JOURNAL OF SCIENCE AND TECHNOLOGY
ISSN: 2229-8460 e-ISSN: 2600-7924

JST

Vol. 15 No. 2 (2023) 11-22
https://publisher.uthm.edu.my/ojs/index.php/jst

Aphrodisiac Potentials of Aqueous Extract of *Azanza Garckeana* Fruit Pulp in Fluoxetine-induced Sexually-impaired Female Rats

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DOI: https://doi.org/10.30880/jst.2023.15.02.002

Article Info

Received: 14 August 2023
Accepted: 18 November 2023
Available online: 13 December 2023

Keywords

Aphrodisiac, *Azanza garckeana*, fluoxetine, reproductive hormones, sexual dysfunction

Abstract

This study aims to investigate the potential of *Azanza garckeana*, a plant traditionally used as an aphrodisiac, to reverse sexual dysfunction induced by an antidepressant 'fluoxetine' in female rats. Aqueous extract of *A. garckeana* fruit pulp was screened for its secondary metabolite compositions. Female rats were induced into sexual dysfunction using fluoxetine, then they were divided into six groups, and treated based on the group with oral administration of varying doses of *A. garckeana* extract (125, 250, and 500 mg/kg body weight) and a reference medication (Tadalafil) for seven days. Sexual behaviour parameters were monitored and hormone levels (progesterone, follicle-stimulating hormone, luteinizing hormone, oestrogen, and prolactin) were measured after the treatment period. Fluoxetine significantly ($p < 0.05$) reduced the frequencies of sexual behaviours in the rats, such as darting, hopping, lordosis, genital grooming, and licking behaviour, but increased the latencies of darting and hopping. However, the extract, at 250 and 500 mg/kg, significantly ($p < 0.05$) reversed these changes in the sexual behavior of fluoxetine-induced rats in comparison to the effects of Tadalafil treatment. Furthermore, the extract significantly ($p < 0.05$) increased the levels of luteinizing hormone, follicle-stimulating hormone, progesterone, and prolactin in the blood but decreased oestrogen levels, especially at a dose of 500 mg/kg. The results of this study suggest that the aqueous extract of *A. garckeana* fruit pulp can improve sexual behavior and reproductive hormone concentrations, thereby potentially restoring sexual competence in sexually-dysfunctioned female rats. These findings provide additional support for the traditional use of *A. garckeana* in managing female sexual dysfunction.

1. Introduction

The female orgasm is a moment of intense pleasure experienced during sexual mating or copulation [1]. To stimulate female orgasm, lotions and oils are made with a variety of substances, including honey, ripe tamarind

fruit, black pepper, camphor, long pepper, and brown jaggery, among others. Aphrodisiac drugs are known to increase virility, the vigor of sexual acts, and the quality of progeny [2]. A significant and well-known source of fruits, drinks, leafy vegetables, nuts, edible oil, and spices are indigenous fruit vegetation [2]. *A. garckeana*, a beloved edible indigenous fruit with many uses and widespread distribution, is a native fruit tree that is locally known as 'goron tula' in Hausa, Nigeria ("Tula" being the town it grows natively in Nigeria while "goro" signifies kola") [3]. Tula Village is a community in Kaltungo Local Government in Gombe State, Nigeria [4]. The fruit has an amazing quantity of carbohydrates, minerals, and vitamins, so it may be eaten right once by biting into its crisp or saved for later use [5]. Additionally, it is used as a sauce before ripening, ingested as juice, prepared as porridge, and utilized as chewing gum for schoolboys [5]. It is frequently used to manage and treat a wide range of conditions including coughs, chest discomfort, stomach aches, menstrual disorders, syphilis, and other sexually transmitted infections including gonorrhea. It is predominantly used as an aphrodisiac [3].

About 33.4% of men and 45.7% of women experience sexual dysfunction, which is characterized by disruptions in sexual desire and the psychophysiological alterations connected to the sexual response cycle [6]. One of the most prescribed antidepressants is selective serotonin reuptake inhibitors (SSRIs such as Fluoxetine and Paroxetine), which are also linked to a high prevalence of sexual adverse effects [7]. The emergence of these sexual adverse effects frequently causes patients to cease taking their medicine before getting relieved from their depressive symptoms [8]. Also, many women find it difficult to use pharmaceutical solutions that are accessible for the treatment of sexual dysfunction probably due to fear of social stigmatization, safety concern, low awareness, lack of funds, etc. Instead, turn to botanicals like *A. garckeana* because of their accessibility, availability, and cost as well as their conviction that it has little or no negative effects. Even though the chemical composition and pharmacological effects of *A. garckeana* has been the subject of several investigations, scientific literature still lacks experimental evidence to substantiate its renowned aphrodisiac effects on female libido.

2. Materials and Methods

2.1 Materials

2.1.1 Plant Material and Authentication

A. garckeana fruits were obtained from a local market in Ilorin West local government, Kwara State, Nigeria. The fruits were then identified and authenticated by a botanist at the University of Ilorin Herbarium, Ilorin, Nigeria, where a voucher sample was deposited under "UILH/001/1494/2022".

2.1.2 Animals

Thirty, healthy, in-bred, sexually active female Wistar rats (*Rattus norvegicus*) weighing 122.83 ± 7.15 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were kept in their cages in a well-conditioned Animal House, at room temperature. They were kept on rat pellets, given free access to tap water, and handled carefully in accordance with the Guidelines of the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.1.3 Drugs, Assay Kits and Chemicals

Tadalafil and Fluoxetine are manufactured by V.S. International Pvt. Limited in Dabhel, Daman, India, and Evans Therapeutic Limited in Isolo, Lagos state, Nigeria, respectively. Progesterone, follicle-stimulating hormones, oestrogen, prolactin, and luteinizing hormone assay kits were made by the Texas-based Elabscience Biotechnology Company Limited. Fortress Diagnostic Limited, based in the United Kingdom, produced assay kits for bilirubin, albumin, alanine, alkaline phosphatase, total protein, alanine and aspartate aminotransferase, creatinine, serum electrolyte, and urea. The rest of the reagents were provided by Sigma-Aldrich Inc. in St. Louis, Missouri, USA.

2.2 Methods

2.2.1 Preparation of Extract

The fruits were sliced into small pieces and cleaned under running tap water to remove the pulp. It was then dried in an oven at 40°C for 48 hours. Prior to extraction, the dried pulp was ground in an electric blender and stored in an airtight container. An aqueous solvent was then used to macerate fifty grams (50 g) of the powdered material for 48 hours at 25°C with periodic shaking before filtering through cheesecloth. A rotary evaporator was further used to evaporate the filtrate to create a sticky residue which was reconstituted in distilled water from which necessary dosages of 125, 250, and 500 mg/kg body weight (determined by combining data from

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earlier research on *A. garckeana*) [3, 9] were obtained. Additionally, mathematical calculations were used to determine the percentage yield (23.35%).

2.2.2 Screening of Secondary Metabolites

Following the procedures outlined by Odebiyi and Sofowora [10], Trease and Evans [11], Sofowora [12], Edeoga *et al*. [13], and Harborne [14], five grams (5 g) of *A. garckeana* fruit pulp extract was dissolved in 40 milliliters of distilled water and subjected to phytochemical screening to check for the presence of flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, and steroids. The following secondary metabolites that were present were further subjected to quantitative analysis using known techniques: saponins [15], alkaloids [16], tannins [17], flavonoids [18], glycosides [19].

2.2.3 Induction of Sexual Dysfunction into Female Rats and Assessment of Their Sexual Behaviour Indices

To induce sexual dysfunction, the oral dosage of fluoxetine (15 mg/kg body weight, prepared daily in distilled water) was administered to 25 female rats (the remaining 5 female rats were not induced into sexual dysfunction, as they serve as control group) for 14 days [20] after which the rats were introduced to male rats on the fifteenth day in separate rectangular wooden cages with wire mesh tops. Mating behaviours were then observed for 30 minutes as reported by Nurudeen and Yakubu [21] and Yakubu and Olutoye [22]. Female rats that exhibited a minimum 25% decrease in their frequencies of darting, hopping, lordosis, genital grooming, and licking behavior as well as a minimum 25% increase in their darting latency and hopping latency were deemed to have sexual impairments, and they were divided into different test groups [21].

2.2.4 Animal Grouping and Administration of Extract and Reference Drug

In a fully randomized design, a total of 30 female rats that had been acclimated for two weeks were divided into six groups (A to F), with 5 animals per group as follows:

- Group A: Rats received 0.5ml of distilled water.
- Group B: Rats induced into sexual dysfunction and administered 0.5 mL of distilled water.
- Group C: Rats induced into sexual dysfunction and administered 0.5 mL of 20 mg/kg body weight of Tadalafil.
- Groups D: Rats induced into sexual dysfunction and administered 0.5 mL of 125 mg/kg body weight of the extract.
- Groups E: Rats induced into sexual dysfunction and administered 0.5 mL of 250 mg/kg body weight of the extract.
- Groups F: Rats induced into sexual dysfunction and administered 0.5 mL of 500 mg/kg body weight of the extract.

Using a plastic oropharyngeal cannula, the different animal groups were treated as described above once daily (08:00–08:45 h) for seven days. After treatment and between the 17:00 and 21:00 h of days 1, 3, and 7, the parameters of female sexual behavior were observed in low light conditions at room temperature (26–28°C), 30 min, and the findings were recorded.

2.2.5 Preparation of Serum

The process outlined by Yakubu *et al*. [23] was followed in the preparation of the serum. Diethyl ether gases were used to induce rats to unconsciousness. Their jugular veins were then sliced to draw 5 ml of blood into sterile, dry centrifuge tubes. The samples were kept at room temperature for 15 minutes to allow the blood to coagulate. After centrifuging at 503 × *g* for 10 min using the Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK), clear serum was then extracted using a Pasteur pipette. In addition, before carrying out the different hormonal tests, the sera were refrigerated.

2.2.6 Determination of Reproductive Hormones

Progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestrogen, and prolactin concentrations in the serum were assessed using the tube-based serum enzyme immunoassay (EIA) technique. The technique described in the manufacturer's instructions (Elabscience Biotechnology Company Limited, Texas, United States of America) was followed for the determination of the hormones.

2.2.7 Statistical Analysis

Five replicated data were used to calculate the mean and standard error of the mean. The result was also subjected to a one-way Analysis of Variance (ANOVA). With the aid of GraphPad Prism version 6.01, they were deemed statistically different at ($p < 0.05$). (GraphPad Software, Inc., San Diego, Califonia, United States).

3. Results

The results of the phytochemical screening of the aqueous extract of *A. garckeana* fruit pulp revealed the presence of Saponins, Flavonoids, Glycosides, Tannins, Phenols and Alkaloids (Table 1). Tannins are the most abundant secondary metabolite detected while flavonoids are the least abundant (Table 1). Following the administration of fluoxetine to female rats, they demonstrated 43.49%, 48.85%, 31.00%, 32.55% and 47.50% decrease in darting frequency, hopping frequency, Lordosis frequency, Genital grooming and Licking behaviour respectively. Also, the Darting Latency, hopping latency and Lordosis latency increased by 30.82%, 38.69%, and 38.29%, respectively, when compared with the control group (Table 2). When compared with the control group, darting frequencies decreased significantly ($p < 0.05$) while darting latencies increased significantly ($p < 0.05$) after fluoxetine administration and distilled water for days 1, 3 and 7. Also, the administration of each 125, 250, 500 mg/kg body weight of the extract and the reference drug, significantly increased ($p < 0.05$) the darting Frequencies and significantly decreased ($p < 0.05$) the darting latencies in a dose-dependent manner for days 1, 3 and 7 (Table 3). Hopping frequencies decreased significantly ($p < 0.05$) while hopping latencies increased significantly ($p < 0.05$) when administered fluoxetine and distilled water for days 1, 3 and 7 when compared with the control group. However, in comparison with the sexually-dysfunctioned group of rats, the reference drug, 125, 250 and 500 mg/kg body weight of the extract significantly increased ($p < 0.05$) the hopping frequencies and significantly decreased ($p < 0.05$) the hopping latencies in a dose-dependent manner for days 3 and 7 while at Day 1 they were comparable to the sexually-dysfunctioned group (Table 4).

When compared with the control group, the lordosis frequencies of the sexually-dysfunctioned group decreased significantly ($p < 0.05$) while the lordosis latencies increased significantly ($p < 0.05$) for Days 1, 3 and 7. Administration of the reference drug, 125, 250 and 500 mg/kg body weight of the extract significantly increased ($p < 0.05$) the Lordosis Frequencies and significantly decreased ($p < 0.05$) the Lordosis latencies in a dose-dependent manner for Days 1,3 and 7 (Table 5). In addition, when compared with the control group the frequencies of genital grooming and licking behaviour decreased significantly ($p < 0.05$) in the sexually-dysfunctioned group for Days 1, 3 and 7. The reference drug, 125, 250 and 500 mg/kg body weight of the extract significantly increased ($p < 0.05$) the genital grooming on Days 3 and 7 and liking behaviour on Day 7 in a dose-dependent manner. Genital grooming on Day 1 and licking behaviour on Days 1 and 3 were comparable to the sexually dysfunctioned group (Table 6).

The concentration of Progesterone, Follicle Stimulating Hormone, Luteinizing Hormone, Oestrogen and Prolactin decreased significantly ($p < 0.05$) in rats administered fluoxetine and distilled water. The reference drug, 125, 250 and 500 mg/kg body weight of the extract significantly increased ($p< 0.05$) the concentration of Progesterone, Follicle Stimulating Hormone, Luteinizing Hormone, Oestrogen and Prolactin in a dose-dependent manner. However, Oestrogen concentration was reduced in the group that was given 500 mg/kg body weight of the extract (Table 7).

Table 1 *Concentration of secondary metabolites (mg/g) in aqueous extract of A. garckeana fruit pulp*

Secondary metabolites	Concentration (mg/g)
Saponins	83.30 ± 0.69
Flavonoids	3.51 ± 0.37
Tannins	235.76 ± 0.28
Phenols	123.94 ± 0.01
Glycosides	62.17 ± 0.65
Alkaloids	106.08 ± 0.06
Terpenoid	Not Detected
Anthraquinones	Not Detected

Values are the mean of 3 replicates ± S.E.M

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Table 2 *Sexual behaviour parameters of female rats administered fluoxetine*

Parameters	Control	Fluoxetine + Distilled Water	Difference as a Function of the Control (%)
Darting frequency (DF)	4.30 ± 0.15 [a]	2.45 ± 0.11 [b]	43.49 β
Hopping frequency (HF)	1.75 ± 0.34 [a]	0.90 ± 0.24 [b]	48.85 β
Lordosis frequency (LF)	1.0 ± 0.01 [a]	0.70 ± 0.04[b]	31.00 β
Darting latency (DL) α	594.30 ± 34.76 [a]	859.03 ± 63.38 [b]	30.82[+]
Hopping latency (HL) α	778.90 ± 43.78 [a]	1269.76 ± 32.62 [b]	38.69[+]
Lordosis Latency (LL) α	834.40 ± 98.76[a]	1352.10 ± 85.47 [b]	38.29[+]
Genital grooming (GG)	8.60 ± 0.34 [a]	5.80 ± 0.45 [b]	32.55 β
Licking behavior (LB)	3.60 ± 0.19 [a]	1.90 ± 0.13 [b]	47.50 β

Data are mean of five determinants ± SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different ($p < 0.05$)

Table 3 *Darting frequency and darting latency of female rats induced into sexual dysfunction by fluoxetine following the administration of aqueous extract of A. garckeana fruit pulp*

Treatment	Darting Frequency			Darting Latency (seconds)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled Water	4.20 ± 0.25 [a]	4.41 ± 0.34[a]	5.78 ± 0.34[a]	590.30 ± 44.15[a]	605.50 ± 87.73[a]	589.20 ± 72.60[a]
Fluoxetine + Distilled Water	2.50 ± 0.15 [b]	2.58 ± 0.21[b]	3.34 ± 0.10[b]	868.11 ± 43.25[b]	834.10 ± 54.37[b]	821.10 ± 87.38[b]
Fluoxetine + 20 mg/kg bodyweight of Tadalafil	3.02 ± 0.22 [c]	5.30 ± 0.21 [c]	6.75 ± 0.35[c]	842.60 ± 68.12[c]	809.30 ± 69.63[c]	785.50 ± 53.76[c]
Fluoxetine + 125 mg/kg body weight of extract	3.22 ± 0.18 [c]	3.50 ± 0.18 [d]	6.50 ± 0.26[c]	815.60 ± 98.23[d]	793.90 ± 87.23[d]	762.20 ± 93.12[d]
Fluoxetine + 250 mg/kg body weight of extract	3.29 ± 0.21 [c]	4.07 ± 0.23 [a]	6.60 ± 0.36[c]	803.40 ± 89.45[d]	780.60 ± 56.34[d]	750.30 ± 96.12[d]
Fluoxetine + 500 mg/kg body weight of extract	3.50 ± 0.27 [b]	3.64 ± 0.33 [d]	4.18 ± 0.34[d]	780.10 ± 53.76[d]	785.70 ± 89.32[d]	770.20 ± 78.47[d]

Data are the mean of five determinants ± SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different ($p < 0.05$)

Table 4 *Hopping frequency and hopping latency of female rats induced into sexual dysfunction by fluoxetine following the administration of aqueous extract of A. garckeana fruit pulp*

Treatment	Hopping Frequency			Hopping Latency (seconds)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled Water	1.80 ± 0.50^{a}	1.60 ± 0.14^{a}	1.80 ± 0.15^{a}	789.50 ± 35.65^{a}	785.20 ± 67.54^{a}	775.30 ± 79.47^{a}
Fluoxetine + Distilled Water	0.90 ± 0.15^{b}	0.95 ± 0.05^{b}	1.00 ± 0.04^{b}	1290.45 ± 34.76^{b}	1130.90 ± 93.97^{b}	1150.30 ± 65.05^{b}
Fluoxetine + 20 mg/kg bodyweight of Tadalafil	0.95 ± 0.17^{b}	1.50 ± 0.02^{c}	2.20 ± 0.07^{c}	1225.30 ± 38.78^{c}	1100.80 ± 75.39^{b}	990.10 ± 75.98^{c}
Fluoxetine + 125 mg/kg body weight of extract	0.95 ± 0.15^{b}	1.10 ± 0.08^{d}	1.80 ± 0.02^{a}	1236.40 ± 74.37^{c}	1187.10 ± 103.42^{c}	1127.80 ± 74.09^{b}
Fluoxetine + 250 mg/kg body weight of extract	1.00 ± 0.05^{b}	1.50 ± 0.06^{c}	2.30 ± 0.08^{c}	1206.50 ± 98.36^{c}	1130.40 ± 93.44^{b}	965.50 ± 84.38^{c}
Fluoxetine + 500 mg/kg body weight of extract	1.10 ± 0.04^{b}	1.60 ± 0.03^{c}	2.20 ± 0.05^{c}	1150.50 ± 84.79^{d}	1120.40 ± 97.98^{b}	1030.50 ± 87.64^{b}

Data are mean of five determinants ± SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different (p <0.05)

4. Discussion

A. garckeana fruit pulp has been used for centuries in Nigerian traditional medicine to treat various ailments, including sexual dysfunction [3]. However, there has been limited scientific research to support its efficacy in treating sexual dysfunction. Research has shown that a decrease of at least 25% in sexual behavior is required before female rats are classified as sexually impaired. One potential cause of reduced libido and sexual impairment in female rats is the use of selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine [21]. Hence, the current study aimed to investigate the potential therapeutic effects of *A. garckeana* fruit pulp in sexually disabled female rats by fluoxetine, an SSRI commonly used to treat depression [24].

This reduction in sexual behavior is typically observed as prolonged darting latency (DL) and hopping latency (HL), as well as a significant decline in genital grooming (GG), licking behavior (LB), lordosis frequency (LF), darting frequency (DF) and Hopping frequency (HF) [21]. These behaviors, being indicative of the animals' sexual impairment, are essential for successful mating. Studies have shown that SSRIs can decrease mesolimbic dopaminergic activity by inhibiting serotonergic midbrain raphe nuclei projections, which can lead to decreased libido and arousal [25].

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Table 5 *Lordosis frequency and lordosis latency of female rats induced into sexual dysfunction by fluoxetine following the administration of aqueous extract of A. garckeana fruit pulp*

Treatment	Lordosis Frequency			Lordosis Latency (seconds)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled Water	1.00 ± 0.25 [a]	1.10 ± 0.03[a]	1.00 ± 0.08[a]	850.40 ± 78.45[a]	810.30 ± 76.45[a]	800.40 ± 75.89[a]
Fluoxetine + Distilled Water	0.70 ± 0.10 [b]	0.80 ± 0.02[b]	0.75 ± 0.03[b]	1360.10 ± 65.25 [b]	1340.90 ± 89.84[b]	1315.20 ± 90.07[b]
Fluoxetine + 20 mg/kg bodyweight of Tadalafil	0.80 ± 0.01[c]	1.00 ± 0.04[a]	1.30 ± 0.04[c]	1295.60 ± 64.35[c]	1130.50 ± 97.76[c]	1010.30 ± 73.92[c]
Fluoxetine + 125 mg/kg body weight of extract	0.80 ± 0.05[c]	0.90 ± 0.03[b]	1.10 ± 0.06[a]	1290.80 ± 103.43[c]	1250.40 ± 121.69[d]	1180.30 ± 98.32[d]
Fluoxetine + 250 mg/kg body weight of extract	0.90 ± 0.04 [c]	1.20 ± 0.03[a]	1.30 ± 0.03[c]	1280.20 ± 112.34[c]	1120.40 ± 126.76[c]	1030.20 ± 99.23[c]
Fluoxetine + 500 mg/kg body weight of extract	0.80 ± 0.03[c]	1.35 ± 0.04[c]	1.45 ± 0.06[c]	1280.10 ± 93.74[c]	1040.70 ± 101.08[c]	950.20 ± 76.36[c]

Data are mean of five determinants ± SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different (p <0.05).

Table 6 *Genital grooming and licking behaviour of female rats induced into sexual dysfunction by fluoxetine following the administration of aqueous extract of A. garckeana fruit pulp*

Treatment	Genital Grooming			Licking Behaviour		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled Water	8.50 ± 1.50 [a]	9.70 ± 0.85[a]	8.70 ± 1.20[a]	3.50 ± 0.35[a]	3.80 ± 0.55[a]	3.20 ± 0.65[a]
Fluoxetine + Distilled Water	5.70 ± 0.30 [b]	6.00 ± 0.43[b]	6.10 ± 0.35[b]	1.85 ± 0.20[b]	1.90 ± 0.20[b]	2.70 ± 0.25[b]
Fluoxetine+ 20 mg/kg bodyweight of Tadalafil	5.90 ± 0.28[b]	7.30 ± 0.47[c]	9.00 ± 0.75[c]	1.90 ± 0.26[b]	2.5 ± 0.50[c]	3.5 ± 0.46[c]
Fluoxetine + 125 mg/kg body weight of extract	5.80 ± 0.43[b]	7.80 ± 0.28[d]	8.30 ± 0.52[a]	1.80 ± 0.24[b]	2.00 ± 0.45[b]	2.9 ± 0.40[b]
Fluoxetine + 250 mg/kg body weight of extract	5.90 ± 0.30[b]	8.00 ± 0.55[d]	8.80 ± 0.21[a]	1.90 ± 0.32[b]	2.10 ± 0.32[b]	3.20± 0.36[c]
Fluoxetine + 500 mg/kg body weight of extract	5.85 ± 0.55[b]	8.40 ± 0.35[d]	8.80 ± 1.33[a]	1.95 ± 0.32[b]	2.00 ± 0.47[b]	2.5 ± 0.65[b]

Data are mean of five determinants ± SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different (p <0.05). Genital grooming and Licking behaviour are expressed in numbers.

Additionally, the inactivation of 5-HT1A receptor-mediated norepinephrine neurotransmission has been linked to decreased libido and arousal in female rats [25]. Therefore, the significant decline in GG, LB, DF, HF, and LF observed in sexually dysfunctional female rats in the current study suggests that fluoxetine administration has reduced libido and impaired sexual behavior in the rats. This reduction in sexual behavior is a critical aspect of sexual dysfunction and can lead to significant health problems in both animals and humans. Several studies have shown that the proceptive, receptive, and orientational behaviors of female rats can be used to assess their sexual function [21, 26]. The increase in proceptive behaviors, such as DF, HF, DL, and HL, and the receptive behaviors, such as LF and LL, indicate that the *A. garckeana* fruit pulp extract has the potential as a management option for sexual dysfunction in females. Additionally, the increase in orientational behaviors, such as GG and LB, further supports this claim [21, 26].

Table 7 *Concentrations of reproductive hormones of female rats induced into sexual dysfunction by fluoxetine following the administration of aqueous extract of A. garckeana fruit pulp*

Treatment	Progesterone (ng/mL)	Follicle Stimulating Hormone (pg/mL)	Luteinizing Hormone (mIU/mL)	Oestrogen (pg/mL)	Prolactin (ng/mL)
Distilled water (control)	34.92 ± 0.63[a]	0.31 ± 0.02[a]	5.73 ± 0.19[a]	16.87 ± 0.61[a]	0.60 ± 0.05[a]
Fluoxetine + Distilled Water	31.49 ± 1.21[b] (12.67%)	0.23 ± 0.02[b] (25.80%)	4.10 ± 0.04[b] (28.45%)	10.23 ±0.72[b] (39.36%)	0.51 ± 0.02[b] (15.00%)
Fluoxetine+ 20 mg/kg body weight of Tadalafil	45.58 ± 1.02 [c]	0.36 ± 0.48 [a]	6.39 ± 0.11[c]	16.54 ± 0.08[a]	0.58 ± 0.08 [a]
Fluoxetine + 125 mg/kg body weight of extract	36.94 ± 0.21[a]	0.29 ± 0.04 [a]	5.53 ± 0.31[a]	15.58 ± 0.77 [a]	0.55 ± 0.02 [a]
Fluoxetine + 250 mg/kg body weight of extract	46.28 ± 1.39[c]	0.32 ± 0.06 [a]	5.60 ± 0.60 [a]	15.52 ± 0.44 [a]	0.59 ± 0.15 [a]
Fluoxetine + 500 mg/kg body weight of extract	66.75 ± 1.49[d]	0.32 ± 0.06 [a]	6.42 ± 0.34 [d]	8.91 ± 0.46 [b]	0.74 ± 0.06[b]

Data are mean of five determinants ± SEM. Test values with superscripts different from the control down the group for each hormone are significantly different (p <0.05)

Furthermore, studies have suggested that sexual behaviors in female rats are regulated by multiple neurotransmitters, such as dopamine, norepinephrine, and serotonin, which interact in a complex manner to regulate sexual function [27, 28]. The ability of the extract to increase the number of genital grooming (GG) and licking behaviors (LB) in sexually impaired female rats may indicate that it affects the norepinephrine mediated neurotransmission pathway positively [29]. The significant increase in proceptive, receptive, and orientational behaviors of normal and sexually improve female sexual function. The potential of the extract to raise dopamine levels in the mesolimbic dopaminergic system may be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and terpenoids in the fruit pulp of *A. garckeana*. These components have been shown to affect dopamine neurotransmission [30]. The increase in DL, HL, and LL in sexually impaired female rats may also be related to fluoxetine's ability to inhibit the nitric oxide synthesis in the female genital tissue, which can result in poor vaginal smooth muscle relaxation in fluoxetine-administered rats [29]. The reversal of sexual dysfunction in the fluoxetine-treated female rats was more evident at the doses, 250 and 500 mg/kg body weight, and the outcome compares favorably with the reference medication. This was demonstrated by the readiness of the female rat to approach their male counterparts, elicit solicitation behavior, and then subsequently display receptive behavior. The demonstration of enhanced sexual behavior in female rats treated with *A. garckeana* fruit pulp extract is comparable to several previous studies. For instance, Aswar *et al*. [31] found that the administration of a friedelin-rich fraction extracted from *Cissus quadrangularis* increased the sexual behavior of female rats. Similarly, Yakubu and Olutoye [22] reported an increase in the sexual behavior of female rats treated with a methanol extract of *Anthonotha macrophylla* leaves. These findings support the notion that natural plant products have the potential to improve sexual function in females.

The medicinal properties of plants are primarily attributed to the presence of secondary plant metabolites and bioactive agents [32]. These plant metabolites are known to have various pharmacological activities, including antimicrobial, anti-inflammatory, and antioxidant properties, among others [33]. In the case of *A. garckeana* fruit pulp, the presence of these bioactive compounds may contribute to its aphrodisiac properties and potentially serve as a natural remedy for sexual dysfunction in females. Several studies have reported the presence of saponins, phenols, flavonoids, and alkaloids in different plant extracts with aphrodisiac properties [34, 35, 36]. These bioactive compounds may stimulate sexual behavior by influencing the levels of sex hormones or by increasing the responsiveness of target tissues to these hormones. For instance, saponins have been shown to stimulate the production of testosterone, which may lead to increased sexual desire and activity [37]. Flavonoids, on the other hand, may act as oestrogen agonists and enhance the activity of nitric oxide synthase, resulting in improved blood flow to the genitalia [38]. Phenolic compounds have been found to increase the activity of tyrosine hydroxylase, which is involved in dopamine synthesis and release, thereby enhancing sexual motivation and pleasure [39]. Furthermore, the presence of alkaloids in the *A. garckeana* fruit

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pulp extract may contribute to its aphrodisiac properties. Alkaloids such as yohimbine and caffeine have been shown to have stimulant effects on the central nervous system and increase sexual arousal and performance [40]. The combination of these bioactive compounds acting centrally or peripherally may thus be responsible for the increased sexual behavior seen in the current study.

Previous studies have established those changes in the levels of reproductive hormones such as progesterone (PG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestrogen (E), and prolactin (PR) can impact sexual behavior in animals [27, 41]. Progesterone, for instance, has been shown to have a modulatory effect on female rat sexual behavior, with low levels leading to decreased sexual motivation and performance [42]. Similarly, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) have been found to play critical roles in the regulation of reproductive function and sexual behavior in both male and female animals [43]. The reduction in reproductive hormone levels observed in fluoxetine-treated female rats could be attributed to the medication's direct effect on the gonads or its indirect effect on the pituitary gland, which is known to control the release of these hormones [44]. In addition, fluoxetine has been shown to modulate the release of hypothalamic hormones such as gonadotropin-releasing hormone (GnRH), which plays a crucial role in the regulation of reproductive function [44].

In the present study, the aqueous extract of *A. garckeana* fruit pulp has been found to have a significant positive impact on female sexual behavior. In sexually impaired female rats, the extract resulted in a substantial increase in blood progesterone levels, which may have lowered the uterine smooth muscle's ability to contract [45]. The extract was also found to activate hormone synthesis by the granulosa cells of the developing follicles in the ovary, improving the release of oestrogen hormone into the bloodstream [46]. This suggests that the extract boosted the mechanism regulating hormone production in granulosa cells, improving the levels of oestrogen and other reproductive hormones at 125 and 250 mg/kg body weight of the extract. Studies have reported that improved levels of oestrogen hormone are associated with the development of female secondary sex traits, including improved vaginal lubrication and sexual receptivity, as well as the triggering of lordosis behavior [47, 48]. Additionally, the increase in oestrogen levels may have made it easier for female sexual behavior to improve. Similar trends have been reported by Nurudeen and Yakubu [21] and Yakubu and Olutoye [22]. The significant drop in the amount of oestrogen in the animals at the highest dosage of the extract administered may indicate that the extract at this dosage reached a critical threshold and began to negatively affect the hormone. In a previous study, it was demonstrated that decreased proceptivity and receptivity in female rats might be attributed to oestrogen receptor inhibition in the ventromedial nucleus of the hypothalamus [49]. Oestrogen receptors are critical for mediating the effects of oestrogen on sexual behavior and fertility [50]. Therefore, the reduction in oestrogen levels at the highest dosage of the extract may have negatively impacted the oestrogen receptors, resulting in a decrease in sexual behavior measures at that dosage.

Furthermore, the dosage-dependent effect of the *A. garckeana* fruit pulp extract on hormone levels is consistent with the concept of hormesis, which suggests that small doses of a stressor can stimulate a positive response, whereas larger doses can be harmful [51]. Therefore, the significant increase in oestrogen levels at the lower dosages of the extract may have stimulated positive effects on sexual behavior measures, while the higher dosage resulted in negative effects due to the possible inhibitory effect on oestrogen receptors.

The administration of 125, 250, and 500 mg/kg BW of the extract has been shown to have a stimulatory effect on the hypothalamic-pituitary axis, which may be responsible for a significant increase in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in female albino rats. The increased levels of LH and FSH may have implications for female reproductive health. For example, the surge of LH triggers ovulation by causing the release of the mature egg from the follicle [52]. Additionally, the LH surge initiates the process of turning the remaining follicle into a corpus luteum, which releases progesterone to prime the endometrium for potential implantation [52]. The progonadotropic effect of natural extracts on female reproductive health has been demonstrated in previous studies as well. For instance, Krishnamoorthy *et al*. [53] reported that administering *Andrographis paniculata* root to female albino rats resulted in increased levels of LH and FSH, suggesting a potential role for this extract in promoting ovulation and corpus luteum formation. Similarly, Yakubu and Olutoye [22] found that administration of *Anthonotha macrophylla* leaves to female albino rats resulted in increased levels of LH and FSH, indicating a potential progonadotropic effect of this extract as well. Prolactin is a hormone that plays a key role in female reproductive function, including sexual behavior and lactation. In sexually dysfunctioned rats, a reduction in prolactin concentration, a condition known as hypoprolactinemia, has been linked to pituitary under activity and sexual dysfunction [54, 55]. In this study, the aqueous extract of *A. garckeana* fruit pulp has been shown to increase prolactin levels in female rats. This increase in prolactin levels may have encouraged female rats to engage in sexual behavior by allowing gonadotropin-releasing hormone (GnRH) to function normally and stimulate the pituitary gland to produce more sex hormones. This finding is consistent with previous studies that have demonstrated the aphrodisiac properties of natural plant extracts on sexual behavior and as well, suggested a dose-dependent relationship between the administration of plant extracts and their therapeutic effects [56].

5. Conclusion

This study highlights the potential beneficial effects of the aqueous extract of the fruit pulp of *Azanza garckeana* on the sexual behavior of female rats. The research findings reveal that the increase in proceptive behaviors, such as DF, HF, DL, and HL, and the receptive behaviors, such as LF and LL, indicate that the *A. garckeana* fruit pulp extract has the potential as a management option for sexual dysfunction in females. The medicinal properties of plants are primarily attributed to the presence of secondary plant metabolites and bioactive agents. In the case of *A. garckeana* fruit pulp, the presence of these bioactive compounds contributed to its aphrodisiac properties, while serving as a natural remedy for sexual dysfunction in females. The combination of these bioactive compounds acting centrally or peripherally may thus be responsible for the increased sexual behavior as reported in this study. The extract also shows the capability of activating hormone synthesis by the granulosa cells of the developing follicles in the ovary, thereby improving the release of oestrogen hormone into the bloodstream, which is an indication that the extract boosted the mechanisms regulating reproductive hormones production. Furthermore, this study reported the most effective dosages of the *Azanza garckeana* extract as 250 and 500 mg/kg body weight, thereby providing compelling scientific evidence supporting the traditional use of *Azanza garckeana* for managing female sexual deficiencies. However, it should be noted that further research is necessary to determine the optimal dosage, potential adverse effects and the specific mechanisms by which the extract affects sexual behavior.

Acknowledgements

The authors appreciate the contribution of Mr. Dele Aiyepeku of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, for the technical assistance provided during this study.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

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