Republic of Iraq
Ministry of Higher Education and
Scientific Research University of Babylon
College of Sciences for Women
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Effect of Berberine Supplement on Some Biochemical Parameters and The Histology of Some Reproductive Organs in Diabetic White Male Rats

A Thesis

Submitted to the Council of College of Science for Women-University of Babylon in partial Fulfilment of the Requirements for the Degree of Master of Science in Biology

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September 2023 A.D **Safar 1445 A.H**

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

وَلَوْلَا فَضْلُ ٱللَّهِ عَلَيْكَ وَرَحْمَتُهُۥ لَهَمَّت طَّآئِفَةٌ مِّنْهُمْ أَن
يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّآ أَنفُسَهُمْ ۖ وَمَا يَضُرُّونَكَ مِن
شَىْءٍ ۚ وَأَنزَلَ ٱللَّهُ عَلَيْكَ ٱلْكِتَٰبَ وَٱلْحِكْمَةَ وَعَلَّمَكَ مَا
لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ ٱللَّهِ عَلَيْكَ عَظِيمًا ﴿١١٣﴾

صدق الله العلي العظيم

سورة النساء

الآية 113

Certificate

I certify that this thesis entitled (**Effect of Berberine Supplement on Some Biochemical Parameters and The Histology of Some Reproductive Organs in Diabetic White Male Rats)** was prepared by **(Dalia Bahaa AL-Jebouri)** under my supervision at the Department of Biology / College of Sciences for Women / University of Babylon **,** as a partial requirement for the degree of master in Biology / Zoology.

Supervisor

Prof. Dr. Manar Mohammad Hasan AL-Murshidi

In view of the available recommendation, I forward this thesis for debate by the examining committee

Dr. Ekhlas AL-Shareefi

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Dedication

I dedicate my effort and my thesis in seeking knowledge to my dear father and dear mother, who used to show me with their prayers, which I feel is the reason for my support in life.

To my dear husband, who gave me the opportunity to complete my studies and create the right conditions for me.

To the beats of my heart, my dear daughter Fadak.

To my dear uncle, Dr. Karim, who helped me in my journey.

To my dear sisters.

To everyone who taught me a message.

To everyone who supported me even with a smile.

To everyone who made my way to knowledge easy for me.

Dalia Bahaa

Acknowledgment

Thanks, and blessing for Allah for what I am blessed with ,and prayers and peace be upon Muhammad the best of mankind, and his family.

My thanks, appreciation, and gratitude to my Supervisor **Prof. Dr. Manar Mohammad Hasan AL-Murshidi** for her support and assistance me to complete this work, words cannot express my gratitude to her .

I would like to express my thanks to the dean of the college **Dr. Abeer Fauzy Al- Rubaee** and the head of department of biology **Dr. Ekhlas Al-Shareefi** for giving me the opportunity to complete my study.

My thanks and appreciations to everyone who supported me and helped me to complete my research.

Dalia Bahaa

Summary:

The present study aimed to investigate the anti- diabetic role of berberine on some biochemical parameters and histology of some reproductive organs of male diabetic rats. The study had been conducted during the period from November 2022 to April 2023. This study had been conducted at the animal house of the College of Sciences / University of Babylon and AL- Hilla General Teaching Hospital. Thirty adult healthy male albino rats aged 10-12 weeks and weighted 230-320 gm. were used in the present study; they were equally randomly grouped into five groups as following: Groups I: The control group consists of 6 rats that received orally normal saline. Group II consists of 6 rats that received intraperitoneal injections of 60 mg\kg body weight of streptozotocine. Group III consists of 6 rats that received orally 200 mg\kg body weight of berberine. Group IV consists of 6 diabetic rats received 200 mg/kg body weight of berberine orally for 45 days. Group V: consists of 6 diabetic rats received 200 mg/kg body weight of berberine orally for 90 days.

After the end of experiment animals were sacrificed by blood and organs were extruded for physiological and histological investigations, The study included measurement of lipid profile and some hormones levels such as: Testosterone hormone (T), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), prolactin and Estradiol hormone (E) as well as effect of berberine on total antioxidants (T.A.O.), and histological changes in testes and epididymis.

Results showed a significant decrease in levels of Triglycerides, Total Cholesterol, Low Density Lipoproteins and a significant increase in High Density Lipoproteins levels in diabetic animals treated with berberine for 45 and 90 days comparing with diabetic groups .Results explained a significant increase ($p<0.05$) in serum total antioxidant levels in berberine treated positive control group rats compared with control, and a significant decrease ($p<0.05$) in

serum total antioxidant levels of diabetic rats. The diabetic rats treated with berberine were exhibited a significant increase in total antioxidant levels for period 45 days (311.6±2.2) and 90 days (320.1±4.7) respectively. Result related to bodies weight explained that diabetic group rats weights were significantly decreased in compare with normal records of controls and berberin group, while diabetic rats treated with berberin for 45 days and 90 days showed a re-establish in body weights. Results exhibited that there was a significant increase ($p<0.05$) in mean levels of serum fasting blood glucose in diabetic animals when compared with control groups animals, And a significant decrease ($p<0.05$) in fasting glucose levels in diabetic rats treated with berberine for 45 days (110±4.1 mg/dl) and 90 days (115± 2.8 mg/dl) respectively compared to diabetic rats , While it explained that the mean levels of insulin in serum of diabetes group rats were significantly decreased ($p<0.05$) compared with control group, While serum insulin levels in diabetes treated rats with berberine for 45 days (0.395±0.09 ng/dl) and 90 days (0.348± 0.4 ng/dl) showed a significant increase compared with that of diabetic group. It produced increase significantly difference ($p\leq0.05$) and ($p\leq0.01$) in both total antioxidants, and hormones level Testosterone hormone, Luteinizing hormone, Follicle Stimulating hormone and significant decrease in prolactin and estradiol hormones in berberine treated rats comparing with diabetic rats.

Histological results were elucidated that diabetes have many necrotic effects on testicular seminiferous tubules with relatively complete impaired spermatogenesis with interstitium fibrosis and vascular congestion with hemorhage and monocyte infilteration.; results that were gradually time dependent restored by berberine.

This study concluded that berberine had anti diabetic ameliorative effects on both physiological and histological studied parameters.

List of Contents

List of Tables

List of Figure

List of Abbreviations

Abbreviation	Full name
1n	Haploid
2n	Diploid
Ab	Antibody
ABP	Androgen-Binding Protein
ADP	Adenosine Diphosphate
AGEs	Advanced Glycation End Products
Akt	protein kinase B
AMP/ATP	Adenosine monophosphate/Adenosine triphosphate
AMPK	Adenosine Monophosphate-activated Protein Kinase
APDs	Action Potential Durations
apoB	Apolipoprotein B
ATP	Adenosine Triphosphate
b.w	body weight
BB	Berberine
BBR	Berberine
BMI	Body Mass Index
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
chol	Cholesterol
CYP1A2	Cytochrome P450 1A2
CYP2C19	Cytochrome P450 2C19
CYP2D6	Cytochrome P450 2D6
CYP2E1	Cytochrome P450 2E1
CYP3A4	Cytochrome P450 3A4
D.P.X.	Distrine Plasticizer Xylene
D.W	Distilled water
DM	Diabetes Mellitus

DNA	Deoxyribonucleic Acid
DQ2	DQ Antigen 2
DQ8	DQ Antigen 8
ED	Erectile Dysfunction
ELISA	Enzyme-Linked Immunosorbent Assay
ERs	Estrogen Receptors
FSH	Follicle-Stimulating Hormone
GAD	Glutamic Acid Decarboxylase
GAD65	Glutamic Acid Decarboxylase 65
GC	Herbal Antioxidant
GDM	Gestational Diabetes Mellitus
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GLUT4	Glucose Transporter Type 4
GnRH	Gonadotropin-Releasing Hormone
GSIS	Glucose-stimulated insulin secretion
H&E	Hematoxylin and Eosin
H2O2	Hydrogen Peroxide
HbA1c	Hemoglobin A1c
HBR	Hydrobromic Acid
HCL	Hydrochloric Acid
HDL	High Density Lipoproteins
HIRD	High Immunoreactivity Dextran
HLA	Human Leukocyte Antigen
HRP	Horseradish Peroxidase
IA-2	Islet Antigen-2
IR	Insulin Resistance
JAK2	Janus Kinase 2

JNK	c-Jun N-terminal kinases
K ATP	ATP-sensitive potassium channels
K+	Potassium ion
KATP	ATP-sensitive potassium channel
KCNH6	Potassium voltage-gated channel subfamily H member 6
Kv	Potassium voltage-gated channel
LADA	Latent Autoimmune Diabetes in Adults
LDL	Low Density Lipoproteins
LH	Luteinizing Hormone
LPL	Lipoprotein lipase
MAPK	Mitogen Activated Protein Kinase
MODY	Maturity-onset diabetes of the young
Nc	Neocuproine
NFκB	Nuclear Factor kappa B
NH4 Ac	Ammonium Acetate
NSB	Non-Specific Binding
O2	Oxygen
OD	Optical Density
OGTT	Oral Glucose Tolerance Test
PCSK9	Proprotein convertase subtilisin/kexin type 9
PEG	Polyethylene Glycol
RBP4	Retinol Binding Protein 4
ROS	Reactive Oxygen Species
RPM	Round Per Minute
SPSS	Statistical Package for the Social Sciences
STsD	Seminiferous Tubules Diameter
STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus

T2DM	Type 2 Diabetes Mellitus
TAC	Total Antioxidant Capacity
TCF7L2	Transcription Factor 7-Like 2
TDM	Types of Diabetes Mellitus
TG	Triglyceride
TMB	Tetramethylbenzidine
TST	Testosterone Replacement Therapy
UGT1A1	UDP-glucuronosyltransferase 1A1
UGT2B1	UDP-glucuronosyltransferase 2B1
VDCCs	Voltage-dependent calcium channels
VGCCs	Voltage-gated Calcium Channels
YY	Peptide YY
ZnT8	Zinc Transporter 8

Chapter One
Introduction

1.1 Introduction

Berberine, a quaternary benzylisoquinoline alkaloid, is found in the plant families Berberidaceae, Papaveraceae, Ranunculaceae, and Rutaceae, among others. The most extensively dispersed sources of berberine are numerous members of the genus Berberis (Singh & Katare, 2020). Berberine-containing plants have been employed in Ayurveda, Chinese, Middle-Eastern, and other traditional remedies to cure a variety of illnesses, including parasite diseases, dyspepsia, jaundice, and bacterial, fungal, and viral infections (Neag *et al*., 2018).

Currently, berberine is one of the most extensively investigated plant chemicals. It has been linked to a variety of activities based on well-known molecular pathways (Sun *et al*., 2009). Due to its hypoglycemic, lipid-lowering, and anti-obesity effects, berberine has been suggested to be helpful in the treatment of diabetes and accompanying metabolic disorders (Imanshahidi & Hosseinzadeh, 2008).

Diabetes mellitus (DM), one of the world's most prevalent chronic diseases, is increasing in prevalence each year. Estimates suggest that by 2040 there will be 642 million persons with DM in the world (Lovic *et al*., 2020). The most common subtypes of the general diagnosis diabetes are type 1 diabetes, type 2 diabetes, gestational diabetes, and diabetes from other causes such as Monogenic diabetes or medications Moody. These account for 90–95% and 5–10%, respectively, of all cases. Hyperglycemia in type 2 diabetes can result from a variety of processes (Kumar *et al*., 2020b).

In addition to diabetes, berberine has been suggested to be beneficial in treating liver diseases, cancer, pain, neurodegenerative diseases, and mood disorders. In experimental settings, berberine has also been shown to have positive effects on musculoskeletal illnesses such osteoporosis, osteoarthritis, rheumatoid arthritis, and promotes bone

regeneration. The benefits of berberine in these conditions are influenced by its antioxidant and anti-inflammatory properties (Shayganfard, 2023).

Berberine, has shown promising protective effects on the male reproductive function, specifically on spermatogenesis in diabetic rats. Studies have revealed that administration of BB improved sperm motility and morphology, and increased the fertility of diabetic rats. This was achieved through the antioxidant effect of BB, which reduced oxidative stress in the testis tissue and inhibited the ROS/JAK2/NFκB pathway, thus decreasing cell injury and apoptosis. The results suggest that BB might play a therapeutic role in diabetic complications linked to oxidative stress, including impaired reproductive function. The translation of these findings to humans proposes potential benefits for diabetic patients with early-onset age and reproductive damage (Song *et al*., 2020b).

1.2 Aims of study

By examining the fluid parameters, the current study established to determine Berberine effects on diabetic abnormalities and the complications in rat reproductive system :

1- Body weights and some biochemical parameters, such as the Fasting glucose level, Lipid profile, Insulin level, Some reproductive hormones levels and Total Antioxidants.
2- Histological investigation of some reproductive organs, including the testis and epididymis

Chapter Two

Literatures Reviews

2. Literatures Reviews

2.1 Diabetes: Definition and diagnosis

Diabetes refers to a class of metabolic illnesses that are distinguished from one another by the presence of hyperglycemia in the absence of medical intervention. The diverse etiopathology comprises disorders in the metabolism of carbohydrates, fats, and proteins as well as problems in insulin secretion, insulin action, or both (Leslie *et al*., 2016). Among other issues, retinopathy, nephropathy, and neuropathy are among the long-term particular effects of diabetes. According to (Zimmet *et al*., 2001), people with diabetes are more likely to develop additional illnesses such as non-alcoholic fatty liver disease, cataracts, erectile dysfunction, peripheral artery and cerebrovascular disease, heart disease, and obesity**.** They also have a higher chance of contracting some infectious diseases, such tuberculosis. Diabetes might manifest with typical symptoms such thirst, polyuria, visual impairment, and weight loss, there are also frequently genital yeast infections (Gianani *et al*., 2010). Ketoacidosis or a non-ketotic hyperosmolar condition, which can result in dehydration, a coma, and, in the absence of adequate treatment, death, are the most serious clinical signs. However, due to the gradual rate at which the hyperglycaemia is increasing, symptoms in T2DM are frequently not severe or may even be absent. In the absence of biochemical testing, hyperglycemia that is adequate to cause pathological and functional alterations may therefore exist for a long period before a diagnosis is made, leading to the presence of problems upon diagnosis (Tuomi *et al*., 2014). According to estimates by Zimmet *et al*., 2001, a sizable portion of diabetes cases between 30 and 80%, depending on the nation go undetected. At the moment, measuring fasting plasma glucose, two hour post-load plasma glucose following a 75 g oral glucose tolerance test

(OGTT), HbA1c, and a random blood glucose are the four diagnostic procedures that are advised for diabetes. People are deemed to have diabetes if their fasting plasma glucose levels are 7.0 mmol/L (126 mg/dl) or higher, their two hours post-load plasma glucose levels are 11.1 mmol/L (200 mg/dl), their HbA1c is 6.5% (48 mmol/mol), or their random blood glucose levels are 11.1 mmol/L (200 mg/dl) or higher (Perry *et al*., 2001).

Repeat testing, ideally with the same test, is advised as soon as is practical on the following day to confirm the diagnosis if elevated results are found in asymptomatic individuals. The potential stigma that a diabetes diagnosis may bring may affect an individual's employment, health and life insurance, ability to drive, social opportunities, as well as other cultural, ethical, and human rights repercussions. Diagnosing diabetes has serious implications for an individual's health (Schabert *et al*., 2013).

2.2 Types of Diabetes Mellitus

2.2.1 Type 1 Diabetes Mellitus (T1DM)

Data on global trends in T1DM prevalence and incidence are not yet available, although data from many high-income nations show that the incidence of T1DM in children has increased by 3% to 4% year (Bellatorre *et al*., 2017).Equally affected are men and women (Patterson *et al*., 2009). Despite the fact that T1DM frequently affects children, it can also develop in adults, and 84% of T1DM sufferers are adults. T1DM reduces life expectancy in high-income countries by roughly 13 years. In nations with insufficient access to insulin, the outlook is far worse. It can be difficult to distinguish between T1DM and T2DM in adults, and mistaking T1DM for T2DM or vice versa could have an effect on

estimates of prevalence and incidence (Atkinson *et al*., 2014).According to a study, 42% of T1DM occurrences arose after the age of 30, and 4% of all cases of diabetes diagnosed between the ages of 31 and 60 years happened among people of European heritage who participated in the UK's Biobank research project. These people had lower body mass indices, used insulin within a year of diagnosis, and had a higher risk of diabetic ketoacidosis as clinical characteristics (Thomas *et al*., 2018). Although it can happen in adults, the fast advancing variant of T1DM is frequently seen in youngsters. Ketoacidosis may be the first sign of the illness in certain patients, especially kids and teenagers (Atkinson *et al*., 2014). Others may have mild hyperglycemia that, in the presence of an infection or another stressor, can quickly worsen to severe hyperglycemia and/or ketoacidosis. Others, especially adults, may still have some -cell function, which is sufficient to fend off ketoacidosis for a very long time. Low or undetectable levels of C-peptide in blood or urine are indicators that there is little to no insulin secretion at the time of the classic clinical presentation with T1DM (Madsbad *et al*., 1980).

Obesity is increasingly prevalent in the general population and this trend is also reflected among patients with Type 1 Diabetes Mellitus (T1DM). It has been observed that between 70% and 90% of T1DM patients develop autoantibodies against glutamic acid decarboxylase (GAD65), islet antigen-2 (IA-2), ZnT8 transporter, or insulin at the time of diagnosis. These developments indicate connections with genes that regulate immune responses. In populations of European heritage, the most frequent genetic correlations are with HLA DQ8 and DQ2 (Eisenbarth, 2007).

Patients who do not exhibit immunological characteristics may suffer from monogenic forms of diabetes, though the exact cause remains unclear. Previously, the classifications Type 1A (autoimmune) and Type 1B (non-immune) were used to describe these two categories of T1DM. However, these terms are no longer widely used or clinically useful (Robertson & Rich, 2018).

An acute onset variant of T1DM in adults, known as fulminant T1DM, is most commonly found in East Asia. It accounts for 7% of acute-onset T1DM in Korea and 20% of acute-onset T1DM in Japan. Despite being rare in those of European descent, it is quite common in China. The primary clinical features of fulminant type 1 diabetes include sudden onset, hyperglycaemic symptoms that typically last less than a week, negligible C-peptide secretion at diagnosis, ketoacidosis, predominantly negative islet-related autoantibodies, increased serum pancreatic enzyme levels, and frequent flu-like and gastrointestinal symptoms preceding disease onset (Imagawa & Hanafusa, 2011).

The rapid death of beta -cells and a heightened immune response to virus-infected islet cells are suggested by cellular infiltration of macrophages and T cells into islets. Measuring islet autoantibodies continues to assist research on the etiology and pathophysiology of T1DM. Despite its limited practical application, testing for islet autoantibodies can be helpful when differentiating between T1DM or Type 2 Diabetes Mellitus (T2DM). However, clinical necessity should take precedence over the presence of such markers when deciding whether or not to administer insulin (Ilonen *et al*., 2019).

2.2.2 Type 2 Diabetes Mellitus

Between 90% and 95% of diabetes cases are caused by T2DM, with the highest prevalence rates occurring in low- and middle-income nations. It is a widespread and critical worldwide health issue that has arisen as a result of quick cultural, economic, and social change, aging populations, and unplanned and growing urbanization (Liu *et al*., 2022). increased consumption of sugar-sweetened beverages and highly processed foods, obesity, decreased physical activity, bad lifestyle and behavioural habits, fetal malnutrition, and increased fetal exposure to hyperglycemia during pregnancy are only a few examples of dietary alterations. The majority of people with T2DM are adults, but a growing number of kids and teenagers are also impacted since T2DM requires dysfunctional cells. Many people with T2DM have relative insulin shortage, and early on in the disease absolute insulin levels rise due to resistance to insulin action (Kahn *et al*., 2014).The majority of T2DM sufferers are overweight or obese, which either contributes to or worsens insulin resistance. Many patients who meet BMI guidelines for obesity have a larger percentage of body fat that is primarily distributed in the abdominal area, indicating visceral adiposity, compared to those who do not have diabetes. However, B-cell dysfunction seems to be more pronounced in some populations than in populations of European ancestry, such as Asians. This is seen in persons of Indian descent living in high-income nations as well as among thinner individuals from low- and middle-income countries like India (Gujral *et al*., 2013).The majority of T2DM patients do not need insulin therapy for survival, although it may be necessary to lower blood sugar levels in order to prevent chronic problems. T2DM frequently goes untreated for a long time because the hyperglycemia is not severe enough to elicit obvious diabetic symptoms. In spite of this, these individuals are more likely to experience macrovascular and microvascular problems

(Yoon *et al*., 2006). Young-onset T2DM is widely recognized as a severe phenotype of diabetes and associated with higher mortality rates, more complications, and unfavorable cardiovascular disease risk factors when compared to T1DM of similar length. Complications are a particular problem in this population. Additionally, young people with diabetes frequently respond poorly to oral blood glucose medicines (Constantino *et al*., 2013). Age, obesity, bad lifestyle choices, and previous gestational diabetes (GDM) are just a few of the many variables that raise the chance of developing T2DM. T2DM is more common in some racial and ethnic subgroups than others, particularly in young and middle-aged persons. There are some communities that are more likely to develop type 2 diabetes than others, such as Native Americans, people from Pacific Island countries, and people from the Middle East and South Asia. It is frequently accompanied by a strong familial propensity that is probably genetic or epigenetic (Stumvoll *et al*., 2005). Although studies indicate that some common genetic variants of T2DM occur among various ethnic groups and populations, the genetics of T2DM are complex and poorly understood. Ketoacidosis is uncommon in T2DM, but when it does occur, it typically does so in conjunction with the stress of another condition, like an infection. Elderly individuals are more likely to get hyperosmolar coma (Pasquel & Umpierrez, 2014).

2.2.3 Hybrid forms of Diabetes

In an effort to separate T1DM from T2DM in adults, new disease categories and nomenclatures have been proposed, such as slowly developing immune-mediated diabetes and T2DM that is prone to ketosis (Atkinson *et al*., 2014). Diabetes with immune-mediated deterioration Long-standing evidence of pancreatic autoantibodies that can react with non-specific cytoplasmic antigens in islet cells, glutamic acid

decarboxylase (GAD), protein tyrosine phosphatase IA-2, insulin, or ZnT8 has been found in adults who initially present clinically with what is initially believed to be T2DM (Kawasaki, 2023). Latent autoimmune diabetes in adults (LADA) is a term that has been used frequently to describe this kind of diabetes. The label "latent" was chosen to distinguish these slow-onset cases from traditional adult T1DM. But this name's suitability has been questioned (World Health Organization, 2019).

This population is initially managed with dietary changes and oral medications; nevertheless, they proceed to needing insulin therapy more quickly than persons with conventional T2DM. This kind of diabetes is more prevalent than traditional, rapid-onset T1DM in several parts of the world. Latent autoimmune diabetes in youth lady is the name given to a related subtype that has been observed in children and teenagers with clinical T2DM and pancreatic autoantibodies (Pan *et al*., 2022). Although there are no accepted criteria for this subtype of diabetes, three factors are frequently used: positivity for GAD autoantibodies, age greater than 35 at diagnosis, and no requirement for insulin therapy in the first 6–12 months following diagnosis. There are regional and ethnic differences in the incidence of autoantibodies to GAD among people with clinically diagnosed T2DM, with autoantibodies being present in 5–14% of cases in Europe, North America, and Asia, with some variation by ethnicity and younger age at diagnosis. 90% of those with autoantibodies in this group had antibodies to GAD, while 18–24% have antibodies to ZnT8 or protein tyrosine phosphatase IA-2. persons with apparent T2DM still had GAD autoantibodies, and one study found that 41% of those persons underwent seroconversion to autoantibody-negative status during the

course of a 10-year follow-up. GAD autoantibodies, even in T1DM, can still be found 10 years after diagnosis (Klingensmith *et al*., 2010).

There has been much debate about whether slowly developing immune-mediated diabetes is a distinct clinical category or merely a step in the development of T1DM. Some have claimed that there is insufficient evidence to support the designation of this as a unique subtype, that methodological issues plague the epidemiology, and that there is no clinical benefit to diagnosing it. Others have suggested for a new definition that incorporates the dual elements of beta-cell autoimmunity and insulin resistance. Obesity, elements of the metabolic syndrome, maintaining greater -cell function, expressing a single autoantibody particularly GAD65, and having the transcription factor 7-like 2 (TCF7L2) gene polymorphism are relative differences between slowly evolving immune-mediated diabetes and T1DM (Cervin *et al*., 2008) .

Type 2 diabetes predisposed to ketosis A new clinical entity known as a ketosis-prone form of diabetes that was first discovered in young African-Americans has evolved during the past 15 years. This subtype has varyingly been labelled as a T1DM or T2DM variation. Some have proposed reclassifying individuals diagnosed with idiopathic or type 1B diabetes as having ketosis.-prone diabetes type 2 Unusual non-immune ketosis-prone type 2 diabetes was first identified in young African-Americans. Subsequently, groups in sub-Saharan Africa were shown to have similar characteristics. Usually, those who are affected show signs of severe insulin shortage and ketosis, but they later enter remission and don't need insulin therapy.90% of these individuals, according to reports, had additional ketotic episodes within ten years. Obese men appear to be

the most vulnerable to this type of diabetes in high-income countries, although a similar (Mauvais-Jarvis *et al*., 2004).

2.3 Berberine

2.3.1 Definition of Berberine

The primary active ingredient of the traditional Chinese herb *Coptis chinensis* is berberine (BBR) (Figure). This herb has been used to treat diabetes in traditional Chinese medicine for countless years. Both animal studies and human clinical trials have supported the glucose-lowering effect of BBR. However, the specific mechanisms underlying BBR's anti-diabetic impact, particularly if it encourages insulin secretion, are still poorly known, which restricts the use of berberine (BBR) in clinical settings (Newman & Cragg, 2016).

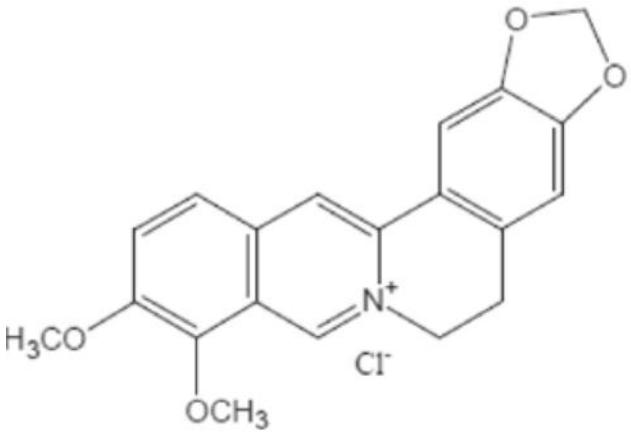

Figure 2-1: Chemical structure of berberine (Hao *et al*., 2022)

2.3.2 Medicinal uses of Berberine

Berberine-containing plants have been used in Ayurvedic, Chinese, Middle-Eastern, and other traditional medicines for the treatment of a variety of illnesses, including parasitic diseases, indigestion, jaundice, and bacterial, fungal, and viral infections (Neag *et al*., 2018). Currently,

berberine is one of the most extensively investigated plant chemicals. Based on well-known molecular pathways, berberine has been linked to a variety of activities. Due to its hypoglycaemic, lipid-lowering, and anti-obesity actions, berberine has been hypothesized to be helpful in the treatment of type 2 diabetes and concomitant metabolic diseases. diseases of the heart and arteries, the liver, cancer, and pain, as well as neurodegenerative illnesses and mood disorders (Fan *et al*., 2019; Och *et al*., 2022). In experimental settings, berberine has also been shown to have positive effects on musculoskeletal illnesses like osteoporosis, osteoarthritis, and rheumatoid arthritis, as well as to speed up bone regeneration. Berberine's effects on the aforementioned illnesses are influenced by its antioxidant and anti-inflammatory properties (Li *et al*., 2014; Zhang *et al*., 2021). Berberine has been demonstrated to positively affect the skeletal system in experimental circumstances, in vitro, and in vivo, through a variety of pathways of antidiabetic activity. It promoted osteogenic differentiation and bone production in vitro while inhibiting osteoclastogenesis and bone resorption. Glucocorticoid-induced osteoporosis in rats, estrogen deficiency-induced osteoporosis in mice and rats, and senile osteoporosis in mice were all prevented in vivo by berberine (Song *et al*., 2020a).

A number of plant species, including *Argemone mexicana*, *Coptis chinensis*, *Berberis vulgaris*, *Berberis aristata*, *Xanthorhiza simplicissima*, *Tinospora cordifolia*, *Berberis aristata*, and *Eschscholzia californica*, among others, can provide berberine, a yellow-colored bioactive chemical. Its principal constituents are basic nitrogen compounds, making it a member of the alkaloid class of chemicals. Berberine has the chemical formula $C_{20}H_{18}NO_4$ and a molecular weight of 336.337g/mol (Cicero & Baggioni, 2016). Alkaloid-containing plants

have been utilized for ages as both therapeutic and fatal medications. It is present in the plant's roots, rhizomes, stems, and bark (Rajaian *et al*., 2006). Young, actively growing shoots have 0.04% berberine, while young, parenchymatous roots contain 1.41% berberine (Srivastava *et al*., 2015). It has a lengthy history of usage in conventional Chinese and Indian medicine, where it was applied to treat a wide range of illnesses. It now provides impressive benefits for a number of various health issues, according to modern science. The majority of alkaloids are typically alkaline and colourless, while berberine is an acidic compound distinguished by its vivid yellow colour. It has a long history of use as a yellow dye in several nations. It has a variety of physiological effects, including as anti-inflammatory, anti-tumour, and broad spectrum antibacterial activity against bacteria, fungi, viruses, and parasites (Preeti *et al*., 2015). The likelihood of contracting the flu or a viral cold as a result of bacterial infection will be decreased because it is potent enough to target antibiotic resistance and have long-term health advantages. Adenosine monophosphate activated Protein Kinase (AMPK) is an enzyme found inside the human body that plays a critical function in regulating metabolism. AMPK activation increases fat burning in the mitochondria. Berberine is one of the few substances known to activate AMPK. Due to AMPK activation, it is normoglycemic and helps with tissue repair, keeping a healthy cardiac rhythm, and controlling lipid and blood sugar levels (Bagade *et al*., 2017).

According to (Sun *et al*., 2009), berberine inhibits the growth and reproduction of some tumor-causing bacteria and viruses, including the helicobacter pylori and hepatitis B viruses. A study suggest that berberine functions by preventing the activity of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9). More LDL is subsequently eliminated

from the bloodstream as a result (Cameron *et al*., 2008). It raises energy expenditure, reduces weight gain, boosts brown adipose tissue activity, and improves cold tolerance. It has been demonstrated that it lowers blood pressure by competitively inhibiting vascular smooth muscle cells' receptors and preventing the enzyme adenylyl cyclase from being released. It encourages the best possible joint health; berberine was found to dramatically improve joint health in rats, By defending mucous membranes all over the body, berberine enhances immunological and digestive health and can reverse severe damage such that brought on by binge drinking (Gong *et al*., 2017; Li *et al*., 2022). Regular berberine consumption increased bone mineral density in both male and female mice, further promoting high bone mineral density (Chen *et al*., 2021). The regular consumption of berberine will increase brain chemicals like dopamine, serotonin, and norepinephrine, all of which are essential for healthy brain functioning and maintaining a positive mood (Kulkarni & Dhir, 2008), according to several studies that have suggested that berberine may help to promote optimal mental health as we age. Berberine is more effective than metformin at achieving more live births with fewer side effects in women with Polycystic Ovary Syndrome (PCOS) who are undergoing in vitro fertilization (An *et al*., 2014). Through its anti-inflammatory properties, berberine pre-treatment greatly lessens the impact of cigarette smoke-induced acute lung inflammation (Lin *et al*., 2013).

2.3.3 Berberine and importance in Diabetes

Glucose-Stimulated Insulin Secretion (GSIS) from pancreatic islet cells is controlled by the electrogenic pathway connected to ATP sensitive K+ (K ATP) channels when berberine is present. Increased intracellular ATP/ADP ratio brought on by high glucose metabolism

results in KATP channel closure, plasma membrane depolarization, calcium influx through voltage-dependent calcium channels (VDCCs), and exocytosis of insulin secretory granules. Sulfonylureas and glinides, two types of insulin secretagogues, have been known to target KATP channels. These medications have been the primary oral anti-diabetic medications used to encourage insulin secretion in patients for the past 50 years (Zhao *et al*., 2021). These substances can produce life-threatening hypoglycemia by directly blocking K ATP channels and increasing insulin secretion in a way that is not dependent on high glucose levels (Amiel *et al*., 2019) . Ion channels may be one such pathway, as in the case of sulfonylureas and glinides, and the finding of disease-associated genetic variants in humans might lead to the discovery of novel biology that may be therapeutic targets. By analysing a large four-generation family with monogenic diabetes, we recently discovered that the voltage-dependent K+ (Kv) channel KCNH6 plays a crucial role in insulin secretion. GSIS from pancreatic islet cells is influenced by high-glucose-dependent repolarization brought on by Kv channels like KCNH6. Indicates that BBR is a glucose-dependent insulin secretagogue for the treatment of diabetes that does not result in hypoglycemia because it blocks KCNH6 channels in a high-glucose-dependent manner or only under hyperglycemic settings (Herrington *et al*., 2006).

The KCNH6 protein and KCNH6 Expedited Channel Closure are linked to BBR. Kv currents leaving through KCNH6 channels are delayed since it was later outlawed after increased glucose, pancreatic islet beta cells repolarize Action potential stimulation, which causes extended APDs. Ca2+ influx increased during gluCob stimulation as a result of forefront-Long APDs (Lu *et al*., 2020). Theoretical explanations for how BBR boosts insulin secretion. (1) By directly binding to the KCNH6

channel and accelerating channel closure, BBR blocks outward Kv channel currents and prolongs APDs in pancreatic islet β-cells. (2) The prolongation of APDs results in an increase in Ca2+ influx through voltage-gated calcium channels (VGCCs), (3) resulting in the accumulation of intracellular Ca2+. (4) Increased intracellular Ca2+ concentrations trigger more insulin secretion. Therefore, BBR may have potential as a new drug treatment for diabetes (Zhao *et al*., 2021).

2.3.4 Chemical properties traditional uses of Berberine

The *berberis* species has a 3000 year history, and formulations based on berberine are frequently utilized in traditional medical systems including Ayurveda and Traditional Chinese medicine (Neag *et al*., 2018). The Berberidaceae family, which includes 12 genera and 600 species globally, includes 77 species that have been identified in India. This family is the primary natural source of berberine (Chander *et al*., 2017). Plants that contain berberine are used to treat a variety of diseases, including inflammation, diabetes, infectious disease, and constipation. The barberry fruit was employed as a blood purifier according to the earliest available data. Plants that contain berberine are used in Ayurveda to treat infections of the eye, ear, quick-healing wounds, antidotes, scorpion stings, and snake bites. Decoctions of Indian barberry and *Emblic myrobalan* combined with honey have been used to treat urinary issues such painful micturition, and they have also been used to treat jaundice. Rasont is a decoction made from the roots, stems, and bark of Indian berberis species. It has traditionally been used to treat skin conditions including ulcers as well as ophthalmic diseases, jaundice, and enlarged liver and spleen (Neag *et al*., 2018).

Berberine's oral bioavailability is incredibly low. From the liver and gut, berberine appears to be vulnerable to P-Glycoprotein-mediated

efflux. P-Glycoprotein in the intestine reduces berberine transportation by 90% and causes berberine to be ejected back into the bowel. However, when combined with P-Glycoprotein inhibitors such cyclosporine A or verapamil, absorption is significantly boosted (Chae *et al*., 2008). Animal research revealed that berberine had a high tissue dispersion following oral administration. There is no human investigation on the distribution of berberine, despite the fact that it is rapidly distributed in the liver, kidney, heart, brain, lungs, muscle, pancreas, and fats (Imenshahidi & Hosseinzadeh, 2019).

The liver is the primary site of berberine metabolism in both rats and humans. CYP2D6 is the human cytochrome P450 that is most important for manufacturing berberine's metabolites, followed by CYP1A2, 3A4, 2E1, and CYP2C19. Due to its involvement in the production of 9% of the metabolite berberrubine (M1) and 8% of thalifendine (M2), CYP2D6 plays a significant role in the metabolism of berberine. When administering berberine, CYP2D6 pharmacogenetics and medication interactions should be taken into account. Phase I demethylation produced phase II metabolites from the conjugation of M1 and M2 berberine metabolites with glucuronic and sulfuric acids. Both UGT2B1 and UGT1A1 are capable of glucuronidating M1 and M2. These glucuronide metabolites are polar and conveniently eliminated (Liu *et al*., 2019).

There is no human research on berberine excretion. Total berberine recovery rate in a rat animal investigation was 22.83%. The main way that berberine is eliminated is through the feces, whereas its main metabolites, such as thalifendine (M1) and berberrubine (M2), are largely eliminated by the bile and urine (Chaudhary & Mittal, 2022; Sun *et al*., 2009).

2.3.5 Berberine mechanism of action

The metabolic syndrome is defined as a set of pathogenic factors including central obesity, insulin resistance, and glucose intolerance. Today, a growing number of younger patients are being affected by the battle against obesity and insulin resistance. It has also emerged as a crucial issue in the struggle against infertility. The berberine T2DM's anti-diabetic effects were originally noted in 1986 (Wang *et al*., 2018). The most well researched possible medicinal use is its antidiabetic action Berberine (Wang *et al*., 2014; Wu *et al*., 2011). Although it has positive impacts and a good security profile, it is unfortunately weak. Its clinical applicability is still constrained by bioavailability. According to earlier studies, berberine reduces alanine and aspartate transaminase levels in T2DM patients as well as insulin resistance and insulin resistance (Och *et al*., 2022). Furthermore, it was demonstrated that berberine decreased insulin resistance while favorably contributing to higher levels of fasting and post-prandial blood glucose and glycosylated hemoglobin. By boosting the activity of glucokinases, enhancing insulin secretion, and preventing gluconeogenesis and adipogenesis in the liver, berberine increases glycolysis. It promotes the translocation of the glucose-4 transporter into the plasma and improves insulin sensitivity in people with insulin resistance by activating 5-adenosine monophosphate kinase (AMPK) .

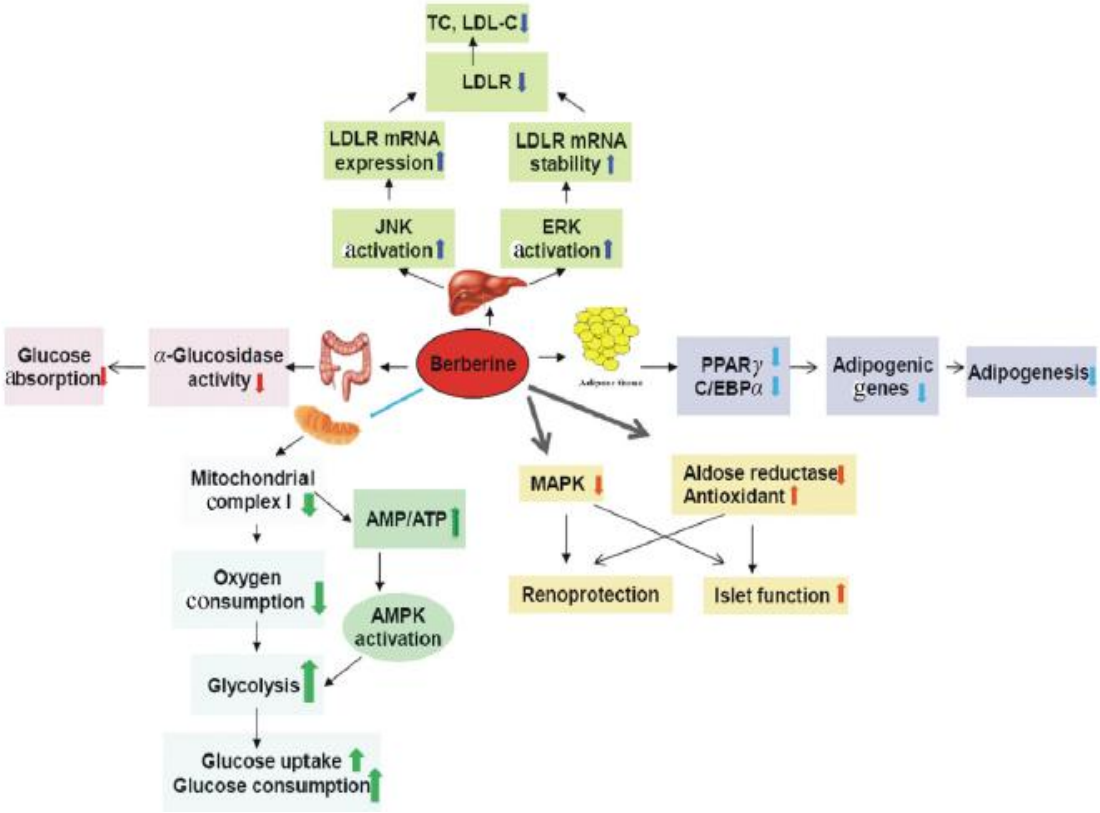

Figure 2-2: Mechanism of berberine in regulation of metabolism (An *et al*., 2014; Wang *et al*., 2018; Yin *et al*., 2012)

It has also been shown that berberine enhances Akt phosphorylation and hence activates Akt, namely through the activation of AMPK, in people with insulin resistance, where the signaling pathway of protein kinase B (Akt) is disrupted. Activation of the AMPK pathway is the most plausible of the several routes by which berberine modulates glucose absorption, according to research reports. Additionally, berberine increases the expression of the AMPK-dependent adipose tissue triglyceride lipase, which is one of the mechanisms of action in the prevention of obesity and is positively associated with long-term weight loss (Wang *et al*., 2018).

Additionally, Using the active berberine has been demonstrated to boost insulin production induced by glucose. The substance raises the level of glucagon-like peptide-1 (GLP-1), which has been demonstrated

to boost insulin secretion in islet cells both in vitro and in vivo (Yu *et al*., 2010). Berberine may lessen glucose transport across the intestinal epithelium because it inhibits glycosidase. As a result, it might have antihyperglycemic effect. After oral administration, it is nonetheless poorly absorbed, and only nanomolar plasma concentrations may be obtained in both humans and animals (Ye *et al*., 2010).

It is contrasted with metformin because of its great therapeutic potential. Berberine also has some advantages in its use, including as in individuals who cannot tolerate metformin therapy, due to its high efficacy and safety profile. Berberine not only seems to regulate blood glucose much more well than metformin, but it also outperforms the advantages of rosiglitazone by raising fasting blood glucose levels. Although more research is required to determine whether berberine is effective when given to T2DM patients, there are promising results when oral hypoglycemic drugs are combined with berberine (Wei *et al*., 2015).

Berberine regulates a number of effectors, including mitogen-activated protein kinase, similarly to metformin (Li *et al*., 2020). In-depth research is now being done on the mechanism of berberine's effectiveness in cases of obesity, particularly those with T2DM. In obese women with polycystic ovarian syndrome, the effect of berberine on insulin resistance was clinically assessed, and improved insulin resistance was found (Zhang *et al*., 2016). The improvement of lipid parameters (total cholesterol, LDL, TG, the cholesterol/HDL ratio, and the TG/HDL ratio), weight loss, and fat mass loss were seen in obese patients with cardiometabolic syndrome risk factors (Kumar *et al*., 2020a). According to Bogoyevitch, excitotoxic neurotransmitters and c-Jun N-terminal kinases (JNK) have a role in the development of insulin resistance in obese people under ischemic conditions. Berberine influences the lipid

metabolic pathways controlled by those kinases and also acts as a neuroprotectant (Bogoyevitch *et al*., 2004).

2.4 Anatomy and histology of male rat reproductive system

Male rats' reproductive system consists of two gonads, the testes, which are mixed glands distinguished by their two primary roles. In this case, the testicle is an exocrine gland, so the first function is the production of sperm from seminiferous tubules. The second function is the production of steroid hormones from the interstitial cells of the testicle, known as leydig cells, which are situated between the tubules that make the sperm. These cells are under the hormonal control of the pituitary gland. The testicle is an endocrine gland since it secretes testosterone, a vital and significant androgen in males. Each testicle has an epididymis border and two extremities: a head extremity that connects to the head of the epididymis and a tail extremity that matches the tail of the epididymis. The epididymis sinus and free border, which make up the epididymis body, are weakly attached to this edge. It is situated on the side of the epididymis border that is opposing, and it is often convex (Mawhinney & Mariotti, 2013).

The testes have two surfaces: a lateral convex surface and a medial almost concave one. Depending on the kind of animal, the testicles might vary in size, weight, and shape. Rodents have enormous testes that are out of proportion to their bodies (Bauer, 2008).

2.4.1 Testes

This mixed glandular installation has an oval form and secretes both internally and externally. Due to the increased blood flow, the left testis is bigger and more functional. It is situated beneath the right testicle. During embryonic development, the testicle grows in the abdominal cavity before descending into the scrotal sac before or after birth. It is a dermal cyst that

causes an external skin dent and is fashioned like an exterior pocket of the body wall. The superficial connective tissue known as the superficial Raphe divides the cyst into two sections. The role of the scrotum is to keep the testicles at a temperature one to one and a half degrees lower than body temperature (34-35 C°). The scrotum is made up of many smooth muscle layers that shrink in the cold and flatten in the heat. The process of sperm production in the testicles cannot function for an extended period of time at body temperature, and the unique muscles that extend between the body and the scrotum sac keep the temperature of the scrotum low (Major *et al*., 2021).

The testicle is surrounded by a serous membrane on all of its front and lateral surfaces, with the exception of the posterior surface known as Tunica Vaginalis, which contracts as the body's temperature drops. Under this tunic, the testicle is covered by a sheath or capsule of dense connective tissue that includes an inner visceral layer and an outside partital layer. A layer of loose connective tissue rich in blood vessels is formed inside the testicles by certain smooth muscle fibers known as the tunica albuginea, which thickens the posterior surface of the testicles, the structure that lies under the tunica albuginea is called Tunica Vasculosa and from the thickened posterior part of the tunica albuginea , fibrous septa extend inside the testicle dividing to (250-300) cubby or pyramidal lounge, which are called testicular lobules , it contains each lobule of (2-5) many of tubules twisted to each other abundantly are called seminiferous tubules (Maina *et al*., 2008).

2.4.1.1 Histological Structure of the testis

The testes Consists of two main parts : -

1. Seminiferous tubules.

2. Interstitial tissue

first : seminiferous tubules :

The Seminal ducts, which have a diameter of 0.2 mm and a length of 30 to 70 cm, are situated between the lobes. Each tubule loses torsion at the apex of the lobule and turns into a straight tubule. These tubules are dispersed throughout the stromal, or primary connective tissue, which is dense with blood vessels and nerves. The spermatic tube either has a blocked end or is mouthful with a neighbouring tubule. Each collecting tube's end opens into the testis Rete, which is adjacent to the epididymis's head (Razi *et al*., 2010).

Seminiferous epithelium, also known as germinal epithelium, lines the seminal tubule. This stratified epithelial tissue is made up of cubic or columnar epithelial cells, and it rests on a thin basal plate that is wrapped in determinant tissue on the outside. It is packed full of smooth muscle fibers , connective tissue cells, and fibers. Convoluted tubules are thought to vary in diameter as a result of smooth muscle cell contraction, which aids in the migration of sperm through the tubule. About 75% of the testes' volume is made up of convoluted tubules, which are internally lined by a stratified epithelium made of two different cell types (Fietz & Bergmann, 2017).

* **Sertoli Cells**
* **Spermatogenic cell**

Sertoli Cell, Also known as the supporting cells, they are huge, long, irregularly shaped, pyramidal to columnar, and located along the spermatic tubule between the generative sex cell. Its broad base lays perpendicular to the basement membrane, and the funnel edge of the tubule is open at the lumen (Pineda & Dooley, 2003).

The cell contains an oval nucleus with one or more big, distinct nucleoli that is located a short distance above the base of the cell. It is situated around the base of the nucleus, and the cytoplasm is made up of several lipid droplets, the Golgi apparatus, glycogen, and the cytoplasm of Sertoli cells in humans, which comprises crystalloids with an unidentified purpose. The Seminiferous tubule's side walls are tightly linked together, and its lateral rims also include tubules and intermediate filaments that are thought to help cells travel from the basal region to the nearby region (O'Donnell, 2014).

Sertoli cells have multiple roles in the regulation of spermatogenesis and its development and continuity by providing the formed sperm with the necessary nutrients, support, and protection, and by consuming the diseased decomposing sperms. Its purpose is to prevent the passage of large particles from interstitial space into the seminiferous tubules. Through the creation of a unique chemical that controls the production of testosterone and the return of some substances, it also permits the transit of some materials required for the generation of sperm. Consequently, the blood-testicular barrier is prepared, preventing the development of antibodies against newly formed Sperm cells (Wong & Khan, 2023).

Under the control of testosterone and FSH, sertoli cells also secrete the androgen-binding protein (ABP), which aids in the regulation of spermatogenesis by moving and concentrating male androgens close to the germ cells and increasing the concentration of testosterone in the spermatic tubule (Sengupta *et al*., 2019).

***Spermatogonia**:

The largest portion of the seminal epithelial tissue is made up of an epithelial layer, and the cells are stacked in many rows (4–8) from cells lining the seminiferous tubule. As they multiply, grow, and specialize, they rush toward the cavity and transform into sperm to separate from the epithelial tissue and become free in the cavity. They are arranged in the form of central layers of different ages, running from the basal region of the tube to its lumen. The following cell types are those that produce sperm from the tubule's basement membrane to the interior (Fayomi & Orwig, 2018):

1-Spermatogonia .

2. primary spermatocytes.

3-Secondary Spermatocytes.

4- Spermatids.

5-Immature Spermatozoa.

6-Mature Spermatozoa

Second : Interstitial tissue:

This tissue can be found inside lobules and in the spaces between seminiferous tubules. It is made up of nerves, lymphatic and blood arteries, loose connective tissue, and Leydig cells, which are endocrine cells. They are also known as interstitial cells because they are large, round or polygonal cells that can be found alone or in groups in the interstitial tissue between the seminiferous tubules. Its nucleus is spherical or oval-shaped and contains color granules and a single nucleolus; two nuclei are more common; the smooth endoplasmic reticulum, which produces hormones, is located in the cytoplasm (O'Donnell *et al*., 2000).

2.4.1.2 Epididymis

The epididymis defined as, it is long often torsional tube approximately 7meters in length, lined with columnar cells and a ciliated pseudo-layer resting on a basement membrane, consists of a loose connective tissue rich in blood vessels and smooth muscle fibers, and the epididymis is located with the testicle inside the scrotal cavity. Sperm go from the testicle to the Vas deferens by a connective tissue made up of tiny efferent ducts, which attaches it to the margin of the testis from behind. The head of the epididymis caput, which is the first of the epididymis' three sections and indicates its expanded front end near the testicle, is attached to the testicle. Each of their 13–23 coiled helical cannulas is 6–8 cm long, has a diameter of 0.05 mm, and is encircled by connective tissue (Cleland, 1957).

These ducts are irregularly undulating separated, have a smooth regular surface, and are lined with simple epithelium made up of columnar ciliated cells and some cuboidal non-ciliated secretory cells that rest on the basement membrane. Due to the different heights of the epithelial tissue cells, groups of long columnar cells frequently exchange with other groups of short cells. The cytoplasm of the long cells' lining is made up of pigment granules and lipid droplets, and these cells have cilia that move sperm through the canals (Johnston *et al*., 2007).

Lysosomes and micro villi are present in the cytoplasm of the short cells, which are also absorbing cells. It serves this purpose by absorbing a significant percentage of the fluid secreted by the Seminiferous tubules, causing the lining to become columnar. The epididymis ducts, which connect the head of the epididymis to the body of the epididymis, a narrow middle region that ends in the tail of the epididymis known as the Cauda, are only at the end of the efferent canals and have a flat lumen.

The cannulas twist on each other to form the head of the epididymis (Mann & Lutwak-Mann, 2012).

The maturation of sperm takes place in the head and body region; it is situated at the bottom and links with the vas deferens. The epididymis' tail is where sperms primarily develop the ability to migrate and fertilize. Suggested that one of the epididymis's most crucial jobs is to store and preserve living sperm since it contains some nutrients required for this activity. Additionally, the epididymis is where sperm develop, mature, and are transferred because this is where they acquire mobility after passing through the epididymis canal (Knez, 2013).

2.4.2 Prostate

The prostate is a little, walnut-shaped gland that surrounds the posterior upper urethra beneath the urine bladder. the urethral segment that passes through it. consists of four lobes: the anterior, dorsal, ventral, and lateral lobes (Aaron *et al*., 2016). The prostatic cluster is a network of veins that surrounds the prostate. The prostate is made up of three glandular regions: the central zone surrounding the ejaculatory duct, the transitional zone enclosing the urethra, and the peripheral zone encircling both of them. The prostate is composed of two sections, the first glandular placed inside the other, which is fibrous muscular tissue (Villers, 1994). It takes role in the production of semen, which contains nutrients that allow sperm to move around and fertilize. Prostate comprises three distinct types of epithelial cells: basal, luminal, and endocrine cells; hyperplasia prostate occurs in the transitional zone whereas cancer develops in the peripheral zone (Hudson *et al*., 2001).

2.4.3 Seminal Vesicle:

The seminal vesicles are a pair of glands that are located behind the bladder, lateral to the vas deferens in front of the rectum and connected to the base of the prostate. The seminal vesicles' function is to release up to 60% of the fluid contained in semen (Tjionas *et al*., 2015). The histological structure of the seminal vesicles is composed of three layers: a layer of pseudo stratified columnar epithelium that includes goblet cells and lamina propria , a layer of muscular outer longitudinal inner circular from smooth muscle, and a layer of adventitial loose connective tissue (Zhang *et al*., 2018). The seminal glands or vesicular glands are other names for the vesicles. The male reproductive system's auxiliary glands are known as seminal vesicles. Between the bladder and the rectum, there are two twisted or deformed tubes (Mikuz, 2019). The vesicle secretes an alkaline fluid that aids in the survival of spermatozoa, fructose also helps sperm survive, and prostaglandins have a role in inhibiting the immunological response of semen in females. The coagulation factors that keep semen in the female reproductive tract functioning are one of the compounds found in vesicular secretion (Druart & de Graaf, 2018) .

2.4.4 Spermatogenesis:

The process of spermatogenesis, which produces mature sperm in seminiferous tubules, takes place during puberty and continues throughout a man's life. It involves a complex series of specialized cell divisions, during which the number of chromosomes is reduced from Diploid (2n) actress of an animal type to half Haploid (1n).Sperm is produced when the sex cells convert and progress through different phases. The first stage of this process involves cell divisions during meiosis and mitosis, which doubles the amount of cells, and the second stage is spermiogenesis (Hickman, 2021).

The division of germ cells to create spermatozoa is the first step in the process of spermatogenesis. The majority of type A spermatogonia are divided to create intermediate spermatogonia, while the majority of type B spermatogonia are divided to create B. Type B spermatogonia go through the last mitosis to form primary Spermatocytes, which contain all of the chromosomes (Johnson *et al*., 2000).

These cells then move away from the basement membrane, enlarge, and get ready to enter the stage of meiosis. Secondary Spermatocytes are then formed from the mediastinum of the chromosomes, and spermatids go through a series of morphological changes to become sperm, a process known as (cellular) The process of spermatogenesis pass through a series of stages and this causes the different arrangement of the spermatogonia, Primary and secondary spermatocytes , spermatids and spermatozoa from across section in the seminiferous tubules to another for this reason absorbed a series of interconnected cells arranged in a certain way along the seminiferous tubule , and the sequential changes in all stages along the length of the seminiferous tubules between two types of cells are called cycle of the seminiferous epithelium . As for the total changes in the cells that start from the activation of the germ cells or sperm progenitors until the release of the sperm into the lumen of the seminal tubule they are called spermatogenic cycle which takes 30 days in rats (Guyton & Hall, 2006; Holstein *et al*., 2003).

2.4.5 Mature Sperm:

A mature human sperm consists of a head, a middle piece and tail. The head consists of the nucleus and the vertical cap containing the terminal body at its front edge and the bulk of the head occupies the

nucleus, which in turn contains the genetic material DNA (Puga Molina *et al*., 2018).

There is a belief that the function of the terminal body is to form a substances of an enzyme nature called sperm lysins in mammals. This enzyme is called (hyaluronidase)as these enzyme degrade the egg membranes in the region where the sperm meets the egg to facilitate the passage of the Sperm to the surface of the egg as for the middle piece separated from the head . By narrow neck , it contains an axis of longitudinal tubules forming what is called a (axial filament complex) surrounded by nine thick fiber and surrounded by a sheath of mitochondria . It is believed that the midsection controls the movement of the tail (Lin *et al*., 1994).

2.4.6 Hormones Control the process of Spermatogenesis:

The process of activating the male reproductive system is involved in three endocrine glands ,which are the hypothalamus which secrete a hormone gonadotropin - releasing hormone (GnRH), pituitary gland and testis, which secrete testosterone and inhibin hormone . The overlap among the functioning of these glands is called the hypothalamic - pituitary – testis axis who is in habited before Puberty (Debieve *et al*., 2000). The hypothalamus is activated upon reaching puberty and secretes a hormone GnRH which is less than ten minutes old and then destroyed by enzymes found in the cells of the pituitary gland then it travels through the hypothalamic - pituitary – portal system by blood vessels it is affects the cells of the anterior part of the pituitary ,which in turn respond to secretion of stimulating hormones FSH , LH (Prevot *et al*., 2010). FSH travels in the blood to the seminiferous tubules and binds to receptors on sertoli cells urging them to secrete androgen – binding protein to male

hormones ABP which binds testosterone leading to an increase in its concentration on the surfaces of Sertoli cells (Howard, 2021).

Thus contributing to the maturation and differentiation of sperm is one of the basic and most important functions of the testicle. As for the LH hormone, it travels in the blood to the testicles to affect the Leydig cells , which are located between the seminiferous tubules to build and secrete the Testosterone which is important in the formation, growth and development of sperm. That's a working mechanism axis hypothalamic - pituitary- testis axis regulated by negative feedback as shown in figure (3), the increase in testosterone inhibits the formation of the (GnRH) hormone. FSH is inhibited by a hormone Inhibin which is secreted by Sertoli cells inside the seminiferous tubules , and when the number of sperm is increased, this hormone is transmitted through blood to affect the pituitary and inhibits the secretion of FSH by the negative feedback process (Tornøe *et al*., 2007).

It should be noted that sexual stimuli send nerve signals through the sacral parasympathetic nerves (the second , third and fourth vertebrae of the spinal cord) which affect the smooth muscles in the arteries (pudendal) the internal nutrients of the penislead to the secretion of nitrogen oxide, which causes relaxation in the muscles of the arteries and their widening and increase the flow of blood from them towards the cavernous bodies where they became engorged with blood , which lead to an erection (McLachlan *et al*., 2002; McNeilly *et al*., 2003).

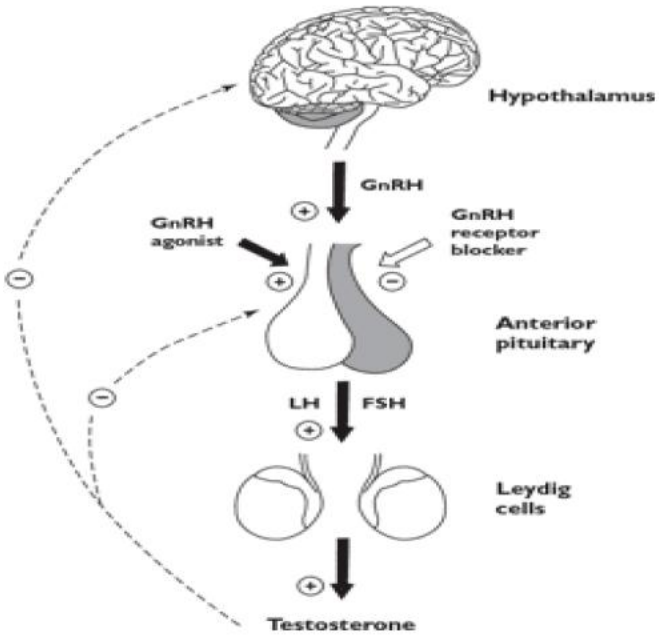

Figure 2-1: showing the mechanism action of the of hypothalamic pituitary-testicular axis (Tornøe *et al*., 2007)

2.4.7 Seminal Fluid:

The prostate and seminal vesicles are responsible for producing most of the semen. The latter is defined as the fluid that work to prepare the food needed for the sperm's metabolism and movement while it forms %60 from the volume of semen , which is secreted from the seminal vesicle and the ratio is 13- 33% the semen has a milky appearance from the prostate gland (Poiani, 2006). The secretion contains citric acid , calcium , acid phosphate and proteolytic enzyme the latter is responsible for dissolving semen and also secretes cholesterol whose function is to protect the sperm from environmental shocks as for the lowest percentage of secreted fluids it is from the epididymis and Cowper's gland at a rate of 5 % (Austin, 2012).

One of the most important qualitative measures of semen characteristics is the movement of sperms , as it is a good indicator that positively correlates with the percentage of normal live sperms, and

negatively with the percentage of dead and deformed sperms , and this movement is evidence of the vitality of the sperms and their ability to penetrate the barriers that they encounter and intercept in the reproductive system for the purpose of reaching the fertilization site. Sperm can live in the ducts for several weeks , but after ejaculation their maximum life span is 72 hours (Poiani, 2006).

Chapter Three

Materials and Methods

3.1 Chemical Materials and Devices:

3.1.1 Chemicals

Table 3-1: Chemicals used in the study with their origins

Chemicals	Origin
Chloroform	England
D.P.X.	India
Distilled water	Iraq
Eosin	Germany
Ethanol	England
Formaldehyde	Iran
Hematoxyline	Germany
Kit measurement of Total antioxidant	China
Kit to measurement of Hormone Insulin,T,FSH, LH, P and E	China
Kit to measurement of Triglycerides, Cholesterol, HDL	France
Methanol	England
Normal physiological saline 0.9%	Iraq
Paraffin wax	Germany
Xylene	Germany

3.1.2 Devices and Tools:

Table 3-2: The Devices and Tools used in Study with Origin

Devices and tools	Origin
Accu-Chek	USA
Centrifuge	USA
Digital camera of Carl Zeiss	Germany
Disposable Insulin Syringes	China
Disposable Syringes	China
Dissecting Set	Pakistan
Electronic Balance	Germany
ELISA device	USA
ELISA reader	USA
Eppendorf tube	China
Gel Tube	Iraq
Hot plate	India
Incubator	Germany
Light Microscope	Japan
Micro pipette	Germany
Rotary Microtome	Germany
Sensitive Scale	Germany
Slides	China
Slides Cover	China
Vortex	China
Water path	Germany
Wells	USA

3.2 Animals:

Thirty matured white healthy male laboratory rats *Rattus norvegicus* , weighing 230-320gm and aged 10 to 12 weeks, were used in the current study. Animals were housed in the animal house of the College of Science University of Babylon, after carrying out the experiment animals were acclimatized for two weeks in a plastic cages with wood chip bedding under controlled and standardized conditions of temperature 22-24 $^{\circ}$C and light/ dark cycle, feed with standard rodent diet and water , in addition to continuous hygiene of cages and water bottles .

3.3 Experimental groups

Animals were randomly grouped into five groups as following:

Groups I: The control group consists of 6 rats that received orally normal saline for 90 days .

Group II consists of 6 rats that received intraperitoneal injections of 60 mg\kg body weight of streptozotocine for 90 days .

Group III consists of 6 rats that received orally 200 mg\kg body weight of berberine for 90 days .

Group IV consists of 6 rats diabetic (streptozotocine injected) rats received 200 mg/kg body weight of berberine orally for 45 days.

Group V: consists of 6 rats diabetic (streptozotocine injected) rats received 200 mg/kg body weight of berberine orally for 45 days.

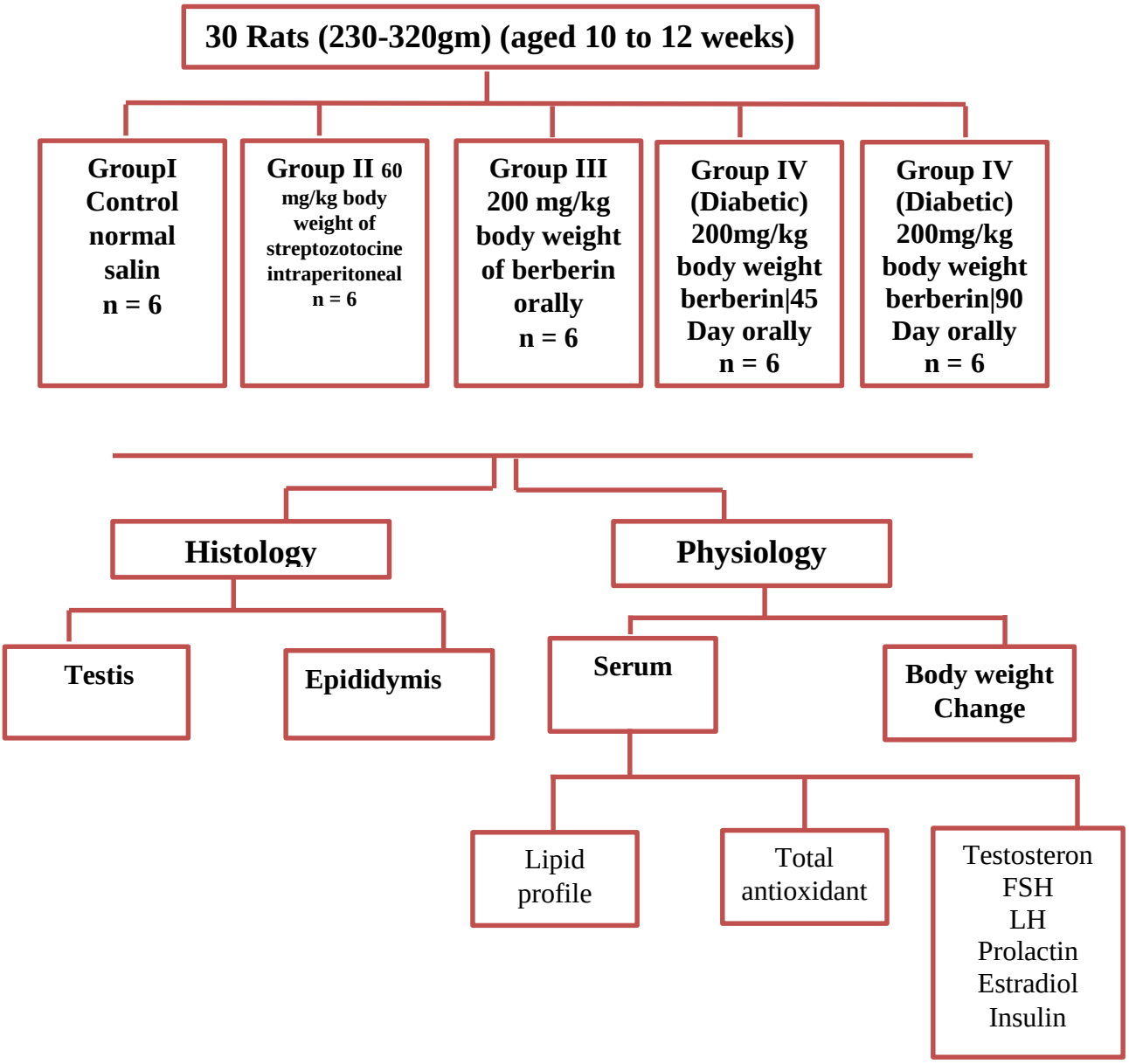

Figure (4): Experimental design

3.4 Streptozotocin and Diabetes induce

Streptozotocin was prepared according to manufacturer instructions. Stock solution using ddH2O as a solvent can be directly diluted with 0.9% NaCl to ready the working solution.

Diabetes was induced in rats by single intraperitoneal injection of freshly streptozotocin 60 mg /kg body weight (Slaughter *et al*., 2013). The animals were fasted for overnight 12 hours before STZ injection. After injection, the rats were kept for next 48 hours on oral 10% glucose solution on top of their chew. Administration of glucose is to prevent hypoglycaemia as STZ is capable of producing fatal hypoglycaemia due to destruction of β cells which in turn results in to massive pancreatic insulin release (Saddala *et al*., 2013).

3.5 Berberine preparation and administration

Berberine dissolved in boiling water (200 mg/kg/day) and administered orally (Zhang *et al*., 2010).

3.6 Collection of blood

At the end of each experiment animals were sacrificed by, 5 ml of blood were obtained from animal heart by heart puncture by using syringe Blood samples were put in anticoagulant free gel tubes , tubes were left for 10 minutes in room temperature , then centrifuged at 3000 RPM for 5 minutes , serum was separated and putted in Eppendorf tubes and freezed at – 20 C for the biochemical tests.

3.7 Collection of organs

At the end of each experiment animals were sacrificed by, organs were isolated, weighted, washed with normal saline , dried and put in tubes contained formalin as fixative for tissue processing .

3.8 Measuring the Body weight Changes:

The average weights of the animals for each group were determined after overnight fasting using an electronic balance before and after the experiment. Then the weight differences were calculated at the end of each experiment.

3.9 Fasting blood glucose measurement

Fasting blood glucose levels at the beginning and ending of the experiment were measured by using Accu-Chek .Aviva\USA apparatus

3.10 Estimation of Insulin hormone levels

Insulin level was measured according to rat insulin ELIZA kit (cosabio Biotech co .)

3.10.1 Test Principle

This assay utilizes the quantitative sandwich enzyme immunoassay technique. An INS-specific antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any INS present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for INS is added to the wells. Following a wash, avidin-conjugated Horseradish Peroxidase (HRP) is added to the wells. After another wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of INS initially bound. The development of color is then halted, and the intensity of the color is measured.

3.10.2 Test Procedure

Test procedure was done according to ELISA kit manufacturer instruction (Cusabio Technology LLC)

1. Referred to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and sealed the ziploc, stored unused wells at 4°C.
2. Added 100µl of standard and sample per well. Covered with the adhesive strip provided. Incubated for 2 hours at 37°C. A plate layout was provided to record standards and samples assayed.
3. Removed the liquid of each well, didn't wash.
4. Added 100µl of Biotin-antibody (1x) to each well. Covered with a new adhesive strip. Incubated for 1 hour at 37°C. (Biotin-antibody (1x) may have appeared cloudy. Warmed up to room temperature and mixed gently until solution appeared uniform.)
5. Aspirated each well and washed, repeating the process two times for a total of three washes. Washed by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step was essential to good performance. After the last wash, removed any remaining wash Buffer by aspirating or decanting. Inverted the plate and blotted it against clean paper towels.
6. Added 100µl of HRP-avidin (1x) to each well. Covered the microtiter plate with a new adhesive strip. Incubated for 1 hour at 37°C.
7. Repeated the aspiration/wash process for five times as in step 6.
8. Added 90µl of TMB Substrate to each well. Incubated for 15-30 minutes at 37°C. Protected from light.
9. Added 50µl of Stop Solution to each well, gently tapped the plate to ensure thorough mixing.

10. Determined the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction was available, set to 540 nm or 570 nm. Subtracted readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction corrected for optical imperfections in the plate. Readings made directly at 450 nm without correction may have been higher and less accurate.

3.10.3 Calculation of results

A standard curve created by reducing the data by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and drew a best fit curve through the points on the graph. The data could have been linearized by plotting the log of the INS concentrations versus the log of the O.D. and the best fit line could have been determined by regression analysis.

3.11 Estimation of Lipid profile levels

3.11.1 Total Cholesterol

Procedure

Let stand reagent and specimens at room temperature.

Reagent	1000 µL
Blank, Standard, Control or specimen	10 µL
Mix. Let stand for 10 minutes at room temperature or 5 minutes at 37°C. Record absorbances at 500 nm (480-520) against reagent blank. Color is stable for 1 hour	

Calculation

Result = Abs (Assay) x Standard concentration (Tietz, 1999, 2006)
Abs (Standard)

3.11.2 Triglycerides

Procedure

Let stand reagent and specimens at room temperature.

Reagent	1000 µL
Blank, Standard, Control or specimen	10 µL
Mix. Let stands for 5 minutes at 37°C (10 minutes at room temperature). Record absorbance at 505 nm against reagent blank. Reaction is stable for 1 hour.	

Calculation

Result = Abs (Assay) x Standard concentration (Tietz, 1999, 2006)
Abs (Standard)

3.9.3 High density lipoprotein (HDL) cholesterol

Procedure

Let stand reagent and specimens at room temperature.

Set up the instrument to read micro-volumes.	Blank	Calibrator	Assay
Reagent R1	300 µL	300 µL	300 µL
Calibrator		3 µL	
Specimen			3 µL
Mix vigorously, let stand for 5 minutes at 37°C. Record absorbance A1 at 600 nm against reagent blank			

Add	Blank	Calibrator	Assay
Reagent R2	100 µL	100 µL	100 µL
Mix vigorously, let stand for 5 minutes at 37°C. Record absorbance A2 at 600 nm against reagent blank			

Calculation

Result = $\frac{\text{Abs (Assay)}}{\text{Abs (Standard)}}$ x Standard concentration

(Badimón *et al*., 1990; Tietz, 2006)

3.9.4 LDL-cholesterol

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDLcholesterol according to the relationship: [LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL (Crook, 2013).

3.12 Estimation of testosterone hormone levels

3.12.1 Test principle

This ELISA kit employs the Competitive-ELISA principle. The micro ELISA plate included in this kit has been pre-coated with T. During the reaction, T in the sample or standard competes with a fixed amount of T on the solid phase supporter for sites on the Biotinylated Detection Ab specific to T. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Following this, a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a stop solution, and the color change is measured spectrophotometrically at a wavelength

of 450 nm ± 2 nm. The concentration of T in the samples is then determined by comparing the OD of the samples to the standard curve (Mooradian *et al*., 1987).

3.12.2 Test procedure

Test procedure was done according to ELISA kit manufacturer instruction (Elabscience Biotechnology Inc.)

1. The Standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (50 uL for each well). The samples were added to the other wells(50 uL for each well). Immediately, 50 µL of Biotinylated Detection Ab working solution was added to each well. The plate was covered with the sealer provided in the kit and incubated for 45 min at 37℃.

2. The solution from each well was aspirated or decanted, then 350 uL of wash buffer was added to each well. It soaked for 1~2 min and then the solution from each well was aspirated or decanted and patted dry against clean absorbent paper. This wash step was repeated 3 times.

    2. 100 µL of HRP Conjugate working solution was added to each well. The Plate sealer was then used to cover it. It was incubated for 30 min at 37°C.

    3. The solution from each well was aspirated or decanted, then the wash process was repeated five times as conducted in step 2.

    4. 90 µL of Substrate Reagent was added to each well. A new plate sealer was used to cover it. It was incubated for about 15 min at 37°C while protecting the plate from light.

    5. 50 µL of Stop Solution was added to each well.

6. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

3.12.3 Calculation of results

The duplicate readings for each standard and samples were averaged. A four-parameter logistic curve was plotted on log-log graph paper, with the standard concentration on the x-axis and OD values on the y-axis.

3.13 Measuring the Level of Follicle-Stimulating Hormone (FSH)

3.13.1 Test Principle

This ELISA kit operates on the Sandwich-ELISA principle. The micro ELISA plate provided in this kit is pre-coated with an antibody specific to Rat FSH. Samples or Standards are added to the wells of the micro ELISA plate and combined with the specific antibody. Following this, a biotinylated detection antibody specific for Rat FSH and an Avidin-Horseradish Peroxidase (HRP) conjugate are successively added to each well and incubated. Free components are then washed away. The substrate solution is added to each well. Only those wells that contain Rat FSH, the biotinylated detection antibody, and Avidin-HRP conjugate will appear blue. The enzyme-substrate reaction is halted by the addition of a stop solution, causing the color to turn yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat FSH. The concentration of Rat FSH in the samples can be calculated by comparing the OD of the samples to the standard curve.

3.13.2 Test procedure

Test procedure was done according to ELISA kit manufacturer instruction (Elabscience Biotechnology Inc.)

1. Wells for diluted standard, blank, and sample were determined. 100 µL of each dilution of standard, blank, and sample were added into the appropriate. The plate was covered with the sealer provided in the kit and incubated for 90 min at 37℃. Note: solutions were added to the bottom of the micro ELISA plate well, avoiding touching the inside wall and causing foaming as much as possible.
2. The liquid from each well was decanted, without washing. Immediately, 100 µL of Biotinylated Detection Ab working solution was added to each well. The plate was covered with a new sealer and incubated for 1 hour at 37°C.
3. The solution from each well was decanted, followed by addition of 350 µL of wash buffer to each well. The wells were soaked for 1 min then aspirated or decanted, and patted dry against clean absorbent paper. This wash step was repeated 3 times. Note: a microplate washer was used in this step and other wash steps. The tested strips were used immediately after the wash step. The wells were not allowed to dry.
4. 100 µL of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer and incubated for 30 min at 37°C.
5. The solution from each well was decanted, and the wash process was repeated for 5 times as conducted in step 3.
6. 90 µL of Substrate Reagent was added to each well. The plate was covered with a new sealer and incubated for about 15 min at 37°C, protected from light. Note: the reaction time could be shortened or extended according to the actual color change, but not more than 30

min. The Microplate Reader was preheated for about 15 min before OD measurement.

7. 50 µL of Stop Solution was added to each well. Note: The stop solution was added in the same order as the substrate solution.
8. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

3.13.3 Results calculation

The duplicate readings for each standard and samples were averaged, then the average zero standard optical density was subtracted. A four-parameter logistic curve was plotted on a log-log axis, with the standard concentration on the x-axis and OD values on the y-axis.

3.14 Measuring the Level of Luteinizing Hormone (LH)

3.14.1 Test Principle

This ELISA kit utilizes the Sandwich-ELISA principle. The provided micro ELISA plate in this kit has already been pre-coated with an antibody specific to Rat LH. Samples or Standards are then added to the wells of the micro ELISA plate, where they combine with the specific antibody. A biotinylated detection antibody, also specific for Mouse LH, and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each well on the plate and incubated. Any free components are subsequently washed away. The substrate solution is then added to each well. Only those wells containing Rat LH, the biotinylated detection antibody, and the Avidin-HRP conjugate will appear blue. The enzyme-substrate reaction is terminated by adding a stop solution, which turns the color yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is

directly proportional to the concentration of Rat LH. This allows for the calculation of Rat LH concentration in the samples by comparing their OD to that of the standard curve.

3.14.2 Test procedure

Test procedure was done according to ELISA kit manufacturer instruction (Elabscience Biotechnology Inc.)

1. Wells for diluted standard, blank, and sample were determined. 100 µL of each dilution of standard, blank, and sample were added into the appropriate wells. The plate was covered with the sealer provided in the kit and incubated for 90 min at 37℃.
2. The liquid from each well was decanted, without washing. Immediately, 100 µL of Biotinylated Detection Ab working solution was added to each well. The plate was covered with a new sealer and incubated for 1 hour at 37°C.
3. The solution from each well was decanted, and 350 µL of wash buffer was added to each well. It was soaked for 1 min and then the solution from each well was aspirated or decanted and patted dry against clean absorbent paper. This wash step was repeated 3 times. Note: a microplate washer was used in this step and other wash steps. The tested strips were made in use immediately after the wash step. Wells were not allowed to dry.
4. 100 µL of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer and incubated for 30 min at 37°C.
5. The solution from each well was decanted and the wash process was repeated for 5 times as conducted in step 3.
6. 90 µL of Substrate Reagent was added to each well. The plate was covered with a new sealer and incubated for about 15 min at 37°C,

protected from light. Note: the reaction time could be shortened or extended according to the actual color change, but not more than 30 min. The Microplate Reader was preheated for about 15 min before OD measurement.

7. 50 µL of Stop Solution was added to each well. Note: adding the stop solution was done in the same order as the substrate solution.
8. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

3.14.3 Results calculation

The duplicate readings for each standard and samples were averaged, then the average zero standard optical density was subtracted. A four-parameter logistic curve was plotted on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

3.15 Determination of Prolactin hormone:

3.15.1 Test Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat PRL. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then, a biotinylated detection antibody specific for Rat PRL and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat PRL, biotinylated detection antibody, and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm.

The OD value is proportional to the concentration of Rat PRL. You can calculate the concentration of Rat PRL in the samples by comparing the OD of the samples to the standard curve.

3.15.2 Test Procedure

Test procedure was done according to ELISA kit manufacturer instruction (Elabscience Biotechnology Inc.)

1. Determined wells for diluted standard, blank, and sample. Added 100µL each dilution of standard, blank, and sample into the appropriate wells. Covered the plate with the sealer provided in the kit. Incubated for 90 min at 37℃. Note: solutions were added to the bottom of the micro ELISA plate well, avoiding touching the inside wall and causing foaming as much as possible.
2. Decanted the liquid from each well, did not wash. Immediately added 100 µL of Biotinylated Detection Ab working solution to each well. Covered the plate with a new sealer. Incubated for 1 hour at 37°C.
3. Decanted the solution from each well, added 350µL of wash buffer to each well. Soaked for 1-2 min and aspirated or decanted the solution from each well and patted it dry against clean absorbent paper. Repeated this wash step 3 times. Note: a microplate washer was used in this step and other wash steps. Made the tested strips in use immediately after the wash step. Did not allow wells to be dry.
4. Added 100µL of HRP Conjugate working solution to each well. Covered the plate with a new sealer. Incubated for 30 min at 37°C.
5. Decanted the solution from each well, repeated the wash process for 5 times as conducted in step 3.
6. Added 90µL of Substrate Reagent to each well. Covered the plate with a new sealer. Incubated for about 15 min at 37°C. Protected the plate from light. Note: the reaction time could have been shortened or

extended according to the actual color change, but not more than 30min. Preheated the Microplate Reader for about 15 min before OD measurement.

7. Added 50µL of Stop Solution to each well. Note: adding the stop solution was done in the same order as the substrate solution.
8. Determined the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

3.15.3 Results calculation

The duplicate readings for each standard and samples were averaged, then the average zero standard optical density was subtracted. A four parameter logistic curve was plotted on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

3.16 Determination of Estradiol hormone:

3.16.1 Test Principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with E2. During the reaction, E2 in the sample or standard competes with a fixed amount of E2 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to E2. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of E2 in the samples is then determined by comparing the OD of the samples to the standard curve.

3.16.2 Test Procedure

Test procedure was done according to ELISA kit manufacturer instruction (Elabscience Biotechnology Inc.)

1. The Standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (50 uL for each well). The samples were added to the other wells (50 uL for each well). Immediately, 50 µL of Biotinylated Detection Ab working solution was added to each well. The plate was covered with the sealer provided in the kit and incubated for 45 min at 37℃.
2. The solution from each well was aspirated or decanted, then 350 uL of wash buffer was added to each well. It soaked for 1~2 min and the solution from each well was again aspirated or decanted and patted dry against clean absorbent paper. This wash step was repeated 3 times. Note: a microplate washer was used in this step and other wash steps.
3. 100 µL of HRP Conjugate working solution was added to each well. It was covered with the Plate sealer and incubated for 30 min at 37°C.
4. The solution from each well was aspirated or decanted, the wash process was repeated five times as conducted in step 2.
5. 90 µL of Substrate Reagent was added to each well. It was covered with a new plate sealer and incubated for about 15 min at 37°C. The plate was protected from light. Note: the reaction time could be shortened or extended according to the actual color change, but not more than 30min.
6. 50 µL of Stop Solution was added to each well. Note: Adding the stop solution was done in the same order as the substrate solution.

7. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

3.16.3 Result Calculation

The duplicate readings for each standard and samples were averaged. A four-parameter logistic curve was plotted on a log-log graph paper, with the standard concentration on the x-axis and OD values on the y-axis.

3.17 Total Antioxidant Capacity Assay

3.17.1 Principle:

According to the test's guiding principle (Apak *et al*., 2005)

Total antioxidants $Cu^{+2} \longrightarrow Cu^{+}$

Maximum at 450 nm: Cu + (2,9-dimethyl-1,10-phenanthroline complex)

3.17.2 Reagents:

1- Chloride of copper (II), one CuCl2. 2H2O, weighing 0.4262g, was dissolved in water, and 250 mL of water was added to create a solution with a concentration of 10-2 M.

2- Dilution Buffer: Ammonium acetate (NH4 Ac) buffer with a pH value of 7.0 was made by dissolving 19.27g of NH4 Ac in water and completing 250 milliliters are the total volume.

3- Trolox the Standard Solution of Sample antioxidant were Prepared at $1.0*10^{-3}$ M Trolox

4- Stop Solution: Neocuproine (Nc) {2,9- dimethyl- 1.10 Phenanthroline} Solution at a Concentration of 7.5*10-3M was prepared by dissolving 0.039 g Nc in 96% Et OH, the Volume was Completed to 25ml with Ethanol

3.17.3 Procedure:

Copper Solution, dilution buffer, and stop solution 30 minutes should pass for the assay to reach room temperature before use. In the dilution buffer, dilute both the standards and the sample 1:40 (for example, 15 mL serum + 585 ml buffer).

1- Fill each well with 50 l of diluted samples or Standards.
2- For a reference measurement, read the plate at 450 nm.
3- Add 1mL of the Cu Solution to each well, then let it sit at room temperature for 30 minutes.
4- Add 1mL to the Stop Solution.
5- Next time 450 nm reading of the plate Test tubes were vortexed a spectrophotometer was used to measure the absorbance at 450 nm after being prepared and incubated for 30 minutes at 37°C.

3.17.4 Calculation :

Total antioxidant levels = $Absorbance_{test}$/ $Absorbance_{standard}$ * $Concentration_{standard}$ (mmol/L)

3.18 Histological Study

Steps were performed on it depending on method described in (Suvarna & Layton, 2013).

3.18.1 Dehydration

The tissue transferred through Series of concentrated ethyl alcohol (70% , 80% , 90%, 100%,100%) to remove all water, for an (1 hour) at each concentration.

3.18.2 Clearing

The samples placed in Xylene for removing alcohol for a period (2 minute).

3.18.3 Infiltration

The tissue then placed in melted paraffin until completely infiltrated with substance Paraffin wax (57-60°C) melting point the process repeated 2-3 time for a period (1hours) inside an electric oven at a temperature of 60 in order to keep the wax melted the number of times of wax change depends on the type of sample so that it decreases whenever the sample is soft and increases whenever the sample is solid . Wax gives it a strong support to prepare it for microtome cutting and help to keep tissue along time with any harm.

3.18.4 Embedding

The paraffin - infiltrated tissue is placed in melted paraffin and allowed to harden in room temperature, inside iron molds or special capsules in which the models were buried.

3.18.5 Sectioning

Used for Cut the models (Rotary Microtome) is fixed and cutes to a thickness of 5 micrometers then the strips of the Sections placed in a water bath (45- 50 c°) for (1-2 min) and then were carried on glass slides and put the slides on hot plate to dry left in room temperature 37C" for (1 hour).

3.18.6 Staining and Mounting

The dyeing was done using two dyes Haematoxylin - Eosin stain, the slides are placed in xylene to clean slides from wax residue for (5 minutes) and then passed on series from descending ethyl alcohol (100%, 100% , 90%, 80%, 70%) and for two minutes in each concentration then stained of the histological Sections.

Table 3-3: Hematoxylin Stain components

Material	Quantity
Hematoxylin Powder	**2.5gm**
AIK $(So4)_2$. $12H_20^2$ or NH_4Al $(S04)_2$. $12H_2o$	**50gm**
Absolute ethanol	**25mL**
D.W	**500 mL**
Mercuric Oxide (red)	**1.25gm**
Glacial Acetic acid	**20ml**

Hematoxylin preparation according to (Suvarna & Layton, 2013) dissolved the hematoxylin powder by absolute alcohol, then it is added to the AlK $(S0_4)_2$ -12 H_2O in warm distilled water, and Add red mercury oxide to the mixture after bringing it to a boil. Cool the mixture by placing it in a beaker of cold water and then add glacial Acetic acid but filter the mixture before use it.

Table 3-4: Eosin Stain components

Material	Quantity
Eosin Powder	1 gm
Glacial Acetic acid	1 mL
Alcohol 70% Ethel	99mL

Dissolved the Eosin Powder by Ethel alcohol and add Glacial Acetic acid but filter the mixture before use it according (Suvarna *et al.*, 2013). Transfer the slide to containers containing the dyes, where they are colored with hematoxylin dye for one minute then it was washed with distilled water for two minutes then dipped in alcohol two or three times to remove the excess dye, then it was colored with eosin and then transferred to an ascending chain of ethyl alcohol (70%, 80%, 90%,

100%, 100 %) for two minutes except for the last concentration put in it for 5 minutes then clearing with Xylene for 10 minutes and then we Perform mounting process by using (D.P.X.) Distrine plasticizer xylene to fixed cover on slide and left the slide to dry on hot plate for 8 hours to be ready for examination

3.19 Photo Micro Graph :

The tissue Sections were taken after preparation and the glass slides were examined and changes in the studied tissue sections were determined using the light microscope type Olympus equipped with digital camera of Carl Zeiss type, the pictures was taken and checked at the appropriate locations of them, then cell checked by objective lens on magnification 40X,10X.

3.20 Statistical Analyses :

The data was analyzed using SPSS(version 23,SPSS Inc .Chicago, Illinois, USA). Descriptive statistics(mean ,standard deviation),and differences were compared by one-way ANOVA ,by using Duncan‘s test .As well as ,it was carried out using student‘s-t test, followed by chi-square. A statistically significant result was one with a($p<0.05$). Analyses of statistical according (Duncan, 1955)

Chapter Four

Results and Discussion

4.1 physiological Study

4.1.1 Effect of berberine 45 days /90 days on body weight / gm in male rats

In Table 4.1, the difference in body weight levels in grams (gm) was observed thought different groups: control, diabetics, berberine, diabetics treated with berberine for 45 days, and diabetics treated with berberine for 90 days. The control group showed a significant increase in body weight by 48 gm, while the diabetic group experienced a significant decrease of 34 gm. Interestingly, the berberine group showed a slight increase of 6.5 gm. Diabetic groups treated with berberine for both 45 and 90 days showed a re-establish in body weights resulted in a decrease of body weight by 29 gm and 26 gm respectively. These results suggest that berberine has an influence on body weight management particularly in diabetic rats, and the effects appear to be more pronounced with the duration of berberine treatment.

Table 4-1: Effect of berberine on fasting (Body weight / gm) levels in study groups treated with berberine for 45 and 90 days rats

Groups/ n=6	Body weight / gm [mean ± S.E.]		Weight difference %
	Initial	Final	
Control	230± 7.5	278±7.1	48 d
Diabetics	225±10.4	191±9.2	-34 b
Berberine	270.5±2.6	277±5.4	6.5 c
Diabetics Berberin\45days	320±13.2	291±11.9	-29 a
Diabetics Barberine\90days	315±5.2	289± 2.4	-26 a

Different letters refer to significancy $p<0.05$

Many studies were established for the investigation of the effects of berberine on body weight , Berberine at a dose of 100 mg/kg/day to 500 mg/kg/day chemically attenuates with the synthesis of enteroendocrine peptides involved in the glucose and energy homeostasis of obese people. It modulates gut microbiota by elevating intestinal peptides such as GLP-1, GLP-2 and peptide YY and decreasing gastric inhibitory polypeptides (Ilyas *et al*., 2020).

Berberine has also show significant efficacy in the treatment of diabetes mellitus, enhancing body weight in diabetic rats duo to its hypoglycemic effect is attributed to increased insulin secretion, improved insulin resistance, and amelioration of dyslipidemia (Khashayar *et al*., 2021).

4.1.2 Effect of berberine 45 and 90 days on the levels of Fasting blood glucose and insulin in male rats

Table 4-2 show the results of fasting blood glucose and insulin in study groups. The fasting blood glucose and serum insulin levels in the control and diabetic groups showed significant differences. The diabetic group demonstrated considerably higher fasting blood glucose levels (460±22.7 mg/dl) compared to the control group (122±2.9 mg/dl). Similarly, a significant reduction in serum insulin levels was observed in the diabetic group (0.157±0.07 ng/dl) compared to the control group (0.301±1.1 ng/dl), thus indicating the effects of diabetes on these parameters.

On comparing the Berberine group with the control, a significant reduction in fasting blood glucose levels was observed in the Berberine group (81.4±3.6 mg/dl) compared to the control. However, there was no significant difference in serum insulin levels between these two groups,

as both groups reported similar values (0.187±0.04 ng/dl for Berberine group, 0.301±1.1 ng/dl for control group).

When comparing the Diabetic Berberine groups (both 45 days and 90 days) with the Diabetic group, it was found that treatment with Berberine for both time periods significantly reduced fasting blood glucose levels, bringing them closer to control levels. Moreover, both Diabetic Berberine groups displayed significantly higher serum insulin levels than the Diabetic group, with the 45-day treatment group showing the highest increase (0.395±0.09 ng/dl), signifying the potential therapeutic effect of Berberine in managing diabetes.

Table 4-2: Effect of berberine on Fasting blood glucose (mg/dl) levels and serum insulin (ng/ dl) levels in study groups treated with berberine for 45 and 90 days rats

Groups\n=6	Fasting blood glucose (mg/dl)	Serum insulin (ng/dl)
	Mean ± SE	
Control	122±2.9 b	0.301±1.1 b
Diabetics	460±22.7 c	0.157±0.07 a
Berberine	81.4±3.6 a	0.187±0.04 a
Diabetics Berberine\45days	110±4.1 ab	0.395±0.09 c
Diabetics Berberine\90days	115± 2.8 b	0.348± 0.4 b

Different letters refer to significancy $p<0.05$

The results of the current study consistent with the result of several previous studies which also found similar effect for berberine on diabetic rats (Tang *et al*., 2006; Wang *et al*., 2009; Zhou *et al*., 2009). Berberine,

recognized for its efficacy in treating diabetes mellitus, operates by several mechanisms to reduce plasma glucose and elevate insulin levels. It stimulates insulin secretion, ameliorates insulin resistance, and improves dyslipidemi. Berberine affects glucose and lipid metabolism through the AMPK/p38 MAPK/GLUT4, JNK, and PPAR pathways (Zhang *et al*., 2012).

Additionally, it promotes the activity of the glucose transporter (GLUT1), an insulin-insensitive glucose transporter, via p38 MAPK and ERK-dependent pathways (Cok *et al*., 2011). Berberine also escalates insulin receptor (InsR) expression and InsR mRNA expression in human liver cells and rat skeletal muscle cells in a dose- and time-dependent manner, achieved through PKC-dependent activation (Zhang *et al*., 2010a).

Berberine minimizes gluconeogenesis in the liver, leading to increased peripheral glucose utilization. It inhibits pyruvate transport into mitochondria, reducing fatty acid oxidation and consequently lowering acetyl CoA levels (Wang *et al*., 2017).

Moreover, berberine boosts the activity of the SIRT1 signaling pathway by elevating the NAD+/NADH ratio, which assists in mitigating diet-induced insulin resistance (Yin *et al*., 2008). Berberine also improves insulin sensitivity and hyperglycemia by inhibiting mitochondrial dysfunction triggered by a high-fat diet and hyperglycemia (Gomes *et al*., 2012).

4.1.3 Effect of berberine 45 and 90 days on the levels of Lipid profile and Total Antioxidants in male rats

It is evident from Table 4.3 that the mean Total Cholesterol (TC) in mg/dl significantly differs among the groups. The Diabetic group had the

highest levels of TC (183.4±2.2), which were significantly higher than the Control (87.2±1.6) and Berberine (85.8±1.1) groups. Interestingly, the Diabetic groups treated with Berberine for 45 and 90 days showed a reduction in TC levels, with 120.6±2.3 and 114.4±3.2 respectively, indicating a significant improvement.

The Low-Density Lipoprotein (LDL) levels were significantly higher in the Diabetic group (177.4±3.) compared to the Control (94.7±0.3) and Berberine (92.5±1.6) groups. However, Diabetic groups treated with Berberine for 45 and 90 days showed a decline in LDL levels, with values of 100.1±2.1 and 110.3±1.9 respectively, suggesting a significant reduction due to Berberine treatment.

Our findings corroborate the existing body of research that supports the cholesterol-lowering effects of berberine in diabetic rats. These results are consistent with previous studies, such as those conducted by (Chen *et al*., 2011; Tang *et al*., 2006; Wang *et al*., 2014; Zhou *et al*., 2008).

BBR significantly inhibits intestinal cholesterol absorption, leading to large decreases of plasma total and nonHDL cholesterol levels. This is another mechanism distinct from the previously reported enhancement of LDLR-mediated liver LDL-C clearance (Kong *et al*., 2004). In the presence of BBR, the content of cholesterol in micelles is significantly decreased. This is due to BBR’s ability to interact with both hydrophobic and hydrophilic molecules of micelles, leading to the formation of agglomerates and reducing the capacity of micelles to incorporate cholesterol (Megyesi & Biczók, 2007).

BBR can also interfere with the passive penetration and/or affect other sterol transporters and proteins involved in intraluminal cholesterol

influx and efflux in the epithelium of the small intestine (Wang *et al*., 2010). This can result in a change of cell membrane properties, reducing its permeability, and leading to a decrease of cholesterol uptake. BBR decreases ACAT2 protein expression by downregulating its gene transcription (Rudel *et al*., 2005). ACAT2 is expressed only in hepatocytes and enterocytes, providing cholesteryl esters for transport in lipoproteins. Therefore, BBR might be an ACAT2 specific inhibitor in the enterocyte. BBR also inhibits cholesterol esterification in Caco-2 cells (Chang *et al*., 2009). This was observed in an in vitro permeability assay where the amount of cholesterol esters in the medium of basolateral chamber was significantly decreased in the presence of BBR and the effect was dose-dependent. This effect was in agreement with the reduced gene and protein expressions of ACAT2 in both the Caco-2 cells and small intestine (Wang *et al*., 2014).

The Triglyceride (TG) levels followed a similar trend to the TC results. The Diabetic group had significantly higher levels of TG (181.3±2.3) compared to both the Control (67.3±1.4) and Berberine (64.1±2.1) groups. However, the Diabetic groups treated with Berberine for 45 and 90 days showed a decrease in TG levels, with values of 78.1±3.1 and 82.3±1.1 respectively, suggesting a significant therapeutic effect of Berberine on TG levels.

These research efforts have consistently demonstrated that berberine exhibits a similar effect in terms of reducing triglyceride levels (Jun *et al*., 2004; Wang *et al*., 2014; Wu *et al*., 2012; Yin *et al*., 2012).

Berberine (BBR) reduces triglyceride levels by stimulating the activity of AMP-activated protein kinase (AMPK), a key regulator of lipid metabolism . It can phosphorylate AMPK in a time- and dose-dependent

manner in Hep G2 cells. The activation of AMPK leads to the inhibition of Acetyl-CoA Carboxylase (ACC), a critical enzyme in fatty acid biosynthesis and oxidation. This reduces fatty acid levels (Jia *et al*., 2008).

Moreover, BBR's activation of AMPK suppresses total cholesterol and triglyceride levels by inhibiting the activity of Glycerol-3-Phosphate Acyltransferase (GPAT) and 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase (HMGR), both of which are rate-limiting enzymes in cholesterol and triglyceride synthesis (Ruderman & Prentki, 2004).

BBR also impacts gene expression involved in lipid metabolism. It inhibits the expression of genes including Fatty Acid Synthase (FAS), HMGR, ACC, and GPAT. Conversely, BBR enhances the expression of Carnitine Palmitoyltransferase-1 (CPT-1) and malonyl-CoA decarboxylase (mCAD), key enzymes in fatty acid oxidation (Kim *et al*., 2009).

BBR's metabolites (particularly M2) also have potent triglyceride-lowering effects. Like BBR, M2 influences AMPK activity and has an impact on the expression of relevant genes. M2 significantly suppresses the expression of FAS, HMGR, ACC, and GPAT. Unlike BBR, M2 only increases the expression of mCAD without affecting that of CPT-1. The triglyceride-lowering properties of BBR and its M2 metabolite are attributed to their ability to down-regulate hepatic fatty acid synthesis genes and partly up-regulate β-oxidation genes. These effects are largely mediated through the activation and modulation of the AMPK signaling pathway (Cao *et al*., 2013).

The High-Density Lipoprotein (HDL) levels among the groups varied significantly. The Diabetic group had the lowest HDL levels (50.4±1.2),

which were significantly lower than that of the Control (93.1±2.1) and Berberine (96.1±2.4) groups. The Diabetic groups treated with Berberine for 45 and 90 days showed an increase in HDL levels, reaching 89.5±1.4 and 83.7±0.3 respectively, indicating a significant positive impact of Berberine treatment on HDL levels.

Previous studies have also supported our findings, indicating that berberine increases HDL levels. (Leng *et al*., 2004; Punitha *et al*., 2005; Tang *et al*., 2006).

Berberine has been found to have several mechanisms that contribute to the increase in HDL (high-density lipoprotein) levels. Firstly, berberine enhances the synthesis and secretion of apo-A1, a key protein involved in HDL functionality. Studies have shown that berberine can increase the concentration of apo-A1 and HDL-C particles while decreasing the apoB/apo-AI ratio in both cell lines and animal models (Sola *et al*., 2014; Zhou *et al*., 2008).

Furthermore, berberine promotes cholesterol efflux, is the process of removing cholesterol from cells and transferring it to HDL particles for transport out of the body. It achieves this by up-regulating the expression of ABCA1, a protein involved in cholesterol efflux, through activation of specific signaling pathways (Yang *et al*., 2020). Berberine also modulates cholesterol metabolism by increasing the expression of LCAT, an enzyme responsible for esterifying cholesterol. Additionally, berberine has been shown to elevate PON1 expression, an enzyme associated with HDL that inhibits the accumulation of oxidized lipids on HDL particles. Lastly, berberine inhibits SphK1 and S1P production, which are involved in various disorders including atherosclerosis, diabetes, and

neurodegeneration (Huang *et al*., 2012; Lan *et al*., 2010; Peng *et al*., 2011).

The Total Antioxidants (T.A.O.) levels were highest in the Berberine group (359.0±0.6), followed by the Control group (340.3±4.1), while the Diabetic group had significantly lower levels (221.7±4.5). However, Diabetic groups treated with Berberine for 45 and 90 days showed an increase in T.A.O. levels, with values of 311.6±2.2 and 320.1±4.7 respectively, indicating a significant improvement due to Berberine treatment.

In agreement with our study, it was observed in several studies that the administration of berberine resulted in a significant increase in antioxidants (Adefegha *et al*., 2022; Kumaş *et al*., 2019; Zhou & Zhou, 2011; Zych *et al*., 2020).

The treatment with berberine significantly restores the levels of both enzymatic and nonenzymatic antioxidants in diabetic animals. This restoration suggests that berberine enhances the capacity for detoxification by increasing the scavenging of oxy-radicals. The overexpression of these antioxidant parameters in diabetic rats treated with berberine highlights the reactivation of potential oxidant defense and the improved ability to counteract oxidative stress (Punitha *et al*., 2005).

Table 4-3: Effect of berberine on Levels of Lipid profile and Total Antioxidants in study groups treated with berberine for 45 and 90 days rats

Parameters Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	T.A.O.
	Mean± SE				
Control	87.2±1.6 a	67.3±1.4 [a]	93.1±2.1c	94.7±0.3 a	340.3±4.1 c
Berberine	85.8±1.1 a	64.1±2.1 a	96.1±2.4 c	92.5±1.6 a	359.0.6 c
Diabetics	183.4±2.2 c	181.3±2.3c	50.4±1.2 a	177.4±3.c	221.7±4.5 a
Diabetics Berberine\ 45 days	120.6±2.3b	78.1±3.1 a	89.5±1.4[bc]	100.1±2.1a	311.6±2.2b
Diabetics Berberine\ 90days	114.4±3.2b	82.3±1.1 b	83.7±0.3 b	110.3±1.9 b	320.1±4.7b

Different letters refer to significances $p<0.05$

4.1.4 Effect of berberine 45 and 90 days on the levels of Testosterone, Luteinizing hormone, Follicle stimulating hormone, Prolactin and Estradiol in male rats

Results in table 4-4 showed the levels of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, and estradiol were measured in male rats treated with berberine for 45 days and 90 days.

Testosterone levels in the control group were 6.25±2.3 ng/mL, while in the berberine group they were slightly higher at 6.91±1.4 ng/mL. However, in the diabetic group, testosterone levels were significantly

lower at 4.01±2.1 ng/mL. In the diabetic group treated with berberine for 45 days, testosterone levels increased to 4.78±2.4 ng/mL, and in the group treated for 90 days, they further increased to 5.22±1.2 ng/mL.

LH levels in the control group were 3.81±2.2 ng/mL, which slightly increased to 4.10±1.5 ng/mL in the berberine group. In the diabetic group, LH levels were significantly lower at 1.12±1.2 ng/mL. After berberine treatment for 45 days and 90 days, LH levels increased to 1.93±2.1 ng/mL and 3.96±1.3 ng/mL, respectively.

FSH levels in the control group were 1.94±1.6 ng/mL, slightly increasing to 2.3±1.2 ng/mL in the berberine group. In the diabetic group, FSH levels were similar to the control group at 1.33±0.6 ng/mL. After berberine treatment for 45 days and 90 days, FSH levels were 1.54±1.02 ng/mL and 1.72±2.2 ng/mL, respectively.

Prolactin levels in the control group were 5.32±0.4 ng/mL, slightly decreasing to 4.61±1.3 ng/mL in the berberine group. In the diabetic group, prolactin levels were significantly higher at 7.67±3.1 ng/mL. After berberine treatment for 45 days and 90 days, prolactin levels decreased to 6.36±1.1 ng/mL and 5.88±2.5 ng/mL, respectively.

Estradiol levels in the control group were 22.8±1.1 ng/mL, which decreased to 17.6±2.1 ng/mL in the berberine group. In the diabetic group, estradiol levels were significantly higher at 90.1±12.4 ng/mL. After berberine treatment for 45 days and 90 days, estradiol levels decreased to 69.9±3.7 ng/mL and 53.6±0.5 ng/mL, respectively.

Table 4-4: Effect of berberine on Levels of Testosterone, Luteinizing hormone, Follicle stimulating hormone, Prolactin and Estradiol in study groups treated with berberine for 45 and 90 days rats

Parameters Groups	T (ng/mL)	LH (ng/mL)	FSH (ng/mL)	Prolactin (ng/mL)	Estradiol (ng/mL)
	Mean ± SE				
Control	6.25±2.3 c	3.81±2.2 b	1.94±1.6 b	5.32±0.4 ab	22.8±1.1 a
Berberine	6.91±1.4 c	4.10±1.5 b	2.3±1.2 b	4.61±1.3 a	17.6±2.1 a
Diabetics	4.01±2.1 a	1.12±1.2 a	1.33±0.6 a	7.67±3.1 d	90.1±12.4 d
Diabetics Berbrine \45days	4.78±2.4 ab	1.93±2.1 a	1.54±1.02 a	6.36±1.1 c	69.9±3.7 c
Diabetics Berberine\ 90days	5.22±1.2 b	3.96±1.3 b	1.72±2.2 ab	5.88±2.5 b	53.6±0.5 b

Different letters refer to significancy $p<0.05$.T, Testosterone . FSH, Follicle stimulating hormone. LH, Luteinizing hormone

Consistent with our findings, a previous study demonstrated that treatment with berberine in diabetic rats with erectile dysfunction (ED) resulted in similar outcomes. Specifically, the study reported an increase in testosterone, luteinizing hormone, and follicle-stimulating hormone levels, along with a reduction in prolactin levels (Adefegha *et al*., 2021).

Berberine has been found to have several effects on hormone levels that are relevant testosterone regulation. Testosterone is a crucial

hormone for erectile function, and berberine has been shown to increase testosterone levels (Al-Roujayee, 2017). In individuals with diabetes, the testosterone level may decrease due to reduced sex hormone-binding globulin and increased aromatase activity in visceral adipose tissue, which converts testosterone to estradiol (Ballester *et al*., 2004). Berberine treatment in diabetic rats has been found to increase testosterone levels, reducing the risk of type-2 diabetes and exhibiting antihyperglycemic properties (Yao *et al*., 2018).

LH is a hormone that stimulates the Leydig cells in the testes to produce testosterone and dihydrotestosterone. In diabetic rats, LH levels may decrease due to altered hypothalamus function caused by the diabetic effect, leading to decreased thyrotropin-releasing hormone (TRH) secretion. Treatment with berberine in diabetic rats with ED has been shown to significantly increase serum LH levels, supporting its antihyperglycemic activity (Vuddanda *et al*., 2010).

FSH is a hormone that regulates the development, growth, and reproductive system. In diabetic rats, FSH levels may be reduced due to the disruption of insulin's modulatory effect on serum FSH levels (Vuddanda *et al*., 2010). Berberine treatment in diabetic rats has been found to improve FSH levels compared to untreated diabetic rats. The improvement in FSH levels was more pronounced with higher doses of berberine (Ballester *et al*., 2004).

Prolactin is a hormone that plays a role in Leydig cell function. In diabetes, there may be a decrease in serum prolactin levels, indicating impairment of Leydig cells (Auriemma *et al*., 2019). Berberine treatment in diabetic rats has been shown to increase prolactin hormone levels,

suggesting its ability to ameliorate hyperglycemia-induced hormonal imbalance (Ballester *et al*., 2004).

4.2 Histological Study Analysis

4.2.1 Effect of berberine for 45 days and 90 days on histological structure of testis in diabetic male rats

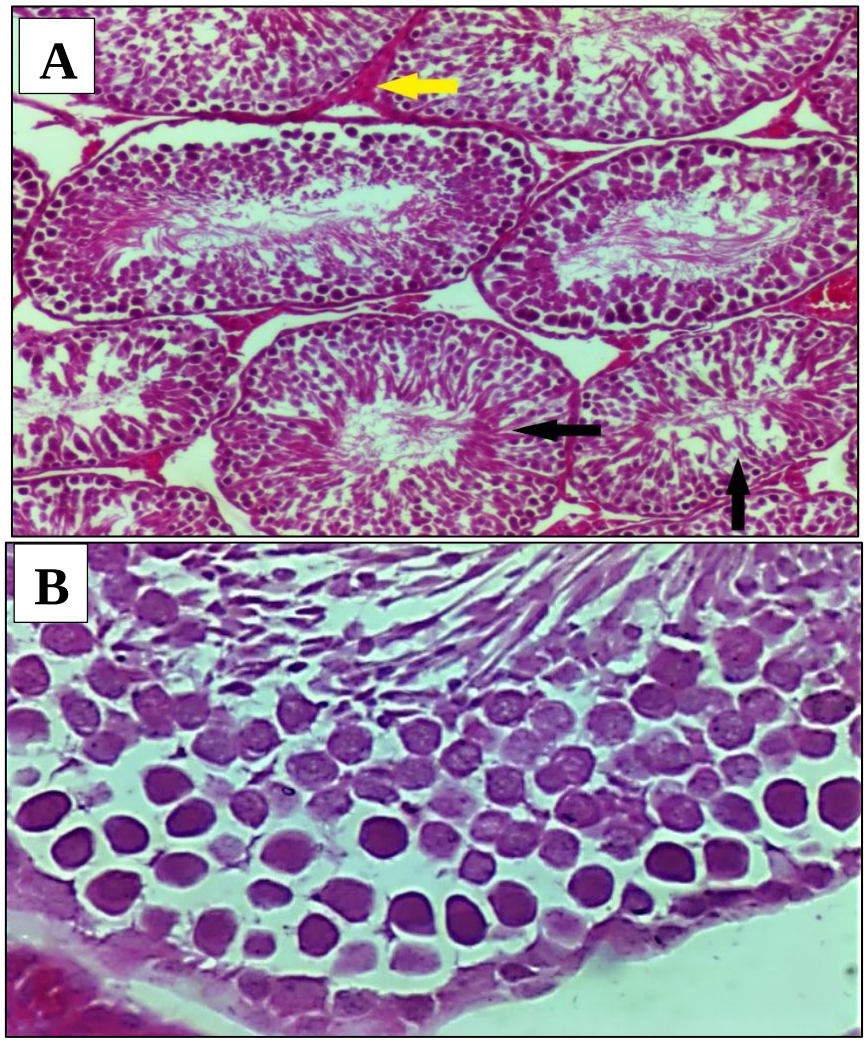

Figure 4-1: Cross section of rat testis from control group rat

A: Explained normal testicular histology architecture. Note the Seminiferous tubules (black arrow) showed normal appearance indicating with presence of all stage of spermatogenesis cells. Also, Leydig`s cells clusters (yellow arrow) were observed between those seminiferous tubules. **H&E 200x. B:** Cross section of seminiferous tubules. **H&E 400x.**

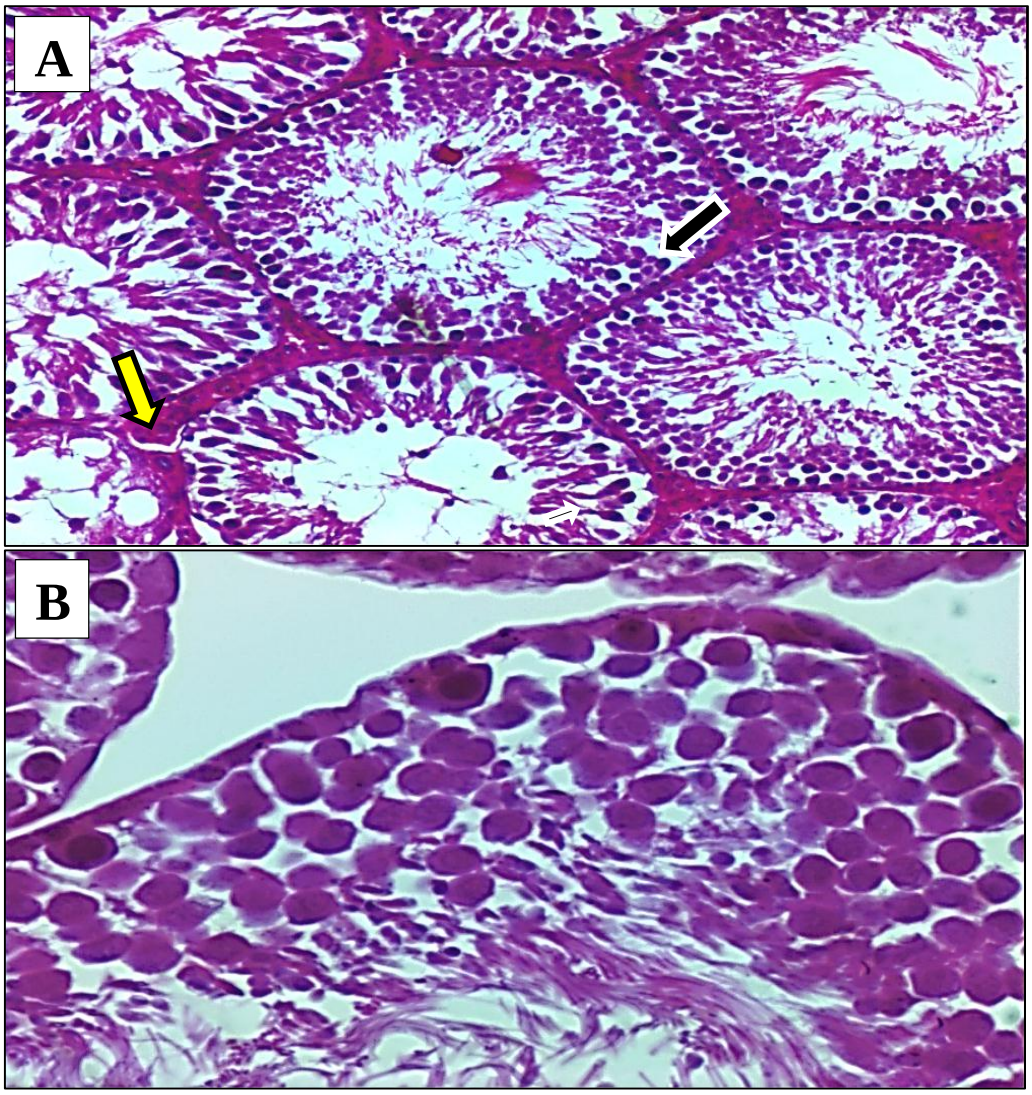

Figure 4-2: Cross section of rat testis from berberine group

A: Normal testicular histology architecture. Note the Seminiferous tubules (black arrow) showed normal appearance indicating with presence of all stage of spermiogenesis process cells. Also, Leydig`s cells clusters (yellow arrow) were observed between theses seminiferous tubules. **H&E 200x. B:** Cross section Seminiferous tubules. **H&E 400x.**

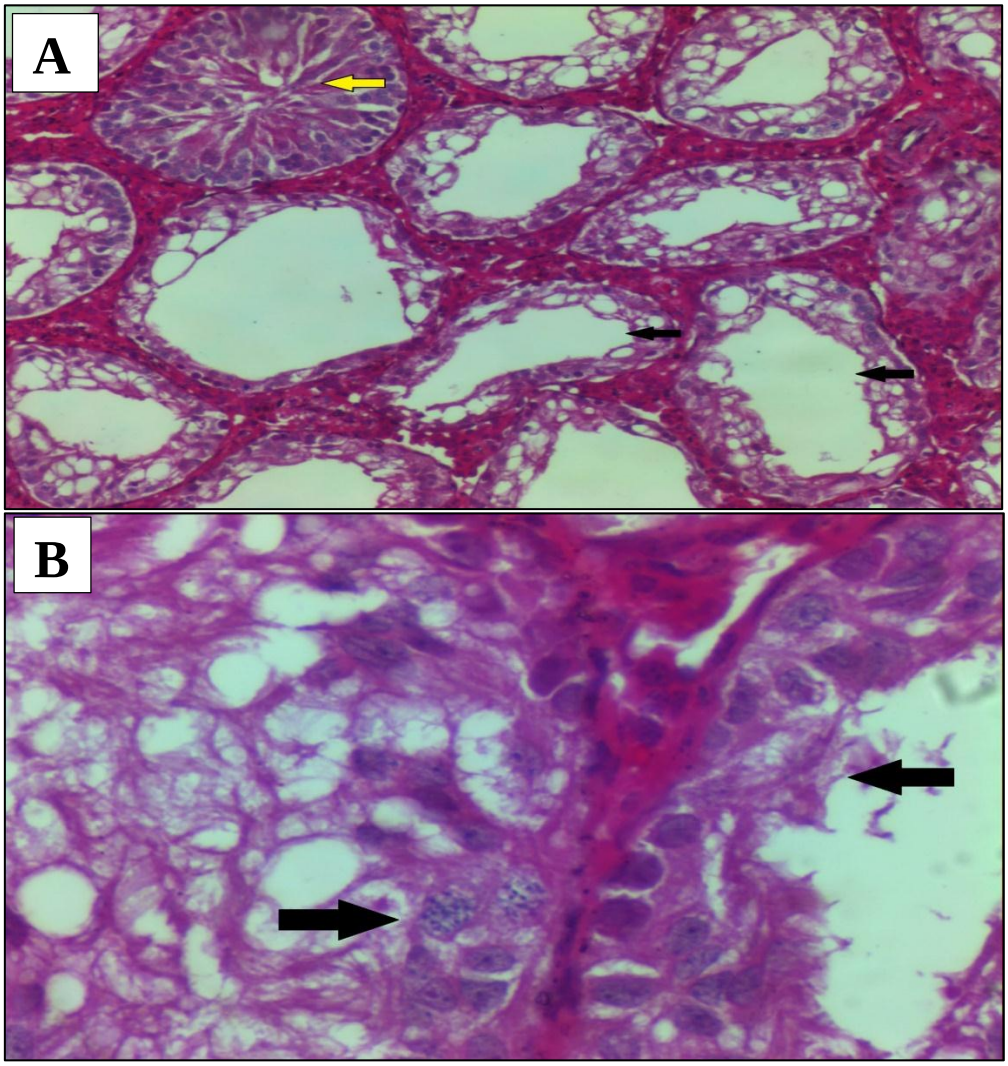

Figure 4-3: Photomicrograph of testes of diabetic rats group

A/ Note all seminiferous tubules (black arrow) was completely empty due to severe destruction of spermatogenesis process cells with presence of dead cells debris in affected area, however only one seminiferous tubule (yellow arrow) showed normal appearance due to presence of spermatogenesis process cells. **H&E 200x**. **B/** Note apoptotic bodies (black arrow) was observed in affected area and spermatogonium cells was under destruction with presence of apoptotic bodies were observed in affected area. **H&E 400x.**

Previous studies have confirmed that STZ-induced diabetes mellitus changes in testis consist of decreased STsD, degeneration of germinal cells, interstitial edema and capillary congestion in rats (Bal *et al*., 2011). Moreover, diabetes effects on the testicular tissue have been attributed to insufficient production of insulin which in turn results in decreasing the endocrine function of both Leydig and Sertoli cells. Diabetes decreased serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone and also FSH can severely affect spermatogenesis and the endocrine function of testis. Other studies showed that reduced insulin levels resulted in decreasing level of FSH in diabetic rats (Ballester *et al*., 2004; Mallick *et al*., 2007). Ballester *et al*. (2004) explained the decreased seminiferous tubular diameter in diabetes by the decreased level of FSH, which in turn, will diminish the response of the epithelium of seminiferous tubules to FSH stimulation. Because of insulin insufficiency insulin-mediated cell proliferation decreases in seminiferous tubules, resulting in a decrease in the number of spermatogonia in seminiferous tubules and thus leading to a decrease in seminiferous tubule diameter. Seminiferous diameter has also been reported to be reduced in STZ-induced diabetes mellitus (Guneli *et al*., 2008)

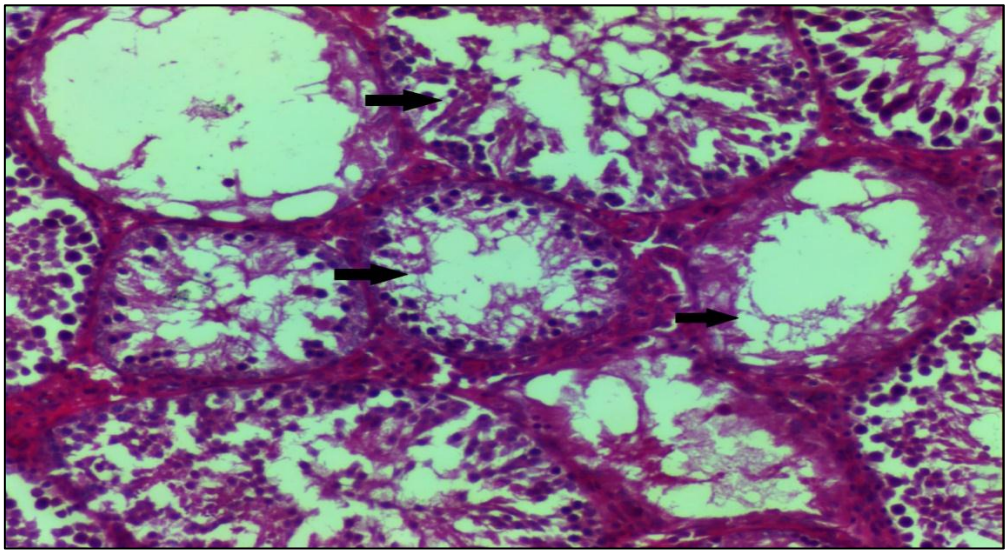

Figure 4-4: Cross section of rat testis from diabetic group treated with berberine for 45 days rat

Note that about 30% of appeared seminiferous tubules (black arrow) were appeared empty due to destruction of spermatogenesis process cells in these tubules. The density of spermatogenesis process cells (black arrow) was higher than in diabetic group. **H&E. A: 200x**.

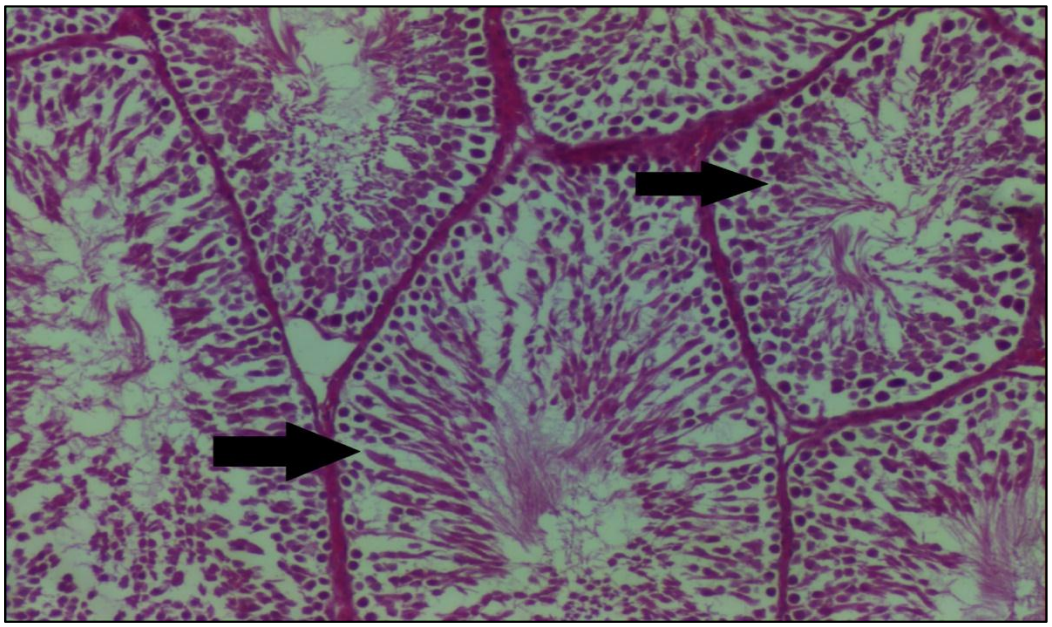

Figure 4-5: Cross section of rat testis from diabetic group treated with berberine for 90 days rat

Seminiferous tubule (black arrow) showed normal appearance due to presence of spermatogenesis process cells. H&E. A: 200x.

The protective effect of berberine on spermatogenesis in diabetic rats is supported by experimental data. BB administration reduces oxidative stress in diabetic rats by decreasing the expression of NOX5 and p22phox and reducing ROS production. This antioxidant effect aligns with current research findings on BB's role in diabetes, where it has been shown to have therapeutic benefits in diabetic complications such as erectile dysfunction and kidney injury (Suresh & Prakash, 2012). Additionally, BB inhibits JAK2 phosphorylation in the testis, leading to a decrease in the activation of the NFκB pathway and apoptosis levels. These findings suggest that BB protects spermatogenesis in diabetic rats by inhibiting the ROS/JAK2/NFκB pathway, thereby reducing cell injury and apoptosis in the testis (Ren *et al*., 2020). Overall, the mechanisms of action of BB involve alleviating oxidative stress, exerting an antioxidant effect, and modulating signaling pathways to promote the health and function of the seminiferous tubules in diabetic rats (Song *et al*., 2020b).

4.2.2 Effect of berberine for 45 days and 90 days on epididymis histology in diabetic male rats

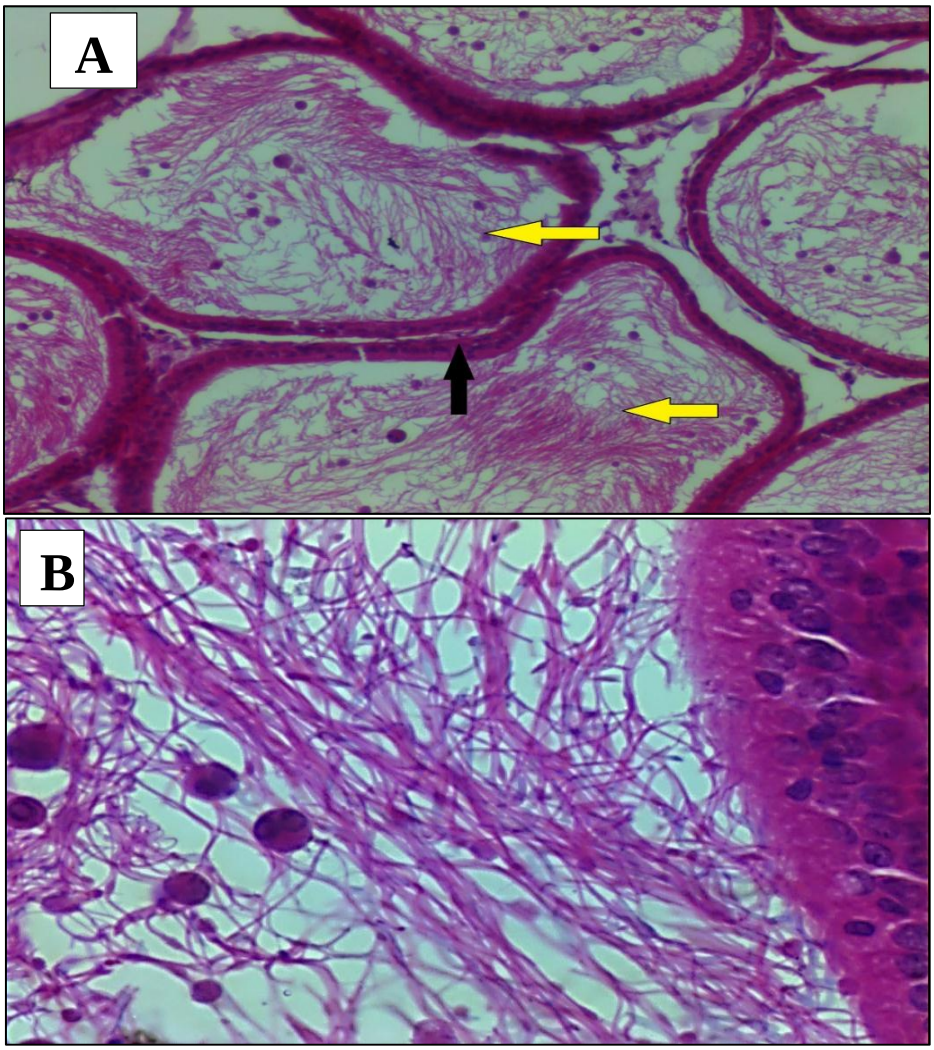

Figure 4-6: Cross section of epididymis of control rats group

A&B/ Normal histological architecture of epididymis. Note the columnar epithelial cells (black arrow) of epididymis ducts, and epididymis lumen was filled with sperms (yellow arrow). **H&E. A: 200x and B: 400x.**

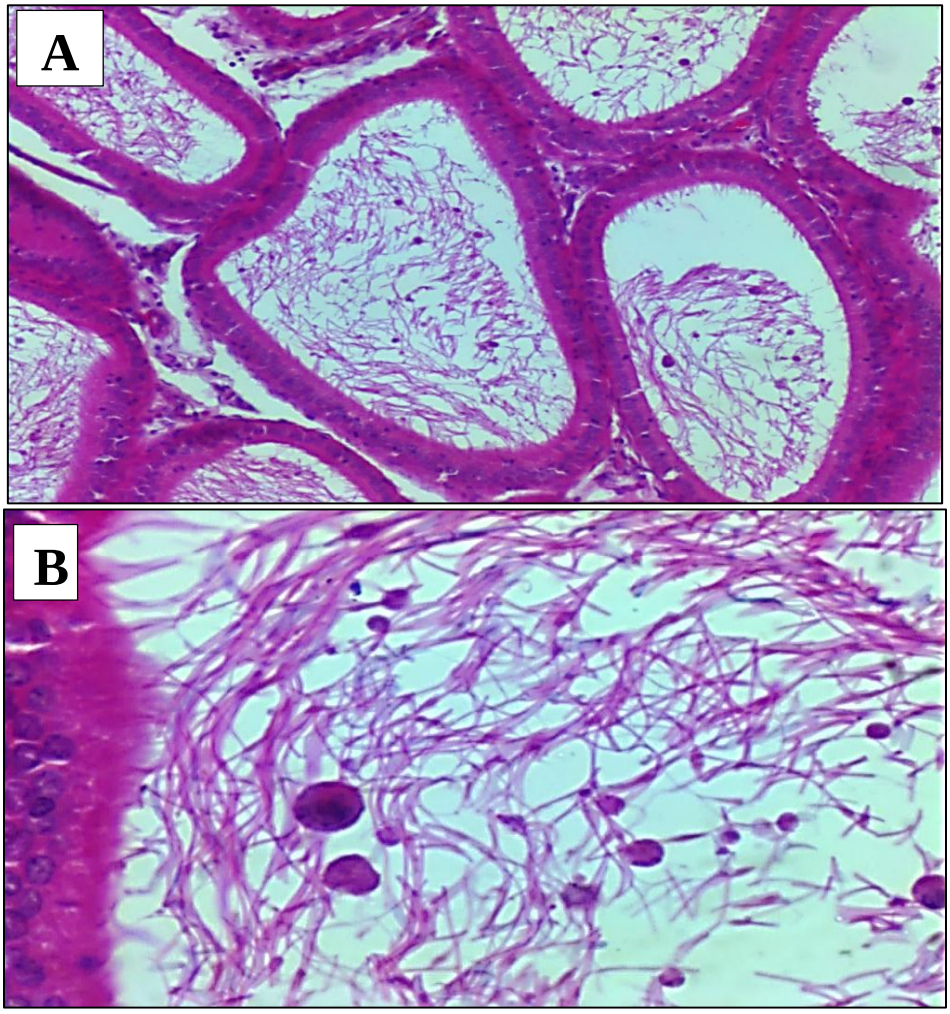

Figure 4-7: Cross section of epididymis of berberine only treated group rat

A&B/ Normal histological architecture of epididymis. Note the columnar epithelial cells of epididymis ducts, and epididymis lumen was filled with sperms. **H&E. A: 200x and B: 400x. Histological Changes in Epididymal**

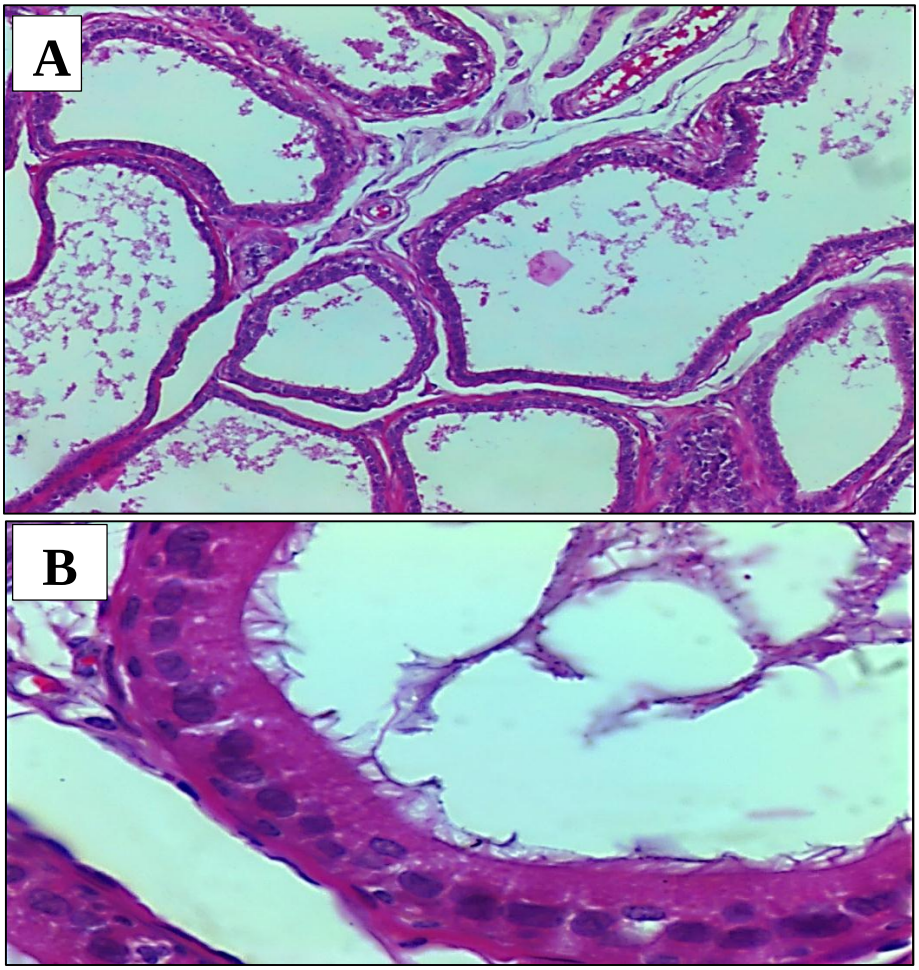

Figure 4-8: Cross section of epididymis of diabetes rat group

A&B/ Note few numbers of sperm was observed in the epididymis lumen, where the lumen of epididymis have few sperms. However, the columnar epithelial cells of epididymis showed normal appearance.

H&E. A: 200x and B: 400x.

We note that in the epididymis of control rats, many large spherical E-vesicles of different sizes and multi-rimmed bodies were found in the cytoplasm above the apical nucleus of the main cells. Bullae protruding from the apical cytoplasm that appear in the lumen are often described as manifestations of endocrine secretion. Such a pattern of histological organization is less common in principal cells of diabetic rats, indicating suppressed secretory activity of these cells. The decreased luminal

volume density of epididymal segments observed in the present study also lends support to the diminished secretory activity of this cell type, as tubular lumen size is largely determined by the amount of fluid or substances secreted by the principal cells and partly by the sperm concentration. Another prominent feature observed in the histological pictures of diabetic rats is the relative increase in the number of undifferentiated cells in the epididymal epithelium, which made the distinction among the different cell types of the epididymis difficult.

We also note that the diabetic animals had a decrease in the diameter of the seminiferous tubules compared to the control group. Pipe structure failure. There was a decrease in the number of spermatocytes, that is, primary and secondary spermatocytes. A decrease in the number of spermatid was also observed. There was a decrease in Sertoli cells, as well as in Leydig cells. An increase in the thickness of the basal membrane of the tubules is observed. Moreover, the spaces between the seminiferous tubules showed less/scarce connective tissue.

Increased blood glucose in diabetic rats created vacuoles and shrinkage in basal membrane. In diabetic rats, epididymal lumen is almost devoid of sperm. This is due to releasing free radicals in diabetic rats. Physiologic cell death is a major factor to increase fibromuscular and interstitial tissue in diabetic rats (Kamani *et al*., 2017).

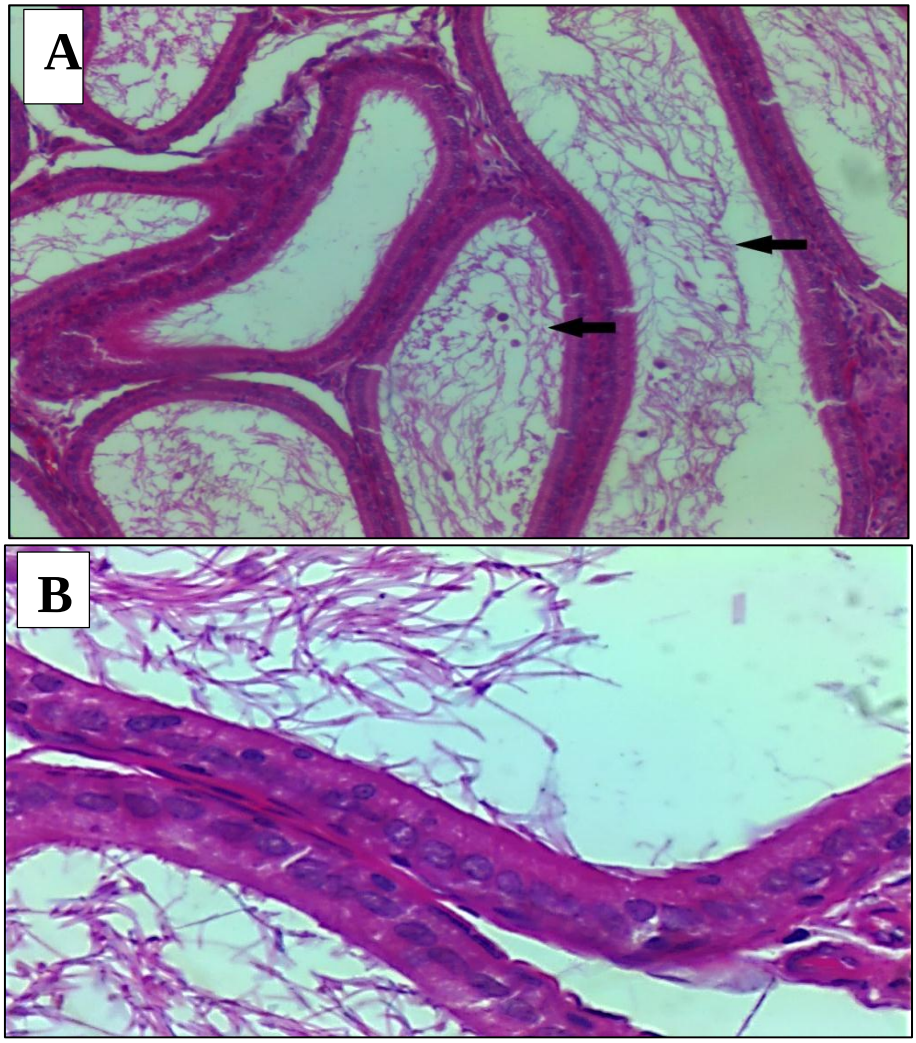

Figure 4-9:Cross section of epididymis from diabetic group treated with berberine for 45 days rat

A&B/ The sperms (black arrow) were observed in epididymis lumen, however the density of sperms in this epididymis lumen was higher than diabetic group. H&E. A: 200x and B: 400x.

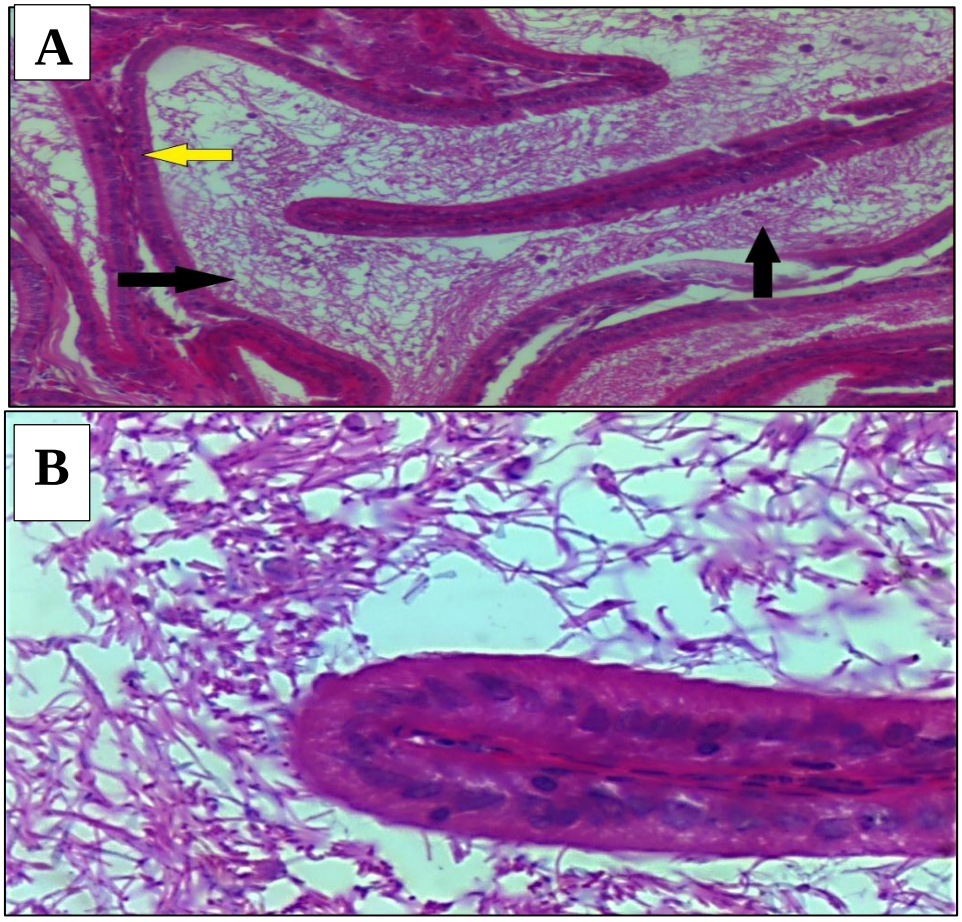

Figure 4-10: Cross section of epididymis from diabetic group treated with berberine for 90 days rat

A&B/ sperms (black arrow) filled the epididymis lumen, with presence of normal columnar epithelial cells. **H&E. A: 200x and B: 400x.**

Similar to its effect on Seminiferous tubule berberine has also been found to enhance epididymis histology and spermatogenesis in diabetic rats. A study investigated the pathogenic mechanism of ROS/JAK2/NFκB pathway in spermatogenic dysfunction caused by diabetes mellitus and evaluated the protective effect of BB on spermatogenesis and reproductive function. The study found that in DM rats, increased expression of NOX5 and p22phox, along with increased ROS content, led to the activation of JAK2, which upregulated the NFκB pathway and increased apoptosis in the testis. This resulted in impaired reproductive

function and infertility in DM rats. However, BB restored reproductive functions in DM rats by reducing ROS production, decreasing JAK2 activity, inhibiting NFκB pathway activation, and preventing apoptosis in the testis (Song *et al*., 2020b).

Further investigations revealed that oxidative stress played a significant role in impairing reproductive function and spermatogenesis in DM rats. Increased ROS content, NOX5, and p22phox expression were observed in the testis tissue of DM rats. The study also found that JAK2 expression and activity increased with elevated ROS levels. Activation of the NFκB pathway was evident through the increased phosphorylation of IκBα and p65. The study confirmed apoptosis in the testis of DM rats through Cleaved-Caspase3 expression and TUNEL staining (He *et al*., 2015; Modesti *et al*., 2005).

Berberine, as a herbal medicine, displayed antioxidant effects in DM. It could reduce ROS production, inhibit JAK2 phosphorylation, and attenuate NFκB pathway activation. This protective effect was consistent with previous research on BB's role in diabetes. Berberine treatment improved reproductive functions in DM rats, as indicated by increased fertility rates, enhanced sperm motility and morphology, and improved seminiferous tubule morphology (Song *et al*., 2020b).

To further understand the mechanisms, a study analyzed Sertoli cells (SCs) and found that JAK2 inhibition reduced cell apoptosis and NFκB pathway activation. Similar results were obtained with BB treatment, demonstrating its inhibitory effect on JAK2 and its ability to alter NFκB pathway activation and apoptosis (Gao *et al*., 2020).

Chapter Five

Conclusions and Recommendations

Conclusions:

This study reached the following conclusions:

1- Diabetes mellitus affects body weight, histology of the testis and epididymis, and can cause hyperlipidaemia when combined with low insulin levels.
2- The total antioxidant levels were decreased in diabetic animals.
3- Berberine exhibited an ameliorative effect on testicular and epididymis histology.
4- Berberine has multiple benefits for diabetes, including restoring insulin hormone levels, balancing fasting blood sugar, improving lipid profile levels, and enhancing antioxidant capacity in diabetic animals.

Recommendations

Based on the result of this study, the following recommendations were included for future work:

1- Study of genetic expression for JAK\2 gene that responsible for the antioxidant activity of berberine
2- Repeating the experiment on female animals and studying the effect of berberine on some hormones and histology of the female reproductive system.
3- Using immunohistochemical study for further assay of berberine histological restoration method .

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الخلاصة :

هدفت الدراسة الحالية إلى معرفة الدور المضاد لمرض السكر لعقار البربارين على بعض المؤشرات الكيموحيوية والنسيجية لبعض الأعضاء التناسلية فى ذكور الجرذان المصابة بداء السكري ، وقد أجريت الدراسة للمدة من شهر نوفمبر/ تشرين الثاني 2022 لغاية شهر ابريل/ نيسان 2022. وقد أجريت هذه الدراسة في البيت الحيواني التابع الى كلية العلوم/ جامعة بابل ومستشفى الحلة التعليمي العام. استخدمت 30 جرذًا ابيض بالغًا من الذكور البالغين الأصحاء الذين تتراوح أعمارهم بين 10–12 أسبوعًا ويزن (230–320) جرامًا. ؛ تم توزيعهم بشكل متساوي وعشوائي علي خمس مجموعات على النحو التالي:

المجموعة الأولى (I): مجموعة السيطرة تتكون من ستة جرذان تلقت محلول ملحي تم تجريعها فمويا. المجموعة الثانية (II): تتكون من ستة جرذان تم حقنها داخل الصفاق 60 مجم / كجم من وزن الجسم من الستربتوزوتوسين. المجموعة الثالثة (III): تتكون من ستة جرذان تناولت 200 مجم / كجم من وزن الجسم من البربرين عن طريق الفم. المجموعة الرابعة (IV): تتكون من ستة جرذان مصابة بداء السكري تناولت 200 مجم / كجم من وزن الجسم من البربرين عن طريق الفم لمدة 45 يومًا. المجموعة الخامسة (V): تتكون من ستة جرذان مصابة بداء السكري تناولت 200 مجم / كجم من وزن الجسم من البربرين عن طريق الفم لمدة 90 يومًا.

بعد انتهاء التجربة تم التضحية بالحيوانات، تم استخراج الدم والأعضاء للفحص الفسيولوجي والنسيجي، تضمنت الدراسة أيضًا قياس مستوى الدهون وقياس المستويات الهرمونية مثل هرمون التستوستيرون (T) ، الهرمون اللوتيني (LH) ، الهرمون المنشط للحوصلة (FSH) ، هرمون

البرولاكتين والإستراديول (E) وكذلك تأثير البربرين على مضادات الأكسدة الكلية (T.A.O.) والتغيرات النسيجية لكل مكون من مكونات الخصيتين والبربخ.

أوضحت النتائج انخفاضًا معنوياً في مستويات الدهون الثلاثية والكولسترول والكولسترول منخفض الكثافة وزيادة معنوية في مستويات الكولسترول عالي الكثافة في الحيوانات المصابة بداء السكري المعالجة بمكمل البربرين لمدة 45 و 90 يومًا مقارنة بمجموعات مرضى السكري ، وأوضحت النتائج حصول زيادة معنوية ($p<0.05$) في مضادات الأكسدة الكلية في المصل في مجموعة الجرذان التي عولجت بمكمل البربرين كمجموعة ضابطة إيجابية بالمقارنة مع المجموعة الضابطة ، مع انخفاضا معنويا ($p<0.05$) في مستويات مضادات الأكسدة الكلية في مصل الجرذان المصابة بداء السكري. أظهرت الجرذان المصابة بداء السكري المعالجة بمكمل البربرين زيادة معنوية في مستويات مضادات الأكسدة الكلية لمدة 45 يومًا (311.6 ± 2.2) و 90 يومًا (320.1 ± 4.7) على التوالي. أوضحت النتائج المتعلقة بوزن الجسم أن أوزان المجموعة المصابة بمرض السكر انخفضت بشكل كبير على عكس مجموعة الضوابط ومجموعة البربرين، بينما أظهرت الجرذان المصابة بداء السكري التي عولجت بمكمل البربرين لمدة 45 يومًا و 90 يومًا إعادة تثبيت في وزن الجسم. اظهرت النتائج ارتفاع معنوي ($p<0.05$) في مستوى الكلوكوز الصيامي في مصل الحيوانات المصابة بداء السكري بالمقارنة مع حيوانات المجموعة الضابطة ، وانخفاض معنوي ($p<0.05$) في مستويات الجلوكوز الصيامي في الفئران المصابة بداء السكري المعالجة بمكمل البربرين لمدة 45 يومًا (110 ± 4.1) و 90 يومًا (115 ± 2.8) على التوالي مقارنة بالجرذان المصابة بداء السكري ، في حين أوضحت أن متوسط مستويات الأنسولين في مصل جرذان مجموعة السكري انخفض بشكل ملحوظ ($P<0.05$) مقارنة مع مجموعة السيطرة ، بينما أظهرت مستويات الأنسولين في الدم في الجرذان المصابة

بالسكري التي عولجت بالبربرين لمدة 45 يومًا (0.395 ± 0.09) و 90 يومًا (0.348 ± 0.4) زيادة معنوية مقارنةً بالمجموعة المصابة بداء السكري.

بينت ايضا زيادة معنوية ($p<0.05$) و ($p<0.01$) لكل من مضادات الأكسدة الكلية ، ومستوى الهرمون هرمون التستوستيرون (T) ، والهرمون اللوتيني (LH) ، والهرمون المحفز للحويصلة (FSH) وانخفاض معنوي في هرمونات البرولاكتين والإستراديول في الجرذان المعالجة بالبربرين مقارنة مع الجرذان المصابة بداء السكري.

اوضحت النتائج النسيجية أن مرض السكري له العديد من التأثيرات النخرية على الأنابيب المنوية للخصية مع ضعف كامل نسبيًا في تكوين الحيوانات المنوية مع التليف الخلالي واحتقان الأوعية الدموية مع نزيف وترشيح الخلايا الاحادية؛ النتائج التي كانت تعتمد على الوقت تدريجيًا تم استعادتها بواسطة البربرين. استنتجت هذه الدراسة إلى أن البربرين له تأثيرات مخففة لمرض السكري على كل من العوامل الفسيولوجية والنسيجية المدروسة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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تأثير مكمل البربرين على بعض المعايير الكيموحيوية وبعض انسجة الاعضاء التكاثرية في ذكور الجرذان البيض المصابة بداء السكري

رسالة مقدمة
مجلس كلية العلوم للبنات- جامعة بابل كجزء من متطلبات نيل شهادة ماجستير العلوم في علوم الحياة

من قبل الطالبة
داليا بهاء الجبوري
بكالوريوس علوم حياة/ جامعة بابل/ كلية العلوم للبنات (2016)

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أيلول 2023م صفر 1445هـ