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EUPHORBIA HIRTA (EUPHORBIACEAE) CRUDEAQUEOUS EXTRACT ENHANCES COPULATORY ACTIVITIES IN SEXUALLY NAÏVE NORMAL MALE ALBINO RATS

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ABSTRACT

Euphorbia hirta has long been used in traditional medicine to treat various ailments, including gastrointestinal disorders, bacterial infections and respiratory conditions. Notably, its potential as an aphrodisiac and fertility enhancer have been reported in ethnoveterinary practices, where it is administered to improve the reproductive performance of male animals. The present study was designed to evaluate the effects of *E. hirta* crude aqueous extract on copulatory activities in sexually naïve normal male albino rats.Twenty-five adult male albino rats weighing between 150 and 300 grams each were subdivided into 5 groups and treated as follows: rats of groups 1, 2 and 3 were administered 300mg/kg, 600mg/kg and 1200mg/kg of the plant extract respectively, those of group 4 received 10 ml/kg distilled water, while those of group 5 received sildenafil citrate: Erekta (5mg/kg). Treatment lasted for 21 days with sexual behavioural parameters recorded on days 1, 7, 14 and 21. Eh_{1200}-treated animals recorded a significant decrease in mount latency from day 1to day 21 compared to the Distilled Water control group. Intromission Latency values decreased in extract-treated groups with the Eh_{1200}-treated animals recording the smallest intromission latency values compared to both control groups. Extract-treated males registered a significant increase in mount and intromission frequencies, penile licking and copulatory efficiency values dose-dependently. Results obtained from our study enables us to say that the crude leaf aqueous extract of *E. hirta*enhances copulatory efficiency by improving on the mating behaviour of male rats.

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INTRODUCTION

Man's quest for sex-enhancers called aphrodisiacs dates as far back as ancient times. Man's desire to improve on sexual interest or appetite (libido), sexual performance and pleasure or satisfaction (orgasm) has led to many studies on different medicinal plants, capable of enhancing these activities. A medicinal plant is onethatis capable of alleviating or curing diseases and has a traditional use as a remedy in a population or community. Its use is one of the oldest practices for treatment, cure and prevention of diseases [Ana and Daniela, 2018]. Presently, the use of medicinal and aromatic plants for the development and preparation of alternative traditionalmedicine and food additives has gained much interest (Bertrand *et al*., 2016). According to World Health Organization (WHO) it is estimated that almost 80% of the people living in the underdeveloped nations depend on traditional and complementary medicines for their basic health care [EGBE *et al.*, 2018]. Plants constitute an important source of medicines and play a key role in the health of a greater portion of the world's population. The use of plants or their products to treat sexual disorders or improve on sexual performance has a long history in most countries, and their investigations in animals have proven that they are effective in improvings exual desire and sexual behavior in male animals (EGBE *et al*., 2019). Natural therapies derived from aphrodisiac plants could reduce dependency on synthetic drugs and lower environmental pollution from pharmaceutical runoff, in

addition to advantages of availability, affordability, tolerance and absence of undesired effects. *Euphorbia hirta*is among the medicinal plants widely used traditionally in Africa and other parts of the world (Ganiyu *et al*., 2022). This plant is widely distributed across tropical and subtropical regions, and has long been used in traditional medicine to treat various ailments, including gastrointestinal disorders, bacterial infections, and respiratory conditions (Ganiyu *et al*., 2022). Notably, its potential as an aphrodisiac and fertility enhancer have been reported in ethnoveterinary practices, where it is administered to improve the reproductive performance of male animals (Ganiyu *et al*., 2022). Phytochemical studies have identified compounds such as flavonoids, alkaloids and saponins in *Euphorbia hirta*, which are known to influence reproductive physiology by modulating hormonal activity, enhancing spermatogenesis and improving antioxidant defence mechanisms (Ganiyu *et al*., 2022; Khursheed *et al*., 2022). Sagar *et al*. (2023) evaluated the phytochemical and pharmacological activity of *E. hirta Linn*. for aphrodisiac purposes. In the present investigation, the aphrodisiac properties of itscrudeaqueous extract were evaluated in sexually naïve normal male albino rats.

MATERIALS AND METHODS

Plant material

Collection: Fresh leaves of *Euphorbia hirta* plant were harvested around the Buea locality (Southwest Region of Cameroon) and were prepared based on a specific extraction process to obtain its aqueous extract.

Preparation of extract: The harvested leaves were thoroughly washed and allowed to air-dry for approximately two weeks at room temperature. The leaves were then ground using an electric blender in order to produce a fine powder. Following the procedure described in our previous works (Egbe *et al*. 2019), 100 g of the powder obtained was macerated into 1000 ml of distilled water and kept for 72 hours. This was accompanied by mechanical agitation at 24-hour intervals. This was then followed by filtration using a laboratory sieve of 80µm diameter pores. The filtrate was later evaporated in an oven at 50°C for a period of 2 days to obtain a semi-solid crude residue. Yield of extraction was calculated to be 5%.

Administrative doses: The administrative doses of the extract were guided by the information obtained from folk users of this plant and the tradi-practitioners as well as a comprehensive review of available literature. Furthermore, according to folk users, about 60 fresh leaves are macerated into 250ml of water and given once a day to an adult male (human) for 21 days who can initiate a penile erection, cannot sustain erection or ejaculates within a short time after penetration. Extensive studies available online provide more quantifiable insights into its pharmacological activities and safety profile. Acute and sub chronic toxicity studies in rodents have generally indicated a high safety margin for *E. Hirta* extracts. For instance, sub chronic toxicity studies involving repeated oral administration for 90 days at doses up to 1000 mg/kg, have also shown no significant changes in body weight, food/water consumption, haematological and biochemical parameters, or organ histopathology, further supporting its relatively safe profile (Ajayi *et al*., 2017). Therefore, the chosen doses were 300, 600, and 1200 mg/kg (Eh_{300}, Eh_{600} and Eh_{1200}) representing a range that gathers traditionally used concentrations while also exploring higher, yet still ostensibly safe, therapeutic levels based on herbal doctors' recommendations, aimed at investigating its dose-dependent effects thoroughly.

Animals

Experimental animals and ethical consideration: All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996) and the Cameroon National Ethical Committee (Yaoundé, Cameroon) for animal handling and experimental procedure: Reg No. FW-IRB00001954 (Egbe *et al*., 2018). The project was approved by the University of Buea Institutional Animal Care and Use Committee with a reference number: UB-IACUC-22/2025.

Raising of animals: Animals used for this research were albino rats of the Wistar strain of either sex and were raised in the Animal Facility of the Department of Animal Biology and Conservation of the Faculty of Science, University of Buea under standard conditions of temperature (25 ± 1°C) and humidity (50%–80%) with a 12/12 hours light/dark cycle and in standard cages. They had free access to food and water. Each animal was used only once. Their laboratory diet was composed as shown in Table 1. The young males were separated from their mother and other females at weaning (21 days old) and raised separated from females until used. This was to ensure that they had no sexual experience before the experiment.

Table 1. Food composition for 1kg standard laboratory diet for laboratory rats

Nutrient	Quantity (g)
Corn flour	600
Roasted soy bean flour	200
Fish flour	150
Bone flour	20
Table salt	10
Vitamin	10

Ovariectomy and induction of oestrus in females: Females were ovariectomised and made receptive following procedures we described in our previous works (Egbe *et al*., 2017; 2018; 2019).

Reagents or chemicals: preparation of other solutions: Products used in this study included sildenafil citrate (Erecta®; strides Pharma Science limited); oestradiol (HEBEI NEW CENTURY PHARMACEUTICAL CO, LTD); progesterone (SYNERGON®); Diclofenac; isoflurane (Pyxus Pharmaceuticals, INDIA); they were all purchased and preserved under recommended conditions until used. Preparation of the penicillin-G solution was done by dissolving 600 mg of powder penicillin-G 1000000IU into 2 ml of distilled water. It was properly homogenised and then used on the ovariectomized females to prevent post-surgical infection. Concerning the diclofenac solution administered, one diclofenac tablet (50mg) was dissolved into 20ml of distilled water. The mixture was properly homogenised and then administered to the animals at the dose mentioned earlier.

Experimental design

Partitioning and treatment of animals: A total of 25 adult males obtained from our breed and weighing between 150 and 300 grams each were subdivided into 5 groups of 5 males each and treated as follows. Rats of group 1, 2 and 3 were administered 300mg/kg, 600mg/kg and 1200mg/kg of the plant extract respectively, while those of group 4 received 10 ml/kg distilled water (negative control) and those of groups 5 received sildenafil citrate (Erekta) at a dose of 5mg/kg; various doses of the plant extract, distilled water and Erekta (standard drug) were administered orally using an Oro-pharyngeal cannula once daily, for a period of 21 days as applied by local herbal doctors.

Mating behaviour testing procedures: Mating behaviour was assessed following the procedure described by (Ratnasooriya & Dharmasiri, 2000) and as in our previous works (Egbe *et al*., 2018 and 2019). Briefly, each male was kept in a separate cage and 30 minutes after administering each substance to its respective group, an oestrous female was introduced into the cage where the male was already placed and observed for mating performance. Treatment lasted 21 days while sexual performance parameters were evaluated on days 1, 7, 14 and 21 in the dark phase of the light-dark cycle under dim light and sound-free conditions. Each observation session was considered terminated once the mount latency (ML) or post ejaculatory interval (PEI) reached 20 minutes. The following performance parameters as seen in Fig. 1 were assessed:

Table 2. Effects of the aqueous leaf-extracts of E. hirta on the Mount (ML) and Intromission (IL) (s) latencies of sexually naïve normal male rats

Parameter	D	Treatment				
		DW	Erekta	Eh_{300}	Eh_{600}	Eh_{1200}
ML (s)	1	107.00 ± 16.33^{ab}	94.00 ± 26.07^{Ab}	97.00 ± 43.20^{Aab}	95.00 ± 21.21^{Aab}	91.75 ± 09.91^{Aab}
	7	90.00 ± 05.52^{ac}	67.50 ± 10.83^{Ac}	80.00 ± 16.33^{Aac}	75.00 ± 0.07^{ac}	70.25 ± 02.07^{ac}
	14	77.00 ± 07.80^{c}	50.00 ± 11.00^{d}	48.00 ± 30.64^{Aad}	47.00 ± 38.89^{Aae}	42.50 ± 02.51^{c}
	21	60.00 ± 09.33^{c}	30.00 ± 13.79^{d}	35.00 ± 14.34^{Aae}	32.00 ± 17.68^{Aae}	27.50 ± 0.17^{Aad}
IL (s)	1	120.33 ± 15.15^{ab}	110.00 ± 46.07^{Ab}	118 ± 43.20^{Aab}	103.00 ± 21.21^{ab}	114.75 ± 10.22^{Aab}
	7	110.67 ± 29.94^{ac}	90.50 ± 48.73^{Ac}	96.00 ± 16.33^{Aac}	90.00 ± 0.23^{Aac}	63.75 ± 13.25^{Aac}
	14	141.67 ± 21.36^{d}	86.00 ± 31.00^{d}	81.67 ± 42.19^{Aac}	65.00 ± 38.89^{Aad}	53.50 ± 16.58^{Aad}
	21	166.33 ± 31.85^{ae}	52.50 ± 0.79^{Ae}	61.33 ± 19.05^{Aad}	40.00 ± 14.14^{Aae}	32.11 ± 06.17^{Aae}

Values presented as Mean±SEM; ML: mount latency; IL: intromission latency; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; s: seconds; D: Day; $p<0.05$.

Mount: This is when the male rat raised the forelimbs and griped the female followed by the movement of its pelvic region towards the vagina of the female. It also occurs when the male's pelvis intentionally came into contact with the female's haunch with accompanying hip movements from the male.

Intromission: when the male mounted and achieved insertion of the penis into the female as marked by deeper than normal thrust usually followed by abrupt movement away from the female, urgent front leg movements and grooming. It is also the thrusting of the pelvic region of the male rat into pelvic region of the female followed by the penetration of the erect penis into the female's vagina.

Penile licking: when the male bends and lick its penis without mounting nor intromission;

Ejaculation: Ejaculation is marked by a more profound thrust than that of regular intromission. It was followed not by movement away from the female, but by sudden limpness and immobility until the female moved away. It is easily noticed when the male grips the female with the latter raising its snout in an upward direction. In rats, it often comes after a series of successive mounts and intromissions.

From these parameters, the following indices were determined or calculated:

Mount Latency (ML): This is the time interval from the introduction of the female into the cage until the first mount. This varies between a few seconds to minutes and measures the degree of arousal or sexual interest of the male. It is often expressed or measured in seconds.

Mount Frequency (MF): This is the total number of mounts preceding ejaculation. It is also a measure of the degree of the male's sexual interest or libido.

Intromission Latency (IL): This refers to the time interval from the introduction of the female into the cage until the first intromission. It ranges from a few seconds to minutes and measures the degree of sexual excitement and erection. It is measured in seconds and varies with the individual and the circumstances.

Intromission Frequency (IF): This represents the number of intromissions preceding an ejaculation. Like intromission latency, it measures the degree of erection.

Ejaculation Latency (EL): It is the time interval from the introduction of the female into the cage until ejaculation; or from the first mount to the next ejaculation within an ejaculatory series. It ranges from a few seconds to minutes and determines the efficiency of copulation in rats.

Post Ejaculatory Interval (PEI): It is the time interval between an ejaculation and the next first mount. Generally, in rats, it ranges from 6 to 10 minutes. It measures the refractory period that comes after ejaculation.

Copulatory efficiency (CE): It is calculated from the intromission (IF) and mount frequencies (MF) as: CE=IF/MFx100/1

Animal Termination/Sacrifice : On day 22 from the beginning of treatment, all animals were terminated in a humane manner. To this effect, each animal was starved on day 21 after recording copulatory parameters, then, terminated by exposing them to an overdose of anaesthesia (Isoflurane).

Statistical analyses

Values were expressed as Mean±SEM and presented per ejaculatory series. Mean values were calculated for each animal and quantitative comparisons between groups established from those means. Analysis of Variance (ANOVA) followed by Duncan test wasused in the SPSS for windows version 20.0 software. Significant levels were tested at $p<0.05$.

RESULTS

Effects of the crude aqueous leaf-extract of *E. hirta*on the copulatory activity of sexually naïve normal male albino rats

Effects on mount (ML) and intromission latencies (IL) (s): Table 2 summarises the effects of the leaf aqueous extract (Eh_{300}, Eh_{600} and Eh_{1200}) of *E. hirta* on mount (ML) and intromission latencies (IL). ML of extract-treated groups generally showed a trend of decreasing ML over the 21-day period, with a most significant decrease induced by the Eh_{1200} from day 1 ($91.75\pm9.91^{)}$ to day21 (27.50 ± 0.17) compared to the DW control group(107.00 ± 16.33, day1 and 60.00 ± 9.33 day21, respectively). For Intromission Latency (IL), AE-treated groups generally exhibited a significant ($p<0.05$) reduction with the Eh_{1200}-treated animals recording the smallest IL values compared to both control groups in which DW-treated animals instead showed a significant increase in this parameter from day1 (120.33 ± 15.15) to day21 (166.33 ± 31.85).

Effects on mount (MF) and intromission frequencies (IF): In a similar manner, compared to the DW-treated males, extract-treated males registered a significant increase in mount frequency values dose-dependently throughout the treatment period, though there was a general decreasing tendency in this parameter from one ejaculatory series (S) to another (Table 3). In addition, all extract-treated males produced 04 ejaculatory series (S) on day 21 of treatment, compared to the DW and Erekta®-treated males which recorded 02 and 03 ejaculatory series (S), respectively. Also, as shown in Table 4, there was a significant increase in MF in a dose-dependent manner among the males treated with the plant extract, compared to both control groups with IF values in Eh1200-treated animals almost equal to MF values.

Effects on Copulatory Efficiency (CE): Copulatory efficiency, which is a function of both mount and intromission frequencies (MF and IF), followed the same trend as MF and IF, with extracted-treated animals recording higher values, especially those administered the 1200 mg/kg dose, though non-significant when compared to the controls (Table 5).

Table 3. Effects of the aqueous leaf-extracts of E. hirta on the Mount Frequency (MF) of sexually naïve normal male rats

Parameter	D	S	Treatment				
			DW	Erekta®	Eh_{300}	Eh_{600}	Eh_{1200}
MF	1	1	16.00 ± 06.66^{ab}	26.00 ± 02.00^{Ab}	23.67 ± 03.88^{ab}	24.00 ± 04.00^{ab}	32.00 ± 04.29^{Aab}
		2	07.50 ± 0.50^{ac}	24.50 ± 03.50^{Ab}	19.00 ± 04.65^{ab}	17.00 ± 02.00^{ac}	29.25 ± 07.85^{ac}
		3	04.50 ± 02.50^{ab}	22.00 ± 05.00^{Ab}	17.00 ± 02.89^{Aab}	21.00 ± 04.00^{ab}	22.25 ± 03.11^{ac}
		4	0.00	24.50 ± 04.50^{Ab}	22.00 ± 02.08^{Aab}	18.00 ± 03.00^{Aab}	26.12 ± 05.00
	7	1	09.00 ± 02.08^{b}	29.25 ± 03.31^{b}	24.00 ± 03.51^{Aab}	27.50 ± 06.50^{Aab}	29.00 ± 05.83^{Aab}
		2	08.33 ± 01.20^{b}	27.00 ± 04.15^{b}	20.33 ± 01.76^{ab}	26.00 ± 04.17^{c}	26.25 ± 06.85^{c}
		3	06.03 ± 0.88^{b}	31.50 ± 05.50^{b}	19.33 ± 01.33^{Aab}	27.00 ± 06.00^{c}	28.00 ± 04.41^{c}
		4	06.33 ± 0.93^{b}	25.00 ± 06.00^{b}	21.33 ± 02.33^{Aab}	24.50 ± 04.50^{Aab}	29.75 ± 04.38^{Aac}
	14	1	16.33 ± 06.84^{b}	32.25 ± 06.89^{b}	31.67 ± 01.33^{Ab}	32.00 ± 08.00^{Ab}	35.25 ± 03.35^{Aab}
		2	08.00 ± 0.58^{c}	29.75 ± 08.25^{b}	32.33 ± 01.20^{Aab}	26.88 ± 04.50^{Aac}	29.75 ± 04.93^{Aac}
		3	05.33 ± 01.33^{ac}	28.25 ± 07.93^{Ab}	28.00 ± 02.08^{Ab}	26.23 ± 04.52^{ac}	31.25 ± 08.18^{Aac}
		4	06.33 ± 01.86^{ac}	25.00 ± 10.00^{Ab}	26.00 ± 0.58^{ab}	23.17 ± 03.11^{ac}	24.50 ± 04.19^{Ac}
	21	1	12.33 ± 11.14^{ab}	19.00 ± 04.83^{Ab}	23.33 ± 03.18^{Aab}	21.50 ± 02.50^{Aab}	30.00 ± 05.71^{ab}
		2	11.50 ± 07.50^{c}	16.25 ± 04.48^{b}	19.67 ± 02.33^{b}	18.50 ± 06.15^{b}	26.00 ± 03.29^{c}
		3	0.00	16.67 ± 06.76^{Ab}	13.00 ± 08.02^{Aab}	17.00 ± 03.22^{Ab}	24.25 ± 03.93^{Aac}
		4	0.00	0.00	10.33 ± 04.91^{Aab}	09.50 ± 0.50^{Aab}	20.25 ± 03.65^{Aac}

Values presented as Mean±SEM; MF: mount frequency; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; S: ejaculatory series; D: Day; $p<0.05$.

Table 4. Effects of the aqueous leaf-extracts of E. hirta on the Intromission Frequency (IF) of sexually naïve normal male rats

Parameter	D	S	Treatment				
			DW	Erekta®	Eh_{300}	Eh_{600}	Eh_{1200}
IF	1	1	11.33 ± 02.96^{ab}	20.00 ± 02.00^{Ab}	20.00 ± 01.00^{ab}	19.50 ± 02.50^{ab}	22.50 ± 03.71^{Aab}
		2	05.00 ± 01.00^{c}	18.50 ± 01.50^{b}	17.00 ± 01.73^{ab}	16.50 ± 01.50^{b}	21.00 ± 05.41^{ac}
		3	02.50 ± 03.50^{ab}	20.00 ± 01.00^{Ab}	14.67 ± 02.60^{ab}	17.50 ± 03.50^{ab}	20.75 ± 04.48^{ac}
		4	0.00	20.50 ± 01.50^{Ab}	18.67 ± 0.67^{Ab}	15.00 ± 01.00^{Aab}	21.53 ± 05.00
	7	1	05.33 ± 01.76^{b}	25.50 ± 07.65^{b}	20.67 ± 02.40^{b}	21.00 ± 03.00^{b}	24.50 ± 03.71^{Aab}
		2	05.33 ± 0.33^{b}	25.67 ± 04.20^{b}	18.00 ± 0.580^{b}	18.50 ± 0.50^{b}	24.10 ± 02.41^{c}
		3	03.33 ± 0.67^{b}	27.00 ± 06.00^{b}	15.67 ± 01.20^{b}	25.50 ± 0.50^{b}	25.25 ± 01.25^{c}
		4	02.33 ± 0.88^{b}	20.00 ± 02.00^{b}	16.00 ± 01.53^{Aab}	20.50 ± 0.50^{Aab}	23.35 ± 02.31^{Aac}
	14	1	08.0 ± 5.03^{b}	27.00 ± 01.29^{b}	25.00 ± 0.00^{b}	26.00 ± 02.00^{b}	29.25 ± 02.02^{Aab}
		2	03.33 ± 0.88^{c}	23.50 ± 0.96^{c}	24.33 ± 0.33^{c}	22.00 ± 02.11^{b}	24.25 ± 01.11^{Aab}
		3	02.00 ± 0.58^{c}	24.75 ± 0.48^{c}	22.33 ± 0.88^{c}	23.00 ± 02.17^{b}	29.75 ± 0.63^{Aac}
		4	03.67 ± 0.67^{c}	21.50 ± 01.50^{c}	20.00 ± 0.00^{c}	20.00 ± 01.63^{b}	22.50 ± 0.96^{Aab}
	21	1	06.33 ± 02.96^{b}	17.75 ± 01.25^{b}	15.00 ± 0.58^{b}	16.00 ± 01.44^{b}	26.25 ± 01.49^{Aab}
		2	03.50 ± 01.5^{b}	12.75 ± 0.48^{b}	13.33 ± 0.33^{b}	13.00 ± 01.00^{b}	20.00 ± 01.22^{Aac}
		3	0.00	13.67 ± 0.33^{Ab}	08.67 ± 0.33^{Ab}	12.00 ± 01.06^{Ab}	20.75 ± 01.31^{Aac}
		4	0.00	0.00	05.00 ± 01.73^{Aab}	05.00 ± 01.05^{Aab}	18.75 ± 01.03^{Aac}

Values presented as Mean±SEM; IF: intromission frequency; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; S: ejaculatory series; D: Day; $p<0.05$.

Table 5. Effects of the aqueous leaf-extracts of E. hirta on the Copulatory Efficiency (CE) of sexually naïve normal male rats

Parameter	D	S	Treatment				
			DW	Erekta®	Eh_{300}	Eh_{600}	Eh_{1200}
CE	1	1	68.00 ± 12.96^{ab}	76.92 ± 12.00^{Ab}	84.49 ± 11.00^{ab}	81.25 ± 12.50^{ab}	70.31 ± 11.71^{Aab}
		2	66.67 ± 11.00^{c}	75.51 ± 11.50^{b}	89.47 ± 11.73^{ab}	97.05 ± 11.50^{b}	71.79 ± 09.41^{ac}
		3	44.44 ± 13.50^{ab}	90.91 ± 13.00^{Ab}	86.29 ± 12.60^{ab}	83.33 ± 09.50^{ab}	93.26 ± 10.48^{ac}
		4	0.00	83.67 ± 14.50^{Ab}	84.86 ± 09.67^{Ab}	83.33 ± 09.00^{Aab}	82.43 ± 09.00
	7	1	59.22 ± 11.76^{b}	87.17 ± 11.65^{b}	86.13 ± 10.40^{b}	76.36 ± 08.00^{b}	84.48 ± 10.71^{Aab}
		2	63.98 ± 10.33^{b}	98.78 ± 14.20^{b}	88.54 ± 10.580^{b}	71.15 ± 08.50^{b}	91.81 ± 12.41^{c}
		3	55.22 ± 10.67^{b}	85.71 ± 11.00^{b}	81.07 ± 08.20^{b}	94.44 ± 10.50^{b}	86.50 ± 10.25^{c}
		4	36.81 ± 0.88^{b}	80.00 ± 12.00^{b}	75.01 ± 08.53^{Aab}	83.67 ± 10.50^{Aab}	78.49 ± 10.31^{Aac}
	14	1	48.98 ± 15.03^{b}	83.72 ± 12.29^{b}	78.94 ± 10.00^{b}	81.25 ± 12.08^{b}	82.98 ± 12.02^{Aab}
		2	41.63 ± 10.88^{c}	79.00 ± 10.96^{c}	75.26 ± 10.33^{c}	81.85 ± 11.00^{b}	81.51 ± 11.11^{Aab}
		3	40.00 ± 08.58^{c}	87.18 ± 09.48^{c}	79.75 ± 10.88^{c}	87.65 ± 11.15^{b}	95.20 ± 10.63^{Aac}
		4	57.98 ± 11.67^{c}	86.00 ± 09.50^{c}	76.92 ± 08.00^{c}	86.31 ± 07.20^{b}	91.84 ± 10.96^{Aab}
	21	1	51.33 ± 09.96^{b}	93.42 ± 11.25^{b}	64.29 ± 07.58^{b}	74.41 ± 07.18^{b}	87.5 ± 11.49^{Aab}
		2	30.43 ± 07.5^{b}	78.44 ± 08.48^{b}	67.77 ± 07.33^{b}	70.27 ± 07.11^{b}	76.92 ± 08.22^{Aac}
		3	0.00	82.00 ± 10.33^{Ab}	66.69 ± 08.33^{Ab}	70.59 ± 06.25^{Ab}	85.57 ± 09.31^{Aac}
		4	0.00	0.00	48.40 ± 05.73^{Aab}	52.63 ± 05.30^{Aab}	92.59 ± 11.03^{Aac}

Values presented as Mean±SEM; CE: Copulatory Efficiency; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; S: ejaculatory series; D: Day; $p<0.05$.

Table 6. Effects of the aqueous leaf-extracts of E. hirta on the Ejaculation Latency (EL) of normal male rats

Parameter	D	S	Treatment				
			DW	Erekta®	Eh_{300}	Eh_{600}	Eh_{1200}
EL (s)	1	1	1200.00±458.26^{a}	180.00±0.00^{A}	483.67±209.17^{Aa}	521.00±139.00^{Aa}	667.25±54.40^{Aa}
		2	360.00±180.00^{a}	240.00±60.00^{A}	300.00±34.52^{Aa}	406.50±193.50^{Aa}	188.50±18.39^{Aa}
		3	1020.00±720.00^{a}	120.00±0.00^{A}	95.67±43.23^{Aa}	372.00±168.00^{Aa}	324.00±17.58^{Aa}
		4	0.00	210.00±90.00^{A}	380.00±131.15^{Aa}	415.00±185.00^{Aa}	0.00
	7	1	420.00±124.90^{a}	1125.00±546.83^{A}	820.00±401.50^{Aa}	1386.50±473.50^{Aa}	411.25±19.47^{Aa}
		2	300.00±60.00^{a}	380.00±174.36^{A}	440.00±156.20^{Aa}	323.00±37.00^{Aa}	188.50±18.39^{Aa}
		3	147.67± 44.33^{a}	180.00±60.00^{A}	360.00±91.65^{Aa}	198.50±41.50^{Aa}	206.50±13.18^{Aa}
		4	619.33±410.67^{a}	120.00±60.00^{A}	393.33±121.29^{Aa}	240.00±60.00^{Aa}	236.75±13.18^{Aa}
	14	1	475.33±206.06^{a}	737.25±207.06^{A}	520.00±156.20^{Aa}	600.00±360.00^{Aa}	534.00±18.49^{Aa}
		2	203.00±41.58^{a}	461.00±117.80^{A}	220.00±87.18^{Aa}	150.00±30.00^{Aa}	432.00±17.61^{Aa}
		3	170.00±55.68^{a}	412.50±131.87^{Aa}	220.00±52.92^{Aa}	121.50±1.50^{Aa}	269.00±13.06^{Aa}
		4	238.00±60.35^{a}	283.50±146.50^{A}	240.00±91.65^{a}	169.00±11.00^{Aa}	428.50±18.71^{Aa}
	21	1	1783.33±401.34^{a}	942.00±286.47^{A}	299.67±158.43^{Aa}	522.50±282.50^{Aa}	606.50±23.45^{Aa}
		2	458.50±21.5^{a}	499.50±162.24^{A}	260.00±100.00^{Aa}	300.00±120.00^{Aa}	185.75±41.10^{Aa}
		3	0.00	244.67±91.98^{A}	400.00±255.34^{Aa}	153.00± 33.00^{Aa}	277.25±31.11^{Aa}
		4	0.00	0.00	247.67±176.29^{Aa}	150.00±30.00^{Aa}	318.75±17.01^{Aa}

Values presented as Mean±SEM; EL: ejaculation latency; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; s: seconds; S: ejaculatory series; D: Day; $p<0.05$.

Table 7. Effects of the aqueous leaf-extracts of E. hirta on the Post Ejaculation Interval of normal male rats

Parameter	D	S	Treatment				
			DW	Erekta®	Eh_{300}	Eh_{600}	Eh_{1200}
PEI (s)	1	1	0.00	0.00	0.00	0.00	0.00
		2	210.00±30.00^{a}	390.00±30.00^{A}	400.00±52.92^{A}	1219.00±201.00^{Aa}	476.50±34.85^{Aa}
		3	480.00±60.00^{a}	420.00±60.00^{A}	420.00±60.00^{A}	613.50±96.50^{Aa}	712.25±74.85^{Aa}
		4	0.00	0.00	580.00±52.92^{Aa}	600.00±92.00^{Aa}	0.00
	7	1	0.00	0.00	0.00	0.00	0.00
		2	400.00±40.00^{a}	660.00±21.71^{A}	535.00±105.83^{Aa}	533.50±64.50^{Aa}	498.50±44.85^{Aa}
		3	500.00±40.00^{a}	510.00±90.00^{A}	540.00±34.52^{Aa}	607.00±67.00^{Aa}	582.75±82.65^{Aa}
		4	672.33±79.57^{a}	662.00± 60.00^{A}	466.67±29.06^{Aa}	586.00±61.00^{Aa}	571.00± 21.27^{a}
	14	1	0.00	0.00	0.00	0.00	0.00
		2	440.00±52.92^{a}	387.75±43.65^{A}	460.00±20.00^{Aa}	420.00±72.00^{Aa}	477.25±24.68^{Aa}
		3	520.00±52.92^{a}	533.75±78.99^{A}	580.00±40.00^{Aa}	540.00±60.00^{Aa}	472.75±29.54^{Aa}
		4	690.00±62.45^{a}	542.00±14.00^{A}	500.00±230.65^{Aa}	448.50±28.50^{Aa}	457.25±27.41^{Aa}
	21	1	0.00	0.00	0.00	0.00	0.00
		2	475.00±65.00	483.00±51.98	360.00±60.00^{Aa}	420.00±60.00^{Aa}	360.75±25.63^{Aa}
		3	0.00	572.67±59.18^{A}	500.00±52.92^{Aa}	360.00±74.00^{Aa}	589.50±48.27^{Aa}
		4	0.00	0.00	680.00±170.88^{Aa}	450.00±50.00^{Aa}	467.75±29.85^{Aa}

Values presented as Mean±SEM; PEI: post-ejaculatory latency; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; s: seconds; S: ejaculatory series; D: Day; $p<0.05$.

Table 8. Effects of the aqueous leaf-extracts of E. hirta on the Penile Licking (PL) of normal male rats

Parameter	D	S	Treatment				
			DW	Erekta®	Eh_{300}	Eh_{600}	Eh_{1200}
PL	1	1	16.00±7.37^{ab}	7.00±1.00^{Ab}	13.00±3.00^{Aab}	13.00±1.00^{Aab}	33.25±3.54^{Aab}
		2	4.50±0.50^{ac}	10.0±5.00^{Ab}	8.67±1.33^{Ab}	11.00±0.00^{Ab}	10.25±0.75^{Ac}
		3	11.50±2.50^{ab}	6.00±3.00^{Ab}	7.33±2.73^{Ab}	13.50±3.50^{Aab}	13.25±1.31^{Aac}
		4	0.00	11.00±7.00^{Ab}	9.33±1.76^{Ab}	18.00±5.00^{Aab}	0.00
	7	1	9.67±2.60^{ab}	12.75±2.78^{Ab}	16.67±6.74^{Aab}	17.50±6.50^{Aab}	29.50±0.96^{Aab}
		2	9.33±0.88^{b}	11.33±3.53^{b}	12.00±0.58^{Ab}	5.50±0.50^{Aac}	10.25±0.75^{Ac}
		3	7.33±0.88^{b}	7.50±2.50^{b}	12.67±0.33^{Aab}	7.00±1.00^{c}	12.50±0.65^{Aac}
		4	11.00±3.51^{ab}	7.00±1.00^{Ab}	13.33±1.76^{ab}	12.50±0.50^{ac}	12.00±0.41^{ac}
	14	1	15.00±6.00^{ab}	19.75±4.48^{Ab}	11.67±1.86^{Aab}	11.50±4.50^{Aab}	23.25±1.89^{Aab}
		2	10.00±0.58^{ac}	15.75±1.55^{Ab}	7.33±1.2^{Aab}	7.00±1.00^{Aab}	18.25±1.89^{Aab}
		3	8.00±1.53^{ac}	12.50±1.44^{Ab}	11.33±1.33^{Ab}	9.00±4.00^{ab}	8.75±1.65^{ac}
		4	10.67±0.88^{c}	9.50±4.50^{c}	8.00±2.08^{b}	7.50±3.50^{Ab}	14.75±1.03^{Aac}
	21	1	29.33±9.35^{ab}	14.50±2.78^{Ab}	12.33±3.93^{Aab}	13.50±0.50^{Ab}	29.25±1.11^{ab}
		2	15.00±7.00^{c}	13.25±2.46^{b}	11.00±2.52^{Ab}	10.50±2.50^{Aab}	14.25±0.48^{c}
		3	0.00	9.67±1.76^{Ab}	11.33±4.84^{Aab}	7.00±1.00^{Ab}	13.50±1.19^{Aac}
		4	0.00	0.00	6.50±3.50^{Aac}	9.00± 0.00^{Aab}	13.50±1.19^{Ac}

Values presented as Mean±SEM; PL: penile licking; DW: distilled water; $Eh_{300:}$ *Euphobiahirta* 300mg/kg; Eh_{600}:*Euphobiahirta* 600mg/kg; Eh_{1200}:*Euphobiahirta* 1200 mg/kg; On the same row: A-significant when compared with Negative control (DW) and a-significant when compared with Positive control (Erekta); On the same column, values with the same letters (b-e) are not significantly different; on the same column, values with different letters (b-e) are significantly different; S: ejaculatory series; D: Day; $p<0.05$.

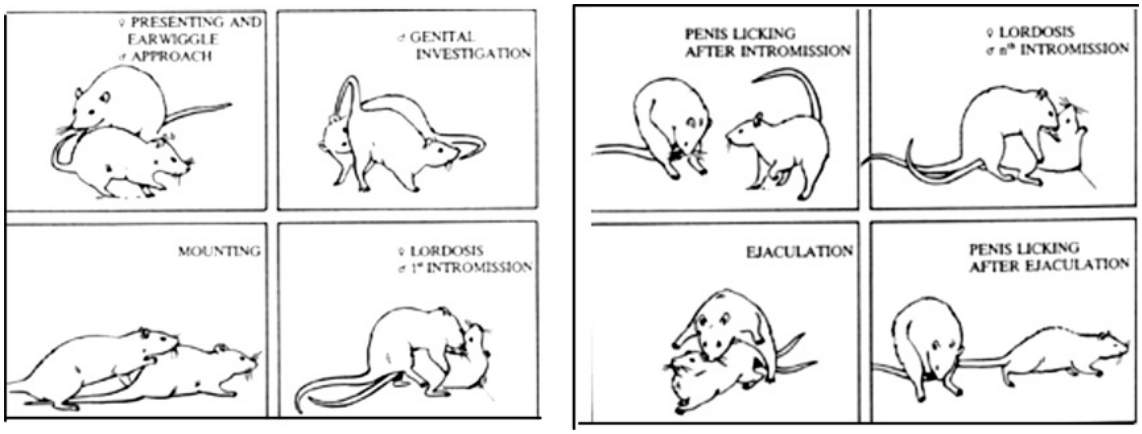

Figure 1. Rats Sexual Behaviour

Effects on ejaculatory latency (EL) (s): Results for Ejaculation Latency (EL), obtained from our studies are illustrated in Table 6. The aqueous leaf extract-treated groups generally showed a significant decrease in EL compared to the group that received DW. Its action was non-significant when compared to Erekta- treated group. Although EL decreased from one ejaculatory series (S) to another and the duration of treatment, EL values recorded in Eh_{1200}-treated males were higher than those in all other groups, whether controls or extract-treated.

Effects on Post-Ejaculatory Interval (PEI) (s): Subjection of sexually naïve normal male rats to either dose of the leaf-aqueous extract of *E. hirta* induced a non-significant effect on PEI amongst extract-treated groups, but when compared to the control groups (DW and Erekta), the effect is significant throughout the treatment period (Table 7).

Effects on Penile Licking (PL): Data obtained on the effects of the leaf-aqueous extract of *E. hirta* on penile licking (PL) of sexually naïve normal male rats is presented in Table 8. PL values produced by Eh_{1200}-treated group showed a significant increase compared to those that received distilled water. Compared to the Erekta treated group, values produced were statistically non-significant.

DISCUSSION

Studying the aphrodisiac properties of a given medicinal plant involves examining their ability to improve libido, sexual performance and as a whole enhance the reproductive health. *Euphorbia hirta,* a common medicinal plant of the family Euphorbiaceae has been commonly used due to its immense potential, notably, because it possesses antibacterial, anti-inflammatory, antioxidant just to state a few (Huang *et al*., 2012; Vijay *et al*., 2016; Khursheed *et al*., 2022). This study was designed to investigate the aphrodisiac properties of *Euphorbia hirta* crudea queous leaf-extract on the mating behaviour of sexually naive normal male albino rats. Phytochemical screening of this plant extract from other studies had revealed the presence of: saponins, tannins, flavonoids, cardiac glycosides, anthraquinone, phenols and alkaloids (Ganiyu *et al*., 2022). The phytoconstituents found in the aqueous extract of this plant suggests that they may be the basis of its pro-copulatpry properties since alkaloids, saponins, flavonoids and phenolic acids have been proved to favour libido, vigour and potency. According to Pittler & Ernst (1998), Gauthaman *et al*. (2002), Yakubu *et al*. (2005), Gauthaman *et al*. (2008), saponins found in *Fadogiaagrestis (Schweinf. Ex Hiern), Tribulusterrestris (Linn.)* and alkaloid from *Pausinystalia yohimbe*are responsible for the aphrodisiac effects produced by these plants. In the same way, sexual enhancing property of *Mondiawhitei* Hook has also been attributed to its steroid and triterpene contents (Drewes *et al*., 2003). Both mount (ML) and intromission (IL) latencies are indicators of male sexual arousal, desire or libido.

There was a significant decrease in ML and IL values noted in the extract-treated animals, which show that there was reduction in the hesitation time of the male rats towards the receptive females: a clear indication of sexual excitement. This high level of sexual excitement noticed in extract-treated animals could be attributed to alkaloids, saponins, flavonoids and phenolic acids found in the extract. These phytoconstituents as mentioned earlier have been proved to favour desire (libido), vigour and potency. Same observations were made by (Fouche *et al*., 2015) where a similar decrease in ML and IL was produced by the aqueous extract of the aerial parts of *Monsonia angustifolia* in the treated animals (at doses of 30 and 300mg/kg). Also, mount (MF) and intromission (IF) frequencies are parameters that translate vigour, libido and potency. MF reflects sexual motivation whereas increase in the IF shows the efficiency of erection, penile orientation as well as the ease by which ejaculatory reflexes are activated (Ratnasooriya *et al*., 2000; Yakubu *et al*., 2005). The significant increase in MF and IF recorded in extract-treated animals suggests an improvement in sexual vigour and libido. The increase in IF noted in the extract-treated animals indicates that penile erection was effective and efficient, an action that could be attributed to the alkaloids and saponins present in the extract which according to Ratnasooriya *et al*. (2000) and Fouche *et al*. (2015), possess ergogenic properties in vasodilation of the blood vessels and hence, penile erection. Furthermore, both mount (MF) and intromission frequencies (IF) are important indicators of copulatory efficiency (CE). Copulatory efficiency in a male rat is a crucial determinant of reproductive success and a valuable measure in research for evaluating sexual motivation, performance as well as neurobiological function.

Copulation is not merely about achieving ejaculation quickly, but about the quality and timing of sexual behaviors that ensure successful fertilization and pregnancy induction in the female. CE is a valuable measure in our study since animals used were sexually naive. Successful reproduction in rats requires a specific pattern of vaginocervical stimulation, achievedthrough a series of intromissions before ejaculation. An appropriate number of intromissions is essential to trigger the female's neuroendocrine responses, specifically the pro-gestational state whichis a prerequisite for blastocyst implantation and the maintenance of pregnancy. Inefficient copulation characterized by too few intromissions or poor placement of the copulatory plug can lead to pre-implantation losses and a reducedlitter-size. In Eh_{1200}-treated males, CE values increased significantly between day 1 and 21, which shows thatthese males were highly sexually motivated and displayed a high sexual performance that probablyled to a successful sexualactivity. Unfortunately, the females used were ovariectomized and socould not befollowed-up to seeevolution of the pregnancy and subsequently assess the litter-size. The significant reduction in Ejaculatory latency (EL) from one ejaculatory series to another experienced by the extract treated animals demonstrated that these animals required a shorter time interval to achieve ejaculation. This finding suggests a potential pro-ejaculatory effect of the extract, even though, the efficacy of the

extract on EL was not statistically significant when compared to the reference drug, Erekta, implying that while the extract shortens ejaculatory latency, its potency may be comparable to or less pronounced than a known standard drug. However, high EL values recorded in Eh_{1200}-treated animals following 21 days of uninterrupted administration of the extract could be a therapeutic advantage in treating premature ejaculation indicating that the extract possesses a time-dependant effect on this parameter. PEI is an important parameter for evaluating the effect of administered extract on erectile function and the rate of recovery from exhaustion after first series of mating (Tajuddin *et al*., 2004; Egbe *etal*., 2018). The non-significance of the Post Ejaculatory Interval (PEI) of the extract-treated animals compared to both the distilled water (DW) and the Erekta-treated animals suggests that while the extract influences the time of ejaculation (EL), it does not appear to significantly enhance or impair the overall erectile function of the extract-treated animals.

CONCLUSION

In this study, we aimed at investigating the aphrodisiac properties of *Euphorbia hirta* crudeaqueous leaf-extract on the mating behaviour of sexually naive normal male albino.

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Conflict of Interest: Authors declare that they do not have any conflict of interest.

Authors' contributions

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