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Aqueous extract of *Hybanthus enneaspermus* exhibited aphrodisiac potentials in fluoxetine-induced sexually-impaired female rats

Muhammad A. Dikwa [1], **Muhammed R. Asinmi** [2], **Mansurat B. Falana** [3]
Quadri O. Nurudeen [2 *] and **Musbau A. Akanji** [4]

[1] Department of Microbiology and Biotechnology, Federal University, Dutse, Nigeria
[2] Department of Biological Sciences (Biochemistry Unit), Al-Hikmah University, Ilorin, Nigeria
[3] Department of Biological Sciences (Microbiology Unit), Al-Hikmah University, Ilorin, Nigeria
[4] Department of Biochemistry, Kwara State University, Malete, Nigeria
[*]Author to whom correspondence should be addressed

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Abstract: *Hybanthus enneaspermus*, traditionally used as an aphrodisiac was investigated for its potential to reverse antidepressant-induced sexual dysfunction in female rats. The aqueous extract was evaluated for secondary metabolite, amino acid and mineral constituents. Alkaloids, tannins, flavonoids, anthraquinones, steroids, terpenoids, phenolics, calcium, potassium, sodium, glutamine and leucine are some of its notable constituents. 60 healthy, sexually responsive female albino rats (144.7±5.9 gm) were divided into six groups (A-F) of 10 rats each; of which 50 were induced into sexual dysfunction. Rats in group A were administered distilled water throughout the experimental period. They served as a control group, while rats induced into sexual dysfunction (Groups B-F) by fluoxetine were given water, the reference medication (Tadalafil) and oral doses of the extract (250, 500, and 1000 mg/kg body weight) once daily for seven days, respectively. When administered to sexually active rats, fluoxetine significantly decreased the frequency of darting, hopping, lordosis, genital grooming, and licking behavior by 57.4%, 42.5%, 43.9%, 64.0%, and 41.8%, respectively. However, the latency of darting, hopping and lordosis were significantly increased by 50.6%, 47.7%, and 54.9%, respectively. *Hybanthus enneaspermus* aqueous extract administered at 250, 500, and 1000 mg/kg significantly reversed fluoxetine-mediated changes in all sexual behavior parameters. The extract's ability to reverse the characteristics of sexual behavior at 1000 mg/kg was comparable to those of tadalafil-treated rats. Additionally, all the extract dosages reversed the levels of blood luteinizing hormone, follicle-stimulating hormone, progesterone, prolactin and estrogen after it has significantly been altered by fluoxetine. The results indicated that the aqueous extract of *Hybanthus enneaspermus* improved the proceptive, receptive and orientational behavior of rats. The extract also enhanced reproductive hormone concentration by restoring sexual competence in sexually-impaired female rats. The findings of this study provide further evidence in favor of *Hybanthus enneaspermus* widespread usage in the management of female sexual dysfunction.

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Introduction

Aphrodisiacs are medicines or herbs that are used to enhance libido, sexual attraction, desire, enjoyment, behavior and orgasm [1]. Pharmaceutical prescriptions such as flibanserin and bremelanotide, which are exclusively authorized for use in premenopausal women, are among the several forms of aphrodisiac pills that are sold [1]. Varieties of lotions and oils that contain components including honey, ripe tamarind fruit, black pepper, camphor, long pepper, and brown jiggery are also used as aphrodisiacs [2]. An increase in virility, sexual vigour, and the quality of offspring is acknowledged to be possible with aphrodisiac medications [1]. *Hybanthus enneaspermus* (*H. enneaspermus*) Linn F. Muell, commonly called *ewe abiwere* among the Yoruba-speaking people of Nigeria, is a traditional medicinal herb that belongs to the Violaceae plant family [3, 4]. Its native range extends over tropical and subtropical parts of the world, encompassing Africa, Australia, and Asia, with a concentration in the sultry regions of India. *H. enneaspermus* is a perennial plant with hairy twigs, solitary pink spade-shaped flowers, linear-lanceolate leaves, and a wooden base for the stem. Many commercial products made from *H. enneaspermus* are sold in powder or pill form and are natural libido boosters designed to increase female desire and arousal. This plant possesses a wide range of medicinal properties. It has been reported to possess anticonvulsant and free radical scavenger [5], nephroprotective [6], antiarthritic [7], larvicidal [8], hepatoprotective [9], and male aphrodisiac [10] properties. *H. enneas-permus* has also been reported to contain some amino acids and phytochemicals such as flavonoids, alkaloids, steroids, carbohydrates, saponins, volatile oil, and terpenoids [11].

Fluoxetine (selective serotonin reuptake inhibitor, SSRI) is a popularly used antidepressant known to have a high frequency of undesirable sexual effects. Since these adverse effects frequently cause patients to stop taking their drug too soon, their depression symptoms are not relieved [12]. A considerable proportion of the population suffers from sexual dysfunction [12]. Disorders in sexual desire and psychophysiological alterations connected to the sexual response cycle impact around 35.0% of men and 45.0% of women [13]. Many women turn to herbal remedies like *H. enneaspermus* for sexual dysfunction because taking medications can be difficult. This predilection stems from several variables, including affordability, availability, and the perception that botanicals have few or no negative effects. There is a noticeable lack of scientific experimental support for *H. enneaspermus* purported aphrodisiac benefits on female libido in the literature, despite plethora of research examining the chemical makeup and pharmacological effects of the plant.

Materials and methods

Plant material and authentication: H. enneaspermus leaves were obtained from a local market in Ilorin West local government, Kwara State, Nigeria. At the University of Ilorin Herbarium in Ilorin, Nigeria, a botanist carried out identification and authentication to guarantee correctness, while a voucher sample was deposited under a reference number (UIH 001/1092).

Animals: Sixty healthy and in-bred, sexually active, female Wistar rats (*Rattus norvegicus*) were obtained from the local Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Rats weighed 144.7±5.90 g. The rats were housed in clean, well-maintained cages in an Animal House at a controlled room temperature (26-28°C). They were fed rat pellets and allowed unlimited access to tap water. The European Convention for the Use of Laboratory Animals for Scientific Purposes (ETS-123) and the National Institutes of Health's (NIH Publication No. 80-23) rules were scrupulously followed during the investigation. The animal care and usage rules of the institution were strictly adhered to guarantee the animals' welfare and proper treatment during the investigation.

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Preparation of the extract: After thoroughly washing the leaves under running water, they were dried for 48 hr at 40°C in an oven. The dried leaves were then crushed in an electric blender and put into an airtight container for storage. An aqueous solvent was used to macerate 100 g of the powdered material throughout 48 hrs at 27°C. The maceration process involved frequent shaking and was filtered using cheesecloth. A sticky residue was produced after the filtrate was subjected to evaporation in a rotary evaporator [14]. This residue was reconstituted in distilled water to get the necessary doses of 250, 500, and 1000 mg/kg body weight. Information gathered from an ethnobotanical survey was used to calculate the dosages, and 500 mg/kg was used as the most commonly stated amount. 250 and 1000 mg/kg were chosen to be half and twice the 500 mg/kg estimated dose, respectively, [15].

Screening of secondary metabolites: Following the guidelines provided previously [16-20], 5 g of *H. enneas-permus* extract was diluted in 40 ml of distilled water. To find out if there were any steroids, flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, cardenolides, and anthraquinones, phytochemical screening. Following identification, the secondary metabolites were subjected to quantitative analysis using established methods for the quantification of phenols, alkaloids, flavonoids, terpenoids, steroids, and anthraquinones [21-27].

Induction of sexual dysfunction in female rats and assessment of sexual behaviour indices: Following the protocol described by Sarkar et al. [28], fifty female rats were given an oral dosage of fluoxetine (15 mg/kg, prepared daily in distilled water) to induce sexual dysfunction [28, 29]. The rats were given fluoxetine for 14 days. The male and female rats were placed in separate rectangular hardwood cages with wire mesh tops on the 15th day. For 30 min, mating behaviours were monitored by the previous guidelines [30-31]. The sexual impairments in female rats were defined as a least 25.0% decrease in the frequency of lordosis, darting, hopping, licking behaviour, and genital grooming and a minimum 25.0% increase in the latency of darting and hopping. These rats were then divided into several groups.

Treatment protocol: A total of 60 female rats that had been acclimated for 2 weeks were split into 6 groups (A-F), each with 10 rats, in a randomized design. The grouping is as follows; Group A: rats that received 0.5 ml 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 of tadalafil, group D: rats induced into sexual dysfunction and administered 0.5 ml of 250 mg/kg of the extract, group E: rats induced into sexual dysfunction and administered 0.5 ml of 500 mg/kg of the extract and group F: rats induced into sexual dysfunction and administered 0.5 ml of 1000 mg/kg of the extract. The different rat groups were treated as described above once daily for seven days using a plastic oropharyngeal cannula. Observations of female sexual behaviour parameters were made between 17:00 and 21:00 hrs. on days 1, 3, and 7.30 min. After treatment, the results were recorded in dim light at room temperature.

Preparation of serum: To prepare the serum, the procedure as described by Yakubu et al. [32] adhered to diethyl ether fumes and was used to anaesthetize the rats. After cutting their jugular veins, 5 ml of blood was extracted and put into dry, sterile centrifuge tubes. To give the blood time to coagulate, the samples were left at room temperature for 15 min. After centrifuging using the Uniscope Laboratory Centrifuge, for 10 min at 503 × g, a Pasteur pipette was then used to obtain clear serum. The sera were refrigerated before the various hormone tests were performed.

Determination of reproductive hormones: The tube-based serum enzyme immunoassay (EIA) method was used to measure the amounts of progesterone (P), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen (E), and prolactin (Pl) in the serum. The method was carried out in full compliance with the manufacturer's instructions (Elabscience Biotechnology Company Limited, Texas, USA).

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Statistical analysis: After calculating the mean and standard error of the mean from 10 replicated data points, a one-way analysis of variance (ANOVA) was performed, followed by Dunnett's test to compare every mean of the group with the mean of the control using GraphPad Prism version 8.0. Statistical significance was ascertained, and $p<0.05$ was used as the threshold for differences to be deemed significant. Data are mean±SEM.

Results

The results of the phytochemical screening of the aqueous extract of *H. enneaspermus* revealed the presence of flavonoids, tannins, phenols, steroids, terpenoids, anthraquinones and alkaloids (**Table 1**). Tannins are the most abundant secondary metabolite detected while anthraquinones are the least abundant. Aqueous extract of the *H. enneaspermus* leaves contained 17 amino acids with leucine having the highest concentration followed by glutamine, while methionine was the least abundant in the plant leaf. In **Table 2**, further amino acids found include alanine, arginine, asparagine, cysteine, glycine, histidine, isoleucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine. Analysis of the mineral constituents of the aqueous extract of *H. enneaspermus* leaves revealed the presence of calcium, chromium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc (**Table 3**). Calcium was the most abundant whereas Lead was the least abundant.

Table 1: Secondary metabolites of *H. enneaspermus*

Secondary metabolite	Concentration (mg/g)
Alkaloids	10.50±0.98
Tannins	22.70±0.31
Flavonoids	20.60±0.71
Anthraquinones	2.80±0.23
Steroids	15.40±0.52
Terpenoids	8.30±0.11
Phenolics	12.66±0.63
Saponins	Not detected
Cardenolides	Not detected
Cardiac Glycosides	Not detected

Table 2: Amino acid composition of *H. enneaspermus*

Amino acids	Conc. (g/100 ml)
Alanine	6.20
Arginine	5.78
Asparagine	7.90
Cysteine	4.55
Glutamine	10.25
Glycine	6.10
Histidine	2.54
Isoleucine	5.75
Leucine	11.88
Lysine	5.03
Methionine	1.60
Phenylalanine	6.40
Proline	4.65
Serine	5.35
Threonine	5.45
Tyrosine	4.42
Valine	6.15

Table 3: Mineral contents *H. enneaspermus* leaves

Minerals	Conc. (mg/100 ml)
Calcium	375.00±5.67
Chromium	8.30±0.09
Copper	10.75±0.19
Iron	30.74±21.26
Argon	5.32±0.02
Magnesium	80.91±1.42
Manganese	18.50±0.38
Phosphorus	30.24±1.23
Potassium	257.51±3.78
Sodium	160.79±3.07
Zinc	10.23±0.55
Lead	3.32±0.03
Cadmium	Not detected
Nickel	Not detected

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In **Table 4**, the administration of fluoxetine to sexually active female rats significantly decreased the darting frequency (DF), hopping frequency (HF), lordosis frequency (LF), genital grooming (GG) and licking behavior (LB) by 57.4%, 42.5%, 43.9%, 64.0%, and 41.8% respectively. Whereas, the darting latency (DL), hopping latency (HL), and lordosis latency (LL) were significantly increased by 50.6%, 47.7%, and 54.9%, respectively. In all the cases, the percentage of change was higher than the 25.0% baseline, which suggests that the animals have been induced into sexual dysfunction (**Table 4**).

Table 4: Sexual behavior parameters of female rats administered fluoxetine

Parameters	Control group	Fluoxetine-treated rats	Percentage change
Darting frequency	9.85±0.12^{a}	4.20±0.09^{b}	57.36#
Hopping frequency	4.35±0.28^{a}	2.50±0.19 ^{b}	42.53#
Lordosis frequency	2.85±0.03^{a}	1.60±0.05^{b}	43.86#
Genital grooming	16.25±0.29^{a}	5.75±0.37^{b}	64.00#
Licking behavior	9.10±0.15^{a}	5.30±0.11^{b}	41.76#
Darting latency*	812.15±27.49 ^{a}	1222.80±41.67^{b}	50.55^{+}
Hopping latency*	955.20±39.62^{a}	1411.45±29.83^{b}	47.65^{+}
Lordosis Latency*	970.75±73.28^{a}	1505.60±92.15^{b}	54.94^{+}

Data are mean±SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different. a is assigned as the mean of the control group while b shows that the mean (of the test group) is significantly different from the mean of the control), # means percentage reduction in parameter, + means percentage increase in parameters

In **Table 5**, the administration of fluoxetine significantly decreased the DL, HL and LF of the rats throughout the exposure period. On the first and third days of treatment, there was no significant difference in the DF, HF and LF of the fluoxetine-induced sexual dysfunction female rats when compared with the group given 250 mg/kg and 500 mg/kg, however, there was a significant increase at 1000 mg/kg dosage. In contrast, on day 7 of treatment, the extract at all the doses evaluated (250, 500, and 1000 mg/kg) produced a significant increase in the DF, HF and LF of the fluoxetine-induced sexual dysfunction female rats. Furthermore, treatment of the animals induced into sexual dysfunction with 1000 mg/kg of extract on day 7 resulted in DF, HF and LF that compared favorably with the reference drug (tadalafil) and the control animals (**Table 5**). The administration of fluoxetine significantly decreased the GG and LB of the rats throughout the exposure period (**Table 6**). The extract at all doses (250, 500 and 1000 mg/kg) produced a significant increase in the GG and of the fluoxetine-treated animals when compared with the control sexual dysfunction female rats (**Table 6**). On day 3 of treatment, the extract at all the doses evaluated (250, 500 and 1000 mg/kg) produced a significant increase in the GG and LB of the fluoxetine-induced sexual dysfunction in female rats, while the significant increase by the extract was more profound on day 7 of treatment when compared with the distilled water-treated fluoxetine-induced sexual dysfunction female rats (**Table 6**). Furthermore, treatment of the rats induced into sexual dysfunction with 1000 mg/kg of extract on day 7 resulted in GG and LB that compared favorably with the reference drug (tadalafil) and the control rats.

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Table 5: Darting, hopping and lordosis frequencies of female rats induced into sexual dysfunction by fluoxetine following the administration of *H. enneaspermus*

Treatments	Darting frequency			Hopping frequency			Lordosis frequency		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control	9.10±0.12^{a}	9.70±0.11^{a}	9.30±0.13^{a}	4.10±0.03^{a}	3.95±0.07^{a}	4.05±0.02^{a}	2.95±0.02^{a}	2.85±0.03^{a}	3.00±0.06^{a}
Fluoxetine-treated	4.90±0.05^{b}	5.50±0.06^{b}	5.20±0.05^{b}	2.30±0.07^{b}	2.20±0.04^{b}	2.40±0.10^{b}	1.75±0.05^{b}	1.95±0.08^{b}	1.95±0.03^{b}
Fluoxetine+tadalafil	6.40±0.06^{c}	7.00±0.07^{c}	8.50±0.08^{a}	2.70±0.10^{b}	3.10±0.05^{c}	4.00±0.03^{a}	2.00±0.07^{b}	2.80±0.02^{a}	3.05±0.07^{a}
Fluoxetin+250 extr.	5.10±0.04^{b}	5.90±0.06^{b}	6.40±0.05^{c}	2.30±0.13^{b}	2.50±0.12^{b}	2.60±0.05^{b}	1.80±0.03^{b}	1.85±0.07^{b}	2.05±0.09^{b}
Fluoxetine+500 extr.	5.20±0.04^{b}	5.80±0.05^{b}	6.60±0.08^{c}	2.40±0.06^{b}	2.70±0.11^{b}	3.10±0.09^{c}	1.95±0.09^{b}	1.90±0.02^{b}	2.50±0.11^{c}
Fluoxetine+1000 extr.	6.15±0.03^{c}	7.35±0.07^{c}	8.90±0.10^{a}	2.90±0.02^{b}	3.25±0.13^{c}	3.75±0.03^{a}	2.05±0.02^{b}	2.70±0.02^{a}	3.10±0.05^{a}

Data are mean±SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different. a is assigned as the mean of the control group while b and c showed that the mean (of the test groups) are significantly different from the mean of the control

Table 6: Genital grooming and licking behavior of female rats induced into sexual dysfunction by fluoxetine following intake of *H. enneaspermus*

Treatments	Genital grooming			Licking behavior		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control	16.20±0.29^{a}	14.90±0.24^{a}	16.40±0.31^{a}	9.40±0.12^{a}	9.70±0.14^{a}	9.10±0.09^{a}
Fluoxetine-treated	5.70±0.05^{b}	5.30±0.08^{b}	5.60±0.06^{b}	5.20±0.03^{b}	5.80±0.04^{b}	5.60±0.04^{b}
Fluoxetine+Tadalafil	9.50±0.06^{c}	11.70±0.08^{c}	15.10±0.17^{a}	5.50±0.03^{b}	7.60±0.04^{c}	9.70±0.08^{a}
Fluoxetin+250 extr.	7.00±0.05^{d}	8.80±0.06^{d}	11.10±0.08^{c}	5.30±0.05^{b}	6.10±0.03^{b}	7.40±0.06^{c}
Fluoxetine+500 extr.	7.90±0.05^{d}	10.10±0.06^{c}	13.10±0.13^{c}	5.50±0.04^{b}	7.30±0.05^{c}	8.70±0.05^{c}
Fluoxetine+1000 extr.	10.30±0.06^{c}	12.60±0.15^{c}	16.70±0.23^{a}	5.30±0.03^{b}	7.30±0.04^{c}	9.00±0.06^{a}

Data are mean± SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different. a is assigned as the mean of the control group, while b and c showed that the mean (of the test groups) are significantly different from the mean of the control

In **Table 7**, the administration of fluoxetine significantly increased the DL and HL of the animals, when compared with the control rats. On day one of treatment, the doses of 500 and 1000 mg/kg produced a significant decrease in the DL and HL of the animals induced into sexual dysfunction by fluoxetine when compared with the distilled water-treated fluoxetine-induced sexual dysfunction female rats. Furthermore, on days 3 and 7 of treatment, the extract at all the doses evaluated (250, 500 and 1000 mg/kg) produced a significant decrease in the DL and HL of the sexual dysfunction female rats, when compared with the distilled water-treated fluoxetine-induced sexual dysfunction female rats. The decrease produced by 1000 mg/kg at day 7 compares favorably with the reference drug (tadalafil) as well as the control rats.

Table 7: Darting and hopping latencies of female rats induced into sexual dysfunction by fluoxetine following the administration of *H. enneaspermus*

Treatments	Darting latency			Hopping latency		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control	855.80±14.76^{a}	878.20 ±15.62^{a}	861.40±13.89 ^{a}	913.40±11.24^{a}	941.70±11.35^{a}	934.20±11.02^{a}
Fluoxetine-treated	1289.60±22.03^{b}	1275.20±21.28^{b}	1247.30±20.15^{b}	1443.70±18.01^{b}	1481.90±17.05^{b}	1439.50±16.83^{b}
Fluoxetine+tadalafil	1132.50± 18.74^{c}	980.90±17.46^{c}	899.20±15.75^{a}	1131.40±16.38^{c}	1093.20±14.12^{c}	946.80±12.16^{a}
Fluoxetin+250 extr.	1275.40±20.31^{b}	1115.70±16.84^{d}	1041.80±14.22^{c}	1352.60±17.44^{b}	1265.80±15.23^{d}	1136.90±14.68^{c}
Fluoxetine+500 extr.	1178.00±18.61^{c}	1082.50±14.92^{d}	967.30±12.21^{c}	1315.60±17.28^{b}	1148.90±15.31^{d}	1024.60±13.36^{c}
Fluoxetine+1000 extr.	1029.60±16.13^{c}	966.90±12.49^{c}	892.10±11.34^{a}	1124.50±16.82^{c}	1039.40±11.89^{c}	954.70±10.98^{a}

Data are mean±SEM. Test values with superscripts different from the control down the group for each day and parameter are significantly different. a is assigned as the mean of the control group, while b, c and d showed that the mean (of the test groups) are significantly different from the mean of the control

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In **Table 8**, the administration of fluoxetine to sexually active female rats significantly reduced the levels of P, FSH, LH, and E by 25.4%, 33.6%, 41.3%, and 39.5%, respectively, while the levels of Pl increased significantly by 58.6% when compared with the distilled water treated rats. The reduced levels of the hormones in the fluoxetine-treated animals were significantly increased following the administration of the aqueous extract of *H. enneaspermus* and the significant increase was more pronounced in the group administered 1000 mg/kg of the extract, and compared favorably, both with those administered the reference drug (tadalafil) and those of the control group. The elevated level of prolactin in the fluoxetine-treated animals was significantly decreased following the administration of all the doses (250, 500 and 1000 mg/kg) of the aqueous extract of *H. enneaspermus*, when compared with the distilled water-treated fluoxetine-induced sexual dysfunction female rats.

Table 8: Concentrations of reproductive hormones of female rats induced into sexual dysfunction by fluoxetine following the administration of *H. enneaspermus*

Treatment	Progesterone (nmol/L)	Follicle Stimulating Hormone (mIU/mL)	Luteinizing Hormone (mIU/mL)	Estrogen	Prolactin
Control	47.28±0.74^a	1.28±0.03^a	7.92±0.21^a	20.12±0.68^a	1.57±0.04^a
Fluoxetine-treated	35.24±1.15^b **(25.42%)**	0.85±0.02^b **(33.59%)**	4.65±0.05^b **(41.25%)**	12.09±0.8^b **(39.85%)**	2.49±0.02^b **(58.60%)**
Fluoxetine+tadalafil	45.35±1.09^a	1.31±0.05^a	6.18±0.12^a	19.79±0.07^a	1.69±0.07^a
Fluoxetin+250 extr.	37.42±0.19^b	1.07±0.03^c	5.71±0.28^c	15.82±0.76^c	2.10±0.02^c
Fluoxetine+500 extr.	40.56±1.34^c	1.19±0.05^a	6.78±0.57^c	16.75±0.42^c	1.68±0.14^a
Fluoxetine+1000 extr.	46.32±1.39^a	1.29±0.05^a	7.20±0.32^a	19.35±0.47^a	1.50±0.06^a

Data are mean±SEM. Test values with superscripts different from the control down the group for each hormone are significantly different. a is assigned as the mean of the control group, while b and c show that the mean (of the test groups) are significantly different from the mean of the control

Discussion

Numerous plants are proven to increase libido, sexual potency, and/or sexual pleasure, which may have an impact on sexual functioning [33]. Secondary metabolites produced by plants act peripherally by increasing nonadrenergic/noncholinergic neurotransmitters, such as nitric oxide (NO) and vasoactive intestinal polypeptide, which are involved in smooth muscle relaxation and improved genital blood flow [34]. Alkaloids as yohimbine, can function as α-2 adrenergic receptor antagonists and stimulate norepinephrine release, enhancing the female rats' arousal and sexual desire. Some alkaloids cause vasodilation, which relaxes blood vessels in the vaginal region and improves blood flow to this crucial part of the female rats' copulatory system [35]. Antioxidants named tannins help to maintain NO, which is necessary for healthy vaginal blood flow and appropriate blood vessel dilatation for erotic enjoyment and performance [36]. Although flavonoids are known for their antioxidant qualities, they improve sexual health. They can prevent free radicals from degrading NO, maintain sustained NO for effective vascular relaxation, and enable regular vaginal lubrication and sexual performance [37]. By altering the balance of sex hormones (testosterone and estrogen), several plant steroids can affect sexual function through hormonal control, potentially enhancing sexual desire and performance [38]. Certain terpenoids can affect neurotransmitters, thereby, enhancing mood and mental arousal and enhancing sexual pleasure. The terpenes, linalool and limonene are perhaps the most well-known compounds that have been linked to increased libido and sex drive [39]. Numerous chemicals, including resveratrol and lignans, are phenolic compounds and can have an impact on sexual functions [40]. Resveratrol is a polyphenol found in red wine, grapes, and berries that has been shown to improve sexual function [41]. Other phenolic-rich extracts have been reported to enhance sexual

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function in animal studies [42]. Phenolics support healthy blood vessel dilation and sexual function by avoiding oxidative damage to the NO molecule, which helps sustain NO. Some phenolics have the potential to behave as phytoestrogens, which can affect hormonal balance and improve sexual function [42].

Protein (amino acids) makes up a sizable component of our body's cells, muscles, and reproductive system tissues [43]. When arginine is converted to citrulline by NO synthase, NO, an active free radical, is created. Arginine can boost sexual desire and improve sensitivity to sexual stimulation by boosting blood flow to the reproductive area [44]. These improve the sense of sexual stimulation and increase desire with the potential of reaching orgasm [45]. In addition, it has been reported to enhance healthy uterine function during implantation, boost cervical mucus, and support endometrial secretions [46]. Protein synthesis requires asparagine, especially for producing structural proteins which are essential for preserving the structural and functional integrity of reproductive tissues and organs [47]. Dietary minerals work as supplements and are essential for a variety of complex biological processes, including hormonal, enzymatic, and neurological responses that are necessary for a healthy sex life [48]. The contraction of muscles, particularly the muscles utilized for sexual activity, depends critically on calcium. It directly impacts the muscular contraction apparatus and causes muscle contraction by its interaction with regulatory proteins, including the troponin system [49]. Muscle contraction, particularly those of the muscles used for sexual activity, depends on calcium and potassium. It assists in the rhythmic contractions that take place during orgasm and sexual desire.

Fluoxetine is commonly prescribed to treat bulimia, premenstrual dysphoric, and major depressive disorders. Fluoxetine has antidepressant action in mice by a significant reduction in the duration of the immobility time and enhanced swimming [50]. It aids in the restoration of a more balanced neurotransmitter profile, which can lessen the signs and symptoms of depression by boosting the availability of serotonin [51]. More than one-third of women using fluoxetine for therapeutic purposes may experience sexual dysfunction as a result of consuming fluoxetine, thus, may experience libido loss, anorgasmia, and/or decreased vaginal lubrication. Although the mechanisms underlying drug-induced sexual dysfunction in females are still unclear, it is thought to be connected to the complex neural and endocrine loops in the female reproductive cycle. Elevated serotonin can affect sexual function by inhibiting some neurotransmitters. Sensations of arousal and desire are known to be correlated with dopamine and norepinephrine. Overproduction of serotonin can diminish norepinephrine and dopamine, hence lowering desire and sexual arousal, thus, noted to lessen genital sensation. This loss of sensation might make it difficult to have orgasms and sexual pleasure. A crucial chemical in sexual response is NO. NO, which helps with blood flow to the vaginal region can be inhibited by high levels of serotonin making it difficult to establish and maintain arousal. A variety of sexual side effects, including lower libido, difficulty in eliciting orgasm, and diminished sexual enjoyment, can occur when fluoxetine upsets this equilibrium by raising serotonin levels while decreasing other neurotransmitters [52]. The reduction in GG, HF, LF, DL, and HL, as well as the increase DL and HL may be signs that the rats have been induced by sexual dysfunction. The proceptive phase, which is characterized by actions like DL and HL, is the initial behavior of a female rat to initiate sexual interaction, whereas the receptive phase, which is characterized by lordosis is a useful indicator in the assessment of libido, sexual vigor, arousability, performance, and motivation [52]. Following the administration of fluoxetine, female rats' sexual behaviors were reduced, which is a sign of lower libido. This decline is probably due to the neurotransmitter systems being affected by fluoxetine. The balance of serotonin is frequently disturbed by fluoxetine. Because fluoxetine indirectly reduces the mesolimbic dopaminergic pathway, which is essential for motivation and reward. In addition, serotonin's inactivity effect on norepinephrine neurotransmission mediated by 5-HT_{1A} receptors might also contribute to decreased arousal and libido, which together impair sexual desire

ISSN: 2789-1895 online
ISSN: 2958-3101 print

and responsiveness in female animals [53]. When compared to rats treated with fluoxetine, the observed reversal of sexual behavior indices in female rats after extract intake suggested that the effects of the extract gradually improved sexual behavior. This improvement might be a result of the extract's capacity to control the mesolimbic dopaminergic system's dopamine. This might be accomplished either by boosting dopamine uptake or by antagonistically interacting with serotonin subtype-2 receptors, which would then enable the suppression of the serotonin-induced drop in dopamine downstream. The reduction of NO generation in female genital tissue in fluoxetine-treated rats may result in poor vaginal smooth muscle relaxation, affecting genital sensitivity and arousal [54]. The ability of the extract to reduce significantly the HL and DL in female rats with sexual dysfunction showed that it improves sexual arousability, enthusiasm, vigor, and receptivity. The fact that the extract was able to enhance the quantity of genital licking and grooming behaviors in female rats with sexual dysfunction suggests that they had increased propensity and responsiveness.

Hormonal indicators of female sexual behavior include P, LH, FSH, E, and Pl [55]. The effect of fluoxetine on the hypothalamic-pituitary-gonadal (HPG) axis may explain the marked decrease in reproductive hormones that were observed in fluoxetine-induced sexual dysfunction in female rats. Fluoxetine's impact on the serotonergic system interferes with the hypothalamic release of gonadotropin-releasing hormone, which in turn affects the HPG axis causing reduced secretion of the pituitary hormones, which are crucial for controlling the generation of sex hormones. The gonads may be directly impacted by the disruption of this hormonal balance, which would also result in less P and E being produced. The subsequent reversal in serum P and E levels in sexually dysfunctional female rats treated with *H. enneaspermus* extract may be due to the extract's possible modulatory actions on the HPG axis. By possibly altering the expression of E receptors, improving vaginal lubrication, and easing the transition of the uterine lining to a receptive state, these actions may help to restore normal hormonal levels and therefore increase female sexual receptivity. Following treatment with *H. enneaspermus* leaves, LH and FSH increased in sexually dysfunctional animals. These changes might have a variety of impacts on the reproductive system. It might help the release of eggs from ovarian follicles, which would aid in ovulation or begin the process of transforming the remaining ovarian follicles into corpus luteum organs. The corpus luteum generates progesterone, which is crucial for preparing the uterine lining for potential implantation [56]. On the other side, increased Pl has been linked to lower levels of reproductive hormones, possibly as a result of dopamine, a neurotransmitter that plays a role in sexual excitement, being partially counteracted. This explains why fluoxetine-induced sexual dysfunction in female rats increased Pl. The significant decrease in Pl that occurred after the administration of *H. enneaspermus* may help to improve female sexual behavior by releasing gonadotropin-releasing hormone, stimulating the pituitary gland to secrete more sex hormones, and possibly resuming normal reproductive function.

Conclusion: The findings revealed that the aqueous extract of *H. enneaspermus* can improve various aspects of sexual behavior, including proceptive, receptive, and orientation behaviors. This result compares favorably with the reference drug used in the study, highlighting the extract's potential as a management option for sexual dysfunction in females. It is noteworthy that the extract's effectiveness in reversing sexual impairment in fluoxetine-treated female rats was most pronounced at the highest dose. Thus, it presents evidence to support the traditional usage of *H. enneaspermus* for the management of female sexual inadequacies.

ISSN: 2789-1895 online
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