التعليق [DS21]: The title should be revised to (EFFECT OF ZINC SULPHATE ON EFFECTS AND METHANOL EXTRACT OF *SPHENOCENTRUM JOLLYANUM* ROOT ON MALE FERTILITY IN WISTAR RATS).

EFFICACY OF ZINC SULPHATE ON EFFECTS OF METHANOL EXTRACT OF SPHENOCENTRUM JOLLYANUM ROOT ON MALE FERTILITY IN WISTAR RATS

ABSTRACT

Sphenocentrum jollyanum has many traditional uses across West Africa but its adverse effects on male fertility have been reported and this posed the need for an adjuvant. Zinc is known for improving sperm health but its impact has not been studied against the effects of *Sphenocentrum jollyanum*.

Aims: This study evaluated the efficacy of Zinc sulphate on effects of methanol extract of *Sphenocentrum jollyanum* root (MSJR) on male fertility in Wistar rats.
Study design: Thirty six male Wistar rats weighing between 180-200g were assigned into six groups (n=6). Group A served as control, groups B and C were administered with 500 mg/kg and 1000 mg/kg MSJR respectively, group D received 20 mg/kg Zinc sulphate, group E received 20mg/kg Zinc sulphate and 500 mg/kg MSJR and group F received 20 mg/kg Zinc sulphate and 1000 mg/kg MSJR.
Place and Duration of Study: Department of anatomy, Ladoke Akintola University of technology, Ogbomoso, Oyo state Nigeria. Between January 2024 and June 2024.

Methodology: All treatments were administered by oral gavage daily for 56 days and sacrificed by cervical dislocation on 57th day. Blood samples were collected by cardiac puncture for hormonal analysis [Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone (TH)] using ELISA method. Semen samples were collected from caudal epididymis for evaluation of the sperm parameters (motility, count, morphology and viability) by light Microscope. The epididymis was fixed in Bouin's fluid and processed for histological evaluation using Haematoxylin and Eosin and Toluidine blue stains. Data were analyzed using One way analysis of variance followed by Tukey Post-hoc test and $P<0.05$ was taken as accepted level of significant difference.
Results: There was significant decrease in [LH (*P=0.001*), TH (*P=0.0069*), FSH (*P=0.001*), rapid-progressive sperm motility (*P=0.0001*), count (*P=0.0001*), morphology (*P=0.0001*), viability (*P=0.0001*) of group B and C while there was significant increase in [LH (*P=0.001*), TH (*P=0.001*), rapid-progressive sperm motility (*P= 0.0474*) and viability (*P=0.0001*)] of group D, sperm morphology of groups E (*P=0.0289*) and F (*P=0.0027*).
Conclusion: This study concluded that the administration of Zinc sulphate has ameliorated MSJR-induced epididymal damages in Wistar rats

Keywords: Epididymis, hormonal profile, methanol extract, *Sphenocentrum jollyanum* (SJ), root, spermatogenesis, zinc

1. INTRODUCTION

When a male is unable to conceive a fertile female, this is known as male infertility. The majority of the psychosocial impacts, particularly in Africa, are attributed to women because infertility in couples was always thought to be a female-only problem. This is most likely the

result of cultural beliefs, a lack of knowledge, and unclear comprehension. Male infertility is defined as when semen quantity and quality do not meet WHO criteria and recognized female causes of infertility are ruled out 40–50% of human infertility is caused by male factors, according to research (Olooto and Wasiu, 2012)

S. jollyanum is commonly used for its aphrodisiac qualities in traditional medicine (Mbaka *et al.*, 2019). Additionally, research has shown that mixtures prepared from S. Jollyanum root can aid in the treatment of fever, irregular menstruation cycles, muscle soreness, mental health issues, and inflammatory disorders by stimulating the central nervous system (Uka *et al*., 2020).

The plant's root extracts have been shown to stimulate sexual desire, prolong ejaculation, and raise follicle-stimulating hormone (FSH) and testosterone levels (Muko *et al.,* 2007). Its activity diminishes the refractory period, resulting in a reduction in post-ejaculatory latency, climbing behavior, and intromission in male animals (Owiredu *et al*., 2007). Notwithstanding these substantial findings, there exists contradictory evidence concerning its antifertility effects, which are linked to decreased sperm quantity and quality, alongside testicular atrophy (Raji *et al.*, 2006 and Baffoe *et al*., 2021). The negative consequences linked to its use underscore the necessity for the creation of supplements with beneficial characteristics.

Zinc supplementation, an essential trace mineral, has demonstrated a significant impact in male reproductive health by facilitating the development and maturation of spermatozoa. It also influences sperm parameters, including motility, morphology, and membrane stability (Esfiokhi *et a*l., 2023). Research has demonstrated that zinc promotes acrosomal exocytosis in bovine sperm and enhances hyper-activated motility in human sperm (Allouche-Fitoussi and Breitbart, 2020). Zinc also functions as an antioxidant, safeguarding sperm from oxidative stress. Additionally, it plays an essential role in the secretion of gonadotropic hormones and the maintenance of testosterone levels, both of which are crucial for sperm production (Schisterman *et al*., 2019). This study evaluated the impact of Zinc sulphate and methanol extract of Sphenocentrum jollyanum root (MSJR) on reproductive parameters in male Wistar rats

التعليق **[DS22]:** ITALIC

~~**Sphenocentrum jollyanum**~~

~~The perennial understory species Sphenocentrum jollyanum prefers deep shade and develops mostly in dense forest settings. It can be found between sea level and 400 meters above sea level. Regions with an average annual rainfall of 1800 mm or more, a mean maximum temperature of 29°C, and a mean minimum temperature of 20°C are generally ideal for this plant's growth. S. jollyanum has sparse branching and grows to a mean height of 1.5 meters. The leaves have a wedge form, are smooth on both sides, range in width from 5 to 12 cm, and can reach a maximum length of 20 cm before ending in a tiny, arrow-like apex (Tiwari *et al*., 2013). Sphenocentrum jollyanum produces a single large oval-shaped seed and clusters of ovoid ellipsoid fruit. When ripe, it turns orange or bright yellow and is delicious and meaty (Odugbemi, 2006). The roots have a characteristic brilliant yellow color and are sour and acidic, which makes subsequent dishes taste sweet (Oke *et al*., 2002).~~

~~Phytochemical analyses have identified the plant as a significant source of secondary metabolites, including saponins, flavonoids, alkaloids, and tannins. Its pharmacological activities encompass anti-inflammatory, anti-diabetic, anti-viral, anti-bacterial, anti-malarial, anxiogenic, and angiogenic effects (Olorunnisola *et al*., 2017).~~

~~**Taxonomical classification of Sphenocentrum jollyanum**~~.

The plant is classified as follows: Kingdom Plantae, Division Magnoliophyta (Cronquist), Subdivision Magnoliophytina (Frohne and Jensen), Class Ranunculopsida (Brongn), Subclass Ranunculidae (Takht), Suborder Ranunculanae (Takht), Order Menispermales (Bromhead), Family Menispermaceae (Juss.), Genus Sphenocentrum (Pierre), and Species Jollyanum (Schoch *et al*., 2020).

منسّق:الخط: Roman weN semiT، ١٢ نقطة، خط اللغة العربية وغيرها: ١٢ نقطة

Fig. 1. Leaves of Sphenocentrum jollyanum (Olorunnisola *et al*., 2017)

منسّق:الخط: Roman weN semiT، ١٢ نقطة، خط اللغة العربية وغيرها: ١٢ نقطة

Fig. 2. Roots of Sphenocentrum jollyanum (Ekpono *et al*., 2018)

Ethno-Medicinal Uses

In folk medicine, different portions of Sphenocentrum jollyanum have long been used. Men in Ghana frequently utilize the plant's root as an aphrodisiac. In order to

extract the active ingredients, it is steeped in alcohol for a few days. The resulting bitters are then taken to improve penile erection, and the effects are said to continue for a long time (Nia *et al*., 2004). The root's ability to treat inflammatory and mental illnesses, depression, pain, and stimulate the central nervous system (CNS) has also been reported in a number of studies (Oke *et al*., 2002). For the treatment of fever and muscular pain, the dried, powdered root is often combined with other anti-malarial herbs. The plant's aerial parts, such as its fruits and leafy twigs, are commonly utilized along with lime juice and Piper guineense (a West African pepper) to cure fever, coughing, and chronic wounds (Amidu, 2008).

Traditional medicine in Nigeria uses the chewed roots of Sphenocentrum jollyanum to improve digestion, increase appetite, and relieve constipation. According to Odugbemi (2006), every morphological component of the plant is significant in the treatment of sickle cell disease (SCD). The therapeutic qualities of the roots have also been recorded by traditional healers in Ghana and Côte d'Ivoire to cure diabetes mellitus, breast tumors, irregular menstrual cycles, and high blood pressure (Kayode *et al*., 2009). Furthermore, to relieve stomach pain, the ground roots are eaten with salt, Elaeis guineensis (African oil palm), and Aframomum melegueta (alligator pepper) seeds (Egunyomi *et al*., 2005). In addition to being used as an anti-fatigue snack, charred fruits are used to treat fibroids. Additionally, it has been reported that the leaves' extracts are used to prevent blood spitting and to eradicate intestinal parasites (Kayode *et al*., 2009).

Phytochemical Constituents of Sphenocentrum jollyanum

Most of the documented pharmacological and biological activity has been ascribed to the phytochemical components and bioactive principles of Sphenocentrum jollyanum preparations. Terpenoids and flavonoids were among the components found in the ethanol root extract, according to a thorough phytochemical analysis; alkaloids were found to be the most prevalent chemical ingredients (Amidu *et al*., 2008). Alkaloids, tannins, saponins, and terpenes were also found in different fractions of methanol extracts from the stem bark by Nia *et al.* (2004) in their phytochemical studies. The most active of these was the chloroform fraction, which tested positive for flavonoids and alkaloids (Nia *et al*., 2004).

Aboaba and Ekundayo (2010) used gas chromatography-mass spectrometry (GC-MS) to thoroughly analyze the essential oil from the root of Sphenocentrum jollyanum and found 19 compounds in total: guaia-6,9-diene-4α-ol, α-pinene, α-ylangene, guaiene-11-ol, globulol, isocaryophyllene, α-eudesmol, selina-4(15),6-dien, aromadendrene, γ-terpinene, E-β-isocaryophyllene, epi-zonarene, γ-humulene, δ-amorphene, 1,8-cineol, β-pinene, camphene, d3-carene, and p-cymene.

According to Ibironke and Olusola (2013) phytochemical examination of the seed extracts, free anthraquinone and phylobatannin were not present, while alkaloids, saponins, and flavonoids were bioavailable. The seed extract's composition, as determined by proximate analysis, was crude protein (48.09%), carbohydrates (16.79%), moisture (16.70%), crude fat (9.65%), ash (3.26%), and fiber (5.51%). Its energy value of 1460 kcal/100 kg indicated that the fruits are a significant source of

~~energy and nutrients. Significant amounts of macro and microelements necessary for both human and animal growth were found in the mineral composition of Sphenocentrum jollyanum seeds, according to investigations using flame photometry and atomic absorption spectrophotometry (AAS).These elements included calcium (8.92 mg/L), iron (0.22 mg/L), magnesium (0.44 mg/L), potassium (4.26 mg/L), zinc (1.38 mg/L), manganese (0.19 mg/L), and sodium (4.70 mg/L). These results highlight the plant's function in promoting bone health, energy production, and other bodily metabolic processes (Ibironke and Olusola, 2013). Three furanoditerpenes—isocolumbine, columbin, and fibeucin—as well as the alkaloid protoberberine—from the fruit (Moody *et al*., 2006).~~

~~**Aim of study**~~

~~This study evaluated the impact of Zinc sulphate and methanol extract of Sphenocentrum jollyanum root (MSJR) on reproductive parameters in male Wistar rats.~~

2. MATERIALS AND METHODOLOGY

A. Collection and Extraction of Plant Material

The fresh roots of *Sphenocentrum jollyanum* were collected from a farmland in Elebonla, Badeku village, Ona Ara Local Government Area, Ibadan, Oyo State, Nigeria. The plant was identified and authenticated by Professor A. J. Ogunkunle, a taxonomist from the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. A voucher specimen (LHO 529) was deposited at the University Herbarium. The roots were air-dried at room temperature and subsequently ground into a powdered form for further use.

التعليق [DS23]: The authors should include a table listing the qualitative identification of the key active compounds present in the methanolic extract of *Sphenocentrum jollyanum* root.

B. Preparation of Methanol Extract

Cold maceration extraction was performed using Soxhlet extraction method with methanol as the solvent of extraction, after which the filtrate is concentrated to dryness to obtain methanol extract of *Sphenocentrum jollyanum* root (Zang et al., 2018). Five hundred grams (500g) of dried and powdered stem bark of *Sphenocentrum jollyanum* were exhaustively macerated in 100% methanol for 72 hours. The mixture was then filtered using a Büchner funnel and Whatman No.1 filter paper. The resulting filtrate was concentrated under reduced pressure at 40°C and stored at room temperature, between 40–60°C. The extract was further evaporated into the extraction column along with the sample, after which it was allowed to siphon and concentrate. The extraction process was conducted at the Department of Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

C. Animals

Thirty-six (36) male Wistar rats, with an average weight of 200g, were utilized in the research. The rats were sourced from the central animal facility of the Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso. The animals were housed in plastic cages within a designated research animal section. They were provided with commercially available standard chow (Top Feeds Nigeria

Limited) and allowed to acclimatize for a period of two weeks before the commencement of the study. The animals were weighed prior to the start of the experiment. Throughout the experimental period, the animals had free access to food and water ad libitum. Ethical approval for the research was obtained from the Ethical Research Committee of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology (Approval number: ERCFBMSLAUTECH: 034/05/2024). All experimental procedures were conducted in compliance with the guidelines established by the Institutional Animal Care and Use Committee (IACUC).

D. Experimental Design

A total of thirty-six (36) adult male Wistar rats, each with an average body weight of 200 g, were randomly assigned to six groups, with six animals per group (n=6). The rats were treated for a 56-day period. Group A served as the control group and was fed with standard feed and water ad libitum. Groups B and C were administered methanol extract of *Sphenocentrum jollyanum* root (MSJR) at doses of 500 mg/kg and 1000 mg/kg, respectively. Group D received zinc at 20 mg/kg. Group E was co-administered zinc at 20 mg/kg and MSJR at 500 mg/kg, while group F received co-administration of zinc at 20 mg/kg and MSJR at 1000 mg/kg.

The LD50 of methanol extract of *Sphenocentrum jollyanum* root (MSJR) in rats was 5,000 mg/kg (Ugwu et al., 2023) while that of Zinc was 57.348 mg/kg (Tekuri et al., 2021).

At the end of the experimental period (57th day), final weights of the animals were recorded and animals were sacrificed by cervical dislocation. Blood samples were taken by cardiac puncture for the estimation of levels of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Testosterone. Semen proximate were collected through the epididymal samples to determine sperm count, motility and morphology. Other epididymal samples were carefully harvested, and fixed in Bouin's fluid for routine histological examination using Haematoxylin and eosin (H&E) and Toluidine blue Stains.

E. Collection and Analysis of Sperm Samples

Semen samples were collected from a 10 mm segment of the caudal epididymis, known for its role in storing mature sperm. The epididymal segment was carefully dissected, cut into several pieces, and placed into specimen bottles containing 1 ml of physiological saline. The samples were then homogenized to release the spermatozoa. The specimen bottles were maintained at 37 0C for 15 minutes to allow sufficient diffusion of sperm into the saline. The semen was then extracted from the specimen bottles using a 1 ml pipette, with drops placed onto cleaned microscope slides and covered with cover slips. The slides were examined under a light microscope to assess sperm parameters, including motility, count, and morphology, for all experimental groups.

Sperm Motility

Sperm motility was assessed according to the guidelines established by the World Health Organization (Feferkorn, 2022). Specifically, a 10 µl sample of the culture-sperm mixture was placed on a microscope slide. The motility of a minimum of 200 spermatozoa per specimen was evaluated across at least five different microscopic fields. The analysis quantified the percentage of sperm exhibiting progressive motility, non-progressive motility (in situ) and immobility.

Sperm count

A counting chamber was utilized for determining sperm count. Drops of semen were placed on two edges of the chamber, few drops of eosin blue stains were added, covered with cover slips, and focused under a light microscope for cell counting. This procedure was repeated

five times, and the average count was calculated to ensure accuracy and minimize bias (Rahayu *et al*., 2019).

Sperm morphology

Cells were stained and observed under a light microscope with the oil immersion objective. The percentage of normal and abnormal spermatozoa were quantified and compared (Oliveira *et al*., 2015).

Sperm viability

Sperm viability refers to the ability of spermatozoa to maintain life-sustaining functions post-ejaculation, including motility, membrane integrity, and fertilization potential. The ability of sperm to remain viable after ejaculation is influenced by several cellular mechanisms (Sharma *et al*., 2023).

F. Hormonal Assay

Blood samples were collected from the left ventricle of anesthetised rats in the tube and promptly centrifuged at 3000rpm for 10 minutes to isolate the plasma. Separated serum was confined in -20 0C freezer for the biochemical test levels of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The hormones were analysed using standardized immunoassay techniques by ELISA kits (testosterone ELISA catalog No. TE187S (96 Tests); LH ELISA Catalog No. LH231F (96 Tests): FSH ELISA Catalog No. FS046F (96 Tests). All the kits were ordered from Calbiotech inc., California. The calculations were done based on the instructions and steps provided on the reagent kits in accordance with the methods used by Ghazal *et al*., (2021).

G. Histological Procedure

The epididymis of each of the animals were carefully dissected out and weighed fixed in Bouin's fluid by total immersion for 24 hours after which it was were trimmed to about 3 to 5mm thick sections and processed via paraffin wax embedding method. The tissue was dehydrated at room temperature through ascending grades of alcohol. Dehydrated tissues were cleansed at room temperature in two changes of molten paraffin wax using a multi block plastic embedding mold. The paraffin block tissue was trimmed and mounted on wooden block for sectioning on a rotary microtome (Bright B5143, Huntington, England), the section was transferred to water bath (40C) to allow spreading of the folded sections. These sections were mounted on new clean glass slides which are later dried on a slide drier to enhance adherence of section to slide.

H. Statistical Analysis

A one-way ANOVA (analysis of variance) or a mixed model was employed and differences between groups were assessed using Turkey Post Hoc test with the assistance of GraphPad Prism Software 9.0.0 (121). The results of the statistical analysis were depicted using bar charts with error bars indicating the mean and standard error of mean (M ± SEM). A significance threshold was established at $p<0.05$. ImageJ software (1.48 v/java 1.6.0_20) was used in histomorphometric analysis.

Statistical analysis

Chris Rorden's ANOVA was used to analyze the data collected in one way analysis of variance while comparing within and between groups post-hoc test (Tukey HSD) was used. The results were expressed as mean ± S.E.M. and $p < 0.05$ was taken as the accepted level of significant difference from control.

التعليق [DS24]: The two sections on statistical analysis should be merged.

التعليق [DS25]: A more comprehensive presentation of the results is required.

3. RESULTS AND DISCUSSION

3.1 RESULTS

A. Effect of zinc and methanol extract of Sphenocentrum jollyanum root (MSJR) on the epididymal weight of experimental animal.

The result revealed that compared to the control (A) group, the mean epididymal weight decreased in groups B, C and F while it increased in groups D and E. Compared to group B and C (MSJR), there was an increase in the mean epididymal weight of groups A, D and E while it decreased in groups C and F.

Table 1. Shows the effect of Zinc and methanol extract of *Sphenocentrum jollyanum* root (MSJR) on the Left, Right and mean epididymal weight.

Groups	Weight of Right Epididymis (g)	Weight of Left Epididymis (g)	Mean Epididymal Weight (g)
A. Control	0.48 ± 0.07	0.59 ± 0.10	0.54 ± 0.07
B. MSJR 500	0.43 ± 0.01	0.51 ± 0.06	0.47 ± 0.02
C. MSJR 1000	0.35 ± 0.07	0.43 ± 0.09	0.39 ± 0.08$^{\#}$
D. ZN	0.66 ± 0.04	0.66 ± 0.02	0.66 ± 0.03
E. ZN/MSJR500	0.57 ± 0.01	0.59 ± 0.01	0.58 ± 0.01
F. ZN/MSJR1000	0.38 ± 0.09	0.43 ± 0.09	0.41 ± 0.09$^{\#}$

*Data presented as Mean ± S.E.M, *P < 0.05 vs. control, $^{\#}$P < 0.05 significant difference from MSJR alone, Number of rats per group =6. ZN: Zinc, MSJR: Methanol extract of Sphenocentrum jollyanum*

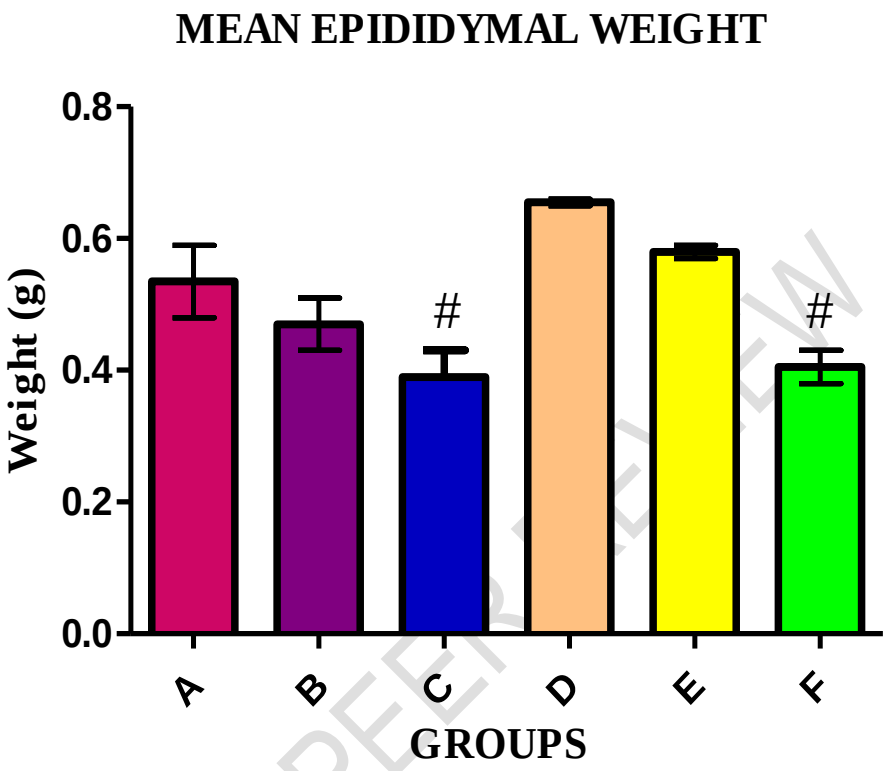


Fig. 3. Effect of zinc and methanol extract of *Sphenocentrum jollyanum* root (MSJR) on the mean epididymal weight.
Each bar represents Mean ± S.E.M, #P<0.05 significant difference from zinc, number of rats per treatment group = 6.

B. Effect of zinc and methanol extract of Sphenocentrum jollyanum root (MSJR) on microscopic Sperm count.

The result revealed that compared to the control (A) group, the sperm count decreased in all animal groups except in group D. Compared to group B and C (MSJR groups), there was an increase in the sperm count of all animal groups (A, D, and F).

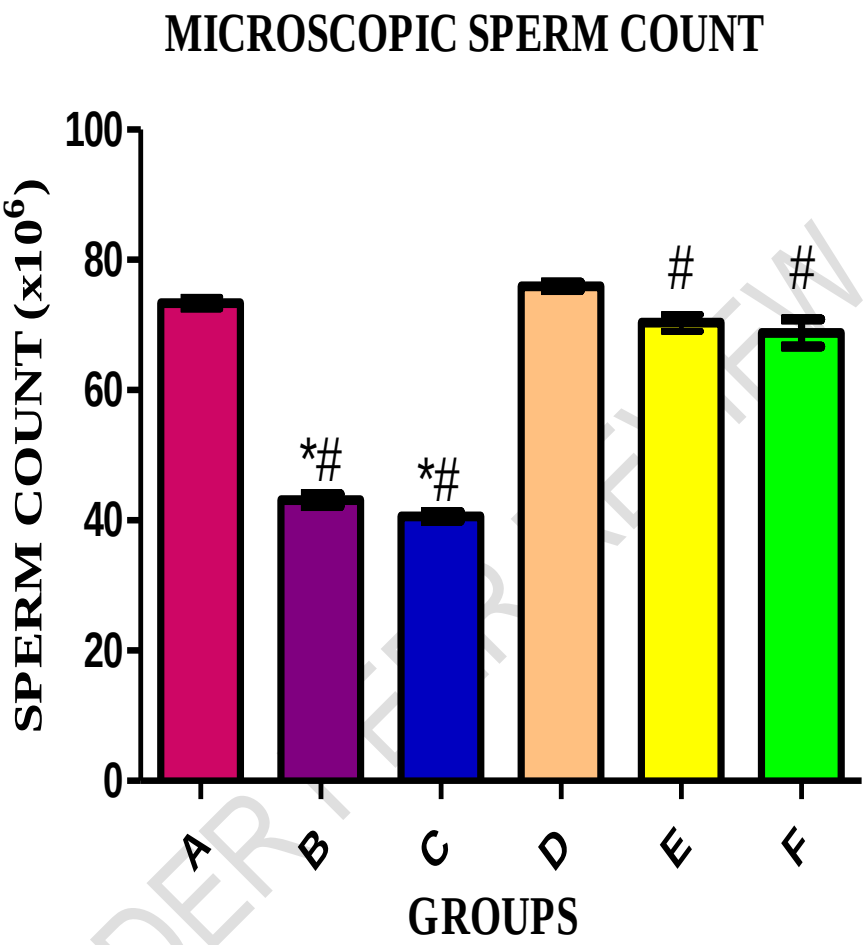


Fig. 4. shows the effect of zinc and methanol extract of *Sphenocentrum jollyanum* root (MSJR) on the percentage Sperm count.
*Each bar represents Mean ± S.E.M, *P < 0.05 vs. control, #P < 0.05, number of rats per treatment group =6.*

C. Effect of zinc and methanol extract of Sphenocentrum jollyanum root (MSJR) on Percentage Sperm motilities.

Compared to the control (A) group, the rapid progressive sperm motility percentage decreased in all animal groups except in group D where significant increase (*P<0.05*) was observed. Compared to group B and C (MSJR groups), there was an increase in the rapid

progressive sperm motility percentage of all animal groups except in group C which decreased.

Compared to the control (A) group, percentage slow progressive sperm motility increased significantly in group F (*P<0.05*) and in all other animal groups except in group D which decreased. Compared to group B and C (MSJR groups), there was a decrease in the percentage slow-progressive sperm motility of all animal groups except in group C which increased.

Compared to the control (A) group, the non-progressive sperm motility percentage increased significantly (*P<0.05*) in group B and in all animal groups (C, E, and F) except in group D which decreased. Compared to group B, there was a general decrease observed in the non-progressive sperm motility percentage of all experimental animals.

Compared to the control (A) group, the dead sperm cell percentage remained the same with groups D and F while it increased in groups E and MSJR groups(B and C). When compared to group MSJR groups, the dead sperm cell percentage decreased in all other groups (D, E, and F).

Table 2. Shows the effects of zinc and methanol extract of *Sphenocentrum jollyanum* root on the percentage Sperm motilities.

*Data presented as Mean ± S.E.M, *P < 0.05 vs. control, #P < 0.05 significant difference from MSJR alone, Number of rats per group =6. ZN: Zinc, MSJR: Methanol extract of*

GROUPS	Rapid Progressive Motile Sperm Cell (%)	Slow Progressive Motile Sperm Cell (%)	Non-Progressive Motile Sperm Cell (%)	Dead Sperm Cell (%)
A. Control	73.35 ± 1.27	15.50 ± 0.20#	6.15 ± 0.66	5.00± 0.23
B. MSJR 500	41.48 ± 0.23*#	30.50 ± 0.20*#	17.52 ± 4.34*	10.50± 0.45*#
C. MSJR 1000	38.75 ± 0.51*#	35.25 ± 0.51*#	15.50 ± 2.60#	10.50± 1.06*#
D. ZN	77.13 ± 0.83*	12.07 ± 0.85	5.80 ± 0.46	5.00± 0.11
E. ZN/MSJR500	71.18 ± 0.32#	15.90 ± 0.37	7.42 ± 1.49	5.50± 0.23
F.ZN/MSJR1000	67.43 ± 0.87*#	20.00 ± 2.04*#	7.57 ± 1.48	5.00± 1.92

Sphenocentrum jollyanum

D. Effect of zinc and methanol extract of Sphenocentrum jollyanum root (MSJR) on Percentage Sperm morphology.

Compared to the control (A) group, the percentage normal sperm morphology decreased across all groups except in group D where it increased. Compared to MSJR groups (B and C), the percentage normal sperm morphology increased across all animal groups (A, D, E, and F).

Compared to the control (A) group, the percentage sperm head defect increased across all groups except in group D where decrease was observed. Compared to group B and C, the percentage sperm head defect decreased across all animal groups

Both percentage sperm mid-piece defect and percentage sperm tail-piece defect remained the same across all groups (A, B, C, D, E and F).

Table 3. Shows the effects of zinc and methanol extract of *Sphenocentrum jollyanum* root on the Percentage Sperm Morphology

*Data presented as Mean ± S.E.M, *P < 0.05 vs. control, #P < 0.05 significant difference from*

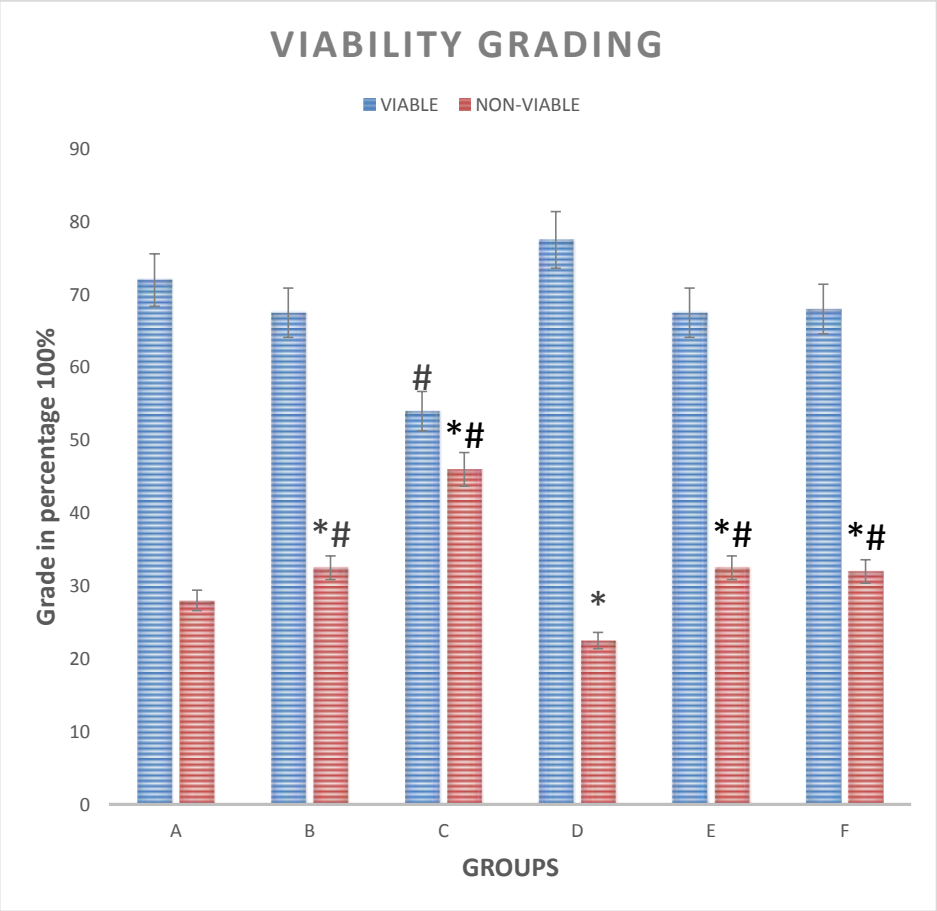
GROUPS	Normal Sperm Morphology (%)	Head Defective Sperm Cells (%)	Mid-Piece & Defective Sperm Cells (%)	Tail Defective Sperm Cells (%)
A. Control	46.17 ± 1.6	43.83 ± 1.75	5.00± 1.50	5.00± 1.00
B. MSJR 500	32.83 ± 0.39*#	57.17 ± 0.96*#	5.00± 0.20	5.00± 0.06
C. MSJR 1000	30.83 ± 0.68*#	59.17 ± 0.48*#	5.00± 0.05	5.00± 0.01
D. ZN	49.50 ± 0.29	40.50 ± 0.29	5.00± 0.01	5.00± 0.01
E.ZN/MSJR500	40.00 ± 0.87*#	50.00 ± 1.16*#	5.00± 0.00	5.00± 0.50
F.ZN/MSJR1000	34.17 ± .77*#	55.83 ± 0.10*#	5.00± 0.00	5.00± 0.10

MSJR alone, Number of rats per group =6. ZN: Zinc, MSJR: Methanol extract of Sphenocentrum jollyanum

E. Effect of zinc and methanol extract of Sphenocentrum jollyanum root (MSJR) on Percentage Sperm Viability and Non-Viability grading.

Compared to the control (A) group, the percentage sperm viability decreased in all animal groups except in group D in which increase was observed. Compared to group B, there was an increase in the percentage sperm viability of groups A, D and F while there was a decrease in group C and had no difference with group E.

Fig. 5. Shows the effects of zinc and methanol extract of *Sphenocentrum jollyanum* root on the percentage Sperm viability and non-viability.



*Data presented as Mean ± S.E.M, *P < 0.05 vs. control, #P < 0.05 significant difference from MSJR alone, Number of rats per group =6.*

F. The effect of zinc and methanol extract of Sphenocentrum jollyanum root (MSJR) on Luteinizing hormone (LH), Follicle Stimulating hormone (FSH), and Testosterone (TEST) in experimental Animal.

The luteinizing hormone levels of the experimental animal decreased significantly Compared to the control (A) group, except in group D (Zinc only) in which increase was observed. Compared to group B and C (MSJR groups), significant increase was observed in groups A, D, E and F animal while LH level decreased significantly in group C.

The levels of Follicle-stimulating hormone (FSH) significantly decreased in all experimental groups (B, C, D, E, F) when compared to the control (A) group, while increased significantly compared to MSJR groups (B and C).

Testosterone level significantly increased in Group D and E while decreased significantly with group B, C and F when compared to the control (A) group, Compared to group B,

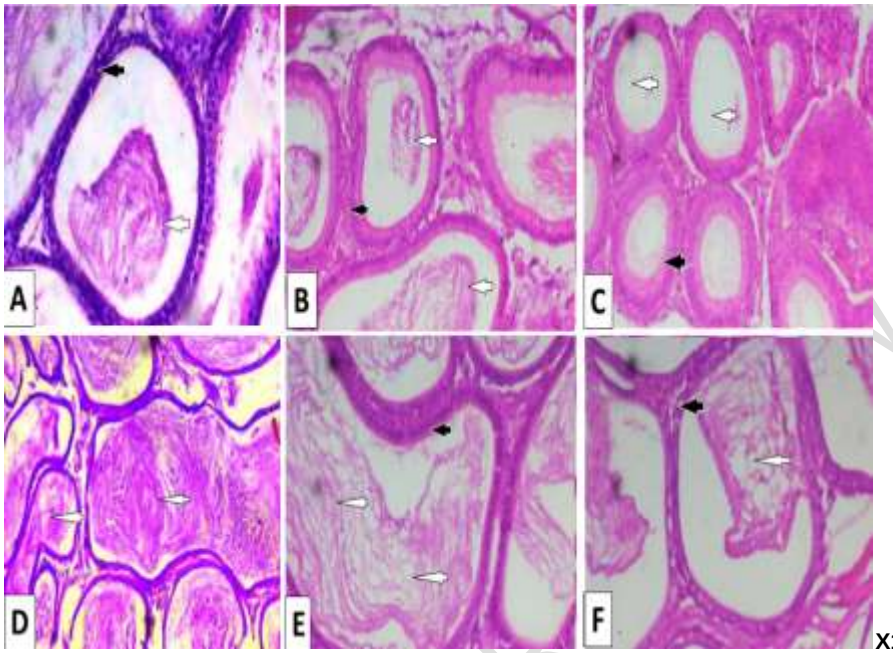
Significant increase in testosterone was observed in all animal groups.

Table 4: shows the effects of zinc and methanolic root extract of *Sphenocentrum Jollyanum* on the levels of Luteinizing hormone (LH), Follicle Stimulating hormone (FSH), Testosterone (TEST).

Groups	LH (MlU/ml)	FSH (MlU/ml)	TEST.(ng/ml)
A. Control	1.68 ± 0.01	3.53±0.87	3.88±0.26
B. MSJR 500	0.75 ± 0.09*#	2.04±0.56*#	3.22±0.68*#
C. MSJR 1000	0.39 ± 0.38*#	2.07±0.04*#	3.54±0.02*#
D. ZN	1.98 ± 0.01*	3.54±0.05	8.97±0.09*
E.ZN/MSJR500	1.01 ± 0.03*#	3.00±0.46*#	7.21±0.48*#
F.ZN/MSJR1000	1.47 ± 0.01*#	3.18±0.82*#	6.35±0.29*#

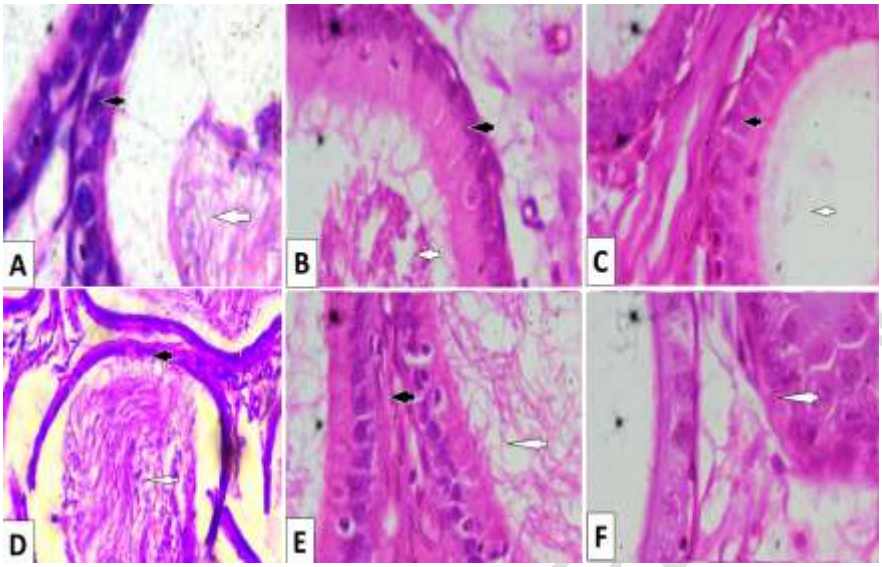
*Data presented as Mean ± S.E.M, *p < 0.05 vs. control, #p< 0.05 significant difference from MSJR alone, Number of rats per group =6. ZN: Zinc, MSJR: Methanol extract of Sphenocentrum jollyanum*

G. Histological Analysis



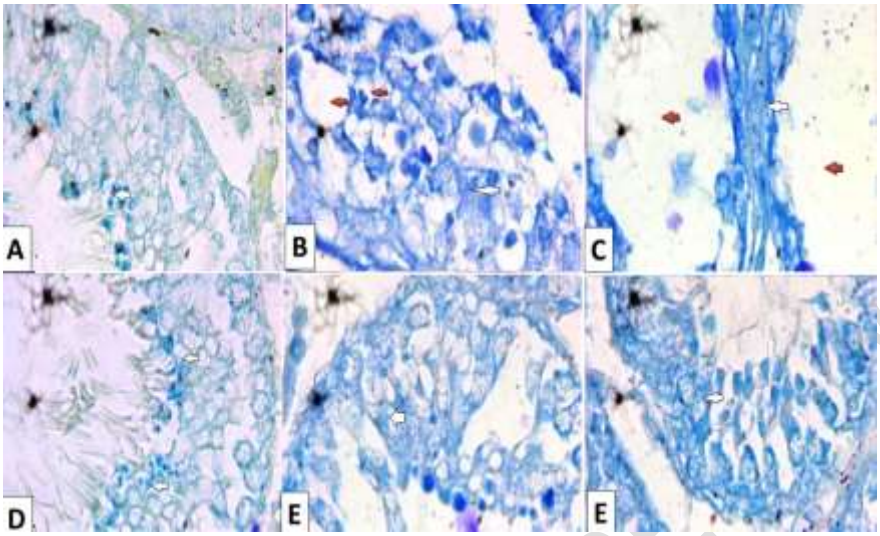
X100

Plate 1. Photomicrographs of Epididymal sections stained by Haematoxylin and Eosin showing normal epididymal ducts (a, d, e, f) lined by normal epithelial layers with normal smooth muscle layer (black arrow). The ducts are seen storing spermatozoa within the lumen (white arrow). The interstitial spaces appear normal while others look degenerated. Some ducts (b) are seen storing reduced volume of spermatozoa within the lumen while (c) appears almost empty (white arrow) indicating reduced spermatogenic activity Mag X100.



X400

Plate 2. Photomicrographs of Epididymal sections stained by Haematoxylin and Eosin showing normal epididymal ducts (a, d, e, f) lined by normal epithelial layers with normal smooth muscle layer (black arrow). The ducts are seen storing spermatozoa within the lumen (white arrow). The interstitial spaces appear normal while others look degenerated. Some ducts (b) are seen storing reduced volume of spermatozoa within the lumen while (c) appears almost empty (white arrow) indicating reduced spermatogenic activity Mag X400.



X400

Plate 3. Photomicrographs of testicular sections stained by Toluidine blue showing normal seminiferous tubules lined by and the lumen lined by normal spermatids (white arrow) as seen (a, d, e, f) with normal spermatids (white arrow), The interstitial spaces appear normal. However, some tubules (b) show vacuolation and the lumen lining show little spermatids (red arrow) while (c) appear almost empty (red arrow). Degenerated germ cells are stained deep blue. Mag. X400.

3.2 Discussion

The effects of the plant extract on organ weight examined in this study showed that increased dose of methanol extract of *Sphenocentrum jollyanum* root resulted in the significant reduction of the testes and epididymides' weight which is in tandem with the study by Raji *et al*., (2006) and Baffoe *et al*., (2021). Since the testis and epididymis weight is synonymous to the process of production and storage of spermatozoa in the testis and epididymis respectively, it's safe to say that administration of high dose of *Sphenocentrum jollyanum* root thus caused decrease in testicular and epididymal weights as also reported earlier (Raji *et al*., 2006; Baffoe *et al*., 2021). However, zinc helped reverse the effects by increasing the testes and epididymides weights, this can be observed in the zinc 20mg/kg treated animal group in this study in which testicular and epididymal weights increased. This was supported by Ebaid *et al*., (2024) who reported that Zinc significantly helped restore epididymal normal relative weight after Olanzapin- induced testicular toxicity. WHO reported global Zinc deficiency to be one-third of the total human population, Bashandi *et al.,* (2016) reported that Zinc aids spermatogenesis and improves sperm quality. Also, Sair *et al*., (2022) suggested that Zinc was able to prevent damage of the testes by Indomethacin. Similarly, the effects of zinc when used as an adjuvant with the plant extract on epididymis were also observed in the groups co-administered with Zinc 20mg/kg and *Sphenocentrum jollyanum* 500mg/kg and 1,000mg/kg respectively. A non-significant increase epididymis weights was observed, dose-dependently, and this suggests that the effects of the plant extracts with zinc may have varying effects. The increase could be a result of some factors or interaction. Previous studies reported non-significant increase in testis and epididymis weights (Raji *et al*., 2006; Baffoe *et al*., 2021).

According to the results of this study, the percentage of sperm count, Sperm motility, morphology, and viability were significantly reduced with *Sphenocentrum jollyanum* administration in agreement with a study done by Raji *et al*., (2006), Wopara *et al*., (2020) and Baffoe *et al*., (2021) who both reported the dose-dependent adverse effects of *Sphenocentrum jollyanum* on the sperm parameters.

In this study, it was observed that the percentage sperm count was highest in the zinc 20mg/kg treated animals, followed by the control group animals and the sperm count largely decreased in animals administered with methanol extract of *Sphenocentrum jollyanum* root (500mg/kg and 1,000mg/kg respectively) while 20mg/kg helped reverse the decrease in the sperm count in the animal groups co-administered with Zinc 20mg/kg and methanol extract of *Sphenocentrum jollyanum* root at 500mg/kg and 1,000mg/kg respectively. The increase in sperm count of the zinc treated group was supported by Razavi *et al*., (2019); Adelakun et al., (2022) and Chen *et al*., (2024) while the decrease in sperm count of animals treated with methanol extract of *Sphenocentrum jollyanum* root was in agreement with earlier studies done by Raji *et al*., (2006) and Baffoe *et al*., (2021).

It was also observed that the rapid progressive sperm motility significantly increased following administration of zinc 20mg/kg ($p<0.05$) in accordance with the report of Chen *et al*., (2024). Slow progressive motile sperms were highest in animal group treated with 1,000mg/kg of methanol extract of *Sphenocentrum jollyanum* root, followed by the group treated with 500mg/kg as also reported by Baffoe *et al*., (2021). The non-progressive motile sperms were significantly increased in the animal group treated with 500mg/kg of methanol extract of *Sphenocentrum jollyanum* root, followed by the animal group treated with 1000mg/kg of methanol extract of *Sphenocentrum jollyanum* root which may indicate varying effects of the extract at different dosage (Baffoe *et al*., 2021). The dead sperm cells were high in both groups treated with methanol extract of *Sphenocentrum jollyanum* root at 500mg/kg and 1,000mg/kg while it remained the same across other animal groups. The Zinc 20mg/kg treated group had the highest percentage of viable normal sperm morphology and the least number of defective sperms, possibly attributed to the spermatogenic effect of Zinc (Chen *et al*., 2024), while the groups treated with methanol extract of *Sphenocentrum*

jollyanum root had reduced percentage of sperms with normal morphology and increased number of defective non-viable sperms (Baffoe *et al*., 2021). The reduced level of the antioxidant markers in this study could be a factor for the adverse effects of *Sphenocentrum jollyanum* on the integrity of sperm parameters. However, the co-administration of the extract and zinc in this study shows ameliorative effects of zinc when compared to the group that received the plant extracts alone. The Sperm count, motility, morphology, and viability were fairly improved with zinc administration.

Phytochemical screening of *Sphenocentrum jollyanum*, revealed the presence of secondary metabolites such as alkaloids, saponins, flavonoids, and tannins. Alkaloids as a major constituent of Sphenocentrum jollyanum contains isoquinoline alkaloids such as berberine which has been associated with various pharmacological activities, including potential stimulation of sexual behavior, Owiredu *et a*l., (2007) also stated that isoquinoline can influence neurotransmitter systems like Gonadotropin releasing hormone (GnRH), which may initially enhance libido and sexual performance. The initial release of LH and FSH by the action of GnRH thus leads to the production of Testosterone which aids spermatogenesis and penile erection as reported by Owiredu *et al*., (2017). However, Raji *et al*., (2006) slams at alkaloids in his findings where he examined the effects of the plant extracts in albino mice. According to Böhm and Koller, (2016) flavonoids present in *Sphenocentrum jollyanum* are known for their antioxidant properties and have been linked to improved blood circulation and relaxation of blood vessels, which can enhance erectile function and overall sexual health. The continued production of Testosterone in proceeding weeks of administration of extract of *Sphenocentrum jollyanum* tends to lead to the negative feedback mechanism on the hypothalamus through the HPG axis; thus, leading to reduction in the level of FSH, LH and then Testosterone produced as evident in this study, this was supported by the reports of Raji *et al*., (2006) and Baffoe *et al*., 2021.

The hormonal results from this study are consistent with Wopara et al., (2020), Raji *et al*., (2006) and Baffoe *et al*., (2021) who reported that the *Sphenocentrum jollyanum* extracts had detrimental effects observed on the levels of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) thus leading to the decreased level of Testosterone in animals administered with the plant extract. However, a study by Owiredu *et al*., (2007) reported that the extract of the *Sphenocentrum jollyanum* when administered, as an aphrodisiac, initially caused increase in the level of testosterone in the second week of administration to increase sexual desires before the level continued to drop in proceeding weeks of administration. In this study is however in agreement with the studies of Wopara *et al*., (2020), on the effects of administration of extract of the *Sphenocentrum jollyanum* in causing decrease in the levels of Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and Testosterone.

Egwurugwu *et al*., (2013) earlier reported that doses of Zinc less than 40mg/kg had beneficial effects on the level of reproductive hormones needed for spermatogenesis. It was observed in this study that the level of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) increased in the Zinc treated groups compared to the control (Egwurugwu *et al*., 2013) while it decreased in the animal groups treated with methanol extract of the *Sphenocentrum jollyanum* root (Raji *et al*., 2006; Baffoe *et al*., 2021) while a recovery increase can be observed in the groups co-administered with Zinc 20mg/kg and methanol extract of *Sphenocentrum jollyanum* root at 500mg/kg and 1,000mg/kg respectively when compared with the groups administered with methanol extract of *Sphenocentrum jollyanum* root only. The testosterone level was increased in the Zinc-treated groups compared to the control due to the spermatogenic effect of Zinc in spermatogenesis (Egwurugwu *et al*., 2013; Ma *et al*., 2020; Esfiokhi et al., 2023) while it decreased in groups treated with methanol extract of *Sphenocentrum jollyanum* root at 500mg/kg and 1,000mg/kg when compared to the control as supported by Raji *et al*., (2006) who reported that level of testosterone decreased with doses higher than 50mg/kg. The report of Baffoe *et al*., (2021) also agrees with this.

The histological examination of the testicular tissue with the usage of the methanol extract of *Sphenocentrum jollyanum* root in this study in tandem with previous reports (Raji *et al*., 2006; Baffoe *et al*., 2021). The Haematoxylin and Eosin (H&E)-stained testicular section of animal groups treated with methanol extract of *Sphenocentrum jollyanum* root revealed several seminiferous tubules with wider lumen indicating spermatogenic arrest and degeneration of the spermatocytes, with the distortion of the interstitial spaces and degenerating Leydig cells. These degenerative changes are suggestive of varying degrees of Oligospermia depending on the dosage of the extract administered. A well-defined normal testicular and epididymal histology is highly required for the process of spermatogenesis as reported and histopathological changes can cause arrest of spermatogenesis, hypospermia, oligospermia etc. (Moore *et al*., (2006) which likely explains the reason for the anti-ferltility effects of the plant extract. Similar effects were observed with the epididymis, suggesting that these phytochemical contents of the plant play a role in disrupting normal spermatogenesis and overall reproductive health. The ducts of animals treated with methanol extract of *Sphenocentrum jollyanum* root are seen with no storage of spermatozoa within the lumen indicating reduced or no spermatogenic activity. The reports of Wopara *et al*., (2020) and Baffoe *et al*., (2021) support this. The Zinc treat animal groups in this study, in contrast had a well-defined normal testicular and epididymal histoarchitecture in accordance with the report of Razavi *et al*., (2019). The animal groups co-administered with Zinc 20mg/kg and methanol extract of *Sphenocentrum jollyanum* root at 500mg/kg and 1,000mg/kg respectively appeared better compared to the methanol extract of *Sphenocentrum jollyanum* root treated groups, which is suggestive of Zinc-ameliorative properties when co-administered with the extract. Toluidine blue-stained testicular sections of the extract treated animal groups also indicated big tubular vacuolation with degeneration of the germinal layer. Toluidine blue-stained testicular sections of the Zinc-treated animal group showed normal seminiferous tubules and the lumen lined by normal spermatids (with their tailpiece, middle-piece and headpiece seen) thus indicating normal sperm morphology. Groups co-administered with Zinc 20mg/kg and methanol extract of *Sphenocentrum jollyanum* root at 500mg/kg and 1,000mg/kg respectively appeared close to normal histomorphology, suggestive of the ameliorative properties of zinc when co-administered with methanol extract of *Sphenocentrum jollyanum* root.

4. CONCLUSION

This study affirms the previous evidence that shows the adverse effects associated with the use of *Sphenocentrum jollyanum* as a traditional medicinal agent. Its wide usage across Sub-Saharan Africa has however been discouraged due to the anti-fertility effect that Sphenocentrum jollyanum has on the male reproductive system as evidenced by recent studies. In this regard, the zinc sulphate adjuvant used in this study showed an ample beneficial impact on the levels of follicle-stimulating hormone, Luteinizing hormone, testosterone, sperm count, motility, morphology, sperm viability, and histomorphology of the epididymis. This information would assist the regulatory bodies in making an objective appraisal of the usage of zinc as an adjunct to the use of Sphenocentrum jollyanum.

This study did not elucidate the exact active phytochemical component of Sphenocentrum jollyanum responsible for its adverse effects. Further research is needed to focus on the exact phytochemicals responsible for its effects. The usage of zinc with Sphenocentrum jollyanum requires a further investigation to ascertain that there is no contraindication of the two substances in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical approval for this research study was granted by the Ethical Research Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria with the approval number **ERCFBMSLAUTECH: 034/05/2024**.

التعليق **[DS26]:** The authors must ensure that all references cited in the text are included in the reference list, and vice versa.

REFERENCES

Aboaba, S.A. & Ekundayo, O. (2010). Constituents, antibacterial activities and toxicological assay of essential oils of Artocarpus communis Forst (Moraceae) and Sphenocentrum jollyanum (Menispermaceae). Int. J. Biol. Chem. Sci., 4, 1455–1461.

Adelakun, S.A., Ogunlade B., Fidelis, O.P. & Omotoso, O.D. (2022). Protective effect of nutritional supplementation of zinc-sulfate against cisplatin-induced spermatogonial and testicular dysfunctions in adult male Sprague-Dawley rats. Journal of Endocrine and Metabolic Science 100116.

Amidu, N. (2008). An Evaluation of the Central and Sexual Behavioral Effects and Toxicity of the Root Extract of Sphenocentrum jollyanum Pierre (Menispermaceae). Ph.D. Thesis, Department of Molecular Medicine, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana.

Baffoe, M., Koffuor, G., Baffour-awuah A. & Sallah l. (2021). Assessment of reproductive toxicity of hydroethanolic root extracts of caesalpinia benthamiana, sphenocentrum jollyanum, and paullinia pinnata. J exp pharmacol. 13:223-234.

Bashandy S.M., Omara E.A., Ebaid, H., Amin, M.M. and Soliman, M.S. (2016). Role of zinc as an antioxidant and anti-inflammatory to relieve cadmium oxidative stress induced testicular damage in rats. Asian Pacific Journal of Tropical Biomedicine.;6(12):1056-64.

Böhm, V., & Koller, M. (2016). Dietary flavonoid intake and incidence of erectile dysfunction. American Journal of Clinical Nutrition, 103(5), 1303-1310.

Chen Y., wang X., Zhou J., Wang G., Gao T., Wei h., Che Y., Li t., Zhang Z., Wang S. & Hu l., renjie l. B. (2024). Association of seminal plasma zinc levels with human semen quality and its toxic effects on sperm motility. Journal of ecotoxicology andenvironmental safety 284.

Ebaid H., Bashandy A. E., Hassan I., Al‐tamimi J., Haredy S. A., Imbabi T., Omara E. A., Bashandy Y. A. & Awad E. M. (2024). The preventive effect of zinc sulfate against olanzapine‐induced testicular toxicity in male rats. https://doi.org/10.1007/s12011-024-04442-8.

Egunyomi, A.; Fasola, T.R. & Oladunjoye, O. (2005). Charring Medicinal Plants: A Traditional Method of Preparing Phytomedicines in Southwestern Nigeria. EthnoBot. J., 3, 261–265.

Egwurugwu J. N., Ifedi C. U., Uchefuna R.C, Ezeokafor E.N. & Alagwu E.A (2013). Effects of zinc on male sex hormones and semen quality in rats. Physiological society of Nigeria.

Esfiokhi S.H., Norouzian M.A. & Najafi A. (2023). Effect of different sources of dietary zinc on sperm quality and oxidative parameters. Journal of Frontiers in Veterinary science. 10:1134244. doi: 10.3389/fvets.2023.1134244

Ekpono, E.U., Aja P. M., Ugwu P. C., Udeozor P. A., Uwa C. U. & Nweke O. L. (2018). Phytochemical and proximate analysis of Sphenocentrum Jollyanum methanol. Journal of Biology, Chemistry and Pharmacy. 2(1): p. 18-17.

Feferkorn I., Azani l., Kadour-peero E., Shrem G., Salmon-divon M., Dahan M. H. (2022). An evaluation of changes over time in the semen parameters data used for the world health organization semen analysis reference ranges, Journal of Andrology. (4):660-668.

Ibironke, A.A. and Olusola, O.O. (2013). Phytochemical analysis and mineral element composition of ten medicinal plant seeds from South-west Nigeria. N. Y. Sci. J., 6, 2–28.

Kayode, J.; Ige, O.E.; Adetogo, T.A. and Igbakin, A.P. (2009). Conservation and Bio derversity in Ondo State, Nigeria. Survey of plant barks used in native pharmaceutical extraction in Akoko region. EthnoBot. Leafl. 13, 665–667

Ma, J., Han R., Li Y., Cui T., & Wang S. (2020). The Mechanism of Zinc Sulfate in Improving Fertility in Obese Rats Analyzed by Sperm Proteomic Analysis. BioMed Research International, 987636.

Moody, J.O.; Robert, V.A.; Connolly, J.D.; Houghton, P.J. (2006). Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of Sphenocentrum jollyanum Pierre (Menispermaceae). J. Ethnopharmacol. 104, 87–91.

Moore, K.L. & Dalley, A.F. (2006) clinically oriented anatomy. 5th edition, lippincott williams and wilkins, baltimore, 556-717.

Muko K.N., Ohiri P.C. & Ezugwu C.O. (2008). Antipyretic and analgesic activities of Sphenoceutrum Jollyanum. Nigerian Journal of Natural Products and Medicine 2, 52-537.

Nia R, D.H. Paper, E.E. Essien, K.C. Iyadi, A.I.L. Bassey, A.B. Antai, G. Franz (2004). Evaluation of the anti-angiogenic effects of Sphenocentrum jollyanum Pierre. Afr. J. Biomed. Res. 7 129 – 132.

Oliveira P. F., Tomás G. D., Dias T. R., Martins A. D., Rato l., Alves M. G. & Silva B. M. (2015). White tea consumption restores sperm quality in prediabetic rats preventing testicular oxidative damage. Journal of reproductive biomed online 31(4):544-56.

Olooto & Wasiu. (2012). Infertility in male; risk factors, causes and management-a review. Journal of microbiology and biotechnology research. 2. 641-645.

Owiredu, W.A.; Amidu, N.; Amissah, F.; Woode, E. (2007). The effects of ethanol extract of root of Sphenocentrum jollyanum Pierre on sexual behavior and hormonal levels in rodents. J. Sci. Technol., 27, 9–21.

Mbaka, **G**., **A**deyemi, **O**., **O**sinubi, **A**., **N**oronha, **C**., & **O**kanlawon, **A**. (2009). The effect of aqueous root extract of sphenocentrum jollyanum on blood glucose level of rabbits. Journal of medicinal plants research, 3(12), 872-877.

Odugbemi T. (2006). Medicinal Plants by Species Names: Outlines and Pictures of Medicinal Plants from Nigeria; Lagos University Press: Ojo, Lagos State, Nigeria.

Olorunnisola, O.S.; Adetutu, A.; Fadahunsi, O.S. (2017). Anti-allergy potential and possible modes of action of Sphenocentrum jollyanum Pierre fruit extracts. J. Phytopharm., 6, 20–26.

Oke, J.M.; Hamburger, M.O. (2002). Screening of some Nigeria medicinal plant Antioxidant Acitivity Using 22-Diphenyl-picryl-hydrazyl radical. Afr. J. Biomed. Res., 5, 77–79.

Raji, **Y**. F., **A**disa, **O**. O., **S**alami, **R**. A., & **S**hakiru, **A**. (2006). Comprehensive assessment of the effect of sphenocentrum jollyanum root extract on male reproductive activity in albino rats. Reproductive medicine and biology, 5(4), 283-292.

Razavi S., Khadivi F, Hashemi F, Bakhtiari A. (2019). Effect of zinc on spermatogenesis and sperm chromatin condensation in bleomycin, etoposide, cisplatin treated rats. Cell. J. 20(4): 521-526. doi: 10.22074/cellj..5522

Sair M., Inam A., Gul S., Adil R., Sadiq M. A. & Kha M.S. (2022**). E**ffect of zinc on spermatogenesis. Pjmhs vol. 16, no.01**.**
Schisterman, E.F., Clemons, T., Peterson, C.M., Johnstone, E., Hammoud, A.O., Lamb, D., Carrell, D.T., & Hotaling, J.M. (2019). Journal of the American Medical Association

Schoch C, Ciufo S, Domrachev M, Hotton C, Kannan S, Khovanskaya R. (2020). NCBI: Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford).

Tekuri S. K., Bassaiahgari P., Gali Y., Amuru S. R. & Pabbaraju N. (2021). Determination of median lethal dose of zinc chloride in wistar rat. Advances in animal and veterinary sciences. 9(3): 393-399.

Ugwu P.C. & Amasiorah V.I. (2020). The effects of crude ethanol root extract and fractions of sphenocentrum jollyanum on the lipid profile of streptozotocin-induced diabetic wistar albino rats. IDOSR Journal of Biology, Chemistry and Pharmacy 5(1)36-46,.

Wopara, I., Mobisson, S. K., Olusegun, G. A., Egelege, A. P., Etim, O. E., & Awarajih, U. C. (2020). The impact of sphenocentrum jollyanum, baphia natida and combined extract consumption on reproductive indices and antioxidants gene of male wistar rats. International journal of innovative science and research technology, 5(4), 442-449.

Rahayi I., Usman E., Reza M. (2019). Effect of vitamin C on testosterone level, sperm count and sperm morphology in gentamicin-induced Wistar rats. International Journal of Research in Medical Sciences 7(2):451

Razavi S., Khadivi F, Hashemi F & Bakhtiari A. (2019). Effect of zinc on spermatogenesis and sperm chromatin condensation in bleomycin, etoposide, cisplatin treated rats. Cell J.; 20(4): 521-526. Doi: 10.22074/cellj.5522

Zhang, Y., Wang, H., Zhang, L., & Wang, Y. (2016). Zinc levels in seminal plasma and their correlation with male infertility: A systematic review and meta-analysis. Scientific Reports, 6, 22473.

Zhang, Z. M.; Li, G. K. (2007). A preliminary study of plant aroma profile characteristics by a combination sampling method coupled with GC–MS. Microchem. J., 86, 29-36.