



Modulatory properties of *Thespesia garckeana* (F. Hoffm.) Exell & Hillc. Aqueous fruit extract on female rats reproductive hormones and tissues histology and potential inhibitory activity of its constituents against SRD5 α 2

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ABSTRACT

Ethnopharmacological relevance: *Thespesia garckeana* (F. Hoffm.) Exell & Hillc. is called *Gorontula* (Kola of Tula) in Nigeria, *Morajwa* in Botswana, and *Thespesia garckeana* in South Africa and is widely distributed across Africa. Its parts reportedly possess multiple medicinal properties and are employed for treating various diseases. In Tula, Gombe State, Nigeria, the ripe fruit decoction is taken as remedy for female infertility as documented by Ochokwu and co in the Journal of Biology, Agriculture and Healthcare in 2015.

Aim of the study: This research examined the effects of aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc. (AFETG) on selected reproductive tissues and hormones in female rats and also evaluated the inhibitory potentials of its phytoconstituents against human 5- α reductase 2 (SRD5 α 2) using *in silico* approach.

Methods: Twenty-five (25) sexually matured female rats were randomized into 5 groups (i.e. A - E). Oestrous in the rats was synchronized (subcutaneous oestradiol conjugate [10 μ g/100 g BW]). Group A received distilled water (control). Group B received standard drug, clomiphene citrate (0.85 mg/kg BW), while groups C, D, and E received AFETG at 50, 100, and 200 mg/kg BW respectively. The animals were treated for five (5) days and sacrificed 24 h after. Their blood was collected and prepared for analysis of serum prolactin, oestradiol (E2), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) while ovaries, uteruses, and oviducts were studied for histological and histomorphometric changes. For the *in silico* study, the target protein, human steroid 5 α -reductase 2 (SRD5 α 2) was prepared and its receptor grid was generated using Optimized Potential for Liquid Simulations-2005. The ligand 2D structures were prepared using LigPrep 2.4 software and docked using Glide. The binding energy of the ligands to the protein receptor was predicted using Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) analysis.

Results: AFETG significantly increased serum levels of E2 but did not alter serum levels of prolactin, LH and FSH when compared with distilled water and clomiphene citrate. AFETG also significantly increased ovarian tertiary follicular diameter, oviductal epithelial height and serosa thickness as well as uterine endometrial epithelial height, endometrial thickness, and myometrial thickness when compared with control. Ovarian secondary follicular diameter and oviductal submucosa thickness and muscular thickness were significantly decreased by AFETG when compared with control. Two compounds in *T. garckeana* (F. Hoffm.) Exell & Hillc.; D-Melezitose

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(−12.55 kcal/mol XP GScore) and 1, 3, 4-trihydroxy-5-oxo cyclohexane-1-carboxylic acid (−9.136 kcal/mol XP GScore) exhibited higher binding affinities for SRD5α2 than the reference ligand, epristeride (−8.096 kcal/mol XP GScore).

In conclusion, the ability of AFETG to increase serum E2 level, thickness of uterine endometrium and ovarian tertiary follicles size can be explored for the treatment of female infertility caused by thinning of the uterine endometrium and reduced follicular size. Two compounds in AFETG (i.e. D-Melezitose and 1, 3, 4-trihydroxy-5-oxo cyclohexane-1-carboxylic acid are potential inhibitors of SRD5α2, thus aiding the biosynthesis of E2. Available evidence therefore corroborate the traditional use of *T. garckeana* (F. Hoffm.) Exell & Hillc fruit as a female fertility enhancer in Northern Nigeria.

1. Introduction

Infertility is a condition that affects either male or female reproductive systems and is characterized by the inability to achieve a pregnancy after at least 1 year of regular unprotected heterosexual intercourse. Between 186 million people and 48 million couples have infertility worldwide. Infertility can have great detrimental impacts on the lives of affected couples (Taebi et al., 2021). Although infertility may occur solely due to male factors, women are more likely to experience anxiety, emotional stress, depression, divorce, low self-esteem, social stigma, and violence (Taebi et al., 2021). Infertility in females can be caused by a variety of factors, including fallopian tube damage or blockage which may be caused by Pelvic Inflammatory Disease (PID), endometriosis, or surgery for an ectopic pregnancy; uterine or cervical abnormalities which may be caused by fibroids, polyps, or congenital abnormalities of the uterus or cervix; age-related factors, lifestyle factors (smoking, excessive alcohol consumption, obesity, and stress), and ovulatory disorders which may be caused by hormonal imbalances and polycystic ovarian syndrome (Abrao et al., 2013; Sharma et al., 2013; Zepiridis et al., 2016; Zhang et al., 2018; Cunha and Póvoa, 2021; Zhang et al., 2021) (see Figs. 4 and 5).

Every individual or couple has the right to have a family of their own. This right is sometimes denied due to the lack of provision of fertility care services in many parts of the world. As things stand, most countries still struggle with the accessibility, availability, and quality of therapies to address infertility (Inhorn and Patrizio, 2015). Prevention, diagnosis, and treatment of infertility are usually not considered a top priority in national policies and therefore barely catered for by public health financing. The unavailability of necessary equipment and trained medical personnel, together with the unaffordability of treatment, are additional impediments to universal access to fertility care. As a result, people in developing countries employ medicinal plants to address their infertility challenges. Examples of some plants employed for the treatment of female infertility in Nigeria include *Spondias mombin* L. leaf and seed, *Xylopiya aethiopica* (Dunal) A. Rich. fruit, *Alstonia boonei* De Wild. leaf, *Cocos nucifera* L. fruit water, *Kigelia africana* (Lam.) Benth. fruit, *Mallotus oppositifolius* (Geiseler) Mull. Arg. leaf, and *T. garckeana* (F. Hoffm.) Exell & Hillc. fruit among others (Ogunlakina and Sonibare, 2019).

T. garckeana (F. Hoffm.) Exell & Hillc. belongs to a class of plants called Magnoliopsida and the family Malvaceae. It is commonly called “tree hibiscus”, “Azanza”, or “snot apple”. It is called *Gorontula* (Kola of Tula) in Nigeria, *Morojwa* in Botswana, and *T. garckeana* in South Africa (Mojeremane and Tshwenyane, 2004; Orwa et al., 2009; Ochokwu et al., 2014). *T. garckeana* (F. Hoffm.) Exell & Hillc. has a wide distribution across east, west, and southern Africa and naturally grows in all woodland types from sea level to about 1700 m above (Mbuya et al., 1994).

The bark, fruit, leaf, roots, and stems of *T. garckeana* (F. Hoffm.) Exell & Hillc. reportedly possess multiple medicinal properties and are employed for treating or managing various diseases and ailments across its distributional range. Its root infusion is taken orally as a remedy for chest pains, cough and menstruation problems in Nigeria, Zimbabwe, and Kenya (Gelfand et al., 1985). In Malawi, Nigeria, and Zambia, a

decoction of its root and stem bark is administered orally to treat gonorrhea, sexually transmitted diseases, and syphilis (Morris and Msonthi, 1996). In Botswana, Kenya, Malawi, and Nigeria, oral administration of the leaf, stem, and root decoctions or ripe fruits are used as a treatment for infertility and liver issues. In Nigeria, fruit poultices are applied on abscesses (Ochokwu et al., 2015) and ripe fruits are taken orally as an aphrodisiac (Dikko et al., 2016).

The antibacterial activity of crude root extract of *T. garckeana* (F. Hoffm.) Exell & Hillc. and several pure compounds obtained from its roots against *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus aureus* were reported by Mutindi (2014) and Masila et al. (2015) while Dikko et al. (2016) similarly reported the antibacterial activities of the ethyl acetate, n-hexane and methanol extracts of its fruit pulp extract against *Enterococci*, *Escherichia coli*, *Helicobacter pylori*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Amuri et al. (2017) reported the hypoglycemic and anti-hyperglycemic activities of its aqueous leaf extract in guinea pigs while Connelly et al. (1996) reported weak antimalarial activities of its aqueous and organic fractions against *Plasmodium falciparum*. The cytotoxicity of the stem bark petroleum ether, ethyl acetate, acetone, methanol and water extracts using the brine shrimp lethality test has been reported (Mshelia et al., 2016) while Ahmed et al. (2016) reported the cytotoxicity of dichloromethane and methanol (1:1) extract of its stem bark on CCRF-CEM leukemia cell line using the resazurin reduction assay.

Since *T. garckeana* (F. Hoffm.) Exell & Hillc. is readily employed in Northern Nigeria for the treatment of fertility problems, it is, therefore, necessary to ascertain the pro-fertility activity claim and to determine the mechanism by which it is elicited. This study evaluated the effects of AFETG on reproductive hormones and the histology and histomorphometry of ovaries, oviducts, and uteruses in female Wistar rats. In addition, constituents of *T. garckeana* (F. Hoffm.) Exell & Hillc. was also docked against human 5-α reductase 2 (SRD5α2) for possible inhibition using *in silico* approach.

2. Materials and methods

2.1. Plant material

Ripe fruits of *T. garckeana* (F. Hoffm.) Exell & Hillc. were obtained from Tula village in Gombe State, Nigeria. They were identified and authenticated at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, where the specimen was deposited (UIL/001/1577/2022).

2.2. Experimental animals

Twenty-five (25) 6–7 weeks old, sexually matured female Wistar rats of average weight 110.50 ± 5.30 g were obtained from the experimental animal-breeding section of the Department of Pharmacology, University of Jos, Nigeria. The rats were accommodated in cages made of plastic and given unrestricted access to recommended rat pellets (Top Feeds Ltd, Ogorode Industrial Estate, Sapele, Delta State, Nigeria) and tap water. The handling of rats in this research was done following

guidelines issued by the University of Jos ethical committee on the care and use of animals for research. This work was allocated ethical clearance number: UJ/FPS/F17-00379.

2.3. Assay kits, reagents, and drugs

Kits for assay of prolactin, E2, FSH, and LH were products of Cal-Biotech Inc., El Cajon, California, USA. Clomiphene citrate (Batch No. 1-29016) was a product of Doppel Farmaceutici SRI, Cortemaggiore (PC), Italy. Ketamine and xylazine were products of Alfasan International Woerden, Netherlands. Alcohol, xylene, paraffin wax, haematoxylin, and eosin were obtained from reputable chemical vendors in Jos, Plateau State, Nigeria.

2.4. Preparation of aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc

One kilogram (1 kg) of fresh ripe *T. garckeana* (F. Hoffm.) Exell & Hillc. fruits were washed, crushed, and extracted in a water bath containing 2 L of distilled water at 40 °C for 12 h. The extracted fruit juice was then filtered with a strainer and the resulting filtrate was concentrated at 50 °C on a water bath to constant weight. The doses (i.e. 50, 100, and 200 mg/kg BW) used in this research was prepared by reconstituting the concentrate in distilled water.

2.5. Animals grouping and treatment administration

Twenty-five sexually matured female Wistar rats were used for this experiment. Oestrous was synchronized in all the rats by administering E2 conjugate (10 µg/100 g BW) through a subcutaneous route 48 h before the commencement of treatment (Amin et al., 1996). Confirmation of the estrous phase in female rats was done by examining their vaginal smears according to OECD-406/407 guidelines (Steinberg et al., 2019). The rats were then randomly assigned into 5 groups (i. e. A - E) consisting of 5 rats each. Group A served as control and received distilled water. Group B was administered a standard drug (clomiphene 0.85 mg/kg), while groups C, D, and E received AFETG at 50, 100, and 200 mg/kg BW respectively. All treatments were administered by oral gavage. The rats were treated for five (5) days and anesthetized by intramuscular administration of ketamin (0.3 mL) and xylazine (0.4 mL) 24 h after the last treatment administration. Dissection of the rats was done, followed by the collection of blood samples by cardiac puncture into plain bottles for hormonal analysis. Rats' ovaries, uteruses, and oviducts were also removed and placed in bottles containing 10% w/v formalin for histological and histomorphometric study.

2.6. Preparation of serum for biochemical analysis

Blood samples collected into plain bottles were left to clot for 1 h at 25 °C and thereafter centrifuged (Allegra X-30, Beckman Coulter Life Sciences, Indianapolis, USA) for 10 min at 3000 rpm. Serum samples were then drawn out of the centrifuge tubes into plain tubes using a Pasteur's pipette and used immediately for the determination of prolactin, E2, LH and FSH.

2.7. Hormonal profile determination

Biochemical assays were done using Absorbance Microplate Reader (Molecular Devices SpectraMax 340PC384, San Jose, California, USA). Prolactin, E2, LH and FSH were determined by following the methods of Vanderpump et al. (1998), McNatty et al. (1976), Cumming et al. (1985) and Kjeld et al. (1976) respectively.

2.8. Histological processing and examination

Histological examination of the ovaries, uteruses, and oviducts was

carried out following the method of Krause (2001). Portions of the tissue were fixed in 10% buffered neutral formalin for 72 h at 25 °C. Increasing concentrations of ethanol (70%, 90%, and 95% v/v) were used to dehydrate the fixed tissues. Following the process of cleaning the tissues using xylene and subsequently impregnating and embedding them in paraffin wax with a melting point of 56 °C, the sections were sliced at a thickness of 5.0 µm using a rotating microtome (Manual Rotary Microtome, STY2260, Jiangsu, China). Clean slides were lightly coated with egg albumin preparation and the cut sections were then floated out on them. The albumin was to prevent the detachment of sections from slides during the staining procedure. The sections were dried in an oven (Carbolite PF 200, Keison Products, Essex, United Kingdom) at 37 °C for 2 h, dewaxed in xylene, and hydrated using decreasing concentrations of ethanol (100%–20%). They were thereafter stained in 1.0% alcoholic eosin, mounted on Canada balsam, and labeled appropriately. The slides were then examined under a microscope (Binocular microscope WKT-LE4, Jiangsu, China) and the photomicrographs were obtained in the bright field at ×400 magnification.

2.9. Tissue histomorphometry determination

The Ovarian, oviductal, and uterine histomorphometric measurements were carried out following the methods of Haslan et al. (2021) and (Adelakun et al., 2022). Briefly, the photographed (× 10 objective lens) sections of the ovary, uterus, and oviduct in each of the rats in the different groups ($n = 5$) treated with AFETG were used. The diameter of the different follicular types (primary, secondary and tertiary) on the ovarian surface of each rat was determined using the modified method of Adelakun et al. (2022). Briefly, five (5) follicles each of the different follicular types were selected from each of the ovaries of the five rats in a group. This bring the total follicles of each primary, secondary and tertiary to 25 follicles per experimental group. In addition, five oviducts and uteruses were chosen per group for determination of variations in epithelial height and submucosa, muscular and serosa tunica thickness of the oviduct, and endometrial epithelial height, glandular epithelial height, glandular luminal diameter, endometrial thickness, myometrial thickness and perimetrial thickness of the uterus. Histomorphometric dimensions were taken using Motic Image Plus, China. The following descriptions were considered during the measurement of the above-stated parameters:

Ovarian follicular diameter: determined from four follicular diameter length coordinates with a common intersection,

Oviductal epithelial height is the distance between the basement membrane and the apical part of the oviductal epithelium,

Oviductal submucosa thickness: is the distance between the basement membrane and the proximal border of the muscular coat layer,

Muscular tunica thickness: is the distance between the distal border of the submucosal layer and the proximal border of the serosal layer,

Serosa tunica thickness: is the distance between the distal border of the muscular tunic and the external border of the entire oviductal tube, and.

Endometrial epithelial height: the distance between the basement membrane and the apical part of the endometrial epithelium,

Glandular epithelial height is the distance between the glandular basement membrane and the apical part of the glandular epithelium,

Glandular luminal diameter: determined from four diameter length coordinates within the glandular lumen with a common intersection,

Endometrial thickness is the space that separates the apical part of the endometrium and its distal border with the myometrial layer,

Myometrial thickness is the space that separates the distal end of the endometrium and the proximal border of the perimetrium, and.

Perimetrial thickness is the space that separates the distal end of the myometrium and its outer boundary (Haslan et al., 2021).

2.10. In-silico analysis

2.10.1. Preparation of target proteins

The protein preparation wizard in Maestro of Schrödinger software was employed for the preparation of the human steroid 5 α -reductase 2 (SRD5 α 2) (PDB ID: 7BW1) protein following its retrieval from the protein database. The Optimized Potential for Liquid Simulations-2005 (OPLS_2005) force field was used to reduce the complex until root mean square deviation (RMSD) of 0.3 was obtained.

2.10.2. Receptor grid generation

The Receptor Grid Generation panel of OPLS_2005 was employed to generate the grid. It specifies receptor structure by eliminating any potential co-crystallized ligands, thus deciding on the position and size of the active site, and representing it with a receptor grid.

2.10.3. Ligand preparation

The 2D structure of the compounds obtained from GCMS analysis of *T. garckeana* (F. Hoffm.) Exell & Hillc. fruit pulp (as reported by Momodu et al., 2022) was prepared using LigPrep 2.4 software (Mahmoud et al., 2021), which can produce a variety of structures with different ionization states, ring conformations, stereochemistries and tautomers from each input structure to exclude molecules based on criteria like molecular weight or the number and type of functional groups present with correct chiralities for each successfully processed input structure. The OPLS-2005 force field was used for optimization, which resulted in the ligand's low-energy conformer (Tripathi et al., 2013).

2.10.4. High throughput virtual screening

The most common methodology for selecting and ranking potential drug candidates from a compound library is virtual screening (Hussain et al., 2021). Based on the active sites of the Human steroid 5 α -reductase 2 (SRD5 α 2) (PDB ID: 7BW1) target, high throughput virtual screening was carried out utilizing compounds from *T. garckeana* (F. Hoffm.) Exell & Hillc.. Using a three-tiered docking technique based on Glide, the compounds were all docked using the steps of High Throughput Virtual Screening (HTVS), Standard Precision (SP), and Extra Precision (XP). Then the best compounds were ranked based on the more precise computational XP GScoring algorithm.

2.10.5. Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) analysis

The docked complexes of the highest-ranking scores including the standard ligands were subjected to MM-GBSA analysis to predict the binding energy using the prime method. The binding energies produced by MM-GBSA OPLS-2005 are thought to be far more accurate than the XP GScore.

$$\Delta G_{\text{(bind)}} = \Delta G_{\text{(solv)}} + \Delta E_{\text{(MM)}} + \Delta G_{\text{(SA)}}$$

Where: $\Delta G_{\text{(solve)}}$ is the difference in GBSA solvation energy of the protein-ligand complex and the sum of the solvation energies for unliganded protein and ligand.

$\Delta E_{\text{(MM)}}$: is a difference in the minimized energies between the protein-ligand complex and the sum of the energies of the unliganded protein and inhibitor.

$\Delta G_{\text{(SA)}}$: is a difference in surface area energies of the complex and the sum of the surface area energies for the unliganded protein and inhibitor.

2.11. Statistical analysis

Data were presented as mean (n = 5) $\pm$ Standard Error of Mean (SEM). Data were analyzed using the IBM® Statistical Package for Social Sciences (SPSS) software version 20. Significant differences were determined by one-way Analysis of Variance (ANOVA) and Post-Hoc

multiple comparisons were carried out using Duncan's multiple range test. The significance level was set at $p < 0.05$.

3. Results

3.1. Reproductive hormones in female Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc

Prolactin levels in rats treated with AFETG (all doses) did not differ significantly ($p > 0.05$) from those of control rats treated with distilled water and those treated with clomiphene citrate (Table 1).

Rats treated with AFETG (all doses) and those treated with clomiphene citrate (all doses) had significantly higher ($p < 0.05$) levels of E2 than those in the distilled water-treated control group. Rats treated with AFETG (at all doses) produced a significantly higher ($p < 0.05$) E2 concentration than the clomiphene citrate-treated rats. Rats that received AFETG at 50 and 100 mg/kg BW showed a significantly lower ($p < 0.05$) E2 concentration than those treated with AFETG at 200 mg/kg BW (Table 1).

AFETG (all doses) and clomiphene citrate did not alter rat serum LH and FSH levels when compared with the distilled water-treated control rats (Table 1).

3.2. Histopathology and histomorphometry of the ovary in Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc

The effect of exposure of rats to different concentrations of *T. garckeana* (F. Hoffm.) Exell & Hillc. is shown in Plate 1. The control rat displayed no visible lesion in the ovarian parenchyma and exhibited noticeable serosa coverings that enveloped the entire ovary. Additionally, there were follicles present (identified by red arrows for primary follicles, a different red arrowhead for secondary follicles, and a black arrowhead for tertiary follicles) in various stages within the ovarian cortex and a clearly defined ovarian medulla (outlined in an elliptical shape) (Plate 1A). For. Clomiphene citrate-treated rat, mild vascular congestion of medullary vessels (red arrow), and moderate focal follicular degeneration (black arrowhead) were observed (Plate 1B). However, the various concentration of AFETG displayed moderate to severe follicular degeneration (red arrow) and depletion of ovarian cortical stroma cells (oval outline) (Plate 1 C-E).

Administration of AFETG (all doses) did not result in any significant alteration ($p > 0.05$) in ovarian primary follicular diameter and is

Table 1

Serum prolactin, estrogen, luteinizing hormone, and follicle-stimulating hormone in female Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

Treatment Group	Serum Prolactin (ng/mL)	Serum Oestradiol (pg/mL)	Serum Luteinizing Hormone (mIU/mL)	Serum Follicle Stimulating Hormone (mIU/mL)
Control (Distilled Water)	0.60 $\pm$ 0.03 ^a	29.04 $\pm$ 1.36 ^a	0.14 $\pm$ 0.01 ^a	0.0010 $\pm$ 0.0000 ^a
Clomiphene citrate (0.85 mg/kg BW)	0.64 $\pm$ 0.02 ^a	34.87 $\pm$ 1.38 ^b	0.14 $\pm$ 0.01 ^a	0.0010 $\pm$ 0.0000 ^a
AFETG (50 mg/kg BW)	0.61 $\pm$ 0.03 ^a	40.61 $\pm$ 1.07 ^c	0.14 $\pm$ 0.01 ^a	0.0012 $\pm$ 0.0002 ^a
AFETG (100 mg/kg BW)	0.66 $\pm$ 0.01 ^a	44.40 $\pm$ 1.48 ^c	0.12 $\pm$ 0.01 ^a	0.0012 $\pm$ 0.0002 ^a
AFETG (200 mg/kg BW)	0.64 $\pm$ 0.02 ^a	55.43 $\pm$ 1.61 ^d	0.14 $\pm$ 0.01 ^a	0.0012 $\pm$ 0.0002 ^a

Data represent means (of 5 replicates) $\pm$ SEM, values carrying different superscripts down the column are significantly different at $p < 0.05$. AFETG: Aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

comparable to distilled water and clomiphene citrate. Rats treated with AFETG at 100 mg/kg BW showed a significantly reduced ($p < 0.05$) ovarian primary follicular diameter than those treated with AFETG at 50 and 200 mg/kg BW (Table 2).

Rats treated with clomiphene citrate and AFETG (all doses) showed a significantly reduced ($p < 0.05$) ovarian secondary follicular diameter when compared with rats in the control group treated with distilled water. Rats treated with AFETG at all doses showed a significantly higher ($p < 0.05$) ovarian secondary follicular diameter than the clomiphene citrate-treated rats while rats treated with 100 and 200 mg/kg BW of AFETG showed a significantly higher ($p < 0.05$) ovarian secondary follicular diameter than those treated with AFETG at 50 mg/kg BW (Table 2). The diameter of ovarian tertiary follicles significantly increased ($p < 0.05$) in clomiphene citrate-administered rats and those administered various doses of AFETG in comparison with the distilled water-administered control rats. AFETG at 200 mg/kg BW significantly increased ($p < 0.05$) ovarian tertiary follicular diameter in comparison with clomiphene citrate while at 50 and 100 mg/kg BW it significantly reduced ($p < 0.05$) ovarian tertiary follicular diameter when compared with clomiphene citrate (Table 2).

3.3. Histopathology and histomorphometry of the oviduct in Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc

The oviduct of the control rat is devoid of lesions and appears normal as characterized by the presence of normal highly folded simple columnar epithelial mucosa lining (black arrow), distinct submucosa (red arrow), muscular (white line) and serosal layer (black line) (Plate 2 A). Similarly, the rats exposed to different doses of AFETG (Plate 2 C - E) and clomiphene citrate (Plate 2 B) shared a similar histological appearance with the control.

Administration of AFETG (100 and 200 mg/kg BW) increased ($p < 0.05$) oviductal epithelial height (EH) significantly in comparison with distilled water-administered control. At 50 mg/kg BW, AFETG did not significantly alter ($p > 0.05$) oviductal EH and was compared favorably with the distilled water (control) and clomiphene citrate (Table 3). Oviductal sub-mucosal thickness (SMT) was reduced significantly ($p < 0.05$) in the AFETG-treated rats (at all doses) and then in the control rats. SMT in the clomiphene citrate-treated rats did not differ ($p > 0.05$) from the control rats. Rats treated with AFETG (at 200 mg/kg BW) showed a significantly lower ($p < 0.05$) SMT than those treated with AFETG at 50 and 100 mg/kg BW (Table 3). Similarly, oviductal muscular thickness (MT) was also significantly decreased ($p < 0.05$) by AFETG in comparison with the control. Oviductal MT of rats treated with clomiphene citrate did not differ ($p > 0.05$) from that of the control rats. Rats treated with AFETG at 100 and 200 mg/kg BW showed a significantly reduced

Table 3

Histomorphometric parameters of the oviduct in female Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

Treatment groups	Histomorphometric parameters			
	EH (μm)	SMT(μm)	MT(μm)	ST(μm)
Control (Distilled water)	110.21 ± 3.32 ^a	292.84 ± 15.06 ^a	367.56 ± 10.60 ^a	40.75 ± 3.12 ^a
Clomiphene citrate (0.82 mg/kg BW)	112.21 ± 6.06 ^a	282.10 ± 8.00 ^a	355.94 ± 14.60 ^a	32.42 ± 2.93 ^b
AFETG (50 mg/kg BW)	119.04 ± 7.16 ^a	252.02 ± 6.98 ^b	267.84 ± 5.37 ^b	41.55 ± 1.00 ^a
AFETG (100 mg/kg BW)	145.8 ± 9.02 ^b	264.34 ± 5.37 ^b	177.14 ± 6.55 ^c	52.44 ± 4.27 ^c
AFETG (200 mg/kg BW)	133.94 ± 6.19 ^b	211.14 ± 6.61 ^c	160.44 ± 9.11 ^c	58.58 ± 1.57 ^c

Values represent mean (of 5 replicate) ± SEM. Values carrying different superscripts down the column are significantly different at $p \leq 0.05$. EH: Epithelial height, SMT: Sub-mucosa thickness ST: Serosa thickness, MT: Muscular thickness, AFETG: Aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

($p < 0.05$) oviductal MT when compared with those treated with AFETG at 50 mg/kg BW (Table 3). Oviductal serosa thickness (ST) was significantly higher ($p < 0.05$) in AFETG-treated rats (100 and 200 mg/kg BW) than in the distilled water-treated control rats while oviductal ST of AFETG-treated rats (50 mg/kg BW) did not differ significantly ($p > 0.05$) from the that of distilled water-treated control rats (Table 3).

3.4. Histopathology and histomorphometry of the uterus in Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc

The uterine parenchyma of the control rat appeared normal and was typified by distinct endometrial epithelial mucosa lined by simple columnar (black arrow), the presence of numerous endometrial glands (red arrowhead), clearly visible muscular (myometrial – black line) and perimetrial coats (red arrow) (Plate 3A). Also, the rats exposed to AFETG (Plate 3 C - E) and clomiphene citrate (Plate 3B) shared a similar histo-architectural appearance with the control.

Administration of AFETG (all doses tested) occasioned a significant elevation ($p < 0.05$) in uterine endometrial epithelial height (EEH) in comparison to the distilled water-treated control. Uterine EEH of clomiphene citrate-treated rats and those treated with AFETG (50 and 100 mg/kg BW) did not differ significantly ($p > 0.05$). Rats administered AFETG (200 mg/kg BW) had significantly higher ($p < 0.05$) uterine EEH than those administered AFETG (50 and 100 mg/kg BW) and clomiphene citrate (Table 4). Administration of AFETG did not induce any alteration in uterine gland epithelial height (GEH). Uterine GEH in AFETG-administered rats (all doses tested) did not differ from that of clomiphene citrate-treated rats (Table 4). Similarly, AFETG did not significantly alter ($p > 0.05$) uterine gland luminal diameter in rats and was compared favorably with distilled water (control) and clomiphene citrate (Table 4). Uterine endometrial thickness (ET) was significantly higher ($p < 0.05$) in rats administered AFETG (at all doses) than in the distilled water-treated control and clomiphene citrate-treated rats. Uterine ET was significantly higher ($p < 0.05$) in AFETG-treated rats (100 mg/kg BW) than in those administered AFETG (50 and 200 mg/kg BW) (Table 4). Uterine myometrial thickness (MT) was significantly higher ($p < 0.05$) in rats administered AFETG (100 and 200 mg/kg BW) than in the distilled water-treated control and the clomiphene citrate-treated rats (Table 4). Uterine perimetrial thickness (PT) was not altered following AFETG (all doses) administration when compared with the control. Clomiphene citrate-treated rats had a significantly higher ($p < 0.05$) PT than the distilled water-treated control and AFETG-treated rats (Table 4).

Table 2

Ovarian primary, secondary, and tertiary follicular diameter in female Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

Parameters (μm)	Control (Distilled water)	Clomiphene citrate (0.83 mg/kg BW)	AFETG (50 mg/kg BW)	AFETG (100 mg/kg BW)	AFETG (200 mg/kg BW)
Primary follicular diameter	33.68 ± 1.52 ^{ab}	33.24 ± 1.53 ^{ab}	35.78 ± 1.67 ^b	29.34 ± 0.71 ^a	36.98 ± 1.71 ^b
Secondary follicular diameter	95.64 ± 1.28 ^a	52.00 ± 0.93 ^b	71.40 ± 1.39 ^c	86.84 ± 1.70 ^d	83.40 ± 0.35 ^d
Tertiary follicular diameter	103.20 ± 1.57 ^a	281.92 ± 1.40 ^b	192.20 ± 2.76 ^c	261.94 ± 2.76 ^d	389.22 ± 3.32 ^e

Data represent means (of 5 replicates) ± SEM, values carrying different superscripts along the row are significantly different at $p < 0.05$. AFETG: Aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

Table 4

Uterine histomorphometric parameters in Wistar rats administered aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc.

	EEH (μ m)	GEH (μ m)	GLD (μ m)	ET (μ m)	MT (μ m)	PT (μ m)
Control	13.20	7.96	18.28	279.08	165.45	9.32
(Distilled water)	$\pm$	$\pm$	$\pm$	$\pm$ 2.44 ^a	$\pm$	$\pm$
Clomiphene	1.46 ^a	0.70 ^a	0.50 ^a	11.98 ^a	1.33 ^a	
citrate (0.85 mg/kg BW)	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
AFETG (50 mg/kg BW)	1.16 ^b	0.72 ^a	2.17 ^a	10.24 ^a	21.10 ^a	2.10 ^b
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
AFETG (100 mg/kg BW)	2.56 ^b	^a	2.21 ^a	38.10 ^b	18.66 ^a	1.42 ^a
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
AFETG (200 mg/kg BW)	19.08	8.38	19.24	397.24	204.74	10.64
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	3.60 ^b	0.43 ^a	1.28 ^a	24.98 ^c	24.53 ^b	1.74 ^a
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	30.08	10.06	18.20	325.96	205.90	10.58
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$ 9.63 ^b	$\pm$
	2.33 ^c	0.68 ^a	1.65 ^a	11.00 ^b		0.92 ^a

Values represent the mean of 5 replicates $\pm$ SEM. Values carrying different superscripts down the column are significantly different at $p < 0.05$. AFETG - Aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc., BW - Body weight, EEH - Endometrial epithelial height, GEH - Gland epithelial height, GLD - Gland luminal diameter, ET - Endometrial thickness, MT - Myometrial thickness, PT - Perimetrial thickness.

3.5. Molecular docking and molecular interaction analysis

Table 5 shows the binding affinities of reference ligand (epristeride) and bioactive compounds of *T. garckeana* (F. Hoffm.) Exell & Hillc. fruit for human 5- α -reductase 2 (SRD5 α 2). Out of all the constituents of *T. garckeana* (F. Hoffm.) Exell & Hillc. fruit as reported by John et al. [26], only four showed good binding affinity (Table 5). D-Melezitose (-12.55 kcal/mol XP GScore/-63.96 kcal/mol MMGBS dG Bind Score) and 1, 3, 4-trihydroxy-5-oxocyclohexane-1-carboxylic acid (-9.136 kcal/mol XP GScore/-43.18 kcal/mol MMGBS dG Bind Score) displayed better binding affinities than the reference ligand, epristeride (-8.096 kcal/mol XP GScore/-35.77 kcal/mol MMGBS dG Bind Score), while 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and 7-(2-Hydroxy-1-methylethyl)-1,4a-dimethyldecahydronaphthalen-2-ol displayed lower binding affinities than epristeride.

Furthermore, we determined the molecular interactions between D-Melezitose and SRD5 α 2 and epristeride and SRD5 α 2. In the interaction of the D-Melezitose as the best hit with SRD5 α 2, thirteen hydrogen bond interactions are formed with amino acid residues TYR³³, ARG⁹⁴, ASN¹⁶⁰, GLU¹⁹⁷, CYS¹¹⁹, ASP¹⁶⁴, GLU⁵⁷, SER²²⁰, ASP¹⁶⁴, TYR¹⁰⁷, GLY¹¹⁵, GLU⁵⁷, and GLU¹⁹⁷ at bond distances 2.03471, 1.818, 1.89486, 1.92909, 2.65016, 1.9263, 2.1446, 1.71103, 2.25646, 1.9783, 2.70309, 2.41143, and 2.84415 respectively (Fig. 1). In contrast, the binding of epristeride; a known inhibitor of SRD5 α 2 forms one electrostatic bond (ARG⁹⁴), three hydrogen bonds (TYR⁹¹ and two ARG¹¹⁴), and eight hydrophobic bonds (LEU¹¹¹, ARG¹¹⁴, LEU²³, TRP⁵³, PHE¹¹⁸, PHE²¹⁹, and two PHE²²³) at 5.37714, 1.86429, 1.86058, 2.07279, 5.30163, 5.19455, 5.14505, 5.41006, 3.66907, 5.44318, 4.26057, and 5.49017 bond distances respectively (Fig. 2) (see Fig. 3).

Table 5

Binding affinities of reference ligand and bioactive compounds of *T. garckeana* (F. Hoffm.) Exell & Hillc. fruit for human 5- α -reductase 2 (SRD5 α 2).

S/N	Compound names	PubChem CID	Docking score (kcal/mol)	XP GScore	MMGBS dG Bind
	Reference ligand	134688837	-8.092	-8.096	-35.77
1	<i>T. garckeana</i> compounds	44135550	-12.55	-12.55	-63.96
	D-Melezitose				
2	1,3,4-trihydroxy-5-oxocyclohexane-1-carboxylic acid	630	-9.136	-9.136	-43.18
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	119838	-6.773	-6.773	-52.57
4	7-(2-Hydroxy-1-methylethyl)-1,4a-dimethyldecahydronaphthalen-2-ol	595888	-6.16	-6.16	-51

4. Discussion

Infertility in females can be caused by tubular, uterine, ovarian and endocrine disorders. Endocrine system problems result in hormonal abnormalities that might affect the menstrual cycle and cause infertility (Naz et al., 2018). AFETG at the doses examined in this study, supported the normal production of prolactin, LH and FSH in female rats which indicates that extract did not interfere with the normal functioning of the hypothalamus-pituitary axis (Barbieri, 2014). Although gonadotropins were not elevated in the extract-treated rats, it is possible that elongation of treatment duration beyond 5 days used in this study might have led to their elevation, especially that of FSH. The recorded elevation of E2 might have been induced by bio-constituents of the extract which could have bound to FSH receptors in the ovaries, leading to cascade of reactions responsible for the biosynthesis of E2. Certain ligands have been reported to mimic natural hormones by binding to common receptors at low levels to mediate a biological effect (Hayes et al., 2011).

Measurements of follicular diameter serve as the sole indicator of follicular maturity in an IVF program (Pohler et al., 2012). AFETG-induced increase in secondary and tertiary follicular diameter indicates extract ability to aid follicular maturity. Follicles with higher diameters have been reported to produce more competent oocytes in several species than those with smaller diameters (Ito et al., 2008; Pohler et al., 2012). Oocytes from large follicles have a higher likelihood of passing through nuclear maturation before becoming fertilized and developing into an embryo (Teissier et al., 2000). The increase in secondary and tertiary ovarian follicular diameter in rats given AFETG, especially at higher doses (100 and 200 mg/kg) seemed to demonstrate the safety of AFETG on ovarian follicular morphological integrity during their developmental phases. The morphometric data recorded in this study further validates the hormonal profile, more particularly the elevated estradiol, which is usually known to rise during ovulation period.

The role of the oviductal epithelial lining cells, particularly the ciliated cells, is to pick up the ovum and subsequent transportation of the zygote (Croxatto, 2002). Therefore, the recorded normal oviductal histo-architectural organization coupled with the significantly increased oviductal epithelial height of rats administered higher concentrations (100 and 200 mg/kg) of AFETG could be attributed to the epithelial growth-stimulating components, most likely the antioxidants reserve in *T. garckeana* (F. Hoffm.) Exell & Hillc.. Cyclic changes in progesterone and E2 that affect oviductal epithelial development, secretory capacity, contractility, and intraluminal hydrostatic pressure gradients have been proposed to be responsible for coordinating the oviduct's ability to transport ova by ciliary action (Brenner, 1994; Harper, 1994). The oviductal histomorphometric evidence buttressed the E2 elevated level recorded in this study.

The differences in uterine coat thickness of rats more specifically endometrial coat have been linked to oedematous stroma showing clear spaces between the cells and could also arise from mitotic proliferation (Dilsiz Yilmaz et al., 2013; Elgamal et al., 2016). Additionally, it has been proposed that oestrogen causes a sudden rise in micro-vascular permeability in the uterus of rodents, culminating in markedly increased stromal edema and uterine wet weight (Shivalingappa et al.,

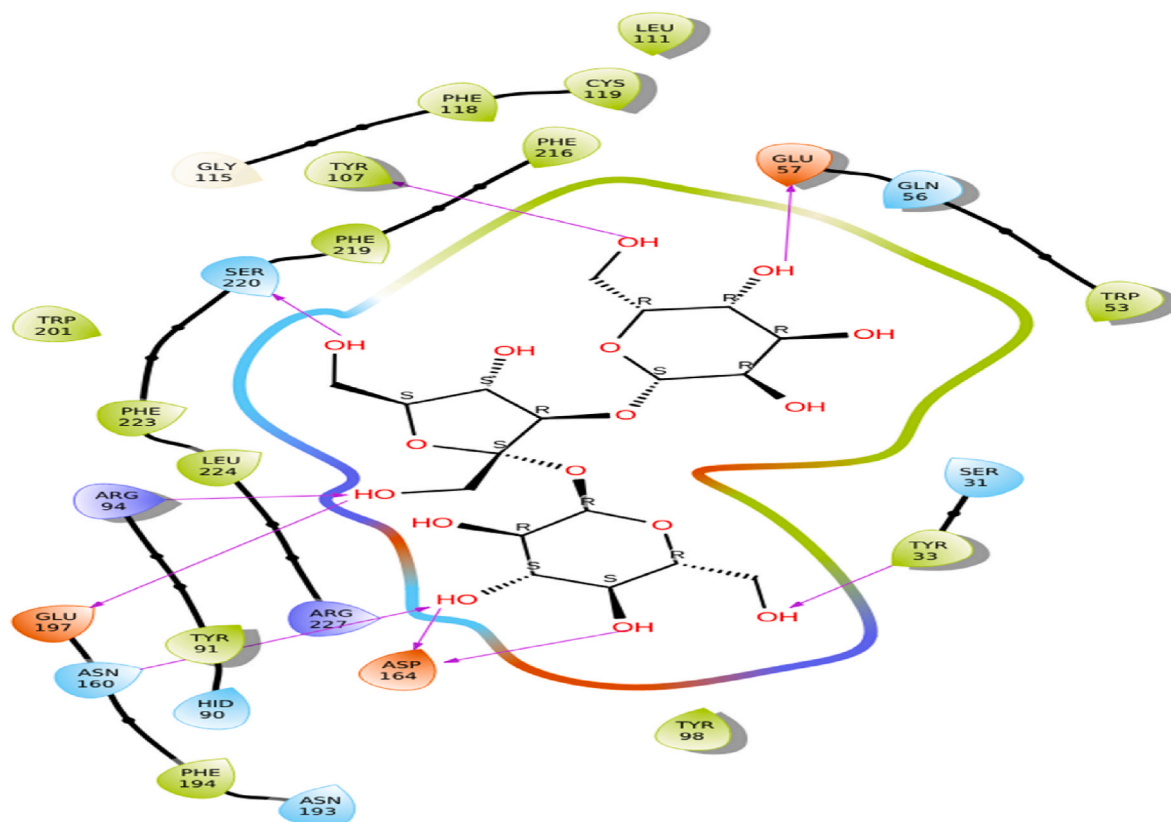


Fig. 1. 2-dimensional representation of Human steroid 5 α -reductase 2 (SRD5 α 2) (PDB ID: 7BW1) in complex with alpha-D-Glucopyranoside from *T. garckeana* (*F. Hoffm.*) Exell & Hillc. at the active site.

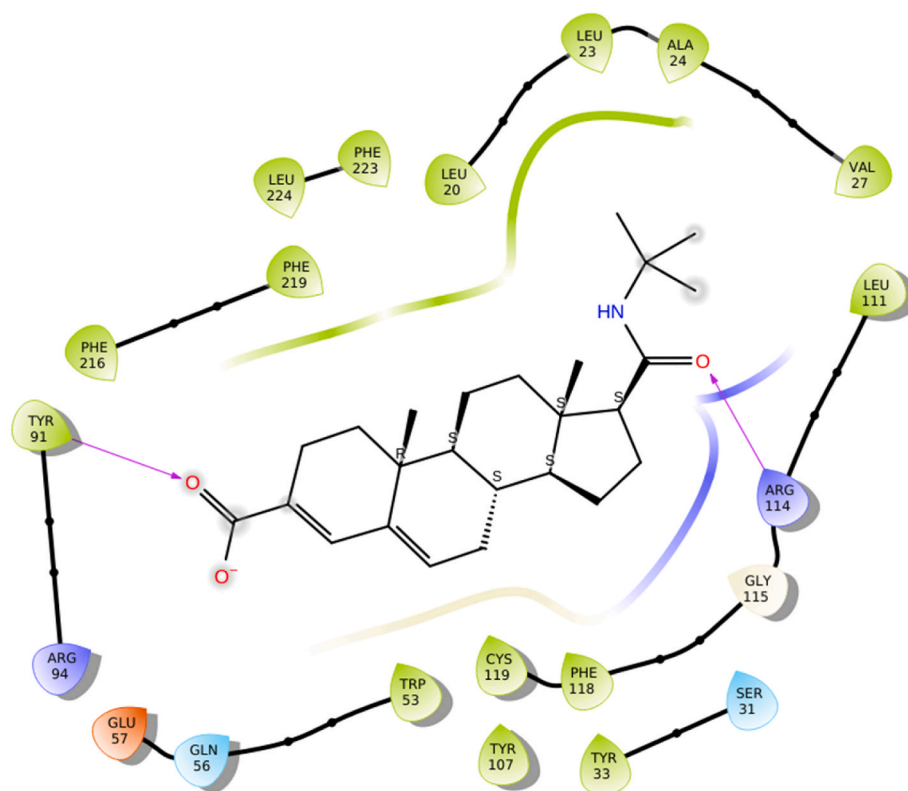


Fig. 2. 2-dimensional representation of Human steroid 5 α -reductase 2 (SRD5 α 2) (PDB ID: 7BW1) in complex with Epristeride at the active site.

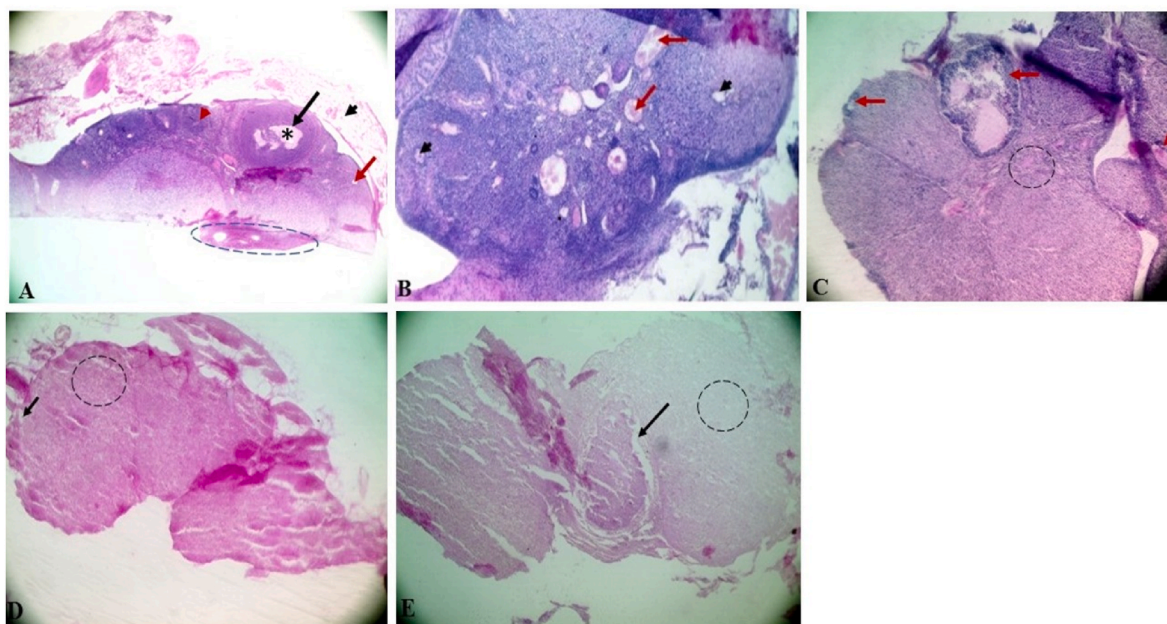


Plate 1. Photomicrographs of the ovary of rats exposed to grades of aqueous fruit extract of *T. garckeana*. (F. Hoffm.) Exell & Hillc. **A: Control (distilled water)**, **B: Clomiphene citrate (85 mg/kg BW)**, **C: AFETG (50 mg/kg BW)**, **D: AFETG (100 mg/kg BW)** and **E: AFETG (200 mg/kg BW)** Stain: Haematoxylin and Eosin; Magnification: x100.

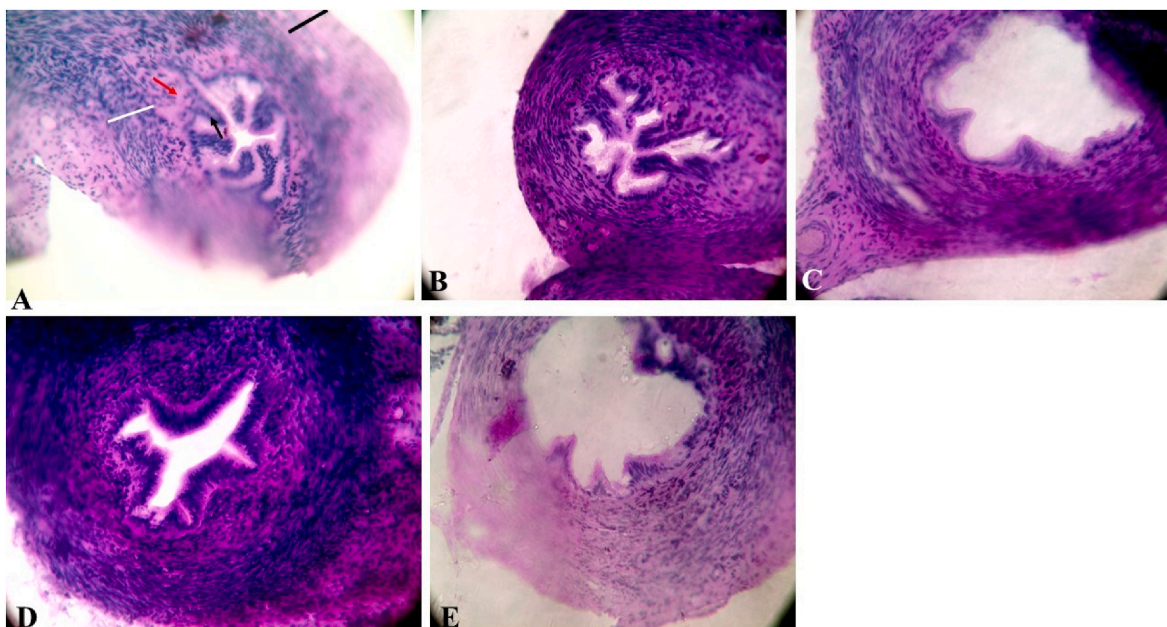


Plate 2. Photomicrographs of the oviduct of rats exposed to grades of aqueous fruit extract of *T. garckeana*. (F. Hoffm.) Exell & Hillc. **A: Control (distilled water)**, **B: Clomiphene citrate (85 mg/kg BW)**, **C: AFETG (50 mg/kg BW)**, **D: AFETG (100 mg/kg BW)** and **E: AFETG (200 mg/kg BW)** Stain: Haematoxylin and Eosin; Magnification: x100.

2002). Therefore, the observed marked increase in uterine endometrial and myometrial thicknesses across the various AFETG-treated groups of rats most especially those treated with the higher concentrations of AFETG confirm the morphophysiological assumptions stated above. The increased uterine coats (endometrium and myometrium) thicknesses appeared to be dose-dependent. The measurement of endometrial thickness is a very important activity carried out to determine the possibility of achieving success with assisted reproductive technologies like *in vitro* fertilization and intracytoplasmic sperm injection and a high endometrial thickness is reportedly correlated with improved pregnancy

rates (Chan et al., 2018; Kasius et al., 2014).

Designing and developing novel drugs requires the use of molecular docking. It correctly predicts the experimental binding mode and affinity of a native molecule within the drug target's binding site (Torres et al., 2019). Virtual screening techniques have been used in the identification of potential lead compounds against a protein target both in ligand-based and structural virtual screening. To explain the recorded increase in E2 level, we carried out molecular docking of *T. garckeana* (F. Hoffm.) Exell & Hillc. compounds against human 5- α reductase 2 (SRD5 α 2) (PDB ID: 7BW1), an enzyme that competes with aromatase for

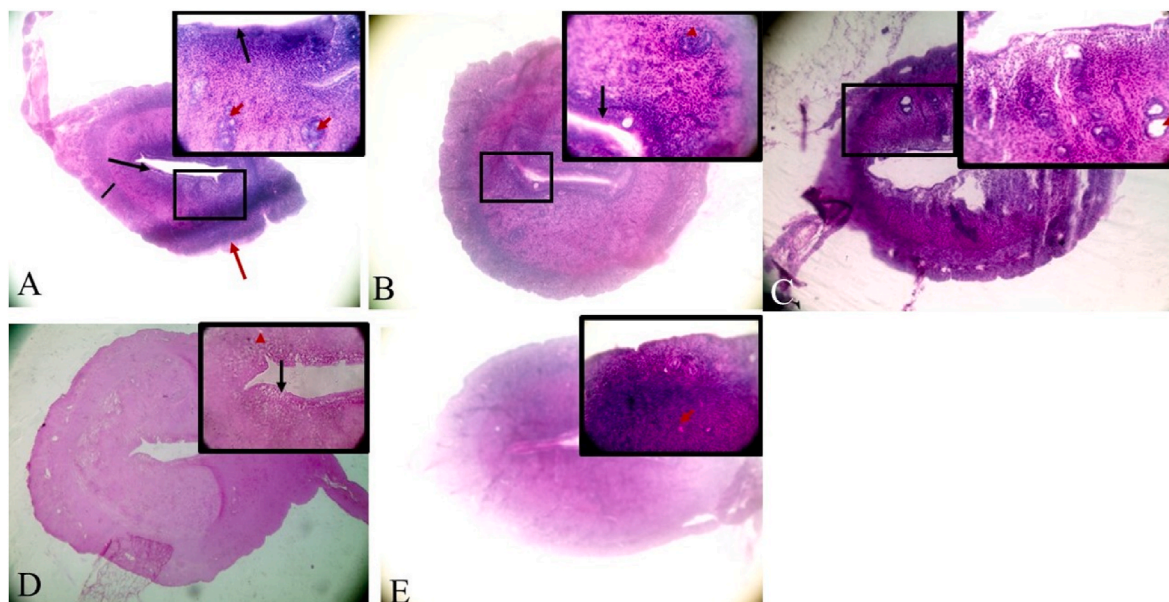


Plate 3. Photomicrographs of the uterus of rats exposed to grades of aqueous fruit extract of *T. garckeana* (F. Hoffm.) Exell & Hillc. **A: Control (distilled water), B: Clomiphene citrate (85 mg/kg BW), C: AFETG (50 mg/kg BW), D: AFETG (100 mg/kg BW) and E: AFETG (200 mg/kg BW)** Stain: Haematoxylin and Eosin; Magnifications: Main photomicrograph (x100), Inset photomicrographs (x400).

available testosterone, and responsible for converting testosterone to dihydrotestosterone (DHT). Inhibition of 5- α reductase makes testosterone available for conversion to E2 by aromatase. The recorded binding affinity of D-Melezitose could be due to the contributions of interacting forces between the ligand and human steroid 5 α -reductase 2 (SRD5 α 2). The lower MMGBSA score of D-Melezitose indicates it binds more strongly to SRD5 α 2 than epristeride. Furthermore, the bond distances recorded in the formation of the D-Melezitose-SRD5 α 2 complex, which was shorter than those of the SRD5 α 2-epristeride complex, indicate that the interaction is much stronger in D-Melezitose-SRD5 α 2 complex than in the SRD5 α 2-epristeride complex, because the shorter the distances the stronger the bond. Thus, D-Melezitose obtained as the best-hit compound may be a potential inhibitor of SRD5 α 2. In conclusion, AFETG increased E2 level, thickened uterine endometrium and increased ovarian tertiary follicles in female rats. D-Melezitose, one of the constituents of AFETG is a potential inhibitor of SRD5 α 2 and may be responsible for the elevation of E2. AFETG can therefore be explored for treating female infertility caused by thinning of the uterine endometrium and reduced follicular size. Results corroborate its traditional use for the treatment of female fertility.

Declaration of generative AI and AI-assisted technology in the writing process

During the preparation of this work the authors used Quillbot in order to reduce the similarity index percentage of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Babasoji Percy Omoniwa: Conceptualization, Writing – review & editing, Supervision, Project administration, Validation. **Jamiu Oye-wole Omirinde:** Writing – original draft, Resources, Investigation, Validation. **Adam Moyosore Afodun:** Investigation. **Charles Obiora Nwonuma:** Resources, Writing – review & editing. **Omolola Soji-Omoniwa:** Formal analysis, Resources, Data curation. **Rhoda Nyamshak Dinmung:** Investigation. **Thaddeus Terdoo Abwa:** Investigation. **Isreal Shilly Dimka:** Investigation. **Godfrey Patrick Abimiku:**

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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