Trop J Nat Prod Res, July 2025; 9(7): 3349 - 3359 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Molecular Docking, Contraceptive Property and Histopathological Changes in Experimental Models by *Digitaria exilis* Grain Extract via Interference with Steroidogenesis at Ovarian Level

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ARTICLE INFO

Article history:
Received 28 April 2025
Revised 05 June 2025
Accepted 08 June 2025
Published online 01 August 2025

ABSTRACT

The use of *Digitaria exilis* in traditional medicine for contraception has been scientifically validated without a report on the mechanism of its possible risk to women's fertility. Therefore, this study evaluated the potential mechanism of contraceptive property of aqueous grain extract of *Digitaria exilis* (AGEDE) in female Wistar rats using molecular docking and histopathological analysis. Twenty female rats were grouped into four (n=5). Group A served as control while groups B-D received (50, 100, and 200 mg/kg BW) of AGEDE for fourteen days. Serum and ovary levels of some reproductive biomarkers and histopathology and molecular docking analysis were conducted. Progesterone, FSH, LH, estrogen, and prolactin levels were altered at different AGEDE dosal levels in the serum and ovary of the animals. The histoarchitecture of ovarian and uterine tissues was altered by AGEDE, with severe infiltration of inflammatory cells, degenerated luteal cells, loosed ovarian stroma with severe vascular congestion, as well as degenerated endometrial glands with distorted hyperplasia, endometrium layer with deranged vascularization and severely fibrotic endometrium, respectively. Phytochemical analyses of AGEDE identified flavonoids, saponins, and tannins. Molecular docking analysis of previously documented compounds with 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) revealed that ethylacetate had the highest docking score (-4.69227982), followed closely by 2,5-dimethylhydrazine (-4.61382103), while pyrazine (-3.80633807) had the least binding affinity. AGEDE demonstrated toxicity to the female reproductive organs via alteration and/or interference with steroidogenesis at the molecular, hormonal, enzymic, uterus, and ovarian levels. These interferences may affect fertility, conception, and pregnancy. Therefore, *Digitaria exilis* may be explored as a fertility-controlling agent.

Keywords: *Digitaria exilis*, Induced fit molecular docking, 3β-hydroxysteroid dehydrogenase, Progesterone, Epostane, Ovarian stroma.

Introduction

Contraception is regarded as a strategy used to prevent pregnancy in women. The contraceptive strategies include hormonal regulation, barrier methods, intrauterine devices (IUDs), sterilization, behavioural techniques, and emergency contraception.[1] Hormone control with the interplay of alterations in female reproductive hormones is an implication in the onset of hormone contraception. Hormone contraception occurs by mimicking the activity of natural hormones like estrogen and progestin, primarily preventing pregnancy by stopping ovulation and altering cervical mucus and uterine lining. This combination prevents sperm from reaching the egg and makes it difficult for a fertilized egg to implant in the uterus. Hormonal contraception works by preventing ovulation, thickening the cervical mucus, and thinning the uterine lining.[2]

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Citation: Adams MD, Manu HA, Enyioma-Alozie S. Molecular Docking, Contraceptive Property and Histopathological Changes in Experimental Models by *Digitaria exilis* Grain Extract via Interference with Steroidogenesis at Ovarian Level. Trop J Nat Prod Res. 2025; 9(7): 3349 – 3359 https://doi.org/10.26538/tjnpr/v9i7.63

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The major types of hormonal contraception include oral contraceptives (pills), hormonal patches, vaginal rings, hormonal injections, implants and IUDs. Synthetic contraception presents with detrimental health effects, including nausea, headaches, breast tenderness, mood changes, weight changes, changes in libido, and acne, as well as spotting or bleeding between periods, among others.[3] Two experimental methodologies to ascertain these likely toxicological effects of hormone contraception are histopathology and *in silico* study (induced fit molecular docking).

Histopathology involves an examination to study tissue, possible tissue disease (pathology), detect abnormalities, and diagnosis of various disease conditions, which are usually carried out through tissue acquisition, tissue processing (sectioning and staining), the use of multi-resolution microscopic analysis, and report detailing the present histoarchitectural picture of the examined tissue.[4,5] The induced fit molecular docking method (IFMDM) is a computational procedure that predicts how a compound (ligand) interacts with an enzyme (target receptor protein). It is a method that supports conformational flexibility between the ligand and target protein where the inherent structure of protein could adjust to accommodate the ligand under study, allowing for better and precise binding mode and affinity prediction.[6] IFMDM brings about a more reliable dock prediction in ligand-enzyme binding and new drug invention. The technique also presents the mechanism of how the amino acids on the receptor proteins bind with the ligands, yielding a two-dimensional (2D)/three-dimensional (3D) structured pattern, a dock score, and the energy of binding between the

participating molecules. The process occurs mainly by predicting binding orientation and evaluating binding affinity while using a virtual assessment tool to screen large libraries of molecules to identify compound lead candidates that bond firmly to a target receptor protein.[7] Despite the toxicological effects of synthetic hormonal contraceptives, many medicinal plants have been recognized to exhibit desirable contraceptive potential.

Digitaria exilis, family *Poaceae*, is popularly recognized as *Hungry Rice* in English. *D. exilis* grain has a few local Nigerian names, including *Acha* (Hausa), *Suuru* (Yoruba) and *Achara* (Igbo).[8] Acha is an annual flower-layered plant mostly found to grow solely as a slender, straight, smooth peduncle standing up to 90 cm in height.[9] It is widely grown in relatively cold weathered states of Nigeria, including Bauchi, Nasarawa, Niger, Kebbi, Jigawa, Kaduna, Plateau, and Taraba, where the grains are prepared into a local delicacy '*Gwate'* (porridge), cake, local drink, flour meal and soup. In some African nations, is known as *Podgi* (Benin), *Apendi* or *Sereme* (Burkina-Faso), *Dibong*, *Findi* or *Monyimonyo* (Gambia), *Kabega* (Ghana), *Kpende* or *Fonio* (Guinea), *Bofinhe* (Guinea-Bissau), *Fini* (Ivory Coast), *Faine* or *Findi* (Mali), *Entaya* (Niger), *Dekole* (Senegal), *Ampindi* (Sierra Leone) and *Figm* or *Tschamma* (Togo). In other nations, *Digitaria* or *Funde* is in Spanish, and Fonio Blanc or Millet Digitaire is in French.[10]

Documented literature on *D. exilis* grains include anti-cardiac properties,[11] glucose-reducing potentials,[12] physical, lipid, chemical constituent analysis and oxygen inhibitory capacity,[13] nutrient and microstructural content assessment,[14] postprandial status/load of *D. exilis* in normoglycemic and infected animals,[15] alteration of carbohydrate hydrolyzing protein by *D. exilis*,[16] proportionate inclusion/mixture of the flour of *D. exilis* with *Cajanus cajan* for inhibitory activity against key carbohydrate digesting enzymes,[17] purgative property,[18] biomolecular assessment of blended food materials of Fonio and Pigeon pea,[19] sugar-lowering action of fermented *D. exilis* starch partly replaced with *Clendendrum volubile*,[20] proximate composition of *D. exilis* grain[21] and the anti-obesity property of hunger rice meal.[22]

The above studies covered several efficacious properties of *D. exilis* grains without any visible documented report on the probable mechanism of the toxicological effect of the plant on female fertility, creating a research vacuum to be filled. This investigation was, therefore, tailored toward reporting the likely contraceptive mode of action of the aqueous grain extract of *Digitaria exilis* in experimental animals through *in vivo* hormonal, histopathological, and induced fit molecular docking studies.

Materials and Methods

Plant collection and identification

White Acha (Fonio) grains were collected on 15th of April 2019 from the National Cereals Research Institute, Badeggi, Niger State (Global Positioning System, GPS: Latitude: 9º 3' 22.790" N Longitude: 6º 8' 34.81" E.), Nigeria. The plant was identified by a taxonomist in the Department of Botany, University of Ilorin, where a voucher specimen code UILH 001/1201 was deposited.

Animals

Female albino rats of Wistar strain weighing 120-170 g were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State. The animals were housed in plastic cages placed in well-ventilated standard housing conditions (temperature: 28-31ºC; photoperiod: 12 hours; humidity: 50-55%). The animals were maintained on tap water and growers' feed.

Assay kits and chemicals

The assay kits for the determination of serum progesterone, estrogen, Follicle Stimulating Hormones (FSH), prolactin, and Luteinizing Hormones (LH) are products of ichroma™ Economical compact immuno-analyzer, USA. All the other reagents, consumables, and chemicals used in the study were purchased from Sigma-Aldrich (Canada) Ltd., Stittsville, Ottawa, Canada and have a purity status of 95%.

Ethical approval

The National Health Research Agency's (NHRA) recommendations for the care and use of laboratory animals were strictly adopted in this study. For the use of animals, an ethical approval with the number (University of Abuja Ethics Committee on Animal Use; UAECAU/2024/021) was granted by the Institutional Animal Care and Ethics (ACE) Committee of UniAbuja.

Phytochemical analysis

Qualitative phytochemical screening of alkaloid, saponin, phlobatannin, flavonoid, tannin, terpenoid and phenolic compounds were analyzed in AGEDE by using the method prescribed by Harborne,[23] Awe and Sodipo,[24] Odebiyi and Sofowora,[25] Trease and Evans,[26] Ganesan and Bhatt,[27] Edeoga *et al*,[28] and El-Olemy *et al*.[29]

Preparation of aqueous grain extract of Digitaria exilis

The procedure earlier described by Adams *et al*.[18] was used. *Digitaria exilis* grains were processed into flour by sorting, drying, milling, and sieving. About 250 g of the resulting powdered flour was weighed and soaked in 1L of distilled water for 48 hours. The mixture was placed on mechanical shaker for uniform content stirring during this period. The mixture was then sieved with Whatman No. 1 Filter Paper (Whatman Plc., Maidstone, Kent, UK). The filtrate (aqueous extract) was steam-dried using a Water Bath (Model: BL-9825S, Germfree Laboratories Incorporated, 4 Sunshine Blvd, Ormond Beach, FL32174, Florida, USA) to give a partly solid extract. A fraction from the stock extract was then added to distilled water to prepare the doses needed for the study (50,100, and 200 mg/kg BW).

Animal grouping and extract treatment

In compliance with the ethical guidelines for proper animal handling procedures provided by the NHRA, UAECAU and Bingham University Karu Nigeria, adequate caution was taken during the sorting of the animals into four groups (A-D; n=5) as detailed below:

Group A: Healthy rats + DW only (Sham Control)
Group B: Healthy rats + AGEDE (50 mg/kg BW)
Group C: Healthy rats + AGEDE (100 mg/kg BW)
Group D: Healthy rats + AGEDE (200 mg/kg BW)
The animals were orally gavaged with AGEDE over 14 days.

Preparation of serum and tissue supernatant

The preparation of supernatants followed the earlier protocol of Adams and Eze.[30] In brief, under anaesthesia using diethyl ether, fur hair was quickly removed from the neck region of the animal, after which the jugular veins were cut open with an aseptic blade when blood cells (6 mL) were collected into plain blood sample container and this was centrifuged at 850 *g* x 15 min with a BioBase Laboratory Centrifuge (Model BD900B, BioBase Scientific {Shandong} Co., Ltd., Jinan, Shandong, China). The serum (supernatant) was pipetted carefully using a dropper and then used for serum hormone determination. Shortly after this, the rats were incised, followed by the collection of ovaries and uterus. The tissues were blotted independently, homogenized, and centrifuged to obtain the tissue supernatants used for ovaries hormone assay. The uterus collected earlier and part of the ovary were fixed for histopathology analysis in 10% formaldehyde.

Determination of hormonal biomarkers in the ovary and uterus
Determination of progesterone

The determination of progesterone in the serum and ovary of the animals was conducted by adopting the earlier procedure of Agoreyo and Onwegbu.[31]

Principle: The assay employs the Competitive Immunodetermination Technique. In this protocol, the material of interest in the test sample is attached to the fluorescence (FL)-marked determination antibody in an assay buffer to produce a complex sample solution. The complexed sample was easily transferred to the nitrocellulose matrix, where the covalently bonded progesterone and bovine serum albumin (BSA) were fixed on the test strip for interference and binding of the material of

interest with the FL-marked antibody. The faster the material of interest remains in the blood, the lower the determination of antibody aggregates, giving rise to a lesser fluorescence signal.
Procedure: About 30 µL of the sample was pipetted into a test tube containing a detection buffer. The detection buffer tube was then covered with a lid and mixed adequately by shaking for 5 minutes. The mixed sample mixture was used immediately. About 75 µL of a mixed sample solution was taken with a pipette and loaded on the sample well of the test cartridge. The samples were loaded on the test cartridge and held at 25 ℃ for 10 minutes. For the ichroma™ tests, the cartridge holder was placed on the instrument to scan the sample-loaded cartridge. The 'Select' button on the Hiwell Diatek ELISA Plate Reader (Model: DR-200Bc, Hiwell Diatek Instruments Co., Ltd., Wuxi, China) was pressed to commence the detection and scanning process for ichroma™ tests. Scanning of the sample-loaded cartridge begins instantly on the Reader for ichroma™ tests. The test result was displayed on the outcome screen of the Reader for the ichroma™ test for reading and recording.

Determination of luteinizing hormone (LH) concentration
The protocol previously illustrated by Kosasa[32] was followed to estimate LH levels in the serum and ovary of the animals.
Principle: The assay is a competitive inhibitory protein immunoassay methodology that involves pre-coating the substrate on a micro-titre plate in the packaged kit.
Assay procedure: All chemicals and consumables were preserved at 25 ℃ before the test's commencement and mixed well before usage. A known quantity (25 µL) of LH control, standards, and serum sample were pipetted into the holder for the exact number of coated strips required, after which 100 µL of conjugated reagent was introduced into all the wells. The plates were mixed on a mechanical shaker for 20 seconds at 500 rpm. This was followed by incubating the mixture for 50 minutes at 25 ℃, and the liquid was immediately removed from all the wells after the incubation process. A buffer solution (1X wash, 300 µL) was used to clean all the wells and drained with an aseptic towel. A known amount (100 µL) of 3',3',5',5'- tetramethylbenzidine (TMB) substrate was added to all the wells, followed by incubation for 10 minutes at 25 ℃. The incubation of all the wells was followed by the addition of 50 µL of a stop solution. After terminating the process with the addition of a stop solution, the absorbance of the mixture was read with a DxL Access Immunoassay Analyzer (Model: DxL 9005 Access; Beckman Coulter Co., Ltd, California, United States) within 10 minutes.

Determination of follicle-stimulating hormones (FSH) concentration
The previous method of Randolph *et al.*[33] was used in this study to determine the concentration of FSH in the serum and ovary of the animals.
Principle: The basic principle guiding the assay procedure involves using a competitive enzymic immunodetection technique.
Assay procedure: All chemicals and consumables used for the assay were preserved at 25 ℃ for 30 minutes before the experiment started. The solutions were also properly mixed before usage. All holders were filled with the exact number of coated strips. A known quantity (50 µL) of FSH standard was added to the selected wells, followed by addition of the sample. After this, 40 µL of the enzyme conjugates were added to fill the wells. Thereafter, the plates were covered with the lid and incubated for 60 minutes at 35 ℃. Shortly after incubation, the plates were read with an HiWell Diatek ELISA Plate Reader (Model: DR-200Bc, Hiwell Diatek Instruments Co., Ltd., Wuxi, China) at 560 nm within 15 minutes, and results were recorded accordingly.

Determination of estrogen
The technique previously used by Blair[34] was adopted in this study to determine estrogen in the serum and ovary of the animals.
Procedure: The initial approach involves the preparation of various work solutions required for the assay, including the number of microwell strips needed, wash buffer estradiol-biotin, and avidin-HRP conjugate. Approximately 40 µL of the control and test samples were measured in duplicate, and the plates were labelled appropriately. About 90 µL of the conjugated work solution was transferred with a pipette into each well. The mixture was placed on a magnetic shaker for homogenous mixing and then incubated at 40 ℃ for 40 minutes. Each well was cleaned thoroughly with 300 µL of dilute wash buffer, and firmly covered with a lid. Approximately 130 µL of TMB precursor solution was added to each well at 30-second intervals. The mixture was appropriately shaken and incubated for 10 minutes at 35 ℃ until a desired blue-black colour was observed. A known volume (40 µL) of terminating solution was added to each well at an interval of 15 seconds, after which the readings were taken with a Hidex Sense Micro Plate Reader (Model: DB750H; Hidex Oy Co., Ltd., Finland) at 480 nm within 15 minutes of adding the solution.

Calculations
- The average absorbance of each calibration/calibrator duplicate was computed.
- A calibration curve was drawn on a graph sheet with the average density of the absorbance on the Y-axis and the calibration levels on the X-axis.
- Again, the average absorbance value of each unknown calibration duplicate was computed.
- The reading of the unknown values was taken and extrapolated from the calibrator curve.

Determination of prolactin
The radioimmunoassay (RIA) protocol previously described by Ohnami *et al.* [35] was used in the current study to determine prolactin in the ovary of the animals.
Procedure: All consumables and chemicals were initially preserved at 25 ^{0}C before the start of the experiment. The ovary supernatant was mixed with 30 µL labelled prolactin (marked/labelled with a radioactive isotope) and an antibody that binds specifically to prolactin. The solution was incubated at 25 ^{0}C to prepare the labelled and unlabeled prolactin to participate competitively for binding to the antibody. This was followed by the separation of unattached prolactin from the antibody-bound prolactin. The concentration of the labelled prolactin attached to the antibody was measured with a gamma counter using a cobas® Radioimmunoassay Analyzer (Model: e807; Roche Diagnostics Ltd, North America). The radioactivity readings were compared with the prolactin standard calibration curve to obtain the prolactin concentration in the sample by extrapolation from the graphical curve, and the results were presented in ng/mL.

Determination of reproductive enzyme activity and cholesterol concentration in ovary
Determination of 3β-hydroxysteroid dehydrogenase (3βHSD) activity
The procedure of Shivanandappa and Venkatesh[36] was adopted in this investigation to determine the activity of 3*β*HSD in the ovary of the animals. The 3*β*HSD was estimated in 0.2 M Tris–HCl buffer (pH 7.5) that contains NAD (400 µM) and the precursor reactant, DHEA or pregnenolone (100 µM), in an overall volume of 2.0 mL. The determination process began by adding the enzyme (40 mL) and then incubating it at 40 ℃ for 30 minutes. The reaction process was terminated by adding 2.0 µL phthalate buffer (pH 4.0). The unclear components of the mixture were removed by centrifuging it at 2000 rpm for 10 minutes while the clearer supernatant was read with a UV-Vis BioBase Spectrophotometer (Model: BT-UV1901; BioBase Group Manufacturers, Zhangqiu, Shandong, China) at 500 nM. The enzyme activity was computed by extrapolation from the standard curve of NADH, and the result was expressed in units/mg of tissue/h.

Determination of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity
The protocol outlined by Akira *et al.* [37] that is dependent on stable isotope dilution method by Liquid Chromatography-Tandem Mass Spectrometry using Electrospray Ionization in positive mode was adopted to test for HMG CoA reductase activity in the ovary of the animals. Briefly, mevalonic acid, the final yield of HMG-CoA

reductase formation, was transformed to mevalonolactone (MVL) in the incubating solution, filtered out by the method of salting-out, to generate an intermediate product: mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide, and it was then cleaned adequately with a silica cartridge. The mevalonylamide produced was estimated quantitatively by a monitoring reaction using a positive mode Bruker Electrospray Ionization (ESI) Mass Spectrometer (Model: VIP25-HESI, Bruker Manufacturing Co., Ltd, Bremen, Germany). Mevalonylamide was observed to have a detection limit of 240 amol (signal-to-noise ratio = 3), ~833 times more sensitive when compared with those of the MVL estimated by a modern radioisotope (RI) technique (200 fmol). The difference between the prepared sample and readings obtained by this technique were quantified by a one-directional layout method and computed to be 3.3% and 1.9%, respectively. Post-experiments were conducted using an incubation solution that peaked at 0.78–2.32 nmol MVL/mg protein and was confirmed by a polynomial formula. These readings indicated that the estimated concentration within a 95% confidence limit was 0.48 ± 0.09 nmol/mg protein, which correlated with the observed $\bar{X}_0$ nmol/mg protein with an average recovery of 95.7%.

Determination of cholesterol concentration

The level of cholesterol concentration was estimated in the ovary of the animal by the "CHOD-PAP" technique previously reported by Li *et al.*[38]

Principle: The assay depends on the principle that after enzymatic breakdown, a metabolite called Quinoneimine is produced from hydrogen peroxide and 4-aminoantipyrine in a reaction catalyzed by peroxidase at 546 nm with a phenol substrate.

Procedure: The test tubes for the assay were marked as B (Blank), TS (Test Sample), and STD (Standard). A known amount (0.03 mL) of distilled water, ovary sample (supernatant), and standard water was collected with a pipette and dropped into test tubes marked as B, TS, and STD. A known quantity (3 mL) of the work (stock) reagent was picked with a pipette and placed into each of the test tubes marked as (Blank, Test Sample, and Standard). The solutions were thoroughly mixed and incubated at 25 ℃ for 10 minutes. The homogenous mixtures were mixed correctly and incubated (at 25 ℃for 5 minutes). Optical activity (absorbance) of the test sample (A_{sample}) and standard ($A_{standard}$) were measured with a UV-Vis BioBase Spectrophotometer (Model: BT-UV1901; BioBase Group Manufacturers, Zhangqiu, Shandong, China) within 30 minutes at a wavelength of 546 nm. The calculations were done using Equation 1.

$$\text{Conc. of Chol. (mg/dL)} = \frac{Absorbance\ of\ Sample\ x\ Concentration\ of\ StandARD}{Absorbance\ of\ Standard} \quad \text{.....Equation 1}$$

Concentration of standard = 12.50 mg/dL

Histopathological examination of the ovary and uterus

The ovary and uterus of the rats were fixed in 10% (v/v) formaldehyde, dried with preferred grades of ethanol (75%, 85%, and 95% v/v), cleaned in xylol, and dipped in paraffin wax (melting point 57 ℃). Each section of the ovary and uterus was then produced following the protocol outlined by Rocha-Pereira *et al.*[39] and stained using hematoxylin/eosin (H&E). The histopathological slides were observed under a light LED camera Magnifier (MIGHTEX, Model: EZ42, Pleasanton, CA 94566, USA). Micrographs representing the ovary and uterus were captured at x100 via Canon! Microsoft Image Capturing Software (Model: Capture Strength A3600, Germany).

Molecular docking studies

Protein selection

The 3D human structure of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) (PDB: 1HW9) was obtained from the Protein Data Bank at http://www.rcsb.org.

Ligand selection

The chemical pattern of the previously documented ligands obtained via GCMS analysis (ethyl acetate, l-hydroxy-2-propanone, pyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine and 2-methylpyrazine)[40] were recovered from the PubChem data bank at www.pubchem.ncbi.nlm.nih.gov, and the retrieved SDF template was changed to PDB pattern using Open Babel application.[41]

Induced fit molecular docking study

Molecular Operating Environment (MOE) Software[42] (Year of Release: 2023) was used for the induced fit molecular docking analysis to interact the documented compounds into the active site of the target proteins. Sequel to the docking of the ligands into the crystal fittings of the target protein, and the highest dock pose was selected for each of the compounds. The highest dock scores were taken as the most stable structure of each compound with their corresponding target proteins. The placement outcomes were evaluated using the MOE-Dock Visualizer 2023.[43] The accuracy of the docking outcomes was done by comparing the best binding affinity derived for the inhibitor/reference compound of 3*β*HSD (Epostane) in its bound structure. This was done by subtracting 3*β*HSD from the protein's active site and re-docking into the binding site using the specific terminals in the protein crystal shape.[44]

Statistical analyses

Statistical tools, one-way analyses of variance (ANOVA), and the Duncan multiple range test were adopted to analyze statistical data from the experiments (n=5). SPSS Version 29 (International Business Machines [IBM] Company; Year of Release: 2022) determined that differences at $p<0.05$ were substantive.

Results and Discussion

Administration of AGEDE at all investigated doses significantly *(p<0.05)* lowered the levels of LH, FSH, progesterone, and estrogen in the serum of the animals when matched with SC. In contrast, oral gavage of all doses of AGEDE significantly *(p<0.05)* heightened the concentration of prolactin in the serum of the animals when compared with SC (Table 1). Notably, the animals that were orally gavaged with 100 mg/kg BW of the extricate produced FSH values that are similar *(p>0.05)* to those that received 200 mg/kg BW of the extract. Interestingly, the extract's 50 mg/kg BW produced the most profound reduction in serum levels of LH, FSH, and progesterone compared to other doses (Table 1).

Table 1: Effect of oral gavage of aqueous grain extract of *Digitaria exilis* on reproductive hormones in serum of female Wistar rats

Treatment Group	LH (mIu/mL)	FSH (mIu/mL)	Progesterone (ng/mL)	Prolactin (ng/mL)	Estrogen (ng/mL)
SC	13.37±2.74^a	13.37±2.74^a	130.15±5.26^a	55.59±2.67^a	2705.98±9.02^a
50 mg/kg	8.85±0.95^b	8.85±0.95^b	47.56±2.92^b	126.68±4.23^b	105.68±3.12^b
100 mg/kg	5.04±0.13^c	5.04±0.13^c	11.28±1.74^c	83.17±3.75^c	902.32±4.23^c
200 mg/kg	1.80±0.02^d	1.80±0.02^d	34.56±0.32^d	222.58±5.03^d	621.64±5.28^d

Outcomes are summation ± SEM of five estimations. Superscripted readings that differ from their respective placebo values down the column are statistically dissimilar ($p<0.05$) SC = Sham Control; LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone

The oral gavage of AGEDE at all studied doses sufficiently *(p<0.05)* lowered the levels of LH, progesterone, prolactin, and estrogen in the animals' ovaries compared with SC. However, compared with SC, all oral gavage doses of AGEDE significantly (p<0.05) raised the FSH level in the animals' ovaries. It is worth noting that the 50 mg/kg BW of AGEDE resulted in the most pronounced reduction in the level of LH and estrogen in comparison with other doses (Table 2). The oral gavage of AGEDE at all examined doses significantly *(p<0.05)* lowered the level of cholesterol and the activities of 3*β*HSD and HMG CoA reductase in the animals' ovary compared with SC. Animals that were orally gavaged with 100 mg/kg BW of AGEDE gave activities of 3*β*HSD in the ovary that are similar *(p>0.05)* to those that received 200 mg/kg BW of AGEDE (Table 3).

Table 2: Effect of oral gavage of aqueous grain extract of *Digitaria exilis* on reproductive hormones in the ovary of female Wistar rats

Treatment Group	LH (mIu/mL)	FSH (mIu/mL)	Progesterone (ng/mL)	Prolactin (ng/mL)	Estrogen (ng/mL)
SC	42.38±3.82^a	3.05±0.03^a	18.02±2.39^a	139.06±4.28^a	1582.56±8.25^a
50 mg/kg	11.02±1.06^b	10.32±1.35^b	14.08±1.68^b	114.26±4.02^b	605.14±3.78^b
100 mg/kg	32.73±3.05^c	6.15±0.18^c	6.16±1.63^c	88.63±3.46^c	818.29±4.39^c
200 mg/kg	17.38±2.53^d	19.77±2.04^d	11.25±1.48^d	81.25±3.25^d	1104.04±7.12^d

Values are mean ± SEM of five samples. Test values with superscripts differing from their respective SC down the column are glaringly dissimilar (p<0.05) SC = Sham Control; LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone

Table 3: Selected reproductive enzyme activity and cholesterol concentration in the ovary of female Wistar rats after oral gavage with aqueous grain extract of *Digitaria exilis*

Treatment Group	3*β*HSD (unit/mg of tissue/h)	HMG CoA red. (HMG-CoA/Mevalonate)	Chol. (mg/dL)
SC	6.45±1.02^a	9.89±1.64^a	22.23±3.27^a
50 mg/kg BW of AGEDE	3.08±0.17^b	2.33±0.03^b	3.45±0.18^b
100 mg/kg BW of AGEDE	0.94±0.04^c	4.04±0.12^c	12.03±1.24^c
200 mg/kg BW of AGEDE	0.79±0.02^c	7.29±1.05^d	8.76±1.03^d

Values are mean ± SEM of five samples. Test values with superscripts differing from their respective SC down the column are glaringly dissimilar (p<0.05) SC = Sham Control; LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone Figures are summation ± SEM of five investigations. Superscripted figures having values different from their respective SC are sufficiently dissimilar down the column (p<0.05) 3*β*HSD = 3*β*-hydroxysteroid dehydrogenase; HMG CoA red. = HMG CoA reductase; Chol. = Cholesterol; BW = Body weight; AGEDE = Aqueous grain extract of *Digitaria exilis*

The administration of distilled water (DW) to animals revealed an ovary section with normal graffian follicles (white arrow) as well as normal theca cells (blue arrow) within the ovarian cortex. Furthermore, the ovarian stroma showed moderate vascularization without congestion (slender arrow) (Figure 1a). Histoarchitectural examination of the uterus section of animals treated with DW showed mild endometrial structure with a normal epithelial layer (white arrow). The endometrium also showed mild to moderate infiltration of inflammatory cells (blue arrow). The endometrial glands were seen to be normal (blue arrow), while the myometrium also appeared normal (black arrow) (Figure 1b). Histological study on the ovary of animals treated with 50 mg/kg BW of the aqueous extract of *Digitaria exilis* grain (AGEDE) showed some graffian follicles with follicular cyst (red arrow) with deranged theca cells (blue arrow) within the ovarian cortex. The ovarian stroma showed severe vascular congestion (white arrow) (Figure 2a). Treating the uterus section of rats with 50 mg/kg BW of AGEDE indicated a degenerated endometrium layer with a distorted epithelial layer (white arrow). The endometrium showed moderate to severe infiltration of inflammatory cells (blue arrow). There were deranged endometrial glands with severe hyperplasia (blue arrow), while the myometrium also appeared distorted (black arrow) (Figure 2b). Histopathological examination of the ovary of animals that received 100 mg/kg BW of AGEDE showed distorted graafian follicles with degenerated follicular cyst (red arrow), others with bizarre morphology (white arrow) within the ovarian cortex, while the ovarian stroma showed severe vascular congestion (black arrow) (Figure 3a). The uterus section of animals treated with 100 mg/kg BW of AGEDE revealed a disintegrated endometrial structure lined with a deranged epithelial layer (white arrow). The endometrium shows severe to chronic infiltration of inflammatory cells (slender arrow), and the endometrial glands are moderately atrophied (blue arrow). At the same time, the myometrium appeared to be severely infiltrated (black arrow) (Figure 3b).

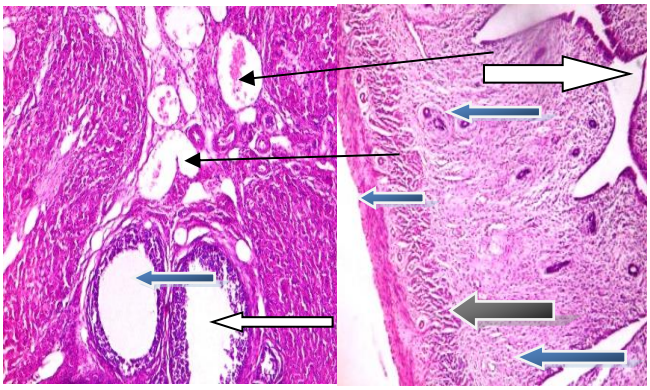

Figure 1a: Cross section of representative of the ovary of rat treated with distilled water (x100, H&E)

Figure 1b: Micrograph of representative of uterus rat that received distilled water (x100, H&E)

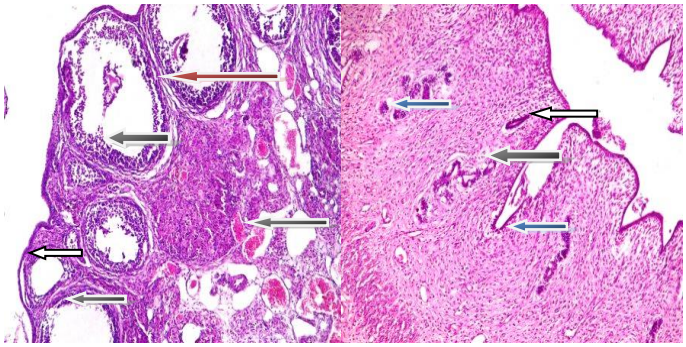

Figure 2a: Photomicrograph of representative of the ovary of a rat treated with aqueous grain extract of *Digitaria exilis* at 50 mg/kg BW (x100, H&E)
Figure 2b: Picture of representative of uterus of rat that took aqueous grain extract of *Digitaria exilis* at 50 mg/kg body weight (x100, H&E)

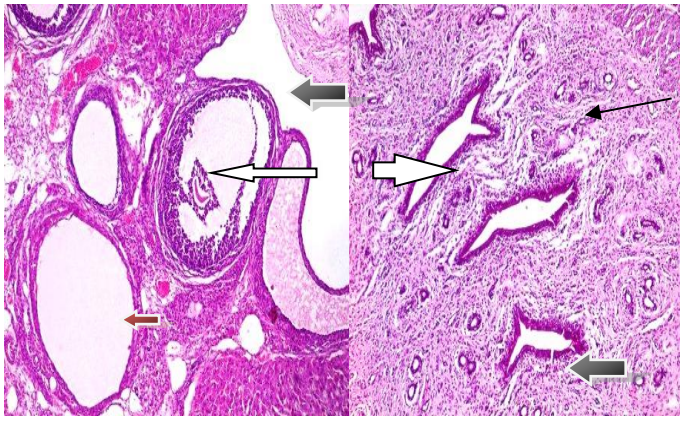

Figure 3a: Photomicrograph of representative of the ovary of rats that took aqueous grain extract of *Digitaria exilis* at 100 mg/kg body weight (x100, H&E)
Figure 3b: Cross section of representative of uterus of rat treated with an aqueous grain extract of *Digitaria exilis* at 100 mg/kg BW (x100, H&E)

The histoarchitectural analysis of the ovary of animals treated with 200 mg/kg BW of AGEDE showed few follicular cysts (white arrow) within the deranged ovarian cortex. Some follicles showed depleted and loosened granulosa (black arrow). The ovarian stroma showed degenerated luteinized cells (slender arrow) (Figure 4a). The uterus of rats that received 200 mg/kg BW of AGEDE showed a deranged endometrium layer with a distorted epithelial layer (white arrow). The endometrium showed severe infiltration of inflammatory cells (slender arrow), while the endometrial glands were seen atrophied and degenerated with glandular cells filled with fat/lipids (blue arrows) (Figure 4b).

Phytochemical analysis of AGEDE identified the presence of flavonoids, saponins and tannins (Table 4). Reports from previously identified compounds, via GCMS analysis, in *Digitaria exilis* by Lasekan and Feijao-Teixeira[40] revealed, among others, these chemical compounds with retention time and peak area (%): ethylacetate (5.67, 17.42); l-hydroxy-2-propanone (6.07, 12.49); pyrazine (10.15, 12.45); 2, 5-dimethylpyrazine (14.68, 19.53); 2,3-dimethylpyrazine (28.12, 6.33) and 2-methylpyrazine (13.30, 1.78) respectively.

Induced fit molecular docking analysis of compounds on binding pocket of the 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) enzyme revealed several bonding interactions including ethylacetate with 3*β*HSD (Figure 5), 1-hydroxy-2-propanone with 3*β*HSD (Figure 6), Pyrazine with 3*β*HSD (Figure 7), 2, 5-dimethylpyrazine with 3*β*HSD (Figure 8), 2-methylpyrazine with 3*β*HSD (Figure 9), 2-ethylpyrazine with 3*β*HSD (Figure 10), Epostane (Reference compound) with 3*β*HSD (Figure 11). Table 5 shows the binding energy and PubChem CID of documented bioactive compounds with 3*β*HSD. Ethylacetate had binding energy and PubChem CID of (-4.69227982, 8857); 1-hydroxy-2-propanone had binding energy and PubChem CID of docking score of (--3.91965556, 8299); Pyrazine had an interaction affinity and PubChem CID of (-3.80633807, 7976); 2, 5-dimethylpyrazine had a interaction score and PubChem CID of (-4.61382103, 31252); 2-methylpyrazine had a docking score and PubChem CID of (-4.0057497; 7976); 2-ethylpyrazine had an affinity range and PubChem CID of (-4.59648943, 26331) while Epostane had an interaction energy and PubChem CID of (-4.16974449, 6917713). Ethylacetate, which has a PubChem CID of (8857) and docking score of (-4.69227982), showed the best fit (binding affinity) on the binding pocket of the enzyme (Table 5). Assessing the possible contraceptive effect of plants can reveal its detrimental impact on the functional and structural well-being of the reproductive structure of an organism.[45] These assessments can be achieved via hormonal, histopathological, and molecular docking studies carried out in this study.

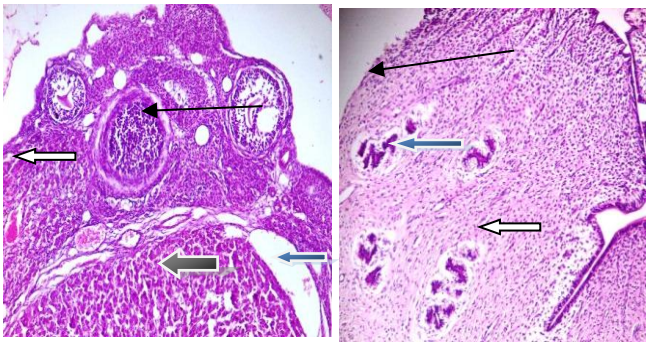

Figure 4a: Micrograph of representative of ovary of rats that took *Digitaria exilis* aqueous grain extract at 200 mg/kg BW (x100, H&E)
Figure 4b: Picture of the representative of uterus of rats treated with aqueous grain extract of *Digitaria exilis* at 200 mg/kg BW (x100, H&E)

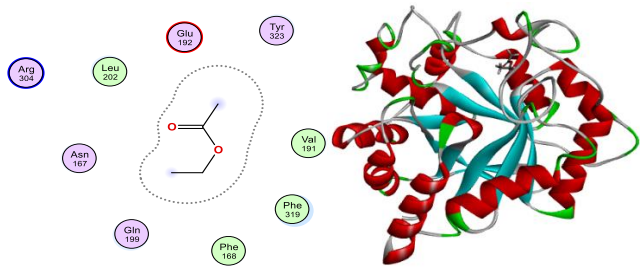

Figure 5: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with ethylacetate (Compound CID: 8857) and amino acid binding

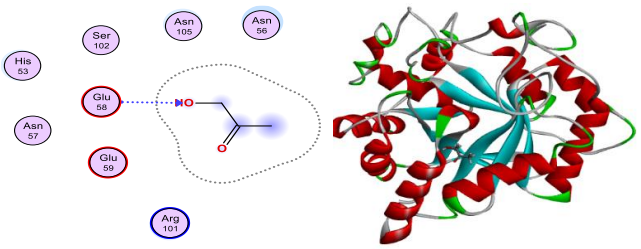

Figure 6: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with 1-hydroxy-2-propanone (Compound CID: 8299) and amino acid binding.

Table 4: Phytochemical analysis of *Digitaria exilis* aqueous grain extract

Phytochemical	Observation
Alkaloids	-
Saponins	+
Phlobatannins	-
Flavonoids	+
Tannins	+
Terpenoids	-
Phenolics	-

Key: (+) = Present; (-) = Absent

Table 5: Binding affinity (kcal/mol) of the six-rated previously documented compounds of *Digitaria exilis* grain by GC-MS analysis docked with 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) in the same binding pocket of the enzyme (Reference Compound: Epostane)

Compound Name	PubChem CID	Docking Score (kcal/mol)
Ethylacetate	8857	-4.69227982
1-hydroxy-2-propanone	8299	-3.91965556
Pyrazine	9261	-3.80633807
2, 5-dimethylpyrazine	31252	-4.61382103
2-methylpyrazine	7976	-4.0057497
2-ethylpyrazine	26331	-4.59648943
Epostane	6917713	-4.16974449

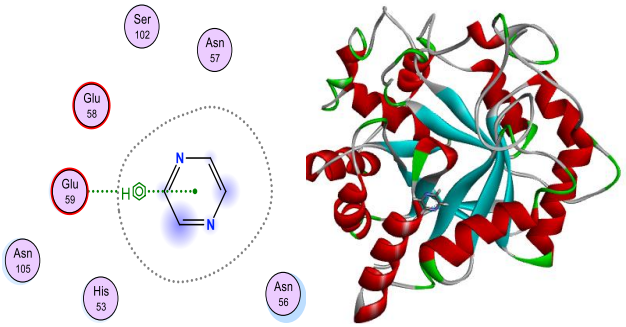

Figure 7: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with pyrazine (Compound CID: 9261) and amino acid binding.

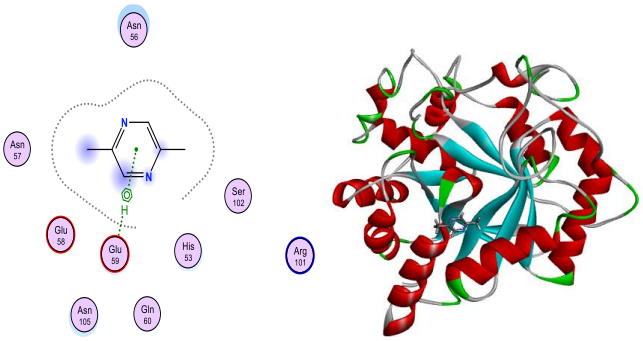

Figure 8: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with 2, 5-dimethylpyrazine (Compound CID: 31252) and amino acid binding.

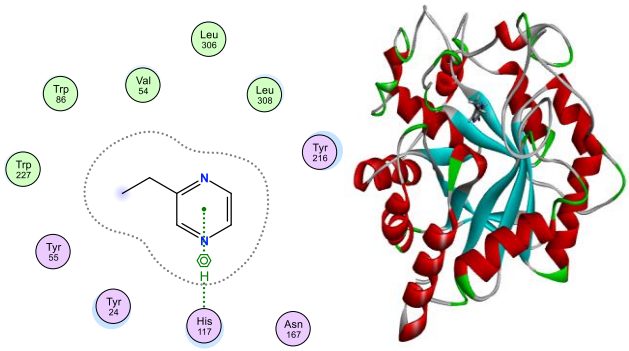

Figure 9: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with 2-methylpyrazine (Compound CID: 7976) and amino acid binding.

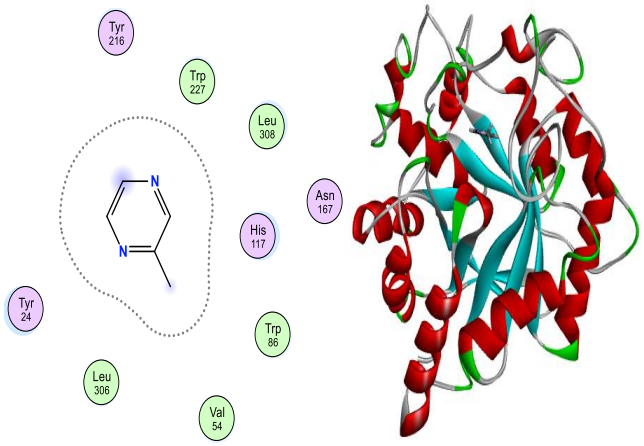

Figure 10: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with 2-ethylpyrazine (Compound CID: 26331) and amino acid binding.

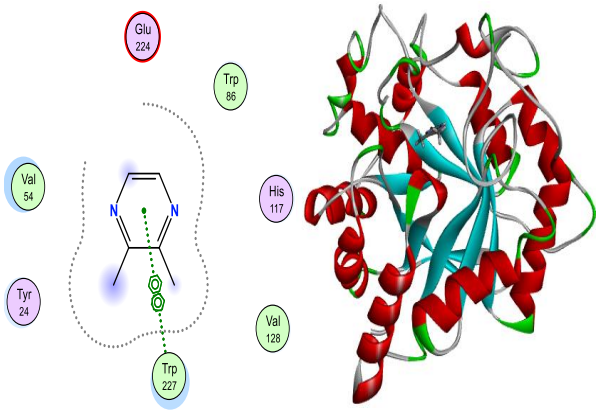

Figure 11: 2D and 3D depiction of the molecular interaction of 3*β*-hydroxysteroid dehydrogenase (3*β*HSD) docked with Epostane [Reference compound] (CID: 6917713) and amino acid binding.

LH is crucial for female fertility, which is key in regulating the menstrual cycle and ovulation. Specifically, LH triggers ovulation, the release of an egg from the ovary, and stimulates the corpus luteum to produce progesterone, which supports early pregnancy. Furthermore, LH is actively involved in follicle development, egg fertilization, and

implantation of the fertilized egg/sperm in the uterus.[46] Therefore, the low level of LH in the serum and ovary of the animals in this study will impair normal menstrual cycle and ovulation. The reduced LH level will also prevent normal follicular development and animal fertilization and conception (implantation).

FSH is essential for regulating the menstrual cycle and the proper functioning of the ovarian structure. It facilitates adequate development and growth of the ovarian follicles where the eggs are domiciled. FSH also prepares the ovum for its release by ovulation, and it stimulates estrogen formation. It is helpful in female fertility, which is required for egg maturation and subsequent release.[47,48] The low level of FSH in the serum and its high level in the ovary indicate an imbalance and may interfere with regular egg and ovarian follicle maturation. This imbalance would also affect the required release of eggs, the entire menstrual cycle and female fertility.

Progesterone plays a central role in female reproductive health by helping the uterus to prepare for pregnancy via thickening of the endometrium (uterine lining) for implantation/conception, inhibition/prevention of uterine contraction, thereby offering support for the onset of early pregnancy, thickening cervical mucus and control of menstrual cycle (menstruation and ovulation). Additionally, progesterone prepares the breasts for milk production, influencing mood and other aspects of female health. Progesterone also supports pregnancy and fetus growth via placenta formation, preparation of the mammary gland for breastfeeding and conferring support for the pelvic bones.[49] The reduced level of progesterone in the serum and ovary at all examined doses would affect steroidogenesis via interference in menstruation and egg release. Furthermore, the low progesterone level would also affect the maintenance of pregnancy in the animals by compromising the thickness of the uterine lining for implantation, pelvic muscle, normal fetus development, and placenta development.

Prolactin plays a significant role in forming breast tissue and signaling the breast to secrete milk after childbirth. It is also involved in controlling fertility by inhibiting the hormones that trigger ovulation.[50] The high serum prolactin level observed in this investigation by the activity of the extract will interfere with the normal formation of the hormones, including progesterone and estrogen. The high-level prolactin will also affect menstruation, leading to irregular periods or eventually causing fertility issues by inhibiting the hormones that trigger ovulation. The low ovary level of prolactin may harm the animals as it may affect steroidogenesis at the ovarian and urethral levels.

Estrogen is involved in female sexual and reproductive wellness by stimulation of pubertal/female reproductive character, regulation of the menstrual cycle and shedding of the endometrial lining. It also supports the release of eggs and preparation of the uterus for probable pregnancy and supports pregnancy by maintaining the integrity of the uterus.[51] The reduced estrogen level in the serum and ovary, by all doses of the extract in this study, might impair fertility by interfering with regular ovulation, reducing the thick uterine lining and probably preventing implantation (conception) of the fertilized ovum. It could also result in alteration in monthly periods, conception issues and female infertility.

3βHSD is a vital enzyme in female fertility that participates in steroidogenesis at the ovarian level. In specific terms, 3βHSD mediates in transforming pregnenolone to progesterone, a substrate for aldosterone and the formation of other associated hormones (estrogen).[52] Therefore, the low level of 3βHSD activity in the ovary of the animals by the extract could result in hormonal imbalance, female sex development challenges and alteration in the steroid hormone formation process. The reduction in ovary 3βHSD activity would also disrupt the steroidogenic pathway via reduced progesterone formation, a hormone required for swift action of the ovarian cycle and implantation (conception).

HMG Co-A reductase is the enzyme that mediates the reaction pathway flow rate in cholesterol formation. It is concerned with fertility in women via its influence on cholesterol concentration, which is needed for steroid hormone formation (progesterone and estrogen). Also, the inhibition of HMG Co-A reductase by statins in polycystic ovary syndrome (PCOS) may facilitate egg release and fertility in women suffering from PCOS. The inhibitory role of statin on PCOS women could offer an antioxidant role, which could confer robust ovarian function and improve fertility in a PCOS state. The reduced HMG Co-A reductase activity, mainly associated with statin use in a PCOS case, can impair women's fertility by lowering cholesterol levels, which is crucial for steroidogenesis. This may lower ovulation, alter the menstrual cycle, and possibly cause difficulty in achieving pregnancy.[53] Without a statin inhibitor, a reduced HMG Co-A reductase activity may lower cholesterol production while disrupting the formation of hormones required for egg release, menstrual cycle and maintenance of a healthy pregnancy. This study's low HMG-CoA reductase activity can negatively impact women's fertility via a reduced cholesterol formation pathway, crucial for steroid hormone production. This can lead to decreased ovulation, menstrual cycle irregularities, and potential difficulties in achieving pregnancy.

Cholesterol is a primary substrate for forming sex hormones (estrogen and progesterone), which are required for regular menstrual cycles and ovulation.[54] The low cholesterol levels of the extract in this study may impair female fertility by affecting egg release, egg quality and hormonal balance.

Medicinal plants with promising contraceptive and female fertility-controlling potentials can be explored as an alternative to conventional/chemical-hormonal contraceptives because they help control the ever-increasing rise in population growth and child spacing. Saponin inhibits the oestrous cycle and reduces fertility and degeneration of the ovarian cortex and graffian follicles in the ovary of the animals.[55,56] Flavonoid is seen to alter follicle development, subsequent egg release, implantation of fertilized egg (conception) and degradation of the endometrium layer of the uterus.[57] Tannin is known to slow down aromatase enzyme action and may hamper probable steroid hormone formation and female reproductive prowess.[58] Therefore, it can be inferred that the phytochemicals contained in *Digitaria exilis* grain contain contraceptive agents that could interfere with steroidogenesis.

Histopathological examination of the female reproductive structure reveals valuable information about the histoarchitectural status of the endometrium, uterus lining, ovary wall, endometrial gland, and its general predisposition to polyps, inflammation or receptiveness to implantation (conception), among others.[59,60] The observation of ovarian stroma with degenerated luteinized cells, minute follicular cyst in the deranged ovarian cortex, graffian follicles with follicular cyst, and presence of follicles with depleted and loosened granulosa in the ovary of the animals may be adduced to contraceptive agent present in AGEDE. The observation of a deranged endometrium layer with a distorted epithelial layer, endometrium with severe infiltration of inflammatory cells, and the endometrial glands with atrophied and degenerations with glandular cells filled with fat/lipids in the uterus of the rats indicates the detrimental effect of phytochemical present in AGEDE. The significant structural damage in the histological sections of the ovary and uterus of the animals by AGEDE further supports the possible infertility impact of the plant on female reproduction. The induction of degeneration by AGEDE on the follicular wall may be responsible, in part, for the significant decrease in serum estrogen levels and other reproductive hormones which support normal follicle development, menstruation, ovulation and conception (pregnancy).[61,62] All these also point to the contraceptive potential of the plant. Induced fit molecular docking is a computational technique employed in contraception research studies to identify and characterize binding interactions between compounds derived (ligand) from medicinal plants with receptor protein target (enzyme) involved in processes like egg maturation, egg release (ovulation), fertilization, implantation and conception/pregnancy. It is a valuable tool to predict how plant-derived compounds (ligands) block the activity of key regulatory enzymes involved in the hormone biosynthetic process, with the blockage interfering/interrupting the formation of hormones usually involved in female fertility. The compound that blocks and interrupts hormone formation could be explored as a potential contraceptive agent.

Epostane, the reference compound used in the induced fit molecular docking study, is a potent inhibitor of 3β-hydroxysteroid dehydrogenase (3βHSD), which operates by blocking the enzyme (3βHSD) from catalyzing the conversion of pregnenolone to progesterone, thus lowering the concentration of progesterone and interfering with egg release (ovulation), fertilization and early

conception (pregnancy) in the animals. This, therefore, confers a probable detrimental role on epostane relative to female fertility. Therefore, the strong binding interaction/affinity of the previously identified compounds: ethyl acetate, 2, 5-dimethylpyrazine, 2-methylpyrazine and 2-ethylpyrazine with the amino acids on the enzyme molecule, which compared well with that of epostane (reference compound), is an indication that the compounds can block 3*β*HSD from converting pregnenolone to progesterone.[63,64] This blockage/inhibition would cause an interruption in progesterone synthesis, thus reducing progesterone levels, whose moderate/high levels are needed for the growth and maintenance of the uterine lining during early pregnancy.[65,66] The potent inhibition in the activity of 3*β*HSD by the identified compounds, which leads to a reduction in progesterone level, could result in obstruction in the menstrual cycle, which may affect ovulation, fertilization and conception.[67] Therefore, ethyl acetate, 2, 5-dimethylpyrazine, 2-methylpyrazine, and 2-ethylpyrazine that are derived from the studied plant, demonstrated good binding properties and could be explored as potential antiprogesterone and contraceptive agents.

Conclusion

The study revealed that *Digitaria exilis* grains had a toxicological effect on the female reproductive organs via alteration and/or interference with steroidogenesis at the molecular, hormonal, enzymic, uterus and ovarian levels. These interferences could impair the animals' ovulation, fertilization, conception and pregnancy. Therefore, *Digitaria exilis* may be explored as a female fertility-controlling agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby affirm that the findings contained in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The research team of this study truly appreciate the technical input of all the laboratory staff members of the Biochemistry Department, Bingham University Karu, Nigeria.

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