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Phytochemical composition and antioxidant properties of *Zingiber officinale* and *Mondia whitei* root ethanolic extracts: effects on reproductive parameters in diabetes-induced infertile male albino rats

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Abstract

Background Medicinal plants are widely utilized as alternatives for the treatment, management, and prevention of a number of illnesses, including diabetes and infertility, because of their accessibility, affordability, and form of ingestion. They are also important sources of phyto-bioactive compounds that are utilized in contemporary medicine. Diabetes is becoming a key risk factor for diabetic induced male infertility; hence, the frequency of infertility is projected to grow. According to the International Diabetes Federation, the global diabetes prevalence is estimated to rise by 55% between 2019 and 2045, with the highest increases forecast in developing countries like Sub-Saharan Africa and Asia. The World Health Organization (WHO) 2021 annual report, promotes the use of medicinal plants in the healthcare system to treat and manage a variety of illnesses. While both *Zingiber officinale* and *Mondia whitei* are known to possess phytochemicals and antioxidant properties, there's a lack of comprehensive data on their ethanolic extracts specifically regarding their phytochemical profiles, antioxidant capacities, and potential therapeutic effects on reproductive health in diabetic conditions. There is little scientific information to compare the effects of ethanolic extracts from the roots of *Z. officinale* and *M. whitei* on diabetic infertile men. While both species have been shown to improve fertility, especially in non-diabetic males, there is little study on their comparative impact on diabetic infertile males. Existing research primarily focuses on individual plants and different extraction methods, rather than comparing ethanolic extracts. The present study focused on the phytochemical analysis and effect of *Z. officinale* rhizome and *M. whitei* root ethanolic extract on reproductive functions in diabetes-induced male infertility in white albino rats.

Methodology Fresh samples of *Z. officinale* rhizomes and *M. whitei* roots were collected from Jubilee Market in Kisumu city, cleaned, chopped into small pieces, weighed, air dried and blended using 70% Ethanol, methanol and aqueous solvent separately for extraction. The mixtures were shaken using an orbital shaker for 24 h, followed by separation using a muslin piece of clothing to form a solution. The solutions were filtered using 125 size Whitman's

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filters followed by air-drying using a vacuum pump/rotary evaporator and hot air oven to obtain the final yield. The data was analyzed using GraphPad Prism version 5.0. Data was presented as mean $\pm$ standard error. Means were compared using one-way ANOVA and statistical differences between groups were determined by Tukey's multiple pairwise test as the post-hoc test at $p < 0.05$.

Results Qualitative phytochemical analysis revealed high levels of alkaloids (15 mg/g), same levels of phenolic compounds (220 mgGAE/g) and lower levels of flavonoids, tannins and saponins in *Z.offinale* (125 mgQE/g, 7.5%(w/w) and 15 mgTAE/g respectively) compared to those of *M. whitei* (140 mg QE/g, 15% (w/w) and 22 mg TAE/g respectively). The antioxidant contents of *Z.offinale* rhizome and *Whitei* root ethanolic extract showed a lower IC_{50} and higher levels of Fe^{2+} equivalents per gram of extract and Trolox equivalents per gram in *Z.offinale* (76 μ g/mL, 400 μ mol and approximately 2500 μ mol respectively) compared to *M. whitei* (120 μ g/mL, 400 μ mol and approximately 2000 μ mol). Administration of 200 mg/kg of *Whitei* also improved all semen parameters measured, and hormonal levels in the present study, which were even significantly higher at 800 mg/kg that are either close to or slightly higher than those of the positive control respectively. The current study analyses illustrated the potential of both plants as sources of beneficial bioactive compounds, which possess both antioxidant and anti-inflammatory properties hence of medicinal value for management and treatment of diabetic induced male infertility.

Conclusion The results provide insights into the phytochemical profiles and antioxidant capacities of *Z. officinale* and *M. whitei*. Further research is, however, required to analyze the mechanisms of action of these therapeutic and nutritional compounds in the user.

Keywords Diabetes, Male infertility, *Zingiber officinale*, *Mondia whitei*, Phyto-bioactive, Antioxidant

Introduction

Millions of individuals worldwide suffer from diabetes-induced male infertility (DIMI), a severe public health concern. Infertility affects around 186 million people, or 15% of couples globally, according to the World Health Organization's (WHO) annual 2021 report. About 40–50% of infertile couples are believed to have male infertility (Elagamey et al., 2023). Approximately 8% of men worldwide suffer from DIMI. Africa has the highest frequency of infertility, with about 14% of males experiencing it (Key et al., 2025). An estimated 12% of males in Kenya are thought to have DIMI, and as a result, these people and their families experience significant social and financial hardship (He et al., 2021). In addition to the financial consequences to individuals, families, and healthcare systems—all of which are already under stress due to a weak economy, DIMI generates substantial psychological and social anguish. According to estimates from the International Diabetes Federation (IDF), between 4 and 5% of Kenyan people have diabetes, with prevalence being higher in cities. The burden of DIMI is anticipated to rise in tandem with the global increases in the prevalence of diabetes mellitus (Latifi et al., 2025). In fact, the International Diabetes Federation predicts that between 2019 and 2045, the prevalence of diabetes would increase by 55% worldwide, with the largest increases expected in Asia and Africa. According to Ni et al. (2025), diabetes is estimated to impact approximately 4.5% of Kenyans and is predicted to increase due to changes in lifestyle and an aging population.

Male infertility brought on by diabetes results from deficits and anomalies in spermatogenesis and

steroidogenesis (Kumar et al., 2023). These included alterations in the deoxyribonucleic acid integrity of spermatozoa, poor sperm morphology, low motility, and low viability, among other changes in semen parameters. Low levels of testicular sex steroids are indicative of decreased steroidogenesis, which is caused by decreased Leydig cell volume. Additionally, DIMI causes morphological and functional changes, such as retrograde ejaculations and vas deferens blockages, respectively (Singh et al., 2025). Reactive oxygen species (ROS), produced when blood sugar levels are high, cause oxidative damage, which is the mechanical cause of the association between diabetes and infertility. The structure, integrity, and functions of the sperm membrane and DNA are disrupted, resulting in a decline in normal sperm parameters (such as motility, counts, morphology, and viability), biological macromolecules like proteins, lipids, and nucleic acids as well as the hypothalamus-pituitary-testicular axis histoarchitecture. The sperm's capacity to fertilize an egg is significantly reduced by these factors (Kumar, Dharendra, et al., 2023). To guard against oxidative stress brought on by ROS, testes often possess a sophisticated and well-developed array of antioxidant enzymes, free radical scavengers, and steroidogenic activities (Sun, et al., 2021). Reactive oxygen species harm sperm DNA, which in turn affects sperm motility, viability, and morphology. According to Elsayy et al. (2023), this process of testicular injury is currently thought to be one of the reasons for the compromised form and function of testicular tissue.

Plants have long been used as alternative medicines particularly in African regions where the poor population lack easy access to medical care and cannot afford

traditional medications (Babalola et al., 2023). Ethnomedicines are said to be effective, economical, easily accessible, simple to use, and have few adverse effects. In these situations, medicinal plants are seen as accessible and reasonably priced treatments. Furthermore, according to reports, they have few adverse effects (Msiska et al., 2021). *Z. officinale*, for instance, is a medicinal plant that has been utilized for millennia for its flavor and therapeutic properties. The anti-inflammatory, anti-oxidant, anti-diabetic, anti-cancer, anti-emetic, and gastroprotective properties of gingerols, flavonoids, phenolic compounds, and essential oils have been reported (Shehu et al., 2022). In men, it has also been shown to improve sperm count, motility, viability, morphology, DNA integrity, and viability in addition to raising testosterone levels in the blood (Li et al., 2024). Accordingly, it might be the ideal medication for treating DIMI (Shakeel, A. et al., 2025). Additionally, it has been shown to enhance male reproductive performance, including sperm count, motility, viability, morphology, DNA integrity, and viability, as well as elevating testosterone levels in the blood (Li et al., 2024). Similar to this, *Mondia whitei* has a rich variety of bioactive phytochemicals, such as phenolics, sterols, flavonoids, alkaloids, tannins, terpenoids, and saponins, which perhaps help explain its historical medical applications. The goal of the current study was to examine the phytochemical components found in these two ethanolic extracts and evaluate the antioxidant qualities that go along with them. White albino rats were used in the study because of their cost-effectiveness, ease of handling, well-characterized physiology, and genetic consistency, which make them perfect for developing accurate, repeatable models to comprehend the underlying mechanisms and possible treatments.

Materials and methods

Collection and preparation of plant materials

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, located in western 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. The samples were cleaned by washing with running tap water, chopped into small 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 Zoology Department, Maseno University.

Preparation of mixtures using solvents

Preparation of the mixture was done following the protocol described by Heinrich et al. (2022), which included air-dried pieces of *Z. officinale* and *M. whitei* blended using a blender (heavy-duty professional blender JTC Omni Blender). Extraction of phytochemicals was done using 70% ethanol as a solvent because ethanol effectively extracts a wide range of phytochemicals, is safe, easy to remove, and compatible with analytical procedures, making it a versatile and practical solvent of choice over others like water, methanol, or non-polar solvents for phytochemical screening. Two hundred grams (200 g) of chopped pieces of *Z. Officinale* and *M. whitei* were blended in 1000 ml of ethanol for approximately 5 min, followed by continuous shaking using an orbital shaker (kj-201bd) for 24 h. The mixtures were then removed and sieved using a Muslin piece of cloth to obtain solutions of *Z. Officinale* and *M. Whitei*.

Filtration was done using circular 125 mm Whatman filter papers. The filtrate obtained was concentrated using a hot air oven at 46.6 °C to obtain crude extract. To obtain the exact weight of the crude extract, an empty tray (plate) was initially weighed and the weight recorded, then the weight of the tray (plate) with crude extract taken; the difference in the two weights was taken as the weight of the crude extract. Distilled water was used as a vehicle. The crude extracts obtained from the different solvents were stored in different labelled, tight air containers for phytochemical analysis and treatment of the diabetic infertile white albino male rats.

Methodology for qualitative and quantitative phytochemical and antioxidant analysis

Qualitative phytochemical screening

Phytochemical tests to detect classes of compounds:

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

Small amount of extract was mixed with specific reagents. Color changes or precipitate formation was observed as the indicative of phytochemicals.

Quantitative phytochemical determination

Total phenolic content (TPC): Total phenolic content was determined calorimetrically by the Folin-Ciocalteu reagent and gallic acid as a standard (Ahmed et al., 2014). The extract (40 µl) was mixed with 3.16 distilled water and 200 µl of the Folin-Ciocalteu reagent. After 8 min, 600 µl sodium carbonate was added, and the mixture incubated at 40 °C for 30 min at room temperature. The absorbances

were measured at 765 nm using a UV–Vis spectrophotometer at different concentrations (0, 20, 40, 60, 80 and 100 µg/ml) of the standard Gallic acid to obtain standard calibration curve. The concentrations of total phenolic contents in *Z. officinale* rhizome and *M. whitei* root extracts were expressed as µg/ml gallic acid equivalents.

Total flavonoid content (TFC): Total flavonoid contents were measured using the colometric method (Aryal et al., 2019). Approximately 1 ml of extract was mixed with 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water. After 30 min of incubation at room temperature, absorbances were read at 415 nm at different concentrations (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) of the standard quercetin reagent to obtain standard calibration curve. Total flavonoid content of *Z. officinale* and *M. whitei* were computed and recorded in mg/ml quercetin equivalents.

Total tannin contents Total tannin content was determined using the Folin-Ciocalteu method, with minor modifications (Haile & Kang, 2019). Approximately 0.1 ml of the extract from each plant was mixed with 7.5 ml distilled water, 0.5 ml Folin-Ciocalteu reagent, 1 ml of 35% sodium carbonate, and diluted to 10 ml. After 30 min of incubation at room temperature, absorbances were measured at different concentrations (0, 20, 40, 60, 80 and 100 µg/ml) of tannic acid standard using a UV spectrophotometer at 700 nm to obtain a standard calibration curve. Tannin concentrations in *Z. officinale* rhizomes and *M. whitei* root extracts were expressed in µg/ml tannic acid equivalents.

Total Saponin content The quantitative determination of saponin content was carried out using the method reported by Ejikeme et al. (2014) with slight modifications. Exactly 100 ml of 20% aqueous ethanol was added to 5 g of either *Z. officinale* or *M. whitei* in a 250 ml conical flask. The mixture was heated over a hot bath for 4 h with continuous stirring at 55 °C. The residue of the mixture was re-extracted with additional 100 ml of 20% aqueous ethanol after filtration and heated for 4 h at a constant temperature of 55 °C. The combined extracts were evaporated to 40 ml over water bath at 90 °C. Twenty millimeters (20 ml) of diethyl ether was added to the concentrate in a 250 ml separator funnel and vigorously agitated from which the aqueous layer was recovered. This purification process was repeated twice and thereafter, 60 ml of n-butanol 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 min, 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:

$$\% \text{Saponin} = \frac{\text{weight of saponin}}{\text{Weight of sample}} \times 100$$

Antioxidant activity assays

DPPH radical scavenging assay The free radical scavenging activity of ethanolic extracts of *Z. officinale* rhizomes and *M. whitei* roots was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique as described by Baliyan et al. (2022) with slight modifications. About 4 mg/ml of DPPH in dimethyl sulfoxide was used as the stock solution. The DPPH stock mixture yielded an absorbance of 0.886 at 517 nm. In test tubes, 2 ml DPPH workable solution (0.24 mg/ml in methanol) was added to 2 ml (20–100 µg/ml in methanol) of ethanolic extracts of either *Z. officinale* or *M. whitei* roots and ascorbic acid in separate tubes. The tubes were then incubated for 30 min (in darkness). the absorbance was determined at 517 nm using UV–Vis spectrophotometer (UV mini-1240, Shimadzu, Japan).

ABTS radical cation decolorization assay ABTS•+ scavenging assay protocol

This assay measures a substance's ability to scavenge the ABTS radical cation, indicated by a reduction in absorbance at 734 nm.

Procedure:

1. Radical Generation: The ABTS•+ was generated by reacting 7 mM ABTS with 2.45 mM K₂S₂O₈ for 12–16 h in the dark. The resulting solution was diluted with PBS to an absorbance of 0.70 (± 0.02) at 734 nm for immediate use.
2. Sample Preparation: A Trolox standard (100 µM) and serial dilutions of *Z. officinale* and *M. whitei* extracts (400 µg/mL) were prepared.
3. Assay Execution: In a microplate, 20 µL of sample/standard was combined with 180 µL of the ABTS•+ working solution. Following a 6-min incubation at room temperature, the absorbance at 734 nm was measured.
4. Controls & Replication: A reagent control (solvent + ABTS•+) and sample blanks (sample + solvent) were run to account for background signal. The entire assay was conducted in triplicate.

$$A = A(\text{sample} + \text{ABTS}) - A(\text{sample blank});$$
$$\% \text{inhibition} = (A \text{ control} - A \text{ corrected})$$
$$/ A \text{ control} \times 100.$$

Converted to Trolox equivalents (TEAC) via Trolox calibration or report IC_{50} . Same solvent for standards and extracts; subtract colored-sample blanks; determine linear range first; report mean $\pm$ SD and units (e.g., μmol Trolox/g extract).

Ferric Reducing Antioxidant Power (FRAP) assay Ferric Reducing Antioxidant Power was assessed as described by Wang et al. (2021), where 1 ml of each extract was added to clean test tubes, mixed with 2 ml of 0.1 M 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 °C for 30 min. The samples were removed and allowed to cool, followed by addition of 2 ml of 10% Tetrachloroacetic acid (TCA), centrifuged at $1000 \times g$ 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 read at 700 nm.

Animal housing and handling

Standard stainless-steel cages were used where all white male albino rats had a minimum cage height of 7 inches (17.8 cm), according to the guidelines (Guide for the Care and Use of Laboratory Animals. Disinfection to the animal house was done prior to introduction of the animals using Lysol disinfectant. The saw dust was used for bedding and was changed daily to avoid build-up of ammonia in the animal house. The animals were allowed free access to water and standard rat pellets throughout the study period. The animal house was well ventilated with light: dark cycle of 12 h: 12 h with room temperature of between 20 and 26 °C (68°F to 78.8°F) 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. The research was conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Ethical clearance for animal use was obtained from the Biosafety, Animal Use and Ethics Committee of the Faculty of Veterinary Medicine (Reference number FVM BAUEC/2023/528).

Experimental study design

A total of 40 (36 male rats and 4 female) white albino rats, aged 7–8 weeks with a body weight of between 150 and 250 g were used in the study. They were divided into 9

groups with 4 males per group; female rats were used for testing fertility, which was confirmed by vaginal smears where presence of cornified epithelial cells indicated estrus, the most fertile phase.

Induction of diabetes mellitus Type I

Hyperglycemia (diabetes) was induced by the use of a single intraperitoneal injection (2 ml) of alloxan monohydrate, which destroys pancreatic beta cells responsible for insulin secretion, thereby causing diabetes. Animals were monitored for several days for changes in blood glucose levels at day 0, 7, 14, 21 and 28. Diabetes was confirmed after strips were inserted in a glucometer (On Call Plus blood glucose Meter) and a drop of blood collected from the puncture on the male albino white rats' tail end put on fixed strips and fasting glucose levels above 126 mg/dl (7 mmol/l) to be considered indicative of diabetes (hyperglycemia). Clomiphene citrate (doses range from 5 to 10 mg/kg body weight administered orally by gavage), was reconstituted in distilled water to a concentration of 5 mg/kg and used as standard drug for positive control. The dosage was arrived at based on literature review and body weight, hence the concentration (e.g., 5 mg/kg) was prepared to facilitate practical, consistent administration; the actual dose per animal was calculated based on body weight and administered volume.

Other than blood glucose levels, symptoms of diabetes that were observed included increased water intake at least two bottles of drinkers (500 ml) per day, increased urination which was observed through wetting of the saw dust, weight loss, and decreased motor activity as show by drowsiness the whole day. Blood glucose levels and body weights were checked on day 0, 7, 14, 21 and 28 following treatments with extracts.

Administration of the ethanolic extracts

The white male albino rats were treated with either 200 mg/kg, 400 mg/kg or 800 mg/kg of *Z. Officinale* for groups 4, 5 and 6 and *M. Whitei* for groups 7, 8 and 9 respectively. The doses were administered orally using gavage needle once daily between 8 and 9 am. After 28 days of treatment, the animals were anesthetized using chloroform and later sacrificed. Blood and sperm samples were collected for analysis (Table 1).

Blood and sperm sampling and processing

The animals were anesthetized using 1% inhaled chloroform (0.05 ml/liter of container volume) and sacrificed by cervical dislocation. The abdomen was opened to expose the heart, then using a 23-gauge needle, 5 ml of blood samples via heart puncture was collected and placed in respective EDTA tubes. Blood samples were gently swirled to mix and centrifuged to separate the plasma and thereafter frozen at -20 °C. Sperm were collected

Table 1 Treatment of white albino male rats with 200, 400, and 800 mg/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 both extracts
2	Induced diabetes and infertile using 1.5 mls of Alloxan alone (negative control group) with no treatment with extracts
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 ZO ethanol extract
5	Diabetic and infertile treated with Alloxan + 400 mg/kg of ZO ethanol extract
6	Diabetic and infertile treated with Alloxan + 800 mg/kg of ZO ethanol extract
7	Diabetic and infertile treated with Alloxan + 200 mg/kg of MW ethanol extract
8	Diabetic and infertile treated with Alloxan + 400 mg/kg of MW ethanol extract
9	Diabetic and infertile treated with Alloxan + 800 mg/kg of MW ethanol extract

according to the procedure described by Al-Alami et al. (2017). Briefly, the left and right cauda epididymis were placed in Petri dishes containing 10 mL of PBS (pH 7.4) maintained at 37 °C and minced using a scalpel blade. The spermatozoa were allowed to diffuse into the buffer for 2 min and swirled to obtain a homogeneous mixture for sperm characteristic analysis.

Hormonal analysis

Plasma levels of follicle-stimulating hormones (FSH), luteinizing hormone (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.

LH bioassay

Before use, all samples and reagents were allowed to equilibrate to room temperature, and standard solutions made in accordance with the protocol on the kit. Briefly, 100 µL of plasma samples and standards were added to the relevant antibody-coated wells in duplicate. The assay also included internal quality controls and blanks for development of the standard curve and extraction of sample readings from the absorbance. After applying a

plate sealer, the plate was incubated for 2 h at room temperature (20–25 °C). To get rid of unbound materials, the wells were cleaned 4 times using the wash buffer. And thereafter each well added 100 µL of an enzyme-conjugated detection antibody. Incubation was done at room temperature for 1 h and thereafter, washed 4 more times with wash buffer.

All the wells were then filled with 100 µL of the substrate solution, incubated at room temperature in the dark for 15 min. To stop the reaction, 50 µL of stop solution was added to each well and mixed gently to ensure uniform color developed. Using a microplate reader, the absorbance was read at 450 nm. The standard curve (absorbance vs. known LH values) was plotted in order to analyze the data. Based on their absorbance, the LH content in plasma samples was computed and expressed in mIU/mL.

FSH assay

The assay procedure used was that provided in the manufacturer's kit. Briefly, 100 µL of standards and samples were pipetted into the relevant anti-body-coated wells in duplicate. The plate was gently shaken and incubated for 1 h at room temperature. To get rid of unattached proteins, the wells were washed 4 times using the wash buffer. Thereafter, each well was added 100 µL of an enzyme-conjugated detection antibody, incubated for 1 h at room temperature 100 µL of substrate solution added to all wells followed by incubation for 15 min at room temperature in the dark. To stop the enzymatic process, 50 µL of stop solution was added to each well Followed by gentle mixing. Absorbance was measured with a microplate reader at 450 nm and the standard curve generated for absorbance of standards against their known concentrations. The FSH concentration in the samples was determined from the standard curve and expressed in mIU/ML.

Testosterone assay

All samples and reagents are equilibrated to room temperature, which is between 20 and 25 °C and aliquoted into the wells following the kit's instructions. Briefly, 100 µL of standards and samples were aliquoted to the appropriate antibody-coated wells in duplicate, sealed and incubated for 1 h at room temperature. In order get rid of unbound testosterone, the wells were washed 4 times using the wash buffer and thereafter, 100 µL of enzyme-conjugated detection antibody added to each well, incubated for 1 h at room temperature. This was followed by washing 4 times to get rid of the unbound detection antibody, addition of 100 µL of substrate solution to each well, and incubation at room temperature in the dark for 15 min.

In order to halt the reaction and guarantee consistent color development, 50 μL of stop solution was added to each well and then gently mixed. A microplate reader was used to measure the absorbance at 450 nm. A standard curve was generated for absorbance against testosterone levels and this was used to determine the sample concentrations and expressed in ng/mL.

Sperm characteristics

Sperm count and motility analysis

This was done following the procedure of Koprulu et al., 2022. The harvested epididymis was dissected at the caudal end by cutting small pieces and placing in a 3 ml of 37 °C neutral buffered formalin solution at a ratio of 1:1 for exactly 15 min, followed by mixing in 1% Trisodium citrate. Thereafter, 1 ml of the solution was placed on a hemocytometer, covered with a cover slip and viewed under a microscope at 400 $\times$ magnification in order to record sperm count as well as determine the sperm motility. The sperm count was determined by simply counting all the sperm in a field for three different fields, three times per sample for each individual and calculating the mean.

The total motility was determined by:

$$\begin{aligned} &\text{Percentage motility} \\ &= \frac{\text{number of motile sperm}}{\text{total number of all the sperm}} \times 100\% \end{aligned}$$

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 determined using Nigrosine-Eosin staining. Nigrosine-Eosin staining differentiates live from dead sperm based on membrane integrity. Live sperm had intact membranes, preventing dye entry, and thus remained unstained or appeared white. Dead sperm showed compromised membranes as a result of allowing eosin to penetrate, staining the sperm head pink or red.

The mixtures of Nigrosine (black dye) with Eosin (pink dye) typically in a 1:1 ratio was prepared. This was followed by mixture of small volume of sperm suspension with the Nigrosine-Eosin stain (10 μL of sperm with 10 μL of stain), and incubated briefly for about 1–3 min at room temperature. A drop of the stained mixture was put on a clean glass slide then covered with a coverslip gently and then observed under a light microscope

at 400 $\times$ magnification. At least 200 spermatozoa were counted for statistical accuracy. Viable sperm counting and calculation was based on unstained or white (membrane intact) or non-viable sperm: stained pink or red (membrane compromised).

Determine Viability Percentage :

$$\begin{aligned} &\text{Viability (\%)} \\ &= \left(\frac{\text{Number of unstained (viable)}}{\text{sperm/Total sperm counted}} \right) \times 100 \end{aligned}$$

Sperm were considered viable if they do not take up eosin stain and remain white. 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.

Statistical analysis

The data was analyzed using GraphPad Prism version 5.0. Hormonal data and quantitative data on sperm parameters are presented as mean $\pm$ SEM. Means were compared using one-way ANOVA and statistical differences among groups determined by Tukey's multiple pairwise comparison test as the post-hoc test at $p < 0.05$.

Results

Phytochemical analysis of Zingiber officinale rhizome and Mondia whitei 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 mgGAE/g) and lower levels of flavonoids, tannins and saponins in *Z. officinale* (125 mgQE/g, 7.5% (w/w) and 15 mgTAE/g respectively) compared to *M. whitei* (140 mg QE/g, 15% (w/w) and 22 mg TAE/g respectively) (Table 2).

Antioxidant analysis of Zingiber officinale rhizome and Mondia whitei root ethanolic extract

The antioxidant contents of *Z. officinale* rhizome and *M. whitei* root ethanolic extract showed a lower IC_{50} and higher levels of Fe^{2+} equivalents per gram of extract and Trolox equivalents per gram in *Z. officinale* (76 $\mu\text{g}/\text{mL}$, 400 μmol and approximately 2500 μmol respectively) compared to *M. whitei* (120 $\mu\text{g}/\text{mL}$, 400 μmol and approximately 2000 μmol) (Table 3).

Table 2 Phytochemical characteristics of ethanolic extract of *Zingiber officinale* rhizome and *Mondia whitei* root ethanolic extracts

Group	Phytochemical	Result			
		Qualitative		Quantitative	
		<i>Z. officinale</i>	<i>Whitei</i>	<i>Z. officinale</i>	<i>M. whitei</i>
1	Alkaloids	+	+	15 mg/g	8 mg/g
2	Flavonoids	+++	+++	125mgQE/g	140 mg QE/g
3	Tannins	+	+	15mgTAE/g	22 mg TAE/g
4	Saponins	+	+	7.5%(w/w)	15% (w/w)
5	Phenolic Compounds	+	+	220 mg GAE/g	220 mg GAE/g
6	Glycosides	+	+		

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

Table 3 Antioxidant analysis results of *Z. officinale* and *M. whitei* ethanolic extracts

S/N	Assay	Concentrations	
		<i>Z. officinale</i>	<i>M. whitei</i>
1	DPPH	IC ₅₀ values 76 µg/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 equivalents per gram	Approximately 2000 µmol Trolox equivalents per gram

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

Weight and fasting blood glucose changes in white albino male rats according to the different treatments received

By day 0, the mean blood glucose levels in all the treatment groups were between the normal range, (3.44 ± 0.13 to 3.824 ± 0.12 mmol/l) (Table 4). However, the FBG levels rose to significant levels in groups 2,3,4,5,6,7,8 and 9 compared to the normal control. All treatment groups had an increase in body weight during the study period. However, negative control animals had the least change

in body weight (21.2 ± 2.85 g) and group 5 had the highest (52 ± 1.30 g).

Effect of ethanolic rhizome extracts of *Z. officinale* and *M. whitei* on the serum levels of reproductive hormones in DIMI white albino male rats.

Plasma levels of follicle stimulating hormone

Induction of diabetes in the negative control significantly decreased ($p < 0.05$) the plasma levels of FSH (2.20 ± 0.29 mIU/ml), compared to the control group (6.30 ± 1.78 mIU/ml). There was a dose-dependent increase in plasma FSH levels among treatment groups of rhizome extracts of *Z. officinale* and *M. whitei*. For instance, treatment of DIMI rats with 800 mg/kg (6.65 ± 0.84 mIU/ml) of the *Z. officinale* increased the levels of FSH from 2.20 ± 0.29 mIU/ml ($p < 0.01$) to significantly higher levels than those of the normal control rats (6.30 ± 1.78 mIU/ml). 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 negative control at $p < 0.05$, with the FSH levels increasing close to those of the normal control (6.30 ± 1.78 mIU/ml). These levels of FSH were lower but close to those in positive control (7.72 ± 1.61 mIU/ml) (Fig. 1).

Plasma levels of luteinizing hormone

Induction of diabetes in the negative control also significantly decreased the levels of LH (1.28 ± 0.37 mIU/ml), compared to the normal control (3.98 ± 1.07 mIU/ml) at $p < 0.01$. However, in treatment groups of both *Z. officinale* and *M. whitei* there was increased the levels of plasma LH in a dose-dependent manner (Fig. 2). Treatment of the diabetic mice with 400 and 800 mg/kg of *M. whitei* significantly increased the LH levels to 3.53 ± 0.16 ($p < 0.05$) and 4.55 ± 0.14 mIU/ml ($p < 0.01$), respectively, compared to the normal and negative controls. Additionally, administration of 200 mg/kg of *M. whitei* only slightly increased the levels of LH, but less so compared to those of normal control. However, at 800 mg/kg dose there were increased plasma levels of LH to 3.50 ± 0.18 mIU/ml, that were not significantly different from those

Table 4 Weight and Fasting Blood Glucose (FBG) levels at day 0 and day of sacrifice of the DIMI white albino rats

Group	FBG Levels (Mmol/l)		Body weight (grams)		
	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

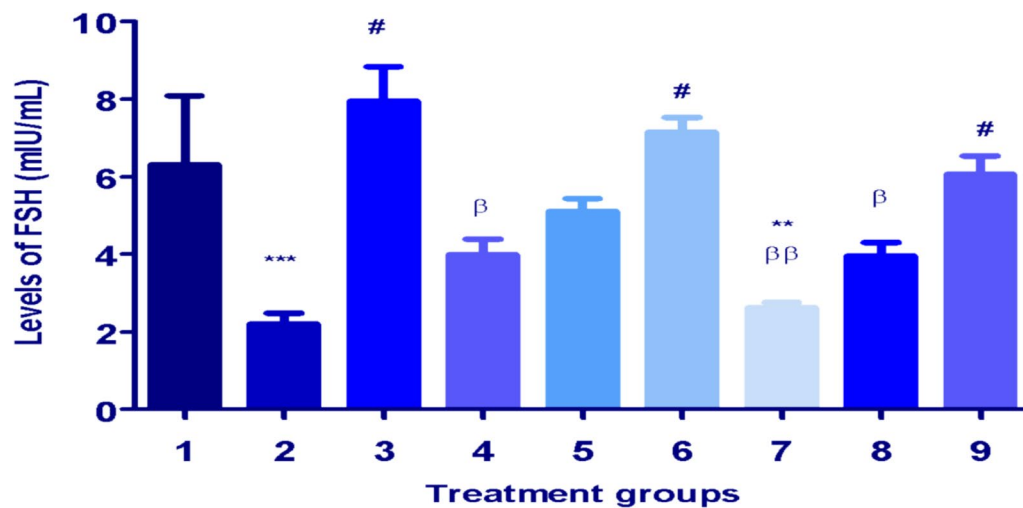


Fig. 1 Effect of *Z. officinale* and *M. whitei* ethanolic root extracts on Follicle Stimulating Hormone levels in white albino male rats over 28 days experimental period. Note that*, # and β indicate statistical difference compared to group 1 (normal control) and group 2 (negative control) and group 3 (positive control) respectively, * or # mean $p < 0.05$, ** mean $p < 0.01$ and *** mean $p < 0.001$

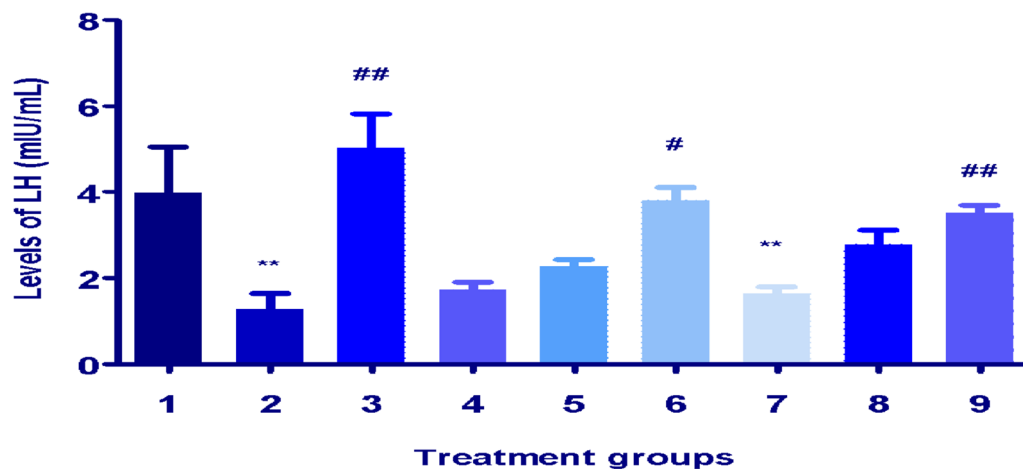


Fig. 2 Effect of escalating doses of *Z. officinale* and *M. whitei* ethanolic extract on Luteinizing Hormone levels in white albino male rats over the 28-day experimental period. Note that* and # indicate statistical difference compared to group 1 (normal control) and group 2 (negative control) respectively, * or # mean $p < 0.05$, ** or ## mean $p < 0.01$

of the normal control and the positive control (5.03 ± 0.37 mIU/ml) but significantly higher than those of negative control ($p < 0.05$).

Plasma levels of testosterone

Figure 3 shows the effect of administration of rhizome and root extracts of *Z. officinale* and *M. whitei* on plasma testosterone levels in DIMI white albino male rats. Induction of diabetes significantly suppressed ($p < 0.001$) the plasma levels of testosterone (71.53 ± 6.84 ng/dl) when compared to those of the normal (509.9 ± 64.97 ng/dl) and positive control (584.9 ± 6.843 ng/dl) rats. The plasma levels of testosterone at 200 mg/kg and 400 mg/kg of *Z. Officinale* dose levels and 200 mg/kg, 400 mg/kg and 800 mg/kg of *M. whitei* extracts-treated groups remained significantly lower compared to those of the

normal control. However, at 400 mg/kg and 800 mg/kg of *Z. officinale* and 800 mg/kg of *M. whitei* there was significant increase ($p < 0.001$) in plasma testosterone levels compared to the negative control (350.1 ± 20.71 ng/dl, 509.8 ± 29.38 ng/dl and 321.6 ± 27.35 ng/dl respectively). However, with 800 mg/kg *Z. officinale* extract treatment having plasma testosterone levels (582.2 ± 0.89 ng/dl) that were not significantly different from those of the positive control.

Effect of ethanolic rhizome extracts of *Z. officinale* and *M. whitei* on the different sperm parameters in DIMI white albino rats

Sperm characteristics, including sperm count, morphology and motility were significantly decreased by induction of diabetes (102.00 ± 14.20 , 25.00 ± 2.86 and

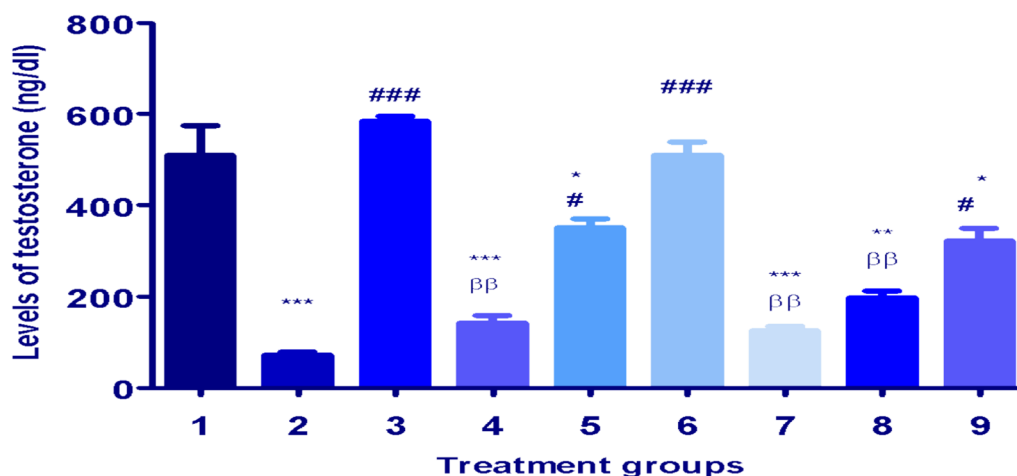


Fig. 3 Effect of *Z. officinale* and *M. whitei* ethanolic root extracts on testosterone hormone levels in white albino male rats over 28-day experimental period. Note that * and # indicate statistical difference compared to group 1 (normal control) and group 2 (negative control) respectively, * or # mean $p < 0.05$, ** or ## mean $p < 0.01$ and *** or #### or ββ mean $p < 0.001$

Table 5 Sperm analysis of rats following exposure to escalating doses of *Z. officinale* and *M. whitei* ethanolic extracts over 28 days experimental period

Group	Sperm count/ ml $\times 10^6$ /ml	Sperm morphol- ogy (%) Structural Normality	Sperm mo- tility (%)
1	203.3 $\pm$ 17.05	55.00 $\pm$ 4.98	51.02 $\pm$ 5.41
2	102.00 $\pm$ 14.20 [#]	25.00 $\pm$ 2.86 [#]	12.54 $\pm$ 2.74 [#]
3	240.01 $\pm$ 12.66 [#]	53.25 $\pm$ 4.09 [#]	52.31 $\pm$ 3.52 [#]
4	149.8 $\pm$ 14.06	27.50 $\pm$ 2.21 [#]	35.00 $\pm$ 2.30
5	173.3 $\pm$ 5.66	37.00 $\pm$ 1.87	42.29 $\pm$ 6.21 [#]
6	210.5 $\pm$ 7.92 [#]	45.00 $\pm$ 3.87	49.55 $\pm$ 3.27 ^{##}
7	228.8 $\pm$ 8.49	58.75 $\pm$ 2.93 [#]	50.58 $\pm$ 6.22
8	232.6 $\pm$ 7.23 [#]	61.57 $\pm$ 4.65 [#]	51.31 $\pm$ 2.66 [#]
9	239.4 $\pm$ 5.67 [#]	64.32 $\pm$ 8.34 [#]	54.43 $\pm$ 4.38 ^{##}

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

12.54 $\pm$ 2.74 respectively) as shown in the negative control when compared to those of the normal control (203.3 $\pm$ 17.05, 55.00 $\pm$ 4.98 and 51.02 $\pm$ 5.41) (Table 5). 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 200 mg/kg of *Z. officinale* slightly improved all the parameters (149.8 $\pm$ 14.06, 27.50 $\pm$ 2.21 and 35.00 $\pm$ 2.300 respectively). This improvement was more enhanced at 400 mg/kg (173.3 $\pm$ 5.66, 37.00 $\pm$ 1.87 and 42.29 $\pm$ 6.21) and 800 mg/kg (210.5 $\pm$ 7.92, 45.00 $\pm$ 3.87 and 49.55 $\pm$ 3.27), with sperm motility for both extract treatments showing significant increase when compared to the negative control. Similarly, administration of 200 mg/kg of *M. whitei* also improved all sperm parameters measured, which were even significantly higher at 800 mg/kg (239.4 $\pm$ 5.67, 64.32 $\pm$ 8.34 and 54.43 $\pm$ 4.38) that were either close to or slightly

higher than those of the positive control (240.01 $\pm$ 12.66, 53.25 $\pm$ 4.09 and 52.31 $\pm$ 3.52 respectively).

Discussion and conclusion

The quantitative and qualitative phytochemical analysis of the ethanolic extracts of *Z. officinale* rhizome and *M. whitei* root revealed the presence of various secondary metabolites. Specific analyses showed that the *Z. officinale* extracts contain significant amounts of flavonoids, trace amounts of alkaloids, tannins, saponins, phenolic compounds and glycosides. Similarly, *M. whitei* contains a complex mixture of bioactive phytochemicals—including saponins, alkaloids, flavonoids, tannins, phenolics, sterols, and terpenoids. Other phytochemical compounds that have been isolated from *Z. officinale* include terpenes, polysaccharides, lipids, raw fiber and organic acids as well as gingerols and shogaols (Mao et al., 2019). The findings of the present study on *M. whitei* are in agreement with those of chloroform extract of *M. whitei* showing moderate amounts of terpenoids, and trace amounts of flavonoids, cardiac glycosides, saponins and phytosterols but with the absence of alkaloids and saponins (Shehu et al., 2022). The variance on the results cannot be clearly explained but probably, may be ascribed to the method of extraction on the phytochemicals obtained.

Zingiber officinale has better antioxidant abilities compared to *M. whitei* due to a lower IC₅₀, implying that smaller quantities of the ethanolic extract of the rhizome of *Z. officinale* are required to achieve 50% of a particular effect compared to those of the ethanolic root extract of *M. whitei* (Berrouet et al., 2020). The main antioxidants isolated from *Z. officinale* included gingerol, shogaol, paradols and gingerone; they work through a variety of methods to produce their antioxidant benefits. Reactive oxygen species (ROS) and free radicals are neutralized by

these antioxidants thus preventing cellular damage (Mustafa & Chin, 2023). Their mode of action includes lipid peroxidation inhibition and thereby safeguard cell membranes. They increase the activity of endogenous antioxidant enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase (SOD). They affect signaling pathways like Nrf2, which controls the expression of antioxidant response elements and strengthens the body's defenses. They cause anti-inflammatory effects by lowering inflammation as a result of reducing oxidative stress indirectly. On the other hand, tannins, alkaloids, saponins, and phenolic chemicals (including flavonoids and phenolic acids) are the main antioxidants isolated in *M. whitei*. Their mode of action includes free radical scavenging by directly neutralizing free radicals and ROS thus lessen oxidative stress. The findings of our study on antioxidant effects of *M. whitei* have also been determined elsewhere (Gbadamosi & Erinoso, 2015). In *M. 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 catalyze the production of free radicals and lipid peroxidation. These findings are in agreement with those of Defo Deeh et al. (2024) on phytochemical analysis and antioxidant potential of *M. whitei* and *Guibourtia tesmannii* against H_2O_2 -induced cytotoxicity in PC3 cells.

The polyphenolic compounds, especially flavonoids and tannins have the ability to activate enzymes including glutathione peroxidase, catalase, and SOD (Defo Deeh et al., 2024). They also have the ability to modulate cell signaling pathways; These substances have the ability to affect Nrf2 and other pathways, which can raise the expression of genes that produce antioxidants. The phenolic compounds, tannins, and other phytochemicals found in *M. whitei* extracts mainly work as antioxidants by scavenging free radicals, chelating metals, and boosting the body's natural antioxidant defenses (Defo Deeh et al., 2024).

The normal functionality of the testes, especially the Sertoli and Leydig cells is dictated, to a large extent, by the levels of stimulating hormones from the pituitary gland (Li et al., 2024). Upon binding to their receptors on the surfaces of these cells, FSH and LH stimulate spermatogenesis and steroidogenesis respectively (Li et al., 2024). Results of the present study show that the levels of FSH, LH and testosterone were significantly suppressed in male white albino rats in the negative control group. However, administration of ethanolic rhizome extract of *Z. officinale* and root extract of *M. whitei* to all the test groups treated with alloxan monohydrate improved levels of FSH, LH and testosterone in a dose-dependent manner. The increase was relatively higher for groups treated with *Z. officinale* when compared with the values

measured in *M. whitei* -treated groups. Results of this study are in agreement with previous studies on Damask rose (*Rosa damascena*) showing that phytochemicals present in this plant including phenolics, flavonoids, tannins, saponins, and other bioactive compounds improved serum levels of FSH, LH and testosterone levels in a dose-dependent manner by stimulating the hypothalamo-pituitary axis in male rats (Jahromi et al., 2016). It is probable that that *Z. officinale* and *M. whitei* possess phytochemical compounds with endocrine enhancing effects on the hypothalamo-pituitary-gonadal axis that then led to the observed effects in the present study. In a related study, a mixture of extracts from three medicinal plants (*Eruca sativa*, *Apium graveolens* L., and *Nigella Sativa*) increased the levels of all three hormones and enhanced anti-oxidant activities in rats treated with lead acetate, indicating the role of hormones and antioxidants in ameliorating infertility associated with oxidative stress (Al Chalabi et al., 2020). However, *Cleistanthus collinus*, a medicinal plant was found to, not only lower the levels of LSH and LH but also deplete the protective mechanisms against oxidative stress in testicular cells because phytochemicals or toxic compounds in the extract might generate ROS, leading to oxidative stress that damages sperm cells and testicular tissue, impairing sperm count, motility, and morphology by inducing cytotoxic effects on Leydig and Sertoli cells within the testes (Umamaheswari et al., 2022).

The results of the present showed improvement of sperm characteristics of sperm count, sperm morphology and motility in *Z. officinale* and *M. whitei* ethanolic root extract treated animals in a dose-dependent manner. The animals in all groups, except normal control, had been treated with alloxan monohydrate to induce diabetes. Alloxan has been shown to induce testicular morphological and physiological changes that lead to dysfunction in the seminiferous tubules, including Sertoli cells and the process of spermatogenesis. The reversal effects could be attributed to the phytochemical constituents of the ethanolic extract of the rhizome and root extract of *Z. officinale* and *M. whitei*. For instance, flavonoids coupled with trace quantities of alkaloids, glycosides, tannins, saponins and phenolic compounds improve sperm count, morphology and motility (Roy et al., 2022). Evidently, *M. whitei* improved these parameters better than *Z. officinale* owing to the more phytochemical constituents as compared to *Z. officinale*. Flavonoids are known to protect against sperm damage induced by ROS, which are known to damage sperm cells (Unuofin et al., 2021).

However, high levels of some alkaloids lead to decreased sperm cell metabolism, which translates into reduced sperm count, motility and morphology (Derbak et al., 2023). This may explain the reduced sperm parameters observed in *Z. officinale*-treated groups where the

results showed more flavonoids and less alkaloids compared to those in *M. whitei*. However, the high levels of saponins in *M. whitei* compared to *Z. officinale* contradict the former's effect on semen characteristics. It has been reported that saponins disrupt the cell membrane of sperm cells and therefore not only negatively affect their count, motility and morphology but also kill or reduce their viability irreversibly (Shakeel et al., 2025).

Comprehensive statistical analysis (ANOVA) revealed significant differences between non diabetic groups and the diabetic control group, validating the effectiveness of the *Z. officinale* and *M. whitei* extracts treatments. Trends demonstrate that higher doses produce the most significant hormonal levels when compared to lower doses, reflecting a dose–response relationship. The findings from the present study will help to demonstrate the potential reproductive benefits of *Z. officinale* and *M. whitei* in combatting diabetes-induced infertility, with significant improvements in hormone levels and testicular functions.

These medicinal plants extract contain beneficial phytochemicals that scavenge free radicals, which has testicular protective effects that preserve the testicular architectural integrity of Leydig and Sertoli cells essential for hormonal synthesis and sperm generation and production. *Zingiber officinale* and *M. whitei* in diabetic infertile white albino male rats increased gonadotropin releasing hormone, FSH, LH and testosterone due to hormonal restoration balance along the hypothalamus–pituitary–testicular axis, which was altered due to diabetic effects. Plasma hormonal concentration enhanced testicular activities and functions which included both spermatogenesis and steroidogenesis especially in male diabetic fertility.

Conclusions

The research reveals that extracts from *Z. officinale* and *M. whitei* have notable protective effects against male infertility caused by diabetes. These herbal treatments improved sperm quality, and affected oxidative stress markers, indicating their potential as natural therapeutic options. The results reinforce the traditional applications of these plants in addressing reproductive complications linked to diabetes thus emphasizing their potential for further clinical research and development into supportive therapies for male infertility under diabetic conditions.

Abbreviations

<i>Z. officinale</i>	<i>Zingiber officinale</i>
<i>M. whitei</i>	<i>Mondia whitei</i>
GnRH	Gonadotropic Releasing hormone
LH	Luteinizing hormone
FSH	Follicle stimulating hormone
ELISA	Enzyme-linked immunosorbent assay kits
DIMI	Diabetes induced male infertility
WHO	World Health Organization

IDF International Diabetes Federation

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study received ethical approvals from University of Nairobi Scientific and Ethics Review Committee (REF: FVM BAUEC/2024/571) before commencement.

Consent for publication

Was consented by supervisors. All data and materials used for research are available and conserved at Maseno University zoology laboratory. No competent interest.

Competing interests

The authors declare no competing interests.

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