ISSN: 2476-8642 (Print)
ISSN: 2536-6149 (Online)
www.annalsofhealthresearch.com
African Index Medicus, Crossref, African Journals Online, Scopus, C.O.P.E & Directory of Open Access Journals

Annals of HEALTH RESEARCH

(The Journal of the Medical and Dental Consultants' Association of Nigeria, OOUTH, Sagamu, Nigeria)

Volume 11 | No. 4 | Oct. - Dec., 2025

IN THIS ISSUE

PUBLISHED BY THE MEDICAL AND DENTAL CONSULTANTS ASSOCIATION OF NIGERIA, OOUTH, SAGAMU, NIGERIA.
www.mdcan.oouth.org.ng

Annals of Health Research

(The Journal of the Medical and Dental Consultants' Association of Nigeria, OOUTH, Sagamu, Nigeria)

Volume 11, Issue 4: 384-398
December 2025
doi:10.30442/ahr.1104-04-302

ORIGINAL RESEARCH

Restorative Effects of Aqueous *Vernonia amygdalina* Leaf Extract on Sperm Parameters and Testicular Integrity in Metronidazole-Induced Infertility in Male Rats

Odufuwa Kuburat T[1], Osonuga Ifabunmi O[2], Adepeju Deborah B[2], Ogunlade Albert A[2], Okebule Babatunde O[2], Edema Victoria B[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Ogun State, Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Ogun State, Nigeria.

Correspondence: Dr Osonuga Ifabunmi O. Department of Physiology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu, Nigeria. E-mail: osonuga.bunmi@oouagoiwoye.edu.ng ;
ORCID - https://orcid.org/0000000159768901.

Citation: Odufuwa KT, Osonuga IO, Adepeju DB, Ogunlade AA, Okebule BO, Edema VB. Restorative Effects of Aqueous *Vernonia amygdalina* Leaf Extract on Sperm Parameters and Testicular Integrity in Metronidazole-Induced Infertility in Male Rats. Ann Health Res 2025;11:384-398. https://doi.org/10.30442/ahr.1104-04-302.

Abstract

Background: *Vernonia amygdalina* (VA) is a potent medicinal treatment of regulating endocrine disruptors, such as metronidazole (MTZ), a drug used for the slightest bowel dysfunction. Prolonged metronidazole use alters hormonal balance, affecting reproductive hormones.
Objectives: To examine the ameliorative effect of aqueous leaf extract of VA on the pituitary-gonadal axis, testicular antioxidant levels, and male reproductive organs of metronidazole-induced infertility in male Wistar rats.
Methods: Thirty-five male Wistar rats weighing 160g on average in five groups (n = 7 each) were given 500 mg/kg body weight of metronidazole for 14 days and 300 mg/kg body weight of aqueous leaf extract of VA treatment for another 14 days. Group A (Control group), Group B (Metronidazole only), Group C (Metronidazole + aqueous seed extract of VA), Group D (Metronidazole Recovery), and Group E (Metronidazole + aqueous seed extract of VA + Recovery) were used for the experiment.
Results: Administration of VA treatment increased plasma Luteinising Hormone (LH) and testosterone levels, but FSH levels did not differ. These led to significant increases in sperm count, motility, and viability across VA-treated rats. The tissue antioxidant assay of the testes showed statistically significant increases in the levels of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) across study groups, while malondialdehyde (MDA) decreased. Improved cellular integrity was also deduced in the testicular photomicrograph of VA-treated rats. The histological analysis also revealed improved pituitary and testicular cells that had been previously damaged by metronidazole following VA treatment.

Conclusions: The aqueous leaf extract of VA showed restorative and anti-inflammatory effects. It also mitigates oxidative stress. Treatment with VA improved sperm parameters and testicular structural analysis, suggesting a regenerative influence on male reproductive health.

Key words: Metronidazole, Pituitary-gonadal axis, Testicular antioxidants, Testosterone, *Vernonia amygdalina*.

Introduction

Infertility represents a worldwide health issue, characterised by the failure of a sexually active pair, who do not use contraceptives, to achieve conception within a year of regular unprotected sex. [1, 2] The prevalence of infertility has emerged as a significant public health concern, impacting more than 15% of partners universally. [3, 4] Several factors have been identified as contributing to the decline in male fertility, including an increase in scrotal temperature, endocrine inconsistencies, inherited abnormalities, acquired or congenital urogenital defects, immunological causes, malignancies, and low semen quality. Chemically induced endocrine disruption and testicular dysfunction may manifest as a result of the administration of toxic agents or potentially harmful medications. The medical manifestations of chemically induced testicular dysfunctions encompass a range of irregularities in both the structural and physiological aspects of the organ. [5, 6] Endocrine disruptors, a class of xenobiotics, can alter the functional activities of biological structures, either by facilitating hormonal balance or by adversely affecting the biological roles and activities of organs that regulate or control hormones, particularly within the reproductive system. [7, 8]

For over 50 years, metronidazole has been widely used to treat infections caused by protozoans and anaerobic bacteria. The synthetic drug is produced from 1-8-hydroxyethyl-2-methyl-5-nitroimidazole, and its efficacy as an antimicrobial agent has been thoroughly researched and affirmed. [9, 10] Metronidazole can be administered through oral, intravenous, rectal, and intravaginal routes. [9] Generally, the side effects of the drug are limited to mild gastrointestinal symptoms such as nausea and diarrhoea. It is commonly prescribed for up to seven days, but in more complex cases, such as deep neck infection and joint infections, the drug may be taken for a more extended period of 4-8 weeks. Unfortunately, prolonged use of metronidazole may lead to male infertility, according to reports [11, 12]. Previous research has suggested that metronidazole (MTZ) can compromise spermatogenic activity and fertility in males. [13] Specifically, prolonged administration of MTZ can have a detrimental impact on male mice's reproductive systems. [14]

Several parts of the brain, including the pineal, pituitary, and hypothalamus, are well-known for their roles in regulating the reproductive system. The hypothalamus helps to maintain a delicate balance of hormone levels in the body, which is essential for proper functioning. Indeed, the hypothalamus plays a vital part in ensuring overall reproductive health. [15, 16] The hypothalamic-pituitary-gonadal (HPG) axis covers the hypothalamus, pituitary gland, and gonads, namely the testicles and ovaries, which are interconnected via feedback loops. This axis acts as a crucial connection between the brain and the reproductive system. [17] The HPG axis begins with the secretion of gonadotropin-releasing hormone (GnRH) by specific hypothalamic neurons, which subsequently triggers the neuro-hormonal activity of the axis. Upon reaching the anterior pituitary (adenohypophysis), GnRH stimulates the gonadotrophs to induce the secretion of luteinising hormone (LH) and

follicle-stimulating hormone (FSH). These hormones regulate the growth and function of the gonads. Gonadotropins, the collective term for two hormones, are responsible for the stimulation of androgen production and gametogenesis in the gonads. [17] In females, ovarian function is regulated by follicle-stimulating hormone (FSH) and luteinising hormone (LH). In contrast, in males, the production of testosterone by specific cells in the testes (Leydig cells) is primarily stimulated by luteinising hormone (LH). [18] On the other hand, follicle-stimulating hormone (FSH) plays a significant role in regulating the seminiferous tubules and spermatogenesis by acting on Sertoli cells. Gonadotropins are essential hormones that regulate reproductive functions, and their precise action is crucial for the maintenance of fertility and overall reproductive health. [18]

Phytochemicals, naturally occurring compounds present in plants of medicinal value, have been recognised as vital sources of biologically active natural products. The potential of phytochemicals in medicine is increasingly recognised, with an extensive range of medicinal properties. [19 - 23] *Vernonia amygdalina*, commonly known as bitter leaf in Nigeria, is a widely consumed plant despite its bitter taste. The documented biological and pharmacological actions, including anticarcinogenic, anti-inflammatory, antioxidant, and antimutagenic activities of these active agents isolated from plants, have attracted research attention toward developing more effective drugs to treat a variety of ailments. *Vernonia amygdalina* has been extensively researched for its pharmacological effects, including antimalarial, [24] antidiabetic, [25] anticancer, [26] hepatoprotective, [27] nephroprotective, [28] analgesic and antipyretic, [29] antibacterial, [30] and antioxidant properties. Jakuta *et al.* reported on the therapeutic potentials of *Vernonia amygdalina* in the management of various disorders. [23] However, there is a pressing need for further exploration of the possible mechanism of action by which the aqueous leaf extract of *Vernonia amygdalina* ameliorates chemically-induced infertility. The findings may highlight the potential reproductive risks associated with the use of MTZ, particularly over an extended period.

Methods

Study design

An experimental, animal-based study

Ethical Approval

Ethical considerations were ensured in consonance with the regulations for the use and care of rats in research. Approval was obtained from the Research Ethics Committee of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria, under reference OOUTH/HREC/682/2023 AP.

Animal care

Adult male Wistar rats were acquired from the animal house of Obafemi Awolowo College of Health Science, Sagamu, Ogun State, Nigeria. Thirty-five mature male Wistar rats, weighing an average of 160g, were utilised in this study. The rats were housed in plastic and wire gauze cages within the animal house of the institution. The rats were allowed 14 days of acclimatisation. The rats were on a standard pellet diet and had unrestricted access to water, following a 12-hour light-dark schedule. The management and handling of the rats were conducted in accordance with the guiding principles for research involving animals, as recommended by the Declaration of Helsinki and the Guiding Principles in the Use of Animals. [31]

Animal grouping

The rats were picked randomly and divided into five groups (seven per group) as follows:

1. Control: received standard pellets and water only.

2. MET: received standard pellets and received 500 mg/kg of metronidazole for 14 days to induce infertility.
3. VA+ MET: received standard pellets and 500 mg/kg of metronidazole for 14 days. Then aqueous leaf extract (300 mg/kg) was administered for another 14 days before sacrifice.
4. MET recovery: received the same treatment as MET only and allowed to recover for 14 days before sacrifice.
5. VA+ MET recovery: received the same treatment as VA + MET only and allowed to recover for 14 days before sacrifice.

Collection of leaves

Fresh *Vernonia amygdalina* leaves were collected during the dry season from a local plantation in Sagamu, Ogun State. The leaves were confirmed and authenticated by the Federal Research Institute, Ibadan, and were assigned voucher number 112938. The sample was deposited in the institute's herbarium.

Preparation of the extract

To prepare the extract, the leaves of *Vernonia amygdalina* were air-dried in the shade at room temperature, with regular turning to prevent molding, until they became crispy. The dried leaves were then ground into fine particles with the aid of a mill. A quantity of 60 mg of the fine particles was soaked in 400 mL of distilled water for 24 hours. The resulting blend was strained with a clean white cloth, and the residue was shrivelled and weighed. By deducting the dry weight from the original weight, the quantity of *Vernonia amygdalina* that dissolved was determined. The compound was orally administered to the rats at a dosage of 300 mg/kg. [32]

Preparation of Metronidazole

The drug (Metronidazole) was obtained from Forever Pharmacy Company, Nanjing Xin'gang Development Zone, Qixia District, Nanjing City. A dose of 500 mg/kg of metronidazole was used in this experiment. [33] A stock solution of metronidazole was prepared by dissolving 600 mg of metronidazole in 240 mL of 0.9% saline solution, yielding a concentration of 2.5 mg/mL.

Animal sacrifice and the collection of organs

Twenty-four hours after the last dose of each group, the rats in each group were sacrificed by cervical dislocation. The rats were immediately placed on a clean, flat surface and pinned down, exposing their ventral region. A deep incision was then made from the anus to the thorax, and the rib cage was cut to expose the thoracic and abdominal viscera. A needle was inserted, aiming at the heart, to draw a blood sample into a syringe. Some quantity of the blood was placed in plain bottles for serum, and the rest in anticoagulant bottles for plasma, which were later used for further analysis. The testes were removed from the scrotal sac. Using a pair of scissors, the head was removed entirely from the carcass, with the skin and surrounding muscles removed. The skull was then carefully opened to collect the brain tissue. The collected tissues were placed in a container of 10% neutral buffer formalin. [34] The testes and epididymis were removed from the scrotal sac.

Reproductive Hormonal Assay

Luteinizing Hormone (LH) concentration

Serum Luteinizing Hormone (LH) concentration was measured using the Microplate Enzyme Immunoassay protocol provided with the kit (ELISA).

Procedure: The microplate's wells were formatted for each calibrator and the sample to be assayed, using the required number of wells. Calibrators or samples (25 µL) were added to each well, and enzyme conjugate (100 µL) was also added. The microplate was gently shaken for 30 seconds to ensure proper mixing, then it was covered and incubated at 37°C for 60 minutes. After decanting the plate's contents, the plate was washed 5 times

with wash solution (350 µL per wash). Subsequently, the substrate (100 µL) was put into each well, and the plate was covered and incubated at ambient temperature in the dark for 20 minutes. Lastly, the stop solution (50 µL) was added to the wells, and the absorbance of each well was measured at 450 nm.

Follicle Stimulating Hormone concentration

A Microplate Enzyme Immunoassay measured serum Follicle Stimulating Hormone concentration following the manufacturer's procedure (ELISA).

Procedure: The microplate wells were correctly formatted to accommodate the calibrators and samples—each well received 25 µL of calibrator or sample and 100 µL of enzyme conjugate. After gentle shaking for 30 seconds, the plate was incubated at 37°C for 60 minutes. The microplate's contents were then discarded by decanting, followed by the introduction of wash solution and subsequent decanting four more times. Substrate was added to the well, and the plate was covered and incubated in the dark at 18-25°C for 20 minutes. Finally, the stop solution was added to the well, and the plate was jiggled for 15-20 seconds before measuring absorbance at 450 nm.

Testosterone concentration

Serum testosterone concentration was measured by a Microplate Enzyme Immunoassay using a protocol designed by the kit's producer (ELISA).

Procedure: The microplate wells were prepared for the calibrators and samples by determining the required number of wells and formatting them accordingly. Each well was then filled with 50 µL of calibrators or samples, and 50 µL of incubating buffer was also added. Gentle shaking for 10 minutes ensures proper mixing. Next, 50 µL of enzyme conjugate was added to each well, and the microplate was gently shaken for 30 seconds. The plate was enclosed and incubated for 60 minutes at 37°C. After discarding the contents of the microplate, it was washed five times with 350 µL of wash solution. The substrate was then introduced into the well, and the plate was covered and incubated in the dark at ambient temperature for 20 minutes. To conclude, 50 µL of stop solution was added to the well, and the plate was shaken for 15-20 seconds to ensure complete colour change. The absorbance of each well was then read at 450 nm.

Procedures for antioxidant determination

Determination of Superoxide Dismutase (SOD) activity

The determination of Superoxide Dismutase (SOD) activity was determined according to the outlined methodology. [35, 36]

Measurement of Catalase (CAT) enzyme functions

Catalase serves a dual role by decomposing hydrogen peroxide (H_2O_2) into water (H_20) and oxygen (O_2), while also oxidising hydrogen donors. In the ultraviolet spectrum, the absorption of H203 increases steadily as the wavelength decreases. The decline in absorbance at 240 nm directly indicates the breakdown of hydrogen peroxide (H2O2). Catalase activity can be determined by monitoring changes in absorbance over time. [37, 36]

Determination of Malondialdehyde (MDA)

The amount of lipid peroxidation in testicular tissue was assayed using the thiobarbituric acid method, which detects malondialdehyde products. [38]

Determination of Reduced Glutathione (GSH) activity

The technique for measuring GSH in tissue homogenate will be performed as Jollow *et al.* [39]

Histological Examination

The tissues were prepared and processed for histological and histochemical techniques at the Histology Laboratory of the Department of Anatomy, Olabisi Onabanjo University, Sagamu Campus. The specimens from the pituitary gland

and testes were fixed in 10% neutral buffer formalin and dehydrated in ascending grades of alcohol. Then they were cleared in xylene, cast, block-cut at 4-5 µm thickness, and stained with haematoxylin-eosin for microscopic examination. [40, 41]

Sperm analysis

Semen collection

The caudal epididymis was isolated from the testes, dried on filter paper, and sectioned to obtain sperm.

Sperm Motility

The sperm were extracted from the caudal epididymis and diluted with 4 mL of saline. Two to three drops of the sample were pipetted onto a clean, prewarmed microscope slide. The microscope slide was gently covered with a coverslip to prevent air bubbles. Subsequently, the slide was scrutinised at a magnification of x400 under the microscope to assess sperm movement of the sperm. The motility of the sperm was categorised into three groups: motile, sluggish, or immotile. The percentage of motile sperm was determined by dividing the number of motile sperm by the total sperm count. [42, 43]

Sperm viability

To evaluate sperm viability, two warm drops of Eosin stain were introduced to the semen on a prewarmed slide. The stained slide was smeared and air-dried after ensuring a uniform distribution of the stain. Subsequently, the slide was observed at a magnification of x400 under the microscope. Live sperm cells remained unstained, while the stain was absorbed by dead sperm cells. The number of sperm with or without stain was counted to calculate the percentage of viable sperm. [42 - 44]

Sperm count

The calculation of sperm count was done using *Naubauer's Hemocytometer* counting chamber. Drops of diluted sperm were put in the chamber and covered with a coverslip. The chamber was then observed under a light microscope, and sperm cells in each square were counted. [42, 43]

Calculation:

Sperm count (sperm/ ml) = Number of sperm counted x dilution factor/ volume x 1000

Statistical analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS), and the results were. The data were summarised as Mean ± Standard Error of the Mean (SEM), and comparisons were performed using the One-Way Analysis of Variance (ANOVA). A probability level of $p < 0.05$ was regarded as statistically significant.

Results

Table I shows the mean serum levels of luteinising hormone (LH), follicle-stimulating hormone (FSH), and testosterone across all study groups. LH in VA-treated groups showed a significant increase ($p = 0.039$compared with the metronidazole-only untreated group and the control group. The table also revealed that FSH levels in the VA-treated group showed no statistical difference ($p = 0.68$) compared with the control group and the metronidazole-only group. In addition to the above, there was a statistically significant increase ($p = 0.049$) in serum testosterone levels compared with the untreated metronidazole-only and control groups.

Table II shows the sperm parameters across all groups. VA-treated rats showed a significant increase in sperm count ($p = 0.009$) compared with metronidazole-induced only and a significant decrease compared with the control group. A significant increase ($p = 0.03, 0.028$) was also observed in sperm motility and viability respectively in VA-treated rats when compared to the untreated metronidazole-only and control groups.

Table I: Comparison of the mean serum levels of Pituitary-Gonadal Hormones across the study groups

Group	*Luteinising hormone Mean ± SEM (MIU/mL)*	*FSH Mean ± SEM (MIU/mL)*	*Testosterone Mean ± SEM (MIU/mL)*
A: Control	3.96 ± 1.63	5.23 ± 2.14	7.06 ± 3.26
B: MET	1.20 ± 0.82	0.89 ± 1.14	3.15 ± 1.55
C: VA + MET	3.98 ± 1.71	4.17 ± 2.23	8.54 ± 3.49
D: MET recovery	3.86 ± 5.70	3.51 ±3.14	9.58 ±3.39
E: VA + MET recovery	6.19 ± 2.54	4.74 ± 2.03	11.45 ± 2.88
p-value	0.039	0.68	0.049

VA - ***Vernonia amygdalina*****, MET – Metronidazole, FSH – Follicle-Stimulating Hormone.**

Table II: Comparison of mean values of sperm parameters across the study groups

Group	*Sperm Count (million per mL) Mean ± SEM*	*Motility (%) Mean ± SEM*	*Viability (%) Mean ± SEM*
A: Control	24.0 ± 2.77	84.4 ± 2.45	85 ± 1.03
B: MET	3.40 ± 3.40	44.6 ± 4.56	54.56 ± 4.54
C: VA + MET	10.60 ± 4.71	75 ± 3.41	77.4 ± 2.15
D: MET recovery	8.40 ± 4.12	64.68 ± 1.03	67.76 ± 2.07
E: VA + MET recovery	18.60 ± 2.60	80 ± 3.04	82.4 ± 1.26
p-value	0.009	0.03	0.028

VA - ***Vernonia amygdalina*****, MET – Metronidazole.**

Figure 1 shows the effect of VA on serum Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Reduced Glutathione (GSH) levels in Metronidazole-induced infertility in male Wistar rats.

Discussion

The utilisation and dependability of herbal products have gained prominence over the past twenty years, because of the positive impacts and complexities of numerous chemical and synthetic drugs. These various medicinal herbs, such as *Vernonia amygdalina*, aid multiple systems, such as the reproductive system, in a compromised adverse effect state of common drugs in Africa upon the slightest bowel disruption. Male reproduction is intricately controlled by hormones, including follicle-stimulating hormone (FSH), luteinising hormone (LH), and testosterone, which play pivotal roles. The outcome of this study demonstrated a decline in LH and FSH levels in metronidazole-treated rats only. The study revealed increased follicle-stimulating hormone (FSH), luteinising hormone (LH), and testosterone levels in VA-treated male Wistar rats. The rise in hormonal levels was particularly pronounced in the VA-treated rats.

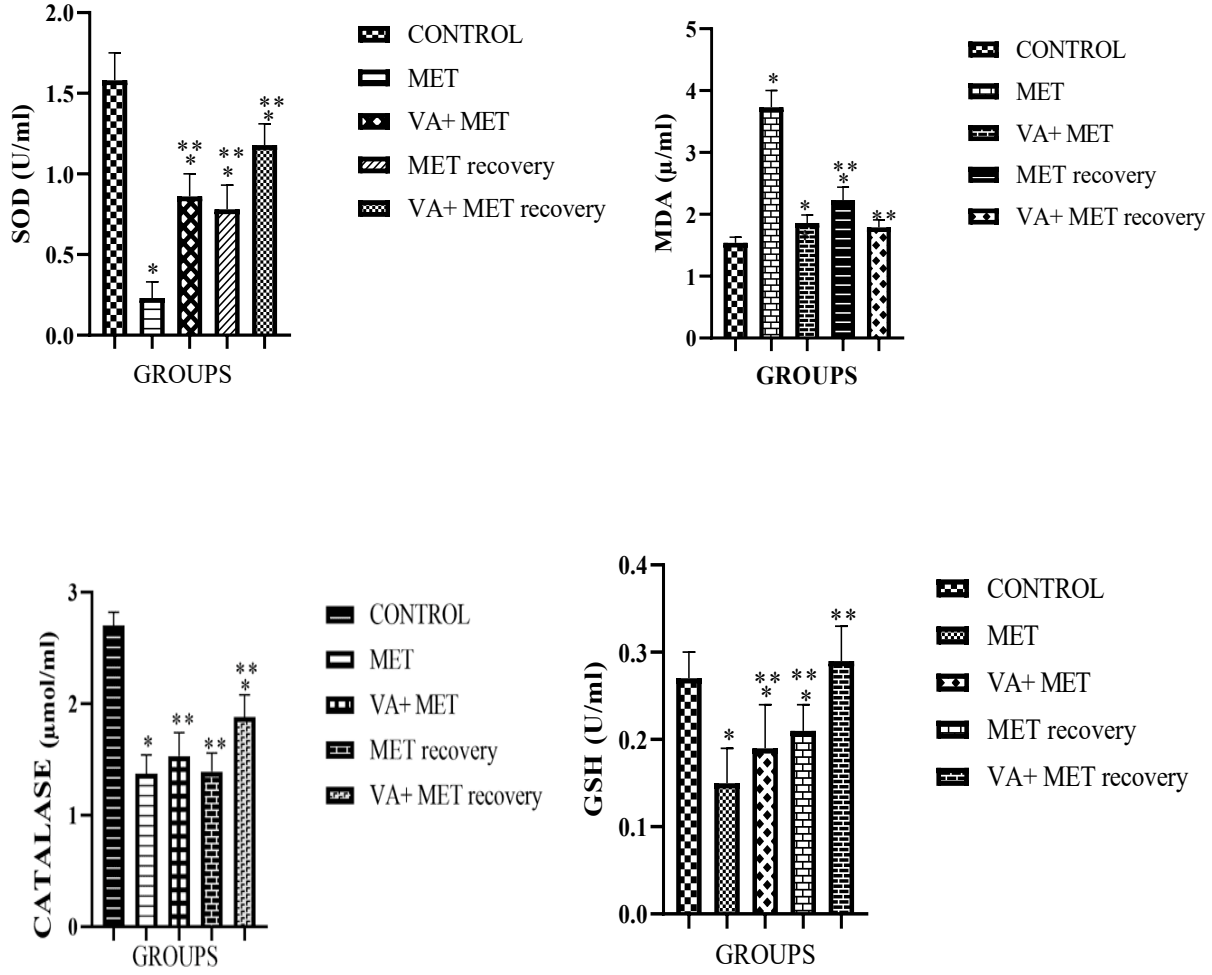

Figure 1: Effect of VA on serum Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Reduced Glutathione (GSH) levels in metronidazole-induced infertility in male Wistar rats.

The observed increase in luteinising hormone (LH) levels in VA-treated and the recovery groups suggests a potential interaction with the HPG axis. VA may influence the hypothalamus, a key regulatory centre for reproductive hormones. Understanding how VA interacts with the hypothalamus (Plate 1) and modulates the release of gonadotropin-releasing hormone (GnRH) could provide insights into its effects on the HPG axis. As LH stimulates testosterone production in the testes, the rise in LH levels may contribute to the observed increase in testosterone, indicating a positive modulation of hormonal feedback loops. [45] The observed increase in testosterone levels in the VA-treated group underscores the potential of VA to modulate male reproductive hormones positively. Testosterone, a key male sex hormone, plays a central role in various aspects of male reproductive health, including spermatogenesis, libido, and the maintenance of secondary sexual characteristics. [46] *Vernonia amygdalina* is rich in bioactive compounds, including flavonoids, alkaloids, and terpenoids. These compounds have demonstrated antioxidant, anti-inflammatory, and immunomodulatory properties. [47] The overall phytochemical composition may contribute to its potential positive effects on male reproductive hormones. Antioxidants within VA may contribute to the

protection of Leydig cells in the testes, which are responsible for testosterone production. By mitigating oxidative stress, VA could enhance the viability and functionality of Leydig cells, leading to increased testosterone synthesis.

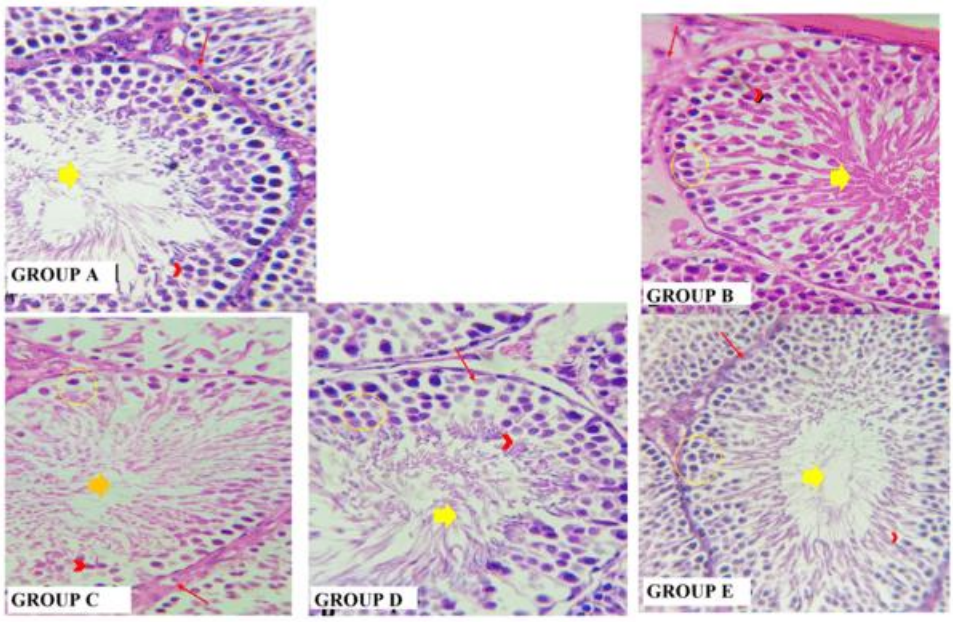

Plate 1: Photomicrograph of testicular tissue (H/E X400): A. [Control] showed well-differentiated and normal spermatogonia cells (yellow circle), lumen (yellow thick arrow), Sertoli cells (red arrowhead), and Leydig cells (red thin arrow) on the interstitial layer. B. [MET] showed the distortion of the interstitial layer with reduced Leydig cells (red thin arrow), constricted lumen (yellow thick arrow), reduced Sertoli cells (red arrowhead), and spermatogonia cells (yellow circle). C. [VA + MET] showed distinguished Leydig cells (red thin arrow), Sertoli cells (red arrowhead), lumen with spermatocytes (yellow thick arrow), and spermatogonia cells (yellow circle). D. [MET] showed well-organised spermatogonia cells (yellow circle), lumen (yellow thick arrow), Sertoli cells (red arrowhead), and Leydig cells (red thin arrow) on the interstitial layer. E. [VA + MET recovery] showed well-differentiated spermatogonia cells (yellow circle), Sertoli cells (red arrowhead), lumen with spermatocytes (yellow thick arrow), and Leydig cells (red thin arrow).

The anti-inflammatory properties of VA may mitigate inflammation, contributing to the proper regulation of gonadotropins such as luteinising hormone (LH) and follicle-stimulating hormone (FSH). Inflammation can negatively impact the testicular microenvironment and hormonal regulation. [48] The anti-inflammatory properties of VA may help create a more conducive environment for testosterone synthesis by reducing inflammation-induced disruptions in the hypothalamic-pituitary-gonadal (HPG) axis. VA may influence the sensitivity of receptors involved in hormonal regulation. This modulation could enhance the responsiveness of Leydig cells to gonadotropins, ensuring efficient testosterone production in response to hormonal signals. This finding agrees with a previous study, [49] which reported that VA enhances androgen biosynthesis, thereby promoting hormonal activity.

The anterior pituitary gland plays a crucial role in regulating male reproductive endocrine function and fertility. This study reveals structural variations in the gland's cells that affect hormone production and may alter male

reproductive hormone levels. The gonadotrophic cells, also known as basophilic cells, secrete essential hormones. General control, grouped rats, were used as a baseline for normal physiological tests in this study. Metronidazole exhibited irregularities in cell size and shape, potentially disrupting cell maturation. Metronidazole-induced DNA damage affected acidophilic and chromophobe cells, leading to irregular hormone secretion and potentially affecting male reproductive hormone production.

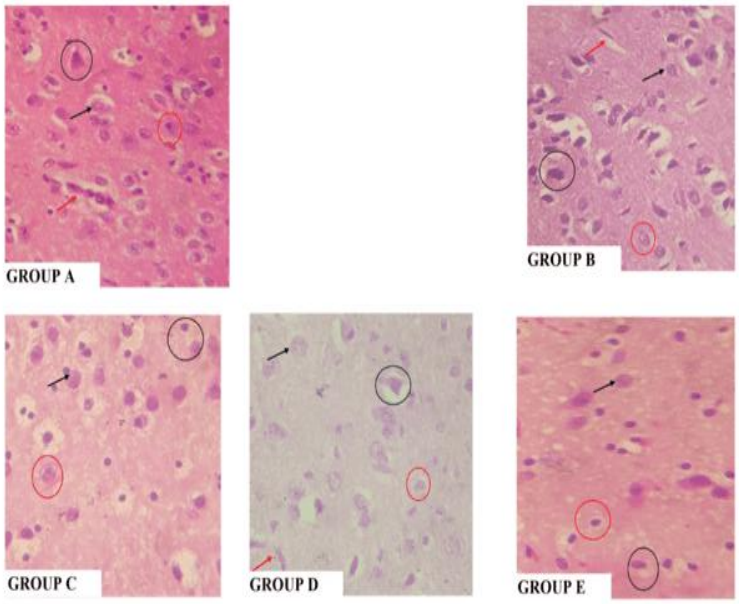

Plate 2: Photomicrograph of pituitary tissue (H/E X400): A. [Control] showed well-differentiated and organisedorganised pituitary cells, blood vessels (red thin arrow), acidophilic cells (red circle), basophilic cells (black circle), and chromophobes (black thin arrow) B. [MET only] showed pleomorphic-hyperchromatic basophilic cells (black circle), acidophilic cells (red circle), and chromophobes cells (black thin arrow). C. [VA + MET] showed well-differentiated cells, the chromophobes (black thin arrow), acidophilic cells (red circle), and basophilic cells (black circle). D. [MET recovery] showed differentiated pituitary cells, chromophobe cells (black thin arrow), basophilic cells (black circle), blood vessels (red thin arrow), and acidophilic cells (red circle). E. [VA + MET recovery] showed differentiated scanty cells, the chromophobes (Black thin arrow), acidophilic cells (red circle), and basophilic cells (black circle) and well-organised.

However, VA-treated rats showed improvement, with well-differentiated cell types indicating a more balanced hormonal profile. *Vernonia amygdalina*, known for its anti-inflammatory and antioxidant properties, may protect against cellular damage and promote a more ordered structure. This plant could modulate signalling pathways involved in cell differentiation and hormone regulation, thus restoring hormonal balance. The presence of differentiated cells in the VA-treated rats suggests a potential restoration of hormonal balance, which could positively impact male reproductive physiology. The recovery of organised structures and the balanced presence of hormone-secreting cells may collectively influence testosterone synthesis and regulation, crucial for male fertility. The *Vernonia amygdalina* recovery group showed slight improvement due to an autoimmune response, while *Vernonia amygdalina* mitigated the damage induced by metronidazole, as observed in a previous study. [49] Also, *Vernonia*

amygdalina was reported to minimise the damage caused by metronidazole in hepatic cells. [50]

Metronidazole-only induced rats showed potential disruption in the testicular structure, including reduced Leydig cells, lumen constriction, and decreased presence of Sertoli cells and spermatozoa. The decrease in Leydig cells suggests a hindrance in spermatogenesis, while alterations in the tubular structure may affect sperm maturation efficiency. A reduction in Sertoli cells disrupts the nurturing environment required for proper sperm maturation, potentially leading to decreased sperm production. The reduced number of spermatozoa suggests a potential impairment in the final stage of spermatogenesis. [51] Well-differentiated Leydig cells were observed in the VA-treated group, suggesting a possible protective effect on testosterone-producing cells. The VA-treated group showed improved lumen with the presence of spermatocytes and spermatogonia, enhancing the conductive environment for proper sperm development. Proper tubular architecture facilitates the transport of developing sperm cells and supports spermatogenesis. The observation of healthy cells in the VA-treated group indicates a potential regenerative influence on the supportive environment for sperm development. A healthy testicular environment supports the production of mature, functional sperm cells, ultimately supporting male fertility. Recovery days further reduced the damage caused by metronidazole, as seen in the VA recovery-treated group, and had a slightly positive impact. This agrees with a previous study. [49]

This study delves into the impact of Metronidazole-induced oxidative stress on testicular tissues and explores the potential protective effects of *Vernonia amygdalina* (VA) in rats. The antioxidative defence system, comprising enzymes such as catalase (CAT), Glutathione (GSH), and superoxide dismutase (SOD), plays a pivotal role in safeguarding against oxidative stress. The study reveals a significant increase in SOD and GSH levels in VA-treated groups, indicating that VA mitigates metronidazole-induced oxidative stress. Additionally, the study suggests that VA may help preserve Leydig cell integrity and testosterone synthesis by influencing mitochondrial function and supporting antioxidative defences during recovery phases. Furthermore, the study evaluates sperm parameters and shows substantial improvements in sperm count, motility, and viability in VA-treated groups, particularly during the recovery phase. Testicular structural analysis reveals disruptions induced by metronidazole, including reduced Leydig cell numbers and altered tubular structure.

In contrast, VA-treated groups exhibit well-differentiated Leydig cells and improved tubular structure, suggesting a potential regenerative influence. The findings of this study agree with a previous study [52] that reported an increase in sperm quality following bitter leaf administration. Another study [53] found that a low dose of VA improves sperm quality in immunosuppressed Wistar rats.

The observed enhancements in sperm parameters in VA-treated rats suggest that *Vernonia amygdalina*, especially when coupled with recovery, positively influences male reproductive health. VA's antioxidative and anti-inflammatory properties likely contribute to the improvements in sperm quality, creating a conducive environment for efficient spermatogenesis. This finding agrees with another study [49], which reported that the methanolic leaf extract of *Vernonia amygdalina* eradicated the adverse effects of nitrobenzene on antioxidant enzymes, markers of testicular oxidative damage, endocrine markers, and testicular structure in rats. The study underscores the potential of VA in supporting

spermatogenesis, hormonal regulation, and overall male reproductive health.

Conclusion

Overall, these findings highlight the multifaceted positive impact of *Vernonia amygdalina* on male reproductive hormones, pituitary gland structure, and testicular integrity, offering a promising avenue for therapeutic interventions in male reproductive health. Further investigations are needed to explore the molecular mechanisms, specific signalling pathways, and gene expression patterns underlying the observed effects. Additionally, a long-term evaluation of the impact of *Vernonia amygdalina* on the reproductive system is essential to understand the sustainability and effectiveness of the extract over time.

Authors; Contributions: OKT, OIO, ADB, OAA conceived and designed the study and did the literature review. OBO and EVB also participated in the literature review. OIO, ADB and OAA curated, analysed and interpreted the data and drafted the manuscript. OIO and OAA revised the draft for sound intellectual content. All the authors approved the final version of the manuscript.
Conflicts of Interest: None.
Funding: Self-funded.
Publication History: Submitted 22 January 2025; **Accepted** 30 June 2025.

References

1. World Health Organization (WHO). Manual for the standardized investigation and diagnosis of the infertile couple. Cambridge: Cambridge University Press; 2000. https://www.who.int/publications/i/item/9780521431361.
2. Practice Committee of the American Society for Reproductive Medicine (PCASRM). Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015;103:e18-e25. https://doi.org/10.1016/j.fertnstert.2014.12.103
3. Sabanegh E, Agarwa A. Male infertility. In: Wein A, Editor. Campbell-walsh Urology. 10th ed. Philadelphia: Elsevier Saunders; 2011. p.616-647. https://link.springer.com/book/10.1007/978-1-4939-1040-3#overview.
4. Leslie SW, Soon-Sutton TL, Khan MAB. Male Infertility. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK562258/
5. Abd El-Fattah AA, Fahim AT, Sadik NAH, Ali BM. Resveratrol and curcumin ameliorate di-(2-ethylhexyl) phthalate induced testicular injury in rats. Gen Comp Endocrinol 2016;225:45-54. https://doi.org/10.1016/j.ygcen.2015.09.006.
6. Wang YJ, Yan J, Yin F, Li L, Qin YG, Meng CY, *et al.* Role of autophagy in cadmium-induced testicular injury. Hum Exp Toxicol 2017;36:1039-1048. https://doi.org/10.1177/0960327116678300.
7. Daston G, Cook J, Kavlock R. Uncertainties for Endocrine Disrupters: Our View on Progress. Toxicol Sci 2003;74:245-52. https://doi.org/10.1093/toxsci/kfg105.
8. Darbre PD. The history of endocrine-disrupting chemicals. Curr Opin Endocr Metab Res 2019;7:26-33.
9. Agarwal A, Kanekar S, Sabat S, Thamburaj K. Metronidazole-Induced Cerebellar Toxicity. Neurol Int 2016;8:6365. https://doi.org/10.4081/ni.2016.6365.
10. Patel L, Batchala P, Almardawi R, Morales R, Raghavan P. Acute metronidazole-induced neurotoxicity: an update on MRI findings. Clin Radiol 2020;75:202-208. https://doi.org/10.1016/j.crad.2019.11.002.
11. Sohrabi D, Alipour M, Melati AA. Effect of metronidazole on spermatogenesis, plasma gonadotrophins and testosterone in male rats. Iran J Pharm Res 2007;6:279-283.
12. Brezina P, Yunus F, Zhao Y. Effects of Pharmaceutical Medications on Male Fertility. J Reprod Infertil 2012;13:3-11.
13. Kumari M, Singh P. Tribulus terrestris improves metronidazole-induced impaired

fertility in the male mice. Afr Health Sci 2018;18:645-652.
14. Al-Timimi ZK. Metronidazole Induces Significant Pathological Alterations in the Male Reproductive System of Mice. Iraq J Agric Sci 2021;52:1375-1381. https://doi.org/10.36103/ijas.v52i6.1477.
15. Chrousos GP. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. N Engl J Med 1995;332:1351-1363.
16. Nutan, Kanwar AJ, Bhansali A, Parsad D. Evaluation of hypothalamic-pituitary-adrenal axis in patients with atopic dermatitis. Indian J Dermatol Venereol Leprol 2011;77:288-293. https://doi.org/10.4103/0378-6323.79697.
17. Falcón J, Zohar Y. Photoperiodism in Fish. In: Skinner MK, editor. Encyclopedia of Reproduction (Second Edition). Vol. 6. Academic Press; 2018. p. 400-408. https://doi.org/10.1016/B978-0-12-809633-8.20584-0.
18. Kaprara A, Huhtaniemi IT. The hypothalamus-pituitary-gonad axis: Tales of mice and men. Metabolism 2018;86:3-17. https://doi.org/10.1016/j.metabol.2017.11.018.
19. Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH, Willett WC, *et al.* Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am J Clin Nutr 2000;72:922-928. https://doi.org/10.1093/ajcn/72.4.922.
20. Gulcin I, Mshvildadze V, Gepdiremen A, Elias R. Antioxidant activity of a triterpenoid glycoside isolated from the berries of Hedera calchica: 3-O-(b-D-glucopyranosyl)-hederagenin. Phytother Res 2006;20:130-134.
21. Köksal E, Gülçin I, Beyza S, Sarikaya O, Bursal E. In vitro antioxidant activity of silymarin. J Enzyme Inhib Med Chem 2009;24:395-405. https://doi.org/10.1080/14756360802188081.
22. Vostálová J, Tinková E, Biedermann D, Kosina P, Ulrichová J, Rajnochová Svobodová A. Skin Protective Activity of Silymarin and its Flavonolignans. Molecules 2019;24:1022. https://doi.org/10.3390/molecules24061022.
23. Ijaz MU, Anwar H, Iqbal S, Ismail H, Ashraf A, Mustafa S, *et al.* Protective effect of myricetin on nonylphenol-induced testicular toxicity: biochemical, steroidogenic, hormonal, spermatogenic, and histological-based evidences. Environ Sci Pollut Res Int 2021;28:22742-22757. https://doi.org/10.1007/s11356-020-12296-5.
24. Bihonegn T, Giday M, Yimer G, Animut A, Sisay M. Antimalarial activity of hydromethanolic extract and its solvent fractions of Vernonia amygdalina leaves in mice infected with Plasmodium berghei. SAGE Open Med. 2019;7. https://doi.org/10.1177/2050312119849766.
25. Du-Bois A, Henneh IT, Acheampong DO, Kyei F, Adokoh CK, Ofori EG, *et al.* Anti-inflammatory, anti-nociceptive and antipyretic activity of young and old leaves of Vernonia amygdalina. Biomed Pharmacother 2019;111:1187-1203. https://doi.org/10.1016/j.biopha.2018.12.147.
26. Bingham HC, Johnson WK, Pervin S, Izevbigie EB. Recent perspectives on the anticancer properties of aqueous extracts of Nigerian Vernonia amygdalina. Botanics (Basel) 2015;5:65-76. https://doi.org/10.2147/BTAT.S62984.
27. Imafidon CE, Olukiran OS, Ogundipe DJ, Eluwole AO, Adekunle IA, Oke GO. Acetonic extract of Vernonia amygdalina (Del.) attenuates Cd-induced liver injury: potential application in adjuvant heavy metal therapy. Toxicol Rep 2018;5:324-332. https://doi.org/10.1016/j.toxrep.2018.02.009.
28. Constance N, Joy DN, Nkeiruka OA, Ahamefula E, Chidimma A, Oluchi AA. Protective effects of bi-herbal formulation of aqueous extracts of Vernonia amygdalina and gongronema latifolium against gentamicin induced nephrotoxicity and liver injury in rats. Asian J Res Biochem 2020;7:12-20.
29. Tijjani MA, Mohammed GT, Alkali YT, Adamu TB, Abdurahaman FI. Phytochemical

analysis, analgesic and antipyretic properties of ethanolic leaf extract of Vernonia amygdalina Del. J Herbmed Pharmacol 2017;6:95-99. https://www.herbmedpharmacol.com/Article/JHP_20170619003608

30. Valizadeh H, Abdolmohammadi-Vahid S, Danshina S, Gencer MZ, Ammari A, Sadeghi A, *et al.* Nano-curcumin therapy, a promising method in modulating inflammatory cytokines in COVID-19 patients. Int Immunopharmacol 2020;89:107088. https://doi.org/10.1016/j.intimp.2020.107088.
31. World Medical Association, American Physiological Society. Guiding principles for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol 2002;283:R281-R283. https://doi.org/10.1152/ajpregu.00279.2002.
32. Igbokwe UV, Eze ED, Adams MD, Felicia C. Aqueous extracts of Vernonia amygdalina and Ocimum gratissimum protect against electrolyte derangement in salt-loaded rats. Int J Pharm Sci Res 2020;11:1889-97. https://doi.org/10.13040/IJPSR.0975-8232.11(4).
33. el-Nahas AF, el-Ashmawy IM. Reproductive and cytogenetic toxicity of metronidazole in male mice. Basic Clin Pharmacol Toxicol 2004;94:226-231. https://doi.org/10.1111/j.1742-7843.2004.pto940505.x.
34. Amemiya K, Hirotsu Y, Oyama T, Omata M. Relationship between formalin reagent and success rate of targeted sequencing analysis using formalin fixed paraffin embedded tissues. Clin Chim Acta 2019;488:129-134. https://doi.org/10.1016/j.cca.2018.11.002.
35. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-3175.
36. Adedara I, Umin-Awaji SG, Sule J, Mike MA. Influence of atrazine and diclofenac co-exposure on hypothalamic-pituitary-testicular axis function in pubertal rats. Arch Basic Appl Med 2021;9:59-68.
37. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-126. https://doi.org/10.1016/S0076-6879(84)05016-3.
38. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-358. https://doi.org/10.1016/0003-2697(79)90738-3.
39. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974;11:151-169. https://doi.org/10.1159/000136485.
40. Bancroft D, Stevens A, Tumer R. Theory and practice of histological technique. 4th ed. Edinburg London Melbourne: Churchill Living Stone; 1996. p. 47-67. https://www.sciencedirect.com/book/9780702068874/bancrofts-theory-and-practice-of-histological-techniques.
41. Hamadouche N, Nesrine S, Abdelkeder A. Lead Toxicity and the Hypothalamic-Pituitary-Testicular Axis. Not Sci Biol 2013;5:1-6.
42. Osonuga IO, Osonuga OA, Osonuga AA. Gonadotoxicity evaluation of oral administration of zidolam in male albino rats. Maced J Med Sci 2010;3:378-382. https://doi.org/10.3889/MJMS.1857-5773.2010.0125.
43. Adewale OO, Oduyemi OI, Ayokunle O. Oral administration of leaf extracts of Momordica charantia affect reproductive hormones of adult female Wistar rats. Asian Pac J Trop Biomed 2014;4:S521-S524. https://doi.org/10.12980/APJTB.4.2014C939.
44. Oyedeji KO, Oshatimi A, Abidoye D, Adeleke KO. Effect of Metronidazole on Reproductive Parameters in Male Wistar Rats. Int J Pharm Sci Rev Res 2015;35:186-190.
45. Okafor IA, Okpara UD, Ibeabuchi KC. The Reproductive Functions of the Human Brain Regions: A Systematic Review. J Hum Reprod Sci 2022;15:102-111. https://doi.org/10.4103/jhrs.jhrs_18_22.
46. Oyovwi MO, Ben-Azu B, Irikefe SO, Oghenetega OB, Emojevwe V, Faith FF, *et al.* Testosterone: The Male Sex Hormone.

IntechOpen 2023.. https://doi.org/10.5772/intechopen.110657.
47. Ugbogu EA, Emmanuel O, Dike ED, Agi GO, Ugbogu OC, Ibe C, *et al.* The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant-Vernonia amygdalina L (bitter Leaf). Clin Complement Med Pharmacol 2021;1:100006. https://doi.org/10.1016/j.ccmp.2021.100006.
48. Ye L, Huang W, Liu S, Cai S, Hong L, Xiao W, Diao L. Impacts of immunometabolism on male reproduction. Front Immunol 2021;12:658432.
49. Oladele JO, Oyeleke OM, Oladele OT, Babatope OD, Awosanya OO. Nitrobenzene-induced hormonal disruption, alteration of steroidogenic pathway, and oxidative damage in rat: protective effects of Vernonia amygdalina. Clin Phytoscience 2020;6:1-9. https://doi.org/10.1186/s40816-020-00161-4
50. Iwo MI, Sjahlim SL, Rahmawati SF. Effect of Vernonia amygdalina Del. Leaf Ethanolic Extract on Intoxicated Male Wistar Rats Liver. Sci Pharm 2017;85:16. https://doi.org/10.3390/scipharm85020016.
51. Adamczewska D, Słowikowska-Hilczer J, Walczak-Jędrzejowska R. The Fate of Leydig Cells in Men with Spermatogenic Failure. Life (Basel) 2022;12:570. https://doi.org/10.3390/life12040570.
52. Oyeyemi MO, Oluwatoyin O, Leigh OO, Adesiji TF. The spermiogram of male wistar rats treated with aqueous leaf extract of Vernonia amygdalina. Folia Vet 2007;2:126-129.
53. Kadir RE, Ibrahim A, Ibrahim BA, Gwadabe SM, Jaji-Sulaimon R, Adigun MF, *et al.* Low dose bitter leaf improves sperm quality disrupted in immunosuppressed Wistar rats: An experimental study. Int J Reprod Biomed 2020;18:215-226. https://doi.org/10.18502/ijrm.v18i3.6720.