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Effects of *Zingiber Officinale* Rhizomes and *Mondia Whitei* Ethanolic Root Extracts on the Hypothalamus, Pituitary and Testicular Histology in Infertility-Induced Diabetic White Albino Male Rats

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Abstract

Treatment of diabetic mellitus (DM) and related complications is available although cost of conventional medicines is relatively high and accompanying side effects beg for alternative therapeutic and management methods. Previous studies demonstrated the antioxidant and anti-inflammatory qualities of *Zingiber officinale* and *Mondia whitei* although the scientific knowledge on their efficacy in ameliorating DM-induced infertility is obscure. This study investigated the histomorphometric and histoarchitectural impacts of ethanolic *Z. officinale* and *M. whitei* root extracts on the hypothalamus, anterior pituitary and testis (HPT) in diabetic male albino rats. Thirty-six rats were assigned nine groups of six treatment groups and three controls groups. All animals except normal control were administered with alloxan monohydrate to induce DM. Treatment groups were administered with *Z. officinale* or *M. whitei* at 200, 400, 800 mg/kg for 28 days, normal control (normal saline), diabetic control (alloxan monohydrate alone) and positive control (alloxan monohydrate and clomiphene citrate). Testicular weight/volume, seminiferous tubule diameter/epithelial height, Leydig cell volume for histomorphometry as well as hypothalamic, anterior pituitary and testicular histology were done. Neuronal degeneration and gliosis in the hypothalamus, decreased number of gonadotropes in the pituitary, disruption of seminiferous epithelium, and Leydig cell atrophy were observed in diabetic controls. Leydig cell volume (501±92.3 μm^3), testicular weight (1.02±0.3 ml), and testicular volume (1.11±0.22 ml) all showed significant decrease ($p<0.01$) in comparison with the normal control group testicular weight (2.71 ± 1.2), testicular volume (2.98 ± 0.71) and Leydig cell volume LCV (1250 ± 212.4). At 800 mg/kg, *Z. officinale* improved testicular weight: 2.51±0.5 ml; Leydig cell volume: 1291±236.3 μm^3, normalized hypothalamic and anterior pituitary histology. Similarly, *M. whitei* at 800 mg/kg improved testicular weight: 3.38±1.9 ml; Leydig cell volume: 1432±346.7 μm^3; $p<0.001$ vs. diabetic controls with testicular weight 1.03±3ml; leydig cell volume 501±92.3μm^3, improved pituitary vascularization, and hypothalamic histology. *Mondia whitei* at 800 mg/kg

performed better than *Z. officinale* specifically regarding Leydig cell metrics, testicular weight, and volume. ***Conclusion***: Both *Z. officinale* and *M. whitei* demonstrated pro-reproductive effects along the HPT axis. However, at 800 mg/kg, *M. whitei* exhibited greater restorative effects, on histoarchitecture and testicular histomorphometry.

Keywords: Zingiber officinale; Mondia whitei; Diabetic infertility; restorative; Testicular histomorphometry; Hypothalamic-pituitary-testicular axis

Introduction

In males, *Diabetes mellitus* (DM) can cause major structural damage along the HPT axis, especially if it is persistent and poorly managed. Previous studies reported decreased libido, erectile dysfunction, infertility and hypogonadism in diabetic males (Huang et al., 2024). Oxidative stress, elevated glucose levels, advanced glycation end products (AGEs), chronic inflammation, hypoinsulinemia and insulin resistance, and altered leptin and kisspeptin signalling (especially at the hypothalamic level), induced by DM, can harm hypothalamic neurons, including those that secrete gonadotropin-releasing hormone (GnRH) (Singh et al., 2025). There is a decrease in the quantity and functionality of neurons in the arcuate nucleus that secrete GnRH during prolonged hyperglycaemia, which leads to neuroinflammation and reactive activation of astrocytes and microglia (Gandhi et al., 2023). *Diabetes mellitus* also damages the gonadotropes of the anterior pituitary gland, limiting the synthesis and secretion of Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH), which are important in the normal functioning of the male reproductive system. The anterior pituitary gonadotropes may exhibit atrophy, apoptosis due to oxidative stress and impaired responsiveness to hypothalamic signals, which lowers LH/FSH output (Zhu et al., 2023). The associated microangiopathy induced by DM reduces blood supply to the pituitary gland, further damaging its structure and limiting the flow of pituitary hormones into the blood and subsequently, to target tissues (Key et al., 2025). At the testicular level, DM results in histopathological damage within the seminiferous tubules that results in the loss of germ cells, characterised by a decline in spermatogonia, spermatocytes, and spermatids. *Diabetes mellitus* - induced vacuolization, decreased tight junction integrity, and structural anomalies cause Sertoli cell dysfunction (Singh et al., 2025). The tubular atrophy results in impaired spermatogenesis and steroidogenesis as a result of Leydig cell atrophy and death. Testicular blood flow is compromised by microvascular disease, which is caused by decreased vascularization, increased collagen deposition and interstitial fibrosis (Fan et al., 2024).

The protective and restorative effects of extracts from *Z. officinale* and *M. whitei* on the structural damage caused by diabetes along the HPT axis in male subjects have been studied. Anti-inflammatory and antioxidant properties of *Z. officinale* have neuroprotective effects in the hypothalamus (Elsawy et al., 2023). Specifically, it protects hypothalamic neurons, particularly those in the arcuate nucleus by reducing reactive oxygen species (ROS) and enhancing insulin sensitivity, and boosting GnRH secretion. On the other hand, *M. whitei* exhibits neuroprotective and adaptogenic properties, restoring the hypothalamus to its normal structure, although the mechanisms for these effects are less understood (Mabonga, 2021). *Zingiber officinale* protects gonadotropes against inflammation and oxidative stress and in, doing so, normalizes the synthesis and secretion of FSH and LH, followed by enhanced downstream testicular functions (Unuofin et al., 2021). It protects Leydig cells, enhances testicular antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and Glutathione (GSH) while lowering lipid peroxidation (3,4-Methylenedioxyamphetamine (MDA levels); It also improves seminiferous tubule structure and spermatogenesis, and restores testosterone synthesis in the testes (Boroujeni et al., 2022). In contrast, *M. whitei* increases the anterior pituitary's responsiveness to hypothalamic GnRH through enhanced vascularization and improved cellular structure especially in non-diabetic males. In non-diabetic models, it also raises the levels of gonadotropins in the blood (Mabonga, 2021). *Mondia whitei* improves testicular histoarchitecture (normalizing Sertoli cells and germinal epithelium), stimulates spermatogenesis, increases steroidogenesis (Leydig cell activity), supports angiogenesis and improves vascular integrity in testicular tissue (Kyarimpa et al., 2023). Despite the foregoing literature, there is scarce scientific information on efficacy of this medicinal plants on the reproductive function. The present study aimed at determining the potential ameliorative effects of ethanolic extracts of *Z. officinale* rhizome and *M. whitei* root extracts on the histolology and histomorphometry of the hypothalamus, pituitary

and testicular tissue in diabetic white male albino rats. We hypothesized that the ethanolic extracts of *Z. officinale* rhizomes and *M. whitei* roots essentially restore histoarchitecture and greatly improves testicular morphometry due to oxidative damage of tissue cells along HPT axis arising from DM.

Materials and Methods

Plant materials, processing and preparation of extracts

Fresh samples of *Z. officinale* rhizomes and *M. whitei* roots were collected from Jubilee market in Kisumu City located at approximately 0°05'S latitude and 34°46'E longitude, located in western Kenya. The samples were collected in the month of June 2025. After purchase from the market, the samples were taken for identification at the Depatment of Botany, Maseno University. The samples were cleaned by washing with running tap water, chopped into small pieces of approximately 1 mm in length, weighed using an electronic compact scale weighing balance (Radwag model wlc.0,6/b1), air- dried and then packed in zip-locked bags for at least 7 days before processing.

Preparation of the mixture was done following protocol of Heinrich et al. (2022). Two hundred grams (200 g) of chopped pieces of *Z. officinale* and *M. whitei* were separately blended in 1000 ml of 70% 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 to obtain the crude extract. In order to measure 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 was 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 ethanolic solvent was stored in different labelled, airtight containers for phytochemical analysis and administration to the diabetic infertile white albino male rats.

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 Vetenary Medicine, University of Nairobi, transported to Maseno University animal house and housed in groups of approximately same size. Disinfection to the animal house was done prior to introduction of the animals. Wood shavings were used for beddings and were changed daily to avoid build-up of ammonia and microbes in the animal house. The animals were allowed free access to water and standard rat pellets bought from Kisumu animal feeds stores. The animal house was well ventilated with light: dark cycle of 12h: 12h with room temperature of between 20°C to 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. Ethical clearance for animal use in the study was obtained from the Biosafety, Animal Use and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi (Reference number FVM BAUEC/2024/571). The research was conducted in accordance with internationally accepted principles for laboratory animal use and care as stated in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

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 nine 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 breeding to test 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 inducing 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-treatment. A drop of blood was collected by slight venipuncture at the tip of the tail of each rat and put on strips and inserted in a glucometer (On Call Plus blood glucose Meter). Fasting glucose levels above 7mmol/l was indicative of diabetes (hyperglycemia). Clomiphene citrate 10 mg/kg body weight administered via intraperitoneal injection). was reconstituted in distilled water to a concentration of 5 mg/ml and used as standard drug for positive control. 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 escalating doses of 200 mg/kg, 400 mg/kg and 800 mg/kg of *Z. officinale* for groups 4, 5 and 6 and, using the same dose levels *M. whitei* for groups 7, 8 and 9 respectively. The normal control was given normal saline using the same regimen as for treatment groups. All administrations were done in volumes of 2 ml of extracts orally using gastric gavage needle once daily between 8 am and 9 am for 28 days. At the end of the treatment period, the animals were anesthetized using chloroform and later sacrificed by cervical dislocation and a ventral laparotomy done to harvest testes, hypothalamus and pituitary gland tissues and immediately immersed in 10% formaldehyde for fixation.

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 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.

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

The embedded tissues were sectioned in a transverse plane at a thickness of 5 µm using a rotary microtome, mounted on clean microscope slides, dried on a heating plate (40°C) and rehydrated using descending concentrations of ethanol and deparafifinized using xylene. The slides were, thereafter, stained using hematoxylin and eosin and viewed and analysed under light microscope (primo star model) fitted with a digital camera and a computer.

Results

Effects of ethanolic rhizome and root extracts of Z. officinale and M. whitei on the histology of the hypothalamus, anterior pituitary and testis in diabetic infertile white albino male rats

Histology of the hypothalamus of the normal control rat showed healthy neurons with structural integrity including intact basement membrane of arcuate nucleus capillaries with numerous glial cells. Neurons and glial cells were seen distributed normally throughout the parenchyma with normal-looking nuclei and cellular outlines that are well-defined (Figure 1 A). However, there were notable pathological alterations in the hypothalamic tissue of the negative controls evidenced by condensed, darkly pigmented nuclei, which are indicative of neuronal degeneration. Vascular congestion and hyalinization patches that point to significant tissue damage were also observed among negative control animals (Figure 1 B). The positive control was necessary to test the reversal effects of the stan-

dard known drug and compare the tissue changes with those of escalating doses of *Z. officinale* rhizomes and *M. whitei* root ethanolic extracts. There was protective or restorative effect, indicated by the increasing population of healthy, well-defined neurons with normal cellular and nuclear morphology (Figure 1 C). Treatment with increasing doses of *Z. officinale* ethanolic rhizome extracts (Figure 1 D, E, F) and ethanolic root extracts of *M. whitei* (Figure 1 G, H, I) showed a dose-dependent trend of hypothalamic architectural restoration evidenced by a decrease in pyknotic nuclei and hyalinization and a corresponding rise in neurons with normal morphological outline. Both *Z. officinale* and *M. whitei* extracts showed a dose-dependent ameliorative effect on alloxan-induced hypothalamus damage with the significant effect at high doses of both extracts.

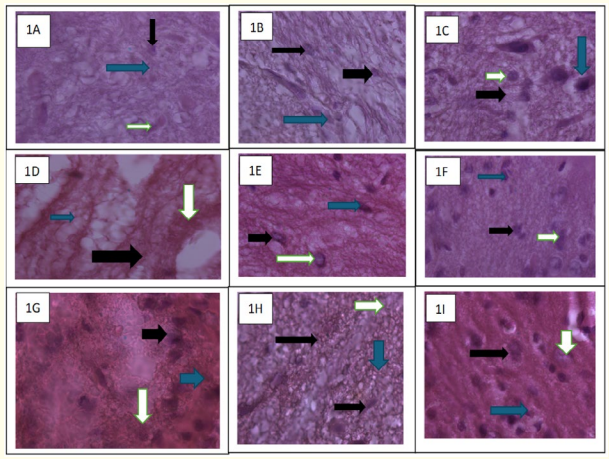

Figure 1

The histology of the hypothalamus of experimental rats showing normal control (A), negative control (B), positive control (C) and treatment groups exposed to escalating doses of Z. offinale (D - F) and *M. whitei* (G - I). The normal control shows neuronal cellular outline with normal distribution of glial cells and neurons scattered throughout the hypothalamic tissue; negative control treated with alloxan monohydrate alone showing condensed, dark nuclei (black arrows), vascular congestion and hyalinization (light blue arrows); positive control showing increased number of healthy, well-defined neurons with normal morphology (black arrows) containing nuclei with normal morphological outline: Escalating doses of Z.offinale (D -F) and (G-I) appears to regaining normal structural alignment within hypothalamus similar to features of the normal control:1000x.

The histology of the anterior pituitary gland of the normal control rat showed cellular outline of an active gland. The histological picture was characterized by clusters and islands of cells separated by thin sinusoidal spaces and slightly larger vascular profiles. The anterior pituitary parenchyma consisted of predominantly acidophils with rare numbers of basophils and chromophobes. Acidophils appeared uniform in size, stained deeply eosinophilic, and laden with cytoplasmic granules. Some of the acidophils exhibited degranulation. Basophils stained slightly basophilic and chromophobes showed pale cytoplasm. The sinusoids were lined by single layer of endothelium and appeared slightly widened in some areas. Fairly dilated sinusoids were distinguished as an effect of fixation and processing but not of any morphological significance (Figure 2 A). In contrast, the negative control showed the gland with pronounced hypocellularity and a notable decrease in the overall distribution of several glandular cells (Figure 2B). The positive control showed improved vascularization within the tissue and a greater predominance of gonadotroph cells, indicative of structural amelioration owing to diabetic tissue effects (Figure 2C).

At low dose (200 mg/kg) of *Z. officinale* ethanolic rhizome and *M. whitei* root extracts (Figure 2 D and G), there was a decreased anterior pituitary gland cell population distribution similar to those observed in negative control. At medium dose (400 mg/kg) of both extracts, (Figure 2 E and H), there was apparent structural restoration to a more active glandular as evidenced by increasing cellularity, which is defined by a greater distribution of acidophilic, basophilic, and chromophobic cells. At high dose (800 mg/kg) of both extracts, (Figure 2 F and I), the pituitary histoarchitecture possessed features that compare favorably to those of the normal control with accompanying large number of acidophilic, basophilic, and chromophobic cells. Additionally, there was evidence of increased vascularization, confirming the increasing glandular activity. Both *Z. officinale* and *M. whitei* extracts showed a dose-dependent restorative effect on the DIMI changes in the anterior pituitary gland with the greatest recovery seen at the highest dose, which was marked by improved vascularity and normalized cellular distribution.

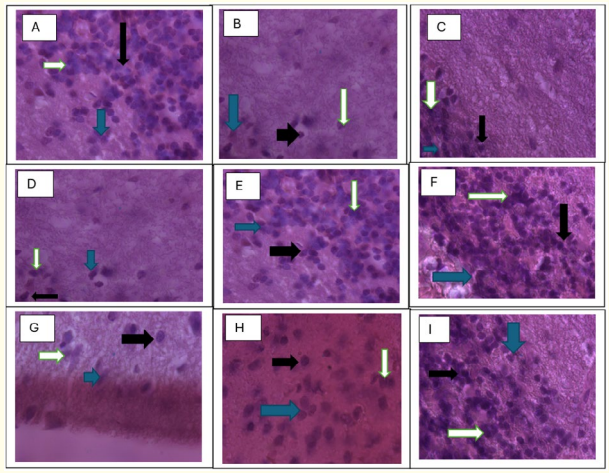

Figure 2

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 showing an active gland with numerous acidophilic cells (black arrows), light staining chromophobic cells (colorless arrows) and darker staining basophilic cells (light blue arrow). Note the negative control showing less distribution of glandular cells while positive control showing increased gonadotroph cells (black arrows) with enhanced vascularization. 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 and the same cell populations indicating an active gland. At 800 mg/kg of *Z. officinale* (F) and *M. whitei* (I), the cellular organization appears more improved with numerous acidophilic cells, chromophobes cells, basophilic cells, and enhanced vascularization: Hematoxylin and Eosin stain at 1000x.

The testicular tissue of controls comprised of seminiferous tubules and sparsely distributed interstitial tissue. The seminiferous tubules showed the basement membrane on which Sertoli cells and cells of the basal compartment of the seminiferous epithelium rested. Various forms of spermatogenic cells occurred in the lateral recesses formed by Sertoli cell branches. Type A spermatogonia, characterized by oval to round nuclei, were present and frequently found lying with their long axis parallel to and contacting the basement membrane. The zygotene spermatocytes were identified by their thick condensed strands of chromatin material surrounded by pale cytoplasm while pachytene spermatocytes appeared comparatively larger with indistinct nuclear membrane. Elongate spermatids were also observed in the tubular wall. The interstitial tissue comprised of Leydig cells, which appeared mostly polyhedral or polyangular in shape, among which were few scattered blood and lymphatic vessels, fibroblasts and macrophages. The Leydig cells

contained a large, ovoid nucleus with peripherally marginated heterochromatin. Electron dense bodies, possibly lipid droplets, were observed in the cytoplasm (Figure 3A). Among the negative control, there was noticeable general disruption in the seminiferous tubular outline. The seminiferous epithelium, comprising of Sertoli cells and spermatogenic cells at different stages of development exhibited considerable disarray and a marked decrease in population density in comparison to the normal control. The seminiferous tubular histology revealed a decreased epithelial cell population and dispersion of these cells within the tubules (Figure 3B).

Treatment of animals with *Z. officinale* at low and medium doses (Figure 3E) showed increased population of the seminiferous epithelium, suggesting recovery of spermatogenic activity. At high dose of Z. offinale (Figure 3F) and *M. whitei* (Figure 3I), the tubular architecture appeared similar to that of the normal control. For *M. whitei* at low dose, there is a decrease in the distribution of spermatozoa, spermatogonia, and Sertoli cells (Figure 3 G) while at medium dose (Figure 3H) of Z. officinale, there appeared to be a rise in the seminiferous epithalial cell count. The histological assessment indicates a dose-dependent ameliorative effect of both *Z. officinale* and *M. whitei* extracts on DIMI testicular damage, with the highest dose (800 mg/kg) demonstrating the most complete restoration to normal testicular architecture and spermatogenesis.

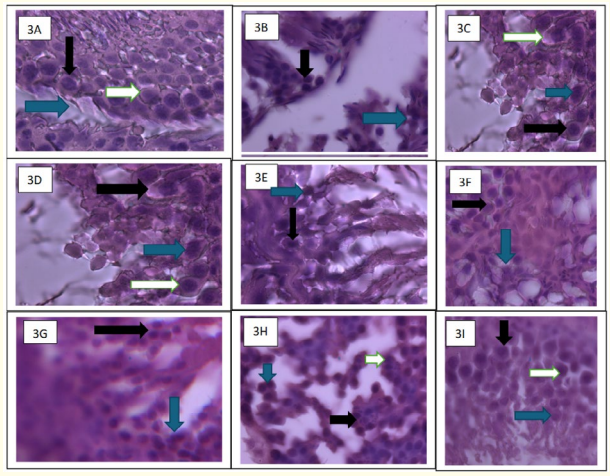

Figure 3

The testicular histology of the rats showing seminiferous tubules of 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). Note spermatogonia (black arrow) resting on basement BM, Sertoli cells (green arrow) and spermatocytes (colorless arrow) of rats treated with alloxan monohydrate alone. At lower dose (200 mg/kg) of *Z. officinale* (D) and *M. whitei* (G) there was reduced distribution of Sertoli cells, spermatogonia and spermatozoa. At medium doses (400 mg/kg) of *Z. officinale* and *M. whitei* (H), there appears to be increased number of seminiferous epithelium while at higher dose (800 mg/kg) of *Z. officinale* (F) and *M. whitei* (I), normal distribution of Sertoli cells, spermatogonium and sperm cell is evident, similar to the normal control. Hematoxylin and eosin stain at magnification of 1000x.

Testicular histomorphometric effects of ethanolic rhizome and root extracts of Z. officinale and M. whitei in diabetes-induced infertile white albino male rats

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 μm), seminiferous epithelial height (45.6 ± 3.2 μm) and Leydig cell volume (501 ± 92.3 μm^3), 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 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 1).

Groups	*TW (ml)*	*TV (ml)*	*STD (μm)*	*SEH (μm)*	*LCV (μm³)*
1	2.71 ± 1.2	2.98 ± 0.71	250.3 ± 5.7	85.2 ± 2.1	1250 ± 212.4
2	1.02 ± 0.3***	1.11 ± 0.22***	180.5 ± 4.3**	45.6 ± 3.2**	501 ± 92.3**
3	2.52 ± 2.4##	2.63 ± 0.01#	220.1 ± 6.2	70.3 ± 4.1##	1201 ± 243.1##
4	1.45 ± 2.1**	1.51 ± 0.41**	195.2 ± 5.1	58.4 ± 3.5*	822 ± 113.4*
5	1.50 ± 0.8**	1.73 ± 0.89*	230.5 ± 4.8	75.6 ± 2.8*	1242 ± 351.2##
6	2.51 ± 0.5##	2.58 ± 1.02#	245.8 ± 6.3	82.1 ± 3.2 #	1291 ± 236.3##
7	1.93 ± 1.5*	2.31 ± 0.31#	190.3 ± 4.5	55.3 ± 3.0*	921 ± 219.6*
8	2.57 ± 1.2##	3.01 ± 1.23##	225.6 ± 5.2	72.8 ± 2.5#	1322 ± 221.3##
9	3.38 ± 1.9###	3.53 ± 1.0¹###	248.2 ± 5.9#	84.5 ± 3.1##	1432 ± 346.7###

Table 1: Effect of increasing doses of *Z. officinal* and *M. whitei* ethanoli root extracts on the testicular histomorphometric variables following 28 day exposure.

At 800 mg/kg of *Z. officinale* there was marked improvement of almost 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 similar 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 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 $p<0.05$. Administration of 800 mg/kg of *M. whitei* similarly 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 $p<0.001$, 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 when compared to the negative control. TV - Testicular volume, TW - Testicular weight, STD - Seminiferous tubule diameter, SEH - Seminiferous tubule Epithelial Height and LCV - Leydig cell volume. Values are mean ± SEM. * as compared to Group A and # as compared to Group B. * and # significant at $p<0.05$, ** and ## significant at $p<0.01$, ***and ### significant at $p<0.001$.

Discussion

The current study evaluated the therapeutic potential of the rhizome and root extracts of two important medicinal plants; *Z. officinale* and *M. whitei* in ameliorating diabetes-induced male infertility in white albino male rats. It has been established that diabetes mellitus, especially when uncontrolled or poorly controlled, leads to long-term damage and multiple organ dysfunction, including the hypothalamus, pituitary and testes axis (Gandhi et al., 2023). Glucose plays an important role in cell metabolism and the general health of cells of the male reproductive system, although high blood glucose levels are detrimental to its normal functioning (Long et al., 2018). While the effects of type 1 *diabetes mellitus* (DM1) on male fertility and testicular functioning have received less attention, the association between type 2 *diabetes mellitus* and male hypogonadism is well established (Venditti et al., 2024). According to *ex vivo* and *in vitro* studies, insulin has a positive impact on spermatogenesis and other testicular functions (Salah et al., 2022), and so these functions may consequently be impacted by insulin insufficiency, which is a defining feature of DM1 patients. Although the exact processes by which DM1 impacts male fertility are still unknown, working hypotheses are predicated on how insulin insufficiency and hyperglycemia impact the gonadal axis, seminal levels of ROS and spermatogenesis (Minas et al., 2024). Men affected by DM1 have lower levels of germ cell function biomarkers (like alanine, glucose transporter 1, phosphofructokinase 1, and lactate dehydrogenase) and higher levels of advanced glycation end products and oxidative stress, which can cause mitochondrial and nuclear sperm DNA fragmentation. These reports imply that dysregulation in testicular glucose uptake and metabolism may affect the quantity and quality of semen in men suffering from DM1 (Huang et al., 2024). Furthermore, hyperglycemia and insulin insufficiency may contribute to

urogenital infections and pelvic neurological diseases, both of which might have an impact on seminal parameters and fertility (Huang et al., 2024). However, a meta-analysis of DM1 and sperm characteristics found that findings are inconsistent and equivocal in determining the impact of DM1 on male fertility (Xu et al., 2023). In the present study, induction of diabetes with alloxan monohydrate led to disruption and atrophy of the seminiferous epithelium, with accompanying reduction in the number of spermatogenic cells and in the size of Leydig cells in the interstitium. Indeed, previous studies showed that alloxan leads to disorganised and reduced spermatogenesis and atrophy of both seminiferous tubular epithelia and Leydig cells, through inducing the build-up of ROS, followed by cellular oxidative stress that damages the cells, subsequently leading to injury and death (Arikawe et al., 2017).

In the present study, treatment of DIMI rats with *Z. officinale* and *M. whitei* greatly improved the histoarchitecture of the hypothalamus, anterior pituitary gland and testes in a dose-dependent manner. This was expected since *both Z. officinale* and *M. whitei* are known to posess antioxidant and anti-inflammatory properties. Z. officinale, especially its phytochemicals like gingerols and shogaols, act as free radical scavengers and thus play a protective role to neurons of the hypothalamus, glandular cells of the anterior pituitary gland and testes (Ayustaningwarno et al., 2024). The results on histoarchitectural changes observed in the present study confirm earlier findings, which showed that *Z. officinale* improves structural alterations in the rat brain, including the hypothalamus and anterior pituitary gland, resulting from streptozotocin- induced *diabetes mellitus* (El-Akabawy & El-Kholy, 2014) as well as improvement of cognitive abilities in male rats through antioxidative and anti-inflammatory effects and reduction of neurodegeneration. In the testes, due to the same mechanisms, *Z. officinale* administration leads to improved general histology of the testes and better seminiferous tubules histology, and is, therefore, in agreement with changes observed in our 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 (Arcusa et al., 2022). The findings of the present study showed that 400 mg/kg and 800 mg/kg of both *Z. officinale* and *M. whitei* greatly improves the histology of hypothalamus which habours gonadotropin releasing hormone (GnRH) secretory cells down to anterior pituitary gland. The gonadotrophs under GnRH influence secretes both FSH and LH, which are transported through blood to testes. The findings further showed that both *Z. officinale* and *M. whitei* at higher doses improved the histology and glandular functions of the anterior pituitary gland and hypothalamus. The results showed a gradual increase of both hormones in a dose-dependent manner, consistent with the findings of Abdelfattah et al. (2023) on effects of *Z. officinale* rhizomes on the reproductive aspects in male Japanese Quails (*Coturnix coturnix japonica*). Further, the findings shows that FSH and LH have both steroidogenic and spermatogenic influence on the testes through their influence on Sertoli and Leydig cell functions.

Additionally, the findings of the present study showed that induction of DIMI using alloxan monohydrate led to 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. These findings are consistent with those of earlier studies on the hypoglycemic and pancreatic protective effects of *Portulaca oleracea* extract in alloxan- induced diabetic rats, where its administration in male animals negatively impacted on the testicular histomorphometric parameters due to its toxic effects on testicular cells for example Ramadan et al., 2017. *Diabetes mellitus* is known to increase oxidative stress and promote a state of chronic inflammation within the spermatogenic, Sertoli and Leydig cells, and in almost all other cells of the seminiferous tubule (Maresch et al., 2018). In our study, the administration of increasing doses of both *Z. officinale* and *M. whitei* extracts showed improvement of histological architecture of the hypothalamus, anterior pituitary and testis. The findings agree with those of Ayustaningwarno et al. (2024) who reported anti-oxidative and anti-inflammatory properties of rhizome and root extracts of *Z. officinale* and M. whitei. Its phytocomponents like zingerone, gingerol, paradol and shogaol are known to have these effects. For instance, *Z. officinale* acts as an anti-oxidative and anti-inflammatory agent through activation of the Nrf2/ARE pathway, and protects against toxic molecules like drugs and pollutants (Abdelfattah et al., 2023). It also regulates the activity of several cell signaling pathways like the Mitogen-Activated Protein Kinase (MAPK) and Transforming Growth Factor-beta (TGF)/SMAD (TGF-β/Smad) pathways, which are important in maintenance of testicular cell function and maintenance of its histoarchitecture, which translates into normal cellular processes like spermatogenesis and steroidogenesis (Emil et al., 2025). Previous studies by abdelfattah et al. (2023) showed that higher doses (15 g/kg) of *Z. 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, *Z. officinale* caused distortion of both the germinal epithelium and supporting cells, atrophy and destruction of the Leydig cells and the overall shrinkage of the seminiferous tubules in adult Wistar rats (Obeten et al., 2014). *Zingiber officinale* reverses and protects against seminiferous tubular destruction caused by excessive administration of monosodium glutamate (El Wakeel et al., 2020).

The present study also compared the relative protective properties of *Z. officinale* and M. whitei. The changes in testicular, seminiferous tubules and Leydig cell parameters associated with administration of M. whitei, as reported in the present study, however, contradict those of previous studies. For example, there was hypocellularity within the seminiferous tubules, with less spermatogenic cells, vacuolized Sertoli cells and increases intercellular spaces in rats administered with *M. whitei* (Ochieng' et al., 2021). Similar studies showed that *M. whitei* improves testicular and seminiferous tubular histology only in low quantities. Administration of *M. whitei* for 55 days resulted in the degeneration of the seminiferous tubules and the epididymis and subsequent cessation of spermatogenesis, although these changes were reversible (Minas et al., 2024). The variation in effects observed with *M. whitei* ethanolic extract on testicular and seminiferous tubule histology likely stems from differences in dosage, duration of administration, and possibly geographical location where the extracts are harvested since soil composition primarily affects secondary metabolite concentration in the plants. Because of its antioxidant qualities or ability to stimulate spermatogenesis, low doses of *M. whitei* may have positive or protective effects on testicular tissue (Minas et al., 2024). As seen in the study following 55 days of treatment, toxicity, cellular damage, and seminiferous tubule degeneration can result from high dosages or chronicity of exposure. Particularly, if doses are low, short or moderate durations may allow the tissue to adjust or regenerate. Higher dosages over longer periods of time of exposure (e.g., 55 days) may result in cumulative harm that causes degeneration, hypocellularity, and vacuolization. Certain effects might be reversible after cessation of exposure, especially those brought about by lower dosages or shorter exposure times (Minas et al., 2024). Although studies indicate that certain effects are reversible and that there is a threshold beyond which damage becomes permanent, severe or persistent exposure may result in more permanent structural damage. The results may potentially be impacted by variations in animal models, extraction techniques, or geographical location where the plants are grown. The primary source of the variation is the dosage and length of time that *M. whitei* like extract is administered; lower dosages and shorter exposure times are typically advantageous or reversible, whereas higher dosages and longer exposure times typically result in tissue deterioration and functional impairemtn such as spermatogenesis cessation (Minas et al., 2024).

In conclusion, *Diabetes mellitus* induced by alloxan monohydrate leads to changes in testicular weight and volume, seminiferous tubules diameter and epithelial height and Leydig cell volume, subsequently leading to male infertility. *Z. officinale* and *M. whitei* restore these changes in a dose-dependent manner. Mondia whitei, however, offers significant protective abilities compared to *Z. officinale* since it induces histological changes within the hypothalamus, anterior pituitary gland and testes similar to the normal control. The superior antioxidant and anti-inflammatory properties of M. whitei's phytochemicals, especially flavonoids, polyphenols, alkaloids, saponins, and phenolic compounds, are probably what gives it a stronger protective effect. These compounds also protect the hypothalamus, pituitary gland, and testes. Overall, the results of the present study have shown that *M. whitei* has very good protective abilities to male infertility induced by *diabetes mellitus* compared to Z. officinale. *Mondia whitei* could, therefore, be used for translational research in order to be used in humans to prevent male infertility in diabetic patients.

List of Abbreviations

ZO - Zingiber officinale.
MW - *Mondia whitei*.
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.

Ethical declaration

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

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Authors contribution

1. *Ambrose Barasa*: Carried out research and manuscript writing.
2. *Prof. Patrick Onyango*: Supervisor.
3. *Dr Albert Nyongesa*: Supervisor.
4. *Mr. Adipo Walter*: Laboratory technician.

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