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ORIGINAL RESEARCH

Assessment of Reproductive Toxicity of Hydroethanolic Root Extracts of *Caesalpinia benthamiana*, *Sphenocentrum jollyanum*, and *Paullinia pinnata*

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Purpose: Male sexual dysfunction negatively affects an individual's quality of life and thus its of prime public concern, hence the need to boost reproductive abilities in such individuals. This study assessed the effect of hydroethanolic root extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE), commonly used as aphrodisiacs in Ghana, using male Sprague-Dawley rats.

Methods: Plasma testosterone, follicle-stimulating hormone, and luteinizing hormone were assayed in grouped rats treated orally with 1 mL/kg normal saline, 50 mg/kg monosodium glutamate (MSG), and 100, 300, or 1000 mg/kg CBRE, SJRE, and PPRE, respectively, for 60 days. Epididymis and testis weights were determined. Semen was assessed on spermatozoa count, motility, and morphology. Malonyladehyde formation in lipid-peroxidation assay and histological examinations were performed to assess pathological changes in testes. Testicular testosterone was also assayed.

Results: While MSG, CBRE, SJRE, and PPRE treatments did not result in significant reduction ($p>0.05$) in plasma testosterone, there was significant reduction ($p\leq0.05$ −0.0001) in plasma luteinizing hormone, and follicle-stimulating hormone. The combined mean wet weights of epididymides and testes of all treated groups did not vary significantly ($p>0.05$) from the control. There was significant reduction ($p\leq0.0001$) in sperm motility and count, with significant morphological changes ($p\leq0.05$–0.001), ie, bent necks, tails, and midpieces, and multiple anomalies in the spermatozoa in extract and MSG-treated groups. There was also significant ($p\leq0.0001$) reduction in testicular testosterone among all treatment groups.

Conclusion: Hydroethanolic CBRE, SJRE, and PPRE were found to have detrimental effects on reproductive function with prolonged usage and thus may not be safe to use in healthy males who intend to reproduce.

Keywords: plasma follicle-stimulating hormone, plasma luteinizing hormone, sperm anomalies, sperm motility, testicular testosterone, monosodium glutamate, herbal aphrodisiacs

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Plain Language Summary

In Ghana, the use of herbal aphrodisiacs by majority of young men is gradually becoming the order of the day. This study therefore assessed the safety of the use of root extracts of three common herbal aphrodisiacs: *Caesalpinia benthamiana*, *Sphenocentrum jollyanum*, *and Paullinia pinnat*a. Results revealed that chronic use of these herbals as aphrodisiacs had

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detrimental effects on semen quality and thus could negatively affect the capability of reproductive-age males to produce offspring, even though they enhance copulation and pleasure.

Introduction

Issues of male sexual dysfunction and reproductive inability are of prime public concern and an important part of an individual's quality of life, and thus people continue to search for substances they can use to heighten their sexual abilities. In the Ghanaian society, it is often an ignominy for a man to be identified with sexual weakness; therefore, sexual vitality is of great necessity to many who eventually use aphrodisiacs.[1–3] The introduction of varieties of herbal aphrodisiacs, driven in part by heavy advertising, has caused a flutter of public attention[4] toward their use when the individual does not even have sexual dysfunction.[5] There are increasing numbers of people who may be susceptible to adverse drug effects, should there be any. The risks of use and abuse of aphrodisiacs are also high, as these preparations are now sold in supermarkets, fuel stations, market places, transport stations, commercial centers, and communication centers, among others, without supervision of any kind.

There are claims that some of these drugs could cause impotence, kidney failure, and problems with hearing and sight, among others. Some aphrodisiacs have also been presumed to cause male fertility problems. For example, studies conducted on *Mondia whitei*, a herbal aphrodisiac, have revealed that chronic administration resulted in antispermatogenic and antifertility effects in male albino rats.[6] A market survey in Ghana conducted by the authors revealed *Caesalpinia benthamiana*, *Sphenocentrum jollyanum*, and *Paullinia pinnata* to be among herbs popularly recommended by herbal medical practitioners and used as aphrodisiacs in Ghana. These plants have also been indicated as such in the literature.

C. benthamiana and *Mezoneuron benthamianum* (family Caesalpiniaceae), locally called *akoo boree*, are widespread in West and Central Africa and have been traditionally used in the management of erectile dysfunction, dysentery, urethral discharge, skin diseases and wounds. These species have antibacterial, vasorelaxation, and antioxidant properties.[7,8] *S. jollyanum* (family Menispermaceae) is a shrub native to the tropical forest zones of Sierra Leone, Nigeria, Ghana, Ivory Coast, and Cameroon.[9] It is known locally as *aduro kokoo* (red medicine), *okramankote* (dog's penis), and *krakoo* among the Akan and Asante tribes of Ghana.[10] Although the root of this plant is widely used as a male aphrodisiac, several studies have indicated that *S. jollyanum* has antioxidant, antiangiogenic, anti-inflammatory, antipyretic, antinociceptive, antitumor, antiviral, laxative, stomachic, and tonic activity.[11,12] *P. pinnata* (family Sapindaceae) locally known as t*wantini*, is an African tropical plant whose roots and leaves are used in traditional medicine for many purposes (wound healing, treatment of dysentery, arthritis, malaria), especially for erectile dysfunction.[13,14]

It is thus required that studies be carried out on these herbal aphrodisiacs to determine possible adverse effects associated with their use to get the public and health policy makers informed to enable them to make decisions that will help regulate the exposure of individuals to such drugs.[15]

This study thus assessed the toxicity of hydroethanolic root extracts of *C. benthamiana, S. jollyanum*, and *P. pinnata* in male Sprague-Dawley rats.

Methods

Choice and Collection of Herbal Aphrodisiacs

A total of 20 medicinal herb sellers (17 men and three women) aged 35–60 years in the herbal market section of the Kumasi Central Market, Kejetia, in the Ashanti region of Ghana, were interviewed in their local language using unstructured questionnaires on the patronage of herbal aphrodisiacs. The sellers gave their knowledge on herbs reported by users to boost libido, enhance erection, and increase male virility. From the interactions, information on local names of plants with aphrodisiac properties, the plant part used, their form of preparation and usage, and their mode of activity, among other things, were obtained. The three most frequently mentioned plants — C. *benthamiana*, S. *jollyanum*, and *P. pinnata* — were then selected for this study (Table 1).

Table 1 Eight Frequently Purchased Herbal Aphrodisiacs from the Kumasi Central Market, in the Ashanti Region of Ghana

	Local Name	Part Used	Frequency
Caesalpinia benthamiana	Akoo boree	Root	8
Sphenocentrum jollyanum	Kraman koti	Root	8
Paullinia pinnata	Twantini	Root	7
Mondia whitei	Asaase wham	Root	7
Guilandina bonduc	Oware nhyini	Root	6
Chrysophyllum beguei	Atadwe dua	Root	5
Khaya senegalensis	Mahoghany	Root	5
Vitex grandifolia	Dunsikro	Root	4

Preparation of Hydroethanolic Root Extracts of Selected Plants

Roots of *C. benthamiana, S. jollyanum*, and *P. pinnata* were sun-dried and crushed into coarse powder using a hammer-mill (STEDMAN 20×18, USA). The powders (5 kg each) were extracted separately with 70% v:v ethanol in a soxhlet apparatus for 72 hours. The filtrates obtained were each concentrated at a low temperature in a vacuum rotary evaporator (CMRE-10110) under reduced pressure to obtain a thick mass that was dried in a hot oven (PT2010) at 40°C for 24 hours. Root-extract yields of 10.2%, 11.7%, and 7.4% were obtained for *S. jollyanum, C. benthamiana*, and *Paullinia pinnata*, respectively. These extracts were labeled CBRE, SJRE, and PPRE, respectively, and kept in a desiccator at room temperature for usey.

Experimental Animals and Husbandry

Male Sprague Dawley rats weighing 150–230 g were purchased from the Centre for Plant Medicine Research, Akuapem-Mampong, in the Eastern Region of Ghana and kept in the Animal Facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in stainless steel cages (36×50×20 cm) with wood shavings as bedding, maintained under normal laboratory conditions (24°C–28°C, relative humidity 60%–70%, and ambient light–dark cycle), and given free access to a solid-pellet diet (Agricare, Kumasi, Ghana) and water throughout the study. All animals used in this study were treated in accordance with the US National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* (Department of Health and Human Services publication 85–23, revised 1985).

Establishing Experimental Doses

To establish favorable doses of the extracts to be used, an experiment previously described by Irwin[16,17] was performed. Normal healthy rats were put into three groups (n=5) and treated with 50, 500, or 1,000 mg/kg CBRE daily, observed and assessed for behavior specifically related to neurotoxicity, central nervous systemstimulation or depression, autonomic function, and lethality at 30 minutes, 1, 2, 3, and 24 hours, and subsequently daily for 2 weeks. The same test was done for SJRE and PPRE. The dose for monosodium glutamate (MSG; the reference drug for reproductive toxicity in this study) was selected based on previous research.[18]

Assessing Reproductive Toxicity of SJRE, PPRE, and CBRE

Experimental Grouping and Dosing of Animals

Experimental animals were put into eleven groups of seven animals each. Group A (vehicle-treated control) was administered 1 mL/kg normal saline. Group B (positive control) was treated with 50 mg/kg MSG to induce oxidative stress in various body organs, including the testes, while groups C, D, and E were dosed with 100, 300, and 1,000 mg/kg CBRE, respectively. Groups F, G, and H were treated with 100, 300, and 1,000 mg/kg SJRE, respectively, and groups I, J, and K 100, 300, and 1,000 mg/kg PPRE, respectively. Treatments were given orally and daily over a period of 60 days. Blood samples were taken from animals in each group by cardiac puncture into well-labeled gel-separation Eppendorf (Vacutainer) tubes for plasma-hormone assays. The animals were humanely killed after anesthetization with phenobarbitol (50 mg/kg intraperitoneally) and testes and epididymides removed for weight determination, semen analysis, and testicular testosterone assays.

Plasma-Hormone Assays

Blood samples in the Eppendorf tubes were centrifuged at 2,500 rpm for 5 minutes using a Wisperfuge 1384 (Tamson Instruments) at 25°C to obtain plasma samples, which were assayed for testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) with analytical grade reagents (Syntron Bioresearch) using ELISA.[19]

Testosterone Assays

A 50 µL measure each of calibrator, control, and test samples was placed in the corresponding labeled wells on a microtiter plate (in triplicate) followed by addition of 100 µL conjugate solution. The solution was properly mixed and incubated on a microplate shaker running at approximately 200 rpm for 1 hour at 25–28°C. Each well was washed properly with 300 µL diluted buffer and the plate dried by tapping against absorbent paper. A 150 µL measure of tetramethylbenzidine substrate was added to each well and incubated for 15 minutes at room temperature before the addition of 50 µL stop solution. Absorbance in each well was read at 450 nm within 20 minutes after addition of the stop solution using spectrophotometry (LabTech advanced microprocessor UV-vis single beam 295). A graph of absorbance versus concentration of the calibrator was plotted and used to extrapolate the actual amount of the hormone in the test samples.

Follicle-Stimulating Hormone and Luteinizing Hormone Assays

Adequately coated microtiter plate wells were selected to run 0, 25, 50 and 100 mIU/mL for FSH calibrators, control, and test samples in triplicate. A 50 µL quantity of each sample was pipetted into the corresponding coated well with addition of 200 µL of the enzyme–antibody conjugate solution. The solution was mixed appropriately and incubated at room temperature for 45 minutes. Wells were then washed with a buffer and deionized water and decanted. Substrate-chromogen solutions (100 µL) were added to each well, mixed thoroughly, and incubated for 15 minutes at room temperature. Shortly after, 100 µL 1 N H_2SO_4 was added to each well and appropriately mixed. Absorbance in each well was read at 450 nm spectrophotometrically. A graph of absorbance versus concentration of the calibrator was plotted and used to extrapolate the actual amount of the hormone in the test samples. A similar procedure was used for LH assays.

Determination of Epididymis and Testis Weight

Epididymides and testes were dissected from treated rats and weighed using a top-loading balance (Grand G). Organ weight:body weight ratios were calculated and compared to the control groups.

Semen Analysis

Semen samples from rats in each treatment group were collected by humanely killing the rat, dismembering the epididymis, and squeezing its content into 0.5 mL normal saline. As soon as liquefaction of the semen had occurred (within 15–30 minutes of collection), sperm count, motility, and abnormalities were assessed.

Estimation of Sperm Count

This was carried out using the modified method of Ekualo et al.[20] Semen samples were mixed thoroughly and drawn to the 0.5 mark on the pipette. This was then diluted to the 11 mark on the pipette using semen-diluting fluid (sodium bicarbonate 5 g, formalin 1 mL, and distilled water to 100 mL). A tissue-free aliquot was loaded into a Neubauer hemocytometer (depth 1/10) and allowed to settle for 2 minutes. Five counts were performed for each sample, and the mean calculated and taken as the mean count for each male rat. The sperm count was estimated:

$$\text{sperm count/mL} = n \times 40{,}000$$

where n = total number of sperm cells in five cells of the cytometer.

Evaluation of Sperm Motility

Liquefied semen samples from the treatment groups were placed on a glass slide, covered with a coverslip ringed with petroleum jelly, and viewed under microscopy (BX51, China). Assessment was made from a minimum of five microscopic fields to evaluate sperm motility on at least 200 spermatozoa for each rat. Sperm motility was analyzed for progressive motile sperm, unprogressive motile sperm, and immotile sperm, distinguished by the movement of the sperm cells.[21]

Sperm Morphology

A fraction of each of the sperm suspensions was examined by placing the solution (20:1) on a glass slide, drying, in air, and fixing by heat. Chloramine solution (1%) was added for several minutes to remove excess mucus. The fixate was then washed and dried by blotting on filter paper. It was stained for 5 minutes (Ziehl–Neelsen carbol fuchsin two parts, concentrated alcohol solution of eosin one part, and 95% alcohol one part), washed with water, and counterstained with Loeffler's methylene blue for a few seconds. The slide was then washed, dried, and examined under oil immersion for percentage abnormalities in every 200 spermatozoa seen on each slide, and five air-dried smears were prepared on glass slides for each sample.[22]

Testicular Testosterone Assay

Testes of animals of the treatment groups were collected into glass containers containing 1 g/mL Tris phosphate buffer, pulverized, and allowed 30 minutes to settle. Supernatants were then collected into separately labeled sample tubes and centrifuged (Wisperfuge 1384) at 2,500 rpm for 5 minutes at 25°C to obtain the physiological samples to be assayed for testosterone using analytical grade reagents (Syntron Bioresearch) using ELISA.[19]

Assessment of Pathological Changes in Testes

MDA Formation in Lipid-Peroxidation Assay

Levels of malonyladehyde (MDA) formation in tissue were determined as per Tüközkan et al[23] with slight modification. To 3 mL reagent solution (3 mL 20% TCA containing 0.5% thiobarbituric acid [TBA]), 1 mL homogenate was added in a test tube. This was heated at 95°C

for 30 minutes, cooled immediately, and centrifuged at 5,000 *g* for 10 minutes. Absorbance was firstly read at 532 nm and then 600 nm to correct for aspecific absorbance. The molar extinction coefficient of the MDA–TBA abduct, 155 mM^{-1} cm^{-1}, was used to estimate the levels of MDA:

$$Nmol\frac{MDA}{mg}protein=\frac{Absorbance\,at532nm-Absorbance\,at600nm}{155\times totalprotein}\times 10^{6}$$

Histological Examination

Tissue portions from the epididymides and testes were utilized for histological examinations. Tissue samples were fixed in 10% buffered formalin (pH 7.2), dehydrated through a series of ethanol solutions, embedded in paraffin, and routinely processed for histological analysis. Sections of 2 µm thickness were cut and stained with H&E for examination. The stained tissue samples were observed through an Olympus microscope (BX51) and photomicrographs taken with a charge-coupled camera.

Statistical Analysis

All data collected on sperm and hormonal assays are presented as means ±SEM and subjected to one-way ANOVA using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Statistical significance between treatments and control was established using Dunnett's multiple-comparison post hoc test using a 95% confidence limit. $p\leq0.05$ represented significant variation between variables.

Results

Choosing the Herbal Aphrodisiacs

C. benthamiana, S. jollyanum, and *P. pinnata* were selected for this study based on their frequency of mention in the questionnaire as has having been used successfully by the populace in enhancing libido, inducing erection, and improving male virility (Table 1).

Roots of C. *benthamiana*, S. *jollyanum*, and *P. pinnata* were bought from the market and authenticated by Dr GH Sam, a lecturer and botanist of the Department of Herbal Medicine, KNUST, Kumasi, Ghana. Samples of these plant parts with voucher numbers KNUST/HM1/2017/L011, KNUST/HM1/2017/L012, and KNUST/HM1/2017/L013, respectively, have been deposited at the herbarium of the Department of Herbal Medicine, KNUST, Kumasi, Ghana.

Effect of Extracts on Plasma Reproductive Hormones

Treatments with extracts generally did not result in significant reduction ($p>0.05$) in plasma testosterone, except for groups treated with 100 mg/kg PPRE, which caused a reduction ($p\leq0.05$). The various treatments, however, resulted in significant reductions ($p\leq0.05$–0.0001) in plasma LH at the various doses, except for the 300 and 1,000 mg/kg CBRE treatments. CBRE, SJRE, and PPRE treatments showed significant reductions in serum FSH ($p\leq0.05$–0.0001) (Figure 1).

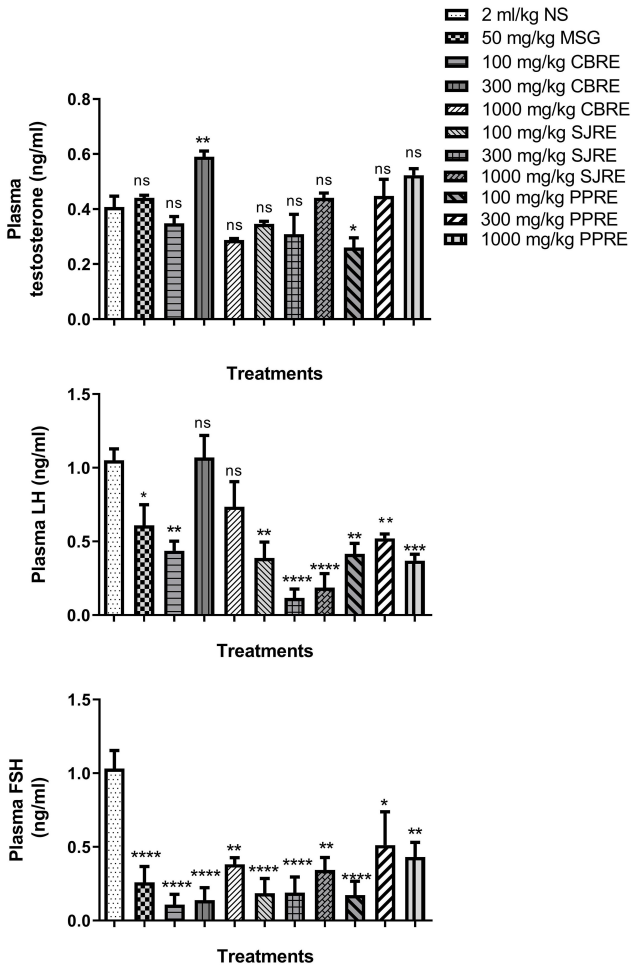

Figure 1 Plasma testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations in rats treated with normal saline (NS), monosodium glutamate (MSG), and hydroethanolic root-bark extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE) for 60 days. Data presented as group means ± SEM, n=7. Significant differences between treatment and control: ns, $p>0.05$; *$p\leq0.05$; **$p\leq0.01$; ***$p\leq0.001$; ****$p\leq0.0001$ (one-way ANOVA followed by Dunnett's multiple-comparison test).

Epididymis and Testicular Weight

The combined mean wet weights of left epididymides and left testes of the treated groups did not vary significantly ($p>0.05$, Table 2).

Semen Analysis

Sperm Count

There were significant reductions ($p\leq0.05–0.0001$) in sperm count in all treatment groups compared to the vehicle-treated group (Figure 2).

Sperm Motility

There was a significant reduction ($p\leq0.0001$) in sperm motility in the extract-treated animals compared to the vehicle-treated groups (Figure 3).

Sperm Morphology

There were varied significant morphological changes ($p\leq0.05–0.001$) in spermatozoa of animals in all treatment groups compared to vehicle-treated groups. The dominant changes occurring in animals treated with CBRE were bent tails and midpieces. PPRE-treated animals showed dominant bent necks, tails, and midpieces. SJRE-treated animals had significant levels of all the deformities recorded. Treatments showed significant levels ($p>0.05$) of multiple anomalies and headless tails (Figure 4).

Assessment of Pathological Changes

Lipid Peroxidation

The effect of each of the extracts on lipid peroxidation of testes of treated rats was measured as the amount of MDA (nmol/mg of tissue protein) formed. There were significant ($p\leq0.001$) increases in MDA concentrations in MSG-, 1,000 mg/kg CBRE–, 300 mg/kg SJRE–, and 1,000 mg/kg PPRE–treated rats. MDA concentration was highest in rats treated with 1,000 mg/kg CBRE (Figure 5).

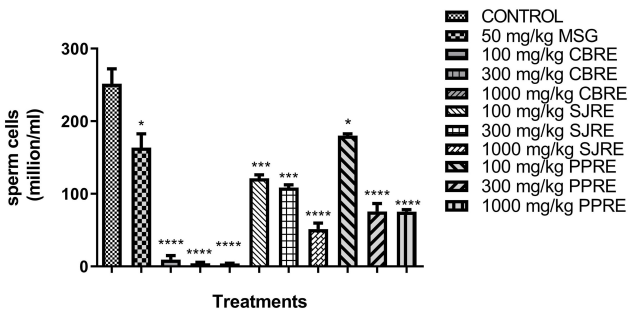

Figure 2 Sperm counts of rats treated with normal saline (NS), monosodium glutamate (MSG), and various doses of hydroethanolic root-bark extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE) for 60 days. Data presented as means ± SEM, n=7. Significant differences between treatment and control: *$p\leq0.05$; ***$p\leq0.001$; ****$p\leq0.0001$ (one-way ANOVA followed by Dunnett's multiple-comparison test).

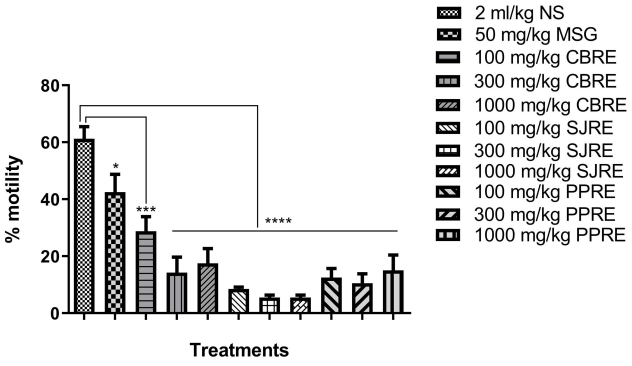

Figure 3 Percentage sperm motility of rats treated with normal saline (NS), monosodium glutamate (MSG), and various doses of hydroethanolic root-bark extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE) for 60 days. Data presented as means ± SEM, n=7. Significant differences between treatment and control: *$p\leq0.05$; ***$p\leq0.001$; ****$p\leq0.0001$ (one-way ANOVA followed by Dunnett's multiple-comparison test).

Table 2 Organ Weight to Body Weight Ratio of Sprague-Dawley Rats Treated with Normal Saline (NS), Monosodium Glutamate (MSG), and various Concentrations of Hydroethanolic Root-Bark Extracts of *C. benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE)

	Weight of Testes	Testes: Body Weight Ratio	Weight of Epididymides	Epididymis: Body Weight Ratio
2 mL/kg NS	1.725±0.14	0.0063±0	0.495±0.05	0.0015±0.0002
50 mg/kg MSG	1.530±0.03^{ns}	0.0062±0^{ns}	0.345±0.07^{ns}	0.0017±0.0001^{ns}
100 mg/kg CBRE	1.288±0.32^{ns}	0.0073±0 ^{ns}	0.3575±0.08 ^{ns}	0.0019±0.0003^{ns}
300 mg/kg CBRE	1.445±0.10^{ns}	0.0069±0^{ns}	0.450±0.05^{ns}	0.0019±0.0003^{ns}
1,000 mg/kg CBRE	1.428±0.06^{ns}	0.0064±0^{ns}	0.460±0.02^{ns}	0.0019±0^{ns}
100 mg/kg SJRE	1.478±0.03^{ns}	0.0071±0^{ns}	0.412±0.01^{ns}	0.0020±0.0001^{ns}
300 mg/kg SJRE	1.693±0.13^{ns}	0.0065±0^{ns}	0.527±0.02^{ns}	0.0017±0.0001^{ns}
1,000 mg/kg SJRE	1.683±0.15^{ns}	0.0069±0^{ns}	0.046±0.02^{ns}	0.0021±0.0001^{ns}
100 mg/kg PPRE	1.713±0.04^{ns}	0.0074±0 ^{ns}	0.465±0.02^{ns}	0.0021±0.0001^{ns}
300 mg/kg PPRE	1.688±0.08^{ns}	0.0068±0^{ns}	0.497±0.01^{ns}	0.0018±0.0002^{ns}
1,000 mg/kg PPRE	1.305±0.11^{ns}	0.0077±0^{ns}	0.407±0.05^{ns}	0.0023±0^{ns}

Notes: Results presented as means ± SEM, n=7. Treatment versus control: $^{ns}p>0.05$ (one-way ANOVA using Dunnett's multiple-comparisons test).

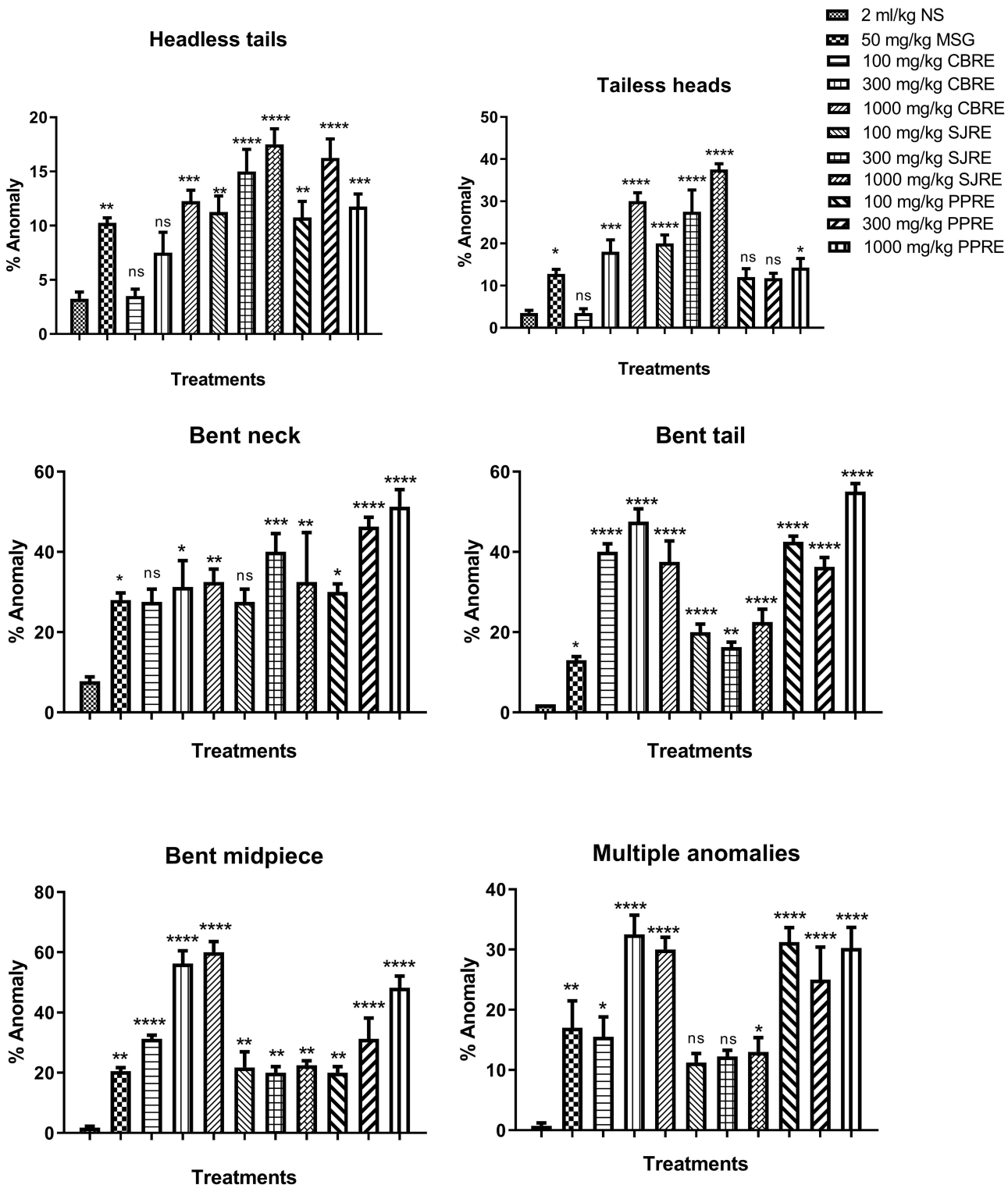

Figure 4 Sperm morphology at the end of 60 days of treatment with normal saline (NS), monosodium glutamate (MSG), and various concentrations of the hydroethanolic root-bark extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE). Values are means ± SEM, n=7. ns, $p>0.05$; *$p\leq0.05$; **$p\leq0.01$, ***$p\leq0.001$; ****$p\leq0.0001$ (one-way ANOVA followed by Dunnett's multiple-comparison test).

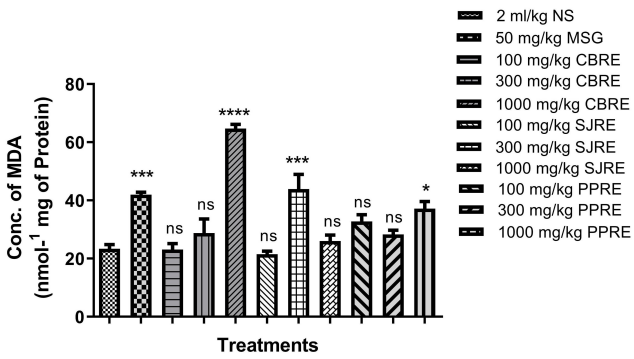

Figure 5 Malondialdehyde concentration indicative of lipid peroxidation of testes after treatment with normal saline (NS), monosodium glutamate (MSG), and various concentrations of hydroethanolic root-bark extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE) for 60 days. Data presented as group means ± SEM, n=7. Significant differences between treatments and control: ns, $p>0.05$; *$p\leq0.05$, ***$p\leq0.001$ (one-way ANOVA using Dunnett's multiple-comparison test).

Effect of Extracts on Testicular Testosterone

There were significant ($p\leq0.0001$) reductions in testicular testosterone for all treatments compared to the normal saline–treated animals (Figure 6).

Histological Studies

Histological assessment of control and drug-treated rats indicated that testes of normal ratsshowed well-layered seminiferous tubules with different stages of spermatogenic cells. Treatment with MSG and high doses (1,000 mg/kg) of CBRE, SJRE, PPRE caused mild–severe degrees of seminiferous tubule atrophy, reduction in interstitial Leydig cells, and destruction (Figure 7, A–E).

Histological assessment of epididymides showed normal epididymides with greatly convoluted tubules embedded in connective tissue. MSG treatment resulted in necrosis of connective tissue. High doses (1,000 mg/kg) of extracts resulted in disorganization of epididymal cells, and although there were greatly convoluted tubules embedded in connective tissue, there was very scanty spermatozoa (Figure 8, A–E).

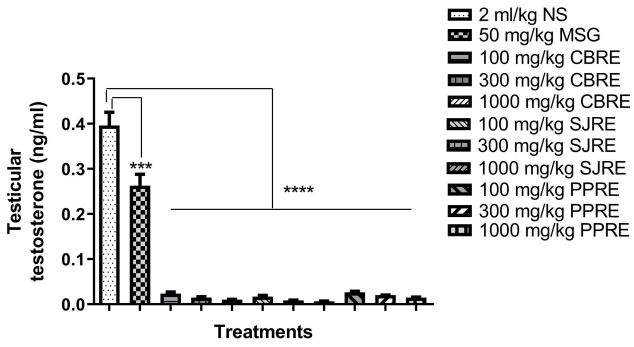

Figure 6 Testicular testosterone concentration inf rats treated with normal saline (NS), monosodium glutamate (MSG), and various concentrations of hydroethanolic root-bark extracts of *Caesalpinia benthamiana* (CBRE), *Sphenocentrum jollyanum* (SJRE), and *Paullinia pinnata* (PPRE) for 60 days. Data presented as means ± SEM, n=7. Significant differences between treatments and control: ***$p\leq0.001$; ****$p\leq0.0001$ (one-way ANOVA followed by Dunnett's multiple-comparison test).

Discussion

Effects of hydroethanolic root extracts of C. *benthamiana*, S. *jollyanun*, *P. pinnata* on reproductive function were studied in male SprageDawley rats. In a survey, these herbs were believed to be effective in increasing sexual desire, inducing erection, and enhancing male fertility, a finding similar to other research.[24] All these plants have been studied for their traditional use as aphrodisiacs.[25,26] The roots of these plants have been used frequently in polyherbal preparations to achieve maximum results.[27]

Oral administration of the C. *benthamiana*, P. *pinnata*, and *S. jollyanun* for 60 days resulted in a significant ($p\leq0.05$) dose-dependent reduction in sperm count and motility and had a detrimental effect on sperm morphology, possibly due to oxidative stress. It has been demonstrated that administration of high concentrations of MSG, induces oxidative stress in different organs, including the testes.[18,28] It has already been established that oxidative stress within the testes can impair their functionality, particularly with respect to the quality of spermatozoa produced.[29] Oxidation of glucose and fructose provides the energy required for spermatozoa motility. The reduction in sperm motility seen in this study is evidence that the extracts used may be inhibiting the uncoupling reaction of oxidative phosphorylation[30] and hence rendering spermatozoa immotile. The susceptibility of sperm cells to oxidative damage is a result of their enrichment with unsaturated fatty acids within sperm plasma membrane.[31] Sanocka and Kurpisz, 2004[32] revealed that increased lipid peroxidation leads to oxidative damage to sperm DNA, modification of membrane function, impairment of mobility, and a possiblly significant effect on spermatogenesis.[29] Lipid peroxidation is one of the major processes in oxidative damage,[33] and this was assessed in this study by the measurement of TBA-reactive substances, particularly MDA. Polyunsaturated fatty–acid degradation is measured by the amount of MDA formed and is thus the common indicator of polyunsaturated fatty–acid oxidative damage.[34]

The effects of CBRE, SJRE, and PPRE, and MSG on sperm count, motility, and morphology could be related to an increased production of free radicals and hence oxidative

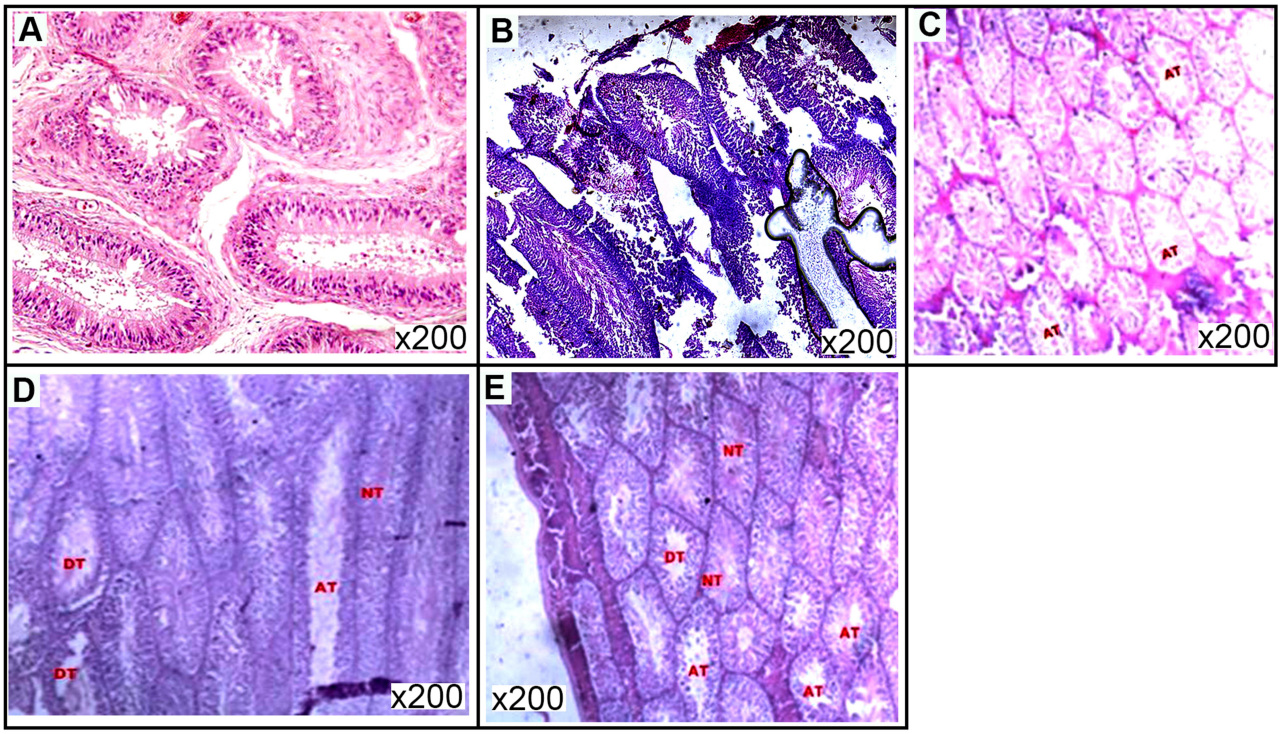

Figure 7 Representative photomicrographs of histological assessment of testes of Sprague-Dawley rats after 60 days of treatment. (**A**) Normal saline–treated rats showing well-layered seminiferous tubules with different stages of spermatogenic cells; (**B**) 50 mg/kg monosodium glutamate (MSG)-treated rats showing severe atrophy in some seminiferous tubules and reduction in interstitial Leydig cells; (**C**) 1,000 mg/kg SJRE–treated rats showing mild atrophy of seminiferous tubules; (**D**) 1,000 mg/kg CBRE–treated rats showing severe atrophy in some seminiferous tubules; (**E**) 1,000 mg/kg PPRE–treated rats showing different degrees of atrophy (transverse section, 200×, H&E stain).
Abbreviations: NT, normal tubule; DT, degenerated tubule; AT, atrophied tubule.

stress in rat reproductive organs. Excessive glutamate metabolism, as in chronic MSG intake, decreased levels of major antioxidant enzymes, and increased lipid peroxidation, can be a source of reactive oxygen species.[35,36] It is possible that CBRE, SJRE, and PPRE, and MSG lead to the excessive production of free radicals and that endogenous antioxidants are insufficient to meet the demand. The increase in free-radical production could lead to sperm-membrane dysfunction, sperm-DNA damage, and impaired sperm movement. The increases in abnormal sperm cells are consistent with findings of testicular damage.

There was no significant difference in testicular and epididymal weight between the normal and extract-treated rats. As reported by Franca and Russell,[37] testicular weight or size generally establishes the normalcy of testes, and thus changes that may be drug-induced can be assessed. The weight or size of an organ correlates partly with the presence of functional units, and thus all functional units were likely to be present after the 60 days of extract treatments, but probably with impaired functionality.

Serum testosterone, FSH, and LH levels were lower in extract-treated rats. The mechanism of action of the antispermatogenic effect of the various extracts could be due to interference in anterior pituitary function and a direct effect on testes. The primary effects of FSH and androgen appear to be similar in rodents, primates, and other mammals, and thus the effect exerted by the various treatments on hormones can be extrapolated to occur also in humans.

The lowered serum-hormone levels would affect spermatogenesis and hence semen quality. It has been established that germ-cell proliferation and survival depends profoundly on gonadotropin-dependent mechanisms.[38,39] FSH exerts its biological effects via G protein–coupled receptors found in the testes. Evidence for the critical role of the LH in initiating and maintaining spermatogenesis has been obtained from several animal models and experimental approaches.[28] Even though FSH, LH, and testosterone have separate roles in the process of spermatogenesis, they also act in a cooperative way to promote quantitative spermatogenesis, likely through the modulation of postreceptor events within Sertoli cells.[38,39] Studies have suggested that testosterone together with FSH promotes spermatogenesis by promoting adhesion of

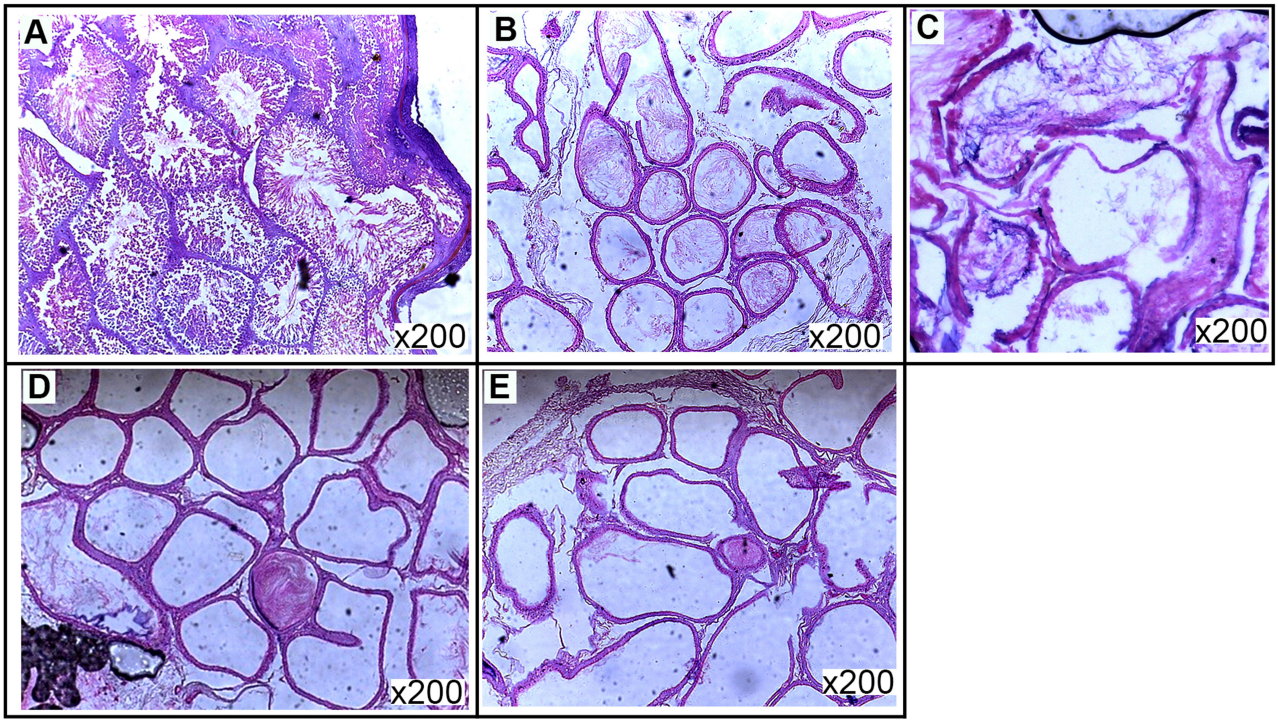

Figure 8 Representative photomicrographs of histological assessment of the epididymides of Sprague-Dawley rats after 60 days of treatment. (**A**) Normal saline–treated rats showing well-layered seminiferous tubules with different stages of spermatogenic cells; (**B**) MSG-treated rats showing necrosis in connective tissue; (**C**) 1,000 mg/kg SJRE–treated rats showing disorganization of cells and epididymitis; (**D**) 1,000 mg/kg CBRE–treated rats showing greatly convoluted tubules, but with very scanty spermatozoa; (**E**) 1,000 mg/kg PPRE–treated rats showing greatly convoluted tubules, but with scanty spermatozoa (transverse section, 200×, H&E stain).

round spermatids to Sertoli cells.[40,41] The combined effect of FSH and testosterone is mainly demonstrated in spermiation.[42] The maturation cessation, which may have been caused by the extracts, could be related to testosterone inhibition, thus leading to the termination of spermatogenesis.

Histology of the testes and epididymides of treated rats revealed changes in the histoarchitecture and decrease in basal lamina, as well as degenerative changes in germ cells, suggestive of mild, moderate, and severe oligospermia with some degree of testicular atrophy from the lowest to the highest treatment doses. The relationship between production of fertile sperm cells and histological integrity of testes and epididymides is well established.[43] Histopathological changes can cause spermatogenic arrest, edema, hypospermia, and decreased basal lamina, likely to be a result of oxidative damage to testicular membrane and tissue.[44]

Conclusion

Hydroethanolic root extracts of *C. benthamiana, S. jollyanum*, and *P. pinnata* were found to have detrimental effects on reproductive function on prolonged usage. Chronic use of these may thus lead to male infertility and hence would not be safe in healthy males who want to reproduce.

Abbreviations

CBRE, *Caesalpinia benthamiana* root extract; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MDA, malondialdehyde; MSG, monosodium glutamate; PPRE, *Paullinia pinnata* root extract; SJRE, *Sphenocentrum jollyanum* root extractTBA, thiobarbituric acid.

Data-Sharing Statement

All data generated or analyzed during this study are included in this published article. The data sets used and/or analyzed are available from the corresponding author on reasonable request.

Ethics Approval

The Committee on Animal Research, Publication, and Ethics (CARPE) of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, approved this study from an ethical point of view (FPPS/PCOL/007/2016).

Consent for Publication

No images, videos, or recordings requiring consent for publication were used in this manuscript.

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Author Contributions

Mavis Sersah Baffoe, Baffour Awuah Agyapong, George Asumeng, and Lorraine Sallah conceived the concept and design of this study. Mavis Sersah Baffoe and Baffour Awuah Agyapong did the literature search, experimental studies, and data acquisition. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval to the version to be published, and agree to be accountable for all aspects of the work.

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