TO EVALUATE THE PHYTOCHEMICAL COMPOSITION AND FERTILITY ENHANCING PROPERTIES OF *ZINGIBER OFFICINALE* AND *MONDIA WHITEI* ETHANOLIC EXTRACTS ON REPRODUCTIVE HORMONES, SELECTED SPERM PARAMETERS AND TESTICULAR HISTOLOGY IN DIABETIC INFERTILE MALE WHITE ALBINO RATS

BY

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DECLARATION

I, Barasa M. Ambrose, declare that this thesis has not been submitted to any other university for a similar or any other academic award. All the information in this thesis is based on my own observations and in case of any consultation, the references are quoted.

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DEDICATION

This Research thesis is dedicated to my beloved wife Doreen Cherop Barasa, my Sons Steven Barasa, Brandon Barasa and Bryden Barasa, my father Mzee Stephen Barasa Kiberenge and my Mother Christine Barasa and my brothers and sisters.

ABSTRACT

Diabetes-induced male infertility (DIMI) affects approximately 48.5 million couples globally and contributes to 20-30% of male infertility cases associated with diabetes. The disease condition arises primarily from chronic hyperglycemia-mediated disruption of the hypothalamic-pituitary-testicular (HPT) axis, leading to impaired hormonal regulation, oxidative stress, testicular dysfunction, and reduced fertility. Although conventional therapies are available, their high cost and associated side effects limit their use. Oxidative stress and inflammation are key drivers of diabetes-induced male infertility; therefore, the antioxidant and anti-inflammatory activities of *Zingiber officinale* and *Mondia whitei* may restore testicular function, although their specific targets and comparative efficacy remain unclear. This study was designed to evaluate the phytochemical composition and antioxidant properties of *Zingiber Officinale* and *Mondia Whitei* ethanolic extracts on reproductive hormones, selected sperm parameters and testicular histology and histomorphometry in diabetic infertile male albino rats. The rhizomes of *Z. officinale* and roots of *M. whitei* were first extracted with ethanol for phytochemical analysis. Total phenolic content, tannins content, flavonoid content, saponins content, terpenoids content and alkaloids content were determined by spectrophotometer while *in vitro* antioxidant activities done using Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging assay and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Thirty-six male albino rats were used for *in vivo* studies and allocated into nine groups comprising a normal control, negative control (alloxan- induced diabetes), positive control (diabetic + clomiphene citrate), and six treatment groups (diabetic) receiving either *Z. officinale* or *M. whitei* at doses of 200, 400, and 800 mg/kg for 28 days. At the end of treatment period, blood samples were collected for luteinizing hormone (LH) bioassay and follicle stimulating hormone (FSH) and testosterone using enzyme-linked immunosorbent assay. Following treatment, the rats were anaesthetized and humanely euthanized, after which sperm from the cauda epididymis was collected for analysis of motility, morphology, count, and viability, while the testes were harvested for histological examination. Median differences among groups were analyzed using the Kruskal-Wallis test. Sperm characteristics were determined using a light microscope while the biochemical assays were assessed spectrophotometrically. The obtained numerical data was subjected to one-way ANOVA followed by Tukey's post hoc test for multiple comparisons of statistical differences in means among groups. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, and glycosides in both extracts. *Mondia whitei* contained higher levels of flavonoids, tannins, saponins, total phenolics (220 mg GAE/g), and greater antioxidant activity (IC_{50} = 76 µg/mL) than *Z. officinale* (IC_{50} = 120 µg/mL), while *Z. officinale* contained higher alkaloid levels (15 mg/g). Diabetes significantly suppressed plasma FSH, LH, and testosterone to 6.30 ± 1.78 mIU/ml, 1.28 ± 0.37 mIU/ml, and 71.53 ± 6.84 ng/dl, respectively ($p<0.001$), compared with normal and positive controls. Treatment with *Z. officinale* increased plasma FSH to 6.65 ± 0.84 mIU/ml and LH to 4.55 ± 0.14 mIU/ml at 800 mg/kg, although testosterone remained low when compared to those of the normal control. In contrast, *M. whitei* restored LH to 3.50 ± 0.18 mIU/ml and FSH to 6.07 ± 0.46 mIU/ml, while testosterone increased significantly and approached values like those in the normal control at 800 mg/kg ($p<0.001$). There were apparent histological alterations of seminiferous epithelium of the negative control with reduced seminiferous epithelial diameter and sloughing of epithelial cells. There was, however, no observable changes in the treatment groups when compared to the controls on this measure. There was also accompanying reduced testicular weight (1.02 ± 0.3 ml), testicular volume (1.11 ± 0.22 ml), and Leydig cell volume (501 ± 92.3 μm^3) in the negative control but these measures were improved in treatment groups, with *M. whitei* producing the greatest restoration of Leydig cell volume (1432 ± 346.7 μm^3; $p<0.001$) and testicular weight (3.38 ± 1.9 ml). In conclusion**,** **t**he findings have pointed at disruptive structural changes along the hypothalamo-pituitary-testicular axis following induction of diabetes that ultimately leads to infertility. The effects have further, shown effects on pituitary gonadotropins and testicular testosterone with accompanying reduced sperm quality manifested by impaired morphology, motility, count and viability. These effects were ameliorated by both *Zingiber officinale* and *Mondia whitei* with *Mondia whitei* exhibiting superior endocrine-restorative and testicular protective effects, particularly at 800 mg/kg. Further pharmacological and toxicological studies are recommended with a view of establishing the therapeutic molecules in these plants that may play key role in the management of diabetes-induced male infertility. The ever-increasing cases of diabetes in the male populations world-wide is a growing challenge that requires cheaper and readily available remedy in managing the condition. This study significantly provides scientific evidence on the comparative efficacy of *Zingiber officinale* and *Mondia whitei* in mitigating diabetes-induced male infertility by restoring reproductive hormones, sperm quality, and testicular structure, thereby supporting the development of affordable, plant-based therapeutic alternatives for managing diabetes-associated reproductive dysfunction.

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ABBREVIATIONS AND ACRONYMS

AZF	Azoospermia Factor
BTB	Blood- Testis Barrier
DIMI	Diabetes- Induced Male Infertility
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FSH	Follicle Stimulating Hormone
GPx	Glutathione Peroxidase
GnRH	Gonadotropin Releasing Hormone
GSR	Glutathione Disulphide Reductase
GST	Glutathione-s- Transferase
HPT	Hypothalamo-Pituitary-Testicular axis
IDF	International Diabetes Federation
KES	Kenya Shillings
LH	Luteinizing Hormone
MW	*Mondia whitei*
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
STD	Sexually Transmitted Diseases
SCs	Sertoli Cells
WHO	World Health Organization
TURP	Transurethral Resection of the prostate
UON	University of Nairobi

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes-induced male infertility (DIMI) is a serious public health issue that affects millions of people globally (Pal et al., 2024). The World Health Organization's (WHO) 2021 annual report estimates that 186 million people, or 15% of couples worldwide, are infertile Sun, H et al., 2021. Male infertility was estimated to impact 56 million persons globally in the same year, accounting for 40–50% of infertile couples (Elagamey et al., 2023). DIMI affects about 8% of men globally. According to Huang et al. (2024), 14% of males in Africa are infertile, which is the highest rate. In fact, the International Diabetes Federation's (IDF) 2024 annual report predicts that between 2019 and 2045, the prevalence of diabetes which is a risk factor for infertility would increase by 55% globally, with the biggest increases expected in Asia and Africa. Lotti et al. (2023) estimate that 4.5% of Kenyans have diabetes, and that number is expected to rise as a result of aging populations and lifestyle changes. Facondo et al. (2022) estimate that 12% of Kenyan men will experience diabetes-related infertility. Infertility causes a great deal of financial and social hardship for individuals and families.

According to Facondo et al. (2023), diabetes-induced oxidative stress, driven by excessive reactive oxygen species (ROS) generation, impairs testicular function by disrupting spermatogenesis and steroidogenesis, thereby contributing to male infertility. This is because severe structural damage along the Hypothalamus-Pituitary-Testicular (HPT) axis is caused by diabetes mellitus, especially if it is chronic and poorly controlled. As a result, male diabetics experience decreased libido, erectile dysfunction, infertility, and hypogonadism (Zhu et al., 2023). Diabetes-induced oxidative

stress disrupts hypothalamic regulation of the Hypothalamus-Pituitary-Gonadal axis, impairing spermatogenesis and steroidogenesis and ultimately causing male infertility (Zhu et al., 2023).

In diabetes mellitus, chronic hyperglycemia disrupts this balance by promoting excessive ROS production and triggering systemic inflammatory responses characterized by increased production of pro-inflammatory cytokines and inflammatory mediators. The resulting oxidative stress and inflammation damage Sertoli cells, Leydig cells, germ cells, and the blood-testis barrier, impairing steroidogenesis, spermatogenesis, and reproductive hormone regulation. Consequently, diabetes-induced oxidative and inflammatory injury compromises testicular function and contributes significantly to the development of male infertility (Gaznee et al., 2023; Venditti et al., 2024).

According to research by Abd-Elkareem et al. (2021), the testes are highly metabolically active organs because spermatogenesis and steroidogenesis require continuous energy production. Consequently, reactive oxygen species (ROS) are continuously generated as natural by-products of mitochondrial respiration and enzymatic activities involving cytochrome P450, xanthine oxidase, and NADPH oxidase (Rotimi et al., 2024). Under normal physiological conditions, ROS participate in cellular signaling, sperm maturation, germ-cell differentiation, and testosterone synthesis. To maintain redox balance and reproductive homeostasis, the testes rely on an integrated antioxidant defense system comprising enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, together with non-enzymatic antioxidants including glutathione, vitamins C and E, and other redox-regulating molecules (Abd-Elkareem et al., 2021). These protective mechanisms preserve cellular integrity, prevent oxidative injury, and support normal testicular function. Germ cells are further protected by the intrinsic antioxidant defense mechanism, which safeguards them against ROS-induced damage and maintains normal cellular development and function (Venditti et al., 2024).

According to Badejogbin et al. (2025), a key component of testicular protection is the blood-testis barrier (BTB), formed by tight junctions between Sertoli cells, which creates an immune-privileged environment essential for spermatogenesis. The BTB regulates nutrient transport, maintains a specialized microenvironment for germ-cell development, and prevents immune recognition of developing germ cells (Elsawy et al., 2023). In diabetes mellitus, chronic hyperglycemia causes excessive ROS production that overwhelms endogenous antioxidant defenses, leading to oxidative stress and disruption of the endocrine, paracrine, and autocrine networks that regulate testicular function (Gaznee et al., 2023). Oxidative damage compromises BTB integrity through injury to Sertoli cells and tight junction proteins, increasing barrier permeability and allowing infiltration of inflammatory mediators and immune cells into the seminiferous epithelium. According to Venditti et al. (2024), this exposes germ cells to immune attack, promotes apoptosis and cellular degeneration, impairs spermatogenesis and testosterone synthesis, and ultimately results in endocrine dysfunction, reduced fertility, and male reproductive impairment.

Male infertility associated with diabetes mellitus is currently addressed through conventional therapeutic approaches, including hormonal supplementation, antioxidant therapy, fertility-enhancing medications, and surgical interventions targeting different levels of the hypothalamic-pituitary-testicular axis. Despite these interventions, treatment outcomes remain inconsistent because many therapies primarily alleviate symptoms rather than effectively reversing the underlying oxidative stress and reproductive dysfunction induced by diabetes. This limitation highlights a critical research gap and underscores the need to explore herbal-based therapeutic alternatives with antioxidant and fertility-promoting properties as potential strategies for restoring

steroidogenesis, spermatogenesis, and overall male reproductive function in diabetic conditions (Venditti et al., 2024).

Despite the widespread traditional use of *Zingiber officinale* and *Mondia whitei* for managing male infertility, there is limited scientific evidence on their phytochemical composition, reproductive efficacy, and mechanisms of action in diabetes-induced male reproductive dysfunction, particularly regarding their ability to restore oxidative balance and by extension, effects on steroidogenesis and spermatogenesis, as well as reproductive hormone impairment associated with testicular dysfunction. Therefore, by conducting a phytochemical analysis of the two plants, it is essential to identify and compare the bioactive compounds present in the extracts, establish a scientific basis for their observed fertility-enhancing properties, and determine whether variations in phytochemical content contribute to differences in their therapeutic efficacy under diabetic conditions. The scientific basis on the efficacy of *Zingiber officinale* and *Mondia whitei* in restoring diabetes-induced male reproductive dysfunction remains unclear, necessitating phytochemical analysis to identify the bioactive compounds underlying differences in their therapeutic effectiveness.

The available information in literature reveals inconsistency in the reported fertility-enhancing efficacy of *Zingiber officinale* and *Mondia whitei*, with variations in findings attributed to differences in extract preparation, dosage, experimental models, geographical origin, and phytochemical composition. Furthermore, while both plants have shown beneficial effects on reproductive parameters in non-diabetic conditions, their efficacy, mechanisms of action, and therapeutic potential in diabetes-induced male infertility remain inadequately validated and inconsistently reported.

Zingiber officinale and *Mondia whitei* root extract are examples of herbal remedies that have been used as alternative medicine therapies, particularly among communities in western Kenya and other African communities where conventional medicines are not readily available (Abd-Elkareem et al., 2021). Although medicinal plants have been explored for their potential to alleviate male infertility, the findings regarding their therapeutic potential remain inconsistent. *Zingiber officinale* and *Mondia whitei* contain diverse bioactive phytochemicals such as flavonoids, phenolic compounds, gingerols, and essential oils that possess antioxidants, anti-inflammatory, antidiabetic, anticancer, antiemetic, and gastroprotective properties beneficial to reproductive health (Venditti et al., 2024). However, limited comparative information exists on the levels of these key phytochemicals and their relative potential as fertility-enhancing agents, highlighting an important knowledge gap that warrants further investigation.

Zingiber officinale and *Mondia whitei* have long been recognized for their antioxidant and fertility-enhancing properties, with studies in non-diabetic models demonstrating improvements in sperm quality, testicular histoarchitecture, and reproductive hormone profiles (Elagamey et al., 2023). Although these plant extracts have shown protective and restorative effects on the hypothalamic-pituitary-testicular axis through enhancement of neuronal function, gonadotropin secretion, Leydig cell activity, antioxidant defenses, and testicular architecture, their effectiveness in diabetes-associated male infertility remains inadequately validated and inconsistently reported across experimental models (Elagamey, et al., 2023). This inconsistency and limited evidence in diabetic conditions highlight a critical knowledge gap regarding the therapeutic potential of *Zingiber officinale* and *Mondia whitei* as interventions for diabetes-induced male reproductive dysfunction (Shehu et al., 2022).

Comparative studies on ***Zingiber officinale*** and ***Mondia whitei*** remain limited, and the molecular mechanisms through which their bioactive phytochemicals influence diabetes-induced male reproductive dysfunction are not fully understood. The scarcity of mechanistic, translational, and clinical evidence highlights a critical knowledge gap and warrants further investigation into their therapeutic potential for managing diabetes-associated male infertility.

1.2. Problem Statement

Sustained hyperglycemia is an emerging public health challenge that contributes significantly to male infertility by disrupting the structural and functional integrity of the hypothalamic-pituitary-testicular axis through oxidative stress, inflammation, and hormonal dysregulation, ultimately impairing spermatogenesis and steroidogenesis. The burden of hyperglycemia-associated male infertility is particularly pronounced in Africa, including Kenya, where the rising prevalence of diabetes, limited access to specialized reproductive healthcare, and the high cost of conventional interventions such as hormonal therapy, antioxidant supplementation, fertility medications, and surgical procedures restrict effective management. Although ***Zingiber officinale*** and ***Mondia whitei*** are widely used in traditional medicine for enhancing male reproductive health, scientific evidence regarding their effectiveness in mitigating the reproductive consequences of sustained hyperglycemia, including their effects on sperm quality, hormonal regulation, and histoarchitecture of the hypothalamic-pituitary-testicular axis, remains insufficient, thereby highlighting a critical research gap in understanding their underlying mechanisms and therapeutic potential. The present study was designed to analyse phytochemical composition and antioxidant properties of *Z. officinale* ethanolic rhizome extracts and *M. whitei* ethanolic root extracts and evaluate their effects on some reproductive hormone profiles by ELISA technique, selected sperm

parameters using light microscopic examination and analysis and accompanying testicular histology and histomorphometry.

1.3 Justification of Study

Diabetes-induced male infertility originates from disruption of the fundamental biological systems that sustain male reproduction (Abdelfattah et al., 2023). Chronic hyperglycemia generates excessive oxidative stress and inflammation, which damages the hypothalamic-pituitary-testicular axis, impair blood-testis barrier integrity, reduce Leydig and Sertoli cell function, suppress testosterone production, and compromise spermatogenesis, ultimately leading to infertility. Given that conventional therapies are often expensive, associated with adverse effects, and primarily manage symptoms rather than reversing the underlying pathological mechanisms, there is growing interest in identifying plant-derived therapeutics capable of restoring reproductive homeostasis **(Mabonga et al., 2021)**. Although *Mondia whitei* and *Zingiber officinale* have demonstrated antioxidant, anti-inflammatory, and fertility-enhancing properties, their efficacy in reversing diabetes-induced reproductive dysfunction remains inconsistently reported and inadequately validated, particularly due to variations in phytochemical composition arising from differences in geographical origin, environmental conditions, extraction methods, and dosage (Akunna & Lucyann, 2023). Therefore, this study employed white male albino rats as a translational model to characterize the phytochemical profiles and evaluate the reproductive efficacy of *Z. officinale* and *M. whitei*, with the aim of providing mechanistic evidence for their therapeutic potential and identifying bioactive compounds that could contribute to the development of affordable interventions for diabetes-related male infertility.

1.4.1 General Objective

To evaluate Phytochemical composition and fertility enhancing properties of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Reproductive Hormones, Selected Sperm Parameters and Testicular Histology in diabetic Infertile white albino male rats

1.4.2 Specific objectives

1. To determine and compare the phytochemical composition of ethanolic extracts of *Zingiber officinale* rhizomes and *Mondia whitei* roots.
2. To determine antioxidant properties of ethanolic extracts of *Zingiber officinale* rhizomes and *Mondia whitei* roots.
3. To evaluate the dose dependent restorative effects of *Zingiber officinale* rhizomes and *Mondia whitei* root ethanolic extract on the synthesis and secretion of plasma Follicle Stimulating Hormone, Luteinizing Hormone and testosterone on infertility induced by diabetes in white albino male rats.
4. To evaluate the dose dependent antioxidant effects of *Zingiber officinale* rhizomes and *Mondia whitei* root ethanolic extracts on sperm morphology, sperm count, motility and viability in on infertility induced by diabetes in white albino male rats.
5. To determine restorative dose dependent effects of *Zingiber officinale* and *Mondia whitei* root ethanolic extracts on the histology of the hypothalamus, anterior pituitary gland and testis on infertility induced by diabetes in white albino male rats.

1.5 Research Hypotheses

1. The ethanolic extracts of ***Zingiber officinale*** rhizomes and ***Mondia whitei*** roots do not possess significant phytochemical constituents that may contribute to ameliorating infertility induced by diabetes in white albino male rats.

2. The ethanolic extracts of ***Zingiber officinale*** rhizomes and ***Mondia whitei*** roots do not possess significant antioxidant properties that may restore infertility induced by diabetes in white albino male rats.

3. Ethanolic extracts of *Zingiber officinale* and *Mondia whitei* do not possess restorative effects on plasma levels of gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone, and testosterone arising from infertility induced by diabetes in white albino male rats

4. Ethanolic extracts of *Zingiber officinale* and *Mondia whitei* have no effect on sperm count, increased sperm motility, viability and morphology arising from infertility induced by diabetes in white albino male rats.

5. Ethanolic extracts of *Zingiber officinale* and *Mondia whitei* have no effect on the histoarchitecture of the hypothalamus, anterior pituitary and testes arising from infertility induced by diabetes in white albino male rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Sustained hyperglycemia is the central pathological event linking diabetes mellitus to male infertility because persistent elevation of blood glucose overwhelms normal cellular metabolism, leading to oxidative stress, chronic inflammation, mitochondrial dysfunction, endocrine disruption, and progressive testicular injury. Diabetes mellitus, a rapidly increasing global metabolic disease affecting over 422 million people worldwide, develops when insulin secretion, insulin action, or both become impaired, resulting in prolonged hyperglycemia and widespread cellular dysfunction (Sun et al., 2022).

The pathophysiology of sustained hyperglycemia begins with excessive intracellular glucose accumulation, which activates pathways such as advanced glycation end-product (AGE) formation, the polyol pathway, protein kinase C signaling, and mitochondrial overproduction of reactive oxygen species (ROS). These changes initiate a self-perpetuating cycle of oxidative stress and inflammation that damages mitochondria, impairs cellular energy production, disrupts insulin signaling, and compromises the structure and function of metabolically active tissues, including the testes. Consequently, chronic hyperglycemia alters the hypothalamic-pituitary-testicular (HPT) axis, suppresses gonadotropin and testosterone production, disrupts blood-testis barrier integrity, impairs Sertoli and Leydig cell function, and interferes with spermatogenesis and sperm maturation (Abd-Elkareem et al., 2021; Lotti et al., 2023, Sun et al., 2022).

As a result, diabetes-induced male infertility emerges from interconnected metabolic, oxidative, inflammatory, vascular, neurological, and endocrine mechanisms that reduce sperm quality, impair sexual function, and compromise reproductive capacity. The increasing prevalence of diabetes

mellitus among men of reproductive age therefore represents a significant contributor to the global burden of infertility, which currently affects approximately 15% of couples worldwide (Lotti et al., 2023; Sun et al., 2022).

2.2 Molecular Mechanism in Sustained Hyperglycemia

Persistent hyperglycemia disrupts testicular glucose metabolism, leading to mitochondrial dysfunction, excessive reactive oxygen species (ROS) production, and oxidative damage that impairs spermatogenesis and sperm maturation (Facondo et al., 2022). Elevated glucose levels also promote advanced glycation product (AGE) formation and inflammatory signaling, causing apoptosis of Sertoli, Leydig, and germ cells, as well as DNA damage that reduces sperm quality and fertility potential. Additionally, diabetes-induced insulin dysregulation disrupts the hypothalamic-pituitary-testicular axis, decreases testosterone production, and contributes to vascular and neurological complications that further impair male reproductive function (Zhu et al., 2023).

2.3 Burden of Diabetic Induced Male Infertility

The main causes of morbidity in diabetes mellitus are systemic problems, and chronic hyperglycaemia is a major contributor by causing the body to experience excessive oxidative stress (Facondo et al., 2022). Elevated levels of oxidative markers show that persistent hyperglycemia weakens antioxidant defenses and promotes the generation of reactive oxygen species (ROS), which causes oxidative damage to biological components, especially DNA (Facondo et al., 2022). Diabetes-related oxidative DNA damage affects germ cells as well as somatic tissues, jeopardizing the integrity and functionality of sperm DNA (Elagamey et al., 2023). Poor glycemic control and reproductive cells damage are directly linked by fasting blood glucose and HbA1c. Consequently,

oxidative DNA damage represents a key mechanistic pathway through which the systemic burden of diabetes contributes significantly to male infertility (Leisegang et al., 2021).

2.4 Effects of Sustained Hyperglycemia

Sustained hyperglycemia is the central metabolic disturbance in diabetes, driving oxidative stress, chronic inflammation, and disruption of cellular pathways that regulate glucose metabolism and tissue function (Sun et al., 2022). In type 1 diabetes, autoimmune destruction of pancreatic β-cells causes absolute insulin deficiency, whereas in type 2 diabetes, insulin resistance and progressive β-cell dysfunction maintain persistent hyperglycemia (Sun et al., 2022). Dysregulation of microRNAs involved in insulin secretion, particularly miR-375, further worsens metabolic imbalance, increasing the systemic burden of diabetes and contributing to long-term complications, including impaired reproductive health (Sun et al., 2022).

2.4.1 Metabolic Shift Linking Sustained Hyperglycemia to Male Infertility

The fundamental metabolic shift in diabetes begins with persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Excess intracellular glucose overwhelms normal metabolic pathways and is diverted into alternative pathways such as glucose autoxidation, protein glycation, and the polyol pathway, leading to excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Zhu et al., 2023). As antioxidant defenses including superoxide dismutase, catalase, and glutathione become depleted, oxidative stress develops and causes cellular damage within the testes, impairing Sertoli cell function, Leydig cell steroidogenesis, and germ-cell survival (Zhu et al., 2023).

Oxidative stress subsequently triggers a pro-inflammatory state that links metabolic dysfunction to reproductive impairment. Elevated ROS activate inflammatory signaling pathways, including nuclear factor-kappa B (NF-κB), protein kinase C (PKC), and mitogen-activated protein kinase

(MAPK), which stimulate the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-18 (IL-18) (Unuofin et al., 2021). At the same time, anti-inflammatory mediators such as IL-10 decline, creating a persistent inflammatory environment that perpetuates metabolic dysfunction and tissue injury.

In the male reproductive system, these pro-inflammatory cytokines directly contribute to infertility by disrupting the blood-testis barrier, inducing apoptosis of germ cells, suppressing Leydig cell testosterone production, and impairing Sertoli cell support of spermatogenesis. TNF-α and IL-1β further increase oxidative stress within testicular tissue, while IL-6 interferes with hormonal regulation of the hypothalamic-pituitary-testicular axis, reducing testosterone synthesis and sperm production. Consequently, chronic hyperglycemia initiates a self-amplifying cycle of oxidative stress and inflammation that impairs sperm quality, decreases fertility potential, and contributes significantly to diabetes-associated male infertility (Zhu et al., 2023; Unuofin et al., 2021).

2.4.2 Mitochondrial Dysfunction in Sustained Hyperglycemia

Mitochondrial dysfunction is a fundamental consequence of chronic hyperglycemia and a key mechanism underlying the systemic burden of type 2 diabetes mellitus (T2D). As the primary regulators of cellular energy production, redox homeostasis, apoptosis, and intracellular signaling, mitochondria are highly vulnerable to prolonged metabolic stress. Persistent hyperglycemia increases substrate flux through the mitochondrial electron transport chain, leading to excessive generation of reactive oxygen species (ROS), impaired ATP production, and disruption of cellular redox balance (Gaznee et al., 2023). The resulting oxidative damage reduces antioxidant defenses and compromises mitochondrial integrity, creating a cycle of metabolic dysfunction and cellular injury hence there is possibility of having autoproteolytic enzymes in HPG-axis at the level of the

median eminence which could break down or prevent biosynthesis of sex hormones. Consequently, impaired mitochondrial bioenergetics contributes to progressive dysfunction of metabolically active tissues, including the heart, liver, skeletal muscle, and endocrine organs, thereby driving the widespread complications and systemic burden associated with T2D (Gaznee et al., 2023).

Persistent hyperglycemia induces mitochondrial dysfunction through excessive reactive oxygen species (ROS) generation and epigenetic alterations, including mtDNA mutations, methylation changes, and mitochondrial RNA modifications, which collectively impair mitochondrial structure, energy production, and cellular function (Abdelfattah et al., 2023). In the male reproductive system, these defects compromise the energy-dependent functions of Sertoli, Leydig, and germ cells, resulting in impaired spermatogenesis, reduced testosterone synthesis, poor sperm quality, and ultimately contributing to diabetes-associated male infertility (Abdelfattah et al., 2023).

2.4.3 Endoplasmic Reticulum Stress in Sustained Hyperglycemia

Endoplasmic reticulum (ER) stress is a key cellular mechanism linking chronic hyperglycemia to reproductive dysfunction in diabetes mellitus**.** Persistent hyperglycemia increases the accumulation of advanced glycation end products (AGEs), non-esterified fatty acids (NEFAs), and reactive oxygen species (ROS), overwhelming the protein-folding capacity of the ER and causing the build-up of misfolded or unfolded proteins that activate the unfolded protein response (UPR) to restore cellular homeostasis (Uzor et al., 2025; Elagamey et al., 2023).

However, prolonged ER stress and sustained UPR activation disrupt cellular adaptation and promote apoptosis, while reciprocal interactions between ER stress and oxidative stress amplify cellular injury through continuous ROS generation (Elagamey et al., 2023). In the male

reproductive system, this process impairs the function and survival of Sertoli cells, Leydig cells, and germ cells, resulting in reduced testosterone production, defective spermatogenesis, increased germ-cell loss, and compromised sperm quality, thereby contributing to diabetes-associated male infertility.

2.5 Effects of Diabetes Mellitus on the Hypothalamo-Pituitary-Testicular Axis

Chronic hyperglycemia and impaired insulin signaling disrupt the hypothalamic-pituitary-testicular (HPT) axis by inducing oxidative stress, neuroinflammation, and proteolytic damage within hypothalamic regulatory regions such as the median eminence, resulting in impaired GnRH secretion and altered LH and FSH release (Lotti et al., 2023; Zhu et al., 2023). Consequently, reduced gonadotropin and insulin signaling compromise Leydig cell testosterone production, Sertoli cell support, and germ-cell development, leading to impaired spermatogenesis, testicular dysfunction, and reduced fertility potential. In addition, hyperglycemia-driven epigenetic modifications, including altered DNA methylation, histone modifications, and microRNA dysregulation, further disrupt reproductive gene regulation, amplifying both the systemic burden of diabetes and the risk of male infertility (Lotti et al., 2023; Zhu et al., 2023).

2.5.1 Testicular dysfunction in Sustained Hyperglycemia

Testicular failure is a major reproductive consequence of diabetes and arises from the direct effects of chronic hyperglycemia on the metabolic, vascular, and immunological environment required for normal spermatogenesis**.** Persistent hyperglycemia promotes oxidative stress and activates inflammatory immune responses within the testis, leading to increased production of pro-inflammatory cytokines that disrupt the immune-privileged status of the testis and compromise the integrity of the blood-testis barrier (Elagamey et al., 2023). This inflammatory milieu impairs

Sertoli cell proliferation and function, reducing their ability to nourish and regulate developing germ cells while exposing spermatogenic cells to immune-mediated injury.

Simultaneously, diabetes-induced damage to seminiferous tubules and testicular microvasculature reduces tissue perfusion, oxygen delivery, and metabolic support, further aggravating cellular dysfunction and germ-cell loss. Consequently, the interaction between hyperglycemia-driven metabolic stress, oxidative damage, vascular impairment, and chronic inflammation progressively compromises spermatogenesis and sperm maturation, culminating in testicular failure and increased male infertility (Elagamey et al., 2023).

2.5.2 Disorders of Glucose Metabolism that Affects Sperm Motility and Fertilization

Normal spermatogenesis depends on efficient glucose transport and metabolism within the testis, particularly across the blood-testis barrier (BTB), a specialized Sertoli cell junction that creates an immune-privileged environment and regulates the movement of nutrients, hormones, and metabolic substrates to developing germ cells (Gaznee et al., 2023). Chronic hyperglycemia alters glucose transporter (GLUT) expression and disrupts glucose utilization, reducing lactate production by Sertoli cells and impairing energy delivery across the BTB, thereby compromising germ-cell development, sperm maturation, and testicular homeostasis. In addition, hyperglycemia promotes AGE-RAGE signaling, oxidative stress, and mitochondrial dysfunction that weaken BTB integrity, expose germ cells to inflammatory and immune-mediated injury, and contribute to impaired spermatogenesis and male infertility (Gaznee et al., 2023; Zhu et al., 2023).

2.5.3 Oxidative Damage of Sperm

Sperm capacitation is a post-ejaculatory maturation process that transforms sperm from a motile cell into one that can fertilize an oocyte**.** It occurs after ejaculation within the female reproductive tract, primarily in the uterus and fallopian tube, and typically takes place during the hours preceding fertilization. During capacitation, sperm undergo membrane remodeling, cholesterol efflux, ion flux activation, increased membrane fluidity, and hyperactivated motility, changes that prepare the sperm for the acrosome reaction and successful penetration of the oocyte.

In diabetes mellitus, chronic hyperglycemia drives excessive mitochondrial production of reactive oxygen species (ROS), shifting ROS from their physiological signaling role to a pathological state of oxidative stress (Zhu et al., 2023). Although low levels of ROS are required for normal sperm functions, including capacitation, motility, maturation, and fertilization, excessive ROS overwhelm the limited antioxidant defenses of spermatozoa, causing oxidative damage to lipids, proteins, and DNA. Consequently, lipid peroxidation of the polyunsaturated fatty acids within sperm membranes disrupts membrane fluidity, cellular transport, energy transfer, and the molecular events required for capacitation, thereby impairing fertilization capacity and contributing to diabetes-associated male infertility (Zhu et al., 2023).

Chronic hyperglycemia-induced oxidative stress damages mitochondrial DNA, impairing oxidative phosphorylation and ATP production required for sperm motility, capacitation, and survival, while ROS-mediated inhibition of the PI3K/AKT/mTOR pathway disrupts autophagy and testicular cell homeostasis, collectively reducing sperm quality, fertility potential, and contributing to male infertility (Lotti et al., 2023).

Sustained hyperglycemia causes excessive oxidative stress that damages mitochondrial DNA (mtDNA) in sperm and testicular cells, leading to mitochondrial dysfunction, reduced ATP

production, impaired spermatogenesis and sperm motility, and ultimately male infertility (Uzor et al., 2025). Compromised blood-testis barrier (BTB) function contributes to male infertility through a mechanism distinct from oxidative damage to sperm cells because the BTB is responsible for creating an immune-privileged and tightly regulated microenvironment necessary for germ-cell development. In diabetes, excessive oxidative stress and dysregulated autophagy impair Sertoli cell function, disrupting BTB integrity and allowing inflammatory mediators and immune cells to gain access to developing germ cells, thereby triggering immune-mediated injury, defective spermatogenesis, and germ-cell loss (Lotti et al., 2023). Concurrently, oxidative stress suppresses Sertoli cell proliferation and Leydig cell steroidogenic function, reducing testosterone production and further impairing sperm development, ultimately resulting in progressive testicular dysfunction and male infertility (Lotti et al., 2023).

2.5.4 Effects of Sustained Hyperglycemia on Histology of the Testis

Spermatogenesis is a tightly regulated process within the seminiferous epithelium in which spermatogonial stem cells proliferate and differentiate into spermatocytes and spermatids, while spermiogenesis transforms haploid spermatids into mature spermatozoa through structural and functional remodeling under the coordinated support of gonadotropins, testosterone, Sertoli cells, and the testicular microenvironment (Uzor et al., 2025; Zhu et al., 2023).

The blood-testis barrier (BTB) is the fundamental architectural and physiological framework that maintains an immune-privileged microenvironment, regulates the transport of nutrients, hormones, and signaling molecules to developing germ cells, and coordinates the endocrine and cellular interactions required for spermatogenesis and steroidogenesis **(Zhu et al., 2023).** In diabetes, chronic hyperglycemia-driven overproduction of reactive oxygen species (ROS) damages Sertoli cells and disrupts BTB integrity, impairing germ-cell protection, metabolic support, and

communication between testicular cells. Consequently, the loss of BTB function alters testicular architecture, suppresses testosterone production by Leydig cells, disrupts spermatogenesis, and compromises overall reproductive function, thereby contributing to male infertility (Zhu et al., 2023).

Steroid biosynthesis is the process by which cholesterol is converted into pregnenolone, the precursor of all steroid hormones, through enzyme-mediated reactions in steroid-producing tissues. Pregnenolone is then transformed into mineralocorticoids (aldosterone), glucocorticoids (cortisol), and sex steroids (testosterone, DHT, estrogens, and progesterone) via distinct metabolic pathways (Elagamey et al., 2023).

Normal male fertility depends on an integrated hypothalamic-pituitary-testicular (HPT) regulatory system, in which hypothalamic gonadotropin-releasing hormone (GnRH) stimulates pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn regulate Leydig cell testosterone production, Sertoli cell function, and the proliferation and differentiation of germ cells during spermatogenesis (Elagamey et al., 2023; Zhu et al., 2023). In diabetes, disruption of this endocrine regulatory system reduces steroid hormone production and impairs testicular function, leading to hypogonadism, defective spermatogenesis, and male infertility (Elagamey et al., 2023).

Leydig cells generate testosterone through steroidogenesis by converting cholesterol into androgens, while Sertoli cells maintain the blood-testis barrier, support germ-cell development, nutrient transport, and testicular homeostasis; therefore, diabetes-induced oxidative stress and reactive oxygen species damage both cell types, disrupting testosterone production, spermatogenesis, and ultimately male fertility (Gaznee et al., 2023; Elagamey et al., 2023).

A complex network of communication between many cell types maintains testicular functions, both histologically and cytologically, and any breakdown of this architecture causes male infertility, as shown in diabetic men (Zhou et al., 2019). Diabetes-related elevated blood glucose levels alter glucose metabolism in testicular tissues. Under high glucose circumstances, the Leydig cells and SSCs are damaged by an increase in ROS, and this damages sperm membranes and reduces testosterone secretion by Leydig cells (Abd-Elkareem et al., 2021). Pulsatile GnRH therapy can restore hypothalamic-pituitary-testicular axis function and improve testosterone production and spermatogenesis in men with hypogonadotropic hypogonadism, but it cannot fully reverse diabetes-induced testicular damage such as oxidative stress, inflammation, mitochondrial dysfunction, blood-testis barrier disruption, microvascular injury, epigenetic alterations, and germ-cell DNA damage (Lotti et al., 2023).

2.6 Potential Therapeutic Measures for Diabetes-Induced-Male Infertility

2.6.1 Drug therapy

Insulin

Insulin therapy reverses diabetes-associated infertility by correcting the primary metabolic defect, restoring cellular glucose utilization, and reactivating the hypothalamic-pituitary-testicular (HPT) axis that regulates male reproduction**.** Through insulin receptor signaling in the hypothalamus, pituitary gland, and testes, insulin normalizes luteinizing hormone (LH), testosterone production, and Sertoli cell metabolic support, thereby restoring the hormonal and energetic conditions required for spermatogenesis (Abd-Elkareem et al., 2021; Elagamey et al., 2023). Furthermore, insulin reduces hyperglycemia-induced oxidative stress, inflammation, epididymal autophagy dysregulation, and testicular structural damage, promoting regeneration of seminiferous tubules,

improving testicular architecture, and enhancing sperm production and overall fertility potential in diabetic males (Abd-Elkareem et al., 2021; Elagamey et al., 2023).

Resveratrol (RES)

Male reproductive dysfunction caused by diabetes has been demonstrated to benefit from resveratrol (RES), a naturally occurring polyphenolic molecule with strong antioxidant and metabolic regulating qualities (Gaznee et al., 2023). Experimental research shows that RES enhances both quantitative and qualitative sperm parameters under diabetic settings while also improving blood glucose control in type 1 diabetic mice. Resveratrol therapy dramatically boosts sperm count and motility and enhances mitochondrial sheath activity in adult rats with diabetes caused by streptozotocin and nicotinamide (Elagamey, et al., 2023). Additionally, when compared to untreated diabetic controls, resveratrol protects sperm DNA integrity in a dose-dependent manner. These results imply that resveratrol helps to maintain and enhance male fertility by reducing oxidative and metabolic damage associated with diabetes (Abd-Elkareem et al., 2021).

Metformin

Because of its insulin-sensitizing properties, metformin is used as an adjuvant for glycemic control in type 1 diabetes and as a first-line treatment for type 2 diabetes mellitus. Metformin dramatically increases sperm count, testicular cell proliferation, and serum testosterone levels in streptozotocin-induced diabetic mice (Gaznee et al., 2023). Increased leptin levels and overexpression of the leptin receptor (Ob-R) in the testes, which improves testicular endocrine function, are partially responsible for these reproductive advantages (Elagamey, et al., 2023). Additionally, metformin increases the number of Leydig cells and improves intratesticular testosterone synthesis by reversing the downregulation of genes linked to steroidogenesis caused by diabetes. Metformin

protects against male infertility caused by diabetes by improving sperm parameters and decreasing sperm nuclear DNA fragmentation (Abd-Elkareem et al., 2021).

2.6.2 Gene therapy

To cure illnesses that do not respond to traditional medication therapy, gene therapy entails inserting functional genes or regulatory genetic elements into cells. It has been thoroughly investigated in diseases like cancer, viral infections, cardiovascular problems, and inherited genetic disorders; several strategies have advanced to successful clinical trials (Gaznee et al., 2023). Emerging gene-based approaches to male infertility concentrate on controlling genes related to spermatogenesis and testicular function, including epigenetic regulators such miR 100 3p that affect DNA synthesis, apoptosis, and Sertoli cell proliferation (Abd-Elkareem et al., 2021).

To treat infertility linked to aberrant testicular and sperm gene expression, experimental techniques including testis mediated gene transfer (TMGT) and sperm mediated gene transfer (SMGT) have been proposed (Abd-Elkareem et al., 2021). These methods provide potential but experimental therapeutic pathways for treating male infertility caused by diabetes, even though their efficacy, tolerability, and germline genetic risk are still major issues (Abd-Elkareem et al., 2021).

2.6.3 Exercise Improves Testicular and Sperm Function

Frequent exercise has been demonstrated to either prevent or mitigate the detrimental effects of diabetes on male fertility, especially in cases associated with sedentary lifestyles and obesity (Abd-Elkareem et al., 2021). By boosting testicular antioxidant defenses, decreasing apoptosis, lowering pro-inflammatory cytokines, and increasing steroid hormone production, exercise improves spermatogenesis and semen quality. The advantages of mixed endurance-resistance training and aerobic exercise on reproductive function are similar (Gaznee et al., 2023). Moderate exercise enhances male fertility by optimizing the hypothalamic-pituitary-gonadal (HPG) axis, stimulating

testicular steroidogenesis and testosterone production, and reducing oxidative stress, thereby supporting healthy spermatogenesis and sperm function (Zhu et al., 2023).

Additionally, research shows that both short-term and long-term exercise can restore tissue perfusion and structural integrity in the testes by reversing the histological changes brought on by diabetes (Gaznee et al., 2023). While combined aerobic and resistance training seems to be the most promising for increasing male fertility, its overall efficacy is still up for question. However, benefits may be increased when insulin therapy is used in conjunction with moderate exercise to prevent testicular apoptosis associated with diabetes (Gaznee et al., 2023).

2.6.4 Mesenchymal Stem Cell Therapy

Mesenchymal stem cells (MSCs) are multipotent, self-renewing cells that, by cellular differentiation and paracrine release of growth factors like HGF, VEGF, and FGF, have anti-inflammatory, anti-apoptotic, and antioxidant properties. Because of their significant therapeutic potential, repeatability, and potential to improve male infertility caused by type 1 diabetes, breast milk-derived MSCs (Br MSCs) are very beneficial (Gaznee et al., 2023).

BM-MSCs may restore fertility by reducing oxidative stress, enhancing antioxidant defenses, repairing testicular tissue, and reactivating the hypothalamic-pituitary-testicular (HPT) axis, thereby increasing LH-mediated Leydig cell steroidogenesis and testosterone production, which support spermatogenesis, improve sperm count, motility, and viability, and facilitate recovery of overall reproductive function (Gaznee et al., 2023).While MSC-based treatments seem safe and promising for erectile dysfunction and diabetes-related reproductive dysfunction, issues such donor variability, immunogenicity, scalability, and long-term safety need to be addressed before routine clinical implementation (Gaznee et al., 2023).

2.6.5 Microbial Therapy

According to research on animals, alginate oligosaccharide–modified fecal microbiota transplantation (A10 FMT) can enhance spermatogenesis and semen quality in type 1 diabetes by improving the systemic and testicular milieu through the gut–testis axis (Zhu et al., 2023). This new strategy offers a viable therapeutic avenue for further investigation and implies that gut microbiota modification may mitigate diabetes-related reproductive abnormalities (Elagamey, et al., 2023). Through macrophage polarization from an anti-inflammatory M2-like phenotype to a pro-inflammatory M1-like condition, diabetes-induced hyperglycemia contributes to chronic inflammation in the male reproductive system, which damages sperm and causes infertility (Zhu et al., 2023).

Testicular macrophages have been successfully converted to an M2-like phenotype in diabetes animals through experimental treatments employing macrophage-specific production of Jumonji domain containing protein D3 (JMJD3) (Zhu et al., 2023). This immunomodulatory change reduces inflammation, enhances sperm motility, and raises intratesticular and serum testosterone levels, suggesting that immunological modulation may be a viable treatment option for male infertility brought on by diabetes (Zhu et al., 2023). Anomalies in any of these variables might affect male fertility; for example, a low sperm count or poor sperm motility may lower the likelihood of conception. Semen analysis is frequently one of the first techniques performed when testing male fertility and can yield valuable information (Zhu et al., 2023).

2.7 Phytochemicals, Antioxidants and Male Fertility

Male infertility is largely caused by oxidative stress, which damages sperm motility, morphology, and genomic integrity by producing too many reactive oxygen species (ROS). Strong antioxidants that neutralize ROS and maintain membrane and mitochondrial function include flavonoids,

phenolics, carotenoids, and alkaloids (Zhu et al., 2023). According to a thorough analysis by Moore et al., 2024, antioxidants have translational promise in reproductive medicine because they not only protect sperm DNA but also enhance clinical fertility results.

Antioxidant supplementation improves male fertility by reducing oxidative stress, restoring cellular redox balance, protecting sperm from DNA and membrane damage, and enhancing sperm count, motility, viability, and fertilization potential, which collectively contribute to higher pregnancy rates in men with idiopathic infertility (Sun et al., 2022). While carotenoids like lycopene reduce DNA fragmentation and improve overall sperm quality, certain phytochemicals like quercetin, resveratrol, and catechins have been demonstrated to increase sperm motility and decrease lipid peroxidation (Elagamey, et al., 2023). Crucially, these protective benefits are enhanced by synergistic interactions between micronutrients and phytochemicals, indicating that complex phytochemical matrices may perform better than isolated substances (Zhu et al., 2023).

Phytochemicals have two functions in diabetes infertility models: they help reproductive organs heal structurally and reduce oxidative damage to sperm cells (Elagamey, et al., 2023). Improved Seminiferous Tubule architecture under Phytochemical treatment is shown by histological data, supporting the molecular connection between tissue-level regeneration and Antioxidant defense (Gaznee et al., 2023). When taken as a whole, these results demonstrate the potential of phytochemicals as therapeutic agents in male reproductive health and establish them as important modulators of sperm integrity (Zhu et al., 2023).

2.7.1 Effects of Phytochemicals in Male Infertility

Through combined metabolic, oxidative, endocrine, and cellular effects, phytochemicals like polyphenols, flavonoids, alkaloids, carotenoids, and terpenoids reduce male infertility brought on by diabetes (Zhu et al., 2023). They boost Sertoli cell energy metabolism and Leydig cell

testosterone synthesis by lowering hyperglycemia and limiting glucose toxicity through increased insulin sensitivity and AMPK signaling activation (Elagamey, et al., 2023). Sperm membranes, mitochondria, and DNA are shielded from oxidative damage by their strong antioxidant actions, which also improve endogenous antioxidant defenses and eliminate excessive reactive oxygen species (Gaznee et al., 2023).

Phytochemicals may alleviate diabetes-associated male infertility by suppressing AGE-RAGE-mediated oxidative stress and chronic inflammation, promoting anti-inflammatory immune responses, preserving blood-testis barrier integrity and HPT-axis function, and thereby improving steroidogenesis, spermatogenesis, sperm quality, and overall reproductive function; conversely, uncontrolled immune activation can directly contribute to male infertility through inflammatory damage to testicular cells and disruption of the immune-privileged testicular environment (Gaznee et al., 2023; Zhu et al., 2023).

2.7.2 Effects of Antioxidants on Male Fertility

By shielding spermatozoa from excessive reactive oxygen species (ROS), which can harm sperm viability and function, antioxidants are essential for male fertility (Abd-Elkareem et al., 2021). Enzymatic mechanisms, mainly Superoxide Dismutase, Catalase, and Glutathione Peroxidase, as well as non-enzymatic and low molecular weight antioxidants that cooperate to preserve redox balance make up the antioxidant defense (Abd-Elkareem et al., 2021).

Superoxide dismutase isoenzymes, which are mostly produced from the prostate, are crucial for neutralizing superoxide radicals and constitute a significant source of antioxidant activity in seminal plasma (Elagamey, et al., 2023). Sperm membranes, mitochondria, and DNA are shielded from oxidative damage by non-enzymatic antioxidants like carotenoids, vitamins E and C, selenium, zinc, and glutathione. Selenium and zinc are necessary for optimal spermatogenesis and

sperm motility, vitamin C protects sperm DNA, and vitamin E stops lipid peroxidation of sperm membranes (Abd-Elkareem et al., 2021).

2.8 Effects of *Zingiber Officinale* and *Mondia Whitei* on Male Fertility

In the medical health care system, WHO promotes the use of traditional medicines (Uzor et al., 2025). The development of natural antioxidants from botanical materials to substitute pharmaceutical drugs has garnered a lot of attention recently (Uzor et al., 2025). Numerous medicinal plants with anti-fertility and fertility-boosting properties may be found all over the world. Medicinal plants and their bioactive compounds can influence libido and fertility by modulating the hypothalamic-pituitary-testicular (HPT) axis, testosterone production, neurotransmitter signaling, sperm quantity and motility, and testicular function, thereby either enhancing or suppressing male reproductive capacity depending on their biological activity (Zhou et al., 2019; Uzor et al., 2025).

The increasing use of herbal medicines reflects the need for therapeutic agents that target the underlying biological mechanisms of disease while minimizing the adverse effects often associated with conventional pharmaceuticals (Uzor et al., 2025). Consequently, rigorous investigation of medicinal plants is essential to identify bioactive compounds with estrogenic, anti-estrogenic, antioxidant, anti-inflammatory, and fertility-modulating properties, and to determine their effects on male reproductive function, hormonal regulation, and overall fertility potential.

2.8.1 Zingiber officinale

Zingiber officinale grows best in the humid tropical rainforest climate of Western Kenya's Kakamega Forest. The forest delivers colder evenings and gentler daytime temperatures around 1,500–1,600 meters above sea level. With an average of 1,742 mm of rainfall, there are only short,

less rainy periods throughout the year. Mean temperatures between 19°C and 22 °C provide stable and productive growing conditions that allow high yields and luxuriant crop development.



Roscoe belongs to the *Zingiberaceae* family of perennial plants (Moore et al., 2024). Growing all over India, it is the most common species of ginger (Moore et al., 2024). The rhizome is white to yellowish to brown, meaty, fragrant, horizontal, and branched. Colic, smooth muscle spasms, vomiting, dyspepsia, flatulence, and other unpleasant symptoms are among the stomach ailments that it frequently helps with (Uzor et al., 2025). Gingerols are among the pharmacologically active metabolites produced in large quantities via the phenylpropanoid pathway (Moore et al., 2024). The odorous vanilloids and paradol are also found in *Zingiber officinale* (Moore et al., 2024).

The physiologically active ingredient gingerol may have a major role in the therapeutic applications of ginger and products made from it (Moore et al., 2024). The antioxidant, anticancer, and anti-inflammatory qualities of ginger are mainly due to its strong constituents, such as gingerol. These symptoms may be alleviated by chewing a piece of fresh ginger. Additionally, the rhizomes can be used to treat coughs and colds (Uzor et al., 2025).

2.8.2. Mondia whitei

Mondia whitei thrives in the humid tropical rainforest conditions of Western Kenya's Kakamega Forest. At 1,500–1,600 meters above sea level, the forest offers milder daytime temperatures and cooler nights. The average annual rainfall is 1,742 mm, evenly spread throughout the year with very sporadic dry spells. Vibrant root and vine development is encouraged by the steady and productive circumstances created by mean temperatures between 19°C and 22 °C.



Mondia whitei contains two species, one of which is *Mondia ecornuta*. The rootstock, known as '*mukombero*' in some regions of Kenya, is widely harvested for medicinal uses (Moore et al., 2024). It has been observed in Guinea, Nigeria, Cameroon, Sudan, Uganda, Kenya, Tanzania, Zimbabwe, Malawi, Mozambique, South Africa, Eswatini, and Angola at elevations ranging from 1000 to 1500 m in damp to wet woodlands and marshy grassland (Uzor et al., 2025). Its roots are extensively harvested for use for different ailments by populations living in endemic areas were grown in Kenya, which often kills the plant (Oketch-Rabah, 2012).

Mondia whitei is a milky latex-producing perennial herbaceous/woody climber from the *Apocynaceae* family (Moore et al., 2024). Some initiatives reproduce the species to fulfil

commercial demand. It works as an aphrodisiac, enhancing sexual desire, sexual performance, sensitivity, and treating erectile dysfunction in men (Uzor et al., 2025). *Mondia whitei's* aphrodisiac properties have been demonstrated not just through its ability to promote testosterone production or relax *corpus cavernosum* erectile tissue but also by increasing human sperm function (Moore et al., 2024). It has been shown to have an erectile dysfunction effect similar to Sildenafil (Viagra), which enhances nitric oxide generation, relaxes muscles, improves blood flow, and generates penile erections (Moore et al., 2024).

To cure impotence and erectile dysfunction, men chew the barks of the root or drink an extract of the roots (Moore et al., 2024). *Mondia whitei* is also used to treat heartburn, indigestion, flatulence, gonorrhoea, stomach pain, and constipation, as well as bilharzia, premature uterine contractions in pregnant women, hunger stimulants, asthma, and hypertension (Moore et al., 2024). It is also rich in micronutrients, including vitamins A, D, E, and K, as well as zinc, iron, magnesium, and calcium(Moore et al., 2024).

2.9 Chemical Induction of Diabetes and Infertility

Chemical induction is an effective approach for investigating the impact of various chemicals on cancer genesis and progression (Uzor et al., 2025). Its significance stems from its capacity to give crucial insights into cancer processes and help create novel therapies for the illness. However, the results must be interpreted cautiously, taking into account the technique's limits and obstacles (Uzor et al., 2025).

2.9.1 Alloxan- Induced Diabetes: Mechanisms and Effects

Mechanisms and effects of alloxan, a powerful oxidizing chemical, destroy the pancreas' insulin-producing beta cells, resulting in a dramatic drop in insulin output. Alloxan causes inflammation in the pancreas, leading to additional damage to beta cells and insulin secretion (Moore et al.,

2024). Alloxan-induced diabetes causes poor glucose tolerance because the body is unable to efficiently use insulin to regulate blood glucose levels. It is reasonably simple to develop in animal models, making it a common choice for diabetes-related research. Alloxan-induced diabetes is reversible, allowing researchers to study how different therapies and interventions affect glucose metabolism and insulin secretion (Moore et al., 2024).

2.9.2 Clomiphene citrate

Clomiphene citrate is widely used in reproductive physiology research as a positive control for induction of the Hypothalamic–Pituitary–Testicular axis. Its mechanisms of action include use of selective androgen receptor modulator (SARM) which acts mainly at the hypothalamus and pituitary gland, interfering with androgen's negative feedback. Clomiphene binds competitively to androgen receptors in the hypothalamus (Moore et al., 2024).

This prevents circulating androgen from exerting its negative feedback effect on Gonadotrophin Releasing Hormone neurons. Because the hypothalamus "senses" low androgen activity, it increases the pulsatile release of Gonadotrophin Releasing Hormone. Elevated Gonadotrophin Releasing Hormone stimulates the anterior pituitary to secrete more Follicle Stimulating Hormone and Luteinizing Hormone. Less frequently used in males, such as for infertility, Luteinizing Hormone activates Leydig cells, which results in higher testosterone levels. Through the activation of Sertoli cells, Follicle Stimulating Hormone promotes spermatogenesis (Zhu et al., 2023).

Because of its well-established mechanism, clomiphene citrate is utilized as a positive control group in experimental research on fertility, diabetes-related testicular injury, or herbal remedies (Zhu et al., 2023). It consistently increases gonadotropins and sex steroids by stimulating the Hypothalamus-Pituitary-Testicular axis. As a SERM, clomiphene citrate inhibits the hypothalamic negative feedback of estrogen, which raises Gonadotrophin Releasing Hormone, increases

pituitary Follicle Stimulating Hormone and Luteinizing Hormone secretion, and promotes gonadal steroidogenesis and gametogenesis (Zhu et al., 2023).

2.10 Traditional Herbal Plants Used as Remedy for Infertility in Kenya.

In Kenya, medicinal plants are utilized as infertility therapies for a variety of causes.

Lack of access to modern healthcare: In certain parts of rural Kenya, modern healthcare facilities and medical experts may be scarce. Traditional herbal remedies may be the only available option for many people. Access to modern healthcare facilities and medical specialists may be restricted. Many individuals may only have access to traditional herbal treatments (Djerrou et al., 2022).

Perceived effectiveness: Many Kenyans think that traditional herbal medicines are beneficial for treating diabetic induced male infertility and have been used effectively for decades. Traditional herbal therapies are generally thought to be safer than contemporary medical treatments since they are natural and non-invasive (Djerrou et al., 2022).

Lack of confidence in contemporary medicine: Some patients may be sceptical to the use of modern medicines and prefer to employ traditional herbal cures instead of what they consider to be "chemical" or "unnatural" therapies (Djerrou et al., 2022).

Accessibility: Many of these plants are widely available in Kenya's rural areas, making them accessible to those who may not have access to contemporary healthcare facilities (Djerrou et al., 2022).

Affordability: Traditional herbal therapies are frequently less expensive than contemporary medical treatments, making them a more accessible alternative for many individuals (Djerrou et al., 2022).

Cultural significance: Traditional herbal therapies for infertility are profoundly embedded in Kenyan culture and traditions. Many Kenyans feel that these therapies are more effective and safer than conventional medical procedures (Djerrou et al., 2022).Traditional use of medicinal plants for male infertility suggests that they may act on fundamental reproductive mechanisms, including

steroidogenesis, hypothalamic-pituitary-testicular (HPT) axis regulation, oxidative stress, inflammation, sperm maturation, and testicular metabolism; however, most evidence remains ethnomedicinal with limited mechanistic validation (Canepa et al., 2023; Djerrou et al., 2022). A major knowledge gap remains regarding how these plants influence diabetes-associated male infertility, particularly their effects on oxidative stress, inflammation, blood-testis barrier integrity, mitochondrial function, epigenetic regulation, and reproductive outcomes, underscoring the need for rigorous studies on their efficacy, safety, dosage, and mechanisms of action.

Table 2.1 **Traditional Herbal Plants Used as Remedy for Infertility in Kenya**

SN	TYPE OF PLANT	USES
1	Ginger plant species (***Z. officinale) and (M. whitei)*** roots extracts	Used in western Kenya, particularly among the Luhya community for the treatment of male infertility. Widely used natural products consumed as spices and medicine for treating *diabetes mellitus*, flatulent intestinal colic, indigestion, fertility, inflammation, insomnia, a memory booster, nausea, rheumatism, stomach ache and urinary tract infections (Canepa et al., 2023).
2.	Maca roots ***(Lepidium Meyenii)*** which grows in Andes Mountain of Peru	In Kenya it's used as herbal remedies to boost fertility and increase libido (Canepa et al., 2023).
3.	Moringa ***(Moringa Oleifera***) it's a native plant to India subcontinents	In Kenya moringa leaves and seeds are used to improve and treat infertility (Canepa et al., 2023).
4.	African potato ***(Hypoxis Hemerocallidea***), native to South Africa.	The plant is traditionally used to treat a range of conditions including fertility. The roots of the plant are believed to contain compounds that may help to improve fertility (Canepa et al., 2023).
5.	Dong quai ***(Angelica Sinensis***) is an herb that is native to China	In Kenya it's used as herbal remedy in regulating menstrual cycles and improving fertility in males (Canepa et al., 2023).
6.	Red clever ***(Trifolium Pratense)***, it's a flowering plant that is native to Europe	Been naturalized in many parts of the world including Kenya; it's used to treat male infertility. The plant contains phytoestrogens which are compounds that may help to balance hormones and improve fertility (Djerrou et al., 2022).
7.	***Warburgia ugandensis*** (Cassia tree)	The bark and leaves of this plant are used to treat infertility and improve fertility (Canepa et al., 2023).
8.	***Piper capense*** (Pepper bush)	The fruit and leaves of this plant are used to treat infertility and improve fertility (Djerrou et al., 2022).
9.	***Hypoxis hemerocallidea*** (Hypoxis)	The rhizome of this plant is used to treat infertility and improve fertility (Canepa et al., 2023).
10.	***Tephrosia vogelii*** (Tephrosia)	The leaves and seeds of this plant are used to treat infertility and improve fertility (Djerrou et al., 2022).
11.	***Gardenia ternifolia*** (Gardenia)	The fruit and leaves of this plant are used to treat infertility and improve fertility (Djerrou et al., 2022).

It's important to note that while traditional herbal remedies may be used by many people in Kenya, there is limited scientific evidence to support their efficacy in treating infertility (Djerrou et al., 2022).

2.11 Biology and Reproductive Behaviour of Experimental Animals

Non-diabetic animals typically exhibit normal reproductive behaviour, including adequate libido, frequent mounting and intromission, successful ejaculation, and high fertility, reflecting intact hypothalamic-pituitary-testicular (HPT) axis signaling, normal testosterone production, efficient steroidogenesis, and healthy spermatogenesis. In contrast, diabetic animals often display reduced sexual motivation, prolonged mount and intromission latency, impaired ejaculation, and decreased fertility due to chronic hyperglycemia-induced disruptions in endocrine regulation, cellular energy metabolism, and testicular physiology (Zhu et al., 2023). Biologically, oxidative stress, inflammation, mitochondrial dysfunction, impaired Sertoli and Leydig cell function, reduced testosterone synthesis, and defective sperm production and maturation collectively compromise reproductive capacity in diabetic males. Therefore, reproductive behaviour serves as an integrated functional indicator of reproductive health, where improvements following treatment suggest restoration of HPT-axis activity, steroidogenesis, spermatogenesis, sperm quality, and overall fertility potential (Gaznee et al., 2023).

Male fertility behaviours are healthy lifestyle practices such as eating a balanced diet, exercising regularly, avoiding smoking, excessive alcohol and drugs, practicing safe sex, managing stress, and seeking timely medical care to maintain optimal reproductive health and sperm quality (Abd-Elkareem et al., 2021; Venditti et al., 2024).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

This study was conducted in the Zoology Laboratory, Department of Zoology, School of Biological and Physical Sciences, Maseno University, Kenya. The laboratory provides a controlled research environment that supports the maintenance and experimental evaluation of laboratory animals under standardized conditions necessary for investigating physiological, biochemical, and reproductive processes. The facility was selected because it enables the assessment of fundamental biological mechanisms linking diabetes mellitus to reproductive dysfunction, including alterations in endocrine regulation, steroidogenesis, spermatogenesis, oxidative stress, and reproductive behaviour. Standardized housing and experimental conditions minimized environmental variation, ensuring reliable comparison of reproductive and metabolic outcomes among the experimental groups.

3.2 Collection and Preparation of Plant Materials

Fresh samples of *Zingiber officinale* rhizomes and *Mondia whitei* roots were collected from Kakamega forest located at approximately 0°05′s latitude and 34°46′e longitude, in Kenya for consumption. the samples were collected in the Month of June 2025. After collection the samples were taken to National Museums of Kenya for Identification at the Department of Botany, EA Herbarium and given collector/voucher numbers as ABK0001 and ABK0002 for *Mondia whitei* and *Zingiber officinale* respectively.

3.3 Plant Materials, Processing and Preparation of Extracts

The samples were washed with running tap water, chopped into small (1mm) pieces using a knife, air- dried in zip-locked bags for at least 7 days in the laboratory at Zoology Department, Maseno

University and weighed using an electronic compact scale weighing balance machine (Radwag model wlc.0,6/b1)

Extraction was performed according to Heinrich et al. (2022) using 70% ethanol because its intermediate polarity enables efficient recovery of a broader spectrum of bioactive phytochemicals, including both water-soluble and moderately non-polar compounds, yielding higher phytochemical extraction than either aqueous extraction alone or highly concentrated ethanol, after which 200 g each of *Zingiber officinale* and *Mondia whitei* were separately extracted in 1000 mL solvent, shaken for 24 hours, and filtered through muslin cloth to obtain crude extracts. Filtration was done using circular 125mm Whatman filter papers. The filtrates obtained was concentrated using a rotary evaporator (Medicare Health Care EA LTD) and a hot air oven (DHG-9053 LAB DRY OVEN) and the resultant slurry weighed and the weight recorded. Distilled water was used as a vehicle. The crude extracts obtained from the ethanolic solvent was stored at -20 °c in different labelled, airtight containers until later used for Phytochemical analysis and administration to experimental white albino male rats.

3.4 Qualitative and Quantitative Phytochemical and Antioxidant Analysis of Zingiber officinale and Mondia whitei

Qualitative Phytochemical Screening of *Zingiber officinale* and *Mondia whitei*

The table below shows standard methods used for phytochemical qualitative screening. Phytochemical screening involves systematic qualitative methods designed to identify major secondary metabolites in plant extracts based on their distinct chemical properties. These tests utilize specific reagent–compound interactions to produce observable changes such as color formation, precipitates, or foam, enabling targeted detection of different phytochemical classes

Table 3.1 Standard Phytochemical Tests to detect classes of compounds

Table 3.1 shows the procedures enabled systematic identification of phytochemical classes through specific, observable chemical reactions. Careful evaluation of reaction outcomes and use of complementary tests ensured accurate differentiation and strengthened the reliability of the overall phytochemical analysis.

Sn	Phytochemical	Test Method
1	Alkaloids	Mayer's, Wagner's, Dragendorff's tests
2	Flavonoids	Shinoda test
3	Tannins	Ferric chloride test
4	Saponins	Froth test
5	Terpenoids	Salkowski's test
6	Phenols	Ferric chloride test

3.4.1 Alkaloids (Mayer's, Wagner's, Dragendorff's Tests)

The plant extract was first acidified with dilute hydrochloric acid to convert alkaloids into soluble salt forms suitable for detection. The acidified extract was filtered to remove insoluble impurities that could interfere with accurate precipitate formation. The filtrate was divided into separate portions and treated with Mayer's, Wagner's, and Dragendorff's reagents to enable multi-reagent detection. The formation of cream (Mayer's), reddish-brown (Wagner's), and orange red (Dragendorff's) precipitates was carefully observed as evidence of alkaloid–reagent complex formation. Consistent precipitate formation across all tests was interpreted as strong confirmation of alkaloids, while a single positive indicated tentative presence and absence of precipitate suggested low or no alkaloid content.

3.4.2 Flavonoids (Shinoda Test)

Total flavonoid levels were measured using the aluminium chloride colorimetric method (aryal *et al*., 2019). Total flavonoid content was determined using a combined analytical approach integrating the aluminium chloride colorimetric method and the Shinoda reaction to exploit flavonoid structural properties. The extract was subjected to reduction with magnesium and hydrochloric acid to generate colored flavilium salts, enabling qualitative confirmation through red, pink, or orange color formation. Subsequently, aluminium chloride was used to form stable flavonoid–reagent complexes suitable for quantitative spectrophotometric analysis. After incubation at room temperature, a UV–Visible spectrophotometer was used to assess the color intensity of the solution. Absorbance was measured at 415 nm, where flavonoid complexes exhibit maximum light absorption. A standard calibration curve was constructed using quercetin at defined concentrations, allowing quantification of flavonoid content based on absorbance intensity. The total flavonoid levels in *Zingiber officinale* and *Mondia whitei* extracts were then calculated and expressed as mg/mL Quercetin Equivalents, reflecting their relative flavonoid composition.

3.4.3 Tannins (Ferric Chloride Test)

Total Tannin content was determined using the Folin-Ciocalteu method, with minor modifications (haile & kang, 2019). Total tannin content was determined using a harmonized approach combining qualitative ferric chloride testing with the Folin–Ciocalteu colorimetric method. Initially, the extract was treated with ferric chloride to verify tannin presence through formation of blue-black or greenish complexes with phenolic hydroxyl groups, allowing differentiation between hydrolysable and condensed tannins. The results were critically interpreted by comparing color intensity to distinguish tannins from simpler phenols, which produce lighter colors. For quantitative analysis, the extract was reacted with Folin–Ciocalteu reagent and sodium carbonate,

enabling oxidation–reduction reactions that produced a measurable-colored complex. After incubation at room temperature, absorbance was measured at 700 nm and compared against a tannic acid standard calibration curve (0–100 µg/mL). The total tannin content of *Zingiber officinale* rhizomes and *Mondia whitei* ethanolic root extracts was calculated and expressed as µg/mL tannic acid equivalents, reflecting their relative tannin composition.

3.4.4 Total Saponin Contents (Foam Test)

The Quantitative determination of saponin content was carried out using the method reported by Ejikeme *et al.* (2014). Exactly 100 ml of 20% aqueous ethanol was added to 5 g of each sample in a 250 ml conical flask. The mixture was heated over hot water bath for 4 hours with continuous stirring at a temperature of 55˚c. The residue of the mixture was re-extracted with additional 100 ml of 20% ethanol after filtration and heated for 4 hours at a constant temperature of 55°c with constant stirring. The combined extract was evaporated to 40 ml over water bath at 90°c. Twenty milliliters of diethyl ether was added to the concentrate in a 250 ml separator funnel agitated after which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. Sixty milliliters of n-butanol was added and extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and dried in an oven to a constant weight. The saponin content was calculated as a percentage

$$\textbf{\% saponin} = \frac{\textbf{weight of saponin}}{\textbf{weight of sample}} \times \textbf{100}$$

3.4.5 Terpenoids (Salkowski Test)

The Quantitative determination of Terpenoids content was carried out using the method reported by Ejikeme *et al.* (2014). The plant extract was dissolved in chloroform to establish a non-polar medium suitable for terpenoid detection. Concentrated sulfuric acid (H_2SO_4) was carefully layered

along the side of the test tube to create two distinct phases. The mixture was left undisturbed to allow a phase-specific interface reaction to occur between the layers. The junction of the layers was critically observed for the formation of a reddish-brown ring. The presence of a reddish-brown interface was interpreted as a positive indication of terpenoids, while its absence suggested either lack of terpenoids or insufficient extraction, emphasizing the importance of the interface reaction rather than bulk mixing.

3.4.6 Total Phenolic Content

The total phenolic content was determined Colorimetrically by the Folin Ciocalteau reagent and gallic acid as a standard (ahmed *et al*., 2014). Total phenolic content was determined using the Folin–Ciocalteu colorimetric method based on the redox properties of phenolic compounds. The plant extract was mixed with Folin–Ciocalteu reagent and, after a defined reaction time, sodium carbonate was added to create an alkaline medium for color development. The reaction mixture was incubated at controlled temperature to allow phenolic compounds to reduce the reagent, forming a blue-colored complex. The intensity of the blue coloration, measured spectrophotometrically at 765 nm, was directly proportional to the concentration of phenolic compounds present. A standard calibration curve was generated using known concentrations of gallic acid to enable accurate quantification. The total phenolic content of *Zingiber officinale* and *Mondia whitei* ethanolic root extracts was then calculated and expressed as µg/mL gallic acid equivalents.

3.5.0 Antioxidant Activity Assays of *Zingiber officinale* and *Mondia whitei* extracts

3.5.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

This was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Defo Deeh et al., 2024). The DPPH free radical scavenging activities of the crude extracts from *Zingiber officinale*

and *Mondia whitei* ethanolic root extracts were evaluated according to a previous method of Zhu *et al.* (2025) with some modifications. first, 10 µl of the adequate diluted samples (dissolved in methanol) or the positive control solutions (ascorbic acid, 31.25–1000 µm) were mixed with 190 µl of methanol solution of DPPH (0.1 mm) in a 96-well plate.

After 30 minutes of reaction in darkness at room temperature, the absorbance of the reaction mixture was recorded at 517 nm with a multifunctional microplate reader (Tecan Infinite M200 Pro, Tecan, Männedorf, Switzerland). Methanol was used as a blank control in this assay, and all the extracts and controls were tested in triplicate. The percentage of inhibition of DPPH free radicals was estimated and computed using the following equation

$$\text{DPPH-free radical scavenging effect (\%)} = \frac{[(\mathbf{odc - ods})}{\mathbf{odc}]} \times \mathbf{100}$$

where, od_c is the absorbance value of the blank control, od_s is the absorbance value of the tested sample or positive control. The 50% inhibitory concentration (IC_{50}) was expressed as the quantity of the extracts that react with 50% of DPPH radicals (Xu *et al.*, 2019).

3.5.2 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Cation Decolorization Assay

The 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay begins with the preparation of a 7 mm ABTS stock solution, which is oxidized using potassium persulfate and incubated in the dark for 12–16 hours to generate the stable $ABTS^{\bullet+}$ radical cation. The radical solution is then diluted with ethanol or buffer until its absorbance at 734 nm reaches approximately 0.70, ensuring assay sensitivity and reproducibility.

Plant extracts and Trolox standard are introduced into this solution, where antioxidants reduce $ABTS^{\bullet+}$, leading to measurable decolorization. The decrease in absorbance at 734 nm is recorded spectrophotometrically after a fixed incubation period, typically six minutes, and reflects the

antioxidant capacity of the sample. Results are expressed as Trolox Equivalent Antioxidant Capacity (TEAC), providing a standardized metric for comparing antioxidant activity across different samples (Defo et al., 2024)

3.5.3 Ferric Reducing Antioxidant Power

The reducing power was assessed as described by Wang et al., (2021), where 1ml of each extract (*Zingiber officinale* rhizomes and *Mondia whitei* ethanolic root extracts) was added to clean test tubes, mixed with 2 ml of 0.1m phosphate buffered saline (pbs) (pH. 6.6) and 2 ml of potassium ferricyanide ($k_3[fe(cn)_6]$) (1% w/v). The mixture was placed in a water bath maintained at 50 ℃ for 30 min. The samples were removed and allowed to cool, followed by the addition of 2 ml of 10% Tetrachloroacetic acid (TCA), centrifuged at 1000 x g at room temperature for 10 min. The supernatant (2 ml) was transferred and mixed with equal volumes of ultrapure water and 0.5 ml of ferric chloride solution ($Fecl_3$) (0.1%, w/v). The mixture was allowed to stand for 10 min, after which the absorbance was read at 700 nm.

3.6 Experimental Animals and Housing

Standard stainless-steel cages of a minimum cage height of 7 inches, according to the animal and housing experimental guidelines, were used. The animals were sourced from the Faculty of Veterinary Medicine, University of Nairobi, transported to Maseno University Animal House and housed in groups of approximately same size. Disinfection of the animal house was done prior to introduction of the animals. Wood shavings were used for beddings and were changed daily to avoid build-up of ammonia in the animal house. The animals were allowed free access to water and standard rat pellets (Unga feeds, kanya). The animal house was well ventilated with light: dark cycle of 12h: 12h, room temperature of between 20°c to 26°c and relative humidity kept between

40% to 60%. Regular health monitoring was done by a qualified veterinarian, and only health animals were used for the research work.

3.7 Experimental Design and Animal Treatment

3.7.1 Experimental Design

Using Tsampoukas et al., 2022 protocol, A total of 36 male rats white albino rats, aged 7–8 weeks and weighing between 150g and 250g were used for this study. The males were randomly assigned 9 groups of 4 animals each for normal control, negative/diabetic control, positive control and 3 treatment groups for *Zingiber officinale and Mondia whitei*, while 3 non-diabetic females for each group were only used for testing male fertility status via mating success examination.

Diabetes mellitus was induced in all animal groups, except normal control, by a single intraperitoneal injection (2 ml) of alloxan monohydrate, a drug known to destroy pancreatic beta cells responsible for insulin secretion (shah & khan, 2014). animals were monitored for changes in blood glucose levels at day 0, 7, 14, 21 and 28 post-treatments. A drop of blood was collected by slight venipuncture at the tip of the tail of each rat and put on strips and inserted in a glucometer (on call plus blood glucose meter). Fasting glucose levels above 126 mg/dl (7 mmol/l) was indicative of diabetes (hyperglycemia).

Clomiphene citrate (dose range from 5 mg/kg to 10 mg/kg body weight administered via intraperitoneal injection), was reconstituted in distilled water to a concentration of 5 mg/ml and used as standard drug for positive control. Animals were then observed for blood glucose levels, water intake levels, frequency of urination, body weight and somatomotor activity. Blood glucose levels and body weights were recorded on day 0, 7, 14, 21 and 28 before commencing treatment with extracts.

3.7.2 Animal Treatment

The extracts were reconstituted using distilled water in a ratio of 1gram in 10mls of distilled water. The treatment groups were administered with escalating doses of 200 mg/kg, 400 mg/kg and 800 mg/kg of *Zingiber officinale* for groups 4, 5 and 6 and for *Mondia whitei* for groups 7, 8 and 9 respectively using the same dose levels. The normal control was given normal saline using the same regimen as for treatment groups. all administrations were done in volumes of 2 ml of extracts orally using gastric gavage needle once daily (8- 9 am) for 28 days. The doses of 200, 400, and 800 mg/kg were selected based on acute toxicity findings and previous studies to evaluate the dose-dependent effects of *Zingiber officinale* and *Mondia whitei* within a safe and pharmacologically effective range.

The experimental design established a controlled framework to evaluate the therapeutic effects of *Zingiber officinale* and *Mondia whitei* extracts on diabetes-induced infertility using defined treatment groups. It incorporated normal, negative, and positive controls alongside graded extract doses to enable systematic comparison of physiological responses.

Table 3.2 Dose Dependant Treatment (200, 400, and 800 mg/kg) of *Zingiber officinale* and *Mondia whitei* extracts.

Table 3.2 shows the grouping strategy that allowed clear differentiation between untreated, disease-induced, and treatment-mediated outcomes across all experimental conditions. Dose-dependent administration of both plant extracts provided a structured basis for assessing their restorative potential relative to standard treatment (clomiphene citrate). Overall, the design enhanced the validity of conclusions by ensuring reproducibility, comparability, and comprehensive evaluation of treatment effects.

Groups n=9	Treatment
Normal control	Nondiabetic and fertile group on water alone (normal control group) with no treatment. which served for comparing effects of both extracts.
Negative control	Induced diabetes and infertility using 1.5 ml of Alloxan alone (negative control group)
Positive control	Diabetic and infertile treated with Alloxan + 5 mg/kg of Clomiphene citrate (positive control group).
Treated with 200mg ZO	Diabetic and infertile treated with Alloxan + 200 mg/kg of *Z. offinale* ethanol extract
Treated with 400mg ZO	Diabetic and infertile treated with Alloxan + 400 mg/kg of *Z. offinale* ethanol extract
Treated with 800mg ZO	Diabetic and infertile treated with Alloxan + 800 mg/kg of *Z. offinale* ethanol extract
Treated with 200mg MW	Diabetic and infertile treated with Alloxan + 200 mg/kg of *M. whitei* ethanol extract
Treated with 400mg MW	Diabetic and infertile treated with Alloxan + 400 mg/kg of *M. whitei* ethanol extract
Treated with 800mg MW	Diabetic and infertile treated with Alloxan + 800 mg/kg of *M. whitei* ethanol extract

After 28 days of treatment, the animals were anesthetized using 1% chloroform (0.05 ml/liter of container volume) and sacrificed by cervical dislocation after confirmation of a deep anaesthetic state that was confirmed by the loss of the righting reflex and pupil dilation. Thereafter, a 23-gauge needle was used to collect 5ml of blood samples via heart puncture and placed in an EDTA tube. Blood was collected, swirled carefully to mix and centrifuged to separate the plasma from the cellular components, decanted and serum frozen at -20^0C. A ventral laparotomy was done to harvest testes and together with the hypothalamus and pituitary gland, immediately immersed in Bijou bottles containing 10% formaldehyde for histological and histomorphometric analysis.

3.8 Hormonal Analysis of the Blood Serum

Serum levels of FSH, LH, and testosterone were measured using enzyme-linked immunosorbent assay (ELISA) kits (Chemoquip Limited Kenya), following the manufacturer's protocol. The technique uses the principle of competition of hormone in sample with enzyme conjugated hormone for limited binding sites on the specific antibody. In all hormone assay procedures, assays were done in triplicate. The optical density of the specimen was measured using a BioTek

PowerWave XS2 microplate spectrophotometer, which measures absorbance at 450 nm with background correction at 620 nm, suitable for 96-well ELISA plates.

3.8.1 Luteinizing Hormone Bioassay

All samples and reagents were equilibrated to room temperature prior to use, and the standard solutions were prepared according to the manufacturer's protocol. Approximately 100 µL of serum samples (triplicate) were aliquoted into the wells, with sample dilution performed as necessary to ensure the concentrations fell within the range of the standard curve. After sealing the plate, it was incubated for 2 hours at room temperature (20–25°C). To remove unbound substances, the wells were washed 5 times using wash buffer. Subsequently, 100 µL of enzyme-conjugated detection antibody was added to each well, and the plate incubated at room temperature for 1 hour in the dark. The wells were then washed again 4 times with wash buffer.

Thereafter, 100 µL of substrate solution was added to each well, and the plate incubated in the dark at room temperature for 15 minutes or until the color development was adequate. To halt the reaction and ensure uniform color development, 50 µL of stop buffer solution was added to each well, followed by gentle mixing. The absorbance at 450 nm was measured using a microplate reader, with a reference wavelength of 620 nm recommended. A standard curve plotting absorbance against known Luteinizing Hormone concentrations was generated to analyze the data. The Luteinizing Hormone concentration in the plasma samples was determined based on their absorbance values, and the results were expressed in milli-international units per milliliter (mIU/mL).

3.8.2 Follicle Stimulating Hormone Assay

Follicle-stimulating hormone (FSH) concentrations were quantified from serum samples using a BioCompare Plus/Biomatik enzyme-linked immunosorbent assay (ELISA) kit according to the

manufacturer's instructions**.** To ensure accurate antigen-antibody interactions, all serum samples, standards, and reagents were equilibrated to room temperature and samples diluted as necessary to fall within the linear range of the standard curve. Briefly, 100 µL of serially diluted FSH standards and serum samples were dispensed in duplicate into antibody-coated microplate wells, gently agitated, and incubated for 2 hours at room temperature, after which the wells were washed four times with wash buffer using a microplate washer to remove unbound proteins. Subsequently, 100 µL of enzyme-conjugated detection antibody was added to each well and incubated for 1 hour, followed by four additional wash cycles; 100 µL of chromogenic substrate solution was then added and incubated for 10 minutes in the dark to allow color development proportional to the amount of bound FSH. The reaction was terminated by adding 50 µL of stop solution to each well, and absorbance was measured at 450 nm using a microplate ELISA reader (spectrophotometer), which detects the optical density generated by the enzyme-substrate reaction. A standard curve was constructed by plotting absorbance values against known FSH standard concentrations, and sample FSH concentrations were determined by interpolation from the curve and expressed as mIU/mL.

3.8.3 Testosterone Assay

All reagents were prepared in accordance with the protocol, and the standard solutions were prepared by serial dilution following the manufacturer's (Bio compare plus, Biomatik) instructions and the expected testosterone concentrations. Samples were diluted as necessary to fall within the range of the standard curve. In duplicate, 100 µL of standards and samples were added to the appropriate wells and carefully sealed.

The plate was incubated for 2 hours at room temperature (approximately 20 to 25°C), as directed. To remove unbound testosterone, the wells were washed 5-times using wash buffer, with gentle shaking. Subsequently, 100 µL of enzyme-conjugated detection antibody was added to each well,

and the plate incubated for 1 hour at room temperature. The wells were then washed again 4 or 5 times to eliminate unbound detection antibody.

Thereafter, 100 µL of substrate solution was added to each well, and the plate incubated in the dark at room temperature for 15 minutes. To stop the reaction and ensure consistent color development, 50 µL of stop buffer solution was added to each well, followed by gentle mixing. The absorbance was measured at 450 nm using a microplate reader, with a reference wavelength such as 620 nm recommended.

A standard curve was generated by plotting the absorbance values against the known testosterone concentrations. The testosterone levels in the samples were determined by extrapolation from the standard curve, and the results expressed in nanograms per milliliter (ng/mL).

3.9 Sperm Characteristics

3.9.1 Sperm Count and Motility

This was performed following the procedure described by Koprulu et al. (2022). Briefly, the left and right epididymis were harvested and dissected at the caudal end, cut into small pieces and placed in a 3 ml of 37 °C neutral buffered formalin solution on a Petri dish at a ratio of 1:1, minced using scalpel blade and allowed to stand for 15 minutes, followed by mixing in 1% trisodium citrate. The spermatozoa were allowed to diffuse into the buffer for 2 minutes and swirled to obtain a homogeneous mixture used for the sperm characteristic analysis. Thereafter, 1 ml of the solution was placed on a hemocytometer, covered with a cover slip and viewed under a microscope at 400x magnification for sperm cell count and motility.

Sperm count was done according to Al-Alami et al., (2017) and, WHO Laboratory Manual for the Examination and Processing of Human Semen, (2021). An aliquot of 10 µL of the homogenous sperm suspension was loaded on each of the chambers of the haemocytometer covered with

coverslip and allowed to settle for 1 minute before counting the sperm cells in the five large squares (four at the corners and the middle one) at 100× total magnification light microscope (Olympus Microscope BH-2, USA). Sperm cells with their heads completely in the square were counted; the counting was done for the two counting chambers and averaged. The concentration of the spermatozoa in 1 mL of the suspension was calculated according to the formula:

$$\boldsymbol{Concentration}\left(\frac{\boldsymbol{Count}}{\boldsymbol{mL}}\right) = (\boldsymbol{Dilution\ factor}) \times (\boldsymbol{Count\ in\ 5\ squares}) \times (\mathbf{0.05 \times 10^6})$$

3.9.2 Sperm Motility

Three metrics were used to access motility: proliferating cells, non-proliferating cells, and dead cells (Delbarba et al., 2025). According to observation, the rapidly motile, slowly motile, and non-motile cells were noted and expressed in percentages. The sperm viability was estimated by adding the progressive and non-progressive motile cells. Non-motile cells were the dead cells with no visible movement seen during microscopic examination. The total motility was determined by:

$$\textbf{Motility}\ (\%) = \frac{\textbf{Number of motile sperms per unit volume}}{\textbf{Total sperm per unit Volume}} \times \mathbf{100}\%$$

For quality control, this process was done by two independent individuals blinded to the treatment given to the different groups. These determined the number of motile sperm three times for each sample, and an average was obtained for the six counts. The mean was calculated for each individual sample.

3.9.3 Sperm Morphology and Viability Analysis

According to protocol by Lotti et al., 2023, Sperm samples were collected from the epididymal reserve at the caudal portion of the epididymis and the sperm viability determined using Nigrosine-Eosin staining. Nigrosine-Eosin staining differentiates live from dead sperm based on membrane integrity. Live sperm have intact membranes, preventing dye entry, and thus remain unstained or

appear white while dead sperm have compromised membranes, allowing eosin to penetrate, staining the sperm head pink or red.

Mixtures of Nigrosine (black dye) with Eosin (pink dye) typically in a 1:1 ratio was prepared as per the protocol. A mixture of small volume of sperm suspension with the Nigrosine-Eosin stain (usually 10 µL of sperm with 10 µL of stain) was incubated briefly (about 3 minutes) at room temperature. A drop of the stained mixture was put on a clean glass slide, gently covered with a coverslip and thereafter observed under a light microscope at 400x magnification. At least 200 spermatozoa were examined for statistical accuracy. Viable sperm counting and calculation was based on unstained or white (membrane intact). Non-viable sperm: stained pink or red (membrane compromised).

Determine Viability Percentage

$$\textbf{Viability}\ (\%)\ = \frac{(\textbf{Number of unstained (viable) sperm}}{\textbf{Total sperm counted}} \times \mathbf{100}\%$$

Sperm stained pink or red were considered non-viable. Counting was performed in multiple fields to ensure accuracy and followed specific protocol for staining concentration and incubation times.

3.10 Tissue Processing for Histology Study

According to Msiska et al., 2020 protocol, Perfusion fixation was performed on animals through the left ventricle with 10% formaldehyde in phosphate buffered saline (pH 7.4) for light microscopy of the testes, pituitary gland and hypothalamus (n = 4 per group). The testes were harvested and testicular volumes calculated using the Scherle method (Msiska et al., 2020), also known as Water Immersion Volumetry. The whole testis of each animal was immersed in a jar containing phosphate buffer of known weight placed on a calibrated weighing balance. The change in weight reading on the balance was taken to be the volume of the immersed testis, based on the Archimede's Principle, that the buoyant force on a submerged object is equal to the weight of the

fluid that is displaced by the object. The mean score on volume was taken after three successive readings of the same sample.

The diameter and cross-sectional area of the seminiferous tubule, volume density of seminiferous tubules and interstitium, number of profiles of seminiferous tubules per unit area of testis, numerical density of seminiferous tubules and length density of seminiferous tubules were all recorded.

Diameter of seminiferous tubule (d)

$$\mathbf{D} = \frac{\textbf{Perimeter of tubule profile}}{\boldsymbol{\pi}}$$

Cross-sectional area of seminiferous tubules (A)

$$\mathbf{A} = \frac{\textbf{Area of the tubule profile}}{\textbf{Number of profiles}}$$

Volume density (Vv) of seminiferous tubules/interstitium

$$\mathbf{Vv} = \frac{\textbf{Number of profiles of the components}}{\textbf{Total number of profiles in the reference space}}$$

After weighing, the harvested tissues were then immersed in the same fixative for 7 days and trimmed into sufficiently small sizes (1 mm^3) to permit proper fixation and processing. The tissues were thoroughly washed in distilled water and dehydrated in ascending concentrations of ethanol (50, 70, 80, 95% and twice in absolute ethanol) and, thereafter, infiltrated and embedded in paraffin wax.

The embedded tissues were sectioned in a transverse plane at a thickness of 5 µm using a rotary microtome, mounted on clean microscope slides, dried on a heating plate and rehydrated using descending concentrations of ethanol and deparafifinized using xylene. The slides were, thereafter, stained using hematoxylin and eosin and viewed and analysed under light microscope (primo star model) fitted with a digital camera and a computer.

3.11 Statistical Analysis

The data was analyzed using Kruskal – Wallis and Chi-square for objective 1, 2 and 3 statistical tools. Quantitative and Qualitative data was presented as median ± standard deviation. Numeric data obtained from Phytochemical analysis, Physiological and Biochemical assays were subjected to one-way ANOVA, followed by multiple mean comparisons using Tukey's post-hoc test. The results on mean difference among groups were considered significant at $p<0.05$.

3.12 Ethical Considerations

The protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Nairobi (REF: FVM BAUEC/2024/571) as well as Maseno University Ethics Committee (REF: FVM BAUEC/2024/571). The experiments were strictly performed in accordance with the internationally accepted standard ethical guidelines for Laboratory Animal Use and Care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986.

CHAPTER FOUR

RESULTS

4.1 Quantitative and Qualitative Phytochemical and Antioxidant Activity Profile

Qualitative phytochemical analysis was performed to identify the major bioactive compounds that may underlie the biological and therapeutic effects of *Zingiber officinale* and *Mondia whitei* extracts. The analysis revealed high levels of flavonoids and low levels of alkaloids, tannins, saponins, phenolic compounds, and glycosides, indicating that flavonoids constitute the predominant phytochemical class and may contribute significantly to the extracts' antioxidant, anti-inflammatory, and reproductive-protective activities. The parameters assessed, namely flavonoids, alkaloids, tannins, saponins, phenolic compounds, and glycosides, are important because they influence key biological processes including oxidative stress regulation, immune modulation, cellular signaling, hormone production, and tissue protection. Therefore, the phytochemical profile suggests that the observed biological effects of the extracts may arise from the combined actions of these compounds, with flavonoids likely playing a dominant role in mitigating cellular damage and supporting reproductive function.

4.1.1 Quantitative and Qualitative Phytochemical Profile of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts

Table 4.1 provides a comparative phytochemical profile of *Zingiber officinale* and *Mondia whitei*, with concentrations expressed using standard equivalents such as QE, TAE, and GAE to ensure analytical consistency. It highlights the relative abundance and trace presence of key bioactive compounds based on qualitative indicators and quantitative measurements.

Table 4.1 Quantitative and Qualitative Phytochemical Profile of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts

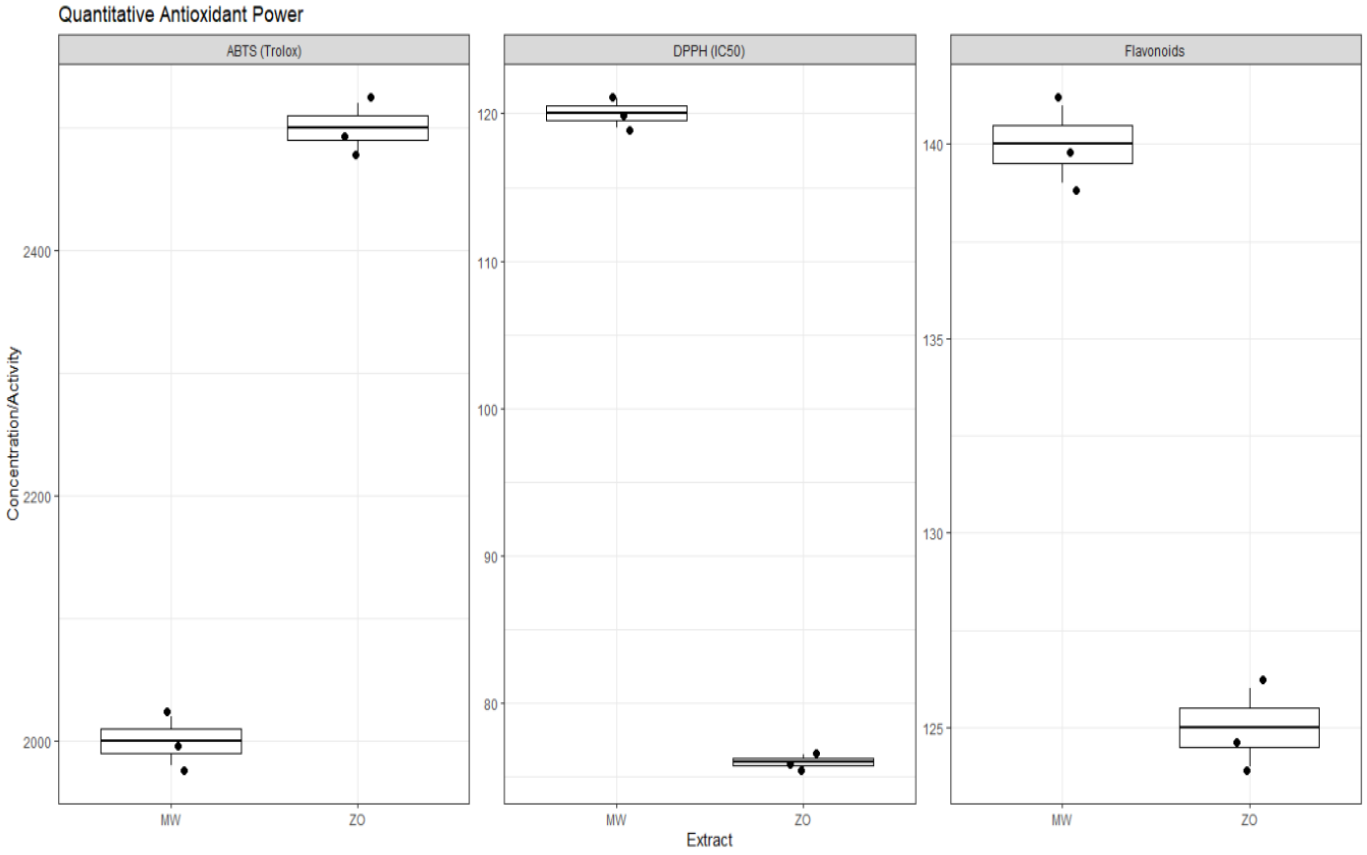
The table 4.1 indicates that *Zingiber officinale* and *Mondia whitei* had trace amount of alkaloids (15mg/g and 8mg/g*, respectively*), large amounts of flavonoids in *both Zingiber officinale* and *Mondia whitei* (125mgQE/g and 140QE/g, respectively), trace amounts of tannins in *both Zingiber officinale* and *Mondia whitei* (15mgTAE/g and 22mgTAE/g, respectively), trace amount of saponins in *both Zingiber officinale* and *Mondia whitei* (7.5%(ww) and 15% (ww)*, respectively*), Phenolic Compounds we have trace amount in both *Zingiber officinale* and *Mondia whitei* (220 mg GAE/g and 220 mg GAE/g, and lastly trace amount of glycosides in *both Zingiber officinale* and *Mondia whitei.*

Grp	**Phytochemicals**	***Qualitative Profile***		***Quantitative Profile***	
		Z. officinale	***M. whitei***	***Z. officinale***	***M. whitei***
1	Alkaloids	+	+	15mg/g	8 mg/g
2	Flavonoids	+++	+++	125mgQE/g	140mg QE/g
3	Tannins	+	+	15mgTAE/g	22mg TAE/g
4	Saponins	+	+	7.5%(w/w)	15%(w/w)
5	Phenolic Compounds	+	+	220mgGAE/g	220mg GAE/g
6	Glycosides	+	+		

Note: Qualitative QE – (quantum efficiency per gram), TAE – (Tannin Acid Equivalent), GAE – (Gallic Acid equivalent) + = typically indicates a small or trace amount of the compound, +++ = signifies a large or abundant amount.

4.1.2 Antioxidant Profile of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts

Figure 4.1 presents the quantitative antioxidant activities of ***Zingiber officinale*** and ***Mondia whitei*** ethanolic extracts as determined by ABTS, DPPH, and FRAP assays, which evaluate their ability to scavenge free radicals, donate electrons or hydrogen atoms, and reduce oxidized substrates. The results indicate that ***Zingiber officinale*** exhibited greater antioxidant potency than ***Mondia whitei***, as evidenced by a higher ABTS value (≈2500 vs. ≈2000 µmol Trolox equivalents) and a lower DPPH IC_{50} value (76 vs. 120 µg/mL), while both extracts showed similar ferric reducing antioxidant power (FRAP) of approximately 400 µmol equivalents/g.

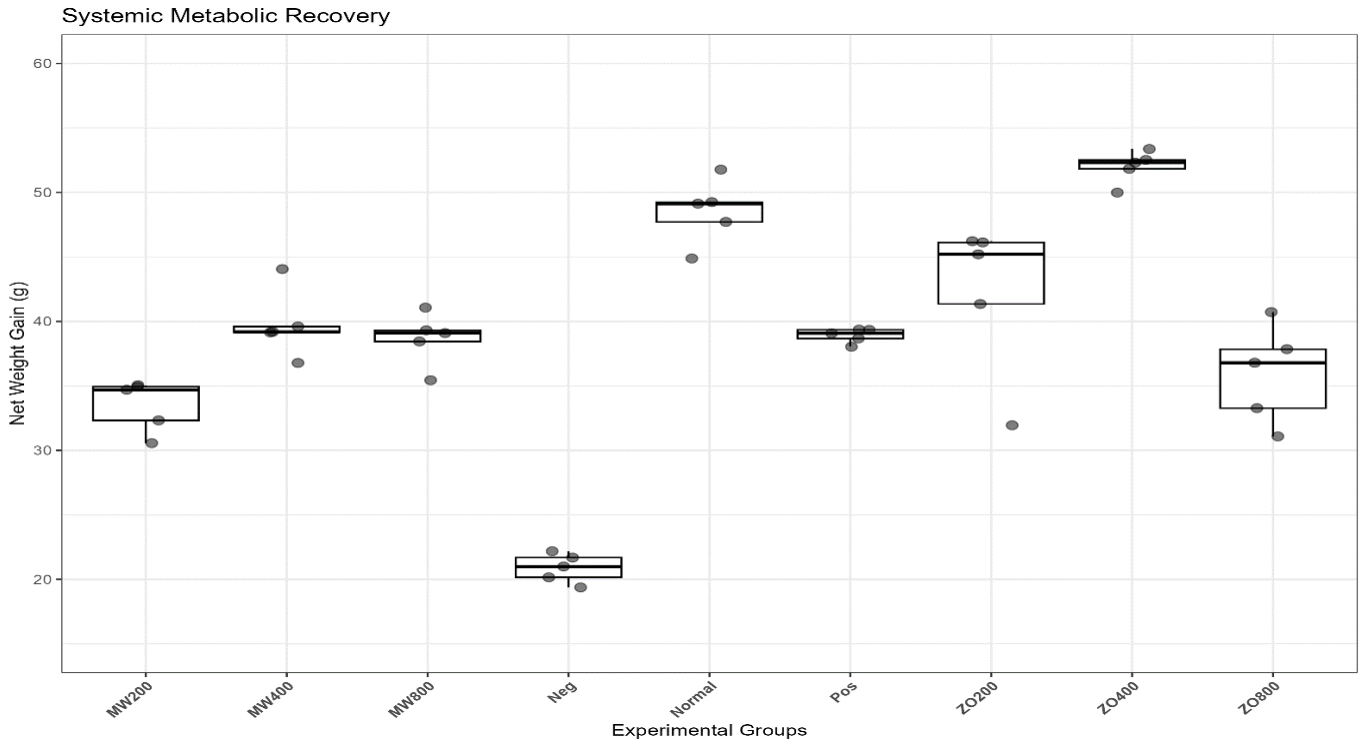


Note: DPPH – (2,2-diphenyl-picryl hydrazyl), IC – (Inhibitory Concentration), FRAP – (Ferric Reducing Antioxidant power), ABTS –2,2'-azinobis (3-ethyl benzothiazoline-6-sulfonic acid), *ZO-Zingiber officinale*, *MW-Mondia whitei*.

4.2 Body Weight

Figure 4.2 shows body weight distribution in both non-diabetes and diabetic rats treated with *Mondia whitei* and *Zingiber officinale*.

The figure 4.2 shows that negative control group exhibits severe metabolic waste, with its distribution consistently below 230g, underscoring the pathological impact of untreated diabetes. In contrast, the treatment groups reveal a graded, dose-dependent restoration of body mass, reflecting the therapeutic potential of phytochemical intervention.



Note- *ZO-Zingiber officinale*, *MW-Mondia whitei*, g-Grams.

4.3 Hormonal Profile

4.3.1 Plasma levels of Follicle Stimulating Hormone

Figure 4.3 shows the plasma follicle stimulating hormones in both non diabetes and diabetic treated groups with *Zingiber officinale* and *Mondia whitei* extracts. It shows that induction of diabetes significantly suppressed plasma FSH levels in the negative control to a median of approximately 2.2 mIU/mL. However, administration of the extracts, particularly at the 800 mg/kg doses for both *Mondia whitei* and *Zingiber officinale*, restored these levels to approximately 6.07 mIU/mL and 6.65 mIU/mL respectively, values that are statistically comparable to the healthy Normal Control (6.30±1.78 mIU/ml).

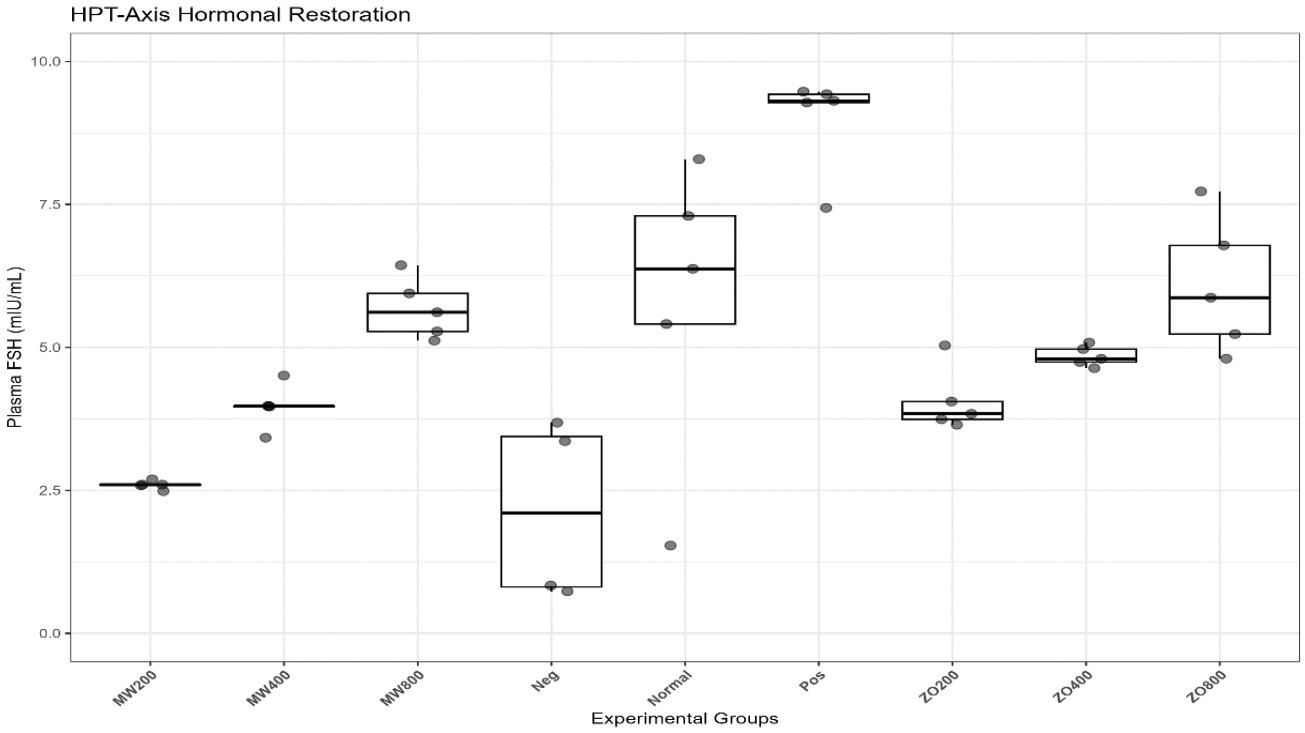


Figure 4.3. Plasma levels of Follicle stimulating hormone

Note; *ZO-Zingiber officinale*, *MW-Mondia whitei* and FSH-Follicle Stimulating Hormone.

4.3.2 Plasma levels of Luteinizing Hormone

Figure 4.4 shows the plasma Luteinizing Hormone in both non diabetes and diabetic treated groups with *Zingiber officinale* and *Mondia whitei* extracts. It shows that the introduction of diabetes significantly ($p<0.01$) decreased the median plasma levels of luteinizing hormone (negative control group) to a median of 1.28 mIU/mL compared to normal rats (3.98 ± 1.07 mIU/ml). When compared to the negative control, luteinizing hormone levels were considerably higher in diabetic rats treated with 400 mg/kg of *Zingiber officinale* (3.53 ± 0.16 mIU/ml) ($p<0.05$) and significantly higher at 800 mg/kg of the sample extracts (4.55 ± 0.14 mIU/ml) ($p<0.01$).

Additionally, the injection of 200 mg/kg of *Mondia whitei* only slightly increased the luteinizing hormone levels, despite the fact that they were significantly ($p<0.05$) lower than those of the normal control. However, luteinizing hormone levels rose to 3.50 ± 0.18 mIU/ml when the dose was raised to 800 mg/kg. These levels were comparable to the normal control but significantly higher than the negative control and close to the positive control (5.03±0.37 mIU/ml) ($p<0.05$).

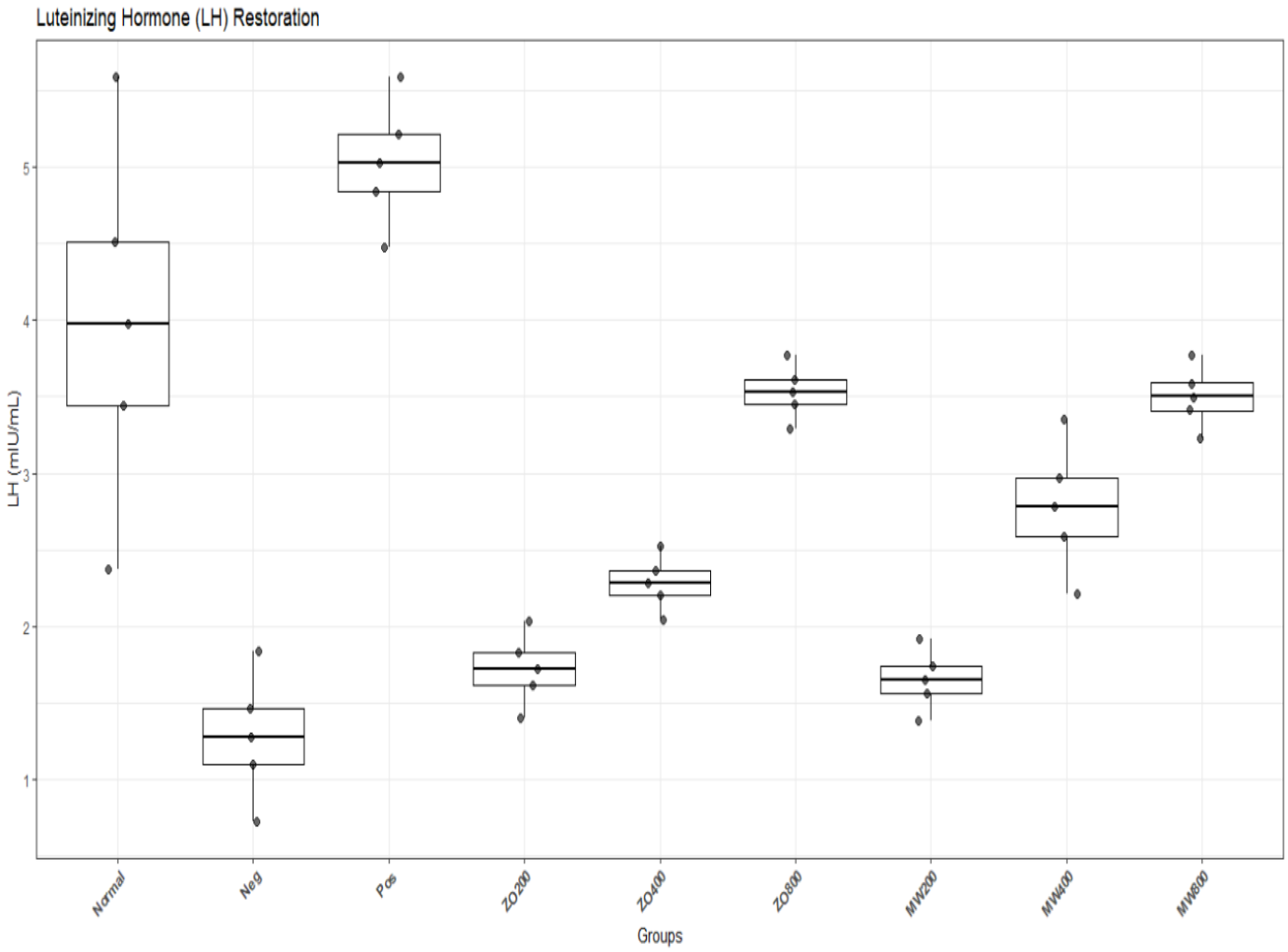


Figure 4.4. Plasma levels of Luteinizing Hormone

Key; *ZO-Zingiber officinale*, *MW-Mondia whitei*

4.3.3 Plasma Levels of Testosterone

Figure 4.5 shows the levels of plasma Testosterone Hormone in both non diabetes and diabetic treated groups with *Zingiber officinale* and *Mondia whitei* extracts. It shows negative control group's plasma levels of testosterone dropped to a median of 71.53 ng/dL, which was substantially lower ($p<0.001$) than those of the positive control group (584.9 ± 6.843 ng/dl) and normal control group (509.9 ± 64.97 ng/dl). Testosterone levels were significantly lower than the normal control at 200 mg/kg and 400 mg/kg for *Zingiber officinale*-treated groups and 200 mg/kg, 400 mg/kg,

and 800 mg/kg for *Mondia whitei* extracts-treated groups. In contrast to the diabetic group, plasma testosterone levels significantly increased to a median of 512.4 ng/dL ($p<0.001$) at 400 mg/kg and 800 mg/kg of *Zingiber officinale* and 800 mg/kg of *Mondia whitei*; treated group 6 had testosterone levels that were comparable to the positive control.

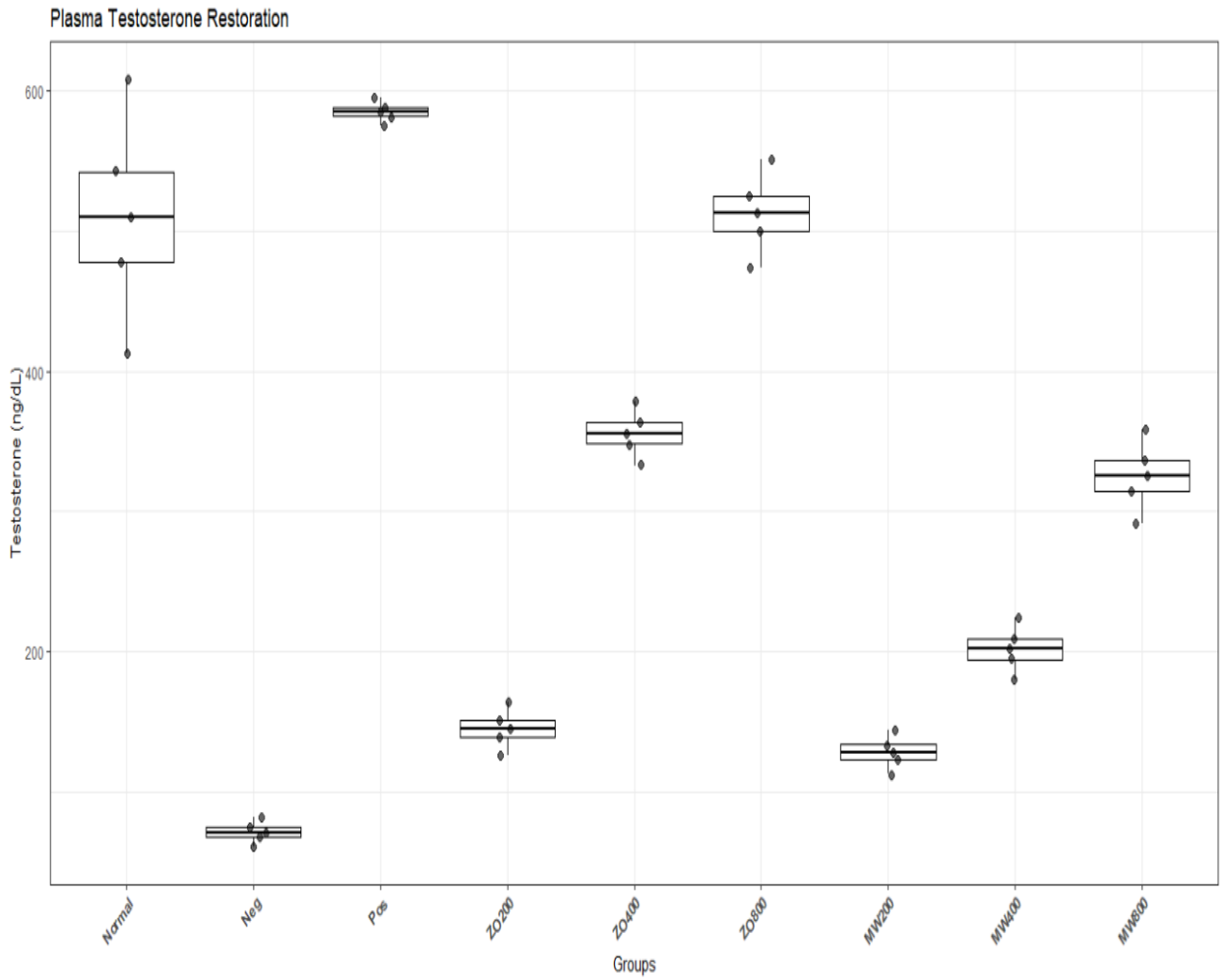


Figure 4.5. Plasma levels of Testosterone Hormone

Note; *ZO-Zingiber officinale*, *MW-Mondia whitei*

4.4 Sperm Parameters

4.4.1 Effects of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts on Sperm Parameters

Table 4.2 shows that Induction of diabetes significantly compromised sperm count, morphology, and motility compared to normal conditions. Treatment with *Zingiber officinale* and *Mondia whitei* extracts progressively restored sperm quality in a dose-dependent manner. At higher doses, the improvements approached or even surpassed normal control levels, indicating strong therapeutic potential. It shows that the induction of diabetes significantly decreased sperm count, morphology, and motility (102.00 ± 14.20, 25.00 ± 2.86, and 12.54 ± 2.74, respectively) compared to the normal control (203.3 ± 17.05, 55.00 ± 4.98, and 51.02 ± 5.41). However, when *Zingiber officinale* and *Mondia whitei* rhizome and root extracts were given, respectively, the sperm parameters; motility, counts, morphology showed restoration in a dose-dependent manner. For example, when 200 mg/kg of *Zingiber officinale* was given, all the readings (149.8 ± 14.06, 27.50 ± 2.21, and 35.00 ± 2.300, respectively) somewhat improved.

In comparison to the negative control, sperm motility increased considerably for both extract treatments at 400 mg/kg (173.3 ± 5.66, 37.00 ± 1.87, and 42.29 ± 6.21) and 800 mg/kg (210.5 ± 7.92, 45.00 ± 3.87, and 49.55 ± 3.27). Administration of 200 mg/kg of *Mondia whitei* also enhanced all assessed sperm parameters at 800 mg/kg (239.4 ± 5.67, 64.32 ± 8.34, and 54.43 ± 4.38), which were either close to or slightly higher than those of the positive control (240.01 ± 12.66, 53.25 ± 4.09, and 52.31 ± 3.52, respectively).

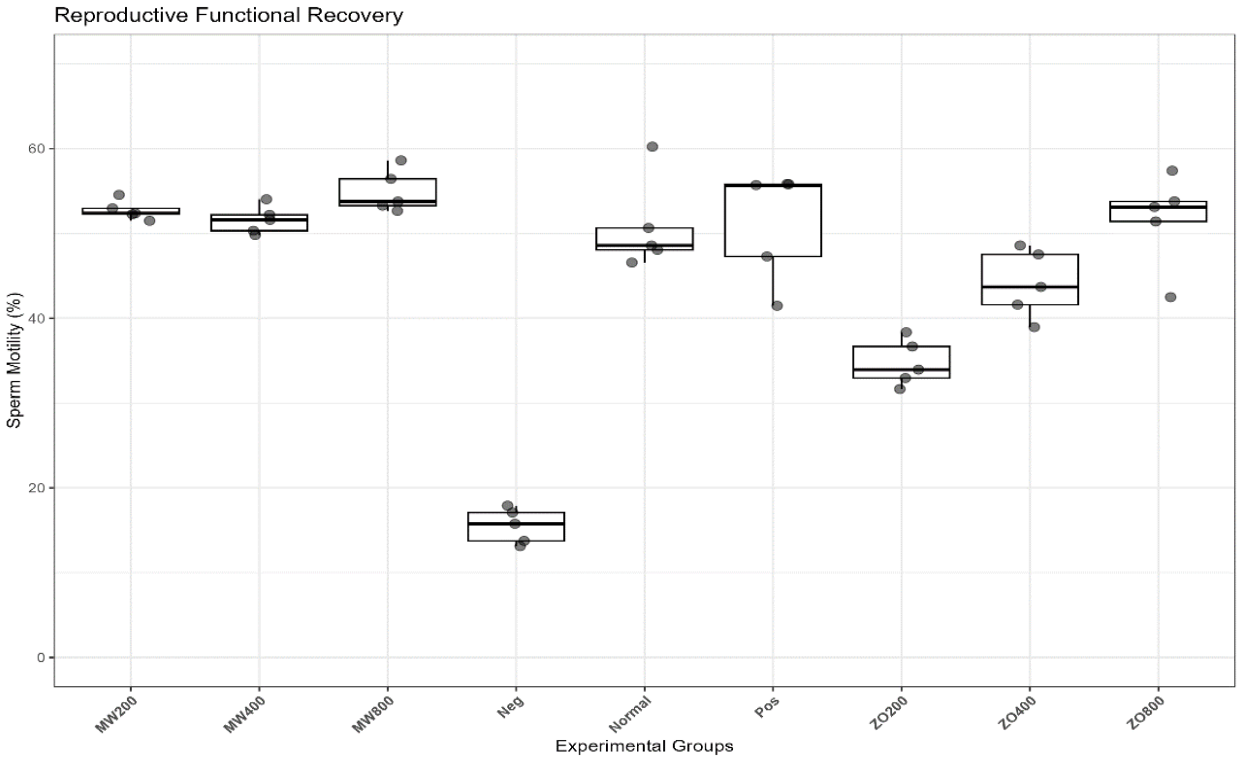
Table 4.2. Effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Sperm Parameters

Group	**Sperm count/ ml × 10^6/ml**	**Sperm morphology (%) Structural Normality**
Normal control	203.3 ± 17.05	55.00 ± 4.98
Negative control	102.00 ± 14.20#	25.00 ± 2.86#
Positive control	240.01 ± 12.66#	53.25 ± 4.09#
Treated with 200mg/kg ZO	149.8 ± 14.06	27.50 ± 2.21#
Treated with 400mg/kg ZO	173.3 ± 5.66	37.00 ± 1.87
Treated with 800mg/kg ZO	210.5 ± 7.92#	45.00 ± 3.87
Treated with 200mg/kg MW	228.8 ± 8.49	58.75 ± 2.93#
Treated with 400mg/kg MW	232.6 ± 7.23#	61.57 ± 4.65#
Treated with 200mg/kg MW	239.4 ± 5.67#	64.32 ± 8.34#

NOTE; #Indicate statistical difference compared to group 1 (normal control) and group 2 (negative control) respectively, # mean $p < 0.05$, ## mean $p < 0.01$

4.4.2 Effects of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts on Sperm Motility

Figure 4.6 showing effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on sperm motility on both non-diabetic and diabetic treated white male albino rats. Figure shows that while the Negative Control sperm motility collapsed to a median of 12.54%, the treatment groups show a dramatic "Functional Recovery". The high-dose *Mondia whitei* 800 mg/kg group reached a median motility of 54.43%, effectively matching the performance of the Normal Control (51.02%) and the Positive Control (52.31%). Treatment, particularly with high-dose *Mondia whitei* (800 mg/kg), resulted in substantial functional recovery, restoring sperm motility to 54.43%, comparable to normal and positive control levels, thereby demonstrating strong therapeutic effectiveness.



Note; *ZO-Zingiber officinale*, *MW-Mondia whitei*

4.4.3. Dose – Response Correlation between *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts on Sperm Motility

Figure 4.7 shows dose – response correlation between *Zingiber officinale* and *Mondia whitei* ethanolic extracts on sperm motility in both non-diabetic and diabetic treated white male albino rats. Figure shows dose–response correlation relationship between extract concentration and sperm motility recovery in diabetic rats. Both *Mondia whitei* and *Zingiber officinale* exhibit linear improvements, confirming that therapeutic efficacy scales with dosage. *Mondia whitei* at 800 mg/kg is particularly noteworthy, achieving a motility rate of 54.43%, which surpasses the baseline of the healthy control group at 51.02%. *Zingiber officinale* shows a steep upward trajectory, rising from 35.00% motility at the lowest dose to 49.55% at the highest, highlighting its strong restorative capacity.

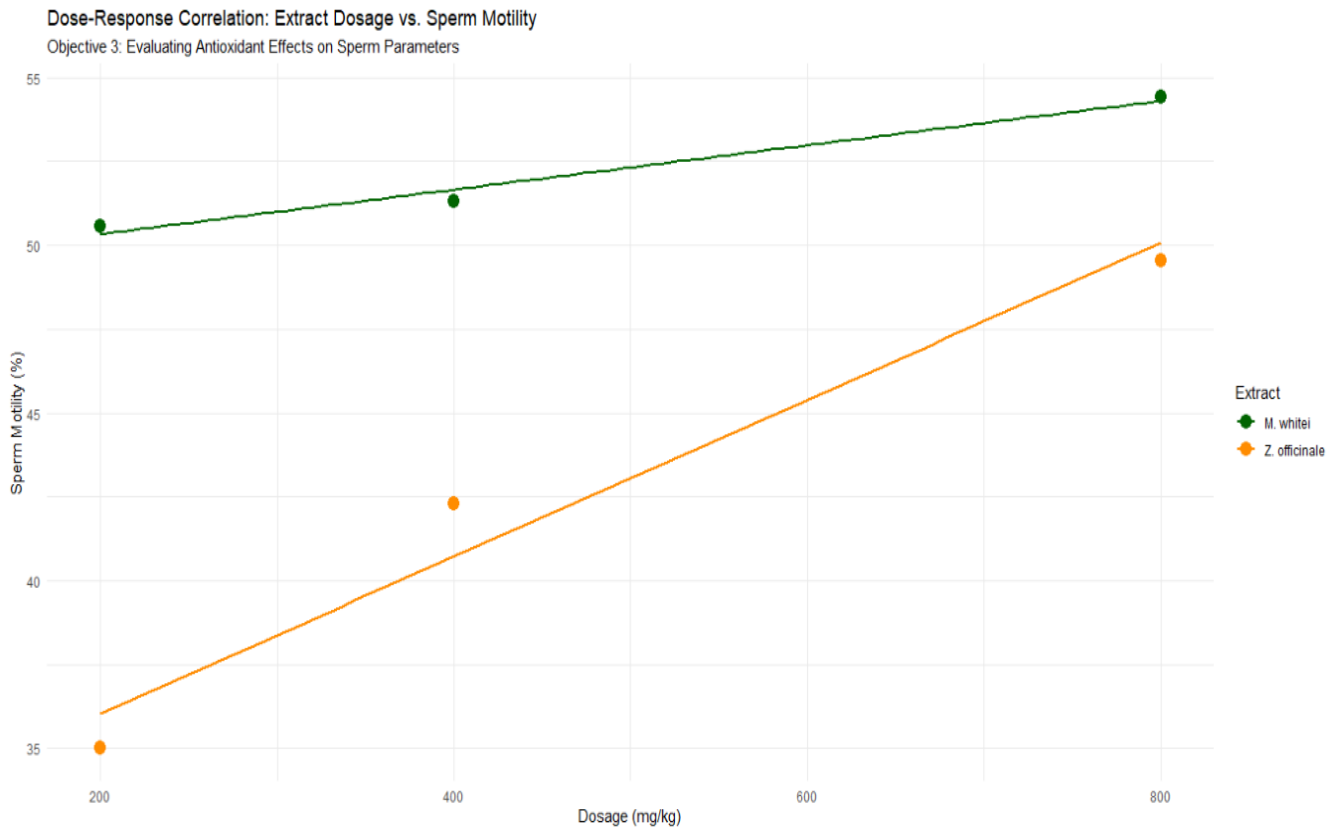


Figure 4.7. Dose – Response Correlation between *Zingiber officinale* and *Mondia whitei* Ethanolic

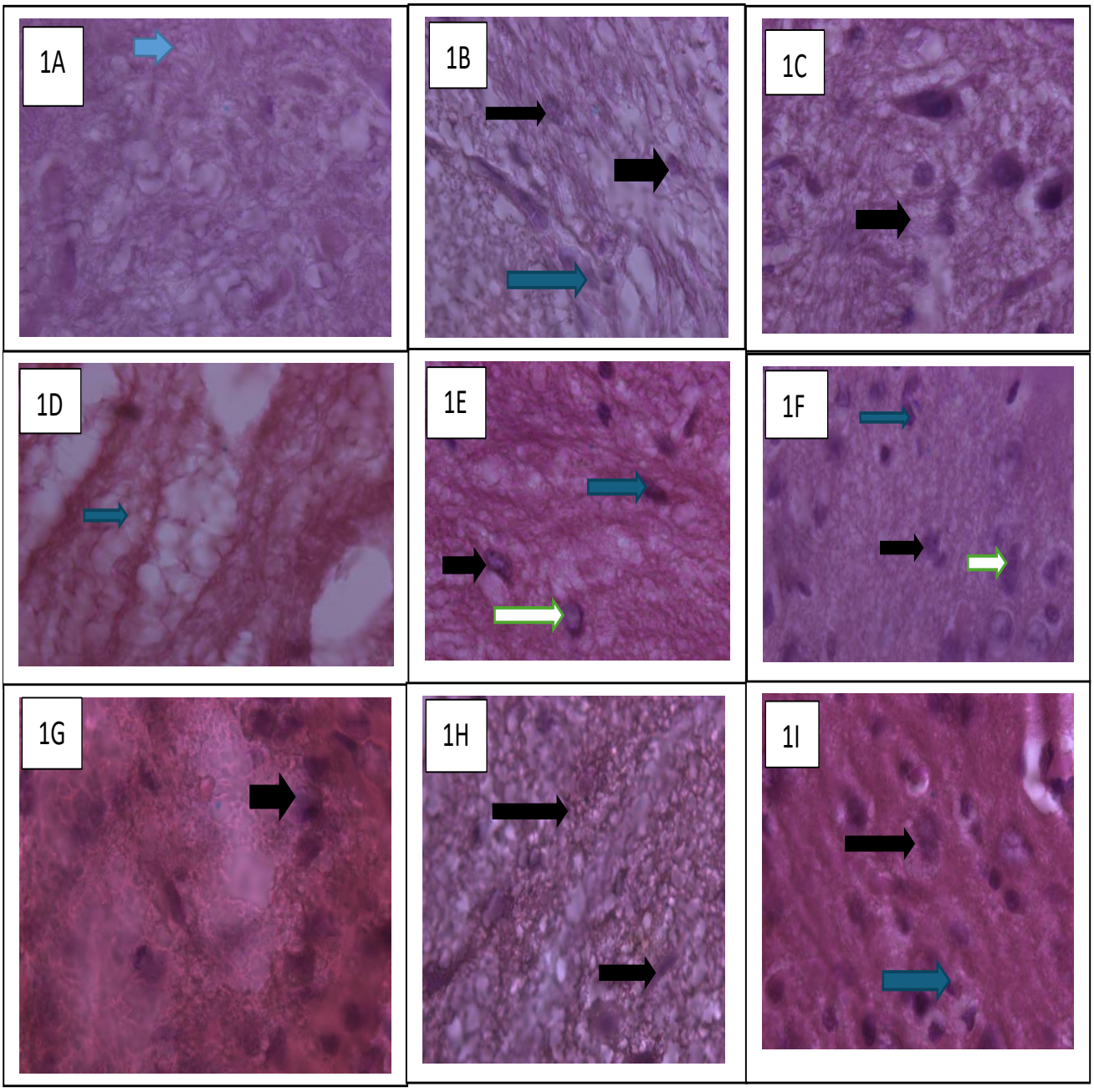
Extracts on Sperm Motility of white male albino rats

4.5 Histology of Hypothalamus, Anterior Pituitary and Testis of white male albino rats

4.5.1 Histological effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Hypothalamus of white male albino rats

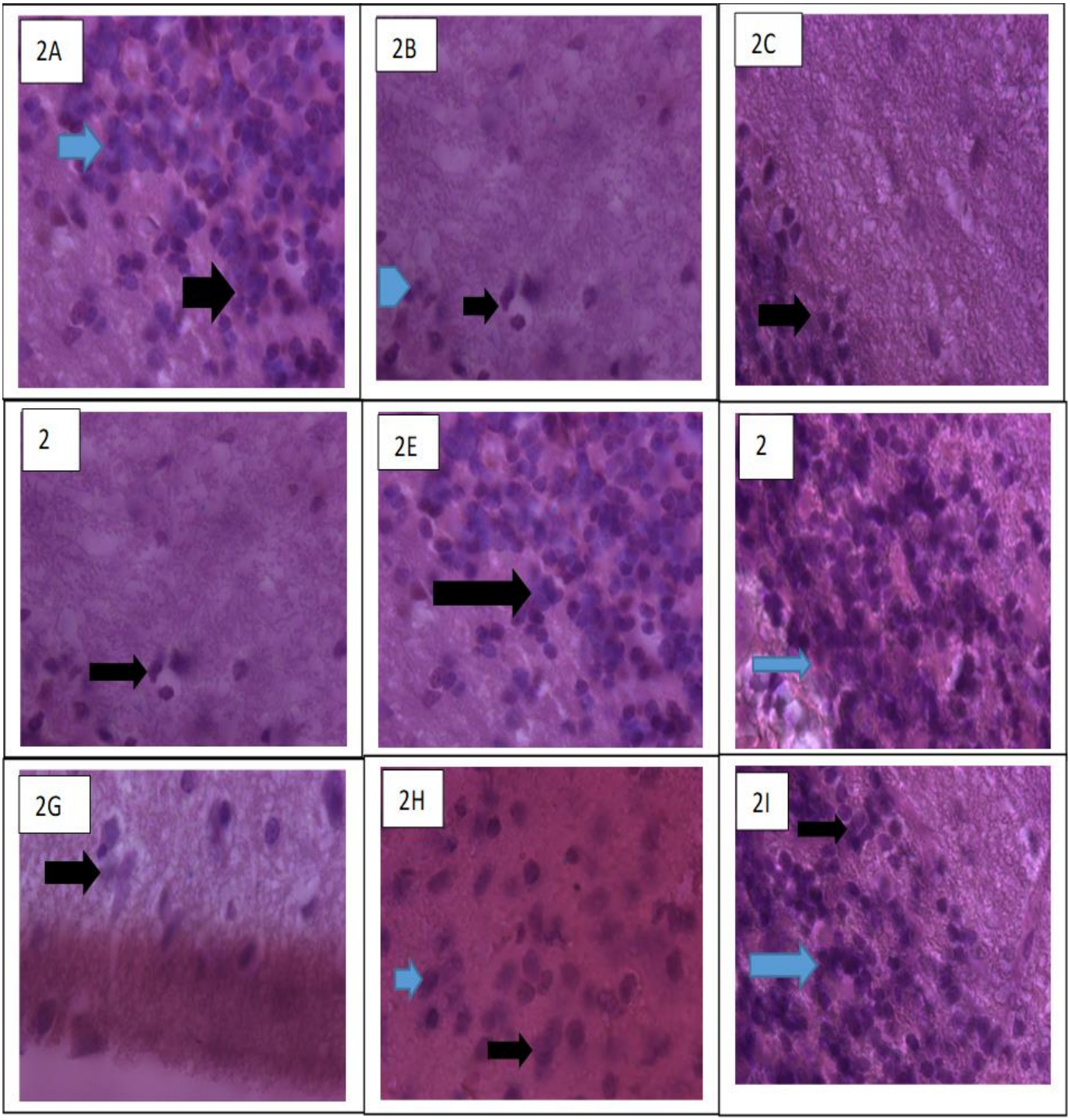
Plate 1 showing histological effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Hypothalamus in both non-diabetic and diabetic treated white male albino rats. It shows plates of the normal control (A), negative control (B), positive control (C), and treatment groups for *Mondia whitei* (G–I) and *Zingiber officinale* (D–F). Examine the histology of the normal control, which has a normal distribution of glial cells and neurons scattered throughout the hypothalamic tissue, whereas the negative control has concentrated, dark nuclei (black arrows) with related vascular congestion and hyalinization (light blue arrows). In the positive control, there are more distinguishable, healthy neurons with nuclei that

have a proper morphological shape (black arrows). The cell populations in the treatment groups for both extracts appear to have normal structural alignment within the hypothalamus, similar to the normal control, according to hematoxylin and eosin 1000x magnification.



4.5.2 Histological Effects on Anterior Pituitary Gland of white male albino rats

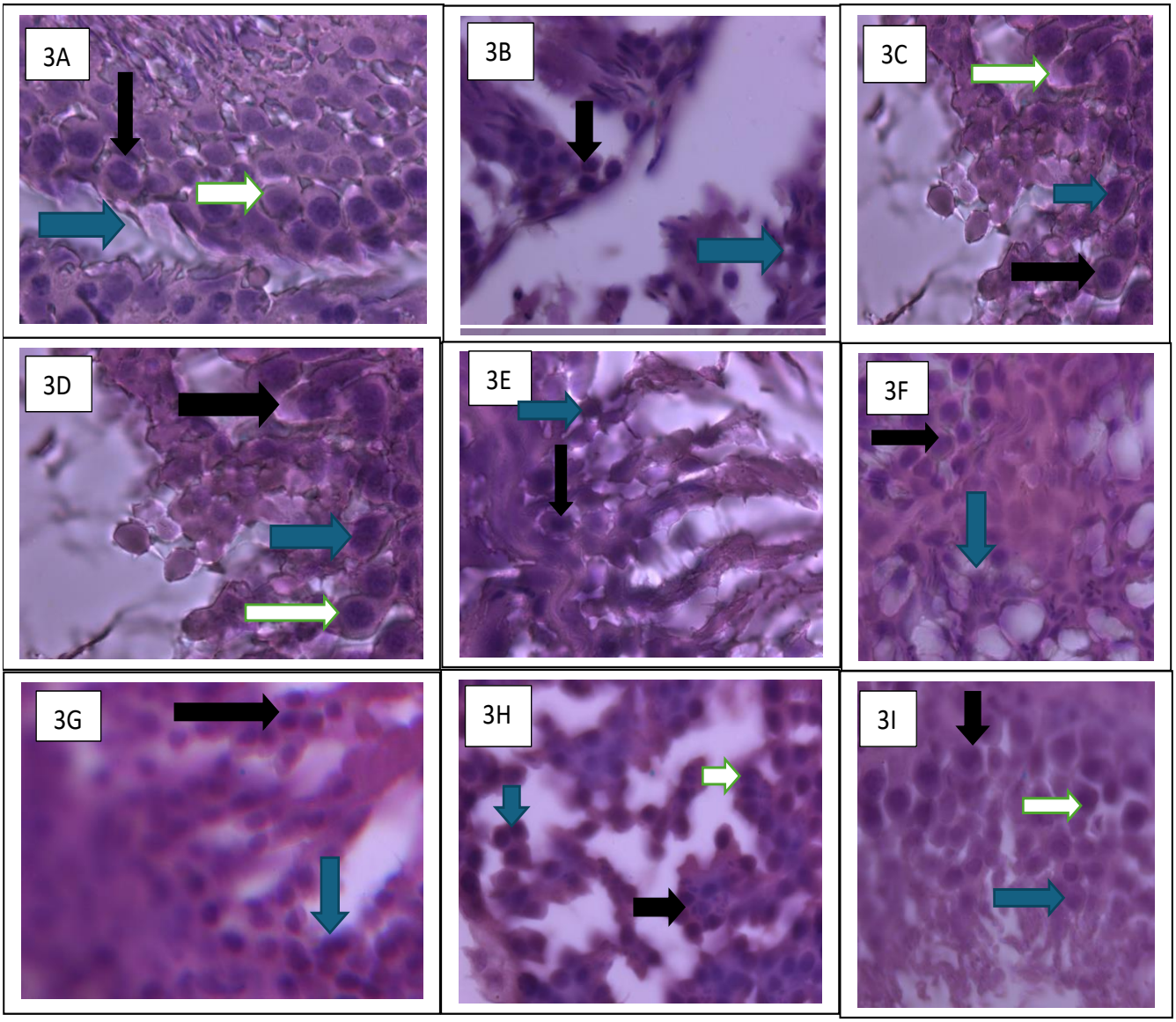
Plate 2 shows histological effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Anterior Pituitary Gland in both non-diabetic and diabetic treated white male albino rats. It shows anterior pituitary gland histology plates on normal control (2A), negative control (2B), and positive control (2C). The positive control (2C) shows more gonadotroph cells (black arrows) with correspondingly higher vascularization; the negative control (2B) shows less glandular cell distribution; and the normal control (2A) shows an active gland with many acidophilic cells (black arrows), chromophobic cells with less staining, and basophilic cells (light blue arrows). At 400 mg/kg of both extracts (2E and 2H), acidophils, chromophobes, and basophils were more extensively dispersed than at 200 mg/kg of *Zingiber officinale* (2D-F) and *Mondia whitei* (2 G-I). At 800 mg/kg (2F and 2I), the tissues have many acidophilic, chromophobic, and basophilic cells, but they also have more gonadotroph cells and better vascularization (1000x magnification).


2A
2B
2C
2
2E
2
2G
2H
2I

4.5.3 Histological Effects on Testes of white male albino rats

Plate 3 shows histological effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Testes in both non-diabetic and diabetic treated white male albino rats. It shows the histology plates of the rats' testes in normal control (3A), negative control (3B), positive control (3C), and treatment groups exposed to increasing doses of *Mondia whitei* (3G–3I) and *Zingiber officinale* (3D–3F). The typical control shows spermatogonia (black arrows), spermatocytes (open arrows), and Sertoli cells (light blue arrows). Note that the negative control has less testicular cells than the positive control, which has more (black arrows).

Following treatment with 200 mg/kg *Zingiber officinale* (3D) and *Mondia whitei* (3G), seminiferous tubular cell populations are less dispersed; but, following treatment with 400 mg/kg *Zingiber officinale* (3E) and *Mondia whitei* (3H), they are more broadly dispersed, indicating ameliorative effects. At 800 mg/kg of *Zingiber officinale* (3F) and *Mondia whitei* (3I), the seminiferous tubular epithelial organization contains more spermagonia, spermatocytes, and spermatozoa. Stain at 1000x magnification using hematoxylin and eosin.


3A
3B
3C
3D
3E
3F
3G
3H
3I

4.6 Histomorphometric Effects on Testes of white male albino rats

The table 4.3 shows histomorphometric effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on Testes in both non-diabetic and diabetic treated white male albino rats.

Table 4.3. Histomorphometric effects of *Zingiber officinale* and *Mondia whitei* ethanolic extracts on testes. It shows a structured evaluation of testicular histomorphometric parameters, including testicular weight, volume, seminiferous tubule diameter, epithelial height, and Leydig cell volume, with statistical significance assessed across experimental groups. It demonstrates that treatment with *Zingiber officinale* and *Mondia whitei* produced dose-dependent restoration in these parameters that appear improving towards normal and positive control levels with significant variation at defined probability thresholds. It shows that all parameters improved in a dose-dependent manner to levels like the normal and positive controls when *Zingiber officinale* and *Mondia whitei* were given. For instance, administering 200 mg/kg and 400 mg/kg of *Zingiber officinale* and *Mondia whitei* at either $p<0.05$ or $p<0.01$ significantly improved all testicular histomorphometric measurements except seminiferous tubule diameter.

Grps	TW (ml)	TV (ml)	STD (µm)	SEH (µm)	LCV (μm^3)
Normal control	2.71 ± 1.2	2.98 ± 0.71	250.3 ± 5.7	85.2 ± 2.1	1250 ± 212.4
Negative control	1.02 ± 0.3***	1.11 ± 0.22***	180.5 ± 4.3**	45.6 ± 3.2**	501 ± 92.3**
Positive control	2.52 ± 2.4##	2.63 ± 0.01#	220.1 ± 6.2	70.3 ± 4.1##	1201 ± 243.1##
Treated with 200mg/kg ZO	1.45 ± 2.1**	1.51 ± 0.41**	195.2 ± 5.1	58.4 ± 3.5*	822 ± 113.4*
Treated with 400mg/kg ZO	1.50 ± 0.8**	1.73 ± 0.89*	230.5 ± 4.8	75.6 ± 2.8*	1242± 351.2##
Treated with 800mg/kg ZO	2.51 ± 0.5##	2.58 ± 1.02#	245.8 ± 6.3	82.1 ± 3.2 #	1291 ± 236.3##
Treated with 200mg/kg MW	1.93 ± 1.5*	2.31 ± 0.31#	190.3 ± 4.5	55.3 ± 3.0*	921 ± 219.6*
Treated with 400mg/kg MW	2.57 ± 1.2##	3.01 ± 1.23##	225.6 ± 5.2	72.8 ± 2.5#	1322 ± 221.3##
Treated with 800mg/kg MW	3.38 ± 1.9###	3.53 ± 1.01###	248.2 ± 5.9#	84.5 ± 3.1##	1432 ± 346.7###

Note: TV – Testicular volume, TW – Testicular weight, STD - Seminiferous tubule diameter, SEH - Seminiferous tubule Epithelial Height and LCV - Leydig cell volume. Values are mean ± SEM. * as compared to Group A and # as compared to Group B. * and # significant at $p<0.05$, ** and ## significant at $p<0.01$, ***and ### significant at $p<0.001$. At $p<0.05$ and $p<0.01$, the testicular weight (1.02 ± 0.3 ml), volume (1.11 ± 0.22 ml), seminiferous tubule diameter (180.5 ± 4.3 µm), seminiferous epithelial height (45.6 ± 3.2 µm), and Leydig cell volume (501 ± 92.3 µm3).

CHAPTER FIVE

DISCUSSION

5.1 Phytochemical Profile and Antioxidant Activity of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts

The phytochemical composition of a medicinal plant reflects the environmental and physiological conditions under which it grows, and these bioactive compounds ultimately determine its therapeutic potential. The quantitative and qualitative analyses in the present study demonstrated that *Zingiber officinale* and *Mondia whitei* contain diverse secondary metabolites, with *Zingiber officinale* characterized by high flavonoid content and lower levels of alkaloids, tannins, saponins, phenolics, and glycosides, while *Mondia whitei* contained flavonoids, alkaloids, tannins, saponins, phenolics, sterols, and terpenoids. These phytochemical profiles are important because compounds such as flavonoids, phenolics, terpenoids, and alkaloids regulate fundamental biological processes including antioxidant defense, inflammation, cellular signaling, steroidogenesis, and reproductive function (Lartey et al., 2023).

Variations in phytochemical composition between studies may arise from differences in soil type**,** climatic conditions, altitude, rainfall patterns, geographical location, plant maturity, and agronomic practices across different regions and counties. Such environmental factors influence plant metabolism and the biosynthesis of secondary metabolites, resulting in differences in both the concentration and diversity of phytochemicals. Additionally, extraction solvent polarity plays a critical role in determining the classes of compounds recovered; for example, ethanol effectively extracts both polar and moderately non-polar compounds, including flavonoids, tannins, phenolics, saponins, and glycosides, whereas chloroform preferentially extracts non-polar constituents such as terpenoids, steroids, and lipophilic compounds. Therefore, understanding regional and

methodological variations in phytochemical composition is essential for interpreting biological activity, standardizing herbal preparations, and validating the therapeutic potential of *Zingiber officinale* and *Mondia whitei* in the management of diabetes-associated reproductive dysfunction (Shehu et al., 2022).

The current study findings revealed that *Zingiber officinale* and *Mondia whitei* had comparable phenolic contents, but *Mondia whitei* ethanolic root extracts had greater concentrations of flavonoids, tannins, and saponins. In addition to extraction techniques, other factors may also have an impact on variations in the phytochemical content of plants. According to Sun et al. (2013), genetic factors, environmental influences, their interplay, and geographic location are the main causes of these variances within a plant species. Essentially, when growing conditions vary, especially nitrogen availability, the number of phenolic compounds can change from plant to plant within the same species. For instance, a lack of nitrogen causes phenolic accumulation in a number of plant parts (Lartey et al., 2023).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is one of the most popular methods for assessing the antioxidant activity of phytochemicals and herbal extracts. Lipid peroxidation inhibition is linked to the capacity to scavenge the DPPH radical, which is known to impede labile hydrogen. According to the current study findings, *Zingiber officinale* had higher antioxidant activity than *Mondia whitei* because of a lower IC_{50}. This suggests that less ethanolic extract of *Zingiber officinale's* rhizome is needed to produce 50% of a given effect than that of *Mondia whitei's* ethanolic root extract (Berrouet et al., 2020).

Zingiber officinale and *Mondia whitei* were found to have IC_{50} values of 76 µg/mL and 120 µg/mL, respectively. The findings imply that the bioactive metabolites of these plants do, in fact, offer therapeutic potential for a variety of diseases linked to oxidative stress. The main antioxidants

found in *Zingiber officinale* include gingerone, paradols, shogaol, and gingerol. These compounds produce their antioxidant effects through a variety of mechanisms. These secondary metabolites prevent cellular damage by neutralizing free radicals and reactive oxygen species (ROS) (Nethengwe et al., 2025).

They shield cell membranes and increase the activity of endogenous antioxidant enzymes including glutathione peroxidase, catalase, and superoxide dismutase (SOD) by stopping lipid peroxidation (Mousa et al., 2024). They affect signaling pathways like Nrf2, which strengthens the body's defensive system and controls the synthesis of antioxidant response elements. This has anti-inflammatory effects by indirectly lowering oxidative stress (Ali et al., 2019).

Tannins, alkaloids, saponins, and phenolic chemicals, such as flavonoids and phenolic acids, are the main antioxidants present in *Mondia whitei*. which, through a mechanism called free radical scavenging, directly neutralize Reactive Oxygen Species and free radicals. According to the current study findings, the ethanolic root extracts of *Mondia whitei* had a significant FRAP (400 µmol Fe^{2+} equivalents per gram in both cases). The FRAP test depends on the samples' antioxidants converting red Fe^{3+} to green Fe^{2+}. The degree of Fe^{3+} reduction is connected with the antioxidant concentration in the samples (Mousa et al., 2024). Chelating transition metals like iron and copper, which use Fenton processes to catalyze the production of free radicals and inhibit lipid peroxidation by preventing the oxidative breakdown of lipids in cell membranes, phenolic compounds in *Mondia whitei*, such as flavonoids and tannins, carry out antioxidant functions.

Hence the current study findings are in line with previous research on the phytochemical analysis and antioxidant potential of *Mondia whitei* and Guibourtia tessmannii against H_2O_2-induced cytotoxicity in PC_3 cells (Defo et al., 2024). Polyphenolic compounds, especially flavonoids and tannins, can activate enzymes such as glutathione peroxidase, catalase, and SOD (Defo et al.,

2024). They can also change cell signaling pathways. These substances have the ability to affect Nrf2 and other pathways, which can raise the expression of genes that produce antioxidants.

According to the current study findings, *Mondia whitei* extracts contain phenolic compounds, tannins, and other phytochemicals that mainly operate as antioxidants by chelating metals, scavenging free radicals, and bolstering the body's natural antioxidant defenses. Alkaloids' has many physiological effects, such as antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity, continue to pique the interest of organic chemists, biologists, biochemists, pharmacologists, and pharmacists use it for the same purposes (Mousa et al., 2024). Saponins are used as dietary cholesterol removal agents, foaming agents in carbonated drinks and cosmetics, emulsifiers in preparations with lipophilic colors or flavors, and preservatives (Mousa et al., 2024). Many terpenes used in medicine have biological properties such as antioxidant, anticonvulsant, antiulcer, anti-inflammatory, antiseptic, anticancer, antiviral, analgesic, antihypertensive, antibacterial, and therapeutic antidiabetic effects (Vuerich et al., 2019). Tannins have been shown to offer a variety of health-promoting properties, such as antiviral, anti-inflammatory, immunological modulator, and antioxidant effects (Manzoor et al., 2020). Male infertility has been treated by medicinal plants in many communities, especially in endemic regions where they are cultivated. One important measure of male reproductive function is sperm parameter analysis (Roozbeh et al., 2021).

5.2 *Zingiber offinale* and *Mondia whitei* Ethanolic Extract's Impact on the Hormonal Profile

Diabetes mellitus disrupts the hypothalamic-pituitary-testicular (HPT) axis, reducing LH, FSH, and testosterone production, while treatment with *Zingiber officinale* partially restores hormonal function, likely through antioxidant protection of hypothalamic and pituitary tissues; although GnRH was not directly measured due to its pulsatile secretion and very short half-life, the observed increases in LH and FSH provide indirect evidence of improved upstream HPT-axis signaling rather than a direct stimulatory effect on testicular cellular steroidogenesis activities.

The findings support the hypothesis that diabetes-induced infertility results from disruption of the hypothalamic-pituitary-testicular (HPT) axis, oxidative stress, inflammation, and testicular dysfunction, and *Mondia whitei* can restore reproductive function by targeting these underlying mechanisms. *Mondia whitei* significantly increased LH, FSH, and testosterone levels, with higher doses restoring testosterone concentrations close to normal values, indicating recovery of pituitary function, Leydig cell steroidogenesis, and HPT-axis activity. Its superior efficacy is likely attributable to phytochemicals such as flavonoids, saponins, and tannins, which reduce oxidative stress and inflammation, preserve mitochondrial function, and protect Leydig and Sertoli cells from diabetes-induced damage. Consequently, *Mondia whitei* exhibits testicular protective properties that support steroidogenesis, maintain spermatogenesis and blood-testis barrier integrity, and may serve as a promising therapeutic adjunct for diabetes-associated male infertility.

The current study findings showed a gradual increase in both hormones at increasing doses, which is consistent with previous studies on the effects of *Zingiber officinale* roots on reproductive traits in male Japanese Quails (Coturnix japonica) (Abdelfattah et al., 2023). The findings also showed that Sertoli and Leydig cells, which have both steroidogenic and spermatogenic effects on the

testes, are impacted by FSH and LH. The results of current study demonstrates that *Mondia whitei* has better testicular protective abilities than *Zingiber officinale* because to its many anti-oxidative and anti-inflammatory properties, which include flavonoids, polyphenols, and tannins.

5.3 Effects of Zingiber officinale and Mondia whitei Ethanolic Extracts on Sperm Parameters

Diabetes mellitus impaired male fertility by disrupting the fundamental cellular processes required for sperm production and survival, as evidenced by significant reductions in sperm count, viability, and normal morphology in the diabetic group**.** The study demonstrated that treatment with *Zingiber officinale* and *Mondia whitei* at doses of 400 and 800 mg/kg restored these sperm parameters, suggesting recovery of spermatogenesis and sperm maturation through protection of the testicular microenvironment. This improvement is likely mediated by the antioxidant phytochemicals identified in the extracts, particularly flavonoids, phenols, tannins, and alkaloids, which counteract hyperglycemia-induced oxidative stress, preserve Sertoli and Leydig cell function, maintain mitochondrial integrity, and reduce germ-cell damage. Consequently, the findings indicate that both extracts possess testicular protective properties capable of mitigating diabetes-induced reproductive dysfunction and improving sperm quality by restoring the cellular and metabolic conditions necessary for male fertility.

Based on the observed differences in sperm parameters between the extract-treated animals and the negative control, the extracts may have either directly scavenged the radicals or increased the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, peroxidases, and catalases, or both. The cellular membranes of the spermatozoa and other testicular components involved in spermatogenesis were shielded from destruction by free radicals. The reversal potential of *Zingiber officinale* rhizomes and *Mondia whitei* ethanolic root

extracts to oxidative-stress-induced infertility can be compared to normal and positive control since statistical analysis revealed no appreciable difference. When compared to conventional drugs, the current study findings illustrated that the extracts' suitability for raising sperm count, motility, and morphology, note that sperm viability in male reproductive function determines the likelihood of egg fertilization by indicating the percentage of live spermatozoa (Abdelfattah et al., 2023).

Sperm viability reflects the integrity of the cellular mechanisms required for fertilization, including membrane stability, mitochondrial energy production, protection against oxidative stress, and the maintenance of normal spermatogenesis. The current study demonstrated a significantly higher proportion of viable spermatozoa in animals treated with *Zingiber officinale* and *Mondia whitei* at 800 mg/kg, indicating that these extracts protected testicular cells and developing sperm from diabetes-induced oxidative injury. Mechanistically, alloxan-induced diabetes increased reactive oxygen species (ROS) generation, leading to lipid peroxidation of sperm plasma membranes, mitochondrial dysfunction, impaired ATP production, and cellular damage that reduced sperm survival and viability. The restoration of sperm viability observed in the treatment groups is likely attributable to the antioxidant phytochemicals present in the extracts, which scavenged excess ROS, preserved membrane integrity, protected Sertoli and Leydig cell function, and maintained the testicular microenvironment necessary for normal spermatogenesis, sperm maturation, and sperm survival.

The current study findings on the Eosin-nigrosin staining method suggests that dead spermatozoa have membrane leakage, which permits eosin to enter the nucleus and result in pink-stained heads. These dyes were used to assess the viability of sperm; dead cells absorbed the dye due to the destruction of the sperm cell membrane, most likely due to lipid peroxidation caused by reactive

oxygen species. Reactive oxygen species (ROS) damage sperm cells by initiating lipid peroxidation, sperm DNA damage, protein oxidative modifications, and mitochondrial dysfunction, all of which result in malformation and the destruction of various sperm cell structures. The membrane of spermatozoa contains high levels of polyunsaturated fatty acids (Abdelfattah et al., 2023). Higher antioxidant enzyme activity and better neutralization of oxidative tissue damage are probably the reasons why these metrics increased with increasing doses of ethanolic root extracts from *Zingiber officinale* and *Mondia whitei*. Previous research has shown that a range of bioactive compounds found in plants can boost the synthesis and secretion of reproductive hormones, improving the morphology, vitality, concentration, and motility of sperm in both fertile and infertile men (Al-Alami et al., 2017). These data corroborate the results of the current study regarding the positive effects of ethanolic root extracts from *Mondia whitei* and *Zingiber officinale* on male infertility by enhancing sperm characteristics.

5.4 Effects of *Zingiber officinale* and *Mondia whitei* Ethanolic Extracts on Histology of Hypothalamo-Pituitary-Testicular Axis

The study findings of the current study show the potential of *Zingiber officinale* and *Mondia whitei* rhizome and root extracts to reverse the effects of DIMI in albino male rats as was evaluated. The findings indicated that diabetes mellitus in the negative control group resulted in long-term damage and multiple organ malfunctions, especially in the hypothalamus, pituitary, and testis, especially when uncontrolled or poorly regulated. The histopathology of these tissues demonstrated this.

Normal glucose homeostasis and insulin signaling are fundamental drivers of spermatogenesis and steroidogenesis because they provide the metabolic and hormonal support required for testicular cell survival and function; consequently, chronic hyperglycemia and insulin deficiency in type 1 diabetes impair cellular metabolism, disrupt testicular physiology, and compromise male fertility

by reducing the capacity of Sertoli and Leydig cells to sustain sperm production and testosterone synthesis (Gandhi et al., 2023; Long et al., 2018; Salah et al., 2022).

Male fertility depends on intact glucose metabolism and insulin signaling because these processes regulate the gonadal axis, control oxidative stress, and support spermatogenesis; therefore, insulin deficiency and chronic hyperglycemia in type 1 diabetes may impair testicular glucose utilization, increase reactive oxygen species (ROS) production, and contribute to reproductive dysfunction, although the precise mechanisms and overall effects on semen quality remain incompletely understood and inconsistently reported in the literature (Minas et al., 2024; Venditti et al., 2024; Xu et al., 2023).

The current study findings showed that alloxan induced diabetes caused seminiferous tubule disruption and atrophy, as well as a reduction in the number of spermatogenic cells and the size of Leydig cells. Alloxan actually causes seminiferous tubular cells and Leydig cells to undergo atrophy and disorganized and reduced spermatogenesis by causing an accumulation of reactive oxygen species, which in turn causes cellular oxidative stress that results in cell damage and death, according to earlier research by Lekan Sheriff et al. (2020).

The current study findings show that *Zingiber officinale* and *Mondia whitei* therapy considerably and dose-dependently enhanced the histoarchitecture of the hypothalamus, anterior pituitary gland, and testes in DIMI rats. Given that *Zingiber officinale* and *Mondia whitei* are both recognized for their anti-inflammatory and antioxidant properties, led to hypothalamus-pituitary-testicular recovery. Free radicals are scavenged by *Zingiber Officinale*, especially by its phytochemicals like gingerols and shogaols, which shield testicular cells, anterior pituitary gland cells, and hypothalamus neurons (Gholami-Ahangaran et al., 2021).

The current study findings on quantitative antioxidant analysis validate the extracts' beneficial benefits. These results are further supported by earlier reports on histoarchitectural changes in the hypothalamus and anterior pituitary gland following higher doses of *Zingiber officinale* administration in alloxan-induced diabetes mellitus, as well as improvements in male rats' cognitive abilities through antioxidant and anti-inflammatory effects and a decrease in testicular neurodegeneration due to the same mechanisms (Gholami-Ahangaran et al., 2021). A disruption in spermatogenesis and, subsequently, steroidogenesis is indicated by the decrease in seminiferous epithelium diameter and vacuolation in spermatogenic cells in the negative control.

The study findings further show the impaired sperm characteristics in the negative control. Where vacuoles are an indication of reduced testicular function, comparative with earlier studies on male New Zealand white rabbits subjected to high doses of khat extract over an extended period (Abdelfattah et al., 2023). According to the current study findings, giving DIMI albino white rats *Zingiber officinale* improved the overall testicular and seminiferous tubule histology. *Mondia whitei* has been shown to improve all testicular markers, including tissue histology, at small doses; however, it may be harmful at higher levels of 800 mg/kg.

The current study findings showed that 400 mg/kg and 800 mg/kg of both *Zingiber officinale* and *Mondia whitei* considerably improved the histology of the hypothalamus, which contains GnRH-releasing cells that extend to the anterior pituitary gland. Under GnRH, the gonadotrophs release both FSH and LH, which are then transported to the testes through the bloodstream. The histology and steroidogenic activities of the brain, anterior pituitary gland, and testicular tissues were improved at high doses of *Zingiber officinale* and *Mondia whitei* (Gholami-Ahangaran et al., 2021).

This outcome is consistent with previous research by Abdelfattah et al. (2023), which shown that Japanese quails given higher dosages (15 g/kg) of *Zingiber officinale* between 7 and 70 days of age had increased germinal epithelium height and seminiferous tubule diameter. However, when *Zingiber officinale* is administered in excess, adult Wistar rats' seminiferous tubules shrink overall, the Leydig cells atrophy and die, and the germinal and supporting cells distort. *Zingiber officinale* reverses and prevents seminiferous tube damage caused by excessive monosodium glutamate administration (Defo et al., 2024).

The current study findings on testicular histopathology shows the similarity and dependance on sperm characteristics. At large doses of *Zingiber officinale* and *Mondia whitei* ethanolic extracts, sperm count, motility, viability, and morphology all improved to the level of the normal control. The study's findings also show that when DIMI was produced using alloxan monohydrate, the male rats' testicular weight and volume, seminiferous tubule diameter, seminiferous epithelial height, and Leydig cell volume drastically decreased when compared to the normal control. This is consistent with earlier studies on the hypoglycemic and pancreatic protective effects of Portulaca oleracea extract in alloxan-induced diabetic rats (Gholami-Ahangaran et al., 2021).

Preservation of male fertility depends on limiting oxidative stress and inflammation while maintaining the structural and functional integrity of Sertoli cells, Leydig cells, and seminiferous tubules; accordingly, flavonoids, polyphenols, and tannins have been shown to restore reproductive hormone levels and protect testicular tissues through antioxidant and anti-inflammatory mechanisms, although the current findings differ from some previous reports in which ***Mondia whitei*** treatment was associated with seminiferous tubular hypocellularity, Sertoli cell vacuolization, increased intercellular spaces, and reduced spermatogenic cell populations, suggesting that its reproductive effects may vary depending on dosage, extract composition, treatment duration, or experimental conditions (Defo et al., 2024, Minas et al., 2024; Venditti et al., 2024; Xu et al., 2023).

CHAPTER SIX

SUMMARY OF STUDY FINDINGS, CONCLUSION AND RECOMMENDATIONS

6.1 Summary of Study Findings

By lowering gonadotropins, testosterone levels, sperm quality, and the histological integrity of the hypothalamus–pituitary–testicular (HPT) axis in white albino rats, the study showed that diabetes had a major impact on male reproductive function. Rich in bioactive phytochemicals, ethanolic extracts of *Zingiber officinale* and *Mondia whitei* demonstrated potent antioxidant qualities that reduced oxidative stress linked to male infertility caused by diabetes. In a dose-dependent way, treatment with both plant extracts increased testicular histoarchitecture recovery, restored reproductive hormone levels, and enhanced sperm count, motility, morphology, and viability.

Mondia whitei has demonstrated superior effects on testicular weight, Leydig cell volume, testosterone restoration, and overall histomorphometric parameters as compared to *Zingiber officinale*, particularly at higher dosages. Overall, the findings support the ability of both plants to protect the testicles and boost fertility, with *Mondia whitei* seeming to be the most promising treatment for male infertility caused by diabetes.

6.2 Conclusion

1. The roots of *Mondia whitei* and the rhizomes of *Zingiber officinale* are rich in bioactive phytochemicals, including flavonoids, phenolics, tannins, saponins, and alkaloids. These substances have potent antioxidant properties and can reduce oxidative stress associated with diabetes-related male infertility.
2. Because diabetes disrupts hormonal regulation along the hypothalamus-pituitary-testicular axis, FSH, LH, and testosterone levels are suppressed; however, treatment with *Zingiber officinale* and *Mondia whitei* ethanolic extracts successfully restored these hormones in a

dose-dependent manner, with Mondia whitei showing superior testosterone restorative potential at higher doses.

3. The ethanolic extracts of both plants significantly improved sperm count, motility, morphology, and viability in diabetic infertile rats, indicating that their cytoprotective and antioxidant properties effectively treat diabetes-induced spermatogenic damage.
4. When *Zingiber officinale* and *Mondia whitei* were administered, normal histoarchitecture and histomorphometric parameters were restored; *Mondia whitei* produced the most pronounced structural recovery. Diabetes significantly reduces testicular mass, seminiferous tubule diameters, Leydig cell volume, and testicular architecture.
5. Overall, the study found that *Mondia whitei* has a greater potential for translational and therapeutic applications than *Zingiber officinale* in reducing diabetes-induced male infertility, even though both plants have fertility-enhancing and testicular-protective qualities.

6.3 Recommendations

1. Based on the demonstrated fertility-enhancing effects of *Zingiber officinale* and *Mondia whitei* in diabetic male rats, it is recommended that further preclinical studies be conducted to evaluate long-term safety, optimal therapeutic dosing, and potential toxicity. Subsequently, well-designed clinical trials should be undertaken to validate their efficacy and safety in human males with diabetes-induced infertility.
2. Future research should investigate the molecular mechanisms underlying the protective effects of *Zingiber officinale* and *Mondia whitei*, particularly their influence on oxidative stress pathways, inflammatory mediators, steroidogenic enzymes, and gene expression along the hypothalamus–pituitary–testicular axis, to provide deeper mechanistic insight.

3. It is recommended that standardized extraction protocols and phytochemical quantification be developed for *Zingiber officinale* and *Mondia whitei* to ensure consistency, reproducibility, and reliability of their therapeutic effects, which is essential for pharmaceutical development and clinical application.
4. The findings support the development of affordable, plant-based adjunct therapies for reversing diabetes-induced male infertility, particularly in resource-limited settings. Collaboration between researchers, pharmacologists, and public health institutions is recommended to translate these findings into accessible herbal formulations.

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