Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Karbala / College of Veterinary Medicine

Physiology, Biochemistry and pharmacology Department

Assessment the role of Ubiquinone (coenzyme Q10) against to toxicity effect of Busulfan in male rats

A thesis

Submitted to the Council of The College of Veterinary Medicine, University Of Karbala In Partial Fulfillment of The Requirements For the Degree of Master of Science In Veterinary Medicine/ Physiology

BY

Rafal Muhammed Hussein Muhammed Reda

2016-2015

Supervise

Asst.prof.Saba Ibrahim Salih

2020 A.D **1442 A.H**

DEDICATION

Firstly of all, I would like to express my grateful thanks to Allah, who has given me everything.
He was Sandy, and he stood with me at all stages of my studies in order to reach this level ... **My father Muhammad Hussein**

To our hope in this life. You are the candle that lights my way. Illiterate..**my mother**.

To the precious jewel that my brothers and I imitate **.... (Rawz) my sister.**

To My brothers, you are the bright moons, you are a blessing from the sky. There are no words to express my thanks to you**…… (Redaa, Ali, Muhammad Jawad, Mumal).**

My sincere thanks and gratitude to my maternal brother and uncle **Hasanein Khazal,** who had a role in bringing the research materials from China.

Rafal al-shibany

Acknowledgements

I thank and appreciate Alla Almighty firstly and finally who guided me to set up this search.

Great thanks for **Prof. Dr. Wifaq Jabbori AL-Bazi** / Dean of College of Veterinary Medicine University of Kerbala for her help in encouragement.

I would like to express my supervisors gratitude and deep appreciation to my sweetheart, mother and honorable one **Assistant. professor. Saba Ibrahim Saleh,** who was a model to emulate. I thank her for standing up and helping me seriously in the research steps.

My sincere thanks and gratitude to the fatherly professor **Asst. Prof. Dr. Ayyed Hameed Hassan AL-Mossawi**.

I would like to extend my thanks and appreciation to **Prof. Dr. Wafaa. Kadhim Jasim and dr.sajaa R.Al-saedi** for her advice and guidance through this work.

I thank people from the bottom of my heart to my friends who have stood next to me during the scientific research stage miss **Huda Abdul Zahra Najy** and miss **Kawther Yahya Jassim AL-Assady**.

Finally, thanks go to every person help me in my research.

Rafal al-shibany

Abstract

Busulfan is a chemotherapy treatment for cancer, while coenzyme (ubiquinone CoQ10) is a coenzyme that works to enhance chemical reactions as antioxidant against free radicals in the body and It helps in decrease the effect of treating by chemotherapy drugs like Busulfan .

This study was conducted at Department of Physiology, Biochemistry and pharmacology in College of Veterinary Medicine / University of Karbala from December 2019 to end of February 2020. The role of ubiquinone was evaluated against the toxic effect of busulfan on sperm in adult albino mice at periods of 28 and 56 days.

Forty eight adult male rats were divided into four groups randomly and equally in each group of (12 rats for each group, animals feeding and the water was available for 8 weeks periods.

The first group was injected with distilled water as control group, second group was injected intraperitonially with single dose10 mg / kg of Busulfan (chemotherapy) ,while The third group injected intraperitonially by single dose of Busulfan 10 mg / kg and the coenzyme ubiquinone (COQ10) 10mg\kg daily ,and the fourth group injected intraperitonially with only CoQ10 10 mg / kg daily to the end of study.

The results showed a significant decrease ($p \leq 0.05$) in hematological parameter (RBC,Hb,Pcv,Plt and WBCs)in both group G2 and G3 While No significant ($p \geq 0.05$) inG4 as comparison to G1 in 28day study period. At 56 day ,the results showed a significant decrease ($p \leq 0.05$) in hematological parameter with improvement in (RBC,Hb,Pcv,Plt and WBCs) in both group G2 and G3 While No significant ($p \geq 0.05$) inG4 as comparison to G1.

The Results of hormones indicated that testosterone ,ADPN,INH-B and ACV-A hormones has a significant decrease ($p \leq 0.05$) in G2, but not significant ($p \geq 0.05$) in G3 and G4 when compared with G1control group. While LH and FSH hormones were significant ($p < 0.05$) increase in G2 and G3,but not significant ($p \geq 0.05$) of G4 when compared with G1 Control group at two peroids study 28 and 56 days.

The Result of liver parameters showed that AST,ALT, ALP ,albumin and bilirubin has a significant increase ($p \leq 0.05$) in G2 and G3, but not significant($p \geq 0.05$) in G4 when compared with control group at two periods study 28 and 56 days. While results showed renal parameters Urea, Uric acid and creatinine has a significant increase ($p \leq 0.05$) in G2 and G3, but not significant ($p \geq 0.05$) in G4 when compared with G1control group at two periods study 28 and 56 days.

The histological study showed a significant decrease in the diameter of the seminal tubes, as well as the occurrence of erosion of the testicle tissue in the animals injected with Busulfan (chemotherapy) compared to the control animals. Also observed changes in the kidneys, which is a decrease in the diameter of the glomeruli in animals treated with Busulfan compared to control animals. But when animals are injected with co 10 we notice an improvement in damaged tissues as a result of chemotherapy toxicity.

List of Abbreviations

Abbreviation term	Call name
A.O	antioxidant
ACV-A	Activin A
ADPN	Adeponectin
ALB	Albumin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
b.w	body wight
BU	Busulfan
DMSO	Dimethyl sulfoxide
EDTA	(Ethylen ediaminetetra acetic acid)
E-H.S	Eosin-Hematoxilin Stain
ELIZA	Enzyme linked immune sorbent assay
ESR	Erythrocyte sedimentation rate
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin –releasing hormone
H&E	Hematoxylin and eosin
HB	Hemoglobin
I.P	Intraperitoneal injection
INH-B	Inhibin B

L.S.D.	**Least significant difference**
LH	**Luteinizing hormone**
mg/kg	**Milligram per kilogram**
mIU/ml	**Microliter international unit per milliliter**
PCV	**Packed cell volume**
Q-10	**CoenzymeQ10(COQ10)**
RBC	**Red blood cells**
RF	**Renal failure**
ROS	**Reactive oxygen species**
SCs	**Sertoli cells**
SD	**Standard deviation**
SEM	**Standard error of mean**
SPSS	**Statistical Program for Social Sciences**
T	**Testosteron**
TM	**Tetramethylbenzedine**
U.A	**Uric acid**
UK	**United Kingdom**
Ur	**Urea**
WBC	**White blood cells**
WHO	**World Health Organization**
µm	**Micron meter**

Contents

Chapter three /Materials & Methods

List of Figures

List of Table

Chapter One
Introduction

Chapter One ……. Introduction

1.Introduction

Infertility is the inability of couples to achieve pregnancy after at least one year of sexual intercourse and Infertility in men constitutes about 50% of infertility cases (Gunes *et al*., 2020) .

Many factors such as life style, drugs, toxicants, and infections lead to infertility(Bordbar H *etal*., 2013) . Some drugs such as cisplatin and busulfan induce reproductive toxicity. Busulfan is used to treat chronic myelogenous leukemia and blood disorders (Amr and Alaaeldin, 2006). It has been revealed that busulfan decreases sperm motility, viability and count. This drug reduces testicular weight, and diameter (Wang DZ, 2010). Other causes like drugs, toxic substances and infection may lead to infertility in men. Some medicines like Busulfan has toxic effects on the reproductive system (Gabrielsen *et al*., 2016). Chemotherapy and radiotherapy in Bu are associated with many changes in the male reproductive system, which is a white crystalline powder, its is an alkylation agent and a cytotoxic drug back to the alkyl sulfonate group(Hassannejad *et al*., 2020).

Busulfan, 1,4-bis [methanesulfonyl-y] butane, is a chemotherapeutic agent **(**Suttorp and Millot, 2010) also, it has been given in higher doses before a bone marrow or stem cell transplant for other types of cancer(Bourgeois A *et al*., 2013) and hematological stem cell transplant for nearly 20 years(Krivoy N *etal* ., 2008) Busulfan is inhibits cell division by sticking to one of the DNA strands(Iwamoto *etal*., 2004). therefore, organs, tissues and cells with high division activities such as testes and germ cells are more susceptible to busulfan side effects. Spermatogenesis involves the passage of diploid germ cells through the reductive divisions of meiosis in order to generate round haploid spermatids(Dun MD *etal*.,

2012). It is clear that cytotoxic therapy such as certain chemotherapy drugs influences spermatogenesis at least temporarily and in some cases permanently(Panahi Y *etal*., 2015).

The negative effect of cytotoxic drugs on spermatogenesis has been extensively investigated and was recently reviewed Most harmful drugs are nitrogen mustard derivates, such as busulphan and melphalan, and alkylating drugs, such as cyclophospfamide and procarbazine(Dohle G. R, 2010). has many side effects on different body parts such as bladder, liver, skin, nervous system, and gonadotropic function, as well as possibly carcinogenic and teratogenic (Parfitt, 2012). Also, it is used in treating of chronic leukemia, cancer of ovary, and before transplantation of bone marrow in patients with cancer (Hassannejad *et al*., 2020). Busulfan has distorting and carcinogenic properties, It kills the stem cells of sperm and disrupts the connections between Sertoli cells and sperm cells in the base layer (Anjum *etal*., 2011).

Coenzyme Q10 is a key element in electron transport in oxidative phosphorylation which occurs in mitochondria. (Littarru and Tiano, 2005) (Belardinelli *etal*., 2008), it is found in almost all tissues of the body, and it is a very effective antioxidant (Sharideh *etal*., 2020). Coenzyme Q10 is an important electronic shuttle for the electron transport system in mitochondria. Additionally, when quantities are less than CoQ10, there can be inhibition of cellular oxidation of fats, proteins and DNA (Ozcan *etal*., 2016). Several well researched studies have shown that antioxidants are effective. These include vitamins C, E, carnitine and coenzyme Q10, which can be considered the first line of treatment(Cannarella *etal*., 2020). It is a powerful antioxidant, membrane stabilizer, and the catalyst for producing adenosine triphosphate through oxidative phosphorylation. Coenzyme

Q10 has been widely applied in nutritional supplements and cosmetic in Japan, USA and many other countries. (Hatanaka *etal*., 2008). CoQ10 deficiency can result in sperm damage, decreased sperm motility, and sperm count. Studies have shown that the use of supplements containing CoQ10 can improve reproductive outcomes in males who have fertility problems (Alahmar *etal*., 2020). Further more, CoQ10 semen concentrations are positively associated with sperm motility and counting. Accordingly, patients treated with CoQ10 had more sperms and formed better sperms compared to those who did not receive CoQ10 (Balercia *etal*., 2009).

Mitochondrial malfunctioning is implicated in the pathogenesis of a variety of disorders, including cancer and multiple neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. Disturbance of mitochondrial vital functions, e.g., production of ATP, calcium buffering capacity, and generation of ROS, can be potentially involved in disease pathogenesis. Neurological disorders caused by mitochondrial deterioration are often associated with cell loss within specific brain regions. In contrast, mitochondrial alterations in tumor cells and the "Warburg effect" might lead to cell survival and resistance of tumor cells to chemotherapy. **(**Gogvadze *etal*., 2009).

1.1 Aims of study

The aimed of the study was designed to evaluate that effects of ubiquinone (coq10) as antioxidant for infertility in rats which caused by busulfan anticancer drug,through at substantial for the fllowing:-

1.Analysis of seminal fluid for estimating counts,viability and abnormality of spermatozoa.

2.Examination of some hematological parameters which include(RBCs,Hb, PCV, Platelets and WBCs).

3.Measurmant the biochemical parameter for liver function tests(AST,ALT,ALP,Albumin and bilirubin). And kidney function tests(Creatinine,urea and uric acid).

4.Assay some levels of hormones which included (Testosterone, FSH, LH, adeponectine, activing A and inhibin B) .

5.Histopathological changes in some internal organs (testis ,liver and kidneys).

Chapter Two
Literature

2.Literature Review

2.1 Male Reproductive System

The male reproductive system consist of the testes and a series of ducts glands and penis. Sperm is produced in the testes and are transported through the reproductive ducts. Which consist of epididymis, delayed duct, ejaculatory duct and urethra (Ndovi *et al.*, 2007).

2.2 The Testes

The testes (singular: testis) are commonly known as the testicles, are a pair of ovoid glandular organs such are responsible for the production of sperm cells and the male sex hormone testosterone (Petersen and Soder, 2006). The testes were produced as many as 12 trillion sperm in a male's lifetime, about 400 million of that are released in a single ejaculation. There testes are protected by the tunica vaginalis, an extension of the peritoneum of the abdomen, and the tunica albuginea, both testis is divided by invaginations of the tunica albuginea that divide it into severaled hundred small segments called lobules, both lobule contains several tightly coiled tubes termed seminiferous tubules (Taylor , 2017).

2.3 Seminiferous Tubules

The seminiferous tubules of the testis consist of two large cell types: the germ cells and the Sertoli cells(SCs). The SCs are uniformly distributed in the seminiferous epithelium along with developing germ cells and they are nourish the germ cells throughout their development. Seminiferous Tubules consist of highly complex epithelial tissue containing sperm cells at different stages of development (spermatogoina ,primary spermatocyte and secondary spermatocyte and spermatids, spermatozoa, choroid cells or sertoli cells).

The epithelium is surround by the basal plate subsist of the basal layer and extracellular layers containing of myofibroblasts responsible for the contraction of spermatozoa that are necessary for the transfered of sperm and testicular fluid , the regular of the process and the construct of the testicular blood barrier (Santoro and Romeo, 2001).

2.4 Epididymis

The epididymis play important role in vertebrate reproduction, mainly in sperm maturation and fertilization.(Rosati *et al*.,2020).When transiting through the lumen of the epididymis, sperm undergo maturation by interacting with proteins synthesized and secreted by the epididymal epithelium ,in addition, the epididymis too functions in sperm transport and concentration, immune protection of sperm, and serves as a sperm storage (Cornwall,2009).Generally the epididymis is divided into four main anatomical sector: the initial segment (only present in rodents)caput, corpus and the cauda, with both epididymis region performing separate functions essential to the different steps of sperm maturation. In support of this view, early studies demonstrated that the caput and corpus provide microenvironments for sperm maturation, and that the cauda region primarily serves as a storage site for functional spermatozoa (Belleannee *et al;*2012).

2.5 Spermatogenesis

In mammalian species, spermatogenesis occurs in the seminiferous tubules of the testis and relies on the appropriate expansion of undifferentiated and

differentiated spermatogonial to the entry of germ cells into meiosis and subsequent spermiogenesis (Ehmcke *et al.,* 2006;Huleihel *et al*., 2007).

The process of spermatogenesis under go to the general control of the endocrine system (Sofikitis *et al*.,2005).The endocrine stimulation of spermatogenesis involves of follicle stimulating hormone (FSH), and luteinizing hormone (LH), and testosterone, produced by the Leydig cells in the testis.Both of these steps represents a key element in the spermatogenic process (i) the multiplication of spermatogonia by the process of mitosis; (ii) meiosis, which reduces the chromosome number from diploid to haploid and commences with the entry of type B spermatogonia into the prophase of the first meiotic division. These cells, now called primary spermatocytes, divide to form secondary spermatocytes, and then divide again to form round spermatids; (iii) the successful transformation of the round spermatid into the complex structure of the spermatoza ,while attaching to sertoli cells, this phase being called spermiogenesis. this regulation occurs at two major levels: (i) hormonal and endocrine and (ii) paracrine/autocrine (Gilbert, 1997).

Various factors such as stress, nutrition and certain drugs may affect of spermatogenesis process and cause azoospermia or infertility. The most common side effects on some cancer drugs is men's azoospermia. Nudell *et al*.,2002; Shetty,2005.

2.5.1 Role of Sertoli Cell in Spermatogenesis

The Sertoli cell was discovered in 1865 by Enrico Sertoli and is also known as the "nurse cell" of the seminiferous epithelium. It is a tall, columnar, polar cell extending from the base to the lumen of the seminiferous tubule, with

many cytoplasmic crypt-like extensions due to the reshaping of the cell by germ cells**(**Wolski,2006).

Without the support of Sertoli cells, spermatogenesis could not be completed. The extensive functions of these cells include (1) providing structural support for the germ cells, (2) aiding in the translocation of the germ cells, (3) secreting many trophic 8 factors and nutrients for the germ cells, (4) phagocytosing dead/damaged germ cells, (5) forming the blood testes barrier (in part to define the polarity of the seminiferous epithelium and in part to create immunity), and other essential functions in spermatogenesis(Wolski,2006).

In the testes of adult male, the number of Sertoli cells in each testis has been determined before adulthood; the number of Sertoli cells determines the volume of the testes and the number of sperm produced per day (Sharpe *et al* ., 2003).

Sertoli cells are joined by tight junctions to form the basolateral blood–testes barrier (BTB), which prevents large molecules from passing from the blood into the seminiferous tubules The junctions barriers serve to protect the developing germ cells from the immune system and exposure to systemic toxins. (Cheng and Mruk, 2010).

Blood-testicular barrier is not the isolation between blood and testicular tissues, but precise boundary formed between haploid and diploid germ cells, It is clear that the number and quality of spermatozoa are determined by the number of functional Sertoli cells. Thus, the size of the testes is usually used as a measure of the number of Sertoli cells, The main function of Sertoli cells is to regulate spermatogenesis and to change the proportion of sperm production (Petersen and Soder ,2006).The secretory

function of Sertoli cells provides transportation for nonmotile spermatozoa from the testis into the excurrent duct system (Filippini *et al.*, 2000).

The functions of Sertoli cells in spermatogenesis have attracted much more attention recently. Normal spermatogenesis depends on Sertoli cells, mainly due to their influence on nutrient supply, maintenance of cell junctions, and support for germ cells' mitosis and meiosis(Ni**,** F *etal*.,2019).

2.5.2 Hormonal Regulation for Spermatogenesis

The gonadotropins, LH and FSH, are the most important pituitary hormones regulating testicular physiology. In mammals, FSH and LH interact with their receptors (FSHR and LHR, respectively) in a highly specific manner with little overlap in biological activities at physiological hormone concentrations . LH regulates Leydig cell sex steroid production; FSH regulates Sertoli cell activities, such as the structural, nutritional, and regulatory (paracrine) support of germ cell development (Huhtaniemi and Themmen,2005).

2.5.2.1Follicle Stimulating Hormone (FSH)

Follicle-stimulating hormone (FSH)is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system**(**Bowen ,2019).

Activity/functions

FSH regulates the development, growth, pubertal maturation and reproductive processes of the human body. (Ulloa etal., 2018)

- In both males and females, FSH stimulates the maturation of primordial germ cells.
- In males, FSH induces Sertoli cells to secrete androgen-binding proteins (ABPs), regulated by inhibin's negative feedback mechanism on the anterior pituitary. Specifically, activation of Sertoli cells by FSH sustains spermatogenesis and stimulates inhibin B secretion.
- In females, FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibin B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle.

Control of FSH release from the pituitary gland is unknown. Low frequency gonadotropin-releasing hormone (GnRH) pulses increase FSH mRNA levels in the rat, but is not directly correlated with an increase in circulating FSHGnRH has been shown to play an important role in the secretion of FSH, with hypothalamic-pituitary disconnection leading to a cessation of FSH. GnRH administration leads to a return of FSH secretion. FSH is subject to oestrogen feed-back from the gonads via the hypothalamic pituitary gonadal axis.(Sharma TP *etal*; 2012)

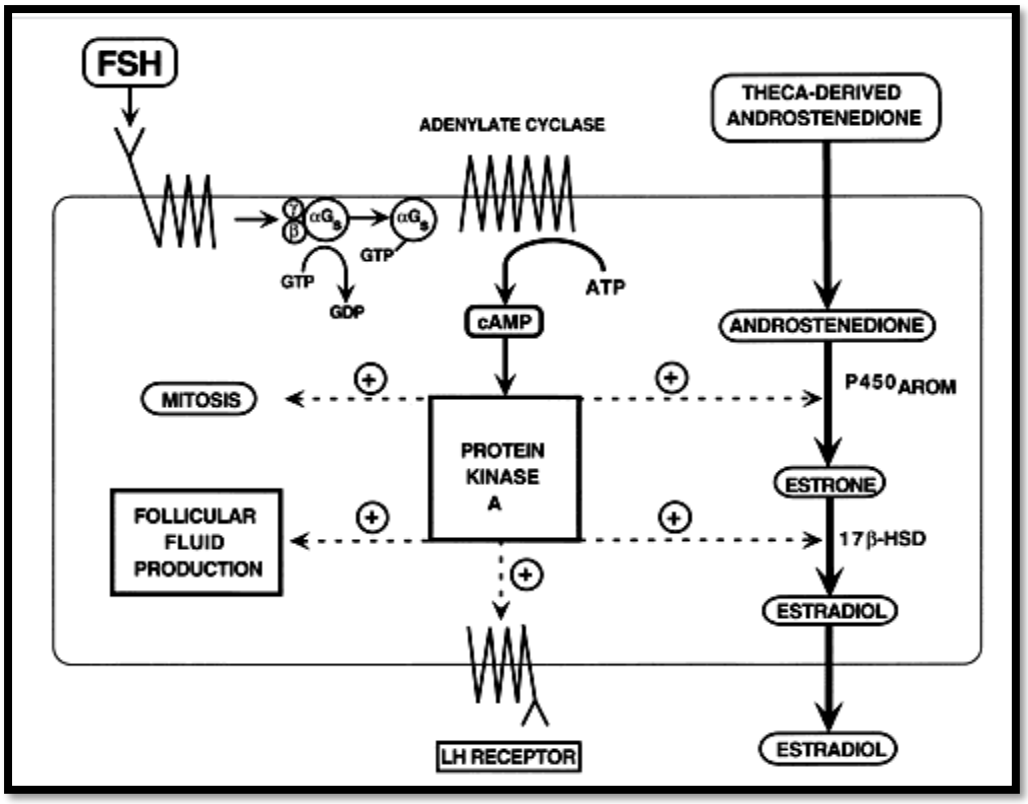
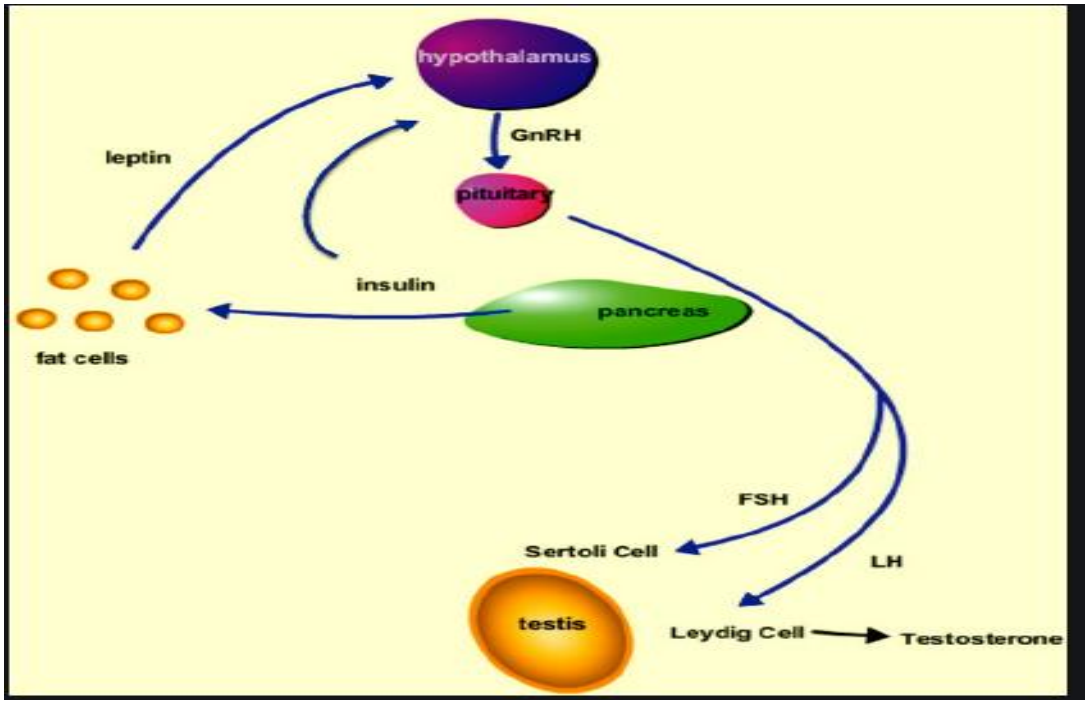


Fig.2.1 Diagram of the follicle-stimulating hormone (FSH) signal transduction pathway in granulosa zszypcells of a dominant follicle. FSH interacts with a receptor protein that has seven transmembrane-spanning domains. The binding event is transduced into an intracellular signal by the heterotrimeric G proteins. The active &agr;Gstimulating (&agr;Gs -GTP) protein interacts with its effector protein, adenylate cyclase, to initiate cAMP formation. cAMP binds to and activates protein kinase A, which phosphorylates substrate proteins that stimulate transcription of the genes encoding P450AROM, 17β-hydroxysteroid dehydrogenase, and the luteinizing hormone and that activate mitosis and follicular fluid formation. (Adapted from Erickson GF: Polycystic ovary syndrome: Normal and abnormal steroidogenesis. In Schats R, Schoemaker J (eds): Ovarian Endocrinopathies: Proceedings of the 8th Reinier deGraaf Symposium. Park Ridge, NJ: Parthenon Publishing, 1994.)

2.5.2.2Luteinizing Hormone (LH)

Luteinizing hormone (LH, also known as lutropin and sometimes lutrophin) is a hormone produced by gonadotropic cells in the anterior pituitary gland. The production of LH is regulated by Gonadotropin Releasing Hormone (GnRH) from the hypothalamus. In females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum. In males, where LH had also been called interstitial cell–stimulating hormone (ICSH), it stimulates Leydig cell production of testosterone. It acts 2synergistically with follicle-stimulating hormone(FSH)(Oduwole *etal*;2018).



Figure(2.2) Representation showing involvement of leptin and insulin in the hypothalamic- pituitary-testis axis (GnRH gonadotropin-releasing hormone, LH luteinizing hormone, FSH follicle- stimulating hormone).

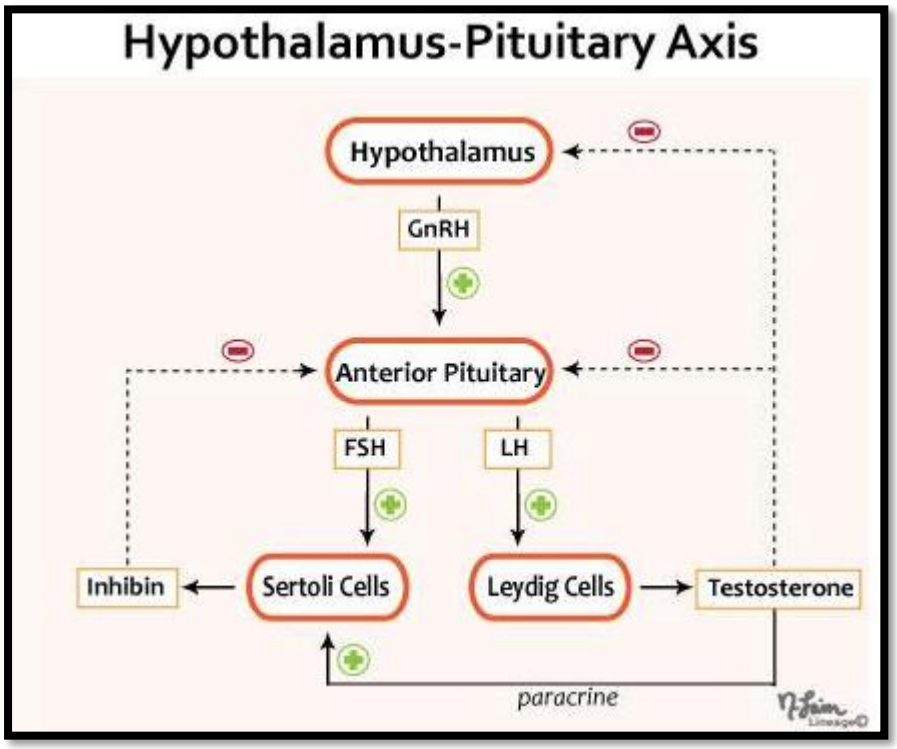
Function

Effects of LH on the body

In both males and females, LH works upon endocrine cells in the gonads to produce androgens.

Effects in males

LH acts upon the Leydig cells of the testis and is regulated by gonadotropin-releasing hormone (GnRH),(Male Medical Fertility Treatment,2015) The Leydig cells produce testosterone under the control of LH. LH binds to LH receptors on the membrane surface of Leydig cells. Binding to this receptor causes an increase in cyclic adenosine monophosphate (cAMP), a secondary messenger, which allows cholesterol to translocate into the mitochondria. Within the mitochondria, cholesterol is converted to pregnenolone by CYP11A1(Zirkin,2018) Pregnenolone is then converted to dehydroepiandrosterone (DHEA)(Akhtar,2005) DHEA is then converted to androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD)(Liu L,2015) and then finally converted to testosterone by 17β-hydroxysteroid dehydrogenase (HSD17B).The onset of puberty is controlled by two major hormones: FSH initiates spermatogenesis and LH signals the release of testosteronean androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis(Oduwole *etal*;2018)



pituitary

- LH
- aka luteinizing horome
- LH → (+) Leydig (interstitial) cells → ↑ testosterone
- LH acts on Leydig cells
- increases activity of cholesterol desmolase enzyme
- increases synthesis and secretion of testosterone via cholesterol
- testosterone has local (paracrine) and distal (endocrine) effects
- locally, testosterone acts on Sertoli cells
- reinforces pro-spermatogenesis action of Sertoli cells

LH is released from the pituitary gland, and is controlled by pulses of gonadotropin-releasing hormone. When bloodstream testosterone levels are low, the pituitary gland is stimulated to release LH, As the levels of testosterone increase, it will act on the pituitary through a negative feedback loop and inhibit the release of GnRH and LH consequently. Androgens (including testosterone and dihydrotestosterone) inhibit monoamine oxidase (MAO) in the pineal gland, leading to increased melatonin and reduced LH and FSH by melatonin-induced increase of Gonadotropin-Inhibitory Hormone (GnIH)(Ubuka etal; 2014) synthesis and secretion. Testosterone can also be aromatized into estradiol (E2) to inhibit LH. E2 decreases pulse amplitude and responsiveness to GnRH from the hypothalamus onto the pituitary.(Pitteloud N *etal*., 2008) .

2.5.2.3 Testosterone

is the primary sex hormone and anabolic steroid in males.[3] In male humans, testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair, in addition, testosterone is involved in health and well-being(Bassil N *etal*., 2009) and the prevention of osteoporosis(Tuck, and Francis, 2009)**.** Insufficient levels of testosterone in men may lead to abnormalities including frailty and bone loss,Testosterone is a steroid from the androstane class containing

a keto and hydroxyl groups at positions three and seventeen respectively. It is biosynthesized in several steps from cholesterol and is converted in the liver to inactive metabolites(Luetjens, and Weinbauer ,2012).

It exerts its action through binding to and activation of the androgen receptor, in humans and most other vertebrates, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females. On average, in adult males, levels of testosterone are about seven to eight times as great as in adult females(Torjesen, and Sandnes ,2004). As the metabolism of testosterone in males is more pronounced, the daily production is about 20 times greater in men, Females are also more sensitive to the hormone(Dabbs , and Dabbs JM , 2000) In addition to its role as a natural hormone, testosterone is used as a medication in the treatment of male hypogonadism, breast cancer in women, and as part of transgender hormone therapy for transgender men(American Society of Health-System Pharmacists,2016),Since testosterone levels decrease as men age, testosterone is sometimes used in older men to counteract this deficiency. It is also used illicitly to enhance physique and performance, for instance in athletes(Institute of Medicine (US) ,2004).

In men, higher levels of testosterone are associated with periods of sexual activity.(Roney JR *etal*., 2003) Men who watch a sexually explicit movie have an average increase of 35% in testosterone, peaking at 60–90 minutes after the end of the film, but no increase is seen in men who watch sexually neutral films. Men who watch sexually explicit films also report increased motivation, competitiveness, and decreased exhaustion, A link has also been found between relaxation following sexual arousal and testosterone levels,Men's levels of testosterone, a hormone known to affect men's mating behaviour, changes depending on whether they are exposed to an ovulating or nonovulating woman's body odour. Men who are exposed to scents of

ovulating women maintained a stable testosterone level that was higher than the testosterone level of men exposed to nonovulation cues. Men are heavily aware of hormone cycles in females.(Miller and Maner , 2010).This may be linked to the ovulatory shift hypothesis, where males are adapted to respond to the ovulation cycles of females by sensing when they are most fertile and whereby females look for preferred male mates when they are the most fertile; both actions may be driven by hormones.

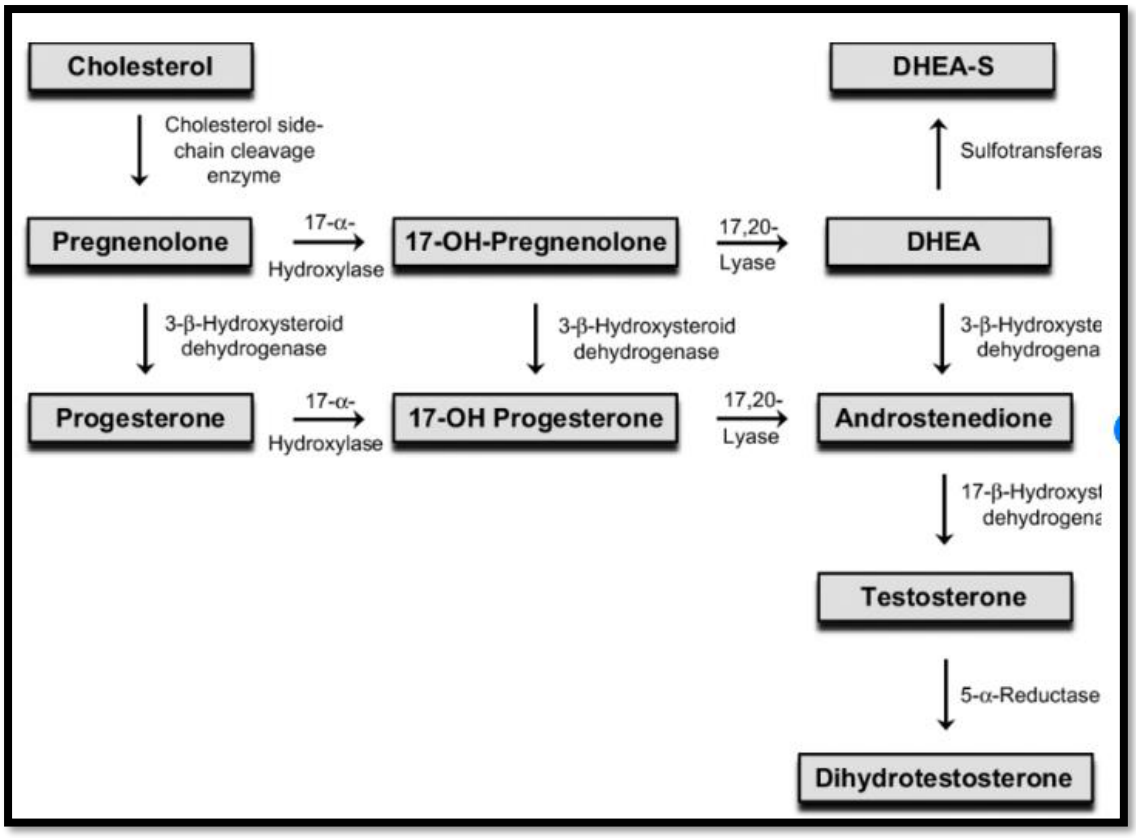


Fig.(2.3)synthesis of testosterone.

It is a steroid hormone that secret from the testicle (Leydig's cells) and is a major influence in the development of secondary male sexual traits after it has been converted to its effective form (Androgen), Dihydrotestosterone, as well as its important role in the process of the emergence of sperm, Testosterone is excreted from the testicles and adrenal gland in males, ovaries and placenta in females ,Testosterone is produced in the form of androgen or

estrogen. Androgen is excreted in dihydrotestosterone while Estrogen is estradiol, It is beyond question that testosterone is needed for adult spermatogenesis, The main targets of testosterone action are meiosis and spermiogenesis, Testosterone is essential for gonadal formation and testicular differentiation, Over the last 5 years, evidence is mounting that testosterone is involved in Sertoli cell differentiation in early postnatal life (Haywood *et al*., 2003)

Testosterone is responsible for the growth and development of the Wolffian duct to the esophagus in fetal, vas deferense, seminal vesicle and Prostate, as well as its responsibility to show secondary sexual characteristics such as coarse sound and hair appearance (Zurita *etal*.,2003). In addition, elimination of testosterone results in a loss of mid-stage round spermatids and mature, elongated spermatozoa, indicating an effect of androgens on spermiation as well as in the transition from round to elongating steps of spermiogenesis Holdcraft and Braun, 2004); Wang *et al.*,2009).

Testosterone also plays a role in the regulation of leydig and myoid cells where it affects steroidogenic functions resulting in anspermatogenic arrest at the round spermatid stage (Wang *etal.*,2009). Androgens are also essential for sexual differentiation (Holdcraft and Braun, 2004).

2.5.2.4Inhibin B

Inhibin subunits are expressed in the human testis from fetal life. At second trimester gestation, both α and β_B subunits are present in Sertoli and Leydig cells, whereas gonocytes express only the β_B subunit and Leydig cells also express the β_A subunit (Anderson *et al.*,2002). However, the β_A subunit serves mainly for the synthesis of its homodimer, activin A (Buzzard *et al.*, 2003)

Inhibins are glycoproteins produced by the granulosa and theca cells of the ovary and by the Sertoli cells of the testis. They are of great importance for the negative feedback control of pituitary gonadotrophin secretion. There are at least two active molecular forms in circulation, inhibin A and inhibin B, which are heterodimers made by an α subunit and either β_A (inhibin A) or β_B (inhibin B) subunits. (Luisi *et al*., 2005).

In male, inhibin B is produced in the testis, principally by the Sertoli cells. Inhibin B expression and secretion are positively correlated with Sertoli cell function, sperm number, spermatogenic status and are negatively correlated with FSH.. The functional relationship between spermatogenesis and serum inhibin B concentrations is nicely demonstrated by the study of patients with variable degrees of spermatogenesis impairment due to a single genetic disorder and presence of microdeletions in the AZFc region of the Y chromosome (Frydelund *et al.*, 2002). In this selected population, serum inhibin B concentrations are absolutely normal in the individuals with bilateral spermatocytic arrest but undetectable in patients with a predominant Sertoli cell-only histological pattern (Frydelund *et al*., 2002).

Sertoli cells also release inhibin B from its apical side into the seminiferous tubules, thus contributing to the seminal plasma inhibin B content. Seminal plasma inhibin B concentrations are substantially reduced in azoospermic men regardless of obstructive, non-obstructive or post-vasectomy azoospermia; and correlate directly with sperm count(Garem *et al.*, 2002).

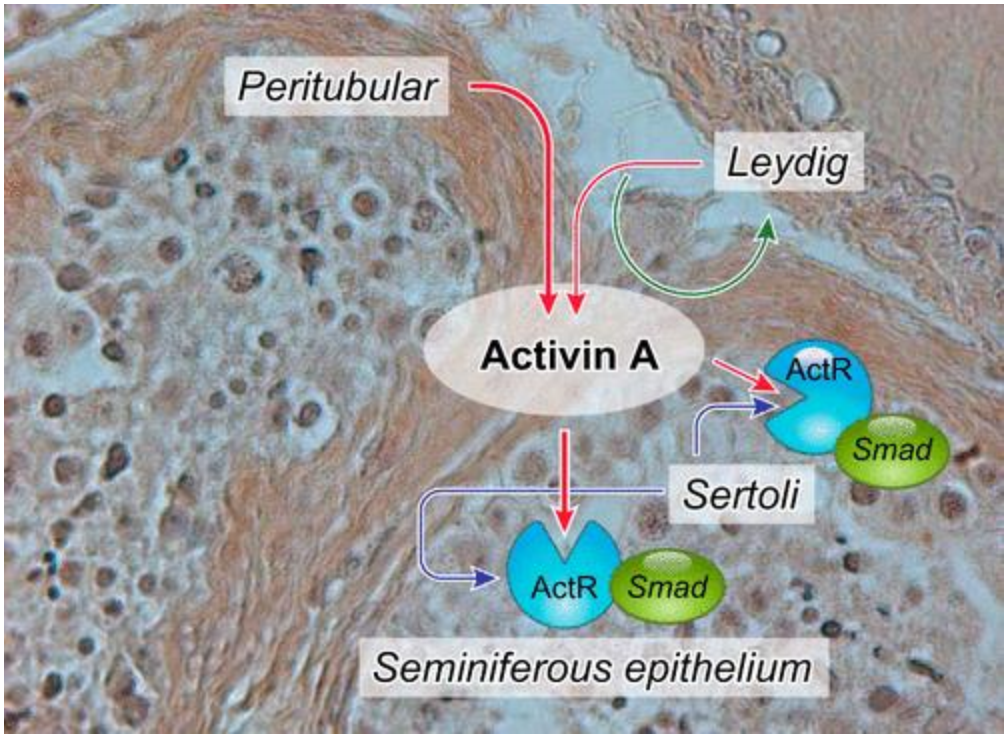
2.5.2.5 Activin A

The discovery of activin A more than 30 yr ago by Wylie Vale at the Salk Institute **(**Citation by Bloise *et al*.,2019). followed the opposite sense of its twin molecule named inhibin. Although inhibin was hypothesized as a reproductive hormone in the 1920s and finally purified and characterized in the 1980s (Makanji *et al*.,2014). The name "activin" was coined by Ling and co-workers for activin AB because this substance was able to stimulate follicle-stimulating hormone (FSH) release from the pituitary gland Activins were originally identified as gonadal proteins that stimulate FSH secretion by the pituitary gland(Bloise *et al*.,2019).

Activin A is a critical growth and differentiation factor during testicular and epididymal development in mammals. The expression of activin βA subunit increases steadily in mouse testis during embryonic development (Mendis *et al*.,2011)The main sources of activin A in mouse testis are the peritubular myoid cells(Barakat *etal.*,2008) and Leydig cells (Archambeault and Yao.,2010), which express the βA subunit mRNA and protein (Archambeault and Yao,2010), Activin A promotes Sertoli cell proliferation (Nicholls *et al*., 2013) and, coherently with this function, its testicular levels peak during mouse perinatal phase, coinciding with Sertoli cell proliferative peak (Buzzard *et al*., 2003). This is followed by a gradual decrease of testicular activin A during childhood and puberty and stabilization at relatively lower levels in adulthood (Barakat *et al.*,2008).

Activin A's role in mouse testis is not only to increase the number of Sertoli cells but also to promote a physiological balance between Sertoli cells and germ cells (Mendis *et al*.,2011). This balance is important in both directions, because overexpression of activin βA subunit in mouse testis leads to sterility (Bloise *et al*., 2019) In addition, germ cells are able to produce their own activin A, as suggested by the localization of βA subunit mRNA in spermatogonia and preleptotene/leptotene spermatocytes (Barakat *et al*.,

2008). but the relevance of this autocrine pathway for the control of spermatogenesis is uncertain In the adult human testis, activin βA subunit is found in Leydig cells and Sertoli cells , whereas activin receptors and their corresponding phosphorylated Smad proteins are mostly found in Sertoli cells and spermatogonia and occasionally in spermatocytes (Dias *et al*., 2009).



FIgure(2.4) Localization and paracrine targets of activin A in adult human testis. The activin βA subunit is localized by immunohistochemistry (*brown staining*) in peritubular cells and Leydig cells, whereas activin receptors (ActR) and their intracellular effectors, Smad proteins, have been previously detected in Sertoli cells and seminiferous epithelium. In addition, in vitro experiments with rat Sertoli cells suggest that they are also capable of releasing activin A (*blue arrows*).

The roles of activin A in the epididymis range from embryogenesis to reproductive age. Activin βA subunit mRNA is expressed in mouse embryos at the mesonephros and in the mesenchyme of the Wolffian duct before it

gives origin to the epididymis (Tomaszewski *et al* ., 2007). Physiological role played by activin A in the prostate gland is the inhibition of tissue growth and branching since embryonic life and throughout

postnatal development In rat postnatal development, activin A from both epithelial and mesenchymal origin reaches activin receptors at the glandular epithelium and inhibits branching morphogenesis either in the absence or in the presence of androgen stimulation (Cancilla *et al ., 2001).*

2.5.2.6 Adiponectin

Human adiponectin contains 244 amino acids, and has the molecular weight of 30 kDa with four different domains: a carboxy terminal globular chain of 137- amino acids, an amino-terminal with 18 amino acids, a very species hypervariable chain with 23 amino acids and an acid collagen domain with 66 amino acids where 22 are repeat motif variables(Nishida *etal.,* 2007)This type of adiponectin generally looks with an extended appearance. Though it contains a smaller version also, that is a fragmented product of elastase enzyme which is produced by the leukocytes such as neutrophils and monocytes. From the collagen domain, numerous proteolytic sites have been identified with different locations. The smaller form of adiponectin conserves its bulbous sphere veracity and forwards its effects with bonding receptors.As compared to human, rat adiponectin contains 247-amino acid sequences,It is produced from the adipose tissues to the main circulation with 3 protein complexes These are trimer with molecular weight of 67 kDa, two trimers of 130 kDa, known as hexamer and a larger molecular weight protein(Waki H *etal*., 2005)

Effect of adiponectin in HPG axis

The HPG axis is considered the prime regulatory endocrine axis in monitoring the functions of the male reproductive system. It is conventionally known that the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus triggers the release of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. These gonadotropins act on their receptors on testicular cells, Leydig cells and Sertoli cells to coordinate the processes of steroidogenesis and spermatogenesis. This regulatory axis receives negative or positive feedback from the testicular and other hormones and factors as per the requirement for physiological homeostasis. Expression of adiponectin and both of its receptors, AdipoR1 and AdipoR2 have been evidenced in the human hypothalamus and pituitary suggesting its importance in the regulatory mechanism of HPG axis(Psilopanagioti *etal*., 2009)Moreover, its deficiency has been suggested to have inhibitory effects over FSH and LH secretion thereby jeopardizing reproductive functions,The influence of adiponectin over the hypothalamic GnRH secretions can be predicted through the study that observed mutation in adiponectin gene significantly reduced the number of GnRH immunoreactive neurons(Cheng *etal*., 2016).

Adiponectin and hypothalamus

Expression of adiponectin receptors in the hypothalamus is evident in different species as well as in humans(Kaminski *etal.*, 2014)Its predominance in the cerebrospinal fluid may indicate its autocrine or paracrine actions over the hypothalamic-pituitary axes(Kusminski *etal*., 2007)It has been shown that in murine models, peripheral intravenous adiponectin administration results in a concurrent increase in adiponectin levels in the cerebrospinal fluid which is suggestive of its ability to cross the blood-brain barrier Adiponectin concentrations also have been reported to elevate during fasting and get reduced after refeeding(Kubota N *etal*., 2007). As discussed earlier,

hypothalamic GnRH neurons are the main regulatory components of the reproductive axis, which controls the secretion and release of pituitary gonadotropins. In vitro studies have demonstrated that adiponectin inhibits hypothalamic GnRH secretions through activation ofAMPK(Cheng XB *etal*., 2011). Experiments in a matured immortalized murine hypothalamic GnRH neuronal cell line (GT1-7 cells) showed that adiponectin inhibited the GnRH secretion along with suppressing the expressions of KISS1 mRNA(Wen JP *etal*., 2011). Kisspeptins, hypothalamic neuropeptides, which by binding to their receptors (KISS1-R) are reported to mediate the mechanism of triggering the physiological onset of puberty by induction of hypothalamic GnRH. Thus, it may be suggested that adiponectin may reduce GnRH secretion by influencing the kisspeptins mediated GnRH inducing signal. A hypothesis of adiponectin actions upon the GnRH neuron suggests that murine GnRH neurons highly express the adiponectin receptor, and AdipoR2 acting through which adiponectin perhaps activate the protein kinase Cζ/liver kinase B1/AMPK signaling pathway to rapidly decrease GnRH neuronal activity(Klenke U *etal* ., 2014).

Adiponectin and pituitary

Alike in the hypothalamic neurons, the adiponectin and its receptors have been found in the pituitary of different speciesincluding human, In human, adiponectin has been found inall the pituitary cells responsible for the production of hormones of reproductive importance, such as LH, FSH, thyroid-stimulating hormone, growth hormone. The adiponectin receptors have alsobeen found to be expressed in these pituitary cells, such as in the gonadotrophs, thyrotrophin, somatotrophs but not were found in the lactotrophs or corticotrophs(Psilopanagioti etal .,2009) Adiponectin has been shown toinhibit the basal and GnRH mediated LH secretion in rat and mouse pituitary cells in vitro(Lu M *etal* .,2009) Moreover, adiponectin has been

reportedto downregulate the gene expressions for GnRH receptor(Rodriguez-Pacheco *etal.*,2007). Theeffects of adiponectin upon FSH release from porcine primary pituitary cells have been shown to be stimulatory Adiponectinalso reportedly could modulate both GnRH and insulin-mediated secretions of LH and FSH. There are observations from two normal nonhuman-primate species, suggesting that adiponectin has no influence upon the LH and FSH release by primary pituitary cell cultures(Sarmento-Cabral *etal* ., 2017). However, from the above discussions, it is clear thatmajority of the studies have unveiled the expressions and actions of adiponectin and its receptors in the hypothalamus and pituitary modulating the secretion and release of key reproductive hormones, GnRH, LH, and FSH. Thus, adiponectin plays important roles in influencing the hypothalamic-pituitary axis in the control of reproductive functions. It has been explained that FSH helps in initiation of spermatogenesis by acting on its receptors on the testicular Sertoli cells, and in combination with high intratesticular testosterone, it plays role in the sustenance of spermatogenesis. It is well known that LH regulates androgen synthesis or steroidogenesis by acting upon its receptors on the testicular Leydig cells

Adiponectin in seminal fluid Semen or seminal fluid is the male body fluid containing spermatozoa and have been reported to contain adiponectin at concentrations of about 66- and 180-folds less that is serum concentrations in men and bulls, respectively(Heinz JF *etal* ., 2015), Moreover, ithas been stipulated that adiponectin concentrations in seminal fluid are positively correlated with that in blood plasma, suggesting it is transferred from the blood to testicular cells traversing the blood-testis barrier Regulation of reproductive functions in different reproductive ages Adiponectin receptor mRNA expressions in chicken displayed modifications during the puberty. Their expressions were found to be increased in adulthood as compared to the levels

of expression in the prepubertal phases(Ocon-Grove OM *etal*., 2008). In Leydig cells in rodents, the expressions ofAdipoR2 protein as well the adiponectin serum concentrations, also showed an increase during puberty(Pfaehler *etal*., 2012). These observations mayindicate that either

physiological changes through the puberty have led to upregulation in expressions of testicular adiponectin and its receptors, or adiponectin may have an essential role in initiating the physiological changes during puberty Adiponectin on sperm functionsKawwass et al had reported that spermatozoa also expressadiponectin receptors(Kawwass *etal*., 2015)Its receptor, AdipoR1 had been shown tobe expressed mainly by the sperm equatorial and acrosome regions, while the AdipoR2 expressions were mostly on the equatorial line and in the sperm head region (Kasimanickam *etal*., 2013)Studies in bulls have shown that adiponectin concentrations in plasma and abundance of its receptor mRNA expressions in spermatozoa positively correlated with the conception rates in the female counterparts,Seminal adiponectin concentration alongwith the presence of its receptors in ram sperm was also found to associate with the sperm motility parameters (Kadivar *etal* ., 2016). In human,seminal adiponectin concentrations positively correlate with semen quality in terms of sperm count, sperm concentration, and sperm morphology,The adiponectin and its receptors reportedlydecrease followed by capacitation, which may suggest a direct role of adiponectin in the regulation of sperm motility. (Thomas S *etal*., 2013).

2.6 Male Infertility

Infertility is one of the major health problems in life, and approximately 30 % of infertilities are due to a male factor (Isidori *et al.*, 2006). An estimated six percent of adult males are thought to be infertile (Purvis and

Christiansen,1992). Infertility is defined by most authorities as the inability to achieve a pregnancy after one year of unprotected intercourse (WH., 2010). Conception is normally achieved within 12 months in 80-85 percent of couples using no contraceptive measures; thus an estimated 15 percent of couples attempting their first pregnancy will have difficulty conceiving. Several conditions can interfere with spermatogenesis and reduce sperm quality and production (Sinclair, 2000).

More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production (Mosher & Pratt,1991). Some cases of male infertility are due to anatomical abnormalities such as varicoceles, ductal obstructions or ejaculatory disorders (Griffin *et al*., 2006).

An estimated 40 to 90% of male infertility is due to deficient sperm production of indefinable origin (Sinclair, 2000). A large proportion of infertile men fail to impregnate their female counterpart because of lack of sperm (azoospermia) or too little sperm (oligozoospermia); infertility may also be due to abnormal sperm morphology (teratozoospermia) and insufficient sperm motility(athenozoospermia) as reported by (Feng, 2003).

Is it purely coincidence that sperm quality has diminished over the last 50 years, while ever increasing amounts of synthetic chemicals and hormones have been introduced to the environment and food supply, perhaps we should consider decreased fertility in men as a physiological early warning system (Sinclare, 2000).

There are various factors that have been reported to be responsible for this increase in male infertility. Primary infertility may result from the use of various drugs. This phenomenon may be the result of an effect on the hypothalamic-pituitary-gonadal axis or a direct toxic effect on the gonads. Antibiotic therapy has been shown to significantly affect spermatogenesis and seminal parameters in both human and animal models (Olayemi, 2010).

More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production (Mosher & Pratt,1991).

Drugs with high risk of infertility include procarbazine, cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine ; drugs like doxorubicin, cisplatin and carboplatin have medium risk while therapies with plant derivatives (such as vincristine and vinblastine), antibiotics (such as bleomycin and dactinomycin) and antimetabolites (such as methotrexate, mercaptopurine and 5-fluoruracil) have low risk of gonadotoxicity (M Brydøy *etal*., 2007).

2.7 Busulfan

2.7.1History and Medication specification of Busulfan

Busulfan was approved by the US Food and Drug Administration (FDA) for treatment of chronic myeloid leukemia (CML) in 1999. Busulfan was the mainstay of the chemotherapeutic treatment of chronic myeloid leukemia (CML) until it was displaced by the new gold standard, Imatinib, though it is still in use to a degree as a result of the drug's relative low cost. Busulfan is used in pediatrics and adults in combination with cyclophosphamide or fludarabine/clofarabine as a conditioning agent prior to bone marrow transplantation, especially in chronic myelogenous leukemia (CML) and other leukemias, lymphomas, and myeloproliferative disorders. Busulfan can control tumor burden but cannot prevent transformation or correct cytogenic abnormalities (Lesurtel *et al*.,2006).

2.7.2Chemotherapy(busulfan):

Many drugs, particularly alkylating agents, have been shown to be gonadotoxic. Indeed, some chemotherapies used in the treatment of lymphoma or in preparation for bone transplantation have been shown to

cause azoospermia, with feedback-raised follicle-stimulating hormone (FSH) levels in over 90% of men following cyclical chemotherapy (Qu, N.,*et al* 2019). Busulfan is a chemotherapeutic agent that is used to treat various malignant diseases, such as chronic myeloid leukemia (Galaup.and Paci,2013). Additionally, busulfan is also commonly used prior to hematopoietic stem cell transplantation (Galaup& Paci,2013). Unlike other chemicals that destroy differentiated spermatogonia, busulfan is a potent agent that preferentially kills spermatogonial stem cells (Qu, N.,*et al* 2019). Consequently, busulfan treatment is the most common method used to prepare recipients of spermatogonial stem cells transplantation (Ogawa, T.2001) and to study spermatogonial stem cells kinetics and fertility recovery(Perez *et al* ., 2011 and Gutierrez *et al* .,2016).

Busulfan can eliminate almost all endogenous germ cells in the recipient, creating an empty space in the spermatogonial stem cells niche; therefore, this drug has been used successfully to prepare recipients in rats Jiang, F.X.;& Short, R.V, (2019);Zhang and Renfree,2003)

Busulfan blocks spermatogenesis in rats and other species by acting on early spermatogonia and germ cells to prevent mitosis; this may ultimately result in sterility ,When given prior to puberty, doses of 10 mg/kg or higher produce sterility, destruction of seminiferous epithelium and abnormal gonadal development and maturation (Zhao *et al*.,2020). Oxidative stress occurs when a system has an imbalance between oxidation and reduction reactions, leading to generation of excess oxidants or molecules that accept an electron from another reactant ,a free radical is a molecule or element with an unpaired electron that is extremely reactive in an attempt to reach an electronically stable state. ROS are free radical derivatives of oxygen (O_2) containing molecules. Some of the clinically important ROS identified include peroxyl ($\cdot ROO^{-}$) and hydroxyl ($\cdot OH^{-}$) radicals, superoxide ($\cdot O_2^{-}$) anion, and H_2O_2. Nitrogen compounds such as nitric oxide (NO) and

peroxynitrite anion (ONOO) also appear to play a role in oxidation and reduction reactions. Common molecules that receive the unpaired electron are lipids in membranes and carbohydrates in nucleic acids This leads to potential cellular membrane and DNA damage when ROS are greater than the antioxidant-carrying capacity(**Fang, and Zhong.,2019)**

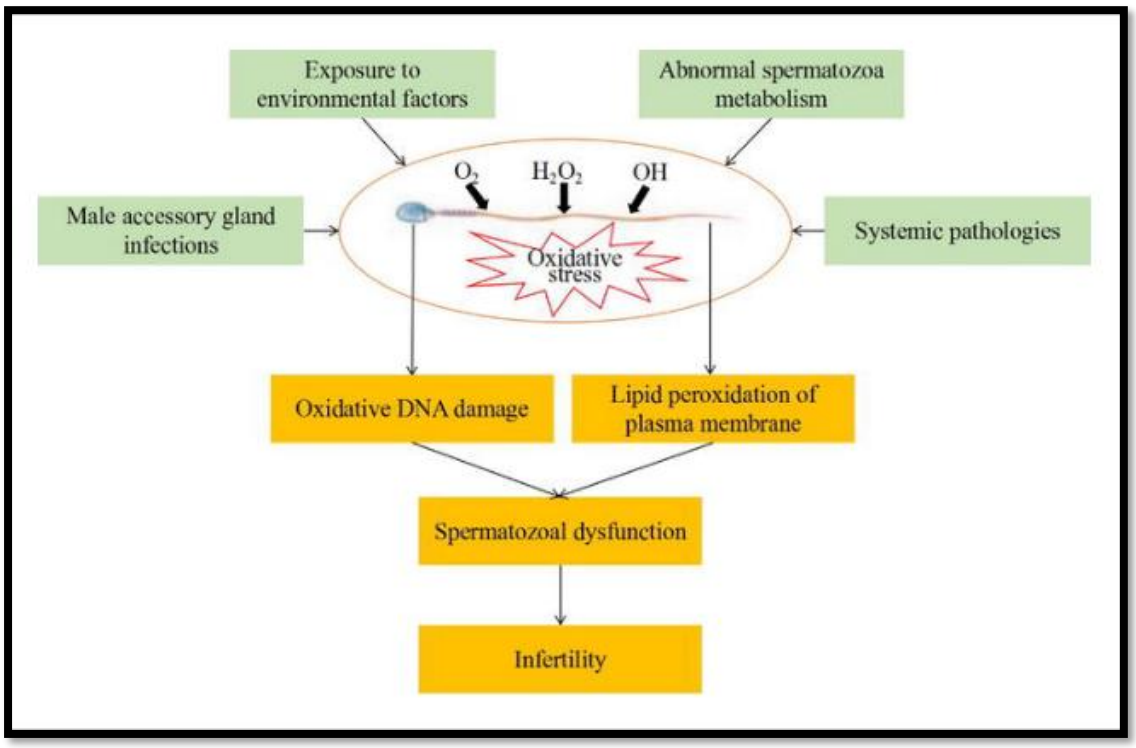


FIG.(2.5)Factors contributing to oxidative stress-induced male infertility.

2.7.3Medication specification of Busulfan

Busulfan, a bifunctional alkylating agent, has been used as a conditioning regimen prior to allogeneic hematopoietic stem cell transplantation (HSCT). Busulfan consists of two labile methane sulfonate groups attached to opposite ends of a four-carbon alkyl chain. In an aqueous solution, busulfan becomes hydrolyzed and releases methanesulfonate groups, which produce reactive carbonium ions that can alkylate DNA.1 Busulfan causes DNA damage by crosslinking the DNA intrastrand at 5'-GA-3' and 5'-GG-3'. These cross-links can be converted into DNA strand breaks, the process of which is responsible for the cytotoxicity of busulfan. (Drugs@FDA., 2016& Iwamoto *et al*., 2007).

2.7.4Pharmacologie of Busulfan

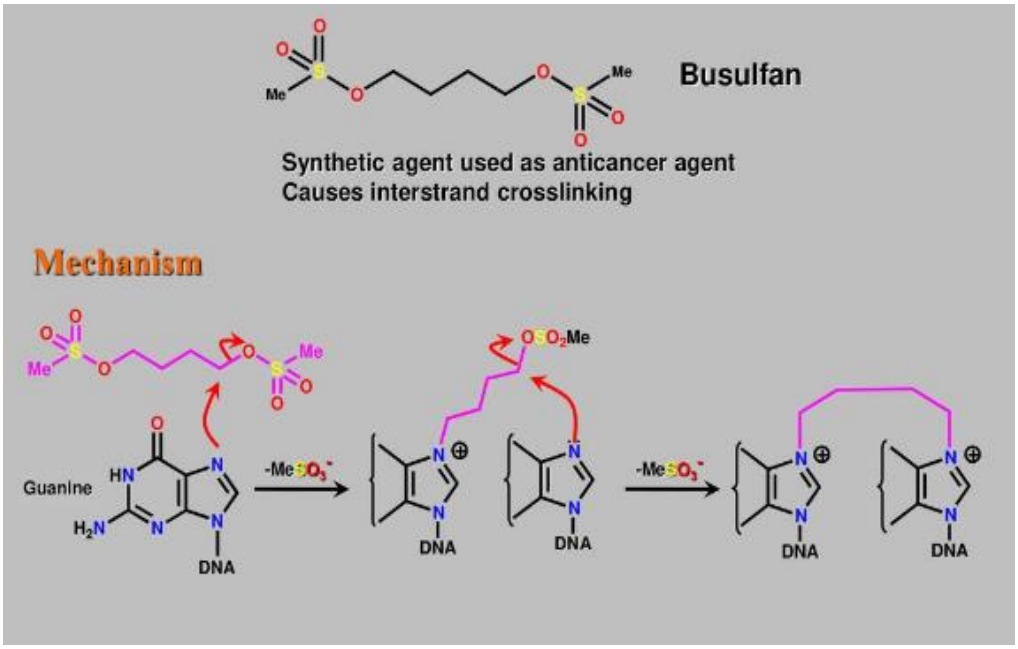


Fig.(2.6) Chemical structure of busulfan (butane-1,4-diyl dimethanesulfonate).

It reaction with nucleic acids 45 (nucleophiles) to form inter-strand bridges and DNA-protein bridges. The lesions are induce of cell death. At low doses, it acts of more specifically on the cells of the granulocytic line, while as high doses its action have been repercussion on all the heopic lines with a very good passage in the cerebrospinal fluid. Busulfan was initially available only in oral form.

2.7.5Absorption, distribution, metabolism, and excretion of busulfan In humans,

upon oral administration, busulfan is readily absorbed from the gastrointestinal tract, binds rapidly to plasma proteins (e.g. albumin) and red blood cells, and rapidly disappears from the blood (GlaxoSmithKline, 2004; Sweetman, 2005; Thomson Healthcare, 2008). Busulfan is reported to have a half-life of 2–3 hours (Sweetman, 2005; Thomson Healthcare, 2008). In the liver, it rapidly undergoes both enzymatic and non-enzymatic transformations, primarily through glutathione-mediated processes, to less active, sulfur-containing metabolites (Thomson Healthcare, 2008). Twelve

metabolites have been isolated including methanesulfonic acid and 3-hydroxytetrahydrothiophene-1,1-dioxide, two major urinary metabolites (Bishop & Wassom, 1986; GlaxoSmithKline, 2004). In spite of its rapid clearance from the blood and its extensive metabolism, radiolabelled busulfan is excreted relatively slowly, with 25–60% of the radioactivity being excreted, primarily as metabolites, within 48 hours after dosing (McEvoy, (1987); GlaxoSmithKline, (2004).

In humans, upon oral administration, busulfan is readily absorbed from the gastrointestinal tract, binds rapidly to plasma proteins (e.g. albumin) and red blood cells, and rapidly disappears from the bklood(GlaxoSmithKline, 2004; Sweetman, 2005; Thomson Healthcare, 2008). Busulfan is reported to have a half-life of 2–3 hours(Sweetman, 2005; Thomson Healthcare, 2008). In the liver, it rapidly undergoes both enzymatic and non-enzymatic transformations, primarily through glutathione-mediated processes, to less active, sulfur-containing metabolites (Thomson, 2008).

Twelve metabolites have been isolated including methanesulfonic acid and3-hydroxytetrahydrothiophene-1,1-dioxide, two major urinary metabolites (Bishop & Wassom, 1986; GlaxoSmithKline, 2004). In spite of its rapid clearance from the blood and its extensive metabolism, radiolabelled busulfan is excreted relatively slowly, with 25–60% of the radioactivity being excreted, primarily as metabolites within 48 hours after dosing (McEvoy, 1987; GlaxoSmithKline, 2004).

2.7.6 Side effect of Busulfan

One of the most common side effects of anticancer drugs is disruption of spermatogenesis process, leading to infertility in many cases (Solomon *et al* .,2019). BUS is a DNA-destruction chemotherapy agent used in low doses for long-term treatment of chronic myelogenous leukaemia and ovarian cancers,

and in high doses for the treatment of bone marrow suppression in patients under bone marrow transplantation (Liu *et al*.,2019) BUS inhibits cell division and has adverse effects on the cells with a high division rate, thereby exerting its highest impact on spermatogonial cells (Hakemi *et al*., 2019). BUS also causes chromosomal sperm anomalies and lethal mutation mostly in sperms (Abofoul *et al*.,2019)

Boujrad N et al., reported that the consumption of BUS induces gonadal dysfunction in pregnant women and reduces somatic and germinal cells in the testis of infants (jalili *et al*.,2020). Application of BUS as a proper pharmaceutical method to evacuate seminiferous tubules has long been taken into consideration in order to study the performance of germinal stem cells (Honaramooz *et al.*,2005). BUS can induce cell death through the production of free radicals (Li B *et al*., 2018), which impairs the lipids, proteins, and nucleic acids of cells (Vafaei *et al* ., 2018). The oxidative stress status due to the production of free radicals decreases the antioxidant enzyme activity, increases ROS level, and induces lipid oxidation consequently (Jalili *et al*.,2018). This phenomenon, in turn, causes the DNA breakdown and inactivation of specific proteins and consequential loss of biologic membranes (Salahshoor *et al*.,2018).

Busulfan is an effective chemotherapy drug widely used for cancer treatment.(Suriapraba *et al*., 2012) Busulfan as a cytostatic agent absorbs from the gastro-intestinal tract and quickly disappears from blood with a half-life of 2 to 3 hr. It is also potentially carcinogenic and teratogenic and has many side effects on gonadal function and different body organs such as skin, bladder, liver and nervous system.(Suriapraba *etal*.,2012) (Soleimanzadeh *et al* .,2018) Spermatogenesis in mammals is a complex process depending on spermatogonial stem cells. Unlike other drugs, busulfan is a potent agent that preferentially kills spermatogonial stem cells. (McClive and Sinclair.,2003 . Kanatsu *et al* .,2003) From the mechanisms by which busulfan can damage

the cells of different organs, production of reactive oxygen species (ROS) are important and they have major impact in development of oxidative stress. (Probin *et al* ., 2007) It seems busulfan inhibits the spermatogenesis process, especially by oxidative damage. Other mechanism suggested that busulfan increases the level of CK-18, a surface marker on Sertoli cell. The elevation of this marker causes spermatogenesis disorder and infertility (Yogev *et al.*,2004).

Busulfan which inhibits cell division by sticking to one of the DNA strands.(Panahi *et al.*,2015) Therefore, organs, tissues and cells with high division activities such as testes and germ cells are more susceptible to busulfan side effects. Spermatogenesis involves the passage of diploid germ cells through the reductive divisions of meiosis in order to generate round haploid spermatids. (Dun *et al* .,2012)

It is clear that cytotoxic therapy such as certain chemotherapy drugs influences spermatogenesissat leasttemporarily andin some cases permanently. Busulfan treated mice exhibited a marked increase in apoptosis and a decrease in testis weight .(Panahi *et al*.,2015) use of busulfan in clinical chemotherapy is limited because of toxicities, including gonadal dysfunction such as amenorrhea, gynecomastia, azoospermia and oligospermia in humans.(Ji, M *etal*.,2007) Therefore, recovery of reproductive function is also considered to be an important issue. Germ cells in the testis, spermatogonia, reside in the basal compartment of the seminiferous epithelium, and they can be divided functionally into undifferentiated (Aundiff) and differentiated type A (Adiff) spermatogonia. The Aundiff spermatogonia consist of the so called Asingle (As), Apaired (Apr) and Aaligned (Aal) spermatogonia. Aal spermatogonia differentiate into differentiating type A1 spermatogonia The type A1 spermatogonia start a series of mitotic divisions generating type A2, A3, A4, intermediate (I) and type B spermatogonia. Ultimately, the type B spermatogonia differentiate into primary spermatocytes, which initiate the

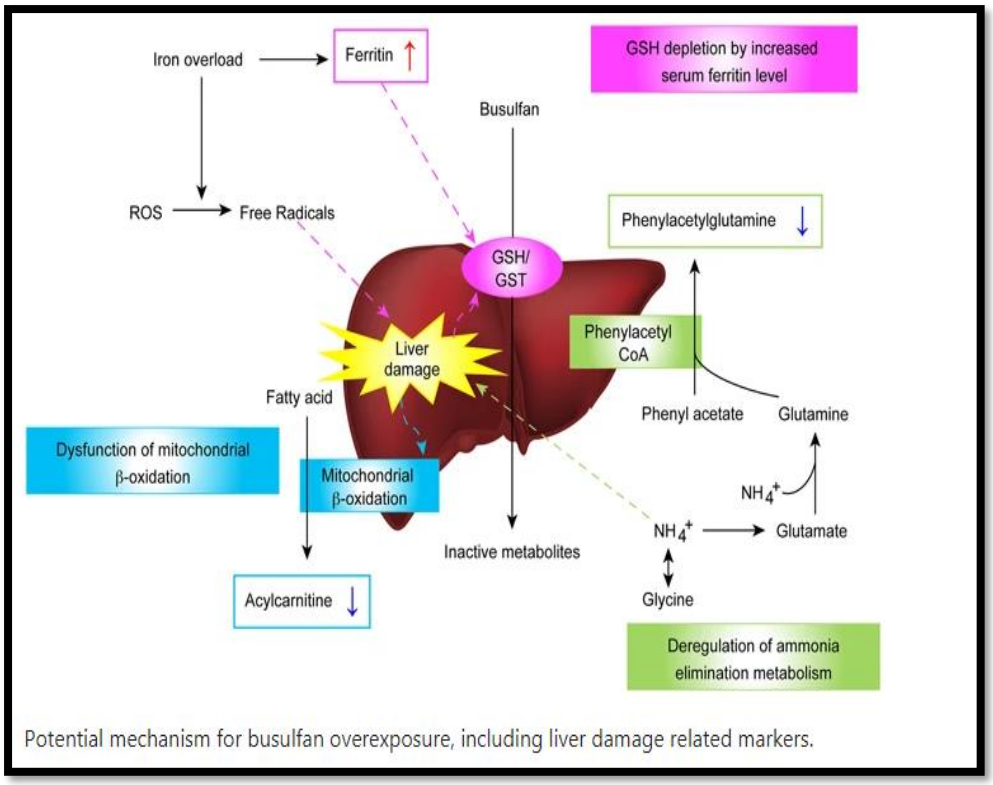
meiotic process and produce round spermatids. These then undergo spermatogenesis to produce morphologically mature spermatozoa (Ji, M *et al* .,2007)

2.7.7 effect busulfan on liver and kidnys

The liver and kidneys play an important role in metabolism, the excretion of harmful drugs and substances, as well as in maintaining balance. Therefore, injuries to the liver and kidneys can be very harmful. Kidney injuries occur for many reasons, such as chemotherapy, trauma, infection, surgery, or the use of antibiotics (Jaeschke *etal.*,2002; Holditch *etal.* &2019). Liver damage is also caused by the use of certain drugs or toxic chemicals, etc. Oxidative stress has been shown to play an important role in causing liver damage, so antioxidants may be effective in treating injuries (Ramachandran A& Jaeschke H.2018)It has a wide range of harmful effects on the body and has harmful effects on many organs including the liver and kidneys (Attari *eta.l,*2018*;*Omidi *etal.*,2008). In this study, busulfan was used to develop a model for liver and kidney injury Busulfan is chemotherapy that may damage different tissues, especially the liver as the main site of the drug Filtration and monitoring due to alkylation and DNA-binding properties and inactivation, causing liver enzymes.(Sadeghi *etal*,.2020). Free radical By binding to protein or lipids, the membrane in the liver cleaves the hydrogen atom in the fat and penetrates the lipid membrane This leads to the appearance of the membrane and necrosis of the liver cell wall at the end. This damage to the wall results in liver enzymes Serum leakage (Imge *et al*., 2008) .

In biochemical tests, liver enzymes are examined for early detection of tissue Liver damage. The most specific effect of busulfan in chemotherapy is liver injury, and laboratory and clinical tests indicate damage to liver cells (Pratt Kaplan, .(Sadeghi *etal*,.2020).

.



(Figure2.7) Two previous population PK studies of busulfan in paediatric patients incorporated ferritin levels as a covariate. Busulfan PKs were not influenced by ferritin levels in these studies (Gaziev *et al* .,2010; Paci *et al* .,2012).

However, one study examined thalassemia patients (Gaziev *et al* .,2010), and the other examined patients with non-malignant diseases (Paci *et al* .,2012). These patient populations are different from the population examined in our study in which the majority had a malignant disease and a history of previous chemotherapy. In addition, those previous studies primarily included patients with pre-existing liver damage and/or iron overload. The differing results regarding the influence of ferritin levels on busulfan PKs observed in the present study may result from differences in the baseline characteristics of enrolled patients.(Kim *etal.,2017*).

2.8 Coenzyme Q10 or ubiquinone-10.

Coenzyme Q, also known as ubiquinone, is a coenzyme family that is ubiquitous in animals and most bacteria (hence the name ubiquinone). In

humans, the most common form is Coenzyme Q_{10} or ubiquinone-10. CoQ_{10} is not approved by the U.S. Food and Drug Administration (FDA) for the treatment of any medical condition(*White, J. 2014).* however, it is sold as a dietary supplement.

It is a 1,4-benzoquinone, where Q refers to the quinone chemical group and 10 refers to the number of isoprenyl chemical subunits in its tail. In natural ubiquinones, the number can be anywhere from 6 to 10. This family of fat-soluble substances, which resemble vitamins, is present in all respiring eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, which generates energy in the form of ATP. Ninety-five percent of the human body's energy is generated this way.(*Hojerová, 2000).*

Coenzyme Q10 is a small lipophilic molecule consisting of a benzoquinone nucleus and an isoprenoid side chain (Figure1), which is synthesized by all cells apart from red blood cells, and shares a common biosynthetic pathway with cholesterol. (Neergheen *et al.,2017)*

Coenzyme Q10 serves as an essential electron carrier within the MRC, transferring electrons derived from complexes I and II to complex III and therefore allowing a continuous passage of electrons within the chain, which is essential for the process of oxidative phosphorylation. In addition to its role as an electron carrier, CoQ10 functions as a lipid-soluble antioxidant, protecting cellular membranes and plasma lipoproteins against free radical–induced oxidation.(Bentinger *et al.*, 2007).

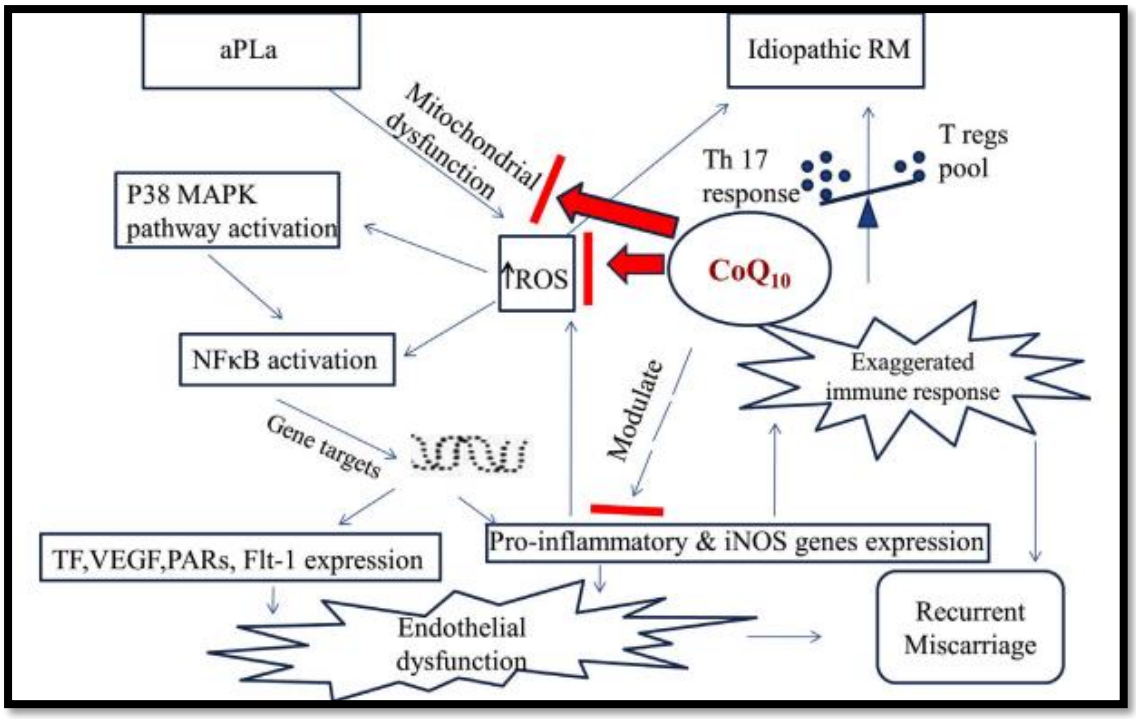
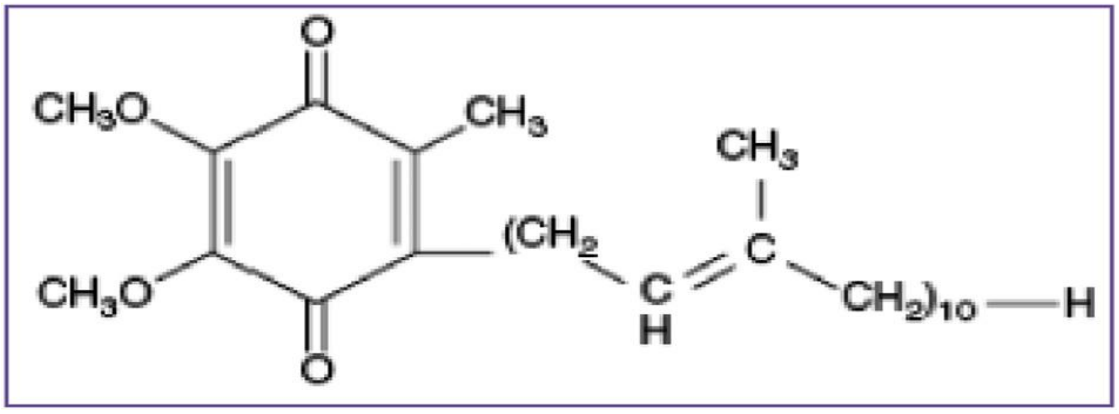


Fig.(2.8).Possible modifiable pathways and site of action of coenzyme Q10 during the event of recurrent miscarriage (RM). Anti-phospholipid antibodies (aPLa) causes mitochonrial dysfuntion leading to increased reative oxygen species (ROS). These ROS cause activation of inflammatory pathway i.e. nuclear factor kappa B (NFκB) which further activates the gene targets responsible for cell surface expression of adhesion molecules viz. tissue factor (TF), vasculo endothelial growth factor (VEGF), protease activated receptors (PARs) and fms like tyrosine kinase (Flt-1); as well as inducible nitric oxide synthase (iNOS) gene expression. These alterations results in endothelial dysfunction as well as excessive immune response which ultimately leads to dysbalance in Th-1/ Th-2 system and Th-17/ T-reg pool. The ultimate consequence of such events leads to RM. The possible sites which can be altered during RM event by coenzyme Q10 (CoQ10) are depicted in the figure. CoQ10 ameliorate mitochondrial dysfunction, increased ROS; and pro-inflammatory & iNOS gene expression.

2.8.1 Chemical form of Coenzyme Q10

CoQ10 is synthesized from the mevalonate cycle, obtained from acetyl-CoA, which goes on to produce cholesterol, dolichol and CoQ10 as the final product (Molyneux *et al* .,2008).

CoQ10 is also known as ubiquinone in its oxidized form and ubiquinol in its reduced form. In humans, ubiquinone (2,3-dimethoxy-5 methyl-6-decaprenyl-benzoquinone) has a chain with isoprene units (Mas E. and Morri T.,2010) and derives from the conjunction of the benzoquinone ring with a chain of hydrophobic isoprenoids, all of them with a double bond and trans configuration(Litarru and Tiano,2010) (Fig. 1).



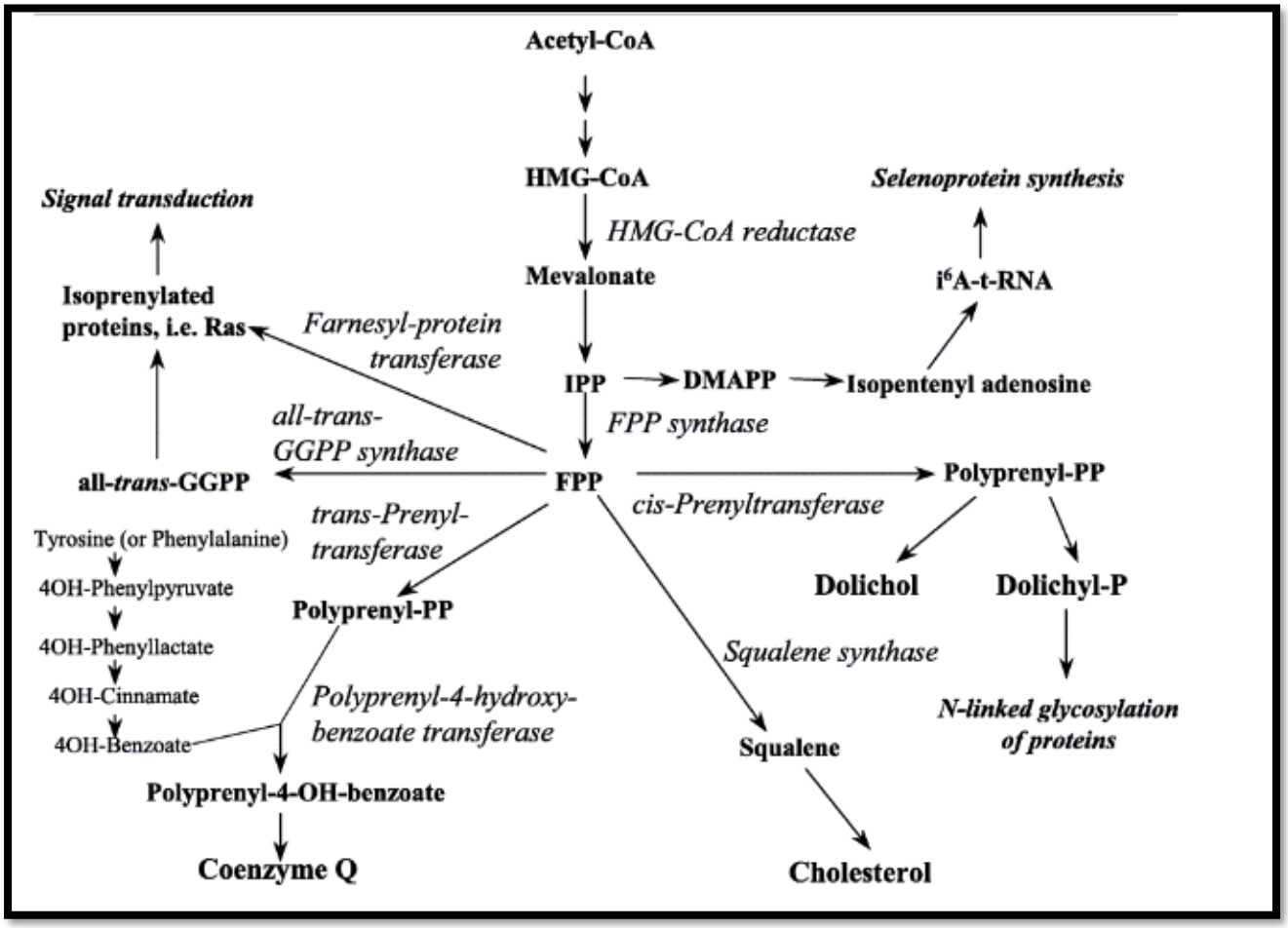
(Fig.2.9) Chemical structure of CoQ10 (reproduced from Prakash *et al., 2010)*

action, supplementation with this enzyme can be an ally in the stabilization and restoration of the natural defenses of the organism. Thus, in view of the above considerations, the objective of the present study was to elucidate what Coenzyme Q10 is, its origin and characteristics, form of absorption, and its relationship with MS and related diseases.

2.8.2The mevalonate pathway of Coenzyme Q10

The mevalonate pathway comprises the reactions starting from acetyl-coenzyme A (acetyl-CoA) and ending up with farnesyl pyrophosphate (FPP), the substrate for the biosynthesis of CoQ, cholesterol, dolichol and isoprenylated proteins (Fig.10)(Paredes-Fuentes *etal*.,2020) Thus, the

conversion of acetyl-CoA to FPP is common for all end-products. This organization is unusual since the biosynthetic sequence is identical for several lipids and one could expect that production of one lipid greatly influences the synthesis of the other lipids. However, the mevalonate pathway lipids are synthesized in highly different rates and amounts, which involves, in addition to the central regulation, a terminal regulation. The regulatory enzymes are probably the branch-point enzymes utilizing FPP. This fact makes the mevalonate pathway very complex in animal cells.



Fig(2.10). The enzymatic conversion and condensation of acetate to farnesyl-PP and subsequent biosynthesis of CoQ, cholesterol and dolichol. Abbreviations: CoA, coenzyme A; DMAPP, dimethylallyl pyrophophate; FPP, farnesyl pyrophosphate; GGPP, geanylgeranyl pyrophosphate; HMG, 3-

hydroxy-3-methylglutaryl; 4OH, 4-hydroxy; IPP, isopentenyl pyrophosphate. Key enzymes are indicated in italic.

2.8.3 Function in the organism

In the respiratory chain, CoQ10 is responsible for electron transport from the protein I complex (NADH dehydrogenase) to the protein II complex (succinate dehydrogenase), and from complex II to complex III (bc1 complex) (Bentinger *et al* .,2010).

When receiving the electrons from both complex I and complex II, it remains in its reduced form as ubiquinol and, after transferring the electrons to complex III it resturns to its oxidized form as ubiquinone (Molyneux *et al*.,2008) The organs that require higher energy concentrations such as the brain, heart, kidneys and liver show higher CoQ10 rates (Zubair *et al.*,2017)

2.8.4 CoQ10 sources

In healthy individuals, normal CoQ10 levels are maintained through two pathways, i.e., the exogenous pathway by food ingestion and endogenous synthesis by the mevalonate cycle. In the endogenousproduction, the mevalonate cycle involves acetyl-CoA as the initial substrate and cholesterol, CoQ10 and dolichol as the final products, the last being crucial for protein glycosylation. In this pathway, the enzyme prenyltransferase is responsible for the synthesis of the isoprenoid side chain of CoQ10, with the later occurrence of another condensation of this chain formed with 4-hydroxybenzoate (Bentinge ,2010).

In the exogenous pathway, CoQ10 is ingested in its oxidized form, being later transformed to its reduced form at the erythrocyte level. It is found naturally in small amounts in different foods, but it occurs in significant amounts in dark vegetables such as spinach and in legumes such as broccoli, grains such as soy and peanuts, oleaginous fruits such as nuts and almonds, and mainly in read meats such as heart and liver and in some fish like mackerel and sardines (Kitano,2006).

However, the dose of CoQ10 that can be obtained from food is 2–5 mg/day and only about 10% of what is ingested is absorbed by the gastrointestinal tract due to the low water solubility and high molecular weight of the enzyme, an insufficient amount to meet the demands of the organism in the presence of redox imbalance (Kumar *et al.*,2009 and Pepe *et al*.,2007) .

2.8.5 Absorption

In healthy individuals, about 95% of the CoQ10 circulating in plasma is in the reduced ubiquinol form (Bhagavan H and Chopra R.,2007).

Because it is hydrophobic and has a high molecular weight, CoQ10 is absorbed from the diet in a slow and limited manner, as is the case for lipids. Plasma CoQ10 levels start to increase 1–2 h after oral intake, with maximum concentration occurring within 6–8 h and with a half-life that may reach 34 h(Miles,2007). CoQ10 is mainly absorbed in the small bowel and is then transported to the liver, forming the lipoprotein complex. For transport, CoQ10 is coupled to the chylomicrons, being taken up by the liver(Miles,2007) and being then incorporated into LDL, which transports 58% of it, and into HDL, which transports 26% of it. CoQ10 is then distributed to various tissues such as the spleen, adrenals, lungs, kidneys, and myocardium (Mas and Morri ,2010). The main pathways of CoQ10 elimination are the bile ducts and the feces and a small fraction of what is absorbed ends up by being eliminated in urine (Bhagavan and Chopra,2006).

2.8.6 Supplementation

Several brands of commercial products containing CoQ10 are available on the market as powders, capsules or oil, in the reduced or oxidized form and in different doses, representing different forms of bioavailability (Molyneux *etal.*,2008). Solubilized CoQ10 formulations have greater bioavailability and are absorbed at faster rates than powders, tablets, capsules or oil powder suspensions (Schulz *et al*,2006). Comparison of the solubilized forms of ubiquinol and ubiquinone has shown that ubiquinol is better absorbed

(Bhagavan and Chopra , 2007). Several clinical trials involving the most diverse diseases have administered a variety of CoQ10 doses and have reported that adverse effects were more common at doses above 1200 mg/day (Hathcock and Shao,2006). with doses of 22–400 mg/day being considered safe (Arizona ,2017).

Cholesterol are synthesized through the mevalonate pathway. The reduction of serum CoQ10 concentrations may be as high as 54%(Strey *et al.*,2005). The magnitude of the reduction of CoQ10 in combination with statins has been shown to be dose related and reversible with the cessation of treatment. It has been hypothesized that this reduction may be the cause of the adverse effects of statins, and CoQ10 supplementation during treatment with statins could be a possible mediator treatment as long as it is properly monitored (Folkers ,1990;Silver ,2004)

2.8.7 Pharmacokinetics of CoQ10

Some reports have been published on the pharmacokinetics of CoQ_{10}. The plasma peak can be observed 2–6 hours after oral administration, depending mainly on the design of the study. In some studies, a second plasma peak also was observed at approximately 24 hours after administration, probably due to both enterohepatic recycling and redistribution from the liver to circulation. (Bhagavan and Chopra,2006). used deuterium-labeled crystalline CoQ_{10} to investigate pharmacokinetics in humans and determined an elimination half-time of 33 hours.(Tomono *et al.*,1986)

2.8.8Improving the bioavailability of CoQ10

The importance of how drugs are formulated for bioavailability is well known. In order to find a principle to boost the bioavailability of CoQ_{10} after oral administration, several new approaches have been taken; different formulations and forms have been developed and tested on animals and humans.(Žmitek *et al.*, 2008).

Reduction of particle sizeNanoparticles have been explored as a delivery system for various drugs, such as improving the oral bioavailability of drugs with poor absorption characteristics. *(Mathiowitz E et al 1997)* However, this has not proved successful with CoQ_{10}, although reports have differed widely.(*HsuCH etal*.,2003;*Joshi etal*.,2003) .

The use of aqueous suspension of finely powdered CoQ_{10} in pure water also reveals only a minor effect(*Ozawa et al .,1989*).

2.8.9 Effect of coq10 in sperm

(CoQ10) is an antioxidant molecule, component of the respiratory chain. Recently there has been growing interest in identifying reversible causes of male infertility, and numerous studies have been performed to investigate whether supplementing infertile men with antioxidants can improve seminal parameters (Showell ,2011)

Among the various antioxidants tested, CoQ10 (as a component of the mitochondrial respiratory chain) appears to play an important role in energy metabolism, as well as functioning as a liposoluble chain-breaking antioxidant for cell membranes and lipoproteins(Showell *et al*.,2011) .

Coenzyme Q10 (CoQ10) is a fat-soluble substance that is primarily present in mitochondria where it plays a role in electron transport chain and plays a part in aerobic cellular respiration and ATP production. Therefore, organs of high energy requirement such as liver, kidney, and heart have higher concentrations of CoQ10 (Oda *et al.*,2018). Seminal plasma and spermatozoa also contain considerable amount of CoQ10 for protection against oxidative

stress and preserving sperm integrity(Mancini and Balercia,2011). , Levels of CoQ10 in seminal plasma correlate significantly with sperm concentration and motility (Alleva *et al.*, 1997). To the best of the authors' knowledge, the protective efficacy of CoQ10 against OXT-induced toxicity has not beeninvestigated previously. Therefore, the present study was planned to assess efficacy of supplementing CoQ10 on oxidative stress status, liver/kidney function, semen quality and histopathology in OXT-intoxicated rats. (Oda *etal*.,2018).

2.8.10 Effect coq10 in liver and kidnys:

The liver plays the role of detoxifying and excreting destructive agents against poisoning the body. Liver injury occurs after pathological changes including degeneration, necrosis, and atrophy of parenchymal cells with interstitial connective tissue as well as increased liver enzymes, such as aspartate aminotransferase / alanine aminotransferase (AST / ALT), alkaline phosphatase (ALP), and total bilirubin level in Plasma (TBili) (Robins *et al*.,2007).

It was achieved that CoQ10 by its anti-oxidant, anti-inflammatory, and anti-apoptotic effects can have a therapeutic role in chemotherapy (busulfan) hepatotoxicity as well as metabolic-stress-induced liver damage (Fouad,2012; Vasiliev *et al*., 2011).

therapeutic effects of CoQ10, reduces metabolic stress by inhibiting hepatocyte apoptosis (Vasiliev *et al.*, 2011). Moreover, it was revealed that CoQ10 through its antioxidant, anti-inflammatory and anti-urination effects could have a role in improving busulfan-induced toxicity. (Fouad,2012).

Metastatic carcinomas are associated with cellular oxidative stress, and during cancer chemotherapy, oxidative stress from excessive drugs can limit therapeutic efficacy and cause a number of side effects, including fatigue, nausea, vomiting, diarrhea and more serious negative effects, such as cardiomyopathy and limb. Neuropathy, hepatotoxicity, and pulmonary

fibrosis. Here we review the hypothesis that the acute and chronic adverse effects of cancer chemotherapy can be reduced by molecular replacement of membrane lipids and enzymatic cofactors, such as coenzyme Q10.(Nicolson and Conklin, 2008).

Chapter Three Materials and Method

3.Materials and Methods:

3.1 Materials:

3.2 Equipments and Instruments:

Table (3.1): The equipments and instruments which were used in this study.

NO.	Equipment & Instrument	Company	Country
1.	Analytical sensitive balance	Sartorius	Germany
2.	Balance for animals	Shimadu company	Japan
3.	Centrifuge	Hettich Roto fix11	Japan
4.	Digital camera	Toup Cam	China
5.	Hematological auto analyzer	Bio Kit	USA
6.	ELIZA reader	Bio Kit	USA
7.	ELIZA washer	Bio Kit	USA
8.	Freezer	Hitachi	Japan
9.	Incubator	BINDER	Germany
10.	Light microscope	Leica	China
11.	Micropipette 100-1000 µl	CYAN	Germany
12.	Micropipette 1-100 µl	CYAN	Germany
13.	Oculometer		

14.	**Optical microscope with table PC**	**OPTICA**	**Italy**
15.	**Rotary macrotom**		**Germany**
16.	**Spectrophotometer**	**Labomed**	**UK**
17.	**Water bath**	**K.F.T.LAB.Equipme**	**Italy**
18.	**Sterile syringes 5, 10 ml**	**PROTON**	**Malaysia**
19.	**Jell tube**	**AFMA-Dispo**	**Japan**
20.	**Latex gloves**	**Great glove**	**Malaysia**
21.	**Latex gloves without powder**	**Great glove**	**Malaysia**
22.	**Funnel**	**HBG**	**England**
23.	**Filter paper**		**China**
24.	**Electronic Balance**	**Metter company**	**Switzerland**

3.3 Chemicals :

Table (3.2): The chemicals were used in this study and their sources:

No.	Chemicals	company	Country
1.	Rat inhibin B(INH-B)Elisa kit	Elisys Uno Human	Japan
2.	Rat adiponectin (ADPN) Elisa kit	Elisys Uno Human	Japan
3.	Rat Activin A(ACV-A) Elisa kit	Elisys Uno Human	Japan
4.	Complete blood count(CBC)kit	Abbott rubyC4000	USA
5.	Architect Testosterone ELISA Kit	Abbott 1000sR	USA
6.	Architect (FSH) ELISA Kit	Abbott 1000sR	USA
7.	Architect Lutinizing Hormone (LH) ELIZA Kit	Abbott 1000Sr	USA
8.	ALT (GPT) Colorimetric. Kit	SPECTRUM	Egypt
9.	AST (GOT) Colorimetric. Kit	SPECTRUM	Egypt
10.	Alkaline Phosphatase (ALP) Kit	SPECTRUM	Egypt
11.	ALBUMIN kit	SPECTRUM	Egypt
12.	BILIRUBIN kit	SPECTRUM	Egypt
13.	Urea kit	SPECTRUM	Egypy
14.	URIC ACID	SPECTRUM	Egypy
15.	CREATININE	SPECTRUM	Egypy

16.	**Busulfan drug (chemotherapy)**	**Xi'an Horlen Bio industries Inc**	**China**
17.	**CoQ10(Coenzyme Q10)**	**AMS(America medic and science)**	**USA**
18.	**EDTA (Ethylen ediaminetetra acetic acid**	**Usb**	**USA**
19.	**Normal saline**	**Labort**	**India**
20.	**Negrosin**	**Merck**	**Germany**
21.	**Paraffin Wax**	**Merck**	**Germany**
22.	**Sodium Citrate**	**Fluka**	
23.	**Chloroform**	**Noorbrok**	**England**
24.	**Xylole**	**Scharlau**	**Spain**
25.	**DMSO**	**LOBA**	**Chemie**
26.	**Eosin-Hematoxilin Stain**	**Merck**	**Germany**
27.	**Ethanol**	**Merck**	**Germany**
28.	**Formalin 10 %**	**TEDIA Company.**	**USA**
29.	**Ethanol 80%**	**Labort**	**India**
30.	**VORTEX-MIXTURE**	**BIONEER**	**E.U**

3.4 Prepare Drug Busulfan (Chemotherapy)

Busulfan is a bifunctional alkylating agent is known chemically as 1,4-butanediol, dimethanesulfonate. BUSULFEX® (busulfan) were intraperitoneally Injection (Benkessou.,2019). the activistic ingredient, a white crystalline powder with a molecular formula of CH3SO2O(CH2)4OSO2CH3 and a molecular weight of 250 g/mole **.** Busulfan 10mg/kg was initially were dissolved in10ml dimethyl sulfoxide(DMSO; Wako, Japan) and further diluted with 10 ml an equal volume of sterile distilled water, the busulfan solution was prepared immediate before use (Terayama, *et al*., 2012).

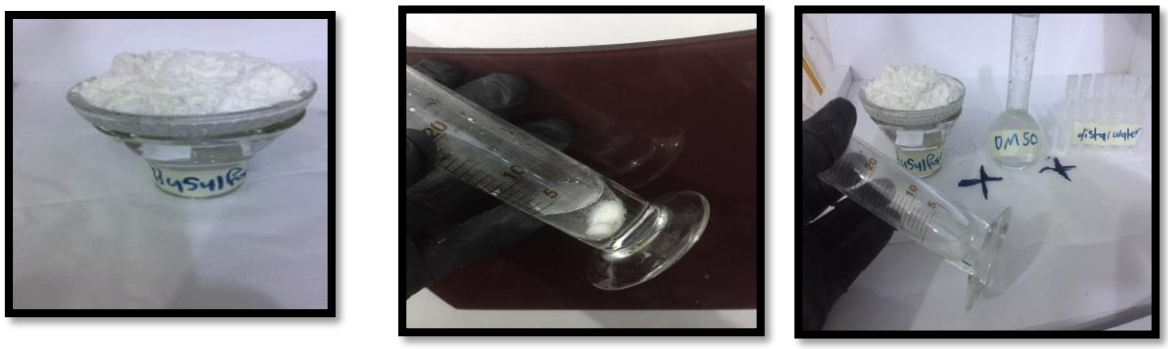


Figure (3.1):The Dose Of Busulfan

3.5 Prepare Drug CoQ10 (ubiquonon) :

Coenzyme Q10 (ubiquinone, ubidecarenone, coenzyme Q) is a component of the electron transport chain and participates in aerobic cellular respiration. Coenzyme Q10 also works as an antioxidant(Lee *etal*., 2017).

CoQ10 AMS(America medic and science) was dissolved in DMSO to prepare 400mg/kg was initially were dissolved in20ml dimethyl sulfoxide(DMSO; Wako, Japan) and further diluted with 20 ml an equal volume of sterile distilled water, the COQ10 solution was prepared immediate before use(Lix *etal*., 2016).

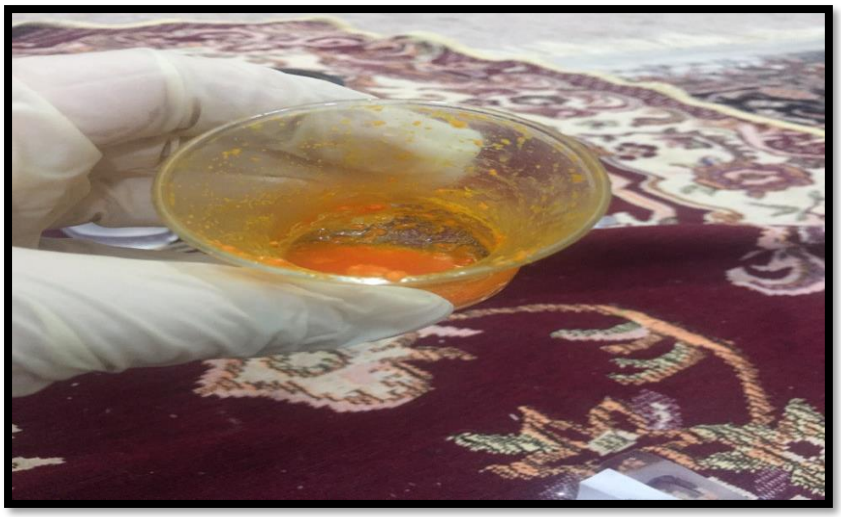


Figure (3.2):The Dose Of CoQ10

3.5 Experimental animals:

the experimental animal model selected for present study is albino rat rattus norvegicus rats were used in this experiment and they were obtained from the laboratory animal unit, faculty of science, university of zakho ,dohuk , iraq. the age were range between (2.5–3month) old with an average body weight (350-390gm) for male. the animals were apparently healthy, kept under hygienic conditions and air-conditioned room. the light system was 14/10 hrs light/dark cycle;22 ±2 c° with a relatively humidity of 30 to 60% from (15/12/2019 to 16/2/2020). the animals were housed in plastic cages , tap water was provided via glassy bottles, rats were fed from alwaha feed factory in karbala city. food and water were offered daily. the animals were accommodated to the laboratory conditions for one weeks before beginning of experiment.

3.6 Experimental Design:

Forty-eight adult male rats weighing (350–390 gm) were randomly divided into Four groups comprising twelve animals for each group as the following:-

*** first group: (Group A)** : twelve male Rats were given **normal saline** (0.2 mL, daily, intraperitoneally injection) and served as the control.

*** second group: (Group B)** : twelve male Rats were given single dose of **busulfan**(10mg/kgIP.)intraperitoneally injection (Wang *et al*., 2010).

*** third group: (Group C)** : twelve male Rats were given single dose of **busulfan** (10mg/kgIP.)**plusUbiquinone**(10mg/kg, daily b.w/IP) intraperitoneally injection. (World Health Organization ,2010).

*** fourth group: (Group D)** : twelve male Rats were given adose Of the **Ubiquinone**(10mg/kg, daily, b.w/IP) intraperitoneally injection(Mazen and Elnegris , 2013).

The drugs were administered intraperitoneally injection for two a period of (28 and 56) days. After 28 days, 6 animals from each there group will be sacrified , and the rest will be sacrified after 56 days.

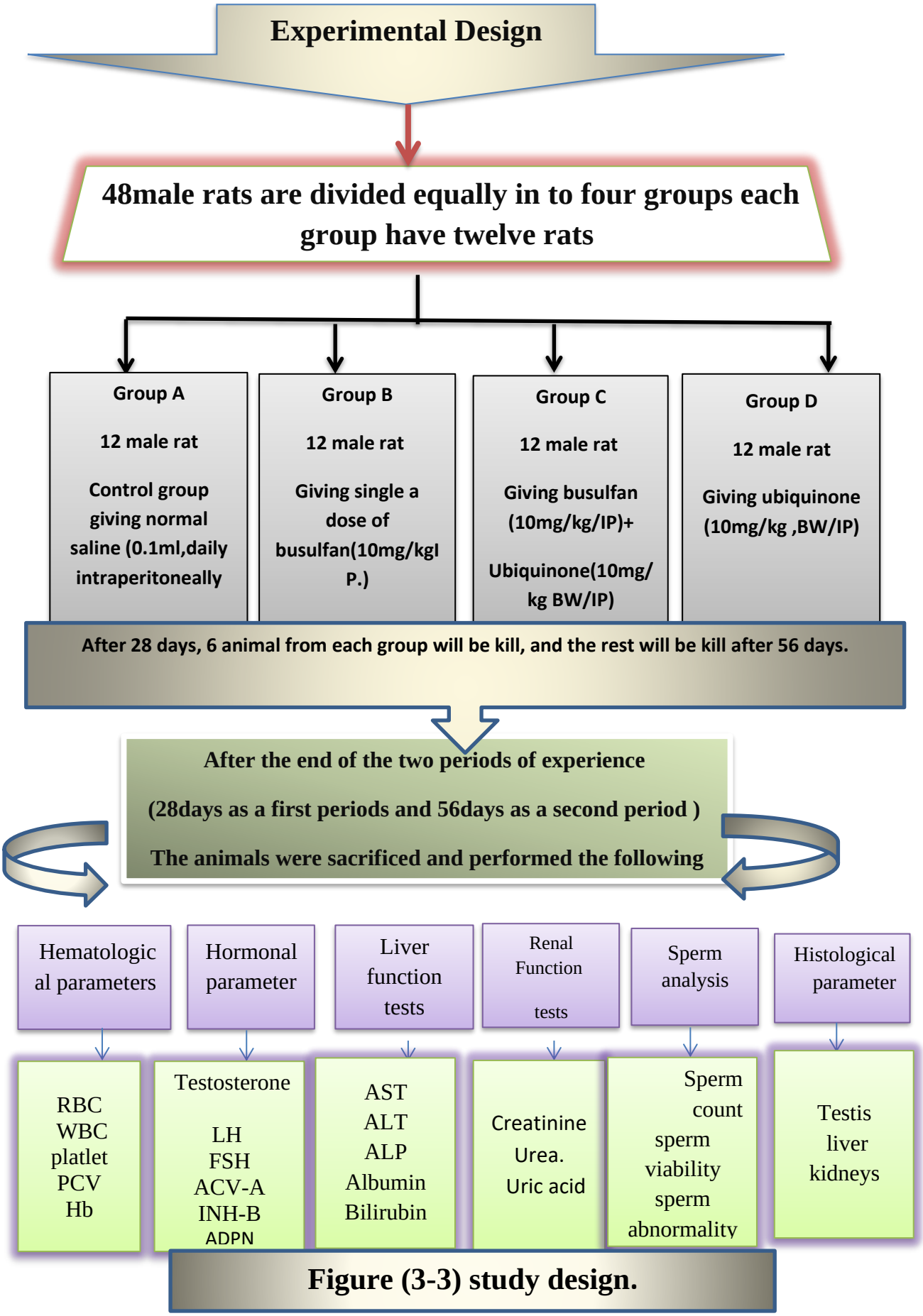


Figure (3-3) study design.

3.7 Preparation and collection of the samples:

At the end of the expe riment, the animals that were (anesthetized by chloramphenicol inhalation). The chest and abdomen were open by thoracotomy and laparotomy. followed by collection of blood to perform Hematological , biochemical and hormonal testing then after sacrificed collection of caudal sperm for caudal sperm analysis and the liver, kidney and testes isolated to histobathological studies**.**

3.7.1- Blood sample :

were collected via cardiac puncture. From each male rat.the blood sample were placed in serum tube and left for 30 minutes. Then the blood sample were drops directly from the heart by using 5 ml disposable syringe the blood putting in the gel tube and then to be centrifuged (3000 rpm for 10 minutes) and kept frozen at -20 °C to obtain .the serum which then were transferred to the ependorf tubes. for assessment of reproductive hormones concentrations in serum (FSH,CBC, LH, testosterone , adiponectin, inhibinB and activinA) **.** All these of tubes were stored at (-4c) until analyzed. All sample were collected at the morning (8:30 AM – 10:30 AM) in the order to minimize the diurnal variation of the hormone levels.

3.7.2- Organs

Liver, kidney and testes were to be removed and The organs were be fixed in to 10 % of formalin for histological examination.

3.8 Methodes:

3.9 The haematological parameters(CBC)

The haematological parameters were done in Laboratory of Research and Studies/ Laboratory of Al daqah / central research laboratory in Karbala by using Hematological auto analyzer (count 60) made in Ruby Abbott C4000 company in USA. The instrument can be measure and calculate 22 different parameter. This instrument used Three reagents only (Dilute and RBC Lyse,

WBC Lyse) and Maintenance reagent (Probe cleanser only) and it has a picture mechanical inside with thermal paper . The haematological parameters estimated by the instrument were (RBC, WBC, MCV ,MCHC,PCV, Hb) .(Mathieleers *et al.*, 2012).

3.10 Hormones Assays in blood serum by Abbott Architect i-1000SR

The ARCHITECT *i*1000SR,USA immunoassay auto analyzer meets your laboratory's high standards by delivering STAT results when you need them. The flexible protocols built into the ARCHITECT *i*1000SR enhance laboratory workflow and allow you to report results with confidence. For *in vitro* diagnostic use only. Use photometric, potentiometer and or CMIA (Chemiluminescent microparticles immunoassay)technology to measure analyze to concentration in samples .

3.10.1Estimation of Follicle Stimulating Hormone (FSH) Concentration (µlU/ml) by using a special kit (Abbott-ARCHITECT LH Kit, USA).:

Measurement of the serum follicular stimulating hormone concentration was generally regarded as valuable tool in the diagnosis of homeostasis of fertility regulation via the hypothalamic –pituitary –gonad axis; the kit were used(Architect abbott,park,IL60064 USA).Clinical and laboratory standareds institute(CLSI),2014. **appendix IX**

3.10.2 Estimation of Hormone (LH) Concentration (ng/ml) by using a special kit (Abbott- ARCHITECT FSH Kit, USA).:

Measurement of the serum follicular stimulating hormone concentration was generally regarded as valuable tool in the diagnosis of homeostasis of fertility regulation via the hypothalamic –pituitary –gonad axis; the kit were used(Architect abbott,park,IL60064 USA).Clinical and laboratory standareds institute(CLSI),2014. **appendix X**

3.10.3 Estimation of Testosterone (T) Concentration (ng/ml) by using a special kit (Abbott- ARCHITECT Testosterone Kit, USA):

Measurement of the serum follicular stimulating hormone concentration was generally regarded as valuable tool in the diagnosis of homeostasis of fertility regulation via the hypothalamic –pituitary –gonad axis; the kit were used(Architect abbott,park,IL60064 USA).Clinical and laboratory standareds institute(CLSI),2014. **appendix XI**

Hormonal assays in blood serum by using appropriate the enzyme–linked immunosorbent assay (ELISA) (Elisys UNOJapane).

The HUMAN ELISA line of reagents and analyzers offers unique solutions for all sizes of laboratories from low to high throughput. All instruments and analyzers come with the HUMAN Plug &Run capability, meaning that all HUMAN ELISA assays are pre-programmed and individually validated. HUMAN ELISAs allow for flexible automation and are designed for intuitive and easy manual processing. The range of ELISA reagents incorporates more than 100 assays for the detection of infectious diseases, hormones, tumor markers, allergy and autoimmune diseases**.** **(**HUMAN -Planck-Ring 21 · 65205 Wiesbaden · Germany)

Fully automated ELISA analyzer for medium and high throughput> Instruments for all kinds of laboratories from low to high throughput > Optimised to meet your needs > Easy to use and flexible > Broad range of parameters, steadily growing > High-quality products > More than 120 assays available for detection of –Infectious Diseases – Hormones –Tumor Markers – Autoimmune Diseases (IMTEC line).

3.10.4 Estimation of rat Activin A (ACV-A)Concentration (ng/ml) by using appropriate the enzyme–linked immunosorbent assay (ELISA) (Elisys UNOJapane). : appendix XII

The kit allows for the determination of ACV-A concentrations in Rat serum, plasma ,tissue homogenates and other biological fluids.

3.10.5 Estimation of rat inhibinB(INH-B)Concentration (ng/ml) by using appropriate the enzyme–linked immunosorbent assay (ELISA) (Elisys UNO Japane). :

The kit allows for the determination of INH-B concentrations in rat serum, plasma, tissue homogenates and other biological fluids. **appendix XIII**

3.10.6 Estimation of rat adiponectin(ADPN)Concentration (ng/ml) by using appropriate the enzyme–linked immunosorbent assay (ELISA) (Elisys UNOJapane). :

The kit allows for the determination of ADPN concentrations in rat serum,plasma,tissue homogenates and other biological fluids. **appendix XIV.**

3.11 Biochemical Test:

3.11 .1 Estimation of Biochemical Renal Function Tests

3.11.1.A Serum urea estimation:

Urea concentration in serum was determined by using a special kit (SPECTRUM- Urea Kit, Egypt-IFUFCC40),and by using device (Spectrophotometer Sesil, England). **described in Appendix I**

3.11. 1.B Serum Creatinine Estimation:

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex (Jaffé method). The rate of complex formation is measured in a short period to avoid interferences. Serum and plasma samples contain proteins that react in a non specific way; nevertheless, the results can be

corrected by subtracting a fixed value. The use of this correction is known as compensated Jaffé method. Creatinine concentration was determined by a creatinine Kit (bioSystems, Spain) (Peake and Whiting, 2006).**show appendix (II)**

3.11.1.C Serum uric acid estimation:

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of dichlorophenol sulphonate (DCBS) and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide to form a quinoneimine dyeproportional to the concentration of uric acid in the sample according to (Fossati *et al*., 1980) as **described in Appendix(III**).

3.11. 2 Estimation of Biochemical liver Function Tests

3.11.2.A Serum Aspartate Aminotransferase Activity (AST):

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction. Aspartate aminotransferase concentration was determined by using a special AST Kit (bioSystems, Spain) (Schumann *et al.*, 2010)**.show appendiX IV.**

3.11.2.B Serum Alanine Aminotransferase Activity (ALT):

Principle of the test

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction. Alanine aminotransferase concentration was determined by using a

special ALT Kit (bioSystems, Spain) (Schumann *et al.*, 2010).**show appendix V**

Alanine + 2 – Oxoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + Glutamate

Pyruvate + NADH +H+ $\xrightarrow{\text{LDH}}$ Lactate + NAD

3.11.2 .C Determination OF concentration ALP

Principle of the test

Alkaline phosphatase concentration was determined by using a special ALP Kit (Agappe Diagnostic LTD., India). Kinetic determination of ALP was done according to the following reaction(Klin , 1980):**show appendix VI.**

3.11.2.D determination of Bilirubin concentration

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubinglucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin the direct is also determined, the results correspond to total bilirubin. The intensity of the color formed is proportional to the bilirrubin concentration in the sample1,2 **Appendix VII.**

3.11 .2.D Determination of Albumin concetration

Measurment of albumin is based on its binding to the indicator dye bromocresol green (BCG) in PH 4.3 to form blue – green colored complex. (Tietz , 1990) **appendix VIII**

3.12 Preparation of Histological Solutions:

3.13 Eosin stain: it was prepared by mixing :

1. Eosin stain (1 gm.)

2. Distilled water (30ml)

3. Ethyl alcohol (70 ml). (AL-Karawi,*et al*., 2019)

3.14:Sperm Count Solutions :

3.15 :Eosin –Nigrosin stain: It was prepared from :

1. (1gm) of Eosin stain soluble in (100 ml) of 3% sodium citrate.

2. (5gm) of Nigrosin stain soluble in (100 ml) of 3% sodium citrate (Hancook, 1951).

3.16 Seminal Analysis:

3.17 Sperm Concentration:

The sperms were counted according to method of (Fernandes *et al*., (1978) by using Neubauer hemocytometer chamber which use for RBC and WBC count.

3.18 Procedure

1)The epididymis were put in a petry dish contained 2ml of 0.9% Normal salin .

2)The epididymis was cut in to 6-10 pieces by using sharp scalpel .

3)The suspension resulted from the previous step was filtered by Clean of piece by gauza in to a test tube .

4)one drop from the filtrate was dropped on the neubauerb chamber Which covered previously with cover slide.

5)The sperms found on the five squares that use for counting the RBCS by using the objective lens (40x) .

6)The sperms were calculated in one mm3 as follwing:

Sperms/cmm=nx10000

N=number of sperms in 5 squires .

3.19 Sperm Motility Percentage:

Number of motile sperms were calculated according to (Lio, *et al*, 1986). Collection of spermatozoa for evaluation of sperm motility. Epididymal spermatozoa were collected by cutting the caudal region of the epididymis into small pieces in 2ml of normal saline pre warmed to 37°C. Sperm was forced out of the caudal epididymis with fine forceps by putting pressure on lower region of caudal epididymis, not forcing out excess material i.e. immature cells. Number of motile spermatozoa were calculated per unit area and expressed as percentage sperm motility. Sperm counts were done using haemocytometer and results were expressed as millions/ml of suspension.

3.20 Percentage of Spermatozoa Viability:

The viability of sperms was counted according to(Graaf and Beilby.,2009).

3.21 Procedure.

1)Diluted semen was dropped on awarm clean slide.

2)warm eosin-negrosin stain was dropped on the semen and mixed together carefully by the use of aglass rod .Asmear was done by using clean slide which was put angularly on the semen slide and was dragged horizontally.

3)The slide was left to dry,and the slide was examed under the light of microscope of using 40x power; Live sperms appeared non stained and the dead sperms appeared stained.

3.22 Percentage of the Abnormal Spermatozoa:

The abnormal spermatozoa were counted in the same slide that was used to measure the dead and live spermatozoa using account 200 sperms under the light microscope using 100 X power. The number of the result sperm divided on 200 and multiple by 100 according to Filler (1993).

3.23 Histological study:

The animals anatomy in the laboratory and the organs of rats were dissected (kidneys, liver and testes) rapidly excised for histological study and rinsed in normal saline then fixed by immersing deeply in a large volume of 10% formalin at least ten times the volume of the tissue as rapidly as was feasible there by keeping postmortem changes at a minimum. The samples were put in a labeled container contain 10% formalin, and shaking of the container gently several times to make certain that the fluid reached all surface and that the pieces were not sticking to the bottom or sides (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom) according to (Al-khalissi., 2014).

3.24 Histopathological Technique:

The liver, kidneys and testes of each animal were quickly removed and rapidly weighed then prepared for histological study according to (Al-khalissi., 2014). with aid of the light microscope as the following steps:

1.Fixation

The specimen was fixated in the natural buffered formalin 10 % for 24 –48 hours.

2.Washing and dehydration

After fixation the specimens were washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

3.Clearing

Bathing the dehydrated fragments in solvent (xylene) for 30 – 60 minutes;this step was repeated 3 times .As the tissues clearing ,they generally became transparent .

4.Infiltration and embedding

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 – 60 oC . The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

5.Sectioning

After hold from the oven the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by the microtome, s steel blade into sections 5 micrometers thick . The sections were floated on water bath (50 – 55 o C) , then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

6.Staining

The histological sections of the studied organs were stained with Hematoxylin - Eosin

3.25 Statistical Analysis:

Statistical analysis of the results was conducted according to SPSS (2016) version 24.00 where one way (ANOVA) was used to assess the significance of changes between the groups' results. The data were expressed as Mean, ± Standard Errors (SE) and P-value ≤ 0.05 and ≤ 0.01 was considered as statistically significant, LSD test was carried out to test the significant levels among means of treatments (Green & Salkind, 2016).

Chapter four

Results

4.Results:
4.1 hematological parameters

The results in table (4-1a) (4-1b) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for both periods 28 and 56 days, they showed significant ($p<0.05$) decrease in the hemoglobin level (HB), Red blood cells count (RBC), White blood cells count(WBC), platelets count and packed cell volume (PCV) in Busulfan group and Busulfan +Co10 when compared with control group and co10 alone group.

Table (4-1a) show the statistical analysis for the effect ofbuselfan, buselfan +co 10 and co 10 alone on some hematological parameters in male rats for 28 days.

Parameter / Group	Hb g/dl	RBC $\times10^6$cell/mm3	WBC $\times10^3$cell/mm3	PLT $\times10^{11}$cell/mm3	PCV %
Control	12.56±2.75 A	6.60±1.04 A	10.02±1.14 A	1080.83±214.25 A	41.083 ± 0.77 A
Buselfan	7.08±0.31 B	3.81±0.19 B	1.69±0.45 C	310.50±88.65 B	21.25±1.32 B
Buselfan+ Co10	8.36±.80 B	4.04± .58 B	3.58±1.61 B	435.33±30.41 B	21.833 ± 1.16 B
Co10	13.88±1.44 A	6.76±0.72 A	10.34±0.70 A	960.50 ± 68.48 A	42.583 ± 1.90 A

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

Table (4-1b) show the statistical analysis for the effect ofbuselfan, buselfan +co 10 and co 10 alone on some hematological parameters in male rats for 56 days.

Parameter / Group	Hb g/dl	RBC×10^6 cell3/mm	WBC×10^3cell/mm3	PLT×10^{11}cell/mm3	Pcv%
Control	12.13±0.97 **B**	6.80± 0.38 **A**	10.16 ±0.86 **A**	00.12± 92.80 **A**	40.38± 0.86 **A**
buselfan	7.53±1.40 **D**	3.92± 0.38 **C**	4.23±0.78 **C**	741.50±65.00 **C**	30.30±1.93 **C**
buselfan+ coQ10	9.46±1.01 **C**	5.59±0.66 **B**	6.56±2.02 **B**	868.83±82.80 **B**	35.03±4.83 **B**
CoQ10	13.93±0.40 **A**	7.23± 0.13 **A**	10.30±1.32 **A**	1125.83±120.44 **A**	41.66±0.42 **A**

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

Table (4-b1) also show significant elevation($p<0.05$) in the hemoglobin level (HB), Red blood cells count (RBC), White blood cells count(WBC), platelets count and packed cell volume (PCV) in Busulfan +co10 group when compare with Busulfan alone group.

4.2.Biochemical parameters

The results in table (4-2a) (4-2b) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for both periods 28 and 56 days, they showed significant ($p<0.05$) increase in the AST, ALT, ALP, Albumin and Bilirubin in Busulfan group when compare with control group, busulfan +c10 group and co10 alone group.

Table (4-2a) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 and CoQ 10 alone on some liver parameters in male rats for 28 days

Parameter / Group	**AST** U/L	**ALT** U/L	**ALP** U/L	**ALBUMIN**	**BILIRUBIN**
Control	50.85±3.04 C	39.50±4.20 C	39.07±2.91 C	2.49±0.18 C	0.19±0.04 C
buselfan	78.47±6.09 A	65.77±3.39 A	72.05±9.49 A	5.16±1.11 A	0.41±0.04 A
buselfan+ coQ10	59.11±5.63 B	47.20±5.67 B	46.86±5.76 B	3.53±0.64 B	0.28±0.04 B
CoQ10	52.83±1.88 C	40.77±1.83 C	38.44±1.58 C	2.44±0.28 C	0.24±0.05 BC

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

Table (4-2b) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 an d CoQ 10 alone on some liver parameters in male rats for 56 days.

Parameter / Group	**AST** U/L	**ALT** U/L	**ALP** U/L	**ALBUMIN**	**BILIRUBIN**
Control	52.28±2.40 A	39.24±3.86 A	38.80±2.71 A	2.44±0.10 B	0.20±0.02 B
buselfan	56.36±5.45 A	43.07±4.06 A	39.16±2.38 A	3.60±0.42 A	0.39±0.04 A
buselfan+ coQ10	53.03±4.20 A	40.62±2.06 A	37.34±1.46 A	2.76±0.26 B	0.24±0.04 B
CoQ10	52.31±1.47 A	39.70±2.51 A	37.67±1.04 A	2.42±0.26 B	0.24±0.06 B

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

The results in table (4-3a) (4-3b) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for both periods 28 and 56 days, they showed significant ($p<0.05$) increase in the Urea, Uric acid and Creatinine in Busulfan group when compare with control group, busulfan +c10 group and co10 alone group.

Table (4-3a) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 and CoQ 10 alone on some Renal parameters in male rats for 28 days.

Parameter / **Group**	**UREA** Mg/dl	**URIC ACID** Mg/dl	**CREATI NINE** Mg/ dl
Control	24.41±4.13 C	2.77±0.44 C	0.78±0.04 BC
buselfan	48.36±2.55 A	5.03±0.86 A	1.65±0.45 A
buselfan+coQ10	30.56±4.79 B	3.80±0.90 B	1.09±0.54 B
CoQ10	24.51±1.25 C	2.74±0.47 C	0.65±0.01 C

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

Table (4-3b) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 and CoQ 10 alone on some Renal parameters in male rats for 56 days.

Parameter / Group	UREA Mg/dl	URIC ACID Mg/dl	CREATI NINE Mg/dl
Control	25.09±3.70 BC	2.61±0.44 B	0.74±0.04 B
buselfan	45.25±2.86 A	4.88±1.07 A	1.60±0.37 A
buselfan+ coQ10	29.35±5.94 B	3.13±0.55 B	0.81±0.08 B
CoQ10	23.58±1.55 C	2.58±0.33 B	0.63±0.04 B

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

4.4 Hormonal parameters

The results in table (4-4a) (4-4b) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for both periods 28 and 56 days, they showed significant ($p<0.05$) decrease in the testosterone level and significant increase ($p>0.05$)in LH &FSH in Busulfan group when compare with control group, busulfan +c10 group and co10 alone group.

Also, there is significant increase in busulfan +co10 group when compare with busulfan alone group in testosterone levels and significant increase in busulfan +co10 group when compare with busulfan alone group in FSH &LH.

Table (4-4a) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 and CoQ 10 alone on some Hormonal Level parameters in male rats for 28 days.

Parameter / Group	Testosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)	adiponectin (ng/mL)	Activin A (ng/mL)	inhibin B (ng/mL)
Control	2.26±0.79 AB	1.58±0.41 C	3.81 ±1.10 C	0.81±0.04 C	7.14±0.38 A	4.22±1.37 A
buselfan	1.13±0.19 C	3.29 ±0.56 A	8.30 ±1.43 A	2.48±0.17 B	1.67±0.63 C	1.29±0.88 C
buselfan+ CoQ10	1.77±0.15 B	2.21 ±0.49 B	5.21 ±0.91 B	4.60±1.02 A	2.92±0.54 B	2.70±0.34 B
CoQ10	2.40±0.58 A	1.64 ±0.32 C	3.83 ±0.68 C	3.07±0.24 B	6.92±0.92 A	4.39±1.03 A

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups ($p \leq 0.05$)

Table (4-4b) show the statistical analysis for the effect of buselfan,buselfan+co 10 and co 10 alone on some Hormonal Level in male rats for 56 days.

- **Values Means ± SD, n = 6**

Parameter / Group	Testosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)	adiponectin (ng/mL)	Activin A (ng/mL)	inhibin B (ng/mL)
Control	2.29±0.73 AB	1.70 ±0.39 C	3.76 ±0.97 C	0.82±0.06 B	7.10±0.53 A	4.08±1.45 A
buselfan	0.92±0.15 C	3.62 ±0.26 A	9.61 ±1.51 A	2.82±0.73 A	0.47±0.14 B	0.77±0.97 B
buselfan+ CoQ10	1.93±0.25 B	2.14 ±0.38 B	4.97 ±0.41 B	2.63±0.72 A	0.45±0.31 B	1.97±0.50 B
CoQ10	2.53±0.49 A	1.58 ±0.33 C	3.59 ±0.77 C	2.44±0.88 A	6.92±0.69 A	4.47±1.13 A

- **The different litters refer to the significant change between groups ($p \leq 0.05$)**

4.5 Sperms Parameters

The results in table (4-5a) (4-5b) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for both periods 28 and 56 days, they showed significant ($p<0.05$) decrease in the sperm count, sperm motility, sperm viability and sperm abnormality in Busulfan group when compare with control group, busulfan +c10 group and co10 alone group.

Also, there is significant increase in busulfan +co10 group when compare with busulfan alone group.

Table (4-5a) show the statistical analysis for the effect of buselfan,buselfan +co 10 and co 10 alone on epididymal sperm Analysis in male rats for 28 days.

Parameter / Group	Sperm count (10^6/ml)	Sperm motility (%)	Sperm viability (%)	Sperm abnormality (%)
Control	66.67 ±8.04 A	67.33±13.35 A	73.17±3.31 A	5.83±1.16 C
buselfan	14.83±5.49 C	20.33 ±5.42 C	19.83±4.44 C	37.67±6.05 A
buselfan+coQ10	28.00±4.73 B	37.83±6.11 B	35.67±2.73 B	12.50±2.07 B
CoQ10	71.83±14.16 A	71.33±7.91 A	75.50±4.41 A	3.67±1.21 C

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)

Table (4-5b) show the statistical analysis for the effect of buselfan, buselfan +co 10 and co 10 alone on epididymal sperm Analysis in male rats for 56 days.

Parameter / Group	Sperm count (10^6/ml)	Sperm motility (%)	Sperm viability (%)	Sperm abnormality (%)
Control	67.33±8.77 B	68.67±3.55 A	71.67±3.44 A	4.83±0.75 BC
buselfan	5.50±3.14 D	11.33±9.22 C	14.17±3.601 C	54.83±7.30 A
buselfan+ CoQ10	42.33±3.20 C	43.83±4.26 B	42.50±6.025 B	8.50±4.27 B
CoQ10	75.33±7.39 A	73.50±3.27 A	76.33±3.44 A	3.33±0.81 C

- Values Means ± SD, n = 6
- The different litters refer to the significant change between groups (($p \leq 0.05$)
-

4.6:The Histological Examinations.

4.6.1 Histological Picture and Changes of the Testes:

Tissue examination of the normal testes of rats shows that the seminiferous tubules are spherical or ovoid shape , lined by germinal epithelium consisting primarly of spermatogenic cells which are in the progressive stages of spermatogenesis (primary spermatocytes, secondary spermatocytes, spermatide and sperms) in the lumen of seminiferous tubule , between seminiferous tubule there are interstitial spaces with the group producing interstitial leydig's cells **Figure (4-6.1).**

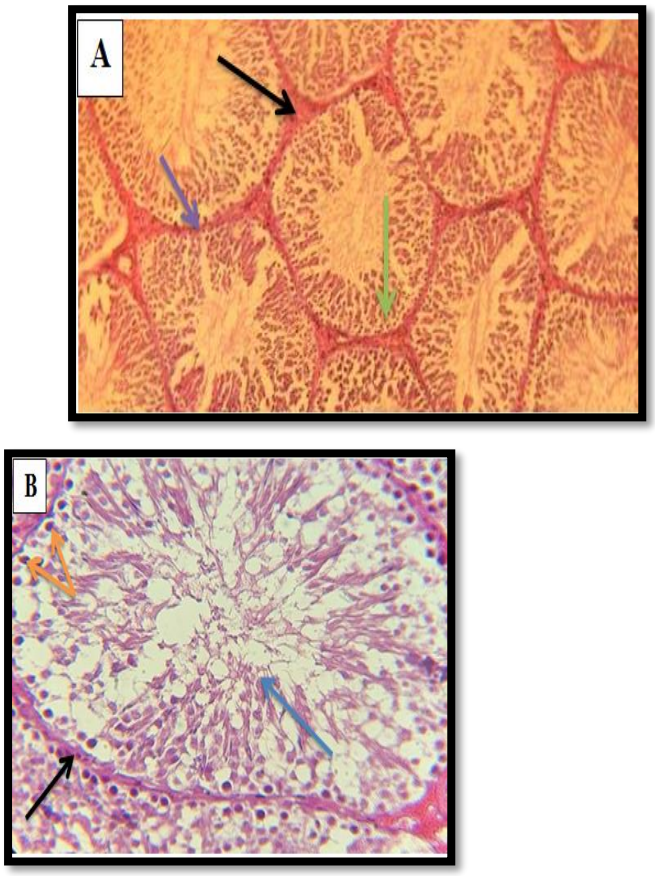


Figure (4-6.1).Testes (control):A normal testes rate the black arrow shows the leydig cells, the green arrow shows the basement membrane and the blue arrow shows the spermatogonia. (X10) .**B** normal testes rate the black arrow shows the sertoli cells, the yellow arrow shows The spermatogonia and the green arrow shows the spermatozoa. (X40).

The histological examination in this study shows a decrease in the diameter of seminiferous tubule,the seminiferous duct tubule and thickness of epithelium of seminiferous. seminiferous tubules suffer from hyperplasia with some absences of spermatogenesis process with in groups of male rats treated with busulfan for both periods 28 and absent of seminiferous duct in 56 days.shows normal seminiferous tubules with increase of spermatogonial and spermatogenesis in groups of male rats treated with busulfan&COQ10 for both periods 28 and 56 days. shows normal seminiferous tubules with increase of spermatogonial and spermatogenesis in groups of male rats treated with COQ10 only for both periods 28 and 56 days.

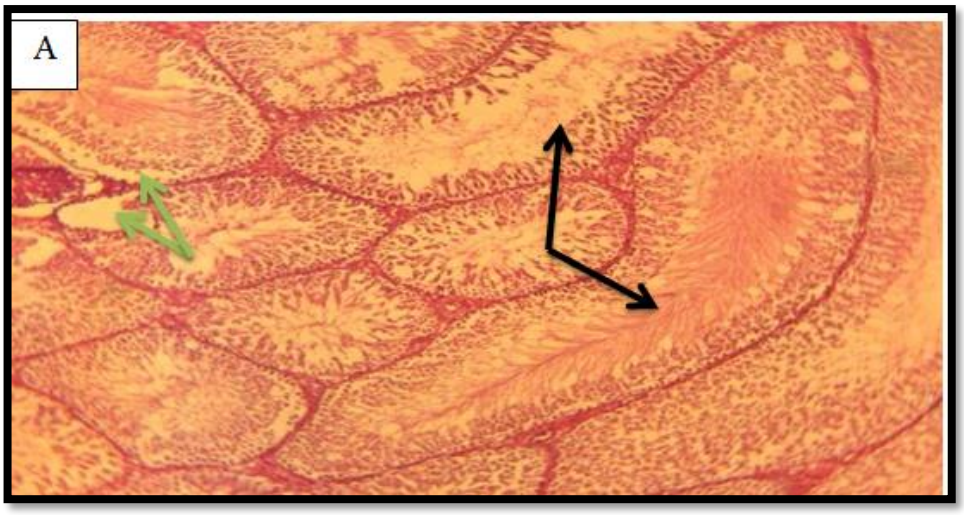


Figure (4-6.2)Testes treated with single dose of busulfan(10mg/kgIP.) 28 days: In this section A the thickness of testicular capsule and decrease in seminiferous tubules diameter the seminiferous tubules suffer from hyperplasia (black arrow), the green arrow show the some absences of spermatogenesis process with decrease the seminiferous duct(X10). H&E staining.

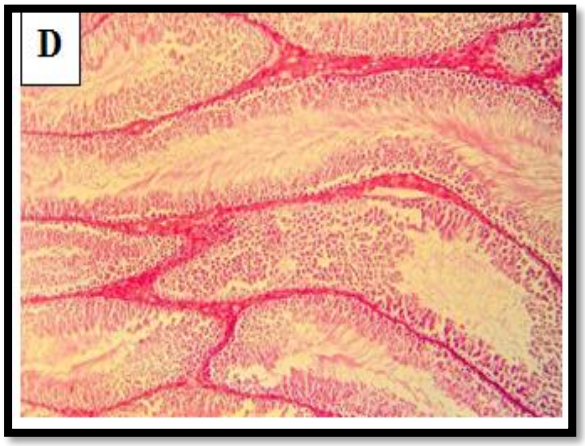


Figure (4-6.3)In this section C single dose of busulfan(10mg/kgIP.) 56 days the seminiferous tubules suffer from hyperplasia (black arrow), with decrease size or absent of seminiferous duct due to treated with busulfan. (X10).

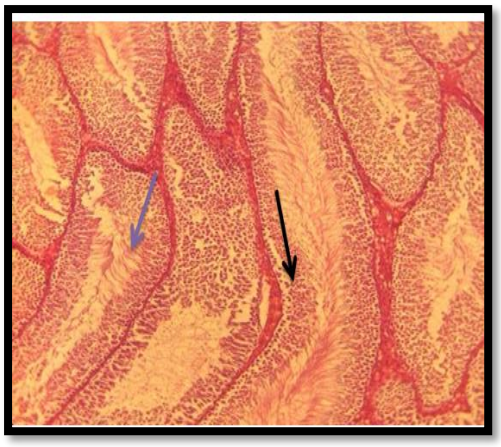


Figure (4-6.4)Testes rate treated with busulfan&CoQ10 28 day: This section D shows the normal seminiferous tubules with increase of spermatogonial and spermatogenesis (X10).

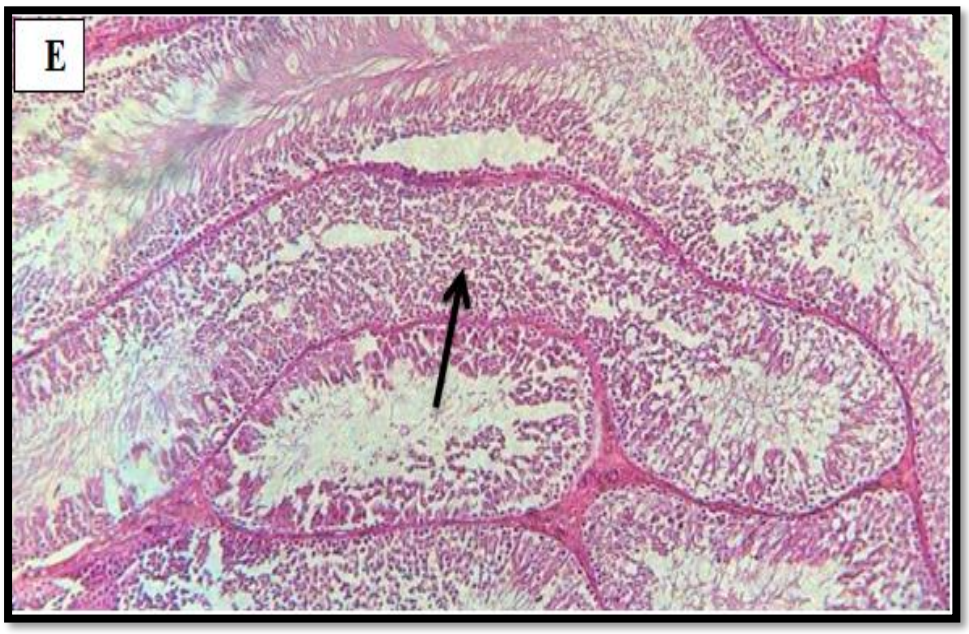


Figure (4-6.5)Testes treated with busulfan&CoQ10 56 days in this section E the seminiferous tubules suffer from hyperplasia (black arrow), with decrease size of seminiferous duct due to treated with busulfan. (X10).

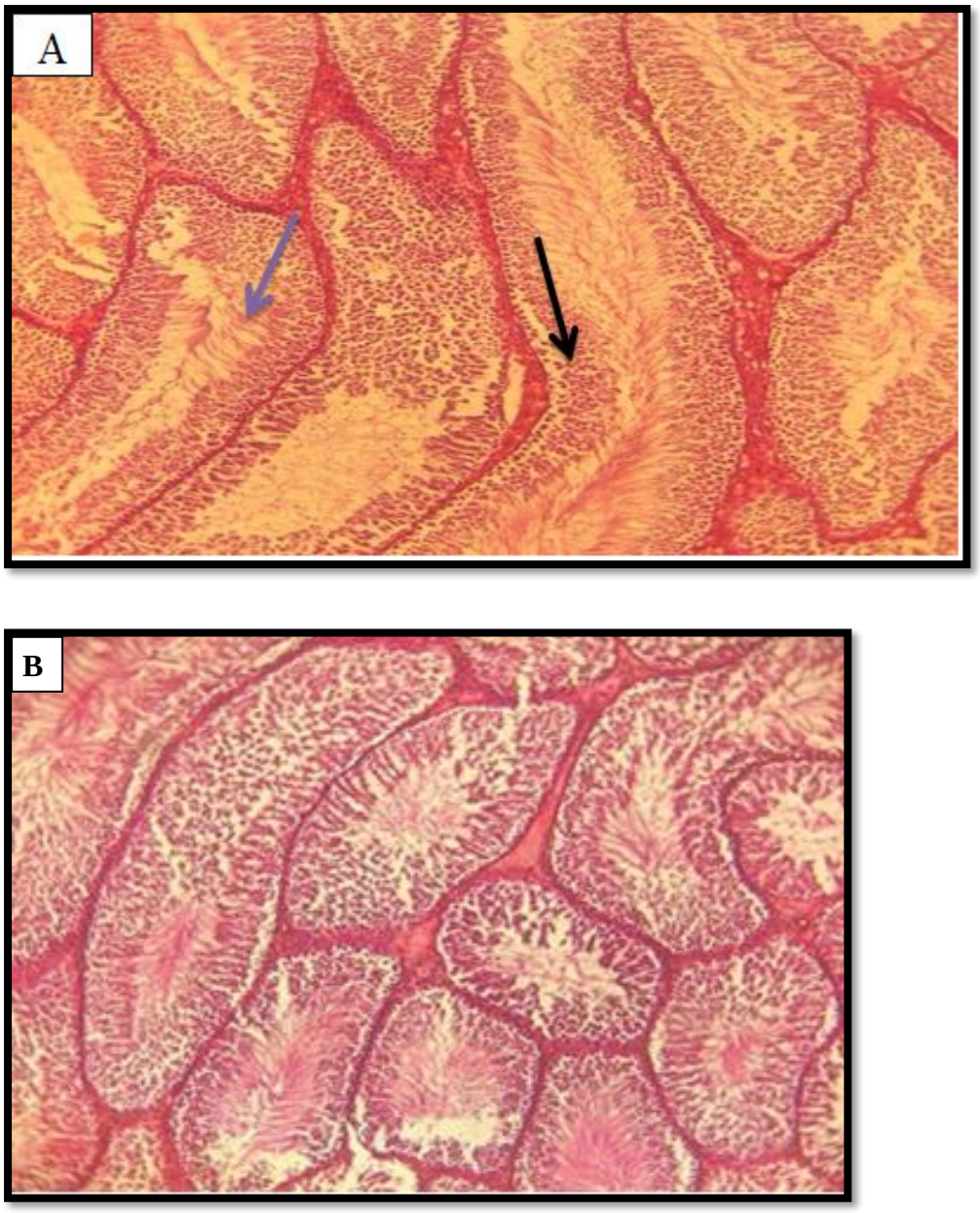


Figure (4-6.6) Testes treated with CoQ10 **28 (A) &56** (B)**day** : In this section the seminiferous tubules suffer from hyperplasia with increase spermatogenesis (black arrow),. (X10).

4.6.2 Histological Picture and Changes of the Kidney:

The histological examination in this study shows a the shrinking glomerulus with increase the glomerular space for period 28 day and in suffer from sever nephrotoxicity and shows sever shrinking of glomerulus with increase of glomerulus space 56 day. In groups of male rats treated with busulfan ,busulfan &coq10.

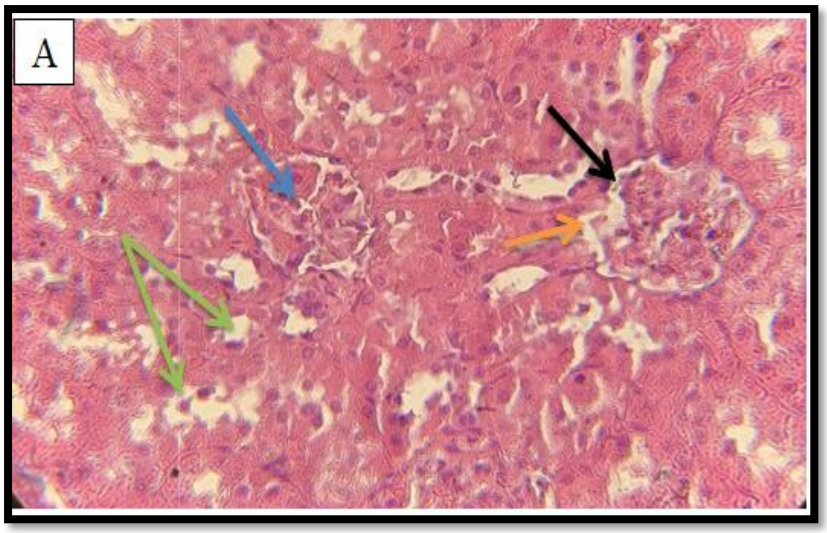


Figure (4-6.7)Kidney (control): A normal kidney paranchemus tissue

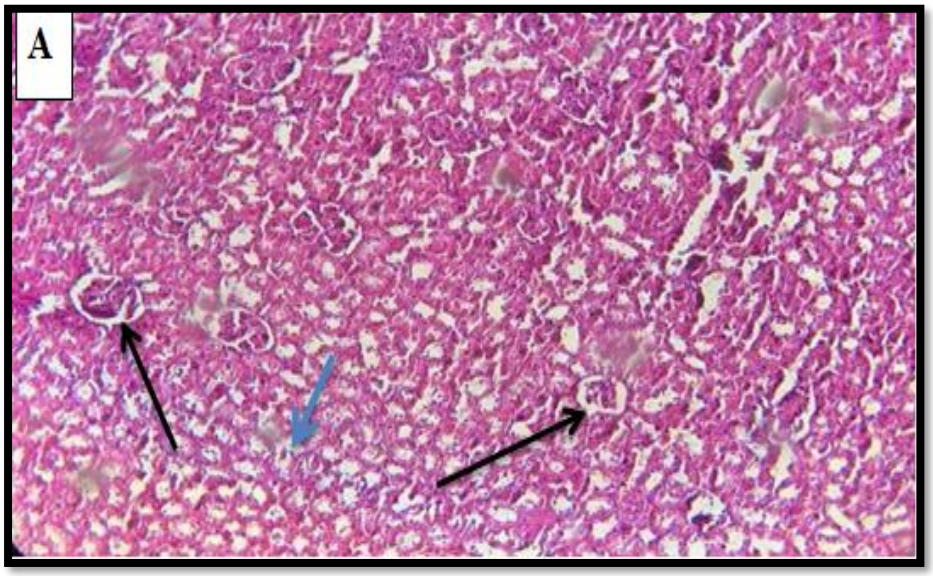


Figure (4-6.8)Kidney treated with busulfan 28 day: In this section A the black arrow shows the shrinking glomerulus with increase the glomerular space and blue arrow show the renal tubules suffer from tubular vacuolization and tubule filled with urea or materials (renal nephritis) and it is commonly interpreted as a sign of drug toxicity and that lead to kidney nephrotoxicity (X10).

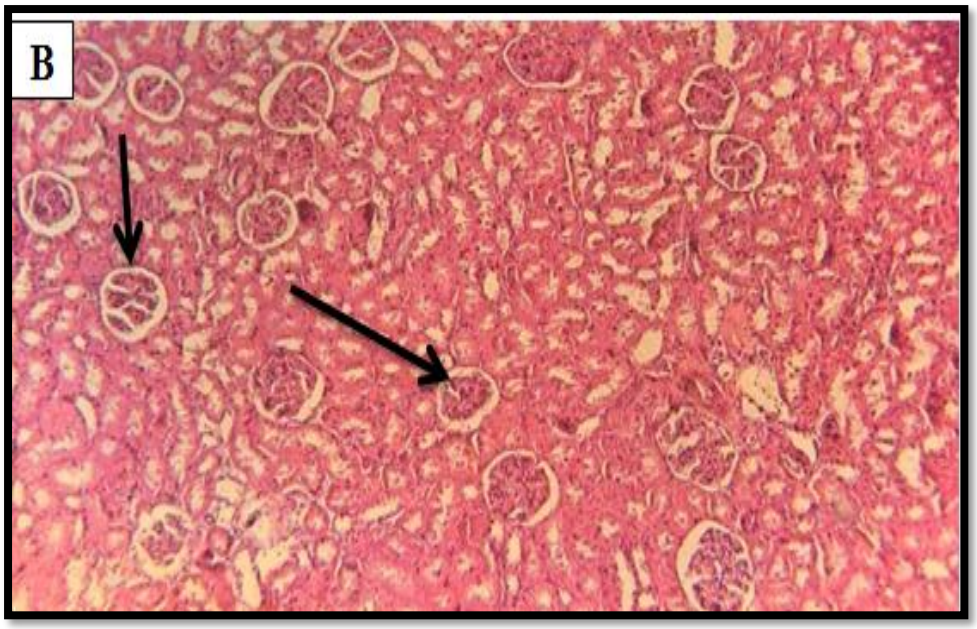


Figure (4-6.9)Kidney treated with busulfan only 56 day: In this section B kidney (cortex and medulla) suffer from sever nephrotoxicity and the black arrow shows sever shrinking of glomerulus with increase of glomerulus space and it is commonly interpreted as a signs of drug toxicity and that lead to kidney nephrotoxicity (X10).

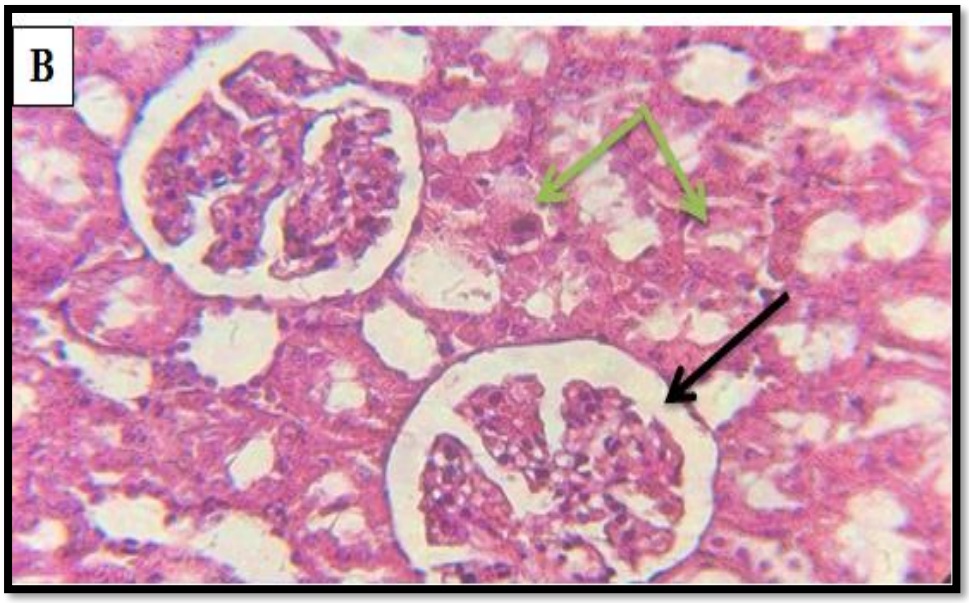


Figure (4-6.10)Kidney treated with busulfan only 56 day: In this section B kidney (cortex and medulla) suffer from sever nephrotoxicity and the black arrow shows sever shrinking of glomerulus with increase of glomerulus space and blue arrow show the decrease renal tubules duct size due degenerative changes and some tubules contain cast of urea and it is commonly interpreted as a signs of drug toxicity and that lead to kidney nephrotoxicity (X100).

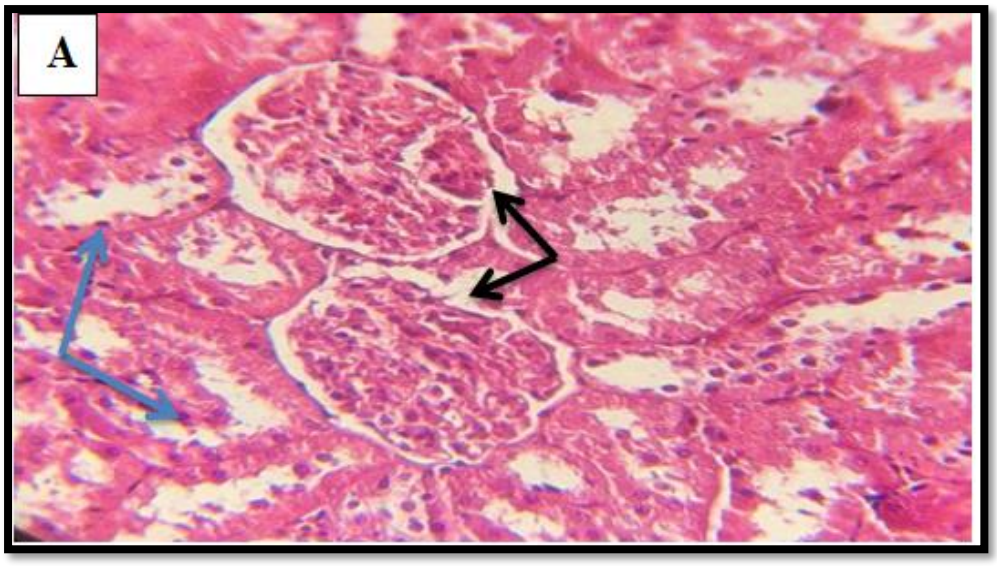


Figure (4-6.11)Kidney treated with busulfan&CoQ10 28 day: In this section A the black arrow shows the shrinking glomerulus and blue arrow show the renal tubules suffer from flattening of the renal tubular cells due to degenerative changes in cells around the tubule and it is commonly interpreted as a sign of drug toxicity and that lead to kidney nephrotoxicity (X100).

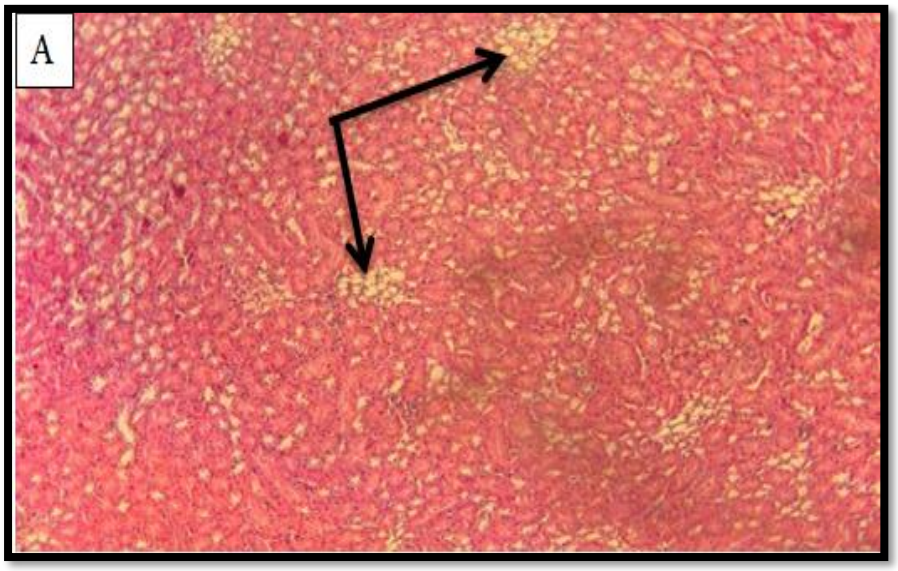
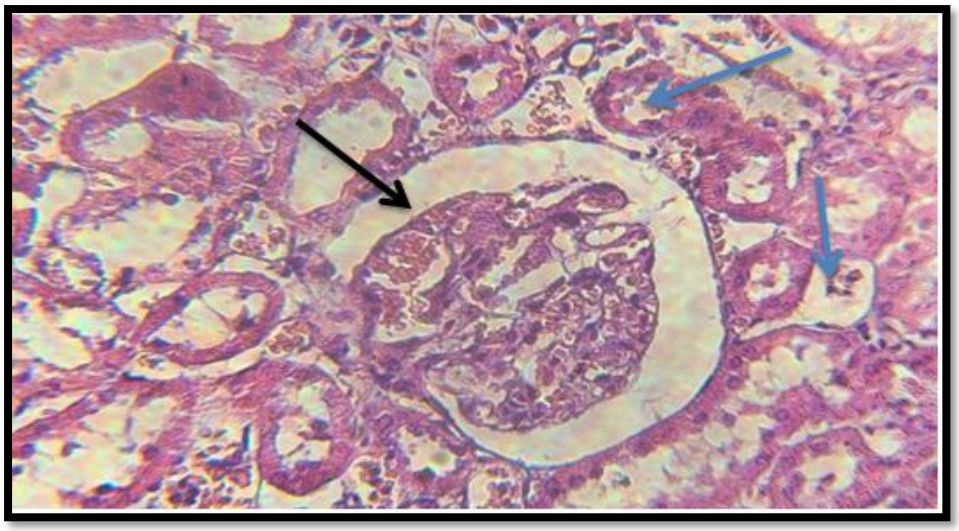


Figure (4-6.12) Kidney treated with busulfan&CoQ10 28 day In this section A shows only the renal tubules suffer from tubular vacuolization (black arrow) and it is commonly interpreted as a sign of drug toxicity and that lead to kidney nephrotoxicity (X10)



4.6.13 Kidney treated with busulfan&CoQ10 56 days: In this section kidney (cortex and medulla) suffer from sever nephrotoxicity and the black arrow shows sever shrinking of glomerulus with increase of glomerulus space and blue arrow show the decrease renal tubules duct size due degenerative changes and some tubules contain cast of urea and it is commonly interpreted as a signs of drug toxicity and that lead to kidney nephrotoxicity (X100).

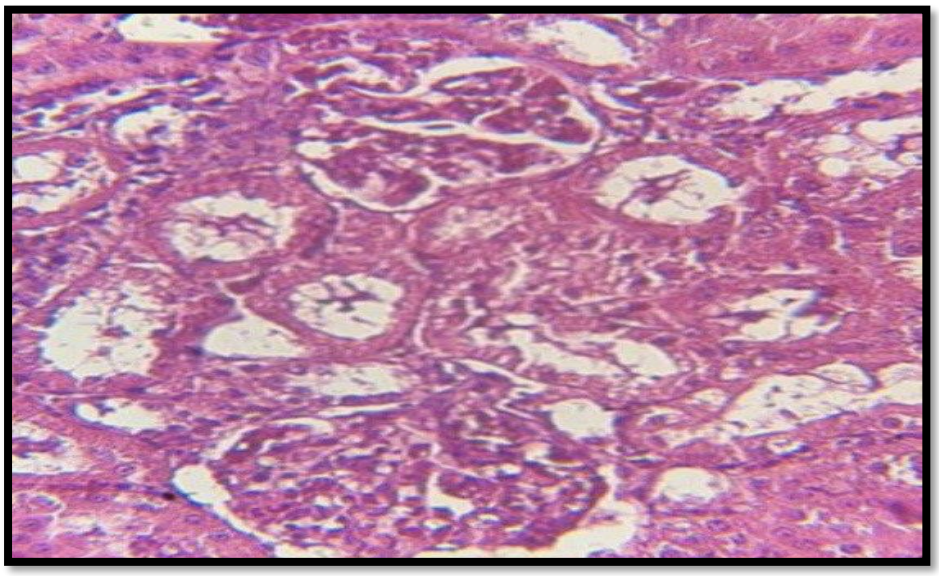


Figure (4-6.14) Kidney treated with CoQ10 group D 28 day: In this section kidney normal glomerulus &renal tubules duct (X100).

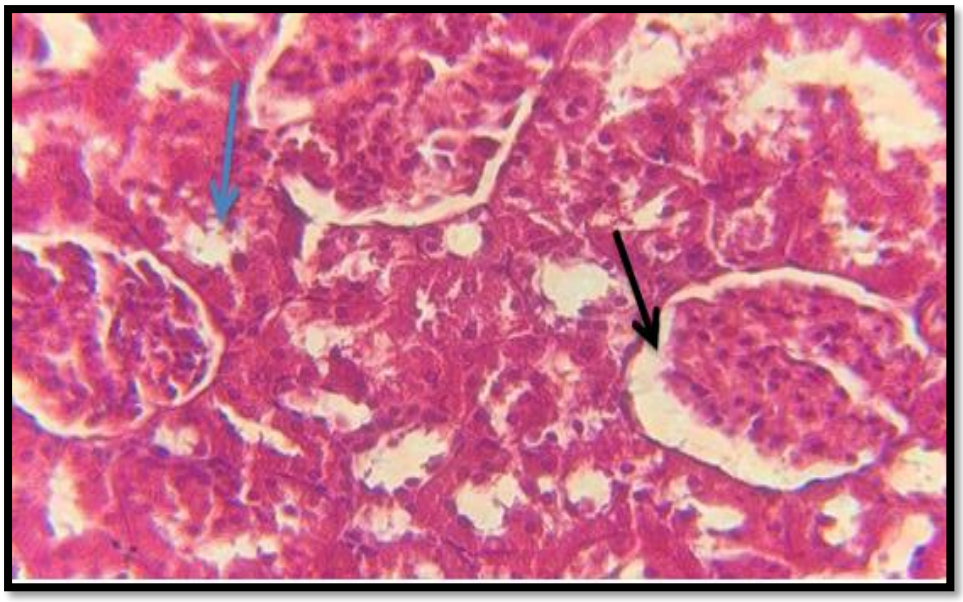


Figure (4-6.15)Kidney treated with CoQ10 56 day paranchemus tissue, show the normal bowman cupsole, show the glomerulus, the green arrow show renal tubule, the show bowman space(X40)

4.6.3 Histological Picture and Changes of the liver:

The histological examination in this study shows liver parenchymal cells suffer from cells injury and apoptosis for both peroids 28-56 days. In groups of male rats treated with busulfan ,busulfan &coq10.

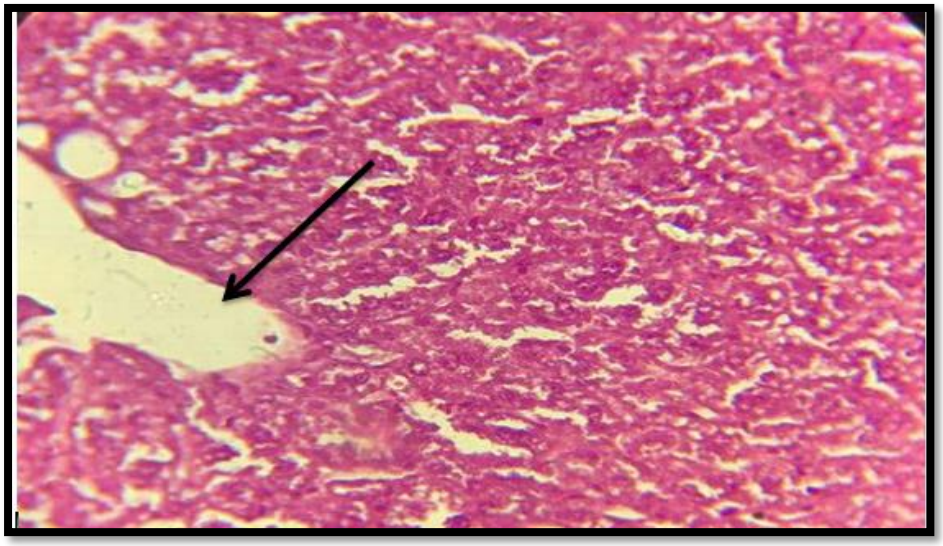


Figure (4-6.16)Liver control: In this section the black shows portal vein branch, and the liver paranchemus cells is normal (X40).

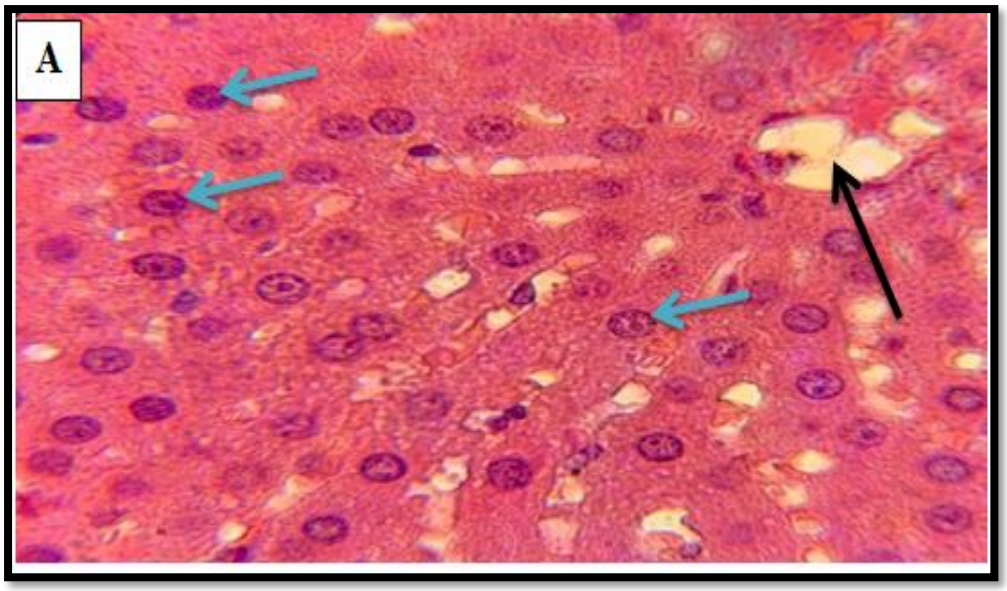


Figure (4-6.17)Liver treated with busulfan 28 days: In this section A the black arrow show the portal vein and most liver parenchymal cells suffer from cells injury and apoptosis (blue arrow) due to treated with Busulfan (X100).

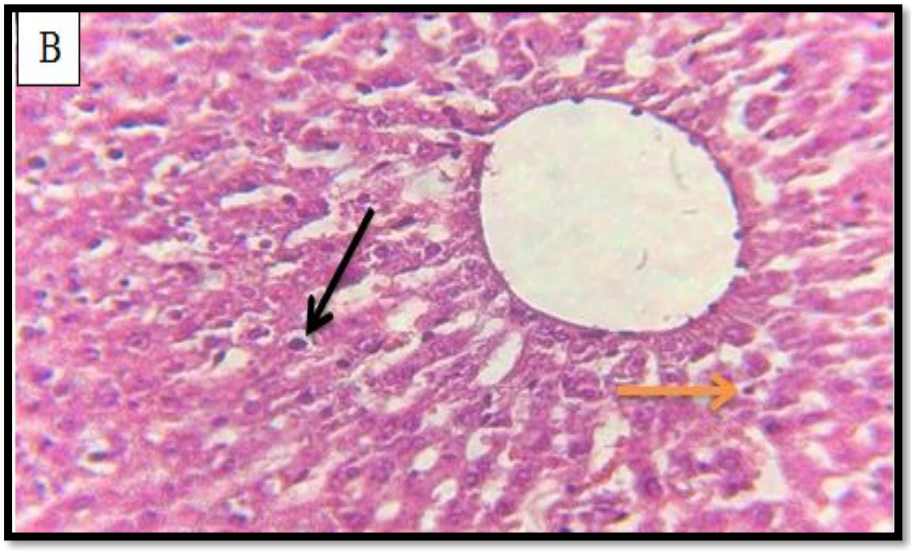


Figure (4-6.18)Liver treated with busulfan 56 days: In this section B liver parenchyma suffer from sever degenerative changes fatty change with fatty infiltration and cloudy swelling, the yellow arrow shows cells suffer from cloudy swelling and black arrow show cells suffer from fatty change due to treated with Busulfan (X40).

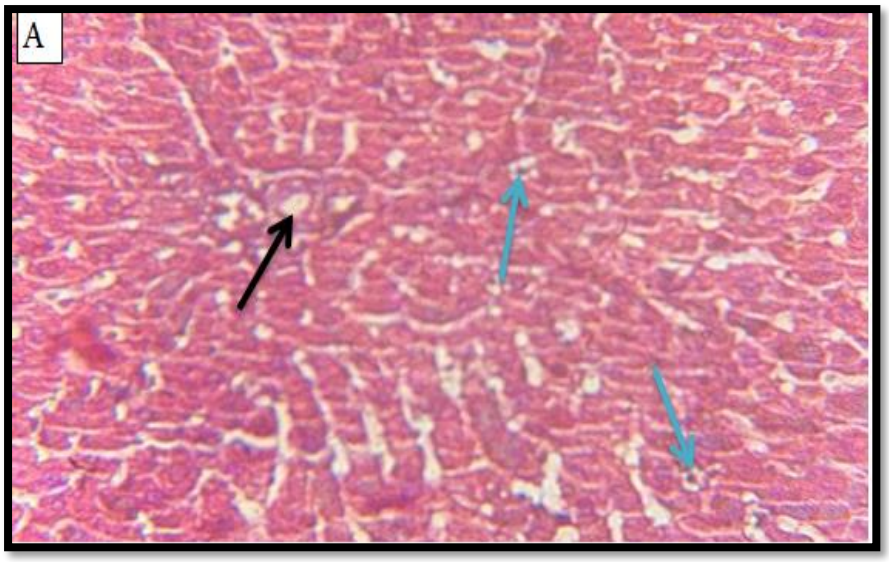


Figure (4-6.19)Liver treated with busulfan&CoQ10 28 days: In this section A the black arrow show the portal vein and the blue arrow show some cells suffer from cell injury (cloud swelling) due to treated with Busulfan (X40).

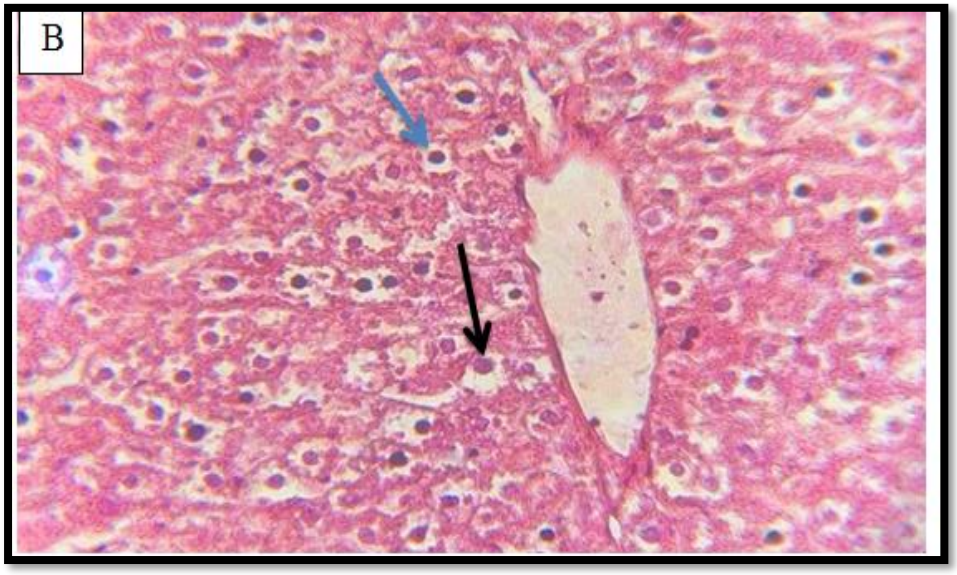


Figure (4-6.20)Liver treated with busulfan&CoQ10 56 days: In this section B liver parenchyma suffer from sever degenerative changes fatty change with fatty infiltration and cloudy swelling, the black arrow shows cells suffer from degenerative changes (fatty change) in liver cells and the blue arrow shows cells suffer degenerative changes (cloudy swelling) due to treated with Busulfan (X40).

Chapter Five
Discussion

5.Discussion:

5.1. Hematological Parameters

From this study, it has been found that some busulfan treated rats has been lost their hair in day 28 but the more lose notice in 56 days treated rats. Hair loos may result either from hair follicle stem cell destruction or from acute damage to the keratinocytes of the lower portion of some follicles. and this is in agreement with (Tosti *et al.*,2005), who found there is diffuse alopecia characterized by greatly reduced hair density with short, thin hair after busulfan treatment.

For the group of male rats treated with the Busulfan only (10mg/kg/BW) and Busulfan +co 10 for 28 days, the results of the present study show a significant decrease($p<0.05$) in the rate of hemoglobin (Hb), packed cell volume (PCV), Red blood cells (RBC), white blood cells (WBCs) and platelets count compared with the control group and co10 treated group and this is agreement with (YuSheng Qin et al., 2016) which found that the values for RBC, Hb ,WBC and platelets of mice in the i.p. injection group after busulfan treatment were significantly lower than those of mice in the control group. The reason for this is two reasons. The first is that blood cells create a type of cell called "stem cells". Stem cells are the master cells that divide and mature into different types of blood cells: red cells, white cells, and platelets. The bone marrow in our body acts as a factory, converting stem cells into blood cells. Every new blood cell contains DNA that carries all the instructions the cell needs to grow and function. Once mature, blood cells leave the bone marrow and enter the bloodstream. Busulfan works by interfering with the production of blood cells' DNA. The drug prevents cells from growing and maturing, which ultimately causes these cells to die. Busulfan is classified as a

chemotherapy because it causes some cells in the body to die (Janes,2014).

The fur became dull and dry, there was a reduction in activity and responses, and an abnormal gait and hunched posture developed. The fur became dull and dry, there was a reduction in activity and responses, and an abnormal gait and hunched posture developed. there was no evidence of BU toxicity during or after drug administration.

there was pronounced marrow depression as shown by significant decreases in erythrocyte values, all leucocytes, and in platelets,

The second reason is low bone marrow caused by busulfan chemotherapy, which may cause aplastic anemia and is low in all blood cells, and this is in agreement with (Gibson et al 2003 and Molyneux et al., 2008) Which found nadir for myelosuppression after a single dose of busulfan . Also(Nilsson et al., 2003) Which found that blood marrow suppression, infection, and bleeding are very common and occur early (day to weeks) after transplantation of hematopoietic stem cells that are given busulfan before bone marrow transplant. Busulfan is an alkyl sulfonate. It is an alkylating agent that forms cross-links within the DNA between the DNA bases guanine and adenine and between guanine and guanine.(Iwamoto et al., 2004) . DNA entanglement prevents DNA replication. Since the cross-links within DNA cannot be repaired by cellular machines, the cell undergoes apoptosis. (*Kramer, 2007).*

Busulfan is a highly cytotoxic and genotoxic agent (Panahi *et al* .,2015] that Various adverse effects, both acute and chronic, such as DNA damage and thus activation of apoptosis or aging in a manner dependent on cell type possibly due to oxidative stress(Iwamoto *et al* .,2004) can occur in many biological organs such as blood diseases. (Albrecht *et al* .,1971)

On day 56, there was a significant increase in the number of Hb, RBC, WBC, PCV and platelets in Busulfan + co10 when compared with the Busulfan group and this result is in agreement with (YuSheng Qin *et al*., 2016)which found that the Hb value, the RBCs, WBCs, and platelets increased. Hematologic parameters gradually until restored to normal levels, we believe that the increased level of haematological parameters in our study may be due to a compensatory response due to a decrease in the effect of busulfan on the bone gland after 56 days. Also, the bioavailability of coenzyme Q10 along with its important antioxidant function makes CoQ10 unique . (Niklowitz *et al* .,2007).

On day 28, the busulfan + CO10 group showed a slight increase in Hb, RBC, WBC and platelets, but this increase did not reach a significant level when compared with the busulfan group alone, and there was also a slight increase in the same level except for the platelet count when comparing the group CO 10 and the control group and also don't reach much level. This results are agreement with (Castro *et al*.,2020 and Geng *et al*.,2004) had reported that, CoQ10 can act as an antioxidant in RBCs membrane and has a protective effect on RBCs membrane structure and expansion and cell engineering.Also (Littarru *et al* .,1994) Which found that erythrocytes previously enriched with exogenous CoQ10 were found to be more resistant to free radical initiator-induced hemolysis. Moreover, CoQ was able to protect the isolated enzymes and the RBCs membrane-bound enzymes from the inactivating effect of free radicals, resulting in an increased RBC count and as a result an increase in Hb and PCV.

Increase in platelets count is agreement with (Niklowitz *et al* .,2007) that found CoQ10 supplementation further increase platelets count (though not statistically significant) after 28 days of

supplementation when compare between Busulfan and Busulfan +co10 group.

On the other hand, Coenzyme Q10 (CoQ10), is a single compound of antioxidant and structurally bound to vitamin K. It may prevent endogenous CoQ10 from opening up the pores of membrane transmission, as it counteracts many apoptotic events, such as DNA fragmentation and cytochrome release. , And the possibility of depolarizing the membrane.

(Coates *et al* .,2010). Previous studies (Andrews *etal.*,1993)have shown.

Previous studies (Andrews et al. 1993) have shown

5.2 Biochemical parameters

Humans and animals alike still face conditions that result from exposure to chemical agents. One of these chemicals is exposure to bosulfan as a chemotherapy . From Table (**4-2a**), it is observed that the ALT, AST, ALP, Albumin and Bilirubin levels of the busulfan group and bosulfan +co10 group are significantly increase than the control group and co10 group and this is in agreement with(Norasteh *et al* ., 2020). This significant increase is an evidence of hepatotoxicity which may be as a result of leakage from the cells through peroxidative damage of membranes. The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver (Yakubu *et al*., 2017). The increase in bilirubin levels may be due to occurrence of VOD (Veno-Occlussive Disease) of the liver which may be occur as a results of busulfan treatment as mentioned by Jung Lim(Lee *et al* .,1999) .

Oxidative stress has been shown to play an important role in causing liver damage, so antioxidants may be effective in wound healing. (Cannistrà et al., 2016; Ramachandran and Jaeschke, 2018). The main

effects of busulfan are to reduce the intracellular content of glutathione and induce oxidative stress (DeLeve and Wang,1996)

Several investigations confirmed that busulfan is metabolized in the liver through conjugation with GSH catalyzed by glutathione S-transferase (GST). The reaction is catalyzed in human liver mainly by GST-A1-1(Czerwinski *et al.*, 1996; Gibbs *et al*., 1996)

Busulfan is a widely used cytotoxic drug in chemotherapy and is commonly used to treat chronic leukemia, ovarian cancer, and bone marrow transplantation in cancer patients. (Attari et al., 2018 ;Omidi et al .,2008). It has a wide range of harmful effects on the body and has harmful effects on many organs including the liver and kidneys (Attari *et al*., 2018 ;Omidi *et al*., 2008)

CoQ10 is a vitamin-like substance with a number of important cellular functions related to liver metabolism and liver disorders. (Mantle and Hargreaves, 2020). Firstly, CoQ10 plays a major role in the biochemical process, providing all cells with the energy they need to function normally; Specifically, CoQ10 is an intermediate in the electron transport system, which generates energy in the chemical form of adenosine triphosphate (ATP), and transfers electrons from the first and second complexes to the third complex of the mitochondrial respiratory chain (Hargreaves, 2003).

Second, CoQ10 is a major fat-soluble antioxidant, and it protects cell membranes, especially those within mitochondria, from oxidative damage caused by free radicals (Hargreaves, 2003). This oxidative stress has been implicated in a number of liver disorders. Moreover, our research found several animal studies that reported the beneficial effects of CoQ10 administration on liver enzymes in the blood of mice. (Fouad & Jresat,2012. . Jimenez *et al* ., 2014; Faddah *et al* .,2010;Esfahani *et al* ., 2013).Our results show the protective effect of

co 10 to the liver by the decrease the effect of busulfan in co10+busulfan group and this in agreement with(Eftekhari *et al* .,2018).

After 56 days **(table 4-2b)** ,the results show a significant increase in busulfan group only and other groups of experiment and this in disagreement with (Hakemi *et al*., 2019) which found there is no difference between treated groups.

Also, as show in (**table 4-3a and 4-3b)** , There is a significant increase in urea, uric acid, and creatinine in the busulfan group and the im busulfan + co 10 group when compared with the control groups and the co10 groups and this corresponds to(Norasteh *et al* .,2020). Kidney injuries occur for several reasons, such as chemotherapy, trauma, infection, surgery, or use of antibiotics. Vitality (Hulse & Rosner,2019). The amounts of free radicals that are exposed to the kidneys can easily overwhelm the body's antioxidant defenses. Left unchecked, this excess oxidative stress contributes to progressive kidney damage and an increased risk of developing chronic kidney disease. (El-Sheikh *et al* .,2012; Asemi *et al* .,2018)mentioned the protective role of co 10 in chemotherapy-induced nephrotoxicity and this is consistent with our results. Plasma CoQ10 concentrations are reduced in CKD patients. (Xu *et al* .,2019)

5.3 Hormonal parameters

In our research as show in (table 4-4a and 4-4b) we found that the LH and FSH levels were significantly ($P \leq 0.05$) increased while testosterone levels decreased in rat treated busulfan as compared with other groups. Busulfan is a drug that significantly affects the testicles. It causes an apparent increase in apoptosis and affects spermatogenesis. Busulfan has toxic effects on the sperm epithelium in rats and causes infertility (Abul-Fotouh *et al*.,2018) mainly by killing sperm stem cells.

The primary gonadal defect is caused by the testicle. Damage has a deleterious effect on spermatogenesis and / or a dysfunction of Leydig cells (Kiserud *et al*., 2009). According to the above, the effect of busulfan, the elevated FSH level in this study may be due to impaired spermatogenesis, whereas the low testosterone levels associated with high LH may occur as a result of Leydig cell dysfunction. (Hassan & Jasim, 2020).

Exposure to cytotoxic chemotherapy and radiotherapy leads to associated elevation of FSH levels with decreased sperm count, and thus, the effects of suppressing testosterone and gonadotrophin analogues on spermatozoa. Moreover, chemotherapy is often used with increased testicular impairment and epithelial germs damage. To make it possible to treat male infertility after cancer treatment (Qu *et al*., 2019). On the other hand, free radical accumulation and thus oxidative injury in Leydig cells in the testis by a specific chemical oxidant (such as busulfan) may degrade their response and performance to testosterone synthesis. (Banihani,2018).

Also, there is a significant increase in the testosterone level in Busulfan +co10 group when compared with busulfan group which is agreement with (Fouad *etal*.,2011, Ghanbarzadeh *et al* .,2014, Mohammad *et al* .,2015). Coenzyme Q10 supplementation has been found to suppress testicular oxidative stress and lipid peroxidation, and restore the antioxidant defense mechanism. (Fouad *et al*., 2011), Which, in turn, could prevent chemical oxidative injury and preserve the function of Leydig cells to produce testosterone (Palmeira *et al*., 2001).

(Banihani,2018) also found there is increase in the level of testosterone but not reach to significance level when compare between co10 group and control group and this agreement with our results.

In 56 days there is a decrease in the level of FSH ,LH in co 10 group when compare with control group and this is agreement with Safarinejad ,2009 which found that long duration treatment with co 10 will cause significant decrease in FSH &LH in serum of male.

In our results, table in (4-4a and 4-4b) there is a significante increase in the levels of adiponectin in all treated group when compare with control group. Several studies have shown that adiponectin hormone and the receptors for adiponectin, AdipoR1 and AdipoR2 are present in various reproductive tissues in both sexes of different species(Rak *et al*.,2017). In rats, adiponectin is mainly present in the Leydig interstitial cells, whereas AdipoR1 is expressed in the seminiferous tubules (Caminos *et al*., 2008). In addition, levels of adiponectin in the plasma were significantly lower in males than those in females in humans and rodents (Nishizawa *et al*., 2002). One explanation is that testosterone (T) could regulate the plasma adiponectin concentration (Nishizawa *et al.*, 2002). So the decrease in testosterone level which caused by busulfan will lead to increased level of adiponectin , also, adiponectin might play a functional role in the local (autocrine) control of testosterone secretion (Caminos *et al*., 2008). High doses of adiponectin (50, 500, or 5000ng/mL) could promote T production from the Leydig cells (Landry *et al*., 2015). So there is an inverse relationship between testosterone and adiponectin levels.(Bjursell et al., 2007 and Lindgren *et al*., 2013) also found that Disruption of AdipoR2 in males leads to seminiferous tubules atrophy and aspermia associated with reduced testes weight.

In our study, there is a significant increase in the level of adiponectin hormone co 10 alone group when compare with control group (in 28 and 56 days treatment) and this is agreement with (Farsi *et al* .,2018) who found that patients who received CoQ10 supplement will increase

levels of adiponectin. (Kishimoto *et al*.,2003) He discovered that peritoneal injection of CoQ10 increased blood adiponectin levels in mice, indicating a mechanism through which the CoQ10 antioxidant could modulate the expression of genes sensitive to reactive oxygen species, such as adiponectin, by reducing the production of reactive oxygen species..

As show in table (4-4a and 4-4b) there is a significant decrease($p<0.05$) in the levels of activin and inhibin in busulfan group when compare with other groups of treatment. The activin and inhibin were first described as regulators of follicle-stimulating hormone (FSH) secretion. The activin were originally recognized for their abilities to augment the gonadotropin-releasing hormone (GnRH)-mediated release of FSH, and were named "activins" because their effects were functionally opposite to those of inhibin in this context (Ling *et al*., 1986a and Vale *et al*., 1986).

Inhibin B is the only form of inhibin produced by the testis and is also the major circulating inhibin (Illingworth *et al.*, 1996; Marchetti *et al*., 2003). FSH stimulates inhibin B expression in Sertoli cells, providing negative feedback for pituitary FSH production (Sharpe *etal*., 1999; Hayes *et al.*, 2001), While LH stimulates the production of androgens from rodent Leydig cells, a process that is augmented by inhibin and diluted by Activin (Hsueh *et al*., 1987). Sertoli cell function is closely related to the biology of Activin and Inhibin. These cells are targeted by the pituitary-derived FSH to regulate their structure, and their levels are the main determinant of male fertility and physiology. .Research has identified specific roles of locally produced activin and ethinbene in testicular development and function.. Kate L. Loveland, Mark P. Hedger, in Sertoli Cell Biology (Second Edition, 2015). Treatments with busulfan specifically target germ cells and

Sertoli cells respectively (with an efficiency close to 100%), and it is very likely that copies will be significantly reduced after each treatment regimen specific to germ cells or Sertoli cells.(Soffientini *et al* ., 2017).

5.4. Sperms Parameters.

Among the most important problems in a couple's life is infertility, its complications, and the causes of some infertility related to men. The most common cause of male infertility is the inability to produce sufficient numbers of active and healthy sperm (Amin & Hamza,2006). Many factors can affect sperm production and the risk of infertility, and among these factors is the use of chemotherapy drugs for cancer and busulfan is one of these drugs that significantly affect the testicles. (Aboul Fotouh *et al* .,2018). These factors can reduce sperm concentration while generating free radicals and oxidation of germ cells in the testicles (Amin and Hamza,2006). Here, we examined the protective effect of ubiquinone (co10) against busulfan-mediated sperm damage in mice, Since the time period from the initiation of stem cell division to spermatogenesis in rats is approximately 56 days (Rooij and Russell, 2000), the time period chosen (4 and 8 weeks) provides sufficient time to monitor the possible recovery of spermatogenesis in the remaining stem cells In animals treated with busulfan. According to(Nagano *et al*., 1999), It took approximately 4 weeks after the busulfan treatment for the effect of busulfan to differentiate the cells secreting from the seminal tubular lumen

Our results showed that administration of busulfan in a single dose and daily administration of ubiquinone (Co10) for 4 and 8 weeks can significantly reduce busulfan-mediated destruction of the testis.

Results showed a significant decrease in busulfan group compared to the other group ($p < 0.05$), (Table 4-5a and 4-5b) in sperm count ,motility, viability and abnormality. Our findings were agreement with

(Moloody *et al* .,2018and Jalili *et al* ., 2020). Unlike other chemicals that destroy differentiated sperm, Busulfan is an effective agent that kills sperm stem cells of several types. However, at higher doses, the drug may kill contrasting strains of sperm resulting in the depletion of sperm cells and sperm as well. (Moloody *et al*., 2018). It has been confirmed that busulfan chemotherapy can induce apoptosis in sperm [Choi *et al* .,2004], increase the production of reactive oxygen species and result in sperm death (Dehghani *et al* ., 2013).

In addition to ensuring the presence of tratospermia, the presence of spermatozoa with abnormal conformation in the semen is another possible outcome of busulfan treatment in mice (Panahi *et al*., 2015).These studies have shown that busulfan is involved in stopping spermatogenesis, although some changes are reversible and are dose dependent Busulfan inhibits spermatogenesis, especially by the oxidative damage that increases the level of ck-18, which is a superficial marker on Sertoli cells. The elevation of this marker caused disturbance of spermatogenesis, sterility and reduced sperm motility by reducing the length of the flagellum(Maymon *et al*., 2004).

Coenzyme Q10 has been used as an attractive intervention approach in a wide variety of diseases or disease disorders, one of which is infertility (Jain *et al*., 2017; Gvozdjakova *et al*., 2015). The successful results of CoQ10 administration in various organ systems prompted us to attempt such a treatment with a model of busulfan-induced testicular disorders. Coenzyme Q10 was first introduced as an ethical drug for patients with heart failure in Japan and other countries. Coenzyme Q10, which works internally in the electron transport chain in mitochondria, can be ingested to suppress free radicals and contribute to antioxidant defenses in vivo (Ikawa *et al*., 2002).

Both the bioenergy and antioxidant role of CoQ10 indicate potential involvement in sperm biochemistry and male infertility. The amount of CoQ10 can be measured in semen, as its concentration is related to sperm count and motility (Aardweg *et al*.,1983). It was found that CoQ10 distribution between sperm cells and seminal plasma was altered in varicocele patients, who also exhibited a higher level of oxidative stress and a lower capacity of antioxidants. The redox state of CoQ10 was also determined in semen: an inverse relationship was found between ubiquinone percentage and hydroperoxide levels and between this ratio and the percentage of abnormal sperm shapes.Then, CoQ10 was administered to a group of patients with idiopathic infertility. The treatment significantly increased CoQ10 concentration, in seminal plasma and sperm cells, and improved sperm motility. (Belardinelli *et al*., 2008).

In a recent study, CoQ10 was shown to improve semen quality (Hassan & Jasim,2020) administration of CoQ10 in Busulfan-treated animals improved sperm quality parameters as well as DAN damage and chromatin condensation indices. The results of the present study indicated that administration of CoQ10 following administration of busulfan could be helpful in improving sperm indices in mice. and this result is agreement with (Moloody *et al*., 2018).

Antioxidants neutralize free radicals and thus the oxidative reactions that cause them. Dietary antioxidants may be helpful in reducing lipid peroxidation and DNA damage in sperms during busulfan therapy (Khak *et al.,* 2009;Safarinejad , 2009) The results showed a positive correlation between the duration of treatment with coenzyme Q10 and the number of sperms, as well as with sperm motility and sperm formation, and this is consistent with our results.

5.5 Histopathological Changes:

5.5.1 Histopathological Effect of Busulfan and COQ10 on Testes Tissue:

The histopathological examination of testicular tissue in our study showed degenerated in seminiferous convoluted tubules with decrease in the diameter of seminiferous tubules and necrosis of spermatids, spermatocytes, epithelial sloughing, absence and degeneration of the spermatogenesis, this result agreed with (Demir *et al.*, 2007) Histomorphometric effect show the thickness of testicular capsule was increased in busulfan groups (P<0.05). Compared to the control group, germinal epithelium height was decreased significantly in busulfan and busulfan & COQ10(P<0.05). There was a significant decrease in seminiferous tubules diameter in busulfan and groups busulfan & COQ10(P<0.05). compared to the control group this is agreement with(Moloodyetal.,2018).

5.5.2 Histopathological Effect of Busulfan and COQ10 on Kidney:

Acute and chronic renal failure are commonly challenged during chemotherapy.(LiuZ *et al*., 2018) These prominence causes include toxic effects on renal cells, DNA and mitochondrial damages, inflammation, oxidative stress, apoptosis, and related pathways(Waseem *etal* ., 2013). In our study, there is a significant increase (p >0 .05). glomerular space increase shrinking glomerulus for period (28&65) day and in suffer from sever nephrotoxicity result of drug toxicity for period 56 day. In groups of male rats treated with busulfan ,busulfan &coq10.this agreement with our results(Topcu-Tarladacalisir *etal*.,2016). In our results.(there is a significant (p >0 .05) decrease renal tubules duct size due degenerative changes and some tubules contain cast of urea for period 56 day In groups of male rats treated with busulfan only agreement with (Abdollahifar *etal*., 2020).in our results

Kidney treated with CoQ10 56&28 day normal paranchemus tissue, bowman cupsule, glomerulus there is a significant to convert the tissue to normal agreement with (Ahmed *etal*., 2015).

5.5.3 Histopathological Effect of Busulfan and CoQ10 on liver

Busulfan has been linked to transient serum enzyme elevations during therapy, to rare cases of cholestatic hepatitis, instances of nodular regenerative hyperplasia and, when given in high doses, to sinusoidal obstruction syndrome which can be severe and fatal (Chabner *etal*.,2011). in our results significant (p >0 .05) Liver treated with busulfan only 28&56 days compare with control . liver parenchymal cells suffer from cells injury and apoptosis Nodular regeneration is thought to be due to damage of small vasculature in the liver. Sinusoidal obstruction syndrome appears to be the result of direct cytotoxicity of busulfan and other agents to the hepatic sinusoidal lining cells, causing their extrusion and obstruction of sinusoids, congestion and centrolobular hepatic necrosis. agreement with(DeLeve *etal*., 2013). Histologically, the liver showed diffuse regenerative hyperplasia with minimal fibrosis. There was sinusoidal infiltration by leukemic cells

.(National Institutes of Health,2017)

Chapter Six
Conclusions
&
Recommendations

Conclusion:

Administration of CoQ10 in busulfan-treated animals improved histological and sperm quality. The curreat study were concluded that :

1. busulfan have toxic effect on sperm.

2.CoQ10 have effects of antioxidant action and protection effects of against oxidative stress, according to the findings of this study, coq10 (ubiquonone) has positive therapeutic and protective effects on testicular tissue and increases sperm production in busulfan-treated rats.

3.Chang able in heamatological parameters effectiveneces by toxic effects of busulfan.

4. Busulfan that toxic effects of liver function and kidney testing in different period time.

Recommendations:

1. Avoid using busulfan (chemotherapy) as drug of choice for male with fertility problem. ,reduced fertility and sometimes sterility.

2.Avoid the use of lipid coq10 by blocking an injection into I.P.

3. the study that we compare between activity of CoQ10 with another and with antioxidant enzymatic(Superoxide Dismutase, Glutathione Peroxidase, catalase). Selenium an effective and powerful antioxidant, helps protect the heart and body from certain types of cancer.

4. Recommend to use coq10 in cases that need to be treated with busulfan for long time and Investigate the mechanism behind the adverse effect of busulfan on the testicles. use coq10 to increses activity reproductive system in male.

5. We Recommend to use coq10 treatment respiratory disease ,mental function disorders, and Alzheimer's disease and schizophrenia Because coenzyme Q-10 supplements have the ability to counteract histamine, so they are useful for people who suffer from allergies, asthma or respiratory illnesses.

6- Study side effects of busulfan drug on other organs such liver, kidney, prostate gland, respiratory system and ovary in female.

7.study of effects of other endocrine gland such as drenal hormone steroids(aldosterone and cortisol).

8. Recommend study of effects of combination with cyclophosphamide as a conditioning regimen prior to allogeneic hematopoietic progenitor cell transplantation for chronic myelogenous leukemia.

References

……..……References ……..

Aardweg, G. V. D., de Ruiter-Bootsma, A. L., Kramer, M. F., & Davids, J. A. G. (1983). Growth and differentiation of spermatogenetic colonies in the mouse testis after irradiation with fission neutrons. *Radiation Research*, *94*(3), 447-463.

Abdollahifar, M. A., Ebrahimi, V., Aliaghaei, A., Raoofi, A., Khosravi, A., Hasani, A. & Asadi, M. (2020). Protective effect of curcumin on busulfan-induced renal toxicity in male rats. Renal Failure, 42(1), 969-977.

Abofoul-Azab M, Lunenfeld E, Levitas E, Zeadna A, Younis JS, Bar-Ami S, Huleihel M(2019). Identification of premeiotic, meiotic, and postmeiotic cells in testicular biopsies without sperm from sertoli cell-only syndrome patients. Int J Mol Sci.;20(3):470.

Agnieszka Rak1, Namya Mellouk2, Pascal Froment2 and Joëlle Dupont2(2017). Adiponectin and resistin: potential metabolic signals affecting hypothalamo-pituitary gonadal axis in females and males of different species. Reproduction 153 R215–R226.

Ahmed, H. H., Aly, H. F., & Ali, S. A. (2015). Potential impact of CoQ10 and vitamin E against (STZ) induced metabolic deterioration in the albino rats. International Journal of Pharmacy and Pharmaceutical Sciences, 7(11), 176-184.

Akhtar MK, Kelly SL, Kaderbhai MA (November 2005). "Cytochrome b(5) modulation of 17{alpha} hydroxylase and 17-20 lyase (CYP17) activities in steroidogenesis". *The Journal of Endocrinology*. 187 (2): 267–74.

Alahmar, A. T., Calogero, A. E., Sengupta, P., & Dutta, S. (2020). Coenzyme Q10 improves sperm parameters, oxidative stress markers and sperm DNA fragmentation in infertile patients with idiopathic oligoasthenozoospermia. The World Journal of Men's Health, 38.

Albrecht M, Tackmann W, Pribilla W: Aplastic syndrome in myleran overdose. Med Klin, 66 (4): 126-130, 1971.

AL-Karawi, M. A. M., & Al-Shammari, S. M. H. (2019, September). Pathological study of kidney in male rats treated with doxorubicin in Diyala province. In Journal of Physics: Conference Series (Vol. 1294, No. 6, p. 062004). IOP Publishing.

Al-khalissi, H. N., (2014). Thesis "Morphohistological Study of the Gills and Kidney" University of Baghdad.

Allouche L, Hamadouche M, Touabti A2009. Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. Exp Toxicol Pathol. Sep; 61(5): 503-10.

Amin A, Hamza AA(2006).: Effects of Roselle and Ginger on cisplatininduced reproductive toxicity in rats. Asian J Androl, 8 (5): 607-612, DOI: 10.1111/j.1745-7262.2006.00179.x .

Amr A, Alaaeldin AH. Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. Asian J Androl . 2006;8:607–612.

Anderson RA, Cambray N, Hartley PS and McNeilly AS (2002) Expression and localization of inhibin alpha, inhibin/activin betaA and betaB and the activin type II and inhibin beta-glycan receptors in the developing human testis. Reproduction 123, 779–788.

Anderson RA, Sharpe RM(2000). Regulation of inhibin production in the human male and its clinical applica- tions. International journal of andrology.; 23(3):136–44. PMID: 10844538.

Andrews CM, Spurling NW, Turton JA. (1993)Characterisation of busulphan-induced myelotoxicity in B6C3F1 mice using flow cytometry. Comp. Haematol. Int.;3:538–546**.**

……..……References ……..

Anjum MR, Sainath SB, Suneetha Y, Reddy PS2011. Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. Ecotoxicol Environ Saf. May; 74(4): 793-9.

Antonella Tosti, Bianca Maria Piraccini, Colombina Vincenzi, Cosimo Misciali.(2005). Permanent alopecia after busulfan chemotherapy. British Journal of Dermatology 152(5):1056-8.

Archambeault DR, Yao HH 2010. Activin A, a product of fetal Leydig cells, is a unique paracrine regulator of Sertoli cell proliferation and fetal testis cord expansion. Proc Natl Acad Sci USA 107: 10526 –10531,. doi:10.1073/pnas.1000318107.

AttariSS, MohammadiS, Ebrahimzadeh A, HosseinzadehH, SoukhtanlooM, Rajabzadeh A2018. Effects of thymoquinone on sperm parameters, apoptosis, testosterone level, and oxidative stress in a mouse model of D-galactose-induced aging. Pharm Sci.;24(3):180-6 .

Ayyed Hameed Hassan and Wafaa Kadhim Jasim,2020. Investigation the Anti-Sterility Role of Ubiquinone-10 Against Procarbazine-Induced Infertility in Male Rats. Indian Journal of Forensic Medicine & Toxicology, January-March 2020, Vol. 14, No. 1.

AzizEftekhari ,Elham Ahmadian ,Aida Azami ,Mohammad Johari-Ahar2018. Protective effects of coenzyme Q10 nanoparticles on dichlorvos-induced hepatotoxicity and mitochondrial/lysosomal injury.Environmental toxicology. Volume33, Issue2 February.Pages 167-177.

Azza A. K. El-Sheikh,1 Mohamed A. Morsy,1 Marwa M. Mahmoud,1 Rehab A. Rifaai,2 and Aly M. Abdelrahman1. Effect of Coenzyme-Q10 on Doxorubicin-Induced Nephrotoxicity in Rats. Advances in Pharmacological Sciences Volume 2012, Article ID 981461, 8 pages.

Balercia G, Mancini A, Paggi F, Tiano L, Pontecorvi A, Boscaro M, et al. Coenzyme Q10 and male infertility. J Endocrinol Invest 2009;32:626–632.

Banihani, S.A.,(2018). Ginger and testosterone. Biomolecules, 8, 119.

Banihani, S.A.,2018,. Role of uric acid in semen. Biomolecules 8, 65.

Barakat B, O'Connor AE, Gold E, de Kretser DM, Loveland KL.,2008. Inhibin, activin, follistatin and FSH serum levels and testicular production are highly modulated during the first spermatogenic wave in mice. Reproduction 136: 345–359,. doi:10.1530/ REP-08-0140.

Bassil N, Alkaade S, Morley JE (June 2009). "The benefits and risks of testosterone replacement therapy: a review". Therapeutics and Clinical Risk Management. 5 (3): 427–48. doi:10.2147/tcrm.s3025. PMC 2701485. PMID 19707253**.**

Belardinelli R, Tiano L and Littarru GP(2008). Oxidative stress,endothelial function and coenzyme Q10. BioFactors,;32:129–33.

Belleannee, C., Thimon, V. and Sullivan, R. (2012) Region-specific gene expression in the epididymis. Cell Tissue Res. 349, 717–731.

Benkessou, F. (2019). Metabolic and pharmacokinetic study to enhance busulphan therapeutic efficacy. Biote., P: 9(20).

Bishop JB & Wassom JS (1986). Toxicological review of busulfan (Myleran). Mutat Res, 168: 15–45. PMID:3713721.

Bjursell M, Ahnmark A, Bohlooly YM, William-Olsson L, Rhedin M, Peng XR, Ploj K, Gerdin AK, Arnerup G, Elmgren A et al.(2007) Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. Diabetes 56 583–593.

Bloise, E., Ciarmela, P., Dela Cruz, C., Luisi, S., Petraglia, F., & Reis, F. M. (2019). Activin A in mammalian physiology. Physiological reviews, 99(1), 739-780.

Bordbar, H., Esmaeilpour, T., Dehghani, F., & Panjehshahin, M. R. (2013). Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. *Iranian journal of reproductive medicine, 11*(6), 467.

Bowen R. "Luteinizing and Follicle Stimulating Hormones". www.vivo.colostate.edu. Retrieved 2019-05-06.

Bucci R and Meistrich, ML.,1987. Effects of busulfan on murine spermatogenesis: ctotoxicity, sterility, sperm abnormalities, and dominant lethal mutations. Mutation Research, ;178: 259-268.

Buzzard JJ, Loveland KL, O'Bryan MK, O'Connor AE, Bakker M, Hayashi T, Wreford NG, Morrison JR, de Kretser DM(2004).. Changes in circulating and testicular levels of inhibin A and B and activin A during postnatal development in the rat. Endocrinology 145: 3532–3541.

Buzzard JJ,. Farnworth, PG, de Kretser DM, O'Connor AE, Wreford NG and Morrison JR (2003) Proliferative phase sertoli cells display a developmentally regulated response to activin in vitro. Endocrinology 144, 474–483.

Caminos JE, Nogueiras R, Gaytan F, Pineda R, Gonzalez CR, Barreiro ML, Castano JP, Malagon MM, Pinilla L, Toppari J et al. (2008)Novel expression and direct effects of adiponectin in the rat testis. Endocrinology 149 3390–3402.

Cancilla B, Jarred RA, Wang H, Mellor SL, Cunha GR, Risbridger GP,(2001). Regulation of prostate branching morphogenesis by activin A and follistatin. Dev Biol 237: 145–158.

Cannarella, R., Lanzafame, F., Condorelli, R., Calogero, A. E., & La Vignera, S. (2020). Antioxidants in the Medical and Surgical Management of Male Infertility. In Male Infertility (pp. 805-816). Springer, Cham.

Cannistrà M, Ruggiero M, Zullo A., Gallelli G., Serafini S, Maria M.,(2016), Hepatic ischemia reperfusion injury: A systematic review of literature

and the role of current drugs and biomarkers. Int J Surg.;33 Suppl 1:S57-70.

Chabner BA, Bertino J, Clearly J, Ortiz T, Lane A, Supko JG, Ryan DP.,(2011). Cytotoxic agents. Chemotherapy of neoplastic diseases. In, Brunton LL, Chabner BA, Knollman BC, eds. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill, , pp. 1677-730.

Cheng L, Shi H, Jin Y, Li X, Pan J, Lai Y, et al. 2016Adiponectin deficiency leads to female subfertility and ovarian dysfunctions in mice.Endocrinology; 157(12): 4875-4887

Cheng XB, Wen JP, Yang J, Yang Y, Ning G, Li XY. 2011GnRH secretion is inhibited by adiponectin through activation of AMP-activated protein kinase and extracellular signal-regulated kinase. Endocrine; 39(1): 6-12.

Cheng, C.Y.; Mruk, D.D. (2010). A local autocrine axis in the testes that regulates spermatogenesis. Nat Rev Endocrinol 6(7):380–395.

Clinical and laboratory standareds institute(CLSI)2014. Protection of laboratory workers from Occupationally acquired infections;approved Guideline-fourth edition .CLSI document M29-A4.wayne,pA:CLSI

Coates, P.M.; Betz, J.M.; Blackman, M.R.; Cragg, G.M.; Levine, M.; Moss, J.; White, J.D., Encyclopedia of Dietary Supplements, 2nd ed.; Informa Healthcare: London, UK, 2010.

Cornwall, G.A. (2009) New insights into epididymal biology and function. Hum. Reprod. Update 15, 213–227.

Cyrus Jalili1 , Amir Abdolmaleki2 , Azita Faramarzi3 , Mohammad Reza Salahshoor.,(2020). Effects of Thymoquinone and Busulfan on Reproductive Parameters in Male Rats: An Experimental Study. Journal of Clinical and Diagnostic Research. Jan, Vol-14(1): AF01-AF04.

……..……References ……..

Czerwinski M, Gibbs JP, Slattery JT (1996). Busulfan conjugation by glutathione S-transferase alpha my, and pi. Drug Metab Dispos ; 24: 1015–1019.

Dabbs M, Dabbs JM (2000). Heroes, rogues, and lovers: testosterone and behavior. New York: McGraw-Hill. ISBN 978-0-07-135739-5.

David Mantle ,Iain P Hargreaves.(2020). Coenzyme Q10 supplementation in non-alcoholic fatty liver disease: an overview. Gastrointestinal Nursing vol 18 no 2 March 2020.

De Rooij DG, Russell LD. All you wanted to know about spermatogonia but were afraid to ask. J Androl. (2000) Nov-Dec;21(6):776-98. PMID: 11105904.

De Rooij, D.G.; Kramer, M.F.,(1970). The effect of three alkylating agents on the seminiferous epithelium of rodents. I. Depletory effect. Virchows. Arch. B. Cell. Pathol., 4, 267–275.

Dehghani, Y., & Dehghani, M. (2013). Effectiveness social skills training on addiction potential reduction in male high school students. *American Journal of Life Science Researches*, *1*(3).

DeLeve LD, Wang X(1996).: Identification of two distinct mechanisms of busulfan toxicity in isolated murine hepatocytes (Abstract). Hepatology 24:464A,

DeLeve LD. Cancer chemotherapy. In, Kaplowitz N, DeLeve LD, eds,(2013),. Drug-induced liver disease. 3rd ed. Amsterdam: Elsevierp. 551.

Demir A., Turker .P, Onol FF., Sirvanci S., Findik A., Tarcan T (2007). Dias VL, Rajpert-De Meyts E, McLachlan R, Loveland KL(20090. Analysis of activin/TGFBsignaling modulators within the normal and dysfunctional adult human testis reveals evidence of altered signaling capacity in a subset of seminomas. Reproduction 138: 801– 811,. DNA intrastrand cross-link at the 5’-GA-3’ sequence formed by busulfan.

Dobrinski I, Avarbock MR and Brinster RL(1999). Transplantation of germ cells from rabbits and dogs into mouse testes. Biology of Reproduction, ;61:1331–1339.

Dohle GR (2010) Male infertility in cancer patients: review of the literature. Int J Urol 17:327–331.

Drugs.com. American Society of Health-System Pharmacists. December 4, 2015. Retrieved September 3, 2016.

Drugs@FDA. [cited 2016 16 Jun]; Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020954s009s010lbl pdf.

Dun MD, Aitken RJ, Nixon B(2012). The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. Hum Reprod Update; 18(4): 420-435.

Ehmcke, J.; Wistuba, J.; Schlatt, S.(2006). Spermatogonial stem cells. Questions, models and perspectives. Hum Reprod Update;12:275–282.

Esfahani SA, Esmaeilzadeh E, Bagheri F, Emami Y, Farjam M.,(2013): The effect of co-enzyme Q10 on acute liver damage in rats, a biochemical and pathological study. Hepat Mon 13:1–2,.

Fang, Y., & Zhong, R. (2019). Effects of Oxidative Stress on Spermatozoa and Male Infertility. In Free Radical Medicine and Biology. IntechOpen.

Farnaz Farsi , MSc,Majid Mohammadshahi , PhD,Pezhman Alavinejad , PhD,Afshin Rezazadeh , PhD,Mehdi Zarei , PhD &Kambiz Ahmadi Engali , PhD.(2018) Functions of Coenzyme Q10 Supplementation on Liver Enzymes, Markers of Systemic Inflammation, and Adipokines in Patients Affected by Nonalcoholic Fatty Liver Disease: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial.

Farzaneh Dehghani1,2 Ph.D., Ashraf Hassanpour2 M.Sc., Aghdas Poost-pasand1,2 Ph.D., Ali Noorafshan1,2 Ph.D., Saeid Karbalay-Doust1,2 Ph.D(2013).. Protective effects of L-carnitine and homogenized testis

tissue on the testis and sperm parameters of busulfaninduced infertile male rats. Iran J Reprod Med Vol. 11. No. 9. pp: 693-704, September

Feng HL (2003). Molecular biology of male infertility. J. Arch. Andr., P: 49: 19-27.

Fernandes, G. S., Arena, A. C., Fernandez, C. D., Mercadante, A., Barbisan, L. F., & Kempinas, W. G. (2007). Reproductive effects in male rats exposed to diuron. Reproductive Toxicology, 23(1), 106-112.

Filippini, A.; Tripiciano, A.; Peluso, C.; Morena, A.R.; Stefanini, Z.M.; Palombi, F. (2000). ECE-1 is cyclically expressed in Sertoli cells and mediates the spatiotemporal control of tubular contractility. Andrologia 32: 61.

Filler R. (1993): Methods for evaluation of rat epididymal sperm morphology. Cited in method in toxicology volume 3, (part A), male reproductive toxicology, Acad. Press Limi. Lon., P: 334-343.

Fotouh, G. I. A., Abdel-Dayem, M. M., Ismail, D. I., & Mohamed, H. H. (2018). Histological study on the protective effect of endogenous stem cell mobilization in busulfan-induced testicular injury in albino rats. *Journal of microscopy and ultrastructure*, *6*(4), 197.

Fouad AA, Jresat I(2012): Hepatoprotective effect of coenzyme Q10 in rats with acetaminophen toxicity. Environ Toxicol Pharmacol 33:158–167,.

Fouad, A.A.; Al-Sultan, A.I.; Yacoubi, M.T(2011). Coenzyme Q10 counteracts testicular injury induced by sodium arsenite in rats. Eur. J. Pharmacol., 655, 91–98.

Fouchecourt S, Lareyre JJ, Chaurand P, DaGue BB, Suzuki K, Ong DE(2003), Identification, immunolocalization, regulation, and postnatal development of the lipocalin EP17 epididymal protein of 17 kilodaltons in the mouse and rat epididymis. Endocrinology, ;144: 887-900.

Frances M. Gibson ,C. Michael Andrews ,Paraskevi Diamanti ,Sian Rizzo ,George Macharia ,Edward C. Gordon-Smith ,Thomas Williams ,John

Turton.(2003). A new model of busulphan induced chronic bone marrow aplasia in the female BALB/c mouse. Int. J. Exp. Path. 84, 31–47.

Frydelund-Larsen L, Krausz C, Leffers H, Andersson AM, Carlsen E, Bangsboell S, McElreavey K, Skakkebaek NE and Rajpert-De Meyts E (2002) Inhibin B: a marker for the functional state of the seminiferous epithelium in patients with azoospermia factor C microdeletions. J Clin Endocrinol Metab 87, 5618–5624.

G.P.Littarru*M.Battino*M.Tomasetti*A.Mordente*S.Santini*A.Oradei†A.Manto‡G.Ghirlanda‡1994 Metabolic implications of Coenzyme Q_{10} in red blood cells and plasma lipoproteinsVolume 15, Supplement 1, 1994, Pages s67-s72.

Gabrielsen JS, Tanrikut C.,(2016). Chronic exposures and male fertility: the impacts of environment, diet, and drug use on spermatogenesis. Andrology.;4(4):648-61.

Galaup, A.; Paci, A.,(2013). Pharmacology of dimethanesulfonate alkylating agents: Busulfan and treosulfan. Expert Opin. Drug Metab. Toxicol., 9, 333–347.

Garem YF, Arini AF, Beheiry AH, Zeid SA and Comhaire FH (2002) Possible relationship between seminal plasma inhibin B and spermatogenesis in patients with azoospermia. J Androl 23, 825–829.

Gemma Molyneux, Frances M. Gibson Matthew Whayman John A. Turton 2008. Serum FLT-3 ligand in a busulphan-induced model of chronic bone marrow hypoplasia in the female CD-1 mouse. Int. J. Exp. Path. Volume89, Issue2.april Pages 159-170.

Geng A.L., Guo Y.M. and Yang Y. (2004). Reduction of ascites mortality in broilers by CoQ10. Poult. Sci. 83, 1587-1593.

Ghanbarzadeh, S.; Garjani, A.; Ziaee, M.; Khorrami, A(2014). Effects of l-carnitine and coenzyme Q10 on impaired spermatogenesis caused by isoproterenol in male rats. Drug Res. (Stuttg.), 64, 449–453.

Gibbs JP, Czerwinski M, Slattery JT .,(1996). Busulfan-glutathione conjugation catalyzed by human liver cytosolic glutathione S-transferases. Cancer Res ; 56: 3678–3681.

Gihan Ibrahim Aboul Fotouh, Menna Mohamed Abdel-Dayem, Dalia Ibrahim Ismail, and Hala Hassan Mohamed.,(2018). Histological Study on the Protective Effect of Endogenous Stem Cell Mobilization in Busulfan-Induced Testicular Injury in Albino Rats. J Microsc Ultrastruct. Oct-Dec; 6(4): 197–204.

GlaxoSmithKline (2004). Prescribing information leaflet Myleran (busulfan) tablets. Available from http:// us.gsk.com/products/assets/us-meyleran.pdf.

Gogvadze, V., Orrenius, S., & Zhivotovsky, B. (2009). Mitochondria as targets for chemotherapy. *Apoptosis*, *14*(4), 624-640.

Graaf de, Beilby, S., K. H., Underwood, S. L., Evans, G., & Maxwell, W. M. C. (2009). Sperm sexing in sheep and cattle: the exception and the rule. Theriogenology, 71(1), 89-97.

Green, S. B., & Salkind, N. J. (2016). Using SPSS for Windows and Macintosh, Books a la Carte. *Pearson Book. AMC LIBRARY*.

Griffin E. Wilson D. Disorders of the Testes. In (2006) : Isselbacher K, Braunwald E, Wilson J, Martin B, et.al, eds. Harrison's Principles of Internal Medicine. 13th ed. J. New York, McGraw Hill; 1994-2017.

Griffin JE, Wilson JD.,(1985). Disorders of the testis and male reproductive tract. In: Wilson JD and Foster DW, eds. Williams Textbook of Endocrinology. Philadelphia Saunders:259-3.

Griswold MD. The central role of Sertoli cells in spermatogenesis. Semin Cell Dev Biol. 1998; 9(4):411–6. doi: 10.1006/scdb.1998.0203 PMID: 9813187.

Gunes, S., Sengupta, P., Henkel, R., Alguraigari, A., Sinigaglia, M. M., Kayal, M., & Agarwal, A. (2020). Microtubular dysfunction and male infertility. The world journal of men's health, 38(1), 9-23.

Gutierrez, K.; Glanzner, W.G.; Chemeris, R.O.; Rigo, M.L.; Comim, F.V.; Bordignon, V.; Gonçalves, P.B. Gonadotoxic effects of busulfan in two strains of mice. Reprod. Toxicol. 2016, 59, 31–39.

Gvozdjakova, A.; Kucharska, J.; Dubravicky, J.; Mojto, V.; Singh, R.B. (2015), Coenzyme Q10, α-tocopherol, and oxidative stress could be important metabolic biomarkers of male infertility. Dis. Markers 827941.

Hakemi SG, Sharififar F, Haghpanah T, Babaee A, Eftekhar-Vaghefi SH.,(2019). Theeffects of olive leaf extract on the testis, sperm quality and testicular germ cell apoptosis in male rats exposed to busulfan. Int J Fertil Steril.;13(1):57-65.

Hancook, J. L. (1951). Astaining technique for the study of temperature shock in serum nature. , 167(4243):323-325.

Hargreaves IP.,(2003). Ubiquinone: cholesterol's reclusive cousin. Biochem.;40(3):207218.https://doi.org/10.1258/000456303321610493.

Hassannejad, S., Ghamari, R., Saremi, M. A., & Baghini, S. S. (2020). The effect of vitamin D treatment on the sperm parameters of mice treated with busulfan. Evaluation, 1(3), 1-3.

Hatanaka J, Kimura Y, Lai-Fu Z, Onoue S and Yamada S. (2008).Physicochemical and pharmacokinetic characterization of water-soluble coenzyme Q10 formulations. International Journal of Pharmaceutics,;363:112–117.

Hayes FJ, Pitteloud N, DeCruz S, Crowley WF Jr, Boepple PA. 2001. Importance of inhibin B in the regulation of FSH secretion in the human male. J Clin Endocrinol Metab 86: 5541–5546.

Haywood, M.; Spaliviero, J.; Jimemez, M.; King, N.J.; Handelsman, D.J. & Allan, C.M. (2003). Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone. Endocrinology 144 509–517.

Heinz JF, Singh SP, Janowitz U, Hoelker M, Tesfaye D, Schellander K, et al. 2015Characterization of adiponectin concentrations and molecular weight forms in serum, seminal plasma, and ovarian follicular fluid from cattle. Theriogenology; 83(3): 326-333.

Hipler UC, Hochheim B, Knoll B, Tittelbach J, Schreiber G. Serum inhibin B as a marker for spermatogenesis. Arch Androl. (2001); 46(3):217–22. PMID: 11339648.

Holditch SJ, Brown CN, Lombardi AM, Nguyen KN, Edelstein CL. (2019).Recent Advances in Models, Mechanisms, Biomarkers, and Interventions in Cisplatin-Induced Acute Kidney Injury. Int J Mol Sci.;20(12). pii:E3011.

Honaramooz A, Behboodi E, Hausler CL, Blash S, Ayres S, Azuma C, (2005). Depletion of endogenous germ cells in male pigs and goats in preparation for germ cell transplantation. J Androl.;26(6):698-705.

Huhtaniemi, I.T.; Themmen, A.P. (2005). Mutations in human gonadotropin and gonadotropin receptor genes. Endocrine 26, 207–217.

Huleihel, M.; AbuElhija, M.; Lunenfeld, E.(2007). In vitro culture of testicular germ cells: Regulatory factors and limitations. Growth Factors; 25:236–251.

Hulse M, Rosner MH. (2019).Drugs in Development for Acute Kidney Injury. Drugs.;79(8):811-821.

Ikawa M, Tergaonkar V, Ogura A, Ogonuki N. Inoue K and Verma IM(2002). Restoration of spermatogenesis by lentiviral gene transfer: offspring from infertile mice. Proceedings of the National Academy of Sciences,;99:7524-7529.

Illingworth PJ, Groome NP, Byrd W, Rainey WE, McNeilly AS, Mather JP, Bremner WJ.(1996). Inhibin-B: A likely candidate for the physiologically important form of inhibin in men. J Clin Endocrinol Metab 81: 1321–1325.

Institute of Medicine (US) Committee on Assessing the Need for Clinical Trials of Testosterone Replacement Therapy, Liverman CT, Blazer DG (2004). "Introduction". Testosterone and Aging: Clinical Research Directions (Report). National Academies Press (US).

Isidori, A. M., Pozza, C., Gianfrilli, D., & Isidori, A. (2006). Medical treatment to improve sperm quality. J. Rep. bio. online, P: 12(6), 704-714.

Iwamoto T, Hiraku Y, Oikawa S, Mizutani H, Kojima M, Kawanishi S (2007). DNA intrastrand cross-link at the 5-GA-3 sequence formed by busulfan and its role in the cytotoxic.

Iwamoto T, Hiraku Y, Oikawa S, Mizutani H, Kojima M, Kawanishi S (May 2004). "DNA intrastrand cross-link at the 5'-GA-3' sequence formed by busulfan and its role in the cytotoxic effect". Cancer Sci. 95 (5): 454–8.

Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ.,(2002). Mechanisms of hepatotoxicity. Toxicol Sci.;65(2):166-76.

Jain, S.; Garg, T.; Kushwah, V.; Thanki, K.; Agrawal, A.K.; Dora, C.P. (2017).α-Tocopherol as functional excipient for resveratrol and coenzyme Q10-loaded SNEDDS for improved bioavailability and prophylaxis of breast cancer. J. Drug Target., 25, 554–565.

Jalili C, Kamani M, Roshankhah S, Sadeghi H, Salahshoor MR.,(2018). Effect of Falcaria vulgaris extracts on sperm parameters in diabetic rats. Andrologia.;50:e13130.

jalili, C., ABDOLMALEKI, A., FARAMARZI, A., & SALAHSHOOR, M. R. (2020). Effects of Thymoquinone and Busulfan on Reproductive Parameters in Male Rats: An Experimental Study. Journal of Clinical & Diagnostic Research, 14(1).

Janes, M in Encyclopedia of Toxicology (Third Edition), 2014. Coates, P.M.; Betz, J.M.; Blackman, M.R.; Cragg, G.M.; Levine, M.; Moss, J.; White, J.D., Encyclopedia of Dietary Supplements, 2nd ed.; Informa Healthcare: London, UK, 2010.

Javier Diaz-Castro 1,2,* , Jorge Moreno-Fernandez 1,2 , Ignacio Chirosa 3 , Luis Javier Chirosa 3 , Rafael Guisado 4 and Julio J. Ochoa 1,2.(2020). Beneficial Effect of Ubiquinol on Hematological and Inflammatory Signaling during Exercise. Nutrients 2020, 12, 424;

Jenke, A., Freiberg-Richter, J., Wilhelm, S., Freund, M., Renner, U. D., Bornhäuser, M., ... & Schuler, U. (2005). Accidental busulfan overdose during conditioning for stem cell transplantation. Bone marrow transplantation, 35(2), 125-128.

Ji, M., Minami, N., Yamada, M., & Imai, H. (2007). Effect of protopanaxatriol saponin on spermatogenic stem cell survival in busulfan-treated male mice. Reproductive medicine and biology, 6(2), 99-108.

Jiang, F.X.; Short, R.V. (2019), Male germ cell transplantation in rats: Apparent synchronization of spermatogenesis between host and donor seminiferous epithelia. Int. J. Androl. 1995, 18, 326–330. Int. J. Mol. Sci. 20, 957 9 of 12.

Jimenez-Santos MA, Juarez-Rojop IE, Tovilla-Zarate CA, Espinosa-Garcıa MT, Juarez-Oropeza MA, Ramon-Frıas T, Bermudez-Oca~na DY,

Dıaz-Zagoya JC., (2014) Coenzyme Q10 supplementation improves metabolic parameters, liver function and mitochondrial respiration in rats with high doses of atorvastatin and a cholesterol-rich diet. Lipids Health Dis 13:1–22,.

Jung Lim Lee, Ted Gooley, William Bensinger, Kathy Schiffman, George B. McDonald. © Veno-occlusive disease of the liver after busulfan, melphalan, and thiotepa conditioning therapy: incidence, risk factors, and outcome 1999 American Society for Blood and Marrow Transplantation.

Kadivar A, Khoei HH, Hassanpour H, Golestanfar A, Ghanaei H.Correlation of adiponectin mRNA abundance and its receptors withquantitative parameters of sperm motility in rams. Int J Fertil Steril 2016; 10(1): 127.

Kadowaki T & Yamauchi T 2005 Adiponectin and adiponectin receptors. Endocrine Reviews 26 439–451. (doi:10.1210/er.2005-0005).

Kaminski T, Smolinska N, Maleszka A, Kiezun M, Dobrzyn K,Czerwinska J, et al. 2014Expression of adiponectin and its receptors in the porcine hypothalamus during the oestrous cycle. Reprod Domest Anim; 49(3): 378-386**.**

Karine Brochu-Gaudreau, Charlotte Rehfeldt, Richard Blouin, V. Bordignon, Bruce D. Murphy & Marie-France Palin. 2010 Adiponectin action from head to toe. Endocrine 37 11–32.

Karstens A, Kramer I (2007). "Chemical and physical stability of diluted busulfan infusion solutions". EJHP Science. 13: 40–7.

Kasimanickam VR, Kasimanickam RK, Kastelic JP, Stevenson JS.Associations of adiponectin and fertility estimates in Holstein bulls.Theriogenology 2013; 79(5): 766-777.

Kawwass JF, Summer R, Kallen CB. Direct effects of leptin andadiponectin on peripheral reproductive tissues: A critical review. Mol Hum Reprod 2015; 21(8): 617-632.

Khaki A, Fathiazad F, Nouri M, Afshin Khaki A, Ozanci CC, GhafariNovin M, Hamadeh M: The effects of Ginger on spermatogenesis and sperm parameters of rat. Iran J Reprod Med, 7 (2): 7-12, 2009.

Kiserud CE, Fosså A, Bjøro T, Holte H, Cvancarova M, Fosså SD.,(2009). Gonadal function in male patients after treatment for malignant lymphomas, with emphasis on chemotherapy. British Journal of Cancer.;100(3):455–463.

Kishimoto C, Nobuyoshi T, Yukie N, Miki M.,(2003).: Anti-oxidant effects of coenzyme Q10 on experimental viral myocarditis in mice. J Cardiovasc Pharmacol 42:588–592,

Klenke U, Taylor-Burds C, Wray S. 2014Metabolic influences onreproduction: Adiponectin attenuates GnRH neuronal activity in female mice. Endocrinology; 155(5): 1851-1863

Kos K, Harte AL, Da Silva NF, Tonchev A, Chaldakov G, James S, etal. 2007Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus. J Clin Endocrinol Metab; 92(3): 1129-1136.

Kramer, J., & Magee, J. (2007, May). Self-managed systems: an architectural challenge. In *Future of Software Engineering (FOSE'07)* (pp. 259-268). IEEE.

Krivoy N, Hoffer E, Lurie Y, et al. 2008 Busulfan use in hematopoietic stem celltransplantation: Pharmacology, dose adjustment, safety and efficacy in adults and children. Curr Drug Saf; 3(1): 60-66

Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H,et al. 2007Adiponectin stimulates AMP-activated protein kinase in thehypothalamus and increases food intake. Cell Metab; 6(1): 55-68.

Kusminski C, McTernan P, Schraw T, Kos K, O'hare J, Ahima R, et al. 2007Adiponectin complexes in human cerebrospinal fluid: Distinct complex distribution from serum. Diabetologia; 50(3): 634-642.

Landry D, Pare A, Jean S & Martin LJ .,(2015) Adiponectin influences progesterone production from MA-10 Leydig cells in a dose-dependent manner. Endocrine 48 957–967.

Le Bourgeois A, Lestang E, Guillaume T, et al.,2013;. Prognostic impact of immune status and hematopoietic recovery before and after fludarabine, IV busulfan, and antithymocyte globulins (FB2 regimen) reducedintensity conditioning regimen (RIC) allogeneic stem cell transplantation (allo-SCT). Eur J Hematol 90(3): 177-186

Lee, S. Q. E., Tan, T. S., Kawamukai, M., & Chen, E. S. (2017). Cellular factories for coenzyme Q 10 production. *Microbial cell factories*, *16*(1), 1-16.

Lesurtel M, Graf R, Aleil B, Walther D, Tian Y, Jochum W, Gachet C, Bader M, Clavien P., (2006). "Platelet-derived serotonin mediates liver regeneration". Science. 312 (5770): 104.

Li B, He X, Zhuang M, Niu B, Wu C, Mu H(2018);. Melatonin ameliorates busulfan-induced spermatogonial stem cell oxidative apoptosis in mouse testes. Antioxid Redox Signal. 28(5):385-400.

Li X, Wang YK, Song ZQ, Du ZQ, Yang CX. (2016)Dimethyl sulfoxide perturbs cell 496 cycle progression and spindle organization in porcine meiotic oocytes. PLoS One. 497; 11(6): e0158074.

Lin, M.T.; Beal, M.F.,(2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature, 443, 787–795.

Lindgren A, Levin M, Rodrigo Blomqvist S, Wikstrom J, Ahnmark A, Mogensen C, Bottcher G, Bohlooly YM, Boren J, Gan LM .,(2013)Adiponectin receptor 2 deficiency results in reduced atherosclerosis in the brachiocephalic artery in apolipoprotein E deficient mice. PLoS ONE 8 e80330.

Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R. 1986a. A homodimer of the β-subunits of inhibin A stimulates the secretion of pituitary follicle stimulating hormone. Biochem Biophys Res Commun 138: 1129–113**.**

Lio, M., Ono, Y., Kai, S., & Fukumoto, M. (1986). Effects of flavonoids on xanthine oxidation as well as on cytochrome c reduction by milk xanthine oxidase. J. Nut. Sci. & vit., P: 32(6), 635-642.

Littarru GP and Tiano L.,(2005). Clinical aspects of coenzyme Q10: an update. Current Opinion in Clinical Nutrition and Metabolic Care,;641: 6-8.

Liu FJ, Dong WY, Zhao H, Shi XH, Zhang YL,.(2019)Effect of molybdenum on reproductivefunction of male mice treated with busulfan. Theriogenology.;126:49-54.

Liu L, Kang J, Ding X, Chen D, Zhou Y, Ma H (2015). "Dehydroepiandrosterone-Regulated Testosterone Biosynthesis via Activation of the ERK1/2 Signaling Pathway in Primary Rat Leydig Cells". *Cellular Physiology and Biochemistry*. 36 (5): 1778–92

LiuZ, HuangP, LawS(2018),*etal* Preventive effect of curcumin against chemotherapy-induced side-effects. Front Pharmacol. ;9:1374.

Luetjens CM, Weinbauer GF (2012). "Chapter 2: Testosterone: Biosynthesis, transport, metabolism and (non-genomic) actions". In Nieschlag E, Behre HM, Nieschlag S (eds.). Testosterone: Action, Deficiency, Substitution (4th ed.). Cambridge: Cambridge University Press. pp. 15–32. ISBN 978-1-107-01290-5.

Luisi, S., Florio, P., Reis, F. M., & Petraglia, F. (2005). Inhibins in female and male reproductive physiology: role in gametogenesis, conception, implantation and early pregnancy. Human reproduction update, 11(2), 123–135.

Makanji Y, Zhu J, Mishra R, Holmquist C, Wong WP, Schwartz NB, Mayo KE, Woodruff TK.,(2014). Inhibin at 90: from discovery to clinical application, a historical review. Endocr Rev 35: 747–794,

Male Medical Fertility Treatment: HCG + LH + Recombinant FSH To Increase Sperm Count Through Spermatogenisis". Archived from the original on February 19, 2015. Retrieved 6 April 2015.[unreliable medical source?].

Marchetti C, Hamdane M, Mitchell V, Mayo K, Devisme L, Rigot JM, Beauvillain JC, Hermand E, Defossez A. 2003. Immunolocalization of inhibin and activin α and βB subunits and expression of corresponding messenger RNAs in the human adult testis. Biol Reprod 68: 230–235.

M Brydøy, SD Fosså, O Dahl, T Bjøro. Acta Oncol, 2007, 46 (4): 480–489.

Maymon BBS, Yogev L, Marks A, Hauser R, Botchan A and Yavetz H. (2004) (Sertoli cell inactivation by cytotoxic damage to the human testis after cancer chemotherapy. Fertility and Sterility,;81(5):1391-1394.

Mazen NF and Elnegris HM.,(2013). Role of coenzyme Q10 in testicular damage induced by acrylamide in weaned albino rats: a histological and immunohistochemical study. The Egyptian Journal of Histology,: 164-174.

McClive PJ, Sinclair AH.,(2003). Type II and type IX collagen transcript isoforms are expressed during mouse testis development. Biol Reprod; 68(5): 1742-1747.

McEvoy GK, editor (1987). American Hospital Formulary Service Drug Information. Bethesda, MD: American Society of Health-System Pharmacists, pp. 426–428.

Mendis SH, Meachem SJ, Sarraj MA, Loveland KL. (2011). Activin A balances Sertoli and germ cell proliferation in the fetal mouse testis. Biol Reprod 84: 379 –391,

Miller SL, Maner JK (February 2010). "Scent of a woman: men's testosterone responses to olfactory ovulation cues". Psychological Science. 21 (2): 276–83.

Mohadeseh Panahi1, Saeideh Keshavarz2, Farhad Rahmanifar3, Amin Tamadon1, Davood Mehrabani1*, Negar Karimaghai1, Masood .,(2015).Sepehrimanesh4, Heydar Aqababa. Busulfan induced azoospermia: Stereological evaluation of testes in rat. Veterinary Research Forum.; 6 (4) 273 – 278.

Mohammad Reza Safarinejad,(2009). Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men J Urol. Jul;182(1):237-48.

Mohammad Reza Safarinejad,ShivaSafarinejad,NayyerShafiei and SabaSafarinejad.(2012). (Ubiquinol) on Semen Parameters in Men with Idiopathic Infertility: a Double-Blind, Placebo Controlled, Randomized Study. The Journal of Urology Volume 188, Issue 2, August Pages 526-531.

Mohammad, N.S.; Arafa, M.H.; Atteia, H.H(2015). Coenzyme Q10 and fish oil synergistically alleviate aluminum chloride-induced suppression of testicular steroidogenesis and antioxidant defense. Free Radic. Res., 49, 1319–1334.

Moloody, M., Shahrooz, R., Razi, M., Zarei, L., & Mohammadi, V. (2018). The Effect of CoQ10 on Testicular Tissue in Rats Treating with Busulfan: Sperm Quality and Histological Changes. Iranian Journal of Veterinary Surgery, 13(1), 29-38.

Molyneux, G., Gibson, F. M., Whayman, M., & Turton, J. A. (2008). Serum FLT-3 ligand in a busulphan-induced model of chronic bone marrow hypoplasia in the female CD-1 mouse. *International Journal of Experimental Pathology*, *89*(2), 159-170.

Mosher WD, Pratt WF (1991). Fecundity and infertility in the United States: incidence and trends. J Fertil. Steril., P: 56:192-3.

Nagano M, Avarboc MR and Brinster RL .,(1999)Pattern and kinetics of mouse donor spermatogonial stem cell colonization in recipient testes. Biology of Reproduction,;60:1429-1436.

National.,(2017).Institutes of Health. Livertox: clinical and research information on drug-induced liver injury,. Available from: Nih. gov https://livertox. nih. Gov.

Ni, F. D., Hao, S. L., & Yang, W. X. (2019). Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis. *Cell Death & Disease*, *10*(8), 1-15.

Nicholls PK, Harrison CA, Rainczuk KE, Wayne Vogl A, Stanton PG. ,(2013). Retinoic acid promotes Sertoli cell differentiation and antagonises activin-induced proliferation. Mol Cell Endocrinol 377: 33– 43,

Nieto Y, Thall P, Valdez B, et al. High-dose infusional gemcitabine combined with busulfan and melphalan with autologous stem-cell transplantation in patients with refractory lymphoid malignancies. Biol Blood Marrow Transplant 2012; 18(11): 1677-1686 Ndovi, T. T.; Parson, T.; Choi, L.; Caffo, B.; Ronde, C. and Hendrix, C. W. (2007). A new method to quantitatively estimate seminal vesicle and prostate gland contributions to ejaculate . BrJ clin pharmacol 63 (4):404-420.

Nieto Y, Thall P, Valdez B, et al., 2012 High-dose infusional gemcitabine combined with busulfan and melphalan with autologous stem-cell transplantation in patients with refractory lymphoid malignancies. Biol Blood Marrow Transplant; 18(11): 1677-1686

Niklowitz, P., Sonnenschein, A., Janetzky, B., Andler, W., & Menke, T. (2007). Enrichment of coenzyme Q10 in plasma and blood cells: defense against oxidative damage. *International Journal of Biological Sciences*, *3*(4), 257.

Nilsson C, Aschan J, Hentschke P.,(2003), et al. The effect of metronidazole on busulfan pharmacokineticsin patients undergoing hematopoietic ste m cell transplantation, Bone Marrow Transplant 31(6):429-35.

Nishida M, Funahashi T, Shimomura I. Pathophysiological significance of adiponectin. Med Mol Morphol 2007; 40(2): 55-67.

Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H, Matsuda M, Kondo H, Furuyama N, Kihara S et al. (2002) Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes 51 2734–2741.

Norasteh H. (MSc)1 ,. Mohammadi. Sh (PhD)∗1 , Nikravesh M. (PhD)1 , Taraz Jamshidi Sh. (MD)2.(2020). Protective Effect of Bene (Pistacia Atlantica) on Busulfan-Induced RenalLiver Injury in Laboratory Mice. J Babol Univ Med Sci 22; 2020. P: 17-23.

Nudell DM, Monoski MM, Lipshultz LI.,(2002). Common medications and drugs: how they affect male fertility. Urol Clin North Am.;29(4):965-973.

Ocon-Grove OM, Krzysik-Walker SM, Maddineni SR, Hendricks GL, Ramachandran R. Adiponectin and its receptors are expressed in the chicken testis: influence of sexual maturation on testicular ADIPOR1 and ADIPOR2 mRNA abundance. Reproduction 2008; 136(5): 627-638.

Oduwole OO, Peltoketo H, Huhtaniemi IT (2018). "Role of Follicle-Stimulating Hormone in Spermatogenesis". *Frontiers in Endocrinology*. Nutrients. 9:
763. doi:10.3389/fendo.2018.00763. PMC 6302021. PMID 30619093.

Ogawa, T. (2001)Spermatogonial transplantation: The principle and possible application. J. Mol. Med., 79, 368–374.

Olayemi, F. O. (2010). review on some causes of male infertility. J. Afr.

Omidi Ha, Omidi He, NaghdiBadi H. (2008);The effect of pistacia atlantica nut powder on liver phosphatidate phosphohydrolase and serum lipid profile in rat. J Med Plants. 2(26):70-8.

Ozcan, P., Ficicioglu, C., Kizilkale, O., Yesiladali, M., Tok, O.E., Ozkan, F., Esrefoglu, M., (2016). Can Coenzyme Q10 supplementation protect the ovarian reserve against oxidative damage? J. Assist. Reprod. Gen. 33, 1223-1230.

Palmeira, C.M.; Santos, D.L.; Seica, R.; Moreno, A.J.; Santos, M.S. (2001).Enhanced mitochondrial testicular antioxidant capacity in Goto-Kakizaki diabetic rats: Role of coenzyme Q. Am. J. Physiol. Cell Physiol., 281, C1023–C1028.

Panahi, M., Keshavarz, S., Rahmanifar, F., Tamadon, A., Mehrabani, D., Karimaghai, N., ... & Aqababa, H. (2015). Busulfan induced azoospermia: Stereological evaluation of testes in rat. In Veterinary Research Forum (Vol. 6, No. 4, p. 273). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Panahi, Y., Hosseini, M. S., Khalili, N., Naimi, E., Majeed, M., & Sahebkar, A. (2015). Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: a randomized controlled trial and an updated meta-analysis. *Clinical nutrition*, *34*(6), 1101-1108.

Paredes-Fuentes, A. J., Montero, R., Codina, A., Jou, C., Fernández, G., Maynou, J., ... & Artuch, R. (2020). Coenzyme Q10 treatment monitoring in different human biological samples. *Antioxidants*, *9*(10), 979.

Parfitt K.(2012).Martindale. The Complete Drug Reference, Journal of the Medical Library Association,;100(1): 75–76.

Perez-Crespo, M.; Pericuesta, E.; Perez-Cerezales, S.; Arenas, M.I.; Lobo, M.O. (2011); Diaz-Gil, J.J. Effect of liver growth factor on both testicular regeneration and recovery of spermatogenesis in busulfan-treated mice. Reprod. Biol. Endocrinol., 9, 21..

Petersen, C. and O. Soder,(2006). The sertoli cell--a hormonal target and 'super' nurse for germ cells that determines testicular size. Horm Res. 66(4): p. 153-61.

Petra Niklowitz,[1] Anka Sonnenschein,[2] Bernd Janetzky,[2] Werner Andler,[1] and Thomas Menke[1] (2007) . Enrichment of coenzyme Q10 in plasma and blood cells: defense against oxidative damage. Int J Biol Sci.; 3(4): 257–262.

Pfaehler A, Nanjappa MK, Coleman ES, Mansour M, WandersD, Plaisance EP, et al.2012 Regulation of adiponectin secretion by soyisoflavones has implication for endocrine function of the testis. Toxicol Lett; 209(1): 78-85.

Pirke KM, Kockott G, Dittmar F (November 1974). "Psychosexual stimulation and plasma testosterone in man". Archives of Sexual Behavior. 3 (6): 577–84.

Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley WF, Hayes FJ (March 2008). "Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men". *The Journal of Clinical Endocrinology and Metabolism*. 93 (3): 784–91Barakat B, O'Connor AE, Gold E, de Kretser DM, Loveland KL.,2008. Inhibin, activin, follistatin and FSH serum levels and testicular production are

highly modulated during the first spermatogenic wave in mice. Reproduction 136: 345–359,. doi:10.1530/ REP-08-0140.

Probin V, Wang Y, Zhou D.,(2007). Busulfan-induced senescence is dependent on ROS production upstream of the MAPK pathway. Free Radic Biol Med; 42(12):1858-1865.

Psilopanagioti A, Papadaki H, Kranioti EF, Alexandrides TK, Varakis JN. Expression of adiponectin and adiponectin receptors in human pituitary gland and brain. Neuroendocrinology 2009; 89(1): 38-4

Purvis, K., & Christiansen, E. (1992). Male infertility: current concepts. J. Ann. Med., P: 24(4), 259-272.

Qu, N., Itoh, M., & Sakabe, K. (2019). Effects of chemotherapy and radiotherapy on spermatogenesis: The role of testicular immunology. International journal of molecular sciences, 20(4), 957.

Qu, N., Naito, M., Li, J., Terayama, H., Hirai, S., & Itoh, M. (2012). Xenogeneic and endogenous spermatogenesis following transplantation of rat germ cells into testes of immunocompetent mice. Reproduction, fertility and development, 24(2), 337-343.

Rak, A., Mellouk, N., Froment, P., & Dupont, J. (2017). Adiponectin and resistin: potential metabolic signals affecting hypothalamo-pituitary gonadal axis in females and males of different species. *Reproduction*, *153*(6), R215-R226.

Ramachandran A, Jaeschke H. (2018) Oxidative Stress and Acute Hepatic Injury. Curr Opin Toxicol.;7:17-21.

Rey RA, Grinspon RP, Gottlieb S, Pasqualini T, Knoblovits P, Aszpis S, (2013)Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. Andrology.; 1(1):3–16.

Roney JR, Mahler SV, Maestripieri D (2003). "Behavioral and hormonal responses of men to brief interactions with women". *Evolution and Human Behavior*. 24 (6): 365–75.

Rosati, L., Prisco, M., Di Lorenzo, M., De Falco, M., & Andreuccetti, P. (2020). Immunolocalization of aromatase P450 in the epididymis of Podarcis sicula and Rattus rattus. European Journal of Histochemistry: EJH, 64(1).

Ross GT. (1985).Disorders of the ovary and female reproductive tract. In: Wilson JD and Foster DW, eds. Williams Textbook of Endocrinology. Philadelphia Saunders:206-58.

Sa A, Faddah L, Abdel-baky A, Bayoumi A., (2010).Protective effect of L-carnitine and coenzyme Q10 on CCl4-induced liver injury in rats. Sci Pharm 78:881–896,

Safarinejad, M. R. (2009). Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. *The Journal of urology*, *182*(1), 237-248.

Salahshoor MR, Haghjoo M, Roshankhah S, Makalani F, Jalili C. (2018).Effect ofthymoquinone on reproductive parameter in morphine-treated male mice. Adv Biomed Res.;7:18-25.

Saleem Ali Banihani.(2018).Effect of Coenzyme Q10 Supplementation on Testosterone. Biomolecules, 8, 172.

Salehinezhad, F., Eshraghi, H., Kadivar, A., Shirian, S., Asghari, A., Aali, E., & Davoodian, N. (2019). Amelioration effects of vitamin E, melatonin, L-carnitine, and atorvastatin, on destructive effects of busulfan in the testes of male rats: A gene expression evaluation. *Kafkas Universitesi Veteriner Fakultesi Dergisi*.

Sarmento-Cabral A, Peinado JR, Halliday LC, Malagon MM, Castaño JP, Kineman RD, et al. 2017Adipokines (leptin, adiponectin,

resistin)differentially regulate all hormonal cell types in primary anterior pituitary cell cultures from two primate species. Sci Rep; 7: 43537.

Schmelzer, C., Lorenz, G., Lindner, I., Rimbach, G., Niklowitz, P., Menke, T., & Döring, F. (2007). Effects of Coenzyme Q_ {10} on TNF-α secretion in human and murine monocytic cell lines. *Biofactors*, *31*(1), 35-41.

Sepideh Ganjalikhan Hakemi, M.Sc,[1] Fariba Sharififar, Ph.D,[2] Tahereh Haghpanah, Ph.D,[1,*] Abdolreza Babaee, M.Sc,[1] and Seyed Hassan Eftekhar-Vaghefi, Ph.D., (2019). The Effects of Olive Leaf Extract on The Testis, Sperm Quality and Testicular Germ Cell Apoptosis in Male Rats Exposed to BusulfanInt J Fertil Steril. Apr-Jun; 13(1): 57–65.

Sharideh, H., Zhandi, M., Zeinoaldini, S., Zaghari, M., Sadeghi, M., Akhlaghi, A., & Peebles, E. D. (2020). Beneficial effects of dietary coenzyme Q10 on the productive and reproductive variables of broiler breeder hens. Animal Reproduction Science, 213, 106256.

Sharma TP, Nett TM, Karsch FJ, Phillips DJ, Lee JS, Herkimer C, Padmanabhan V (June 2012) [Published online before print 2012-03-14]. "Neuroendocrine control of FSH secretion: IV. Hypothalamic control of pituitary FSH-regulatory proteins and their relationship to changes in FSH synthesis and secretion". Biology of Reproduction (published 2012-06-01). 86 (6): 171.

Sharpe RM, Turner KJ, McKinnell C, Groome NP, Atanassova N, Millar MR, Buchanan DL, Cooke PS. 1999. Inhibin B levels in plasma of the male rat from birth to adulthood: Effect of experimental manipulation of Sertoli cell number. J Androl 20: 94–101.

Sharpe, R.M.,(2003). Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction. 125(6): p. 769-84.

Shetty G, Meistrich ML.,(2005). Hormonal approaches to preservation and restoration of male fertility after cancer treatment. J Natl Cancer Inst Monogr. (34):36-39. doi:10.1093/jncimonographs/ lgi002.

Sinclair, S. (2000). Male infertility: nutritional and environmental considerations. Alternative medicine review: J. Clin. Ther., :p5(1), 28-38.

Soffientini U, Rebourcet D, Abel MH, Lee S, Hamilton G, Fowler PA, Smith LB, O'Shaughnessy PJ., (2017)Identification of Sertoli cell-specific transcripts in the mouse testis and the role of FSH and androgen in the control of Sertoli cell activity. BMC Genomics. Dec 15;18(1):972.

Sofikitis, N.; Pappas, E.; Kawatani, A. (2005). Efforts to create an artificial testis: Culture systems of male germ cells under biochemical conditions resembling the seminiferous tubular biochemical environment. Hum Reprod Update;11:229–259.

Soleimanzadeh, A., Mohammadnejad, L., & Ahmadi, A. (2018). Ameliorative effect of Allium sativum extract on busulfan-induced oxidative stress in mice sperm. In Veterinary Research Forum (Vol. 9, No. 3, p. 265). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Solomon R, Shvartsur R, Azab AN.,(2019). The association between psychotropic drug use and fertility problems among male subjects. J Psychiatr Pract.;25(1):22-33.

Suriapraba E, Mani PR, Kumar RA., (2012)et al. Protective effect of NAC on busulfan induced clastogenesis in human peripheral blood lymphocytes. Int J Inst Pharm Life Sci; 2(1): 172-177.

Suttorp M, Millot F. Treatment of pediatric chronic myeloid leukemia in the year 2010: use of tyrosine kinase inhibitors and stem-cell transplantation. Vol. 1: Orlando, USA: Ash Education Program Book 2010: 368-376 **Santoro** , G. and Romeo , C. (2001) . Normal and varicocele testis in adolescent Asian J. Androl . 3:259-262.

Sweetman SC, editor (2008). Martindale: The Complete Drug Reference. London: Pharmaceutical Press.

Taylor, T. (2017). Male Reproductive System , Anatomy and Physiology Instructor. Thomas S, Kratzsch D, Schaab M, Scholz M, Grunewald S,

Thiery J, Paasch U & Kratzsch J 2013 Seminal plasma adipokine levels are correlated with functional characteristics of spermatozoa. Fertility and Sterility 99 1256–1263.e1253.

Thomas S, Kratzsch D, Schaab M, Scholz M, Grunewald S, Thiery J, et al. Seminal plasma adipokine levels are correlated with functional characteristics of spermatozoa. Fertil Steril 2013; 99(5): 1256-1263. e3

Thomson Healthcare (2008). Physicians' Desk Reference62nd ed. Montvale, NJ.

Thomson. Sweetman SC, editor (2008). Martindale: The Complete Drug Reference. London: Pharmaceutical Press. Available at.

Tomaszewski J, Joseph A, Archambeault D, Yao HH.(2007) Essential roles of inhibin beta A in mouse epididymal coiling. Proc Natl Acad Sci USA 104: 11322–11327,.

Topcu-TarladacalisirY, Sapmaz-MetinM, KaracaT. ,(2016)Curcumin counteracts cisplatin-induced nephrotoxicity by preventing renal tubular cell apoptosis. Ren Fail. ;38(10):1741–1748.

Torjesen PA, Sandnes L (March 2004). "Serum testosterone in women as measured by an automated immunoassay and a RIA". Clinical Chemistry. 50 (3): 678, author reply 678–9.

Tosti, A., Hay, R., & Arenas-Guzman, R. (2005). Patients at risk of onychomycosis–risk factor identification and active prevention. *Journal of the European Academy of Dermatology and Venereology*, *19*, 13-16.

Tuck SP, Francis RM (2009). "Testosterone, bone and osteoporosis". Advances in the Management of Testosterone Deficiency. Frontiers of Hormone Research. 37. pp. 123–32

Ubuka T, Son YL, Tobari Y, Narihiro M, Bentley GE, Kriegsfeld LJ, Tsutsui K (2014). "Central and direct regulation of testicular activity by gonadotropin-inhibitory hormone and its receptor". *Frontiers in Endocrinology*. 5: 8

Ulloa-Aguirre A, Reiter E, Crépieux P (August 2018). "FSH Receptor Signaling: Complexity of Interactions and Signal Diversity". Endocrinology. 159 (8): 3020–3035.

Vafaei A, Mohammadi S, Fazel A, Soukhtanloo M, Pour AM, Beheshti F. (2018);Effects of carob (Ceratoniasiliqua) on sperm quality, testicular structure, testosterone level and oxidative stress in busulfan-induced infertile mice. Pharm Sci. 24(2):104-11.

Vanden Aardweg GJMJ, De Ruiter-Bootsma, AL, Kramer MF and Davids JA. (1983)Growth and differentiation of spermatogenic colonies in the mouse testis after irradiation with fission neutrons. Radiation Research,;94:447-463.

Vermeulen A, Verdonck I, Kaufman JM. A (1999)critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab;84:3666–72.

Waki H, Yamauchi T, Kamon J, Kita S, Ito Y, Hada Y, et al. 2005Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. Endocrinology; 146(2): 790-796

Walker, W.H. (2003). Nongenomic actions of androgen in Sertoli cells. Current Topics in Development Biology 56 25–53.

Wang DZ, Zhou XH, Yuan YL and Zheng XM. (2010).Optimal dose of busulfan for depleting testicular germ cells of recipient mice before spermatogonial transplantation. Asian Journal of Andrology,;12:263–270.

Wang, R. S., Yeh, S., Tzeng, C. R., & Chang, C. (2009). Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-

specific androgen receptor knockout mice. J. End Rev., P: 30(2), 119-132.

WaseemM, KaushikP, ParvezS. (2013).Mitochondria-mediated mitigatory role of curcumin in cisplatin-induced nephrotoxicity. Cell Biochem Funct. ;31(8):678–684.

Welshons, W. V., Nagel, S. C., & vom Saal, F. S. (2006). Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology, 147(6), s56-s69.

Wen JP, Liu C, Bi WK, Hu YT, Chen Q, Huang H, et al. 2012Adiponectin inhibits KISS1 gene transcription through AMPK and specificityprotein-1 in the hypothalamic GT1-7 neurons. J Endocrinol; 214(2):174.

Wolski, K. M. (2006). The Sertoli Cell-Spermatid Junctional Complex: A potential avenue for Male contraception.

World Health Organization,(2010);. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva, Switzerland: World Health Organization 21-26.

Yakubu OE, Olawale O, Arowora KA and Imo C. (2017).Biochemical Changes in Haematological and Liver Function Parameters in Intoxicated Male Albino Rats Treated with Hymenocardia acida Leaves Ethanolic Extract.. iMedPub Journals. Vol. 2 No. 2: 10.

Yamauchi T, Iwabu M, Okada-Iwabu M & Kadowaki T (2014) Adiponectin receptors: a review of their structure, function and how they work. Best Practice and Research Clinical Endocrinology and Metabolism 28 15–23.

Yogev L, Marks A.,(2004), et al. Sertoli cell inactivation by cytotoxic damage to the human testis after cancer chemotherapy. Fertil steril; 81(5): 1391-1394.

Yongxing Xu, Juan Liu, Enhong Han, Yan Wang, and Jianjun Gao. (2019).Efficacy of coenzyme Q10 in patients with chronic kidney disease: protocol for a systematic review. BMJ Open.; 9(5): .

Yun-Jung Choi, ,Dueg-Nam Kwon ,Jong-il Chung ,Heung-Cheol Kim ,Sung-Moon Yeo ,Teoan Kim ,Han-Geuk Seo and Jin-Hoi Kim.,(2004). Murine male germ cell apoptosis induced by busulfan treatment correlates with loss of c-kit expression in a Fas/FasL- and p53-independent manner. FEBS Lett. Volume575 , Issue1-3 September 24,. Pages 41-51.

YuSheng Qin1 , Ling Liu1 , YaNan He1 , (2016).Chen Wang3 , MingYuan Liang2 , XiaoLi Chen1 , HaiSheng Hao1 , Tong Qin1 , XueMing Zhao1 , Dong Wang1 *.2016. Testicular Busulfan Injection in Mice to Prepare Recipients for Spermatogonial Stem Cell Transplantation Is Safe and Non-Toxic.

Zatollah Asemi (2018).et al. Coenzyme Q10 supplementation on metabolic profiles of patients with chronic kidney disease. Current Pharmaceutical Design, Volume 24, Issue 31,

Zhang, Z.; Renfree, M.B.; Short, R.V.,(2003). Successful intra- and interspecific male germ cell transplantation in the rat. Biol. Reprod., 68, 961–967.

Zhao, Y., Zhang, P., Ge, W., Feng, Y., Li, L., Sun, Z., ... & Shen, W. (2020). Alginate oligosaccharides improve germ cell development and testicular microenvironment to rescue busulfan disrupted spermatogenesis. Theranostics, 10(7), 3308.

Zirkin BR, Papadopoulos V (July 2018). "Leydig cells: formation, function, and regulation". *Biology of Reproduction*. **99** (1): 101–111

Zurita, M., & Merino, C. (2003). The transcriptional complexity of the TFIIH complex. TRENDS in Genetics, 19(10), 578-584.

الخلاصه

بوسولفان هو علاج كيميائي للسرطان بينما الإنزيم المساعد (يوبيكوينون (CoQ10 هو أنزيم يعمل على تعزيز التفاعلات الكيميائية كمضاد للأكسدة ضد الجذور الحرة في الجسم ويساعد في تقليل تأثير العلاج بأدوية العلاج الكيميائي مثل بوسولفان . أجريت هذه الدراسة في فرع الفسلجة والكيمياء الحيوية والادوية في كلية الطب البيطري / جامعة كربلاء من كانون الأول 2019 إلى نهاية شباط 2020 اذ تم تقييم دور يوبيكوينون مقابل التأثير السام للبوسولفان على الحيوانات المنوية في الفئران البيضاء البالغة في فترات 28 و 56 يومًا. تم تقسيم 48 من ذكورالفئران البالغة إلى أربع مجموعات بشكل عشوائي وبالتساوي في كل مجموعة (12 جرذ لكل مجموعة) تغذية الحيوانات والماء كانت متاحة لمدة 8 أسابيع.

حقنت المجموعة الأولى بالماء المقطر كمجموعة سيطرة ، المجموعة الثانية حقنت داخل الصفاق بجرعة وحيدة 10 ملجم / كجم من بوسلفان (علاج كيميائي) ، بينما حقنت المجموعة الثالثة داخل الصفاق بجرعة وحيدة من بوسولفان 10 ملجم / كجم وأنزيم يوبيكوينون (10 (COQ10 ملغ / كغ يومياً ، المجموعة الرابعة تحقن داخل الصفاق فقط 10 CoQ10 ملغ / كغ يومياً حتى نهاية الدراسة.

أظهرت النتائج انخفاضًا معنويًا ($p \leq 0.05$) في المتغيرات الدموية (عدد كريات الدم الحمراء ، نسبة خضاب الدم ، حجم كريات الدم المرصوصة ، عدد الصفيحات الدموية وعدد كريات الدم البيضاء) في كلا المجموعتين الثانية والثالثة بينما لا يوجد فرق معنوي ($p \geq 0.05$) في المجموعة الرابعة مقارنة مع مجموعة السيطرة في فترة الدراسة 28 يوم. في 56 يومًا ، أظهرت النتائج انخفاضًا معنويًا ($p \leq 0.05$) مع تحسن في المتغيرات الدموية (عدد كريات الدم الحمراء ، نسبة خضاب الدم ، حجم كريات الدم المرصوصة ، عدد الصفيحات الدموية وعدد كريات الدم البيضاء) في كلا المجموعتين الثانية والثالثة بينما لا يوجد فرق معنوي ($p \geq 0.05$) في المجموعة الرابعة بالمقارنة مع المجموعة الاولى.

اشارت نتائج الهرمونات إلى أن هرمونات التستوستيرون و ADPN و INH-B و ACV-A لها انخفاض معنوي ($p \leq 0.05$) في المجموعة الثانية ، ولكن ليس معنويًا ($p \geq 0.05$) في المجموعتين الثالثة والرابعة عند مقارنتها مع مجموعة السيطرة . بينما كانت هرمونات LH و FSH زيادة معنوية ($p < 0.05$) في المجموعتين الثانية والثالثة ، ولكنها ليست معنوية ($p \geq 0.05$)في المجموعة الرابعة بالمقارنة مع مجموعة السيطرة في فترتي الدراسة (28 و56 يوم).

اظهرت نتيجة متغيرات الكبد أن AST و ALT و ALP والألبومين والبيليروبين لديهم زيادة معنوية ($p \leq 0.05$) في المجموعتين الثانية والثالثة ، ولكن ليس معنويًا ($p \geq 0.05$) في المجموعة الرابعة عند مقارنتها مع مجموعة السيطرة في فترتي الدراسة 28 و 56 يومًا. بينما أظهرت النتائج زيادة معنوية ($p \leq 0.05$) في كل من اليوريا وحمض البوليك والكرياتينين في المجموعتين

الثانية والثالثة ولكن ليس معنويا ($p\geq0.05$) في المجموعة الرابعة بالمقارنة مع مجموعة السيطرة في فترتي الدراسة 28 و 56 يوما.

أظهرت الدراسة النسيجية انخفاضاً معنوياً في قطر الأنابيب المنوية وكذلك حدوث تآكل في أنسجة الخصية في الحيوانات المحقونة بالبوسولفان (العلاج الكيميائي) مقارنة بالحيوانات الضابطة. لوحظ أيضًا تغيرات في الكلى ، وهو انخفاض في قطر الكبيبات في الحيوانات المعالجة بالبوسلفان مقارنة بالحيوانات الضابطة. ولكن عندما يتم حقن الحيوانات بـالانزيم المساعد CO 10، نلاحظ تحسنًا في الأنسجة التالفة نتيجة تسمم العلاج الكيميائي.

جمهوريه العراقيه

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء /كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والادوية

تقييم دورأوبيكينون

(الإنزيم المساعد Q10) ضد التأثير السمي للBusulfan في ذكور الجرذان

رسالة مقدمة الى

مجلس كلية الطب البيطري جامعة كربلاء وهي جزء من متطلبات نيل-

درجة الماجستير في علوم الطب البيطري / الفسلجة والادوية والكيمياء الحياتية

من قبل

رفل محمد حسين محمد رضا

بكالوريوس طب وجراحة بيطرية/ كلية الطب البيطري/ جامعه كربلاء

2016-2015

باشراف

أ.م. صبا إبراهيم صالح

كليه الطب البيطري

جامعه كربلاء

1442 ه 2020م