



East African Journal of Health and Science

eajhs.eanso.org

Volume 9 Issue 1, 2026

Print ISSN: 2707-3912 | Online ISSN: 2707-3920

Title DOI: <https://doi.org/10.37284/2707-3920>



EAST AFRICAN
NATURE &
SCIENCE
ORGANIZATION

Original Article

Comparing the Phytochemical Compounds, Antioxidant Effectiveness, and Mitigating Effects of *Mondia whitei* Root Ethanolic Extracts and *Zingiber officinale* Rhizomes on Hormonal and Histomorphological Changes along the Hypothalamus, Pituitary, and Testicular Tissues in Male Albino Rats with Diabetes-Induced Infertility

Ambrose Barasa^{1*}, Albert Nyongesa², Walter Odipo¹ & Patrick Ogolla Onyango¹

¹ Maseno University, P. O. Box 3275-40100, Kisumu, Kenya.

² University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya.

*Author for Correspondence Email: ambrosebarasa2@gmail.com

Article DOI: <https://doi.org/10.37284/eajhs.9.1.4558>

Date Published: **ABSTRACT**

23 February 2026

Keywords:

Healthcare Personnel
Expenditure,
Economic Growth,
Public Healthcare
Spending.

Background. Medicinal plants are widely used due to their affordability, accessibility, and ease of consumption, serving as alternatives for managing diseases such as diabetes and infertility. They are vital sources of phyto-bioactive compounds that contribute to modern medicine. With diabetes recognised as a major risk factor for male infertility, global infertility rates are expected to rise alongside the projected 55% increase in diabetes prevalence between 2019 and 2045, especially in Sub-Saharan Africa and Asia. The World Health Organization's 2021 report supports integrating medicinal plants into healthcare systems. *Zingiber officinale* and *Mondia whitei* are known for phytochemicals and antioxidants, yet detailed information on their phytochemical profiles, antioxidant capacities, and therapeutic effects in diabetic reproductive health remains scarce. Research on ethanolic extracts in diabetic infertile men is limited, with most studies focusing on individual species or alternative extraction methods. Although both plants have shown fertility-enhancing properties in non-diabetic males, comparative studies in diabetic conditions are lacking. Methodology: This study addressed the gap by analysing phytochemical composition and evaluating reproductive effects of ethanolic extracts from *M. whitei* roots and *Z. officinale* rhizomes using male albino rats with diabetes-induced infertility. Fresh samples were collected from Kakamega forest, identified at the National Museums of Kenya, and processed at Maseno University. Extracts were prepared using ethanol, methanol, and aqueous solvents, then analysed. Data were processed using GraphPad Prism with ANOVA and Tukey's post-hoc test. Results. Results showed *Z. officinale* had higher alkaloids (15 mg/g), equal phenolic compounds (220 mg GAE/g), but lower flavonoids, tannins, and saponins compared to *M. whitei*. Antioxidant analysis revealed *Z. officinale* had lower

IC₅₀ and higher Fe²⁺ and Trolox equivalents, indicating stronger antioxidant capacity. Administration of *M. whitei* (200–800 mg/kg) improved semen parameters and hormonal levels, with higher doses approaching positive control values. Conclusion. The findings highlight both plants as sources of bioactive compounds with antioxidant and anti-inflammatory properties, useful for managing diabetes-related male infertility. However, further research is needed to clarify their mechanisms of action.

APA CITATION

Barasa, A, Nyongesa, A., Odipo, W. & Onyango, P. O. (2026). Comparing the Phytochemical Compounds, Antioxidant Effectiveness, and Mitigating Effects of *Mondia whitei* Root Ethanolic Extracts and *Zingiber officinale* Rhizomes on Hormonal and Histomorphological Changes along the Hypothalamus, Pituitary, and Testicular Tissues in Male Albino Rats with Diabetes-Induced Infertility. *East African Journal of Health and Science*, 9(1), 337-362. <https://doi.org/10.37284/eajhs.9.1.4558>

CHICAGO CITATION

Barasa, Ambrose, Albert Nyongesa, Walter Odipo and Patrick Ogolla Onyango. 2026. "Comparing the Phytochemical Compounds, Antioxidant Effectiveness, and Mitigating Effects of *Mondia whitei* Root Ethanolic Extracts and *Zingiber officinale* Rhizomes on Hormonal and Histomorphological Changes along the Hypothalamus, Pituitary, and Testicular Tissues in Male Albino Rats with Diabetes-Induced Infertility". *East African Journal of Health and Science* 9 (1), 337-362. <https://doi.org/10.37284/eajhs.9.1.4558>

HARVARD CITATION

Barasa, A, Nyongesa, A., Odipo, W. & Onyango, P. O. (2026). "Comparing the Phytochemical Compounds, Antioxidant Effectiveness, and Mitigating Effects of *Mondia whitei* Root Ethanolic Extracts and *Zingiber officinale* Rhizomes on Hormonal and Histomorphological Changes along the Hypothalamus, Pituitary, and Testicular Tissues in Male Albino Rats with Diabetes-Induced Infertility", *East African Journal of Health and Science*, 9(1), pp. 337-362. doi: 10.37284/eajhs.9.1.4558

IEEE CITATION

A, Barasa, A. Nyongesa, W. Odipo & P. O, Onyango "Comparing the Phytochemical Compounds, Antioxidant Effectiveness, and Mitigating Effects of *Mondia whitei* Root Ethanolic Extracts and *Zingiber officinale* Rhizomes on Hormonal and Histomorphological Changes along the Hypothalamus, Pituitary, and Testicular Tissues in Male Albino Rats with Diabetes-Induced Infertility", *EAJHS*, vol. 9, no. 1, pp. 337-362, Feb. 2026.

MLA CITATION

Barasa, Ambrose, Albert Nyongesa, Walter Odipo & Patrick Ogolla Onyango. "Comparing the Phytochemical Compounds, Antioxidant Effectiveness, and Mitigating Effects of *Mondia whitei* Root Ethanolic Extracts and *Zingiber officinale* Rhizomes on Hormonal and Histomorphological Changes along the Hypothalamus, Pituitary, and Testicular Tissues in Male Albino Rats with Diabetes-Induced Infertility". *East African Journal of Health and Science*, Vol. 9, no. 1, Feb. 2025, pp. 337-362, doi:10.37284/eajhs.9.1.4558.

INTRODUCTION

Male infertility rates are on the rise due to the global epidemic of diabetes mellitus (Latifi et al., 2025). A multimodal approach that includes medication, lifestyle modifications, early diagnosis, and cutting-edge treatments that target certain biochemical pathways is necessary to address diabetes-induced male infertility in diabetic patients (Uzor et al., 2025). Diabetes causes damage to Leydig and Sertoli cells, decreased spermatogenesis, increased germ cell death, and decreased testosterone synthesis (Singh et al., 2025). Hyperglycemia, oxidative stress, inflammation, apoptosis, and abnormal hormone levels are some of the

mechanisms that contribute to the pathophysiology of diabetes-induced testicular failure (Huang et al., 2024a). Chronic hyperglycemia caused by diabetes mellitus overwhelms endogenous defences (SOD, CAT, GPx) by producing excessive ROS through the polyol pathway flow, mitochondrial electron transport chain overload, advanced glycation end-product (AGE) production, and protein kinase C activation (Badejogbin et al., 2024). This redox imbalance underlies problems in the vascular and reproductive systems by promoting lipid peroxidation, DNA damage, endothelial dysfunction, and inflammatory signalling (e.g., NF- κ B) (Facondo et al., 2022). Systemic hyperglycemia

stress is linked to male infertility because ROS damages Leydig/Sertoli function, steroidogenesis, spermatogenesis, and sperm membrane integrity in testicular tissue (Uzor et al., 2025).

Furthermore, investigating plant-based therapies as alternative treatments has the potential to reduce male infertility in diabetics (Nwakanma et al., 2024). Managing male infertility in diabetics requires a comprehensive strategy that includes pharmacological therapies, lifestyle changes, and diagnostics. In order to successfully address and alleviate male infertility in diabetic populations, future research paths point to the necessity of large-scale clinical trials, personalised medicine approaches, and cutting-edge technologies (Kyarimpa et al., 2023).

Both *Zingiber officinale* and *Mondia whitei* are rich in bioactive phytochemicals that have various physiological effects, such as anti-inflammatory, anti-diabetic, antioxidant, and antifertility properties (Abarikwu et al., 2020). For example, phenolic ketones (gingerols, shogaols, paradols) and volatiles (zingerone, sesquiterpenes) found in *Zingiber officinale* have substantial antioxidant ability throughout assays, with notable FRAP-reducing potency, DPPH/ABTS radical scavenging, and prevention of lipid peroxidation (“Ethnobotany, Pharmacology and Phytochemistry of Medicinal Plants Used for Management of Diabetes Mellitus in Uganda, Kenya, Tanzania and the Democratic,” 2024). Processing affects bioactivity; heat and drying can change gingerols into shogaols, which frequently improve some antioxidant and anti-inflammatory properties because of their increased electrophilicity and membrane permeability (Dolatabadi et al., 2025). On the other hand, *Mondia whitei* roots are abundant in alkaloids, saponins, tannins, and phenolics, including benzaldehydes that resemble vanillin. Together, these classes promote anti-inflammatory signalling, membrane stability, metal chelation, and radical scavenging (Pradeep et al., 2022). Preclinical research indicates antioxidant and anti-inflammatory properties

commensurate with reducing diabetic oxidative stress; traditional use in East and Central Africa stresses vitality and reproductive support. It is anticipated that ethanolic extraction will concentrate phenolics and saponins, increasing redox activity and possibly working in concert with aromatics involved in membrane contacts and enzyme regulation (“Ethnobotany, Pharmacology and Phytochemistry of Medicinal Plants Used for Management of Diabetes Mellitus in Uganda, Kenya, Tanzania and the Democratic,” 2024).

Zingiber officinale and *Mondia whitei* regulate redox and immune pathways mechanistically by activating Nrf2, which increases phase II detoxifying enzymes, while suppressing NF- κ B and downstream cytokines, bridging antioxidant and anti-inflammatory functions related to ROS and chronic inflammation caused by hyperglycemia (Pradeep et al., 2022). Phenolics are regularly identified as significant contributors to overall antioxidant capacity by phytochemical profiling using TLC and other techniques. Ethanolic extracts are a sensible option when increased phenolic loading is required since *Zingiber officinale* and *Mondia whitei* bioactive substances reduce oxidative indicators, enhance antioxidant enzyme activities, and alter glyco-oxidative stress signalling in diabetes situations (Lopes-Ferreira et al., 2025). Saponins and phenolics protect testicular tissue by maintaining steroidogenic pathways and lowering lipid peroxidation, supporting functional recovery under diabetic conditions (Dolatabadi et al., 2025). *Zingiber officinale* and *Mondia whitei* have physiological and pharmacological protective effects for reproductive oxidative stress because of their abundance of bioactive molecules (Dolatabadi et al., 2025). Shogaols may have greater cell permeability and are frequently more lipophilic than gingerols, which could enhance the tissue-level antioxidant effects of ginger ethanolic extracts. The saponins in *Mondia whitei* may improve membrane contacts and affect co-occurring phenolic absorption. *Zingiber officinale* has been shown to modify inflammatory signalling and glycation-

related pathways; *Mondia's* phenolic-rich profile indicates similar ROS interception with potential endocrine-supportive actions in reproductive organs (Mao et al., 2019). Although standardisation and dose-ranging are still crucial for translational studies, *Mondia's* traditional use supports tolerability; *Zingiber officinale* is widely acknowledged as safe in dietary ranges with favourable anti-inflammatory properties. Reproducibility is guaranteed, and dose-response modelling in diabetic models is made possible by marker-based measurement (e.g., 6-gingerol/6-shogaol for ginger; signature benzaldehydes/saponins for *Mondia*) (Unuofin et al., 2021).

Persistent hyperglycemia is a hallmark of diabetes mellitus, a chronic metabolic disease that puts patients at risk for oxidative stress from an excess of reactive oxygen species (ROS) (Lakshmi et al., 2025). The development of diabetes complications, such as nephropathy, cardiovascular disease, and reproductive dysfunction, is greatly aided by oxidative stress (Minas et al., 2024). The search for safer, plant-based substitutes is necessary because current synthetic antioxidants and antidiabetic medications frequently have poor efficacy and unfavourable side effects. Despite the fact that *Zingiber officinale* and *Mondia whitei* have long been utilised for their therapeutic qualities, there is no scientific data comparing their phytochemical makeup and antioxidant capability in relation to oxidative stress brought on by diabetes. Their logical incorporation into therapeutic approaches is hampered by this information gap.

Chronic hyperglycemia, oxidative stress, and endocrine dysfunction are the hallmarks of diabetes mellitus, a metabolic disease. Male infertility brought on by diabetes has become a major reproductive health issue among its numerous consequences (Nethengwe et al., 2025). Excessive production of reactive oxygen species (ROS), weakened antioxidant defence systems, hormonal abnormalities along the hypothalamic-pituitary-

testicular (HPT) axis, and histomorphological damage to reproductive organs are the main causes of the illness. In afflicted males, these pathological alterations result in decreased spermatogenesis, changed testosterone secretion, and poor reproductive outcomes (Fan et al., 2024).

The oxidative and hormonal disturbances that underlie reproductive dysfunction are frequently ignored by conventional treatment methods for resolving diabetes-related infertility (Fan et al., 2024). This has led to an increase in interest in bioactive substances derived from plants that have aphrodisiac and antioxidant qualities as possible substitute treatments. Two medicinal plants that have long been used in Asian and African ethnomedicine to improve male reproductive health are the rhizome of *Zingiber officinale* and the root of *Mondia whitei* (Boroujeni et al., 2022). According to preliminary research, their ethanolic extracts include a variety of phytochemicals, including flavonoids, phenolics, and saponins, which may reduce oxidative stress, adjust hormone levels, and improve testicular function.

The antioxidant effectiveness, phytochemical makeup, and mechanistic effects of *Zingiber officinale* and *Mondia whitei* extracts in the setting of diabetes-induced male infertility, however, have not been thoroughly studied despite their extensive traditional use. In particular, nothing is known about how these extracts affect the histomorphological integrity of hypothalamus, pituitary, and testicular tissues in diabetic male albino rats, as well as hormonal control along the hypothalamus-pituitary-testicular axis. In order to validate their therapeutic potential and direct the development of plant-based therapies for diabetes-related reproductive dysfunction, it is imperative that this knowledge gap be filled. The purpose of this study is to examine and determine the therapeutic potential of ethanolic extracts from *Zingiber officinale* and *Mondia whitei* in reducing oxidative stress, hormonal imbalance, and histomorphological damage linked to male infertility caused by diabetes.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Plant Materials, Processing and Preparation of Extracts

Fresh samples of *Z. officinale* rhizomes and *M. whitei* roots were collected from Kakamega forest near Malava Town, where its normally collected and transported to Jubilee market in Kisumu City, 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 collecting, the samples were taken to the 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. The samples were cleaned by washing with running tap water, chopped into small 1mm pieces using a kitchen knife, weighed using an electronic compact scale weighing balance machine (Radwag model wlc.0,6/b1) and air-dried in zip-locked bags for at least 7 days in the laboratory at the Zoology Department, Maseno University.

Preparation of the mixture was done following the protocol of Heinrich et al. (2022). Extraction was done using 70% ethanol due to its increased polarity

as a result of the water content, and thus offering a more effective, broader range of extraction of polar phytochemicals with accompanying high yield compared to 95% or absolute ethanol. Two hundred grams (200 g) of chopped pieces of *Z. officinale* and *M. whitei* were separately blended in 1000 ml of ethanol for approximately 5 minutes, followed by continuous shaking using an orbital shaker (kj-201bd) for 24 hours. Thereafter, the mixtures were removed and sieved using a Muslin piece of cloth to obtain extracts of *Z. officinale* and *M. whitei* for analysis.

Filtration was done using circular 125 mm Whatman filter papers. The filtrate obtained was concentrated using a rotary evaporator and a hot air oven, and the obtained crude extract was weighed, and the weight was recorded. Distilled water was used as a vehicle. The crude extracts obtained from the ethanolic solvent were stored in different labelled, airtight containers for phytochemical analysis and administration to the diabetic, infertile white albino male rats.

Methodology for Qualitative and Quantitative Phytochemical and Antioxidant Analysis

Qualitative Phytochemical Screening

Used standard phytochemical tests to detect classes of compounds:

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

Procedure:

- A small amount of extract was mixed with specific reagents.
- Colour changes or precipitate formation was observed as an indication of phytochemicals.

Quantitative Phytochemical Determination**Total Phenolic Content (TPC):**

Method: Folin-Ciocalteu assay

Procedure:

- Serial dilutions of the extract were prepared.
- The extract was mixed with Folin-Ciocalteu reagent, then sodium carbonate solution.
- Then incubated for 30 minutes at room temperature.
- Absorbance at 765 nm using a UV-Vis spectrophotometer was measured.
- Phenolic content as gallic acid equivalents (GAE) was calculated using a standard curve.

Total Flavonoid Content (TFC):

Method: Aluminum chloride colorimetric assay

Procedure:

- The extract was mixed with aluminum chloride solution.
- Incubated for 10 minutes.
- Absorbance at 415 nm was measured.
- Flavonoid content was expressed as quercetin equivalents.

Other Compounds:

Tannins and saponins quantified via HPLC methods.

Antioxidant Activity Assays**DPPH Radical Scavenging Assay**

- Principle: Measures ability to scavenge free radicals.

-Procedure:

- DPPH solution (0.1 mM in methanol) was prepared.

- Mixed with various concentrations of extracts.
- Incubated in the dark for 30 minutes.
- Measured absorbance at 517 nm.
- Calculated % inhibition and determined IC₅₀ (concentration required to inhibit 50%).

ABTS Radical Cation Decolourisation Assay

- Similar to DPPH, but used ABTS•+ radical cation.
- Measured absorbance at 734 nm.

FRAP (Ferric Reducing Antioxidant Power) Assay

- Measured reduction of Fe³⁺ to Fe²⁺.
- Incubated extracts with FRAP reagent; read absorbance at 593 nm.

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 the same size in standard rat cages. Disinfection of the animal house was done prior to the introduction of the animals. Wood shavings were used for bedding and were changed daily to avoid the build-up of ammonia in the animal house. The animals were allowed free access to water, and standard rat pellets were bought from Kisumu animal feed stores throughout the study period. The animal house was well ventilated with a light: dark cycle of 12h: 12h with a 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 healthy animals were used for the research work.

Experimental Design and Animal Treatment

A total of 40 (36 male rats and 4 female) white albino rats, aged 7–8 weeks and weighing between 150g and 250g, were used for this study. The males were randomly assigned to 9 groups of 4 animals each for normal control, negative diabetic control, positive control and 3 treatment groups for *Z. officinale* and *M. whitei*, while 1 female for each group was only used for testing male fertility status. Diabetes was induced by the use of a single intraperitoneal injection (2ml) of alloxan monohydrate, a drug known to destroy pancreatic beta cells responsible for insulin secretion (Shah & Khan, 2014), thereby causing diabetes in all groups except the normal control. 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 into a glucometer (On Call Plus blood glucose Meter). Fasting glucose levels above 126mg/dl (7mmol/l) was indicative of diabetes (hyperglycemia). Clomiphene citrate (doses range from 5mg/kg to 10mg/kg body weight administered via intraperitoneal injection) was reconstituted in distilled water to a concentration of 5mg/ml and used as a 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.

The treatment groups were administered with escalating doses of 200mg/kg, 400mg/kg and 800mg/kg of *Zingiber officinale* for groups 4, 5 and 6, and for *M. whitei* for groups 7, 8 and 9 respectively, using the same dose levels as shown in the table below. The normal control was given normal saline using the same regimen as for the treatment groups. All administrations were done in volumes of 2ml of extracts orally using a gastric gavage needle once daily between 8 am and 9 am for 28days. After 28days of treatment, the animals were anaesthetised using 1% chloroform (0.05ml/litre of container volume) and sacrificed by cervical dislocation after confirmation of a deep anaesthetic state, which was confirmed by the loss of the righting reflex and pupil dilation. Then, using a 23-gauge needle to collect 5ml of blood samples via heart puncture and placed in an EDTA tube. Blood collected was swirled carefully to mix and centrifuged to separate the plasma from the cellular components, decanted and the plasma frozen at -20°C. A ventral laparotomy was done to harvest testes and, together with the hypothalamus and pituitary gland, immediately immersed in Bouin's bottles containing 10% formaldehyde for histological and histomorphometric analysis.

Table 1: Showing Treatment of White Male Albino Rats with 200, 400, and 800mg/kg of *Z. officinale* and *M. whitei* Extracts.

Groups n=9	Treatment
1.	Non diabetic and fertile group on water alone (normal control group) with no treatment. which served for comparing the effects of both extracts.
2.	Induced diabetes and infertility using 1.5 ml of Alloxan alone (negative control group)
3.	Diabetic and infertile treated with Alloxan + 5 mg/kg of Clomiphene citrate (positive control group).
4.	Diabetic and infertile treated with Alloxan + 200 mg/kg of <i>ZO</i> ethanol extract
5.	Diabetic and infertile treated with Alloxan + 400 mg/kg of <i>ZO</i> ethanol extract
6.	Diabetic and infertile treated with Alloxan + 800 mg/kg of <i>ZO</i> ethanol extract
7.	Diabetic and infertile treated with Alloxan + 200 mg/kg of <i>MW</i> ethanol extract
8.	Diabetic and infertile treated with Alloxan + 400 mg/kg of <i>MW</i> ethanol extract
9.	Diabetic and infertile treated with Alloxan + 800 mg/kg of <i>MW</i> ethanol extract

Hormonal Analysis

Plasma 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 hormones in a 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.

LH 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 μ L of plasma samples (triplicate) were added to 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 two hours at room temperature (20–25°C).

To remove unbound materials, the wells were washed four or five times using the supplied wash buffer. Subsequently, 100 μ L of enzyme-conjugated detection antibody was added to each well, and the plate was incubated at room temperature for one hour. The wells were then washed four times with wash buffer.

Next, 100 μ L of substrate solution was added to each well, and the plate was incubated in the dark at room temperature for 15 minutes until the colour development was adequate. To halt the reaction and ensure uniform colour development, 50 μ L of stop 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 LH concentrations was generated to analyse the data. The LH concentration in the plasma samples was determined based on their absorbance values, and the results were expressed in milli-international units per millilitre (mIU/mL).

FSH Assay

According to the instructions provided by the kit, the samples were diluted to fit within the range of the standard curve. All samples and reagents were brought to room temperature prior to use. Following the kit's protocol, 100 μ L of standards, typically prepared by serial dilution, were added, and samples were pipetted into the designated wells in duplicate. The plate was then gently shaken and incubated for one to two hours at room temperature. To remove unbound proteins, the wells were washed four times using the supplied wash buffer.

Each well then received 100 μ L of enzyme-conjugated detection antibody, which was incubated for one hour at room temperature. The wells were washed four times with the wash buffer. Subsequently, 100 μ L of substrate solution was added to each well, and the plate was incubated for 10 minutes in the dark at room temperature until the desired colour development was observed. To terminate the enzymatic reaction, 50 μ L of stop solution was added to each well, followed by gentle mixing. The absorbance was then measured at 450 nm (or the specified wavelength) using a microplate reader.

A standard curve was generated by plotting the absorbance of standards against their known concentrations provided by the manufacturer. The FSH concentration in the samples was determined by extrapolating from this standard curve, and the results were expressed in units of mIU/mL.

Testosterone Assay

All reagents were prepared in accordance with the protocol, and the standard solutions were prepared by serial dilution following the kit's instructions and

the expected testosterone concentrations. Samples were diluted as necessary to fall within the range of the standard curve. In duplicate, 100 μ L of standards and samples were added to the appropriate wells. A sealer was then applied to the plate.

The plate was incubated for one to two hours at room temperature (approximately 20 to 25°C), as directed. To remove unbound testosterone, the wells were washed five times using the supplied wash buffer, with gentle shaking. Subsequently, 100 μ L of enzyme-conjugated detection antibody was added to each well, and the plate was incubated for one hour at room temperature. The wells were washed four or five times to eliminate unbound detection antibody.

Next, 100 μ L of substrate solution was added to each well, and the plate was incubated in the dark at room temperature for 10 to 30 minutes or until sufficient colour development was observed. To stop the reaction and ensure consistent colour development, 50 μ L of stop 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 of 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 were expressed in nanograms per millilitre (ng/mL).

Sperm Characteristics

Sperm Count and Motility Analysis

This was done following the procedure as described by Koprulu et al. (2022). Briefly, the epididymis was harvested and dissected at the caudal end by cutting small pieces and placing them in a 3ml of 37 °C neutral buffered formalin solution on a Petri dish at a ratio of 1:1 and allowed to stand for exactly 15 minutes, followed by mixing in 1% trisodium

citrate. Thereafter, 1ml 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. The sperm count was determined by simply counting all the sperm cells in a field for three different fields three times per sample for each individual and calculating the mean.

Sperm count was counted in five of the 16 small squares of the hemocytometer, and the sperm count was expressed as No. x 10⁶/ml. The World Health Organization (1999) method was used for the motility test. One drop of the suspension was aspirated using the Pasteur pipette into a slide and examined under a light microscope. Three metrics were used to assess motility: proliferating cells, non-proliferating cells, and dead cells. 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:

$$\text{Motility (\%)} = \frac{\text{Number of motile sperms per unit volume}}{\text{volume}} \times 100\%$$

Total sperm per unit Volume

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.

Sperm Morphology and Viability Analysis

Sperm samples were collected from the epididymal reserve at the caudal portion of the epididymis, and the sperm viability was determined using Nigrosine-Eosin staining. Nigrosine-Eosin staining differentiates live and dead sperm based on

membrane integrity. Live sperm had 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.

Procedure:

Mixtures of Nigrosine (black dye) with Eosin (pink dye), typically in a 1:1 ratio was prepared as per the protocol. A mixture of a small volume of sperm suspension with the Nigrosine-Eosin stain (usually 10 μ L of sperm with 10 μ L of stain) was incubated briefly (about 1-3 minutes) at room temperature. A drop of the stained mixture was put on a clean glass slide, then covered with a coverslip gently, and thereafter observed under a light microscope at 400x magnification. At least 200 spermatozoa were counted 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:

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

Total sperm counted

Sperm cells were considered viable if they did not take up the eosin stain and remained white. Sperm stained pink or red were considered non-viable. Counting was performed in multiple fields to ensure accuracy and followed a specific protocol for staining concentration and incubation times.

Tissue Processing for Histology and Histomorphometry

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 = 3 per group).

The testes were harvested and testicular volumes calculated using the Scherle method (Scherle, 1970), 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 tissue.

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)

$$D = \frac{\text{Perimeter of tubule profile}}{\pi}$$

π

Cross-sectional area of seminiferous tubules (A)

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

Volume density (V_v) of seminiferous tubules/interstitium

$$V_v = \frac{\text{Volume of seminiferous tubules/interstitium}}{\text{Total volume of testis}}$$

$$V_v = \frac{\text{Number of profiles of the components}}{\text{Total number of profiles in the reference space}}$$

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 (1mm³) 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 μ m using a rotary microtome, mounted on clean microscope

slides, dried on a heating plate and rehydrated using descending concentrations of ethanol and deparaffinized using xylene. The slides were, thereafter, stained using hematoxylin and eosin and viewed and analysed under a light microscope (primo star model) fitted with a digital camera and a computer.

Statistical Analysis

The data was analysed using GraphPad Prism version 5.0 as a statistical tool. Quantitative and Qualitative data was presented as mean $\pm$ standard deviation. Means were compared using one-way ANOVA, and statistical differences were determined by Tukey's multiple pairwise test as the post-hoc test at $p < 0.05$.

Ethical Considerations

All guidelines for the care of laboratory animals were strictly followed. To comply with the ethical standards for using animals in research, animals were housed in standard animal cages and given access to water and pellets throughout the study period, apart from when fasting was required to determine the fasting blood glucose levels. The rats were sacrificed after a state of deep anaesthesia was confirmed as described. The study received two

ethical approvals from the Maseno University Ethics Committee (REF: FVM BAUEC/2024/571) and from the Biosafety, Animal Use and Ethics Committee of the University of Nairobi (UON), Faculty of Veterinary Medicine (Reference number FVM BAUEC/2024/571) before commencement.

STUDY FINDINGS AND PRESENTATION OF RESULTS

Quantitative and Qualitative Phytochemical and Antioxidant

Phytochemical Analysis of Zingiber officinale (ZO) rhizome and Mondia whitei (MW) Root Ethanolic Extract

Qualitative phytochemical analysis of *Z. officinale* and *M. whitei* rhizome and root ethanolic extracts revealed the presence of high levels of flavonoids and low levels of alkaloids, tannins, saponins, phenolic compounds and glycosides. However, quantitative analysis revealed high levels of alkaloids (15 mg/g), same levels of phenolic compounds (220 mg GAE/g) and lower levels of flavonoids, tannins and saponins in *Z. officinale* (125 mgQE/g, 7.5% (w/w) and 15 mg TAE/g respectively) compared to *M. whitei* (140 mg QE/g, 15% (w/w) and 22mg TAE/g respectively (Table 2).

Table 2: Quantitative and Qualitative Phytochemical Analysis of the Ethanolic Extract of *Z. officinale* rhizome and *M. whitei* Root Ethanolic Extracts

Grp	Phytochemical	Method used	Result			
			Qualitative		Quantitative	
			ZO	MW	ZO	MW
1	Alkaloids	Presence noted through the brown precipitate with Wagner's reagent	+	+	15mg/g	8 mg/g
2	Flavonoids	The yellow colour change with NaOH indicates significant flavonoid content	+++	+++	125mgQE/g	140mg QE/g
3	Tannins	Green/blue-black colouration with FeCl ₃	+	+	15mgTAE/g	22mg TAE/g
4	Saponins	Stability in Foam formation suggests Saponin presence	+	+	7.5%(w/w)	15% (w/w)
5	Phenolic Compounds	Colour change upon reaction with FeCl ₃	+	+	220mgGAE/g	220mg GAE/g
6	Glycosides	Results in Colour change and odour	+	+		

The table shows quantitative and qualitative phytochemical analysis of the ethanolic extract of *Z. officinale* rhizome and *M. whitei* root ethanolic extracts. It indicates that *Z. officinale* and *M. whitei* has trace amount of alkaloids (15mg/g for ZO and 8mg/g for MW), flavonoids large amount in both *Z. officinale* and *M. whitei* (125mgQE/g in ZO and 140QE/g in MW), tannins trace amount in both *Z. officinale* and *M. whitei* (15mgTAE/g in ZO and 22mgTAE/g), trace amount of saponins in both *Z. officinale* and *M. whitei* (7.5%(ww) in (ZO) and 15% (ww) in (MW), Phenolic Compounds have trace amount in both *Z. Officinale* and *M. Whitei* (220 mg GAE/g in (ZO) and 220 mg GAE/g in (MW), and lastly trace amount of glycosides in both *Z. Officinale* and *M. Whitei*.

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.

Antioxidant Analysis of Zingiber officinale (ZO) rhizome and Mondia whitei (MW) Root Ethanolic Extract

The antioxidant contents of *Z. officinale* rhizome and *M. whitei* root ethanolic extract showed a lower IC₅₀ and higher levels of Fe²⁺ equivalents per gram of extract and Trolox equivalents per gram in *Z. officinale* (76µg/mL, 400 µmol and approximately 2500 µmol, respectively) compared to *M. whitei* (120 µg/mL, 400 µmol and approximately 2000 µmol) (Table).

Table 3: Antioxidant Analysis Results of *Z. officinale* and *M. whitei* Ethanolic Extracts

S/N	Assay	Concentrations			
		ZO		MW	
1.	DPPH	IC ₅₀ values 76µg/mL		IC ₅₀ value of 120 µg/mL	
2.	FRAP	400 µmol Fe ²⁺ equivalents per gram of extract.		400 µmol Fe ²⁺ equivalents per gram	
3.	ABTS	Approximately 2500 µmol	Trolox	Approximately 2000 µmol	Trolox
		equivalents per gram		equivalents per gram	

The Table shows Antioxidant analysis of *Zingiber officinale* rhizome and *Mondia whitei* root ethanolic extract, where *Z. officinale* indicates that DPPH (IC₅₀ values 76µg/mL), while *M. whitei* (IC₅₀ value of 120 µg/mL), FRAP (400 µmol Fe²⁺ equivalents per gram of extract for *Z. officinale*) and (400 µmol Fe²⁺ equivalents per gram for *M. whitei*, and finally ABTS (Approximately 2500 µmol Trolox equivalents per gram for *Zingiber officinale*) and (Approximately 2000 µmol Trolox equivalents per gram for *M. whitei*).

DPPH – (2,2-diphenyl-picryl hydrazyl), IC – (Inhibitory Concentration), FRAP – (Ferric Reducing Antioxidant power), ABTS –2,2'-azinobis (3-ethyl benzothiazoline-6-sulfonic acid).

Weight and Fasting Blood Glucose Changes in White Male Albino Rats According to the Different Treatments Received

According to the table below, the normal group fasting blood glucose levels was within the normal ranges on day zero and day of sacrifice (3.44±0.13 and 3.824±0.12). In the negative control, there was significantly increased FBG levels (3.72±0.14 and 21.06±1.61***), and in the positive control, on the day of sacrifice, the FBG was almost normal (4.02±0.23 and 08.65±1.80*). In groups 4, 5, 6, 7, 8 and 9, there was an increase in FBG levels across the groups. All groups had an increase in weight during the study period. However, group 2 had the least change in weight (21.2±2.85g), and group 5 had the highest (52±1.30g) (Table 4).

Table 4: Weight and Fasting Blood Glucose Levels at Day 0 and Day of Sacrifice of the DIMI White Albino Rats

Grp	FBG Levels		Body weight		
	Day 0	Day of sacrifice	Day 0	Day of sacrifice	Change
1.	3.44±0.13	3.824±0.12	222.3±8.41	271.3±5.9	49.0±2.44
2.	3.72±0.14	21.06±1.61***	218.8±6.93	240±4.08	21.2±2.85
3.	4.02±0.23	08.65±1.80*	218.8±8.09	258±6.18	39.2±1.91
4.	3.96±1.18	16.72±0.55**	222±7.95	262.3±15.31	40.3±7.36
5.	3.79±0.22	13.74±2.40*	217.3±9.03	269.3±7.73	52±1.30
6.	3.91±0.17	12.13±1.26*	233.5±3.87	269.8±8.52	36.3±4.65
7.	3.81±1.04	18.83±0.15***	231±4.71	265.2±9.84	34.3±5.24
8.	3.93±1.21	16.85±1.22**	234±4.82	272.4±7.66	37.2±3.36
9.	3.73±1.34	14.86±1.28**	241±4.91	285.1±6.48	39.4±2.54

The table shows the fasting blood glucose level on day zero and on the day of sacrifice. In the normal group, the fasting blood glucose levels was within the normal ranges on day zero and day of sacrifice (3.44±0.13 and 3.824±0.12). In the negative control, there was significantly increased FBG levels (3.72±0.14 and 21.06±1.61***), and the in positive control the day of sacrifice, the FBG was almost normal (4.02±0.23 and 08.65±1.80*). In groups 4, 5, 6, 7, 8 and 9, there was an increase in FBG levels across the groups.

*, indicate statistical difference compared to (normal control) and group negative control) and positive control) respectively, * mean $p < 0.05$, ** mean $p < 0.01$ and *** mean $p < 0.001$.

Effects of Ethanolic Rhizome Extracts of *Z. officinale* and *M. whitei* on the Plasma Levels of Reproductive Hormones in DIMI White Albino Rats

Plasma Levels of Follicle Stimulating Hormone

Induction of diabetes in the negative control group, white albino rats, FSH levels significantly decreased ($p < 0.05$), compared to the normal group rats (6.30 ± 1.78 mIU/ml). Treatment with rhizome extracts of *Z. officinale* and *M. whitei* increased their FSH levels in a dose-dependent manner (Figure). For example, treatment of DIMI rats with 800 mg/kg (6.65 ± 0.84 mIU/ml) of the *Z. officinale* increased the levels of FSH ($p < 0.01$) to significantly higher levels than those of the normal control. Likewise, treatment of diabetic rats with 800 mg/kg *M. whitei* improved the levels of FSH to statistically significant levels (6.07 ± 0.46 mIU/ml) compared to the group negative control group at $p < 0.05$, with the FSH levels increasing to close to those of the normal group (6.30 ± 1.78 mIU/ml). These levels of FSH were lower but close to those in positive control rats (7.72 ± 1.61 mIU/ml).

Figure 2: Levels of Follicle Stimulating Hormone

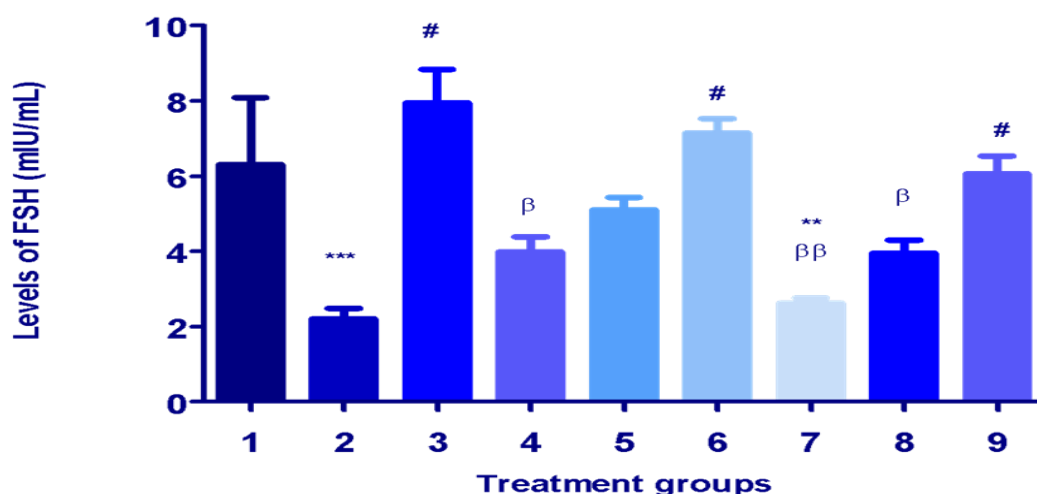


Figure 2 shows the concentration of follicle-stimulating hormone in normal, negative, positive control and treatment groups. In normal group rats (6.30 ± 1.78 mIU/ml), negative control (2.2 ± 1.4 mIU/ml), positive control rats (7.72 ± 1.61 mIU/ml) and showing different values in increasing doses of *Z. officinale* and *M. whitei*. Treated groups (4, 5 and 6) with *Z. officinale* shows gradual increase in plasma concentration of FSH, and the same gradual increase was observed in treated groups (7, 8 and 9) with *M. whitei*.

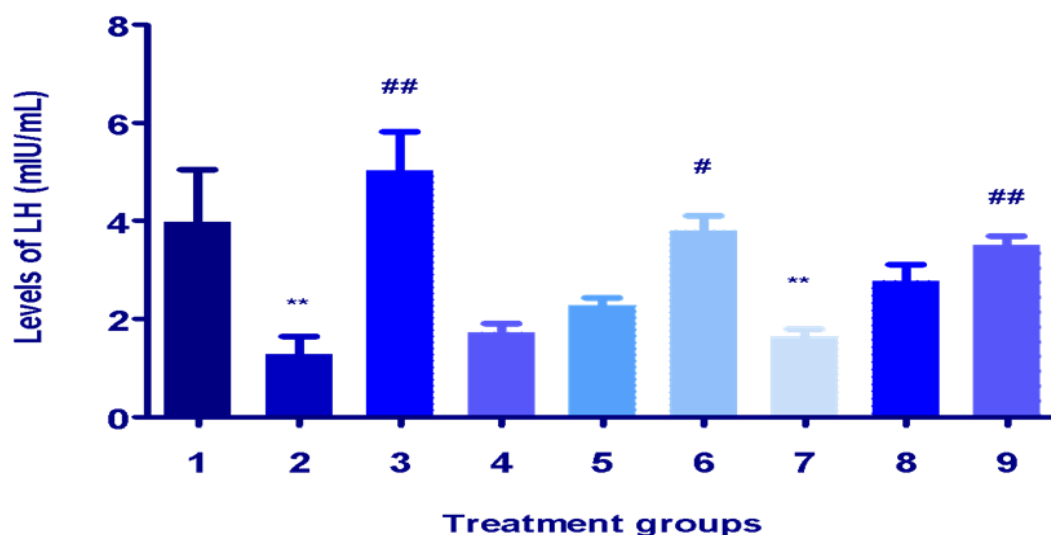
*, # and β indicate statistical difference compared to group 1 (normal control), group 2 (negative control) and group 3 (positive control) respectively, * or # mean $p < 0.05$, ** or ## mean $p < 0.01$ and *** or ### mean $p < 0.001$.

Plasma Levels of Luteinizing Hormone

Induction of diabetes in the negative control rats significantly decreased ($p < 0.01$) the levels of LH

(1.28 ± 0.37 mIU/ml), compared to normal group rats (3.98 ± 1.07 mIU/ml). However, treatment of the diabetic rats with both *Z. officinale* and *M. whitei* increased the levels of LH in a dose-dependent manner (Figure). Treatment of the diabetic rats with 400 and 800mg/kg of *Z. Officinale* significantly increased ($p < 0.05$ and $p < 0.01$, respectively) the LH levels to 3.53 ± 0.16 and 4.55 ± 0.14 mIU/ml, respectively, compared to those of the negative control. Additionally, administration of 200mg/kg of *M. whitei* only slightly increased the levels of LH, with the levels being significantly less ($p < 0.05$) than those of the normal control. However, increasing the dose to 800mg/kg increased the levels of LH to 3.50 ± 0.18 mIU/ml, which were comparable to those of the normal group, significantly higher than those of the negative control group ($p < 0.05$) and close to group 3 (5.03 ± 0.37 mIU/ml).

Figure 3: Levels of Luteinizing Hormone

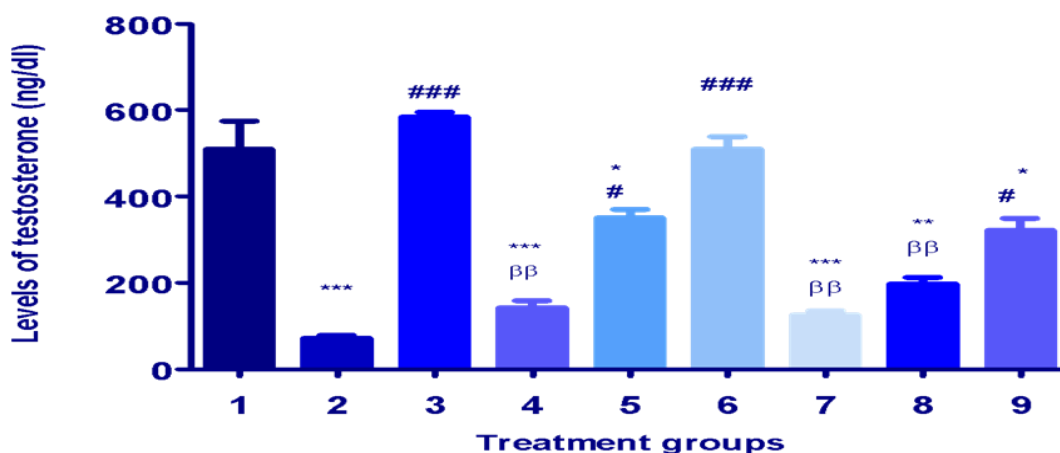


The figure shows the concentration of luteinizing hormone in normal, negative, positive control and treatment groups. In normal group rats (3.98 ± 1.07 mIU/ml), negative control (1.28 ± 0.37 mIU/ml), positive control rats (5.72 ± 1.61 mIU/ml) and showing different values in increasing doses of *Z. officinale* and *M. whitei*. Treated groups (4, 5 and 6) with *Z. officinale* shows gradual increase in plasma concentration of Luteinizing Hormone, and the same gradual increase was observed in treated groups (7, 8 and 9) with *M. whitei*.

*and # indicate statistical difference compared to group 1 (normal control) and group 2 (negative control), respectively, * or # mean $p < 0.05$, ** or ## mean $p < 0.01$ and *** or ### mean $p < 0.001$.

Serum Levels of Testosterone

Induction of diabetes reduced the serum levels of testosterone to 71.53 ± 6.84 ng/dl, which is significantly lower ($p < 0.001$) compared to those of the normal group (509.9 ± 64.97 ng/dl), positive group (584.9 ± 6.843 ng/dl) rats. Levels of testosterone in 200mg/kg and 400mg/kg of *Z. officinale*-treated groups and 200mg/kg, 400mg/kg and 800mg/kg of *M. whitei* extracts-treated groups remained significantly lower compared to those of the normal control (Figure 3). However, 400 mg/kg and 800 mg/kg of *Z. officinale* and 800 mg/kg of *M. whitei* significantly increased ($p < 0.001$) testosterone levels compared to the diabetic group, with the treated group 6 having serum testosterone levels close to those of the positive.

Figure 4: Levels of Testosterone Hormone

The figure shows the concentration of testosterone hormone in the normal, negative, positive control and treatment groups. In the normal group (509.9 ± 64.97 ng/dl), the negative control (128.8 ± 0.37 mIU/ml), and the positive group (584.9 ± 6.843 ng/dl) and showing different values in increasing doses of *Z. officinale* and *M. whitei*. Treated groups (4, 5 and 6) with *Z. officinale* shows gradual increase in plasma concentration of testosterone hormone, and the same gradual increase was observed in treated groups (7, 8 and 9) with *M. whitei*.

*and # indicate statistical difference compared to group normal control and group negative control, respectively, * or # mean $p < 0.05$, ** or ### mean $p < 0.01$, and *** or #### mean $p < 0.001$.

Effects of Ethanolic Rhizome Extracts of *Z. officinale* and *M. whitei* on the Different Sperm Parameters in DIMI White Albino Rats

Semen characteristics, including sperm count, morphology and motility were significantly decreased in negative control (structural normality was $34.2 \pm 14.20^*$ (%), sperm count $25.00 \pm 2.86^* \times 10^6/\text{ml}$ and sperm motility $12.54 \pm 2.74^*$ (%), compared to normal group (structural normality was 82.0 ± 17.05 (%), sperm count $55.00 \pm 4.98 \times 10^6/\text{ml}$ and sperm motility 51.02 ± 5.41 (%), after induction of diabetes

confirming the diabetes induced male infertility. However, administration of *Z. officinale* and *M. whitei* rhizome and root extracts respectively, improved the semen parameters in a dose-dependent manner. For example, administration of 200mg/kg of *Z. officinale* slightly improved all the parameters (structural normality was 44.9 ± 14.06 (%), sperm count $27.50 \pm 2.21^* \times 10^6/\text{ml}$ and sperm motility 35.00 ± 2.30 (%)) respectively). This improvement was enhanced by increasing the dosage to 400mg/kg of *Z. officinale* (structural normality was $51.3 \pm 5.66^*$ (%), sperm count $37.00 \pm 1.87 \times 10^6/\text{ml}$ and sperm motility $42.29 \pm 6.21^{\#}$ (%)) and at 800mg/kg (structural normality was $63.0 \pm 7.92^{\#}$ (%), sperm count $45.00 \pm 3.87 \times 10^6/\text{ml}$ and sperm motility $49.55 \pm 3.27^{\#\#}$ (%), with sperm motility for both being significantly compared to that of the negative control. Additionally, treated with 200mg/kg *M. whitei* (structural normality was $54.8 \pm 8.49^{\#}$ (%), sperm count $58.75 \pm 2.93^{\#} \times 10^6/\text{ml}$ and sperm motility 50.58 ± 6.22 (%), treated group 8 with 400mg/kg *M. whitei* (structural normality was $64.2 \pm 7.23^{\#}$ (%), sperm count $61.57 \pm 4.65^{\#} \times 10^6/\text{ml}$ and sperm motility $51.31 \pm 2.66^{\#}$ (%)) and lastly treated group 9 with 800mg/kg of *M. whitei* (structural normality was $73.9 \pm 5.67^{\#}$ (%), sperm count $64.32 \pm 8.34^{\#} \times 10^6/\text{ml}$ and sperm motility $54.43 \pm 4.38^{\#\#}$ (%)).

Table 5: Semen Analysis of Different Treatment Groups

Grp	Sperm morphology (%) Structural Normality	Sperm Count $\times 10^6/\text{ml}$	Sperm Motility (%)
1.	82.0 $\pm$ 17.05	55.00 $\pm$ 4.98	51.02 $\pm$ 5.41
2.	34.2 $\pm$ 14.20*	25.00 $\pm$ 2.86*	12.54 $\pm$ 2.74*
3.	78.4 $\pm$ 12.66*	53.25 $\pm$ 4.09*	52.31 $\pm$ 3.52*
4.	44.9 $\pm$ 14.06	27.50 $\pm$ 2.21*	35.00 $\pm$ 2.30
5.	51.3 $\pm$ 5.66	37.00 $\pm$ 1.87	42.29 $\pm$ 6.21#
6.	63.0 $\pm$ 7.92#	45.00 $\pm$ 3.87	49.55 $\pm$ 3.27##
7.	54.8 $\pm$ 8.49	58.75 $\pm$ 2.93#	50.58 $\pm$ 6.22
8.	64.2 $\pm$ 7.23#	61.57 $\pm$ 4.65#	51.31 $\pm$ 2.66#
9.	73.9 $\pm$ 5.67#	64.32 $\pm$ 8.34#	54.43 $\pm$ 4.38##

Table showing sperm analysis. Normal group (structural normality was 82.0 $\pm$ 17.05(%), sperm count 55.00 $\pm$ 4.98 $\times 10^6/\text{ml}$ and sperm motility 51.02 $\pm$ 5.41 (%), negative control (structural abnormality was 34.2 $\pm$ 14.20*(%), sperm count 25.00 $\pm$ 2.86* $\times 10^6/\text{ml}$ and sperm motility 12.54 $\pm$ 2.74*(%), positive control (structural normality was 78.4 $\pm$ 12.66*(%), sperm count 53.25 $\pm$ 4.09* $\times 10^6/\text{ml}$ and sperm motility 52.31 $\pm$ 3.52*(%), treated group 4 with 200mg/kg ZO (structural normality was 44.9 $\pm$ 14.06 (%), sperm count 27.50 $\pm$ 2.21* $\times 10^6/\text{ml}$ and sperm motility 35.00 $\pm$ 2.30 (%), treated group 5 with 400mg/kg ZO (structural abnormality was 51.3 $\pm$ 5.66*(%), sperm count 37.00 $\pm$ 1.87 $\times 10^6/\text{ml}$ and sperm motility 42.29 $\pm$ 6.21#(%), treated group 6 with 800mg/kg ZO (structural normality was 63.0 $\pm$ 7.92#(%), sperm count 45.00 $\pm$ 3.87 $\times 10^6/\text{ml}$ and sperm motility 49.55 $\pm$ 3.27##(%), treated group 7 with 200mg/kg MW (structural normality was 54.8 $\pm$ 8.49#(%), sperm count 58.75 $\pm$ 2.93# $\times 10^6/\text{ml}$ and sperm motility 50.58 $\pm$ 6.22 (%), treated group 8 with MW (structural normality was 64.2 $\pm$ 7.23#(%), sperm count 61.57 $\pm$ 4.65# $\times 10^6/\text{ml}$ and sperm motility 51.31 $\pm$ 2.66# (%)) and lastly treated group 9 with 800mg/kg of MW (structural normality was 73.9 $\pm$ 5.67#(%), sperm count 64.32 $\pm$ 8.34# $\times 10^6/\text{ml}$ and sperm motility 54.43 $\pm$ 4.38## (%))

Histology of Hypothalamus, Anterior Pituitary and Testis

Histological Effects of Ethanolic Rhizome and Root Extracts of Z. officinale and M. whitei on the Hypothalamus-Pituitary-Testicular Axis of Diabetes-Induced Infertile White Albino Male Rats

Histological Effects on the Hypothalamus

One of the two parts of the brain that is anatomically linked to the pituitary, an endocrine gland, is the hypothalamus; the other is the pineal gland. The hypothalamus controls almost every bodily function, either directly or indirectly. Although it is evident that the hypothalamus's afferents extend to other parts of the brain, the hypothalamus has a special role in releasing regulatory factors that influence anterior pituitary gland cells and trigger the release of hormones that regulate the body's endocrine glands. Afferents from higher brain centres control the neuroendocrine cells, which are located in particular areas of the hypothalamus. The networks between and among the neuroendocrine cells provide fine control to achieve homeostasis, and integrated function is obviously complex. The structural layout of the neurosecretory zone, which involves glial cells, is probably the reason why hypothalamic neurons that extend from the hypothalamus to the median eminence do not release substances into the hypophyseal portal blood. Distinct nuclei contain the neuroendocrine cells of the hypothalamus, which release

neurohormones into the hypophysial portal system and either stimulate or inhibit the anterior pituitary gland's cells. The distribution of the peptides is specific to neuroendocrine cells, which include the preoptic area, paraventricular nucleus, dorsomedial nucleus of the hypothalamus, arcuate nucleus of the hypothalamus, gonadotropin releasing hormone,

arginine vasopressin, corticotropin releasing hormone, somatotropin release inhibitory hormone or somatostatin, thyrotropin releasing hormone, gonadotropin inhibitory hormone, growth hormone releasing hormone, and dopamine—all of which are localized to different nuclei in other parts of the hypothalamus/brain(Sheng et al., 2024).

Figure 5: Showing Histology of Hypothalamus

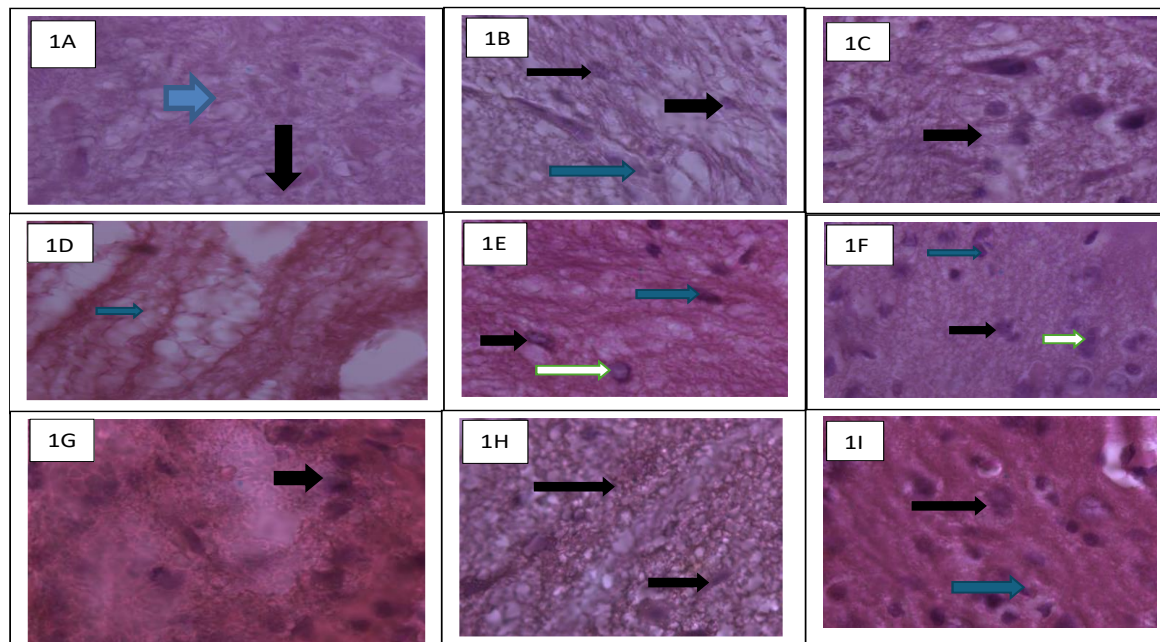


Figure (A, B, C, D, E, F, G, H and I). The histology of the rat hypothalamus treated with escalating doses of *Z. officinale* and *M. whitei* over the experimental period against the control groups. The plates show normal control (A), negative control (B), positive control (C) and treatment groups for ZO (D – F) and MW (G – I). Note the histology of the normal control with normal distribution of glial cells and neurons scattered throughout the hypothalamic tissue, while the negative control shows condensed, dark nuclei (black arrows) with accompanying vascular congestion and hyalinization (light blue arrows). The positive control shows an increased number of healthy, well-defined neurons containing nuclei with normal morphological outline (black arrows). The treatment for both extracts appears to have cell

populations with normal structural alignment within the hypothalamus, similar to the normal control: Hematoxylin and Eosin 1000X magnification.

Histological Effects on Anterior Pituitary Gland

The histology of the anterior pituitary gland of the rats showing normal control (A), negative control (B), positive control (C) and treatment groups exposed to escalating doses of *Z. officinale* (D – F) and *M. whitei* (G – I). The normal control shows an active gland with numerous acidophilic cells (black arrows), light staining chromophobic cells (colourless arrows) and darker staining basophilic cells (light blue arrow). Note the negative control showing less distribution of glandular cells, while the positive control shows increased gonadotroph cells (black arrows) with enhanced vascularisation.

Treatment with 200 mg/kg *Z. Officinale* (D) and *M. whitei* (G) shows less distribution of anterior pituitary gland cell populations, while at 400 mg/kg of *Z. officinale* (E) and *M. whitei* (H), there was increased distribution of the same cell populations, indicating an active gland. At 800 mg/kg of *Z.*

Officinale (F) and *M. Whitei* (I), the cellular organisation appears more improved with numerous acidophilic cells, chromophobe cells, basophilic cells, and enhanced vascularisation: Hematoxylin and Eosin stain at 1000X.

Figure 6: Showing histology of Anterior Pituitary Gland

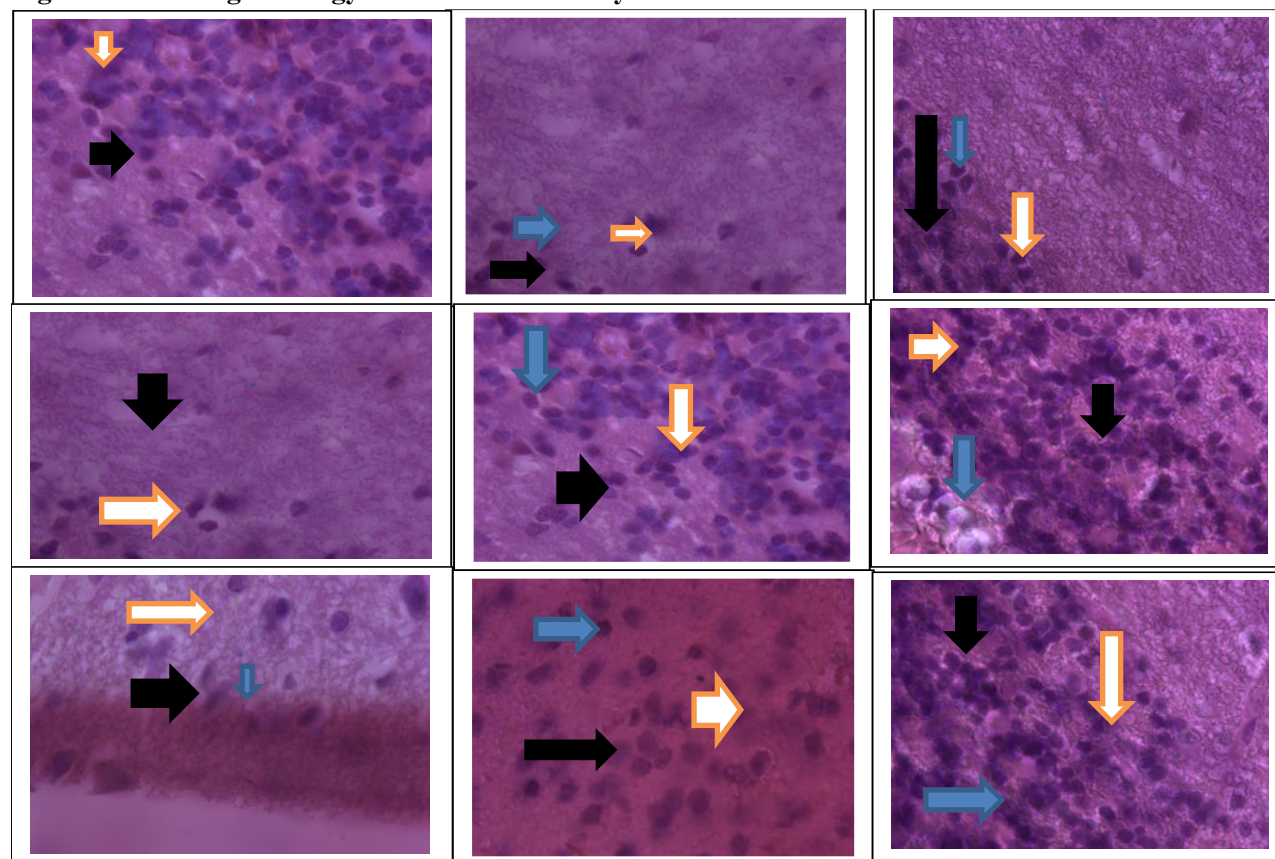


Figure (A, B, C, D, E, F, G, H and I). The Anterior pituitary gland histology of the rats showing normal control (2A), negative control (2B), positive control (2C) and treatment groups exposed to escalating doses of *ZO* (2D – F) and *MW* (2G – I). Note the normal control (2A) showing an active gland with numerous acidophilic cells (black arrows), chromophobic cells with less staining (colourless arrows) and basophilic cells (light blue arrow); Negative control (2B) showing less distribution of glandular cells and positive control (2C) showing increased gonadotroph cells (black arrows),

enhanced vascularisation. Treatment with escalating doses of *ZO* (2D – F) and *MW* (2G – I), there is less distribution of acidophiles, chromophiles cells, and basophiles 200mg/kg (2D and 2G), while at 400mg/kg (2E and 2H) there was increased distribution of acidophiles, Chromophobes and basophiles, and 800mg/kgs (2F and 2I), appears normal with numerous of acidophilic cells, Chromophobes cells, basophilic cells, shows increased Gonadotroph cells and enhanced vascularization: 1000X.

Histological Effects on Testes

Interstitial tissue and seminiferous tubules make up the testes' histology. Sertoli cells and developing germ cells at different stages of spermatogenesis are found in seminiferous tubules, which are tightly wound structures bordered by germinal epithelium (Bharti et al., 2025). A layer of myoid cells and a basement membrane encircle the tubules, aiding in sperm motility. The Leydig (interstitial) cells that produce testosterone are found in the interstitial tissue between tubules. The testes are generally set up to facilitate the secretion of hormones and the generation of sperm (Li et al., 2025).

The histology of the testes of the rats showing normal control (3A), negative control (3B), positive control (3C) and treatment groups exposed to

escalating doses of *Z. officinale* (3D – 3F) and *M. whitei* (3G – 3I). The normal control showing spermatogonia (black arrows), spermatocytes (colourless arrows) and Sertoli cells (light blue arrow). Note the negative control showing less testicular cells, while the positive control shows increased testicular cells (black arrows). Treatment with 200 mg/kg *Z. officinale* (3D) and *M. whitei* (3G) shows less distribution of testicular cell populations, while at 400 mg/kg of *Z. officinale* (3E) and *M. whitei* (3H), there was increased distribution of testicular cell populations, indicating improvement. At 800 mg/kg of *Z. officinale* (3F) and *M. whitei* (3I), the testicular cellular organisation appears more improved with numerous spermatogonia spermatocyte and spermatocyte cells, Hematoxylin and Eosin stain at 1000X.

Figure 7: Showing Histology of Testes

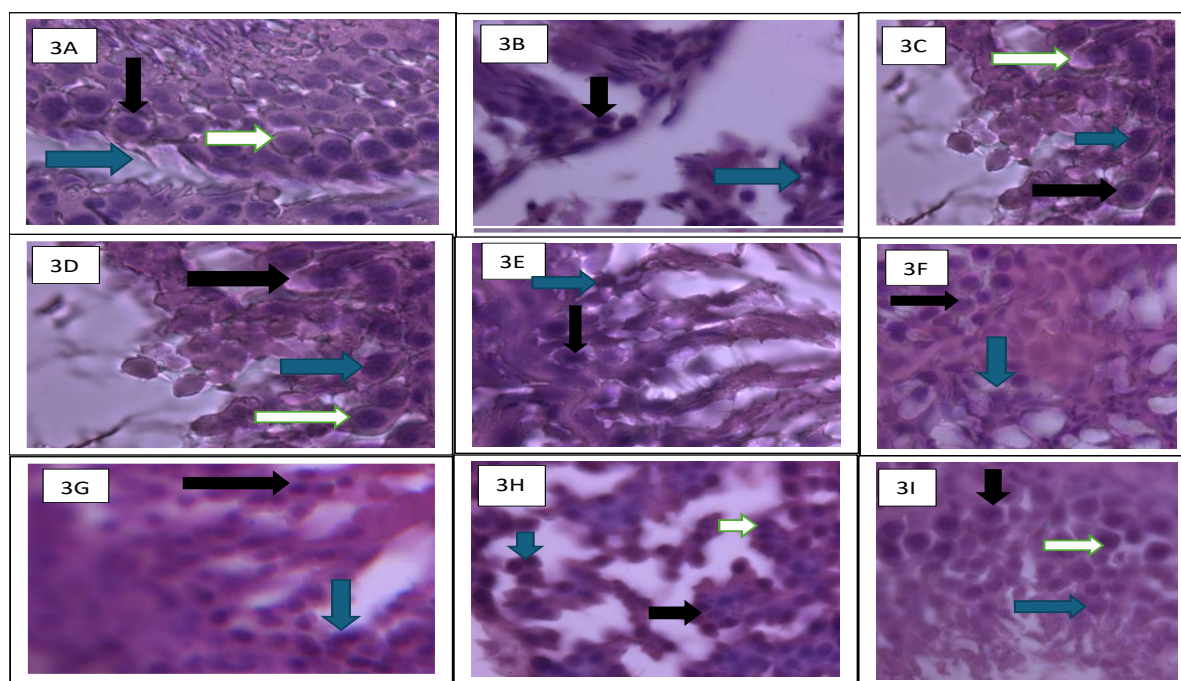


Figure (A, B, C, D, E, F, G, H and I). The testicular histology of the rats showing seminiferous tubules of normal control (3A), negative control (3B), positive control (3C) and treatment groups exposed to escalating doses of ZO (3D – F) and MW (3G – I). Note spermatogonia (black arrow) resting

on basement BM (arrow), Sertoli cells (green arrow) and spermatocytes (colourless arrow) of rats treated with alloxan monohydrate alone. At the escalating doses as shown in histological plates (3D - 3I), at lower doses (200mg/kg) shows reduced distribution of sertoli cells, spermatogonium and sp-sperm cell,

at medium doses (400mg/kg), there is increased number of sertoli cells, spermatogonium and sperm cell, then at higher doses (800mg/kg), it shows normal distribution of sertoli cells, spermatogonium and sperm cell compared to negative control. At high doses of *M. whitei*, the seminiferous epithelium appears to have normal structural alignment within the seminiferous tubule, similar to features of the normal control; 1000X.

Histomorphometric Effects on the Testes

Histomorphometric evaluation of the testis of DIMI rats was carried out using different parameters (Table). Induction of DIMI led to a significant decrease in all parameters including the testicular

weight (1.02 ± 0.3 ml), volume (1.11 ± 0.22 ml), seminiferous tubules diameter (180.5 ± 4.3 μ m), seminiferous epithelial height (45.6 ± 3.2 μ m) and Leydig cell volume (501 ± 92.3 μ m³), compared to values of the normal control at $p < 0.05$ and $p < 0.01$. However, administration of *Z. officinale* and *M. whitei* improved all the parameters to levels similar to those of the normal and positive controls and in a dose-dependent manner. For example, administration of 200 mg/kg and 400 mg/kg of *Z. officinale* and *M. whitei* significantly improved all testicular histomorphometric parameters compared to the normal control at either $p < 0.05$ or $p < 0.01$, apart from the seminiferous tubule diameter.

Table 6: Effect of *Z. officinale* and *M. whitei* Ethanolic Extracts on Testicular Histomorphometric Parameters in DIMI White Albino Rats.

Grps	TW (ml)	TV (ml)	STD (μ m)	SEH (μ m)	LCV (μ m ³)
1	2.71 ± 1.2	2.98 ± 0.71	250.3 ± 5.7	85.2 ± 2.1	1250 ± 212.4
2	$1.02 \pm 0.3^{***}$	$1.11 \pm 0.22^{***}$	$180.5 \pm 4.3^{**}$	$45.6 \pm 3.2^{**}$	$501 \pm 92.3^{**}$
3	$2.52 \pm 2.4^{##}$	$2.63 \pm 0.01^{\#}$	220.1 ± 6.2	$70.3 \pm 4.1^{##}$	$1201 \pm 243.1^{##}$
4	$1.45 \pm 2.1^{**}$	$1.51 \pm 0.41^{**}$	195.2 ± 5.1	$58.4 \pm 3.5^{*}$	$822 \pm 113.4^{*}$
5	$1.50 \pm 0.8^{**}$	$1.73 \pm 0.89^{*}$	230.5 ± 4.8	$75.6 \pm 2.8^{*}$	$1242 \pm 351.2^{##}$
6	$2.51 \pm 0.5^{##}$	$2.58 \pm 1.02^{\#}$	245.8 ± 6.3	$82.1 \pm 3.2^{\#}$	$1291 \pm 236.3^{##}$
7	$1.93 \pm 1.5^{*}$	$2.31 \pm 0.31^{\#}$	190.3 ± 4.5	$55.3 \pm 3.0^{*}$	$921 \pm 219.6^{*}$
8	$2.57 \pm 1.2^{##}$	$3.01 \pm 1.23^{##}$	225.6 ± 5.2	$72.8 \pm 2.5^{\#}$	$1322 \pm 221.3^{##}$
9	$3.38 \pm 1.9^{###}$	$3.53 \pm 1.01^{###}$	$248.2 \pm 5.9^{\#}$	$84.5 \pm 3.1^{##}$	$1432 \pm 346.7^{###}$

Table shows all histomorphometric parameters TW (2.51 ± 0.5 , TV 2.58 ± 1.02 , STD 245.8 ± 6.3 , SEH 82.1 ± 3.2 and LCV 1291 ± 236.3) to values that compare to those of the normal control TW (2.71 ± 1.2 , TV 2.98 ± 0.71 , STD 250.3 ± 5.7 , SEH 85.2 ± 2.1 and LCV 1250 ± 212.4) but significantly higher than those of the negative control rats TW (1.02 ± 0.3 , TV 1.11 ± 0.22 , STD 180.5 ± 4.3 , SEH 45.6 ± 3.2 and LCV 501 ± 92.3) at $p < 0.05$. On the other hand, administration of 800 mg/kg of MW also significantly enhanced the testicular weight, volume and Leydig cell volume to 3.38 ± 1.9 , 3.53 ± 1.01 and 1432 ± 346.7 respectively at $p < 0.001$, as

compared to the negative control, and improved the seminiferous tubule diameter and seminiferous epithelial height (248.2 ± 5.9 and 84.5 ± 3.1) at $p < 0.05$ or $p < 0.01$ respectively.

TV – Testicular volume, TW – Testicular weight, STD - Seminiferous tubule diameter, SEH - Seminiferous tubule Epithelial Height and LCV - Leydig cell volume. Values are mean $\pm$ 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 $p < 0.001$.

DISCUSSION AND RECOMMENDATIONS

Phytochemical Analysis and Antioxidant Activity of *Z. officinale* and *M. whitei* Extracts

The quantitative and qualitative phytochemical analysis of the ethanolic extracts of *Zingiber officinale* rhizome and *Mondia whitei* root in the present study revealed the presence of various secondary metabolites. Specific analyses showed that the *Zingiber officinale* extracts contain significant amounts of flavonoids, trace amounts of alkaloids, tannins, saponins, phenolic compounds and glycosides. Similarly, *Mondia whitei* contains a complex mixture of bioactive phytochemicals—including saponins, alkaloids, flavonoids, tannins, phenolics, sterols, and terpenoids. Previous studies report other phytochemical compounds isolated from *Zingiber officinale*, including terpenes, polysaccharides, lipids, raw fibre, and organic acids, as well as gingerols and shogaols (Defo Deh et al., 2024). However, the findings on *Mondia whitei* in the present study are at variance with those of the chloroform extract of *Mondia whitei*, which showed moderate amounts of terpenoids, and trace amounts of flavonoids, cardiac glycosides, saponins and phytosterols, but with the absence of alkaloids and saponins (Abdelfattah et al., 2023). According to current study findings, *Zingiber officinale* has better antioxidant activity compared to *Mondia whitei*, owing to a lower IC₅₀, implying that smaller quantities of the ethanolic extract of the rhizome of *Zingiber officinale* are required to achieve 50% of a particular effect compared to those of the ethanolic root extract of *Mondia whitei*, which agrees with a study by M Gholami-Ahangaran - Veterinary Medicine 2021, 2021. The main antioxidants isolated from *Zingiber officinale* included gingerol, shogaol, Paradols and gingerone, which work through a variety of methods to produce their antioxidant benefits (M Gholami-Ahangaran et al., 2021). Their mode of action is through inhibition of lipid peroxidation, thus safeguarding cell membranes, which increases the activity of endogenous antioxidant enzymes, such as

glutathione peroxidase, catalase, and superoxide dismutase (SOD) (Boroujeni et al., 2022). In *Mondia whitei*, phenolic compounds, including flavonoids and tannins, carry out antioxidant activities through metal chelation, where substances have the ability to chelate transition metals, such as copper and iron, which use Fenton processes to catalyse the production of free radicals and lipid peroxidation inhibition by halting the oxidative breakdown of lipids in cell membranes (Elsawy et al., 2023).

The current study findings shows that induction of diabetes with alloxan monohydrate led to disruption and atrophy of the seminiferous tubules, with accompanying reduction in number of spermatogenic cells and in the size of Leydig cells which agrees with previous studies by (Huang et al., 2024) who demonstrated that alloxan leads to disorganized and reduced spermatogenesis and atrophy of both seminiferous tubular cells and Leydig cells, through inducing the build-up of ROS, which is followed by cellular oxidative stress that damages the cells, subsequently leading to injury and death. The current study findings showed that treatment of DIMI rats with *Zingiber officinale* and *Mondia whitei* greatly improved the histoarchitecture of the hypothalamus, anterior pituitary gland and testes in a dose-dependent manner. This was expected since both *Zingiber officinale* and *Mondia whitei* are known to possess antioxidant and anti-inflammatory properties. *Zingiber Officinale*, especially its phytochemicals like gingerols and shogaols, act as free radicals' scavengers and thus play a protective role to neurons of the hypothalamus, cells of the anterior pituitary gland and testes (Gandhi et al., 2023).

The results on histoarchitectural changes observed in the current study confirmed earlier findings which showed that *Zingiber officinale* improves structural alterations in the rat brain, including the hypothalamus and anterior pituitary gland, resulting from alloxan induced diabetes mellitus (Badejogbin et al., 2024) as well as improvement of cognitive

abilities in male rats through antioxidant and anti-inflammatory effects and reduction of neurodegeneration in the hypothalamus and pituitary glands, due to the same mechanisms, *Zingiber officinale* administration led to improved general histology of the testes and seminiferous tubules histology, which is in agreement with changes observed by Badejogbin et al., 2024 in his study. *Mondia whitei* has been shown to improve all testicular parameters, including tissue histology, at low doses but may be cytotoxic at higher levels of 800 mg/kg (Nethengwe et al., 2022).

The current study findings show that at 400 mg/kg and 800 mg/kg of both *Zingiber officinale* and *Mondia whitei* greatly improved the histology of the hypothalamus, which harbours gonadotropin releasing hormone cells down to the anterior pituitary gland. The gonadotrophs under gonadotropic releasing hormone influence secrete both FSH and LH, which are transported through the blood to the testes. The findings of the current study show that both *Zingiber officinale* and *Mondia whitei* at higher doses improved the histology and steroidogenic functions of the hypothalamus, anterior pituitary gland, and testicular tissues. This finding is consistent with previous studies by Tsampoukas et al. (2022), which showed that higher doses (15 g/kg) of *Zingiber officinale* administered to Japanese quails from 7 to 70 days of age improved the diameter of seminiferous tubules and height of the germinal epithelium. However, when administered in excessive quantities, *Zingiber officinale* causes distortion of both the germinal and supporting cells, atrophy and destruction of the Leydig cells and the overall shrinkage of the seminiferous tubules in adult Wistar rats (Zhu et al., 2023). According to the current study, *Zingiber officinale* reversed and protected seminiferous tubular destruction caused by reactive oxygen species. It also showed a gradual increase of both hormones at increasing doses, consistent with the previous findings on the effects of Ginger (*Zingiber officinale*) roots on the

reproductive aspects in male Japanese Quails (*Coturnix japonica*) (Badejogbin et al., 2024).

The current study findings show that induction of DIMI using alloxan monohydrate led to a significant decrease in the histomorphometric parameters of the male rats, including the testicular weight and volume, seminiferous tubule diameter, seminiferous epithelial height and Leydig cell volume when compared to those of the normal control. It also showed that *Mondia whitei* has better testicular protective abilities compared to *Zingiber officinale* since *Mondia whitei* has several anti-oxidative and anti-inflammatory properties that include flavonoids, polyphenols and tannins. The current study finding is consistent with earlier studies by Delbarba et al. (2024) on the hypoglycemic and pancreatic protective effects of *Portulaca oleracea* extract in alloxan-induced diabetic rats. In their study, they reported that administration of *Portulaca oleracea* extract in alloxan-induced diabetic rats negatively impacted the testicular histomorphometric parameters due to its toxic effects on testicular cells.

A study by Defo Deeh et al. (2024) stated that flavonoids, polyphenols and tannins improved levels of male fertility-stimulating hormones, reduced oxidative stress parameters and inflammatory cytokines, and protected both liver and testicular tissues from Bisphenol A (BPA)-induced changes. Thus, the actions of alginate/DPP beads make them a promising choice for antioxidant, liver-protecting, and male hormone-enhancing hydrogels. The changes in testicular tissues, seminiferous tubules and Leydig cell parameters associated with administration of *Mondia whitei* as reported in the current study the findings of the current study showed that *Mondia whitei* improves testicular and seminiferous tubular histology only in low quantities.

CONCLUSION

The current study findings illustrated the potential reproductive benefits of *Zingiber officinale* and

Mondia whitei in combating diabetes-induced male infertility, with significant improvements in hormone levels and testicular functions. These medicinal plant extracts contain beneficial phytochemicals that scavenge free radicals, with testicular protective effects which preserves the testicular architectural integrity of Leydig and Sertoli cells. Histological analysis provided essential information about testicular architecture, which enhances spermatogenic structures that had been compromised by diabetes. Beneficial phytochemicals found in these medicinal plant extracts scavenge free radicals and have protective effects on the hypothalamus pituitary testicular axis. They also maintain the architectural integrity of the Leydig and Sertoli cells, which is crucial for testicular function. However, *Mondia whitei* offers better testicular protective abilities compared to *Zingiber officinale* since it induced test histological changes within the hypothalamus, anterior pituitary gland and testes similar to the normal control. This shows that *Mondia whitei* has very good protective abilities against male infertility induced by diabetes mellitus compared to *Zingiber officinale*. *Mondia whitei* could therefore be used for translational research in order to be used in humans to prevent male infertility in people with diabetes.

REFERENCES

- Abarikwu, S. O., Onuah, C. L., & Singh, S. K. (2020). Plants in the management of male infertility. *Wiley Online Library*, 52(3). <https://doi.org/10.1111/AND.13509>
- Abdelfattah, M., ... M. H.-B. V., & 2023, undefined. (n.d.). The effects of Ginger (*Zingiber officinale*) roots on the reproductive aspects in male Japanese Quails (*Coturnix coturnix japonica*). *Bmcvetres.Biomedcentral.Com*. Retrieved July 18, 2023, from <https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-023-03576-6>
- Badejogbin, O. C., Chijioke-Agu, O. E., Olubiyi, M. V., & Agunloye, M. O. (2024). Pathogenesis of testicular dysfunction in diabetes: exploring the mechanism and therapeutic interventions. *Springer*. <https://doi.org/10.1007/S10815-024-03314-3>
- Bharti, N., Yadav, P., Choudhary, S. K., Dhaked, R. K., & Mali, P. C. (2025). Cassia siamea-Derived Silver Nanoparticles: Synthesis and Their Impact on Male Fertility Through Biochemical and Histopathological Insights in Rats: N. Bharti et al. *Springer*. <https://doi.org/10.1007/S12011-025-04658-2>
- Boroujeni, S. N., Malamiri, F. A., Bossaghzadeh, F., Esmaeili, A., & Moudi, E. (2022). 522 The most important medicinal plants affecting sperm and testosterone production: a systematic review. *JBRA Assisted Reproduction*, 26(3), 522–530. <https://doi.org/10.5935/1518-0557.20210108>
- Defo Deeh, P. B., Sathiyaseelan, A., Vishven Naveen, K., & Wang, M. H. (2024). Phytochemical analysis and antioxidant potential of *Mondia whitei* and *Guibourtia tessmannii* against H₂O₂-induced cytotoxicity in PC3 cells. *Taylor & Francis*. <https://doi.org/10.1080/07391102.2024.2335299>
- Delbarba, A., Anelli, V., Bambini, F., Buoso, C., Facondo, P., Gatta, E., Girelli, A., Cappelli, C., & Ferlin, A. (2024). Type 1 diabetes mellitus and sperm quality: A case-control study. *Wiley Online Library*. <https://doi.org/10.1111/ANDR.13681>
- Dolatabadi, S., Oskuei, S. R., Mehri, S., & Hosseinzadeh, H. (2025). A comprehensive review of medicinal plants and their beneficial roles in alleviating bisphenol A-induced organ toxicity. *Springer*. <https://doi.org/10.1007/S00210-025-03795-8>
- Elsawy, H., Fouad, E., Dairy, M. K.-J. of F. and, & 2023, undefined. (2023). Comparative Study between the Effect of Marjoram (*Origanum marjoram*) and Ginger (*Zingiber officinale*)

- Extract on the Fertility of Diabetic Male Albino Rats. *Jfds.Journals.Ekb.Eg*, 14(1), 13–24. <https://doi.org/10.21608/jfds.2023.180204.1087>
- Ethnobotany, pharmacology and phytochemistry of medicinal plants used for management of Diabetes mellitus in Uganda, Kenya, Tanzania and the Democratic. (2024). *Springer*, 6(6), 312. <https://doi.org/10.1007/S42452-024-05970-7>
- Fan, C., Zhang, J., Qiu, D., Barnea, E. R., Lettieri, G., Cui, Z., & Liu, K. (2024). Causal relationship between genetically predicted type 2 diabetes mellitus and male infertility. *Frontiersin.Org*, 15. <https://doi.org/10.3389/FENDO.2024.1357279/FULL>
- Gandhi, G. R., Hillary, V. E., Antony, P. J., Zhong, L. L. D., Yogesh, D., Krishnakumar, N. M., Ceasar, S. A., & Gan, R. Y. (2023). A systematic review on anti-diabetic plant essential oil compounds: Dietary sources, effects, molecular mechanisms, and safety. *Critical Reviews in Food Science and Nutrition*. <https://doi.org/10.1080/10408398.2023.2170320>
- Huang, R., Chen, J., Guo, B., Jiang, C., & Sun, W. (2024a). Diabetes-induced male infertility: potential mechanisms and treatment options. *Springer*, 30(1). <https://doi.org/10.1186/S10020-023-00771-X>
- Huang, R., Chen, J., Guo, B., Jiang, C., & Sun, W. (2024b). Diabetes-induced male infertility: potential mechanisms and treatment options. *Springer*, 30(1), 11. <https://doi.org/10.1186/S10020-023-00771-X>
- Kyarimpa, C., Nagawa, C. B., Omara, T., Odongo, S., Ssebugere, P., Lugasi, S. O., & Gumula, I. (2023). Medicinal plants used in the management of sexual dysfunction, infertility and improving virility in the East African Community: a systematic review. *Wiley Online Library*, 2023(1). <https://doi.org/10.1155/2023/6878852>
- Lakshmi, G., Medicine, S. M.-A., & 2025, undefined. (2025). Diabetes and hypogonadism in males. *Journals.Sagepub.Com*, 22(3), 199–207. <https://doi.org/10.1177/09760016251332088>
- Li, H., Zhang, M., Ma, J., Li, W., Liu, X., Li, Y., Ma, J., Yang, D., Tie, Y., Bai, H., & Wang, S. (2025). Zinc Combined with Metformin Corrects Zinc Homeostasis and Improves Steroid Synthesis and Semen Quality in Male Type 2 Diabetic Mice by Activating PI3K/AKT. *Springer*. <https://doi.org/10.1007/S12011-025-04518-Z>
- Lopes-Ferreira, J., Matos, J., ... F. D.-B. J. of, & 2025, undefined. (n.d.). Protective effects of phenolic phytochemicals on male fertility: a narrative review. *SciELO Brasil*. Retrieved May 20, 2025, from <https://www.scielo.br/j/bjb/a/p35DBYXKxcw46tSryk6TnWN/>
- Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. Bin. (2019). Bioactive compounds and bioactivities of ginger (*zingiber officinale roscoe*). In *Foods* (Vol. 8, Issue 6). <https://doi.org/10.3390/foods8060185>
- Minas, A., Camargo, M., ... M. A.-I. J. of B., & 2024, undefined. (n.d.). Effects of diabetes-induced hyperglycemia on epigenetic modifications and DNA packaging and methylation during spermatogenesis; A narrative review. *Pmc.Ncbi.Nlm.Nih.Gov*. Retrieved June 1, 2025, from <https://pmc.ncbi.nlm.nih.gov/articles/PMC10722482/>
- Nethengwe, M., Okaiyeto, K., Opuwari, C. S., & Oguntibeju, O. O. (2025). The role of hyperglycaemia, oxidative stress, and inflammation in diabetes-related male infertility: Therapeutic properties of medicinal

plants. *Herbmedpharmacol.Com*, 14(3), 265–276. <https://doi.org/10.34172/jhp.2025.52803>

<https://doi.org/10.3389/FENDO.2023.1279058>
/FULL

Nwakanma, E., Karpiuk, U., & Minarchenko, V. (2024). *Comparative Analysis Of Medicinal Plants And Medicines Of Plant Origin In Nigeria And Ukraine*. <http://ir.librarynmu.com/bitstream/123456789/11547/3/NwakanmaEmmanuella-стиснуто.pdf>

Pradeep, M., Kruszka, D., Kachlicki, P., Mondal, D., & Franklin, G. (2022). Uncovering the phytochemical basis and the mechanism of plant extract-mediated eco-friendly synthesis of silver nanoparticles using ultra-performance liquid. *ACS Publications*, 10(1), 562–571. <https://doi.org/10.1021/ACSSUSCHEMENG.1C06960>

Sheng, W., Li, Y., Liu, L., Li, B., Sun, T., Zhu, C., & He, Q. (2024). Tortoise-shell glue ameliorates male infertility through the hypothalamic-pituitary-gonadal axis in obese rats. *Taylor & Francis*, 28(1), 110–122. <https://doi.org/10.1080/19768354.2024.2329684>

Tsampoukas, G., Tharakan, T., Narayan, Y., Khan, F., Cayetano, A., Papatsoris, A., Buchholz, N., & Minhas, S. (2022). Investigating the therapeutic options for diabetes-associated male infertility as illustrated in animal experimental models. *Andrologia*, 54(9). <https://doi.org/10.1111/AND.14521>

Unuofin, J. O., Masuku, N. P., Paimo, O. K., & Lebelo, S. L. (2021). Ginger from Farmyard to Town: Nutritional and Pharmacological Applications. *Frontiers in Pharmacology*, 12. <https://doi.org/10.3389/FPHAR.2021.779352>/FULL

Zhu, X.-B., Niu, Z.-H., Fan, W.-M., Sheng, C.-S., Chen, Q., Guo, K., Xie, H., Lilly, E., States, U., & Li, X. (2023). Type 2 diabetes mellitus and the risk of male infertility: a Mendelian randomization study. *Frontiersin.Org*, 14.