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Research Article

Aphrodisiac Activity of Aqueous Extract of Herbal Formulation (Zainacin dadin duniya) in Female Wistar Rats

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ABSTRACT

Female sexual dysfunction (FSD) presents a significant challenge in maintaining intimate relationships and overall well-being. This study investigates the aphrodisiac activity of aqueous extract of herbal formulation (AEOHF), a commercial herb, in female Wistar rats. The study involved the administration of AEOHF at varying concentrations (100, 200, and 400 mg/kg bwt) to female rats and assessing its impact on sexual behavior and sex hormone levels. Phytochemical analysis revealed the presence of alkaloids, tannins, saponins, flavonoids, and phenols, with alkaloids having the lowest concentration (15.94±0.42). The LD_{50} of the extract is greater than 5000 mg/kg bwt. Behavioral observations indicated a significant ($p > 0.05$) decrease in darting and hoping latency (310.33±4.91 & 462.33±13.92 respectively) in rats administered AEOHF at the dose of 400 mg/kg bwt compared to rats (697.00±4.04 & 905.33±6.39) in the control group. However, a significant ($p > 0.05$) increase was observed in darting and hooping frequency (7.67±0.33 and 6.00±0.58 respectively) in animals administered AEOHF at the dose of 400 mg/kg bw. Also, a significant ($p > 0.05$) increase was observed in sex hormones; follicle-stimulating hormone (FSH: 4.56±0.12), luteinizing hormone (LH; 3.76±0.05), testosterone (0.22±0.00) and estradiol (144.87±0.70) of rats administered AEOHF at the dose of 400 mg/kg bwt as compared to rats in the control group with 2.85±0.06, 2.03±0.02, 0.12±0.00 and 67.24±1.01 values for FSH, LH, testosterone and estradiol respectively. Findings confirm the aphrodisiac activity of AEOHF. This study suggests AEOHF as a viable natural herb for managing FSD and a good therapeutic agent for the treatment of FSD.

Keywords: Herbal Formulation; Aphrodisiac; Testosterone; Estradiol; Sexual dysfunction

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INTRODUCTION

Commitment and emotional connections between partners can be strengthened by sexual relationships, regardless of whether they are monogamous or non-monogamous. Sexual intimacy frequently promotes affection and increases the probability of couples remaining together, with sexual satisfaction being positively correlated with lower divorce rates (Ciritel, 2022). Sexual activity is vital for enduring relationships, offering protection against psychological distress such as anxiety and depression, facilitating deeper partner connections, and improving relationship satisfaction (Cutrer & Glahn, 2020).

Chemical messengers produced by endocrine glands play a crucial role in regulating various physiological processes, such as appetite, sleep, and growth. Among these, sex hormones play a vital role in sexual development and reproduction, primarily synthesised by the adrenal glands and gonads (ovaries in females and testes in males) (Finkielstain *et al*., 2021).

In females, sex hormones such as oestrogen and progesterone are crucial for reproductive health and contribute significantly to overall well-being. Primarily synthesized in the ovaries, with additional input from adrenal glands and adipose tissue, this hormone plays a significant role, affecting sexual desire through its

variations during the menstrual cycle. During ovulation, when oestrogen levels reach their peak, many women may notice an increase in libido (Wieczorek *et al.,* 2023). According to Prior (2020), the involvement of oestrogen in enhancing sexual desire encompasses a range of physiological and psychological mechanisms. The impact on neurotransmitter activity, blood circulation, hormonal equilibrium, variations in the menstrual cycle, and cognitive function all work together to shape its role in influencing sexual desire in women.

The inability to find satisfaction in sexual engagement is referred to as sexual dysfunction. It may manifest at any point within the sexual response cycle, whether during desire, arousal, orgasm, or resolution. In females, this dysfunction is classified into four categories: desire disorders (lack of interest in sexual activity), arousal disorders (inability to achieve arousal), orgasmic disorders (delay or absence of orgasm), and pain disorders (vaginismus and dyspareunia). (Aleixo, 2021). The prevalence of female sexual dysfunction (FSD) is significant, with global rates ranging from 22 to 43 percent. In a study by Abdullahi *et al*. (2019), 86.0% of women visiting a family planning clinic at Aminu Kano Teaching Hospital reported FSD, with desire disorders being the most common at 91.8%.

Aphrodisiacs, categorised historically by their influence on sexual drive, potency, and overall satisfaction, are compounds that boost sexual desire and performance (Yakubu and Olutoye, 2016). Herbal treatments, a fundamental aspect of traditional medicine, have been utilised for ages to tackle sexual dysfunction and improve overall health. These therapies, encompassing herbal remedies, draw upon a variety of health practices and beliefs (WHO, 2019).

In Suleja Market, Niger State, Nigeria, the female aphrodisiac (Zainancindadinduniya) is widely available and most recommended. Despite its popularity, there is a lack of empirical evidence supporting its pharmacological efficacy and safety. To address this, the study aim at investigating the aphrodisiac activity of AEOHF in female rats and to provide a complementary and alternative medicine approach to FSD.

MATERIALS AND METHODS

Collection and Preparation of Sample

The herbal formulation sample (Zainacindadinduniya) was purchased from a popular "Kayan Mata" seller in Suleja market, Niger State, Nigeria. The plant sample was extracted using the cold maceration method described by Sankeshwari *et al.* (2018). The extract was dried and stored in an airtight container until further analysis.

Experimental Animals

Approximately, twenty-five healthy female rats and twenty-five male rats of two (2) weeks were acquired from the animal house of the Department of Biochemistry, Federal University of Technology, Minna, Nigeria. The rats were kept in clean plastic cages within a well-ventilated animal house with unrestricted access to rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water.

Drug, Chemicals and Assay Kits

Drug, assay kit and other chemicals used were of experimental grade.

Phytochemical Analysis

Standard phytochemical screening methods as described by Ibrahim *et al*. (2022) were employed to determine the presence and concentration of the following phytochemicals alkaloids, flavonoids, tannins, saponins, and phenolic compounds.

Experimental Design and Treatment

The method of Giuliano *et al*. (1999) described by Yakubu & Olutoye (2016), was used for the aphrodisiac study, Briefly, about twenty-five (25) female animals were randomly assigned into five groups (A-E) of five animals each. Animals in groups A and B were served 1 mL of distilled water and 1 mL of an aqueous solution containing 6 mg/kg BW of Eve's desire, respectively. Animals in groups C, D, and E were administered 100, 200, and 400 mg/kg BW of the AEOHF, respectively. The duration of the experiment was seven days, the doses were given once daily, and the animals were sacrificed on day 8 after fasting for 12 hrs. The blood samples were collected and placed in plain bottles for biochemical analysis.

Aphrodisiac Activity

Sexual Behavior assessments were observed using the procedure by Giuliano *et al*. (1999), as described by Yakubu & Olutoye (2016), to evaluate changes in sexual receptivity and activity in female rats after administration of AEOHF. The following parameters; Prospective sexual behavior (Darting latency and frequency, hopping latency and frequency), Receptive sexual behavior (Lordosis latency and frequency), and orientational activities (licking behavior, genital and anogenital grooming) were observed and recorded.

Sex Hormones

The serum hormone levels of LH, FSH, E, and testosterone were measured following the instructions from the makers of the assay kits, utilizing microplate immunoenzymometric (EMA/ELISA) detection assays. The concentrations of serum hormones were subsequently estimated by interpolation from their corresponding calibration curves. Calibration and validation of the analyzer were conducted for its application with rat sera.

Ethical Consideration

The study was conducted in accordance with guidelines for the handling and care of experimental animals (National Research Council, 2011). The protocol for experiments was approved by the Animal Ethics Committee of Ibrahim Badamasi Babangida University, Lapai. Letter of Ethical Approval with reference No. IBBUL/02/2023 was issued.

Statistical Analysis

Results were expressed as the mean ± standard error of mean of 3 replicates. Data were analyzed using a one-way analysis of variance, followed by the Tukey's post-hoc test to determine significant differences in all the parameters with Students Package for Social Science, version 26.0 (SPSS Inc., Chicago, USA). Differences with values of $P<0.05$ were considered statistically significant.

RESULTS

Phytochemicals

The phytochemical results of the aqueous extract of herbal formulation (AEOHF) are presented in Table 1. The result reveals the presence of five distinct phytochemicals: alkaloids, tannins, saponins, flavonoids, and phenols, while others (Anthraquinones, Phlobatannins, Steroids, Terpenes, and Cardiac Glycosides) were not dictated. Subsequently, the quantitative assessment established that phenols exhibited the highest concentration at 213.00 ± 0.37 mg/g, followed by flavonoids at 73.27 ± 0.46 mg/g, and saponins at 66.74 ± 0.46 mg/g. Tannins and alkaloids were present in lower concentrations, measured at 29.71 ± 0.52 mg/g and 15.94 ± 0.42 mg/g, respectively (Table 1).

LD_{50} Determination

The results of the acute toxicity (LD_{50}) determined following oral administration of an aqueous extract of AEOHF in female Wistar rats are presented (Tables 2 & 3).

The acute toxicity assessments were conducted in two phases. In the initial phase, no signs of mortality or toxicity were observed at doses of 10, 100, and 1000 mg/kg body weight (Tables 4.2). Subsequently, the second phase of acute toxicity, which involved the administration of 1900, 2500, and 5000 mg/kg body weight of the extract, also showed no signs of toxicity or mortality. This has established the LD_{50} to be greater than 5000 mg/kg body weight (Table 3).

Sexual behavior

Prospective Sexual Behavior

Darting latency and frequency

The darting latency and frequency of female Wistar rats are presented in Table 4. On day 1, the control group showed the highest darting latency, with latency of 705.33 ± 4.33 on day 1 and 697.0 ± 4.04 on day 7. Darting latency displayed significant differences among the tested groups. The group treated with 400 mg/kg BW had the lowest latency at 323.67 ± 5.24, followed by the Eve's Desire group (348.00 ± 4.62), the 200 mg/kg BW group (412.00 ± 6.25), and the 100 mg/kg BW group (622.67 ± 6.12). The control group had the highest latency of 705.33 ± 4.33, as detailed in Table 6. By day 7, latency decreased as the extract concentration increased, with the control group maintaining the highest latency at 697.0 ± 4.04, followed by the 100 mg/kg BW group (598.33 ± 6.64) and the 200 mg/kg BW group (399.33 ± 6.89). The 400 mg/kg BW group and the Eve's Desire group showed no significant difference between them, with latencies of 310.33 ± 4.91 and 324.33 ± 7.44, respectively. The group administered 100 mg/kg body weight (BW) exhibited the lowest darting frequency, with values of 3.67 ± 0.33 on day 1 and 3.33 ± 0.33 on day 7. Darting frequency increased in a dose-dependent manner, reaching a peak in the 400 mg/kg BW group, which recorded frequencies of 6.33 ± 0.33 on day 1 and 7.67 ± 0.33 on day 7.

Table 1 Concentration of phytochemicals in the AEOHF

Phytochemicals	Inference	Concentration (mg/kg)
Alkaloids	Present	15.94±0.42
Tannins	Present	29.71±0.52
Saponins	Present	66.74±0.46
Flavonoids	Present	73.27±0.46
Anthraquinones	ND	ND
Phlobatannins	ND	ND
Phenols	Present	213.00±0.37
Steroids	ND	ND
Terpenes	ND	ND
Cardiac glycosides	ND	ND

ND. Not detected.

LD_{50} determination

Table 2. **Phase 1 acute toxicity test of AEOHF on female Wistar rats**

Dosage (mg/kg Bw)	No. of animals	Mortality
10	3	Nil
100	3	Nil
1000	3	Nil

Table 3. **Phase 2 acute toxicity test of AEOHF on female Wistar rats**

Dosage (mg/kg Bw)	No. of animals	Mortality
1900	3	Nil
2500	3	Nil
5000	3	Nil

Table 4. Darting latency and frequency of female Wistar rats orally administered with varying concentrations of AEOHF

	Darting latency (s)		Darting frequency	
Treatment	**Day 1**	**Day 7**	**Day 1**	**Day 7**
Control	705.33±4.33^{e}	697.0±4.04^{d}	3.67 ± 0.33^{a}	3.33±0.33^{a}
Eve's desire (6 mg/kg BW)	348.00±4.62^{b}	324.33±7.44^{a}	7.33 ± 0.33^{c}	8.00±0.00^{c}
100 mg/kg BW.	622.67±6.12^{d}	598.33±6.64^{c}	4.33 ± 0.33^{ab}	4.00±0.58^{a}
200 mg/kg BW	412.00±6.25^{c}	399.33±6.89^{b}	5.33 ± 0.33^{b}	6.667±0.33^{b}
400 mg/kg BW.	323.67±5.24^{a}	310.33±4.91^{a}	6.33 ± 0.33^{c}	7.67±0.33^{bc}

Values are expressed in mean ± standard error of triplicates Values with the same superscript down the Column have no significant difference at $p < 0.05$

Hopping latency and hopping frequency

The hopping latency data (Table 5) at days 1 and 7 show a notable decrease with increasing extract concentrations. No significant difference was observed between the Eve's desire group and the group administered 400 mg/kg BW of AEOHF on day 1 (500.67±8.19 vs. 481.67±8.51) and day 7 (484.67±3.52 vs. 462.33±13.92). The control group recorded the highest hopping latency, with values of 911.67±6.64 on day 1 and 905.33±6.39 on day 7, followed by the 100 mg/kg BW group with 867.00±4.93 on day 1 and 854.67±6.94 on day 7. On days 1 and 7, the 200 mg/kg BW group had latencies of 781.67±9.46 and 743.67±6.49, while the 400 mg/kg BW group had the lowest latency on both days.

The hopping frequency (Table 5) of female wistar rats administered AEOHF also shows a similar trend at days 1 and 7, with frequency increasing as the concentration of AEOHF increases. There was no significant difference between the control group and the 100 mg/kg BW group on day 1 (2.33 ± 0.33 vs. 2.33 ± 0.33) and day 7 (2.33±0.67 vs. 2.66±0.33). On day 1, Eve's desire group (6.33 0.33) and the 400 mg/kg BW group (5.00 0.33) recorded the highest hopping frequency.

Receptive Sexual Behavior

Lordosis latency and frequency

Table 6 summarizes the data on receptive sexual behavior, specifically lordosis frequency and latency. On day 1, lordosis latency did not differ significantly between the groups treated with Eve's Desire (779.67±12.99), 400 mg/kg body weight (BW) (816.67±14.19), and 200 mg/kg BW (845.67±24.86). However, a significant difference was observed between the control group (1455.33±19.63) and the 100 mg/kg BW group (1355.00±36.86). This trend persisted on day 7, with significant differences noted between the control (1457.00±11.53) and the 100 mg/kg BW group (1250.33±22.30).

On the lordosis frequency on day 1, no significant differences were found among the treated groups, with the control and the 100 mg/kg BW group exhibiting the lowest frequencies (1.33±0.33 and 1.33±0.33, respectively). On day 7, there were no significant differences between the 200 mg/kg BW (2.67±0.33) and 400 mg/kg BW (3.33±0.33) groups, nor between the 400 mg/kg BW group and the group treated with Eve's Desire (4.00±0.00).

Orientational Activities

Genital grooming and anogenital grooming of female Wistar rats

Genital grooming was assessed on day 1 and day 7, with all groups demonstrating a consistent trend. The control group exhibited the lowest grooming activity during the experimental period (5.33±0.33 and 5.67±0.33 for days 1 and 7, respectively). On days 1 and 7, no significant differences were observed between the control group, the 100 mg/kg BW group (5.67±0.33 and 6.67±0.33), and the 200 mg/kg BW group (5.67±0.33 and

7.33±0.33). However, the 400 mg/kg BW group showed grooming activities of 7.67±0.33 on day 1 and 10.00±0.58 on day 7, while the 6 mg/kg BW Eve's Desire group recorded grooming activities of 10.33±0.88 and 12.33±0.88 on days 1 and 7, respectively (Table 7).

Anogenital grooming data, also summarized in Table 9, indicated no significant difference between the control (6.67±0.33) and the 100 mg/kg BW group (7.67±0.33) on day 1. Significant differences were noted among the other groups on day 1: the 200 mg/kg BW group (10.33±0.33) and the 400 mg/kg BW group (13.00±0.58). On day 7, increasing extract concentrations significantly increased anogenital grooming. The highest grooming activity was seen in the Eve's Desire group (12.67±0.33), followed by the 400 mg/kg BW group (14.33±0.33), the 200 mg/kg BW group (12.66±0.33), and the 100 mg/kg BW group (8.67±0.33). The control group had the lowest anogenital grooming activity (7.00±0.57).

Table 5 Hopping latency and frequency of female Wistar rats following oral administration of AEOHF.

	Hopping latency (s)		Hopping frequency	
Treatment	**Day 1**	**Day 7**	**Day 1**	**Day 7**
Control	911.67±6.64^{d}	905.33±6.39^{d}	2.33 ± 0.33^{a}	2.33±0.67^{a}
Eve's desire (6 mg/kg BW)	500.67±8.19^{a}	484.67±352^{a}	6.33 ± 0.33^{d}	7.00±0.58^{c}
100 mg/kg BW	867.00±4.93^{c}	854.67±6.94^{c}	2.33 ± 0.33^{a}	2.66±0.33^{a}
200 mg/kg BW	781.67±9.46^{b}	743.67±6.49^{b}	3.66 ± 0.33^{b}	4.33±0.33^{b}
400 mg/kg BW	481.67±8.51^{a}	462.33±13.92^{a}	5.00 ± 0.33^{c}	6.00±0.58^{c}

Values are expressed in mean ± standard error of triplicates Values with the same superscript down the Column have no significant difference at $p < 0.05$

Table 6 Lordosis latency and frequency of female Wistar rats following oral administration of AEOHF.

	Lordosis Latency (S)		Lordosis Frequency	
Treatment	**Day 1**	**Day 7**	**Day 1**	**Day 7**
Control	1455.33±19.63^{c}	1457.00±11.53^{d}	1.33± 0.33^{a}	1.33 ± 0.33^{a}
Eve's desire (6 mg/kg BW)	779.67±12.99^{a}	737.67±10.71^{a}	3.33± 0.33^{b}	4.00 ±0.00^{c}
100 mg/kg BW	1355.00±36.86^{b}	1250.33±22.30^{c}	1.33± 0.33^{a}	1.00±0.00^{a}
200 mg/kg BW	845.67±24.86^{a}	802.33±9.61^{b}	2.33± 0.33^{ab}	2.67±0.33^{b}
400 mg/kg BW	816.67±14.19^{a}	777.67±12.14^{ab}	2.33± 0.33^{ab}	3.33±0.33^{bc}

Values are expressed in mean ± standard error of triplicates Values with the same superscript down the Column have no significant difference at $p < 0.05$

Table 7 Genital and Anogenital grooming of female Wistar rats following oral administration of AEOHF.

	Genital Grooming		Anogenital Grooming	
Treatment	**Day 1**	**Day 7**	**Day 1**	**Day 7**
Control	5.33±0.33^{a}	5.67±0.33^{a}	6.67±0.33^{a}	7.00±0.57^{a}
Eve's desire (6 mg/kg BW)	10.33±0.88^{c}	12.33±0.88^{c}	11.67±0.33^{c}	12.67±0.33^{c}
100 mg/kg BW	5.67±0.33^{a}	6.67±0.33^{a}	7.67±0.33^{a}	8.67±0.33^{b}
200 mg/kg BW	5.67±0.33^{a}	7.33±0.33^{a}	10.33±0.33^{b}	12.66±0.33^{c}
400 mg/kg BW	7.67±0.33^{b}	10.00±0.58^{b}	13.00±0.58^{d}	14.33±0.33^{d}

Values are expressed in mean ± standard error of triplicates Values with the same superscript down the Column have no significant difference at $p < 0.05$

Licking behavior of female Wistar rats.

The licking behavior of female Wistar rats was assessed following oral administration of AEOHF (Table 8). The control group and the group receiving 100 mg/kg body weight (BW) exhibited the lowest licking behavior on both day 1 and day 7, with mean values of 3.00±0.58, 3.33±0.33, 3.67±0.33, and 4.33±0.33, respectively. There was no significant difference in licking behavior between the control group and the 100 mg/kg BW group, according to statistical analysis. On day 1, there was no significant difference in licking behavior between the Eve's desire group and the groups receiving 200 mg/kg and 400 mg/kg BW. On day 7, the licking behavior was not significantly different between the Eve's desire group (8.33±0.33), the 200 mg/kg BW group (9.00±0.59), and the 400 mg/kg BW group (9.67±0.33), all of which exhibited the highest levels of licking behavior.

Hormonal assay

The serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E), and testosterone (T) in female Wistar rats were assessed following a 7-day oral administration of AEOHF, as detailed in Table 9.

The lowest FSH concentration was observed in the control group (2.85 ± 0.06), followed by 100 mg/kg BW (3.15 ± 0.07), 200 mg/kg BW (3.75 ± 0.05), 400 mg/kg BW (4.56 ± 0.12), and the group administered 6 mg/kg BW of Eve's Desire exhibited the highest FSH concentration (5.26 ± 0.16).

Serum LH exhibited no significant variance between the control group and the 100 mg/kg BW group (2.03 ± 0.02 and 2.18 ± 0.03, respectively). Notably, LH levels significantly escalated at doses of 200 and 400 mg/kg BW (2.98 ± 0.06 and 3.76 ± 0.05, respectively), displaying a dose-dependent increase.

Similarly, the lowest serum testosterone concentration was recorded in the control group (0.12 ± 0.01), followed by the 100, 200, and 400 mg/kg BW groups, and Eve's Desire group (0.13 ± 0.01, 0.17 ± 0.00, 0.22 ± 0.00, and 0.21 ± 0.01, respectively). Levels of estradiol changed with dose. The control group had the lowest level (67.24 ± 1.01), then the 100, 200, and 400 mg/kg BW groups (96.25 ± 0.76, 136.40 ± 0.81, and 144.87 ± 0.70, respectively), and finally the Eve's Desire group had the highest level (156.92 ± 0.16).

Table 8 Licking behavior of female Wistar rats following oral administration of AEOHF.

	Licking Behavior	
Treatment	**Day 1**	**Day 7**
Control	3.00±0.58^{a}	3.33±0.33^{a}
Eve's desire (6 mg/kg BW)	7.67±0.33^{b}	8.33±0.33^{b}
100 mg/kg BW	3.67±0.33^{a}	4.33±0.33^{a}
200 mg/kg BW	7.33±0.67^{b}	9.00±0.59^{bc}
400 mg/kg BW	8.67±0.33^{b}	9.67±0.33^{c}

Values are expressed in mean ± standard error of triplicates Values with the same superscript down the Column have no significant difference at $p < 0.05$

Table 9: Effect of oral administration of AEOHF on sexual hormones of female wistar rats

Treatment	**Luteinizing Hormone (pg/mL)**	**Follicle Stimulating Hormone (ng/mL)**	**Estradiol (mU/mL)**	**Testosterone (mU/mL)**
Control	2.03 ± 0.02^{a}	2.85±0.06^{a}	67.24±1.01^{a}	0.12±0.01^{a}
Eve's desire (6 mg/kg BW)	4.31 ± 0.17^{d}	5.26±0.16^{d}	156.92±0.16^{e}	0.21±0.01^{c}
100 mg/kg BW	2.18 ± 0.03^{a}	3.15±0.07^{a}	96.25±0.76^{b}	0.13±0.01^{a}
200 mg/kg BW	2.98 ± 0.06^{b}	3.75±0.05^{b}	136.40±0.81^{c}	0.17±0.00^{b}
400 mg/kg BW	3.76 ± 0.05^{c}	4.56±0.12^{c}	144.87±0.70^{d}	0.22±0.00^{c}

Values are expressed in mean ± standard error of triplicates Values with the same superscript down the Column have no significant difference at $p < 0.05$

DISCUSSION

Secondary compounds found in plants, such as alkaloids, flavonoids, phenols, and saponins, have been reported to possess aphrodisiac qualities (Enema *et al.*, 2018). Certain chemical compounds have the ability to elevate sex hormone levels, while others can enhance their activity within the body (Yakubu & Olutoye, 2016; Singh & Kumar, 2017). Studies have indicated that saponins may act as building blocks for sexual hormones such as testosterone, progesterone, estradiol, and androgen (Kunjiappan, 2023). An increase in blood levels of LH and FSH can initiate the activation of these reproductive hormones within the body (Santi *et al.*, 2020). Alkaloids have demonstrated estrogenic attributes by affecting receptors that induce sexual behavior in female rats, alongside causing vasodilation in the blood vessels of the uterus and vagina (Kunjiappan *et al.,* 2023). Alkaloids can act like neurotransmitters, promoting relaxation in the smooth muscles of the uterus and vagina. They may also influence the pituitary gland to possibly stimulate the release of luteinizing hormone (LH) (Tijjani *et al.,* 2018). Flavonoids can counteract the effects of free radicals by attaching to iron (II) ions. This antioxidant function could protect dopaminergic, serotonergic, and adrenergic neurons from oxidative damage, possibly increasing their numbers and improving sexual behavior (Rani *et al.,* 2016).

This study reveals the presence of alkaloids, tannins, saponins, flavonoids, and phenols in AEOHF, with phenols being the most concentrated. The absence of toxicity symptoms and the lack of mortality within 24 hours following administration of 5000 mg/kg dose to female Wistar rat indicate that the oral LD_{50} of the extract is greater than 5000 mg/kg body weight.

Female rats show increased readiness for mating during their oestrous cycle, especially after ovulation. This phase involves actively seeking male attention through explicit demonstrations of sexual behaviour when potential mates are present (Pfaus *et al*., 2015; Jennings & De Lecea, 2020). The sexual behaviour of female rats is influenced by the peripheral gonadal hormone produced by the ovaries, which affects both the peripheral and central nervous systems (Rudolph *et al.,* 2016). The increase in proceptive sexual behaviour markers like DF, HF, DL, and HL, coupled with the rise in receptive sexual behaviour indicators LF and LL, as well as enhanced orientation behaviours AG, GG, and LB, in rats administered AEOHF indicates that a higher concentration of the extract enhances sexual behaviours when compared to the untreated female rats. This is demonstrated by the females' readiness to engage with males, initiate solicitation behaviours, and subsequently display receptive behaviours. These findings align with previous research by Yakubu and Olutoye (2016), who observed enhanced sexual behavior in female Wistar rats following the administration of an aqueous extract of *Anthonothamacrophylla* P. Beauv*.* leaves. The sexual behavior observed in this study is also in line with a report made by Anyanwu *et al.,* (2021), who reported a significant increase in hop and dart following the administration of *Citrulluslanatus* ethanolic rind extract for 14 days.

The normal function of the female reproductive system involves the release of LH and FSH from the pituitary gland, triggered by the hypothalamic gonadotropin-releasing hormone (GnRH). (Casteel & Singh, 2020). In females, LH stimulates the theca cells in the ovaries to synthesise testosterone, while FSH activates granulosa cells in developing follicles to produce estrogen through the action of aromatase, an enzyme that converts testosterone into estrogen (Kishi *et al.*, 2018; Cisternas *et al.,* 2018). Consequently, the rise in LH and FSH concentrations in the blood, in line with dosage, may be attributed to their ability to activate the hypothalamic-pituitary axis. This suggests a tendency to enhance reproductive function through increasing dosages. The findings in the present study correlate well with those reported by Krishnamoorthy *et al.,* (2013) who reported an elevation in female sex hormoesa after administration of *Andrographispaniculata*. Yakubu & Olutoye (2016) reported a significant rise in female sex hormone after administration of *Anthonothamacrophylla*; Anyanwu *et al.,* (2021) reported a significant rise in female sexual hormones after administering *Citrulluslanatus* ethanolic extract for 14 days. Although the extract didn't induce significant alterations in serum testosterone levels irrespective of the dose, the peak testosterone level was observed at 400 mg/kg body weight. This increase in testosterone could be due to the animals' inability to completely convert all produced testosterone in the theca cells to estradiol through aromatase, suggesting a possible inhibition of aromatase. Another possibility is ovarian degeneration at this specific dosage. Nonetheless, despite these factors, sexual behavior measurements remained unchanged at 400 mg/kg body weight during the study.

Estradiol, the primary hormone produced by the ovaries in females, plays a vital role in modulating normal sexual behaviour in female rats, mainly functioning within the ventromedial nucleus of the hypothalamus. Increased concentrations of estradiol, influenced by dosage, may be linked to enhanced hormone release from ovarian follicle granulosa cells, thereby leading to enhanced secretion. This indicates that the AEOHF used in the study promoted hormone production in granulosa cells, leading to a release into the bloodstream that is dependent on the dosage. The increase in estradiol levels probably plays a role in the noted enhancement of sexual behaviour in female rats after the administration of the extract. The findings in this study align with Yakubu & Olutoye (2016), who reported that treatment with aqueous extract from *Anthonothamacrophylla* leaves (AEAML) at doses of 25 and 50 mg/kg body weight led to elevated levels of FSH, LH, testosterone, and estradiol in female Wistar rats.

CONCLUSION

Aqueous extract of the commercial herbal formulation (Zainacindadinduniya) is abundantly rich in phenolic compounds. The extract possesses aphrodisiac properties that enhance sexual activity in female wistar rats. Elevation of sex hormones; LH, FSH estradiol and testosterone in rats administered AEOHF suggests that the mechanism of sexual stimulation of the extract is in the enhancement of sex hormones secretion. The sexual enhancement of AEOHF is dose dependent.

REFERENCE

Abdullahi, H. M., Abdurrahman, A., Ahmed, Z. D., & Tukur, J. (2019). Female sexual dysfunction among women attending the family planning clinic at Aminu Kano Teaching Hospital: A cross-sectional survey. *Nigerian Journal of Basic and Clinical Sciences*, *16*(1), 32

Aleixo, I. M. P. (2021). Female sexual response: models, sexual dysfunctions and clinical approach.

Anyanwu, E. G., Atuadu, V. O., Egwuatu, A. A., Christian, E. M., Ozoemena, C. L., & Emmanuel, A. E. (2021). Effect of ethanolic rind extract of *Citrulluslanatus* on hypothalamic-pituitary-gonadal axis structures, sexual

behaviour and hormones of female Wistar rats. *Journal of Pharmacognosy and Phytochemistry*, *10*(4), 60-65.

Barbu, R. M., Ciobîcă, A., Stana, B. A., Popescu, I. R., &Bild, W. (2021). Neurohormonal and pharmacological regulation of oestrogen, progesterone, luteinizing hormone and follicle stimulating hormone over the menstrual cycle–the possible relevance of angiontensin ii. *Farmacia*, *69*(1), 82-89.

Casteel, C. O., & Singh, G. (2020). Physiology, gonadotropin-releasing hormone.

Ciritel, A. A. (2022). Sexual intimacy and relationship happiness in living apart together, cohabiting, and married relationships: evidence from Britain. *Genus*, *78*(1), 32.

Cisternas, C. D., Garcia-Segura, L. M., &Cambiasso, M. J. (2018). Hormonal and genetic factors interact to control aromatase expression in the developing brain. *Journal of Neuroendocrinology*, *30*(2), e12535.

Cutrer, W. R., &Glahn, S. (2020). *Sexual intimacy in marriage*. Kregel Publications.

Enema, O. J., Umoh, U. F., Umoh, R. A., Ekpo, E. G., Adesina, S. K., &Eseyin, O. A. (2018). Chemistry and pharmacology of aphrodisiac plants: a review. *Journal of Chemical and Pharmaceutical Research*, *10*(7), 70-98.

Finkielstain, G. P., Vieites, A., Bergadá, I., & Rey, R. A. (2021). Disorders of sex development of adrenal origin. *Frontiers in Endocrinology*, *12*, 770782.

Giuliano, F., Hultling, C., El Masry, W. S., Smith, M. D., Osterloh, I. H., Orr, M., & Sildenafil Study Group. (1999). Randomized trial of sildenafil for the treatment of erectile dysfunction in spinal cord injury. *Annals of Neurology*, *46*(1), 15-21.

Ibrahim, S. R., Altyar, A. E., Mohamed, S. G., & Mohamed, G. A. (2022). Genus Thielavia: Phytochemicals, industrial importance and biological relevance. *Natural Product Research*, *36*(19), 5108-5123.

Jennings, K. J., & de Lecea, L. (2020). Neural and hormonal control of sexual behavior. *Endocrinology*, *161*(10), bqaa150.

Kishi, H., Kitahara, Y., Imai, F., Nakao, K., &Suwa, H. (2018). Expression of the gonadotropin receptors during follicular development. *Reproductive medicine and biology*, *17*(1), 11-19.

Krishnamoorthy, P., Sivaranjani, K., Rajeswari, K., &Kalaiselvan, D. (2013). Effect of Andrographispaniculata Wall. exNees root extract fractions on estrogen, FSH, LH, Progestrone and ovary of female albino rats, Rattusnorvegicus.

Kunjiappan, S., Pandian, S. R. K., Panneerselvam, T., Pavadai, P., Kabilan, S. J., &Sankaranarayanan, M. (2023). Exploring the Role of Plant Secondary Metabolites for Aphrodisiacs. In *Plant Specialized Metabolites: Phytochemistry, Ecology and Biotechnology* (pp. 1-19). Cham: Springer Nature Switzerland.

Pfaus, J. G., Jones, S. L., Flanagan-Cato, L. M., &Blaustein, J. D. (2015). Female sexual behavior. *Knobil and Neill's physiology of reproduction*, *2*, 2287-2370.

Prior, J. C. (2020). Women's reproductive system as balanced estradiol and progesterone actions—a revolutionary, paradigm-shifting concept in women's health. *Drug Discovery Today: Disease Models*, *32*, 31-40.

Rani, N., Bharti, S., Krishnamurthy, B., Bhatia, J., Sharma, C., Amjad Kamal, M., & Singh Arya, D. (2016). Pharmacological properties and therapeutic potential of naringenin: a citrus flavonoid of pharmaceutical promise. *Current pharmaceutical design*, *22*(28), 4341-4359.

Rudolph, L. M., Bentley, G. E., Calandra, R. S., Paredes, A. H., Tesone, M., Wu, T. J., &Micevych, P. E. (2016). Peripheral and central mechanisms involved in the hormonal control of male and female reproduction. *Journal of neuroendocrinology*, *28*(7).

Saad, K. A., Threaf, M. F., &Tektook, N. K. (2023). Determine levels of Estradiol, Follicle-stimulating hormone, Luteinizing hormone, anti-FSH, and anti-LH in Thyroiditis and Polycystic ovarian syndrome (comparison study). *Al-Nisour Journal for Medical Sciences*, *5*(1).

Sankeshwari, R. M., Ankola, A. V., Bhat, K., &Hullatti, K. (2018). Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against Streptococcus mutans?. *Journal of the Scientific Society*, *45*(2), 67.

Santi, D., Crépieux, P., Reiter, E., Spaggiari, G., Brigante, G., Casarini, L. & Simoni, M. (2020). Follicle-stimulating hormone (FSH) action on spermatogenesis: a focus on physiological and therapeutic roles. *Journal of Clinical Medicine*, *9*(4), 1014.

Tijjani, U., Mabrouk, M. I., Yarube, I. U., & Muhammad, M. (2018). Aphrodisiac effect of aqueous stem bark extract ofFicussycomorus on female wistar rats. *Nigerian Journal of Basic and Applied Sciences*, *26*(1), 70-79.

Wieczorek, K., Targonskaya, A., &Maslowski, K. (2023). Reproductive Hormones and Female Mental Wellbeing. *Women*, *3*(3), 432-444.

World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization.

Yakubu, M. T., & Olutoye, A. F. (2016). Aphrodisiac activity of aqueous extract of Anthonothamacrophylla P. Beauv. leaves in female Wistar rats. *Journal of Integrative Medicine*, *14*(5), 400-407.