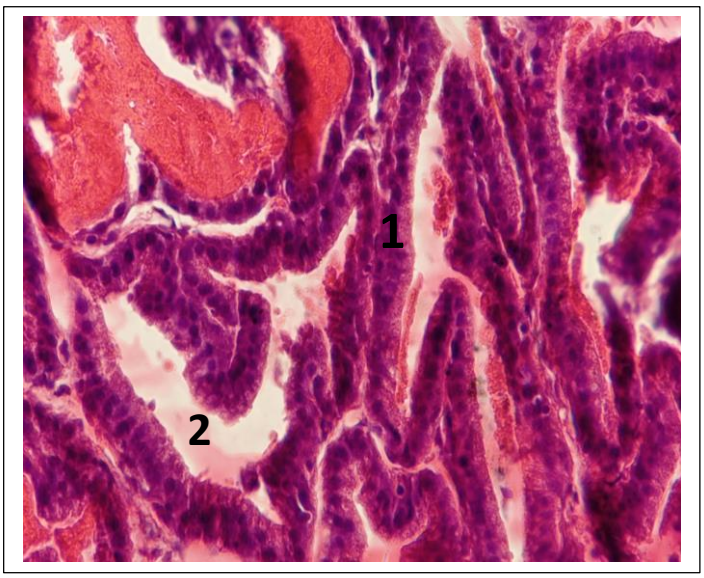

Figure (4-27): seminal vesicle of Escitalopram male mice six week showing, 1 increase the folds, 2 lumens, (H&E, 400 X).

4-7-Histochemical study

4-7-1-The Testis

The result of the study in the second week of the testis for the control group showed that the interaction of the basement membrane of the testis with PAS weak. Figure (4-28), while the testis sections for Amitriptyline and Escitalopram group showed the interaction moderate. Figure (4-29, 4-30).

As for the fourth week, the testis for the control group showed that the interaction of the basement membrane of the testis with PAS was weak Figure (4-31), while the testis sections for the Amitriptyline and Escitalopram group showed the interaction moderate. Figure (4-32, 4-33)

While in the sixth week the testis for the control group showed that the interaction of the basement membrane of the testis with PAS weak Figure (4-34), for the testis sections for Amitriptyline and Escitalopram group showed the interaction strong. Figure (4-35) as shown in the following Figure (4-36).

4-7-2-The Epididymis

The result of the study in the second week, the epididymis for the control group showed that the interaction of the basement membrane of the epididymis with PAS moderate. Figure (4-37), while the epididymis sections for Amitriptyline and Escitalopram group showed the interaction moderate. Figure (4-38, 4-39).

As for the fourth week, the epididymis for the control group showed the interaction of the basement membrane with PAS moderate Figure (4-40), while the epididymis sections for the amitriptyline and Escitalopram group showed the interaction strong. Figure (4-41, 4-42)

While in the sixth week, the epididymis for the control group showed the interaction of the basement membrane with PAS moderate Figure (4-43), while the epididymis sections for the Amitriptyline and Escitalopram group showed the interaction strong. Figure (4-44, 4-45)

4-7-3-The Seminal Vesicles

The result of the study in the second week, the seminal vesicles for the control group showed that the interaction of the basement membrane with PAS strong. Figure (4-46), while the seminal vesicle sections for Amitriptyline showed the interaction weak Figure (4-47), and the Escitalopram group showed the interaction strong. Figure (4-48)

While in the fourth week, the seminal vesicles for the control group showed the interaction of the basement membrane with PAS strong Figure (4-49), while the seminal vesicles sections for the Amitriptyline and Escitalopram group showed the interaction weak. Figure (4-50, 4-51)

As for the sixth week, the seminal vesicles for the control, Amitriptyline and Escitalopram group showed that the interaction of the basement membrane with PAS was strong Figure (4-52, 4-53, 4-54),

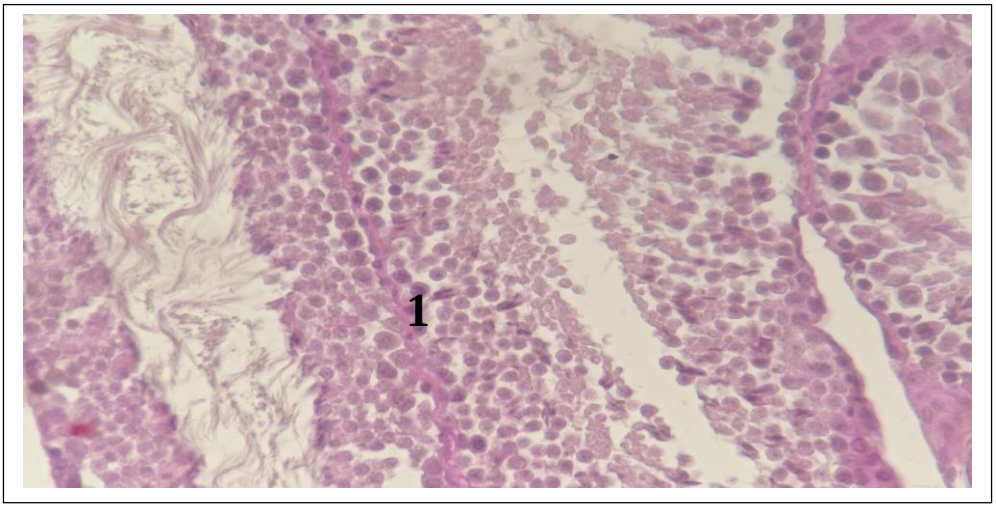

Figure (4-28): testis of control male mice two week showing, 1 basement membrane reaction with PAS weak (400x)

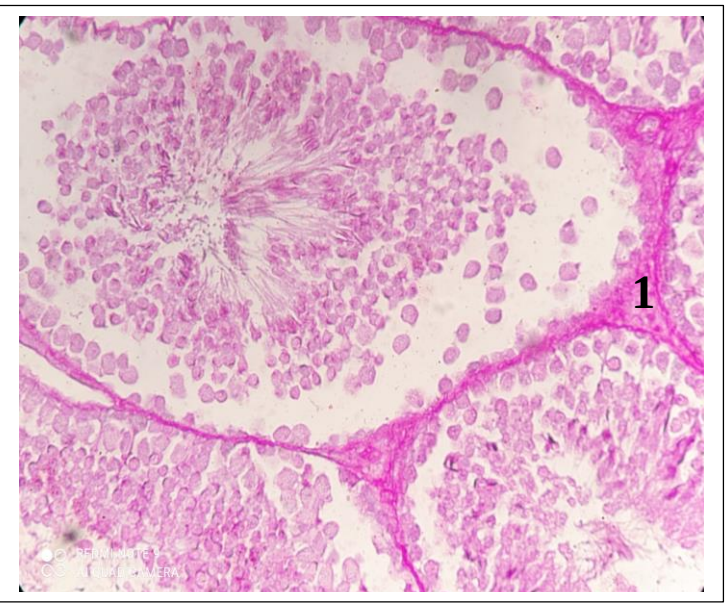

Figure (4-29): testis of Amitriptyline male mice two week showing, 1 basement membrane reaction with PAS moderate (400x)

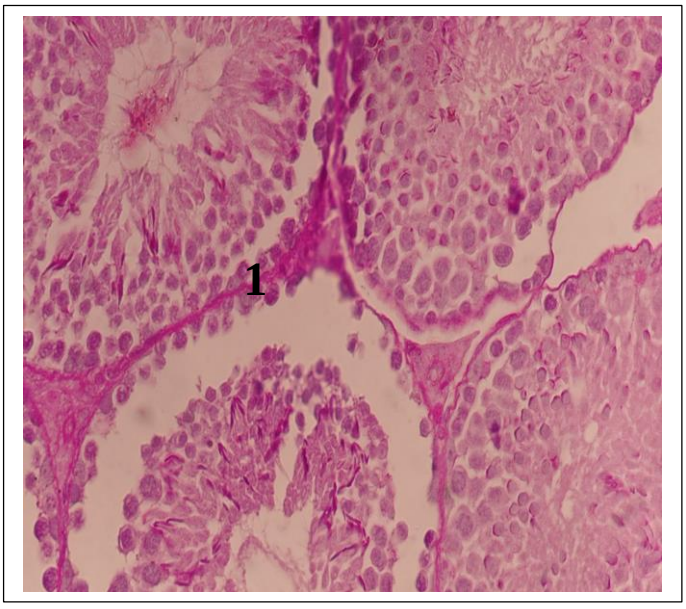

Figure (4-30): testis of Escitalopram male mice two week showing, 1 basement membrane reaction with PAS moderate (400x)

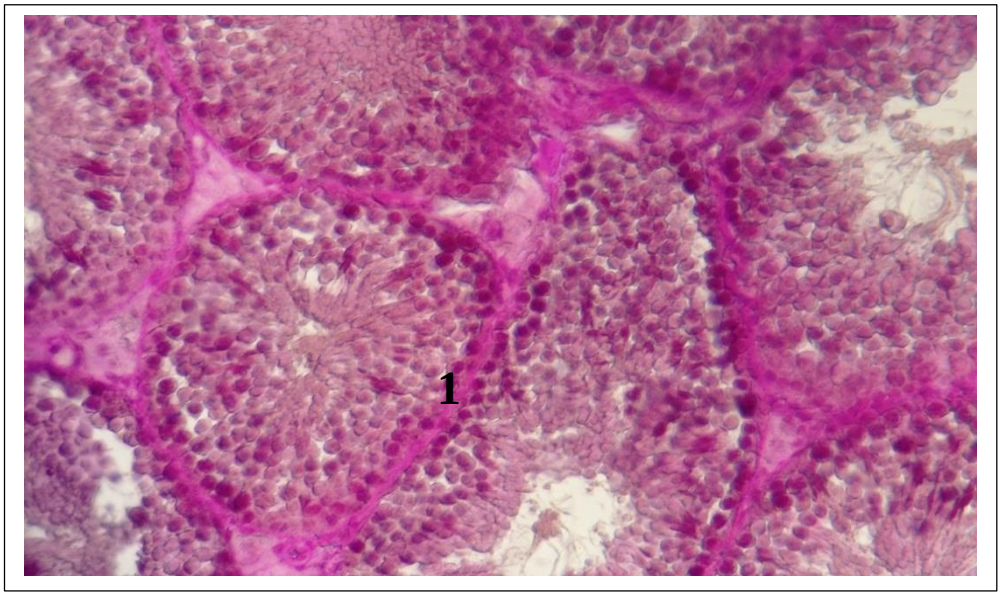

Figure (4-31): testis of control male mice four week showing, 1 basement membrane reaction with PAS weak (400x)

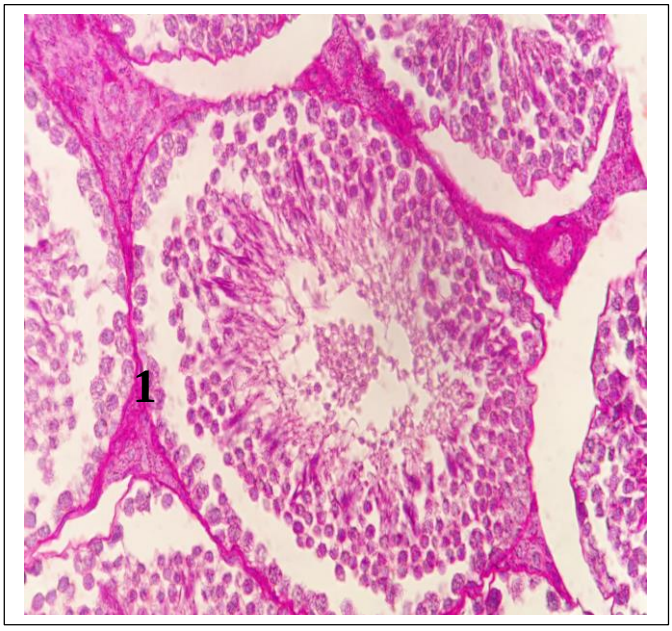

Figure (4-32): testis of Amitriptyline male mice four week showing, 1 basement membrane reaction with PAS moderate (400x)

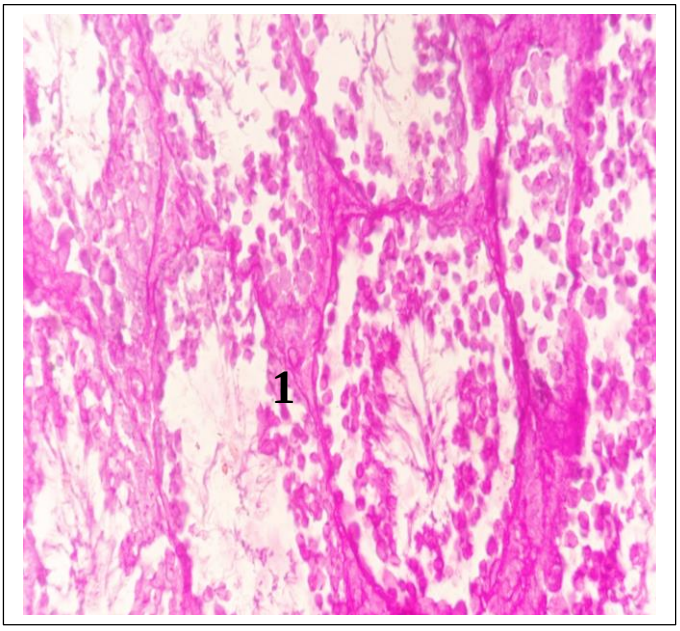

Figure (4-33): testis of Escitalopram male mice four week showing, 1 basement membrane reaction with PAS moderate (400x)

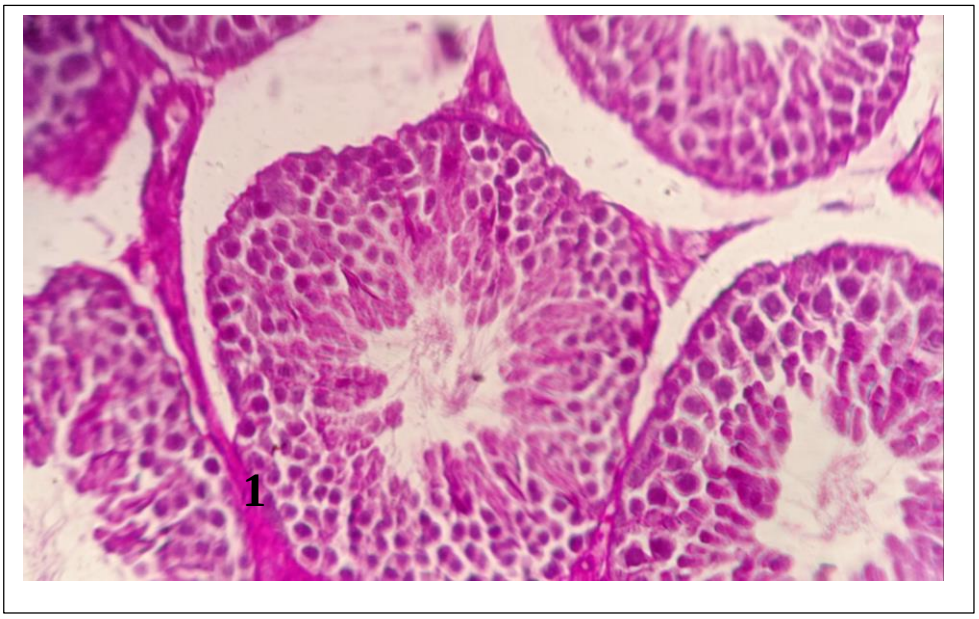

Figure (4-34): testis of control male mice six week showing, 1 basement membrane reaction with PAS weak (400x)

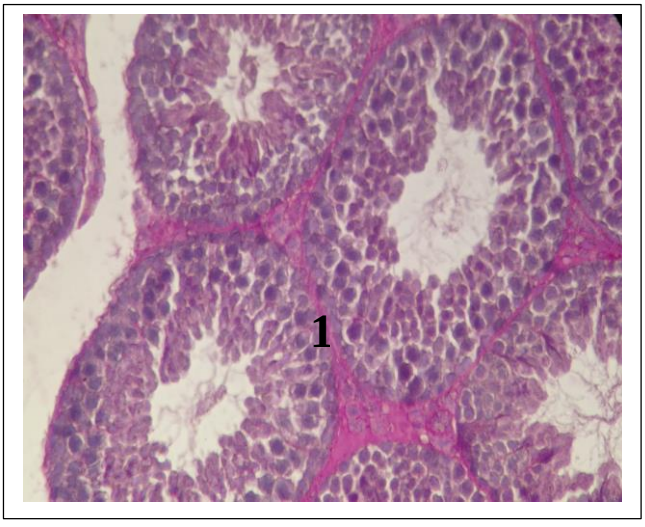

Figure (4-35): testis of Amitriptyline male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

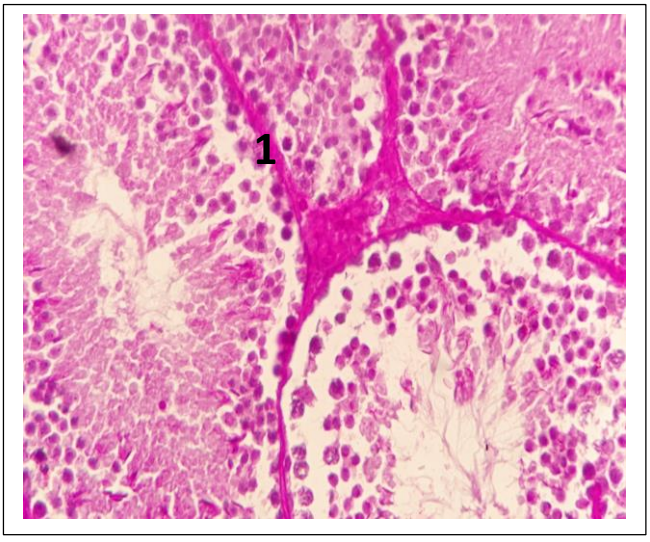

Figure (4-36): testis of Escitalopram male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

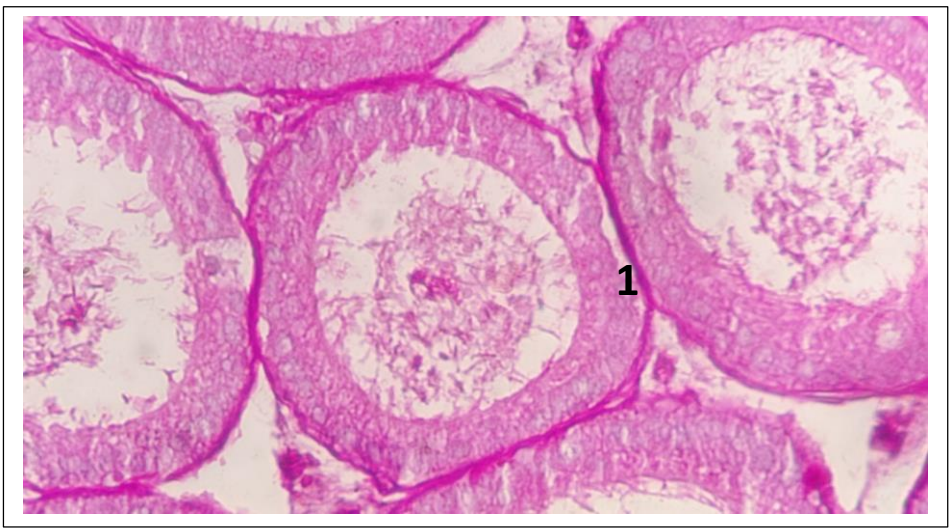

Figure (4- 37): epididymis of control male mice two week showing, 1 basement membrane reaction with PAS moderate (400x)

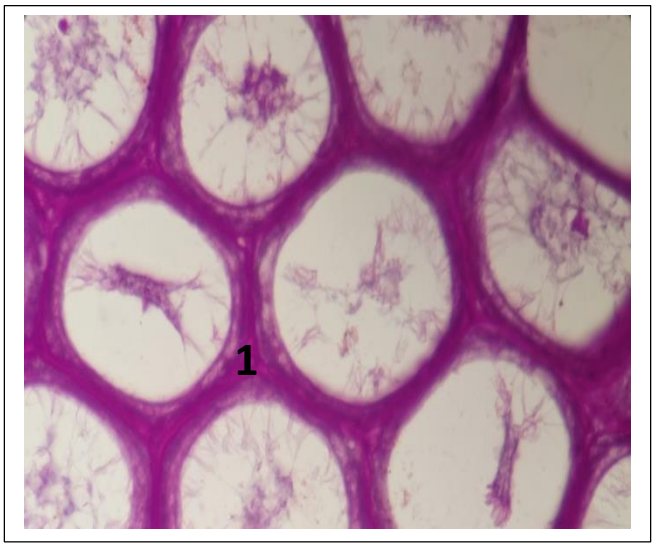

Figure (4-38): epididymis of Amitriptyline male mice two week showing, 1 basement membrane reaction with PAS moderate (400x)

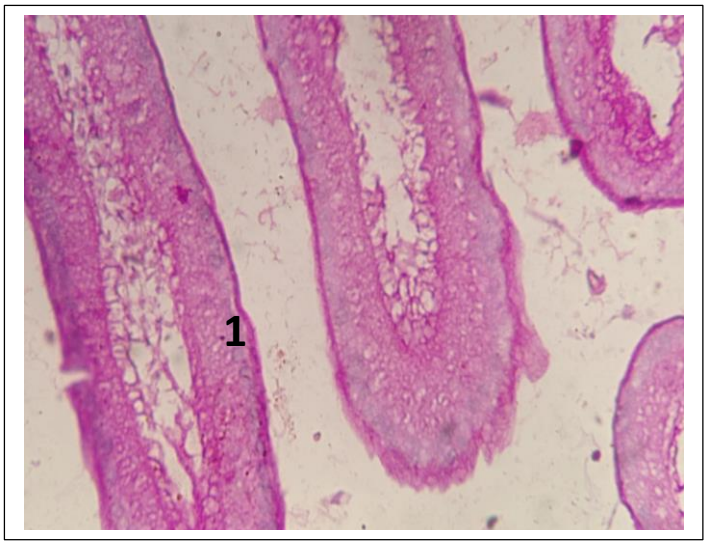

Figure (4-39): epididymis of Escitalopram male mice two week showing, 1 basement membrane reaction with PAS moderate (400x)

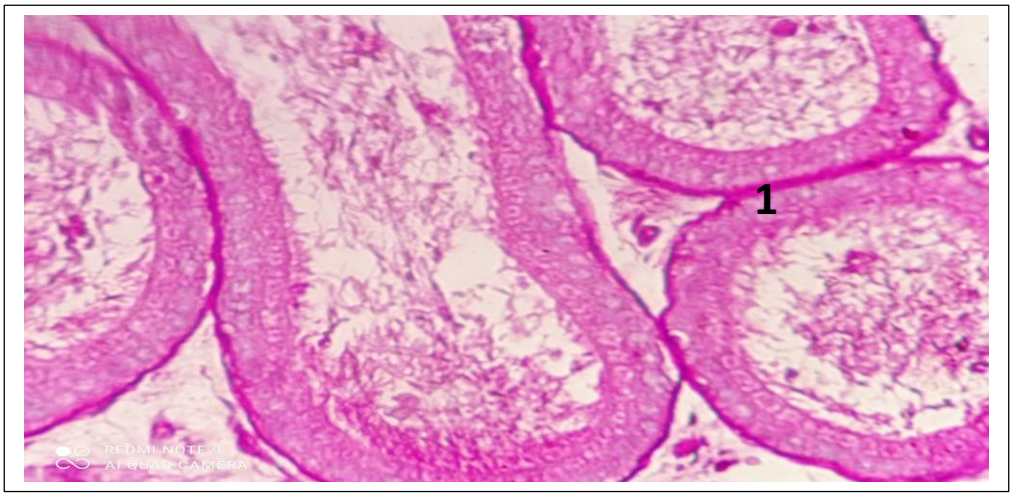

Figure (4-40): epididymis of control male mice four week showing, 1 basement membrane reaction with PAS moderate (400x)

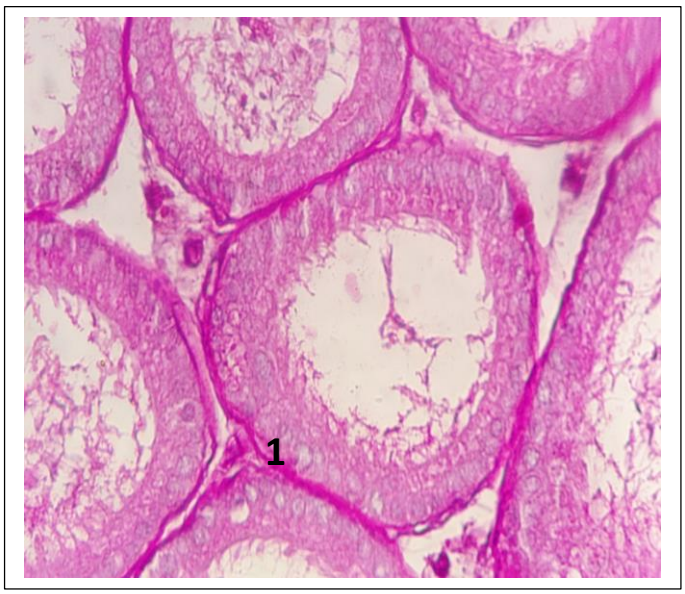

Figure (4-41): epididymis of Amitriptyline male mice four week showing, 1 basement membrane reaction with PAS strong (400x)

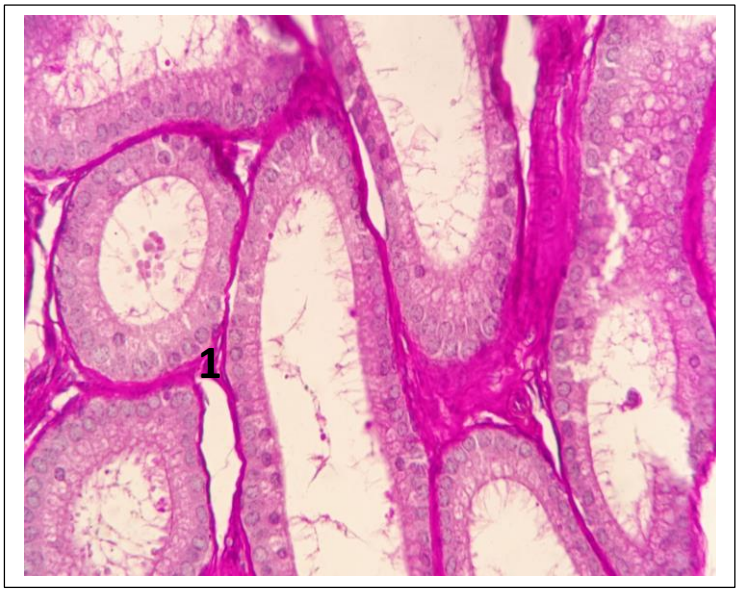

Figure (4-42): epididymis of Escitalopram male mice four week showing, 1 basement membrane reaction with PAS strong (400x)

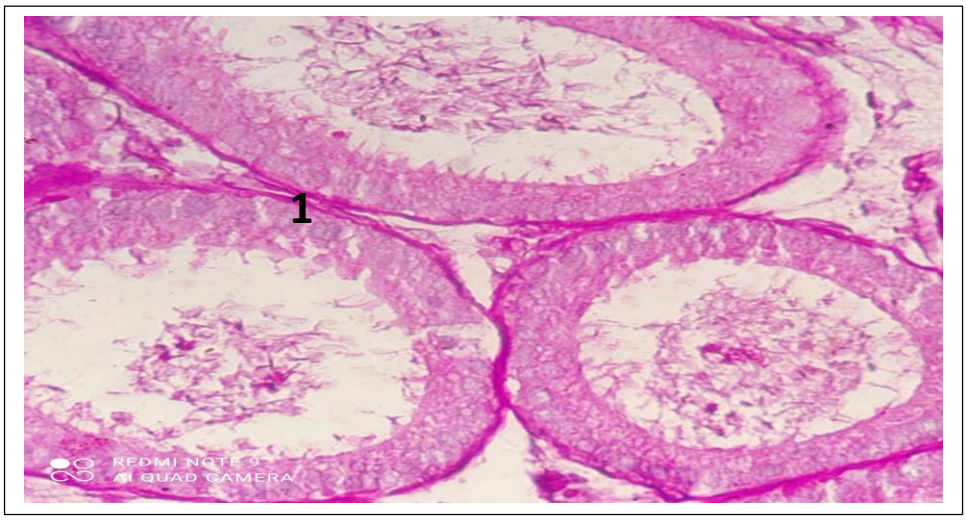

Figure (4-43): epididymis of control male mice six week showing, 1 basement membrane reaction with PAS moderate (400x)

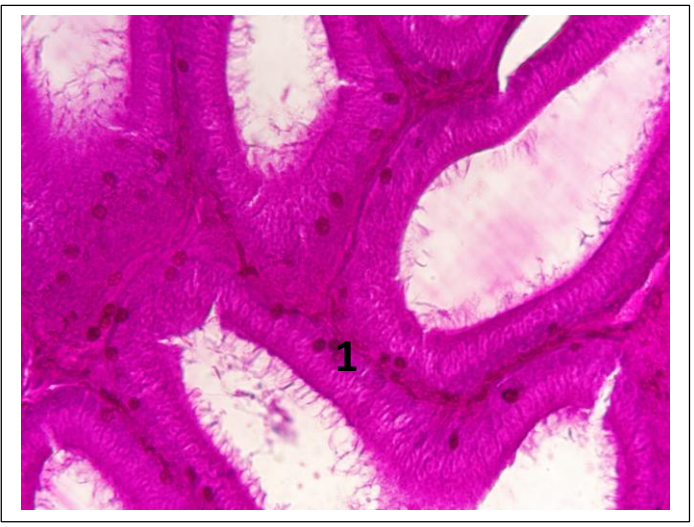

Figure (4-44): epididymis of Amitriptyline male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

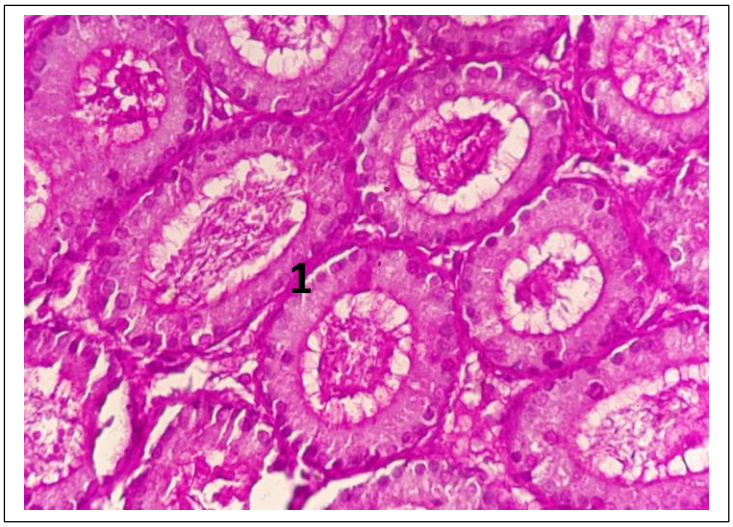

Figure (4-45): epididymis of Escitalopram male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

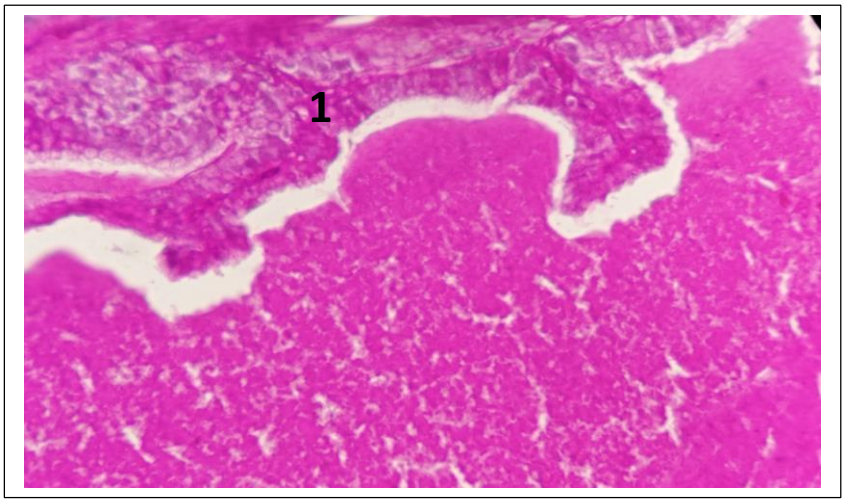

Figure 4-46: seminal vesicle of control male mice two week showing, 1 basement membrane reaction with PAS strong (400x)

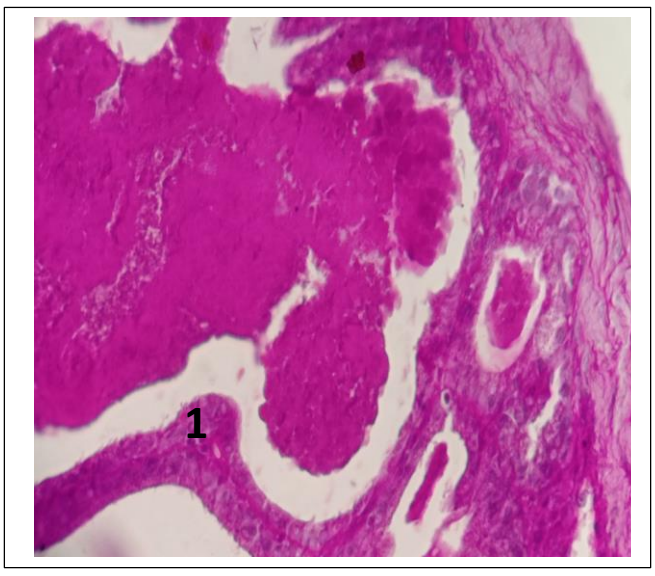

Figure (4-47): seminal vesicle of Amitriptyline male mice two week showing, 1 basement membrane reaction with PAS weak (400x)

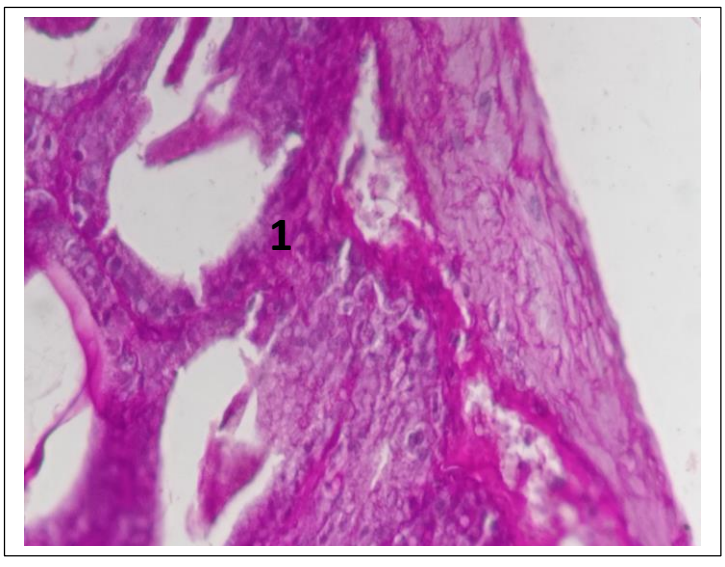

Figure (4-48): seminal vesicle of Escitalopram male mice two week showing, 1 basement membrane reaction with PAS strong (400x)

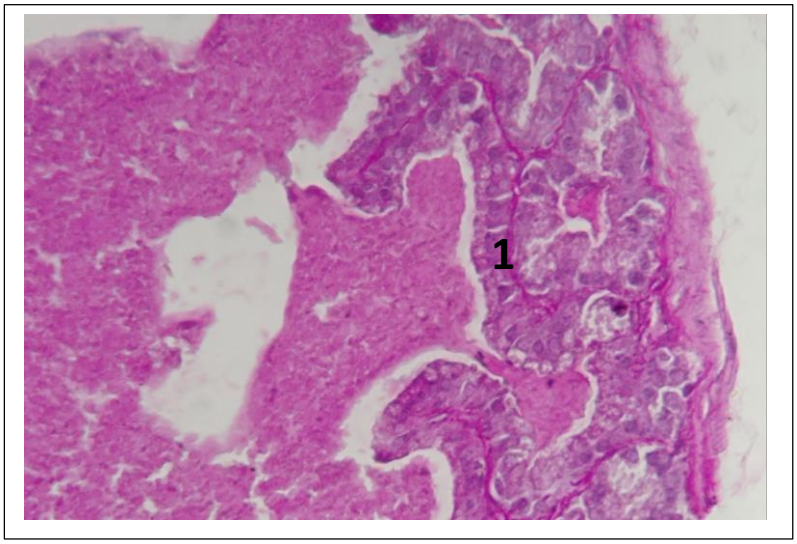

Figure (4-49): seminal vesicle of control male mice four week showing, 1 basement membrane reaction with PAS strong (400x)

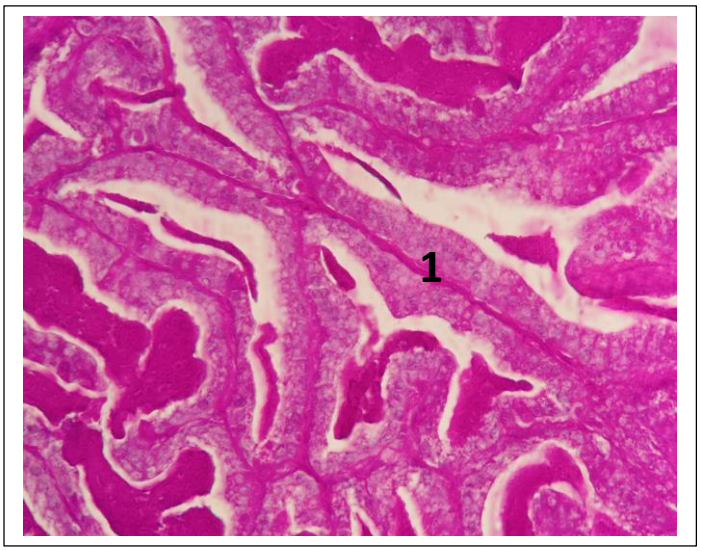

Figure (4-50): seminal vesicle of Amitriptyline male mice four week showing, 1 basement membrane reaction with PAS weak (400x)

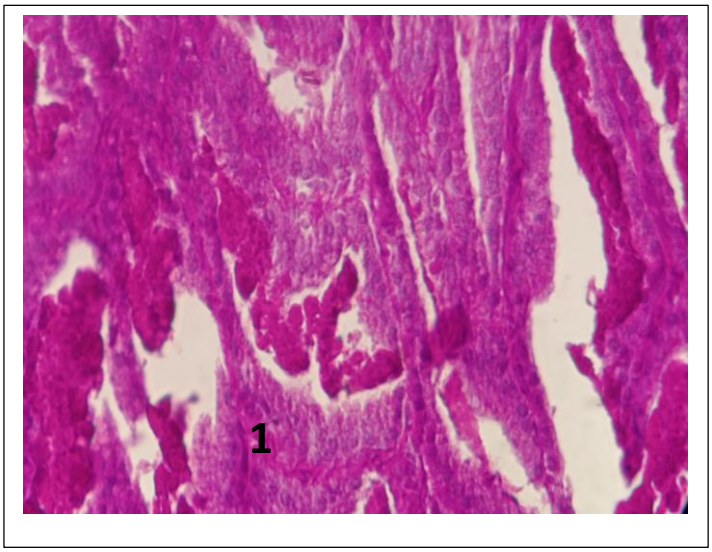

Figure (4-51): seminal vesicle of Escitalopram male mice four week showing, 1 basement membrane reaction with PAS weak (400x)

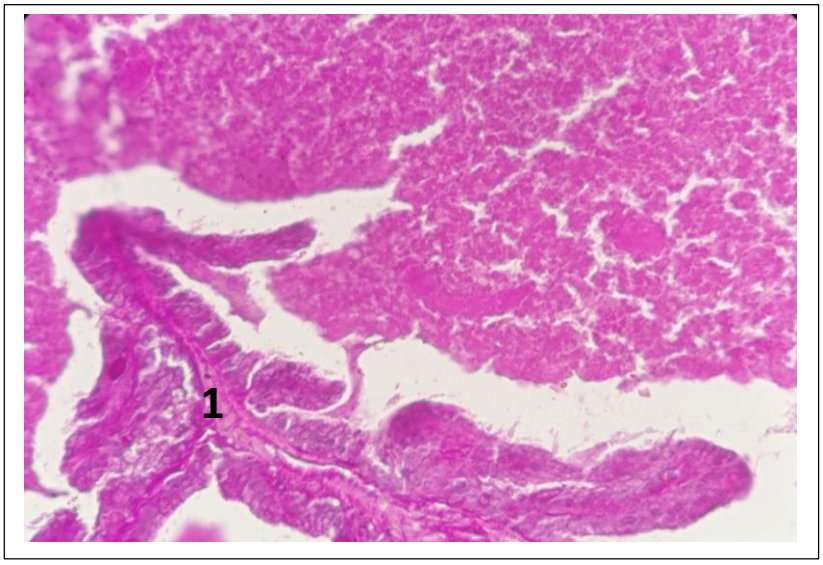

Figure (4-52): seminal vesicle of control male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

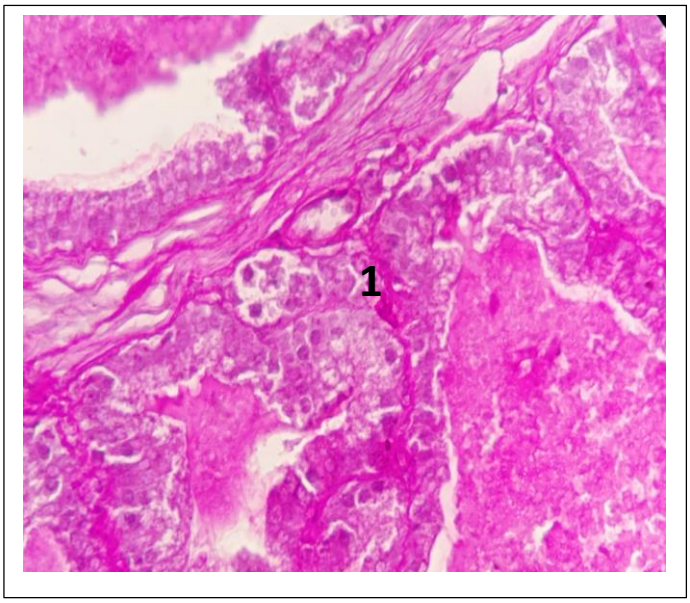

Figure (4-53): seminal vesicle of Amitriptyline male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

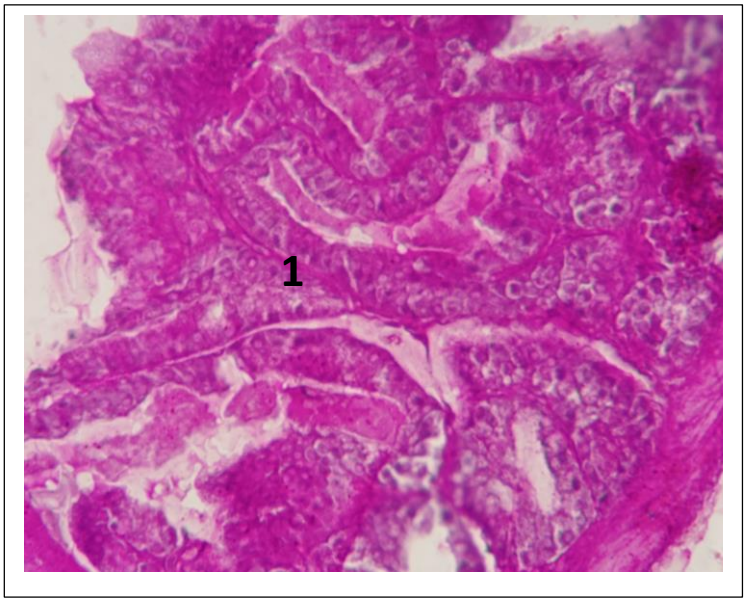

Figure (4-54): seminal vesicle of Escitalopram male mice six week showing, 1 basement membrane reaction with PAS strong (400x)

Chapter Five
Discussion

5- Discussion

Many of the drugs commonly used today have not taken into account the negative effects of drugs on male fertility , have not been properly understood, and have not been sufficiently studied to know the risks of these drugs .Many drugs affect male fertility and lead to a delay or non- fertility , and these effects are in several ways , which are damage to sexual function , damage to spermatogenesis and negative impact on the maturation of the epididymis through the axis (HPG) or oxidative stress methods (Semet *et al*., 2017).

The presence of regular levels of (FSH and LH) is important for the start of spermatogenesis. Changes that occur in the hormone damg Sertoli and Leydig cells. (Al-Hussein, 2021).

The study showed that Amitriptyline causes weight decrease and Escitalopram increases weight in the sixth week of the experiment. The explanation for the weight change due to hormonal disturbance. Kanoski and Grill, (2017) mention that the hypothalamus sending signals to the anterior pituitary gland, and there is direct control of metabolic functions and the secretion of hormones that regulate other glands. Frase and other (2018), mention that Amitriptyline reduces Ghrelin and Cortisol. Ghrelin leads to food intake. (Meier and Gressner, 2004).

High levels of cortisol are associated with weight increase, especially abdominal obesity (Marniemi *et al*., 2002).

Shebak and Varma, (2014), mention that the use of antidepressant drugs causes an increase in weight and cannot be controlled in weight even after regular diet and exercise.

Kraus and other (2002), mention that antidepressants cause an increase in body weight by influencing the level of leptin. Which is a hormone secreted by fat cells

and works to regulate food intake, metabolism, energy balance, and the function of neuroendocrine and immune function (Park and Ahima, 2015).

An important factor in the functional ability of the reproductive organ is the weight organ of the testis. (Dorostghoal *et al*., 2014).

The result of our study showed that both Amitriptyline and Escitalopram caused a decrease in the weight of the testis, epididymis and seminal vesicles, this results is agreement with Garamszegi and other (2005), where it was shown that antidepressants cause a decrease in the weight of the test is due to the low level of testosterone.

To obtain vital information related to the body's reaction to stress, deprivation and damage through the blood profile and to determine the extent of drug damage based on hematological characteristics. (Raza *et al*., 2002; Rahman *et al*., 2001).

The current study showed that both Amitriptyline and Escitalopram caused an imbalance in the blood parameter, where the treatments caused an increase in white blood cells (WBC), Canan and Ataoglu (2009), mention that the reason for the increase of (WBC) because any change in the central nervous system that causes changes in the immunity of the organism and vice versa. Several studies showed that pharmacotherapy of depression can also increase the number and cytotoxic activity of NK cells (Szałach *et al.*, 2015).

The current study showed that both Amitriptyline and Escitalopram caused an increase in red blood cells (RBC). The reason for this increase may be due to the hormone erythropoietin, which regulates the production and maturation of red blood cells in the bone marrow, the liver has a role in secreting this hormone about 10- 15% of the total hormone level. (Machalinska *et al.*, 2002), and antidepressant drugs damge the liver. (Voican *et al*., 2014).

The result of our study showed a decrease in (PLT), this results is agreement with Song and other (2012), which indicated that antidepressants such as venlafaxine cause a decrease in (PLT). To explain this, Ishizuka and other *(*2012), mention that stem cells participating in cell proliferation contain angiotensin receptors that affect the stimulation of DNA synthesis, leading to a change in the number of platelets.

The two most elements important for reproduction are (FSH) and (LH), which are released from the anterior pituitary gland by stimulation from (GnRH) , which is released from the hypothalamus according to the axis (hypothalamus – pituitary – gonads).There are three cells essential in the testis are germ cells that pass through several stages to mature and become sperms , Leydig cells (cell interstitial) are located between the seminiferous tubules, the third type of cells are Sertoli cells which located between the epithelial cells of germ cells called (support cells). Hormone FSH targets Sertoli cells and is motivated to carry out their functions, which produce and release nutrients and molecules essential to the process of spermatogenesis. Hormone LH target Leydig cells, stimulating them to synthesize and secrete testosterone. Inhibin –B is released from Sertoli cells by FSH signals, they are feedback molecules. Testosterone stimulates the maturation of germ cells by binding to its active receptor, the testosterone hormone diffuses between the seminiferous tubules, and testosterone has a negative feedback, as the case with FSH. The spermatogenesis process is controlled by hormones (FSH, LH, and Testosterone). (Aggarwal *et al*., 2013; Corradi *et al*., 2016; Clavijo and Hsiao, 2018; Dutta *et al*., 2019).

Disrupted the synthesis and release processes and the transfer of hormones and metabolism by drugs (Diamanti-Kandarakis *et al*., 2019; Thomas *et al*., 2010).

Can be explained the neurotransmitters in the central nervous system are responsible for the nervous system (Lovinger, 2008). Where many studies have

shown that the regular neuronal and hormonal signals at different levels of the axis (HPG) that control neuroendocrine and reproductive functions (Maffucci and Core, 2009; Oyola and Handa, 2017).

Other study noted the mental drugs are working by modifying the activity of nervous transport on the axis (HPG) (Bhuvaneswar *et al*., 2009).

The decrease in FSH, and LH due to neuroendocrine factors such as serotonin causes increased prolactin hormone by inhibiting dopamine and acting to increase prolactin to suppress GnRH and its decrease causes a decrease in FSH, and LH. (Llgin, 2020).

Increased serotonin causes dopamine inhibition and dopamine has an important role in the capacitation vitality and movement of sperm ability. (Ramirez *et al*., 2009).

D_2 : receptors are present in seminiferous tubules and interact directly with the proliferation of germ cells , antidepressant drugs act on these receptors , which leads to inhibition of D_2.(Goyal *et al*., 2001; Sethi and Chaturvedi, 2009).

Angiotensin receptors: they are receptors present in the Leydig cells in the testis and are considered a regulator of the function of the testis by luteinizing hormone secreted by pituitary gland. Inhibiting these receptors causes a decrease in the secretion of Testosterone from the Leydig cells. (Leung and Sernia, 2003).

In this study, both the Escitalopram and the Amitriptyline caused a decreased in the sperm concentration as well as the treatment caused a decrease in sperm movement compared to the control, as for the dead sperm increased, our study also showed that the increase in serotonin due to taking antidepressant lead to a change in hormone levels (FSH, LH, and Testosterone).

According to a results in agreement with Erdemir and other (2014), mention that the use of antidepressant lead to oxidative stress and production of reactive oxygen species (ROS) in abundance and this is due to the damage the antidepressant dose to the membrane mitochondria. An imbalance in the redox balance can negatively affect cell vitality. It has been found that disturbances in intracellular ROS levels, and lipid peroxidation affect testicular tissues and sperm cells (Aprioku, 2013; Bhardwaj *et al*., 2021). The data indicate that treatment with antidepressants due to oxidative stress leads to damage to cellular components as well as damage to the DNA of sperm cells (Viola *et al.*, 2000; Singh *et al*., 2016).

Some studies showed that impotence and decreased spermatogenesis are due to changes in (FSH, LH, and Testosterone). (Bahmanpour *et al*., 2009).

The membranes of the sperm cell contain unsaturated fatty acids, and the amount of these acids decreases due to the up-regulation of ROS, which causes a decrease in the number of sperm as well as sperm movement, testicular dysfunction, hypogonadism, and abnormal sperm parameters occur due to the negative effect of ROS directly or indirectly on the major enzymes associated with antioxidants for sperm (Doshi *et al*., 2012; Bandegi *et al*., 2018).

Antidepressants cause changes in cellular antioxidants and cause an increase in thiol pool levels (Duda *et al*., 2016). NF-KB is a transcription factor that has an important role in cell growth, development, survival, and proliferation, thus protecting the cell and maintaining ROS levels. NF-KB may be involved in many diseases (Yon *et al*., 2010), increased transcription activity NF-KB may be associated with decreased cell viability (Bartholoma *et al*., 2002). And genes involved in spermatogenesis and development are associated with NF-KB (Morgan and Liu, 2011).

BDNF is factor that protect the cell and its organelles. Some antidepressants affect cells in a neural way apoptosis can be inhibited by an increase in these factors (Ozbeyli *et al*., 2019; Czarnywojtek *et al*., 2020).

MuMa is a protein that has a major role in cell division and depends on the phase of the cell cycle and is distributed by phosphorylation and dephosphorylation (Bhattachrya *et al*., 2013).

When treated with an antidepressant, changes in MuMa level were observed, and this may be associated with disorders of depolymerization and microtubule polymerization, leading to impairment of the spindle system. (Karabasheva and Smyth, 2019).

Numerous studies have shown that oxidative stress damages sperm through various aspects, including induction of DNA damage, lipid peroxidation of the plasma membrane of sperm, morphology and impaired sperm motility (Anbara *et al*., 2018).

Studies have also shown that oxidative stress reduced sperm concentration through apoptosis of immature reproductive cells through DNA damage (Safarinejad, 2008).

The production of energy for the movement of the flagellum and hyperactivity results from the process of oxidation of mitochondria, and therefore the movement of sperm depends on this process and also depends mainly on the flow of calcium, so the effect on the movement of sperm interferes with the structure and function of calcium channels directly or indirectly (Correia *et al*., 2015).

The current study showed that the Amitriptyline and Escitalopram through the histological diagnosis over six weeks of taking the two treatments lead to a decrease in spermatogonia and clear spaces between the spermatogonia layer and

primary spermatocyte layer, proliferation of Sertoli cells, and a decrease in spermatid and lumen wider in testis. Histological epididymis image showed the epithelial cell layer changed from the pseudostratified columnar type to the simple type, and the cell shape changed from the columnar to the cuboidal shape. The explanation for these changes may be due to an imbalance in hormone levels.

According to a results in agreement with Afify and other (2010), mention that the Amitriptyline leads to a decrease in the testicular function as well as explained by Bahmanpour and other (2009), Amitriptyline causes a decrease in testosterone and a decrease in sperm count, which was evident from the morphological changes in the testicular tissue.

Aggrwal and other (2012), mention that the effect of Antidepressants which distorted the seminiferous tubules and this result is also in the current study.The presence of membrane-bound receptors is one of the most important factors on which the side effects of antidepressants depend, and these receptors by antidepressants participate in the cellular response, testicular tissue contains alpha-2a receptors (Mhaouty-Kodja *et al*., 2007; Fujinoki, 2011).

Studies have shown that time and dose are among the factors that reduce metabolic efficacy, cell proliferation rate, and abnormalities in a germ cell (El-Fiky *et al*., 2016), and reverse the inhibition of spermatogenesis (Riggin and Koren, 2015).

Fibroblast growth factors (FGF2) broadly control biological functions including cell differentiation, migration, survival, and reproduction, studies have shown that improving FGF2 synthesis, has been associated with cell proliferation in spermatogenic cells by antidepressants (Caviedes *et al.*,2017).

The mechanism of action of FGF2 is not well understood and needs further studies, but some studies have shown that FGF2 signaling controls the early stages of spermatogenesis (Gonzalez *et al*., 2006; Mallei *et al*., 2002).

And another explanation for the result is that antidepressants cause the induction of lipid peroxidation and this causes the release of free radicals, which leads to membrane disorganization, causing membrane fluidity. (Hanif and Aslam, 2019)

Cell damage in the reproductive organs may be a result of oxidative stress and the production of reactive oxygen species, SSRIs stimulate DNA fragmentation and ROS formation. (Atli *et al*., 2017)

Mohmed and other (2015), mention that the imbalance between the production of reactive oxygen species and defensive antioxidants stimulates oxidative stress, which causes damage to DNA, proteins and lipids, which causes apoptosis and necrosis.

When spermatozoa move from the testis to the epididymis, they are nonfunctional gametes. as it passes through the epididymis, it matures and acquires progressive movement and can fertilize the ova, the reason for the maturation of spermatozoa when they moves to the epididymis are the interaction of spermatozoa with proteins that are made and secreted in the epididymis epithelium .about 40 % of infertility men have an unknown cause that may be the result of disorders of spermatozoa maturation and this confirms understanding of the function of the epididymis (Cornwall, 2009)

Substances secreted from the seminal vesicles are important for the movement of sperm, suppression of the immune activity in the female reproductive tract, semen coagulation and stability of sperm chromatin. The fluid secreted from the seminal vesicles is consist of fructose, which the main nutritional source of sperm in the semen, liquid secreted also has ascorbic acid, other simple sugars, prostaglandins and amino acids. (Aggarwal *et al*., 2014).

Numerous studies have shown that after taking antidepressant drugs, men suffer from problems related to dysfunction of the accessory sex glands, and these

problems are related to fertility, such as a decrease in movement and vitality of sperm. (Gonzales, 2001).

The study showed that the Testosterone hormone affects epithelial cells and acts to reduce basic secretory proteins, this study found a decrease in the level of the testosterone hormone, which leads to the proliferation of folds in the seminal vesicles. (Aggarwal *et al*., 2014)

According to a results in agreement with Giuliano and Clement (2012), the presence of receptors in the seminal vesicles in rodents, which are responsible for the contractile movement of the seminal tract.

The histochemical results of the current study showed the presence of the interaction of PAS stain with basement membranes, and the interaction ranged between weak, moderate and strong.

According to a results in agreement with Soliman and other (2017), the reason for the interaction of PAS with basement membranes is the oxidative stress caused by antidepressant.

El-Din and Abd-El-Aty, (2012) mention that the formation of hydroxyl molecules, the highly reactive oxidizing molecules, caused the oxidation of fats and thus caused damaged to nucleic acid and proteins.

Chapter Six
Conclusions and Recommendations

6- Conclusion and Recommendation

6-1- Conclusions

1. Amitriptyline can be lead to decrease in the body weight, organs weight (testis, epididymis, seminal vesicles), while the Escitalopram cause increase in the body weight and decrease in organs weight.
2. Both Amitriptyline and Escitalopram can be cause increase in the count of WBC. Amitriptyline can be lead to high decrease in the hormones levels (FSH, LH and Testosterone), while Escitalopram canbe lead to low decrease.
3. Amitriptlyine was found to be more damage a than Escitalopram, so the use of Escitalopramis preferable when necessary.

6-2- Recommendations

1. Recommend molecular biological study to determine the effects of Amitriptyline and Escitalopram.
2. A histological study on another organs of male mice such as brain, pituitary and adrenal glands
3. Comparative study of effect (Amitriptyline, Escitalopram) on male and female of different age for long period (3-5) momths on (estradiol, FSH and LH).

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Appendixes

Appendix 1:

Measurement of serum luteinizing hormone (LH):

The architect FSH assay is a tow – step immunoassay to determine the presence of FSH in serum and plasma using CMIA technology with flexible assay protocols, referred to as chemiflex

1- Sample and anti-B LH coated paramagnetic microparticles are combined. The LH present in the sample bind to the anti- B LH coated microparticles.

2- After washing, anti – a LH acridinium – labeled conjugate is added to great a reaction mixture.

3-Following another wash cycle, pre – trigger s solutions are added to the reaction mixture.

4- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of LH in the sample and the (RLUs) detected by the architect I system optics.

Appendix 2:

Measurement of serum follicle stimulating hormone (FSH):

The architect FSH assay is a tow – step immunoassay to determine the presence of FSH in serum and plasma using CMIA technology with flexible assay protocols, referred to as chemiflex

1- Sample and anti-B FSH coated paramagnetic microparticles are combined. The FSH present in the sample bind to the anti- B FSH coated microparticles.

2- After washing, anti – B FSH acridinium – labeled conjugate is added to great a reaction mixture.

3-Following another wash cycle, pre – trigger s solutions are added to the reaction mixture.

4- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of FSH in the sample and the (RLUs) detected by the architect I system optics.

Appendix 3:

Measurement of serum Testosterone hormone:

The architect 2 nd Generation Testosterone assay is a delayed one – step immunoassay for the quantitative determine of Testosterone in serum and plasma using CMIA technology with flexible assay protocols, referred to as chemiflex

1-samples, assay specific diluent and anti- testosterone (sheep, monoclonal) coated paramagnetic microparticles are combined the testosterone present in the sample bind to the anti- testosterone coated microparticles.

2- After incubation, testosterone acridinium – labeled conjugate is added to great a reaction mixture.

3- After further incubation and washing , pre – trigger and trigger solutions are added to the reaction mixture.

4- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of testosterone in the sample and the (RLUs) detected by the architect I system optics.

Appendix 4:

Nigrosine 10% solution procedure:

Weigh 5 grams of nigrosine and add 50ml deionized water.

Dissolve using gentle heat.

Cool the liquid to room temperature and filter using filter paper.

Appendix 5:

Neutral buffered formalin (10%)

Neutral buffered formalin (10%) was prepared by dissolving 6.5 g Na2HPO4 and 4 g KH2PO4 in 900 ml of distilled water, then 100 ml of 37% formaldehyde were added and mixed thoroughly.

Appendix 6:

Hematoxylin stain

Hematoxylin Solution (Ehrlich's)

Ethanol (100%)……...100 mL

Glycerol……100 mL.

Glacial acetic acid……..10 mL

Hematoxylin…….2g

Humotoxylin is mixed with ethanol alcohol, then added glycerol and glacial acetic acid, then added a quantity of potassium alum, these components place in

glass bottle exposed to sunlight. The vial is opened for a period, then closed and shaken. This process is repeated for several weeks until the dye matures.

Appendix 7:

Eosin Y stain

Eosin Y ..... 1 g

Distilled water 20ml 95%
Ethanol..... 80 ml

Mix to dissolve and store in room temperature.

Appendix 8:

Periodic Acid Schiff PAS technique Solutions Periodic Acid Solution:

Periodic acid ..... 1 g

Distilled Water..... 100 ml Schiff Reagent: Basic Fuchsin.....1.0 gm.

Sodium metabisulphite .....2 gm.

Distilled water.....100 ml

Hydrochloric acid..... 2 ml

Charcoal activated 0.3gm

Dissolve basic fuchsin in boiling water, cool at 50 C and filter. Add sodium metabisulphite and HCl.store at dark room at room temperature overnight. Add

charcoal, shake for one minute and filter. Results: Glycogen, neutral glycoprotein: magenta

Appendix 9:

Dosage calculation method

The dose was calculated based on the weight , so the adult person of reproductive age was assumed to be at a weight of (70 Kg), and the dose for this person was (20 mg/day), so the weight was converted to (70000gm) to match the weight of the mouse , whose average weight was(30 mg),then(70000gm)was divide by(30gm) and the result is (2333 mice) , the drug tablet is dissolved in distilled water (tablet 20 in 2 ml), and every(2 ml) equals(2000 µl) ,thus every (2000 µl) is sufficient for (2333) mice, so the dose was(0.84 µl) , and this dose was divided in the morning and evening, so it was (0.42 µl) in the morning and evening. (Bruggeman, and O'Day, 2021).

الخلاصة

الملخص

أجريت هذه التجربة باستخدام (90) من ذكور الفئران وقسمت الى ثلاثة مجموعات كل مجموعة تتكون من (30) فأر ,المجوعة الأولى(الضابطة) تلقت محلول ملحي , المجموعة الثانية جرعت الامتربتلين والمجموعة الثالثة تلقت الاسيتالوبرام . وكانت الجرعة لكل مجموعة(0.42) مايكروليتر لمدة ستة أسابيع مرتان في اليوم. خلال فترة الدراسة تم قياس الاوزان في نهاية الأسبوع (الثاني – الرابع- السادس) , القتل الرحيم وجمع الدم لغرض فحص (CBC) و فحص الهرمونات(الهرمون اللوتيني, الهرمون المنبه للجريب , هرمون التستوستيرون) , وزن الأعضاء (الخصى ,البربخ, الحويصلات المنوية), جمع السائل المنوي من البربخ لغرض فحص (تركيز الحيوانات المنوية , حركة الحيوانات المنوية , الحيوانات المنوية الميتة), تحديد تغييرات الانسجة بسبب الدواء باستخدام نوعين من الصبغات, الهيماتوكسلين- الايوسين ,حامض البريودك – كاشف شيف (PAS),دراسة التغييرات في القياسات النسيجية (قطر الانابيب المنوية, قطر قناة البربخ, عدد الخلايا : سليفات النطف, الخلية النطفية الأولية, الخلية النطفية الثانوية, ارومة النطفة).

أظهرت نتائج الدراسة انخفاض معنوي($P<0.05$) في وزن الجسم في مجموعة الامتربتلين , اما في مجموعة الاسيتالوبرام حصلت زيادة في الوزن($P<0.05$) في الأسبوع السادس مقارنة بالمجموعة الضابطة , وكان هناك انخفاض معنوي ($P<0.05$) في وزن الخصى , البربخ والحويصلات المنوية في الأسبوع الرابع.

لوحظ تغييرات معنوية في معايير الدم, حصل انخفاض معنوي($P<0.05$) في مستوى الهرمون المنبه للجريب في مجموعة الامتربتلين و لا توجد تغييرات في مستوى الهرمون في مجموعة الاسيتالوبرام , انخفاض معنوي في مستوى الهرمون اللوتيني وكان الانخفاض في مجموعة الامتربتلين اكثر من الانخفاض في مجموعة الاسيتالوبرام في الأسبوعين الثاني والرابع, بينما في الأسبوع السادس لم تكون هناك تغييرات في مستوى الهرمون في مجموعة الاسيتالوبرام بينما استمر الانخفاض في مجموعة الامتربتلين, انخفاض في مستوى هرمون التستوستيرون في مجموعة الامتربتلين اكثر من الانخفاض في مجموعة الاسيتالوبرام , لوحظ انخفاض في تركيز وحركة الحيوانات المنوية في مجموعة الامتربتلين اكثر من الانخفاض في مجموعة الاسيتالوبرام وزيادة معنوي في عدد الحيوانات المنوية الميتة في كلا المجموعتين .

لم يحصـل تغير في قطر الأنابيب المنوية وقطر قناة البربخ في كلا المجموعتين وكذلك حصـول انخفاض في اعداد الخلايا (الخلايا الجذعية النطفية - الخلايا الطفية الأولية – الخلايا النطفية الثانوية- النطف) في كل من المجموعتين.

بينت نتائج الدراســة حدوث تغييرات نســيجية في خصــى الفئران في مجموعة الامتربتلين هي وجود مسـافات بين سـليفات النطف , مسـافات بين طبقة سـليفات النطف و الخلايا النطفية الأولية, تكاثر خلاي سرتولي , نقص الخلايا النطفية الأولية, توسع التجويف ونقص في ارومة النطفة .

اما مجموعة الاسـيتالوبرام أظهرت قلة في سـليفات النطف , وجود مسـافات بين طبقة سـليفات النطف وطبقة الخلايا النطفية الأولية ,الطبقات الخلايا (ســليفات النطف- الخلايا الطفية الأولية – الخلايا النطفية الثانوية- ارومة النطف) غير منتظمة في الترتيب , غياب طبقة ارومة النطف, توســع التجويف , غياب التجويف في بعض المقاطع , تغيير في حجم الخلايا الجذعية وكان تفاعل الغشــاء المخاطي مع PAS يتراوح من المتوسط الى القوي في كل من المجموعتين.

حدثت تغييرات في نســيج البربخ في كل من المجموعتين (الامتربتلين- الاســيتالوبرام) , التغييرات في مجموعـة الامتربتلين هي وجود فجوة بين الخلايـا الظهـاريـة ,غيـاب الحيوانـات المنويـة في التجويف والتجويف اصبح ضيق وغير منتظم وتضخم الخلايا الظهارية . اما التغييرات في مجموعة الاسيتالوبرام هي تضــخم الخلايا الظهارية , قلة الحيوانات المنوية في التجويف , وجود حيوانات منوية غير ناضــجة دائرية, تغير الخلايا الظهارية من النوع العمودي المطبق الكاذب الى النوع البسـيط وتغيير شـكل الخلايا من عمودي الى مكعبي وكان تفاعل الغشـاء المخاطي مع PAS يتراوح من المتوسط الى القوي في كلا المجموعتين.

حدثت تغييرات في الحويصــلات المنوية في كل من المجموعتين , التغييرات في مجموعة الامتربتلين هي زيادة عدد الطيات , التقسـيم الطبقي للخلايا الظهارية , تحول النسـيج من ظهاري عمودي الى مكعبي وقلة في افراز الحمضات, اما التغييرات في مجموعة الاسيتالوبرام هي زيادة في عدد الطيات , ضيق التجويف ,قلة في افراز الحمضــات والتقسـيم الطبقي للخلايا ,تضــييق التجويف وكان تفاعل الغشــاء المخاطي مع PAS يتراوح من ضعيف الى قوي.

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دراسة مقارنة نسيجية ، كيمائية نسيجية وفسيولوجية لتأثير (امتربتلين، اسيتالوبرام) على بعض الاعضاء في الجهاز التكاثري لذكور الفئران البالغة (*Mus musculus*)

دراسة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

اسراء عبد الامير نعيم

بكلوريوس تربية / علوم الحياة (2011)

بأشراف

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