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Original Research Article

Aphrodisiac Properties and Reproductive Hormones Concentrations of Ethanol Leaf Extract of *Eremomastax speciosa* in Male Wistar Rats

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ABSTRACT

The effects of *Eremomastax speciosa* leaf extract on reproductive activities was evaluated; antioxidant activities and the phytochemistry of *Eremomastax speciosa* were assayed using standard methods. Thirty-five matured male Wistar rats were used for the experiment and they were divided into five groups of seven rats each; groups 1 and 2 were the positive and normal controls respectively and were given Manix capsule and distilled water respectively. Groups 3, 4 and 5 were administered the ethanol extract at the doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg respectively. Twelve matured female rats were used for the aphrodisiac assessment. The phytochemical screening showed the presence of Alkaloids, Tannins, Saponins, Terpenes and Flavonoids and the leaves showed a significant free scavenging activity with an IC_{50} value of 90 µg/ml. The result showed a dose-dependent significant ($P<0.05$) increase in Testosterone levels and also an increase in Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) which was not significant when compared with the normal control. The result also showed a dose-dependent significant increase in the mount, intromission and ejaculatory frequencies while decreasing their latencies when compared with the normal control. In conclusion, the ethanol leaf extract of *Eremomastax speciosa* possesses aphrodisiac potentials and could boost testosterone level which could enhance reproductive indices.

***Keywords*:** Antioxidant, *Eremomastax speciosa*, Medicinal plants, Phytochemicals, Reproduct Reproductive Hormones.

Introduction

Reproduction is the process by which organisms create descendants.[1] This is a characteristic that all living things have in common and sets them apart from nonliving things. The major function of the reproductive system is to ensure the survival of the species. Other systems in the body, such as the endocrine and urinary systems, work continuously to maintain homeostasis for the survival of the individual. An individual may live a long, healthy, and happy life without producing offspring, but if the species is to continue, at least some individuals must produce offspring. Within the context of producing offspring, the reproductive system has four functions: to produce egg and sperm cells; to transport and sustain these cells; to nurture the developing offspring; to produce hormones.[1] The human reproductive system includes the male reproductive system which functions to produce and deposit sperm; and the female reproductive system which functions to produce egg cells, and to protect and nourish the fetus until birth.[2] Phytochemicals are chemicals of plant origin. [3] They play a role in plant growth or defense against competitors, predators, or pathogens and generally have biological activity in the plant host. [4] Phytochemicals are regarded generally as research compounds instead of essential nutrients due to a lack of possible proof of their health effects. [5, 6]

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Those under research are classified into major categories like polyphenols, which include flavonoids, phenolic acids, and stilbenes/lignans.[5,7] Phytochemicals are chemical compounds formed during the plants' normal metabolic processes. These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids.[8] Aphrodisiac substances are often used to enhance sexual performance and treat sexual dysfunction. The modern definition of aphrodisiac can vary, but it is generally regarded as a substance that increases sexual desire (i.e., libido) and/or sexual pleasure[9] and it include those substances which aid in the proper functioning of the male and female sex organs.[10,11] These substances can vary from foods, drinks, beverages, vitamins, minerals to other natural or synthetic chemicals that were claimed by diverse cultures could improve men stamina, libido, and sexual function.[12] Aphrodisiac is found widely in various plant and animal sources and even though most are not yet scientifically confirmed, many cultures practice that knowledge to treat those with a sexual problem.

Male sexual behaviour comprises a complex pattern of genital and somatomotor responses. Hormones act via receptors in the brain, spinal, and peripheral sites to bias sensory inputs and motor outputs to favour sexual responsiveness. Copulation includes mounts, intromissions, and ejaculations, followed by sexual quiescence. After 6–12 ejaculations, male rats become sexually satiated. Neural controls include chemosensory inputs via the main and accessory olfactory systems to the medial amygdala, which transmits information directly and indirectly to the medial preoptic area (mPOA), which integrates sensory and hormonal information and elicits genital reflexes and copulatory patterns and contributes to sexual motivation. [13]

Sexual behaviour parameters include a wide variety of activities individuals engage in to express their sexuality. [14] The parameters

include mount Latency (ML), intromission Latency (IL), ejaculatory Latency (EL), mount Frequency (MF), intromission Frequency (IF), ejaculatory Frequency (EF).[15,16]

Eremomastax speciosa (Hochst.) Cufod commonly known as "edem iduodut" or "ndadad edem" by Ibibio people and "African blood tonic" in Cameroon belong to the family Acanthaceae. [17] It is a perennial herb found in Africa along the rainforest zone and occurs as a weed. It is cultivated in Cameroon and Akwa-Ibom in Nigeria due to its medicinal values. [18] It is a polymorphous herb that grows up to 2 m high with a remarkable quadrangular stem. [19] The leaf (decoction or infusion or maceration) is used by the natives in the treatment of dysentery, anaemia, menstrual pain, fracture, haemorrhoids and urinary tract infection. [18, 20, 17] The leaves of *Eremomastax speciosa* are also used to enhance fertility and arrest postpartum bleeding in women of reproductive age among the Southern Regions of Cameroun and Nigeria. [21, 22, 23] Hormones are the drivers of human reproduction, responsible for sexual development and controlling the menstrual cycle. Sex hormones are responsible for driving sexual development (puberty) and the main reproductive hormones are oestrogen and testosterone. [24] The male and female reproductive cycles are controlled by hormones released from the hypothalamus and anterior pituitary as well as hormones from reproductive tissues and organs. The hypothalamus monitors the need for the FSH and LH hormones made and released from the anterior pituitary. FSH and LH affect reproductive structures to cause the formation of sperm and the preparation of eggs for release and possible fertilization. [25] In the male, FSH and LH stimulate Sertoli cells and interstitial cells of Leydig in the testes to facilitate sperm production. The Leydig cells produce testosterone, which also is responsible for the secondary sexual characteristics of males. [26] Locally it is reported that the leaves of *Eremomastax speciosa* can have an improved effect on sexual behaviour in males[27] hence, the leaves were evaluated for reproductive activities.

Materials and Methods

Collection and Identification of Plant Material

Eremomastax speciosa was acquired from Apkajo community in Eleme Local Government Area of Rivers State, Nigeria in May 2019. The plant was identified and authenticated by Prof. (Mrs.) Uduak Eshiet of the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Nigeria. It was given the Voucher Number UUPH1 (b) and was deposited at the Department of Pharmacognosy and natural medicine, Faculty of Pharmacy Herbarium.

Preparation and Extraction of Plant Material

The wet method of extraction was used for the extraction.[28] The leaves were plucked from the plant stalk, thereafter the leaves were washed to remove the debris, 500 g of the leaves were cut into pieces and immersed in 2.5 L of 70% ethanol and kept in an amber-coloured bottle for 72hours. At the end of the three days, the mixture was filtered using a cheesecloth and then with Whatman No.1 filter paper. The filtrate was then put in a beaker and kept in a water bath at 30-40^{O}C, it yielded 25g of extract and stored in a refrigerator until needed for analysis.

Experimental Animals (Ethical Approval Number: UU/CH/EC/22/105)

Thirty-five male Wistar rats weighing 150-200g were used. The rats were bought from the University of Port Harcourt, Nigeria and brought to the Department of Biochemistry, University of Uyo. The animals were kept in wooden cages with a wire mesh top and maintained under standard conditions of humidity (50±5%) and temperature (28±2^{O}C) and maintained in a 12hours light/dark cycle. They had free access to feeds and water daily and were acclimatized for 14 days before the commencement of the work. The Ethical Committee for the use of laboratory Animals'[29] Recommendations, University of Uyo, Nigeria was adhered to in the rats' management.

Experimental Design

The animals were divided into five groups of seven rats each and treated as shown in Table 1. Group I was the positive control and administered the Manix capsule at a dose of 9.0 mg/kg, group II was normal control, administered distilled water (5 ml/kg) while groups III, IV and V were administered the extract at a dose of 400 mg/kg, 200 mg/kg and 100 mg/kg respectively. All the administration was done by oral route and it lasted for 14 days. 48 hours before the last day of administration, 17, *β*-estradiol was administered to 12 selected female animals subcutaneously to induce oestrous and enable vaginal opening; furthermore 8 hours before the mating experiment was carried out, progesterone was also administered to these 12 selected female animals subcutaneously to allow for mating to occur on the last day of administration when mounting would be done.

Male Rat Sexual Behavior: Test Procedure

48 hours before the last day of administration of extracts, 17, β-estradiol was administered to 12 selected female animals subcutaneously to induce oestrous and enable vaginal opening; furthermore 8 hours before the mating experiment was carried out, progesterone was also administered to these 12 selected female animals subcutaneously to allow for mating to occur on the last day of administration when mounting would be done as follows;

Mount Latency (ML): This is the time interval between the introduction of the female and the first mount by the male.

Intromission Latency (IL): This is the time interval between the introduction of the female and the first intromission by the male.

Ejaculatory Latency (EL): This is the time between the first ejaculation and the first intromission of the male rats.

Mount Frequency (MF): This is the total number of mounts made by the male rats during the observation period.

Intromission Frequency (IF): This is the number of intromissions from the time of introduction of the female until ejaculation by the male.

Ejaculatory Frequency (EF): This is the number of times semen was ejected from the male copulatory organ. After the sexual behaviour test. [15,16]

Assay Procedure for Serum Hormones

Determination of concentration of Testosterone in serum using assay kits from Monobind Inc.

The desired number of coated wells was secured in a holder. Ten microliter (10 µl) of standards, specimens and control were dispensed into appropriate wells. Anti-testosterone reagent (50 µL) was dispensed into each well and mixed thoroughly.[30]

Table 1: Experimental Design

Groups	No. of Animals	Treatment	Dosage
I (Positive control (PC))	7	Manix	9.0 mg/kg
II (Normal control (NC))	7	Distilled water	5 ml
III (400 mg/kg extract)	7	Crude extract of *Eremomastax speciosa*	400 mg/Kg
IV (200 mg/kg extract)	7	Crude extract of *Eremomastax speciosa*	200 mg/Kg
V (100 mg/kg extract)	7	Crude extract of *Eremomastax speciosa*	100 mg/Kg

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Testosterone HRP conjugate reagent (100 µL) was dispensed into each well and incubated for 90 mins at 37°C. The microwells were then rinsed and flicked 5 times with wash buffer. TMB substrate (1000 µL) was dispensed to each well and gently mixed for 10 seconds. The mixture was incubated at room temperature (18-22°C) for 20mins. The reaction was stopped by addition of stop solution to each well and gently mixed for 30 seconds to ensure a complete colour change. Absorbance at 450nm was read within 15mins with a microtiter plate reader.

Determination of concentration of Follicle Stimulating Hormone (FSH) in serum using assay kits from Monobind Inc

The microwell for each serum reference, control and specimen were formatted and assayed in duplicate. The appropriate serum reference, control and specimen (50 µL) were pipetted into the assigned wells. FSH enzyme reagent solution (100 µL) was added to all wells. The microplate was gently swirled for 20-30 seconds to mix and then covered. The mixture was incubated at room temperature for 60 mins. The contents of microplate was discarded by decantation and the plates blotted dry with absorbent paper. Wash buffer (350 µL) was added to the wells then decanted by tapping and blotting. This was repeated twice for a total of 3 washes. Working substrate solution (100 µL) was added to all the wells. This were done in the same order to minimize reaction time differences between wells. The mixture was incubated for 15mins at room temperature. Stop solution (50 µL) was then added to each well and gently mixed for 20-30 seconds. Finally, the absorbance in each well was read at 450nm in a microplate reader. This was done within 30mins of adding the stop solution [31].

Determination of concentration of Luteinizing Hormone in serum using assay kits from Monobind Inc.

The desired number of coated wells was secured in a holder. Fifty microliter (50 µL) of standards, specimens and control were dispensed into appropriate wells. LH Enzyme reagent (100 µL) was dispensed into each well, swirled thoroughly and allowed to mix for 20-30 seconds. The mixture was allowed to incubate for 60 minutes at room temperature. The contents of the micro wells were discarded by decantation, then rinsed and flicked 3 times with wash buffer (350 µL). Working substrate solution (100 µL) was dispensed to each well. The mixture was incubated at room temperature for 15 minutes. The reaction was stopped by addition of stop solution (50 µL) to each well and gently mixed for 15-20 seconds to ensure a complete colour change. Absorbance at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfection) was read within 30 minutes with a microplate reader [31].

Data Analysis

Data were presented as mean ± standard error of mean (SEM) and analyzed with statistical package of social science (SPSS) version 20 using one-way analysis of variance (ANOVA). Significance was accepted at the level of $p < 0.05$.

Results and Discussion

Phytochemical screening

The phytochemical screening of ethanol leaf extract of *Eremomastax speciosa* plant as shown in Table 2, revealed the presence of the following secondary metabolites: Alkaloids, Tannins, Saponins, Terpenes, Flavonoids and Combined Anthroquinone. The phytochemical screening of the extract, revealed the presence of Alkaloids, Flavonoids, Saponins, Terpenes, Tannins, and Combined Anthraquinones while it showed an absence of free anthraquinones, Cardiac Glycosides and phlobatannin. This result agrees with the works of. [22, 33, 23, 34, 35, 36, 37] These phytochemical compounds are known to play important roles in the bioactivity of medicinal plants. The flavonoids which are predominant compounds in the *E. speciosa* are known to exhibit a wide range of biological activities. [34]

Antioxidant Activity of Eremomastax speciosa Leaves

The free radical scavenging activities of extract and ascorbic acid are as shown in Table 3.

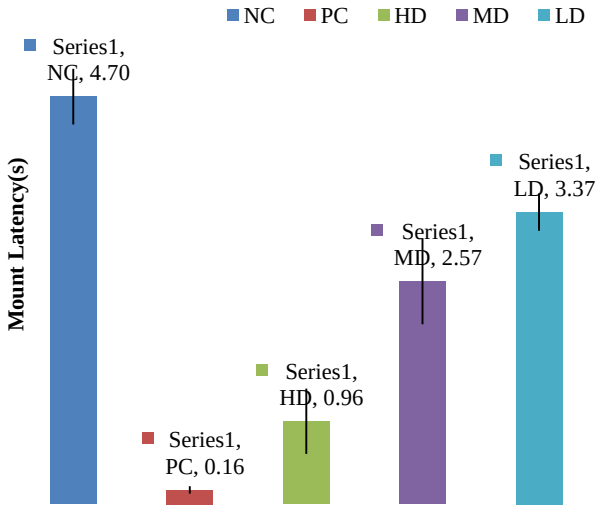

Table 2: Results of Phytochemical Screening of Leaf Extract

Tests	Observation	Inference
Alkaloids	Cream precipitate	+
Keller-Kiliani	No brown ring	-
Flavonoids	Yellow colouration	+
Saponins	Persistent foaming	+
Tannins	Dark green colour	+
Terpenes	Reddish-brown colour	+
Combined Anthraquinones	Pink colouration	+
Free Anthraquinones	No Pink colouration	-
Phlobatannins	No deposition of red precipitate	-

Key: - Absent, + Positive.

Table 3: Results of Antioxidant Screening of Extract

Group	Conc. (µg/ml)	Absorbance (517 nm)	% Inhibition	IC_{50}
Ethanol Extract	Control	1.167 ± 0.003	0	90 µg/ml
	15.625	1.128 ± 0.002	23.34	
	31.25	0.995 ± 0.004	34.70	
	62.50	0.676 ± 0.003	42.10	
	125.00	0.317 ± 0.002	72.80	
	250.00	0.139 ± 0.001	88.10	
Ascorbic Acid	Control	1.368 ± 0.001	0	110 µg/ml
	15.625	0.468 ± 0.001	6.20	
	31.25	0.445 ± 0.001	10.80	
	62.50	0.353 ± 0.001	29.30	
	125.00	0.208 ± 0.002	58.30	
	250.00	0.092 ± 0.001	81.60	

The result showed that the crude ethanol leaf extract had a significant free radical scavenging activity, with IC_{50} 90µg/mL when compared with the IC_{50} of Ascorbic acid (110µg/mL). The results of the DPPH free scavenging activity of the crude ethanol extract of *Eremomastax speciosa* showed that the crude ethanol extract possessed significant free radical scavenging activity, with an IC_{50} value of 90µg/mL,

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comparing with obtained IC_{50} value of ascorbic acid (110 µg/mL) indicated that the crude extract exhibited/possesses antioxidant activity which is similar to the result obtained by.[36,37,38]

Aphrodisiacs are substances that enhance sexual drive/sexual pleasure and can arouse sexual desire or libido.[39] They are substances that can be used to modify impaired sexual functions. In male rats, the extract caused a marked change in sexual behaviour. There was a significant decrease in mount latency, with a corresponding increase in mount frequency, indicating enhanced arousal and sexual vigour. There was also a decrease in intromission latency and an increase in intromission frequency indicative of an increased copulation rate. This result was similar to the work of Kosses[30] on aqueous extract of *Eremomastax speciosa* leaf. These indices of libido, when taken together pointed to the fact that the extract may possess aphrodisiac properties; such increases in the frequencies of mount and intromission, suggest that libido, sexual vigour and sexual performance were enhanced. [40] This potential of the leaf extracts could be due to the abundance of flavonoids in the leaf extract; flavonoids are involved in penile erection onset and improve sexual performance.[41,42,43]

Effects of Extract on Sexual Behavior of Male Wistar Rats

There was a significant increase ($p<0.05$) in mount, intromission and ejaculatory frequencies of extract groups when compared with the normal control while, there were significant decreases ($p<0.005$) in mount, intromission, ejaculatory latencies and penile erection latencies (Figure 1-8). In the hormonal assay result, testosterone and luteinizing hormones' levels increased in a dose-dependent manner; with that of 200mg/kg treated rats increasing but declining in the 400 mg/kg group thereby, suggesting that 400 mg/kg administration of the extract could be harmful to the male reproductive system. Testosterone is a primary sex hormone that promotes the development of the reproductive organs and secondary sexual characteristics in males,[39] luteinizing hormones, on the other hand, promotes male reproductive functions via stimulation of testosterone production from Leydig cells in the testis.[44,39] This may be the reason for the increase in testosterone concentration in the low and moderate dose treated groups as LH are increased. Observations in declined testosterone concentration following high-dose treatment may have resulted from either the direct effect of the extract on the Leydig cells or indirectly as a consequence of decreased luteinizing hormone level in the group. Testosterone concentration decline is reportedly a major sign of infertility.[45] Follicle-stimulating hormones (FSH) increased in concentration following treatment with the extract thus suggesting that the extract may have improved reproductive function in the treated male rats. FSH in males, regulates sexual development, growth, pubertal maturation and reproductive qualities by enhancing the induction and maintenance of normal sperm production.[46,47] Although, FSH concentration's excessive increase as observed in the high dose treated rats may affect reproduction negatively due to its effect on spermatogenesis. It is established that the most common endocrine abnormality associated with male infertility or subfertility is elevated FSH concentration, which generally indicates the impairment of spermatogenesis and primary testicular defect. [48]

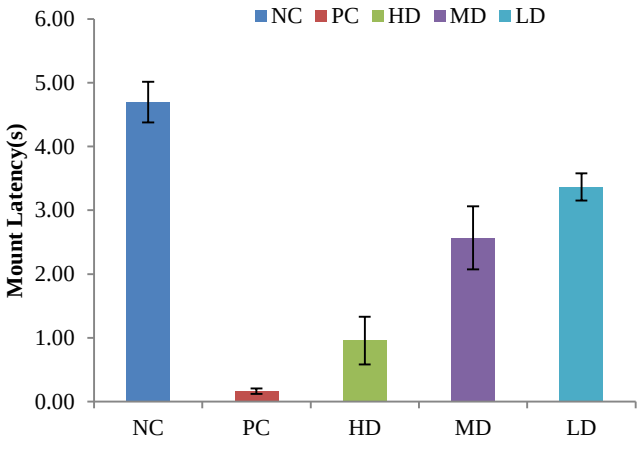

Figure 1: The effect of the extract on the Mount Latency
Where HD = 400 mg/kg, MD = 200mg/kg, LD = 100 mg/kg

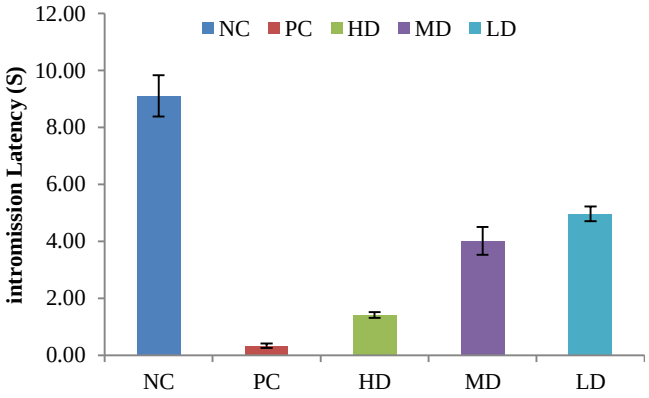

Figure 2: The effect of the extract on the Intromission Latency
Where HD = 400mg/kg, MD = 200mg/kg, LD = 100mg/kg

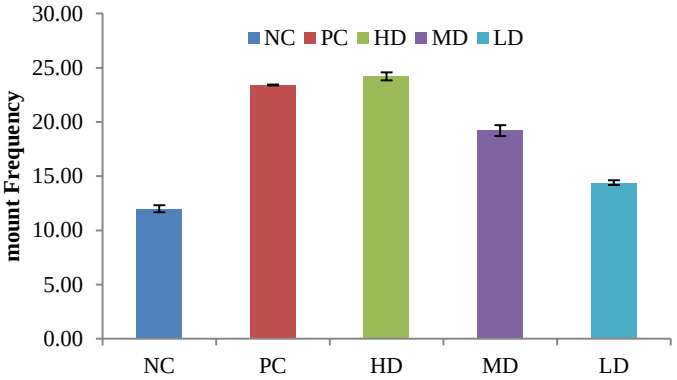

Figure 3: The effect of the extract on the Mount Frequency
Where HD = 400mg/kg, MD = 200mg/kg, LD = 100mg/kg

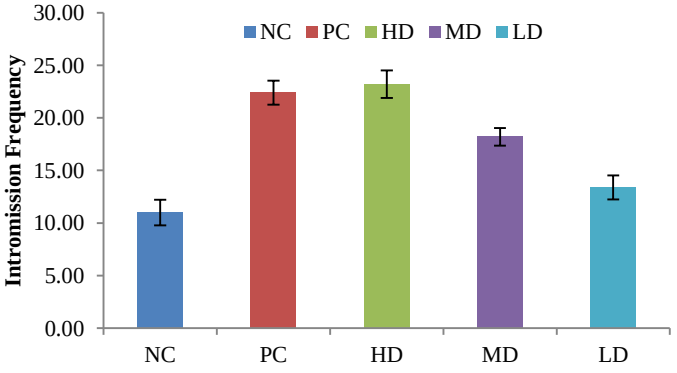

Figure 4: The effect of the extract on the Intromission Frequency. Where HD = 400mg/kg, MD = 200mg/kg, LD = 100mg/kg.

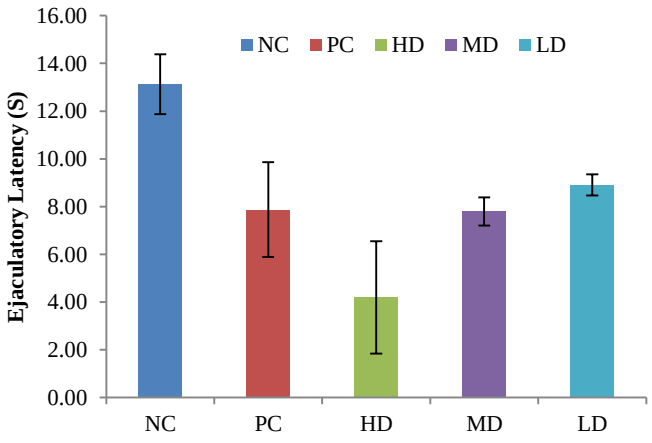

Figure 5: The effect of the extract on the Ejaculatory Latency
Where HD = 400 mg/kg, MD = 200 mg/kg, LD = 100 mg/kg

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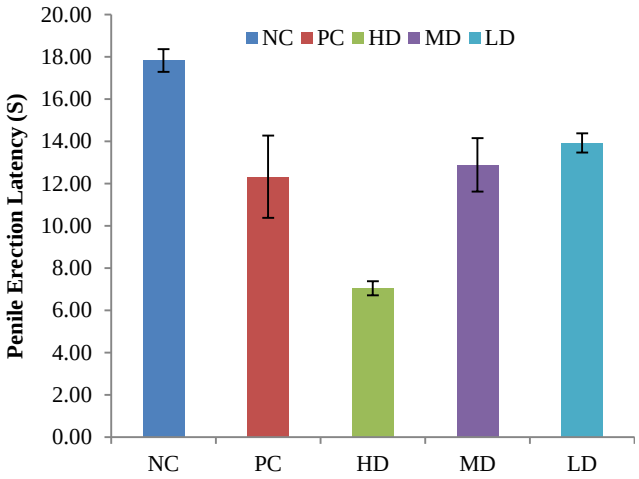

Figure 6: The effect of the extract on the Penile Erection Latency. Where HD = 400 mg/kg, MD = 200 mg/kg, LD = 100 mg/kg

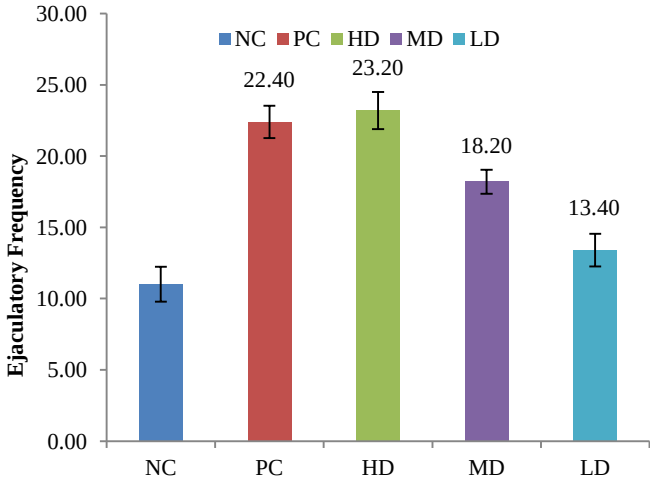

Figure 7: The effect of the extract on the Ejaculatory Frequency**.** Where HD = 400 mg/kg, MD = 200 mg/kg, LD = 100 mg/kg

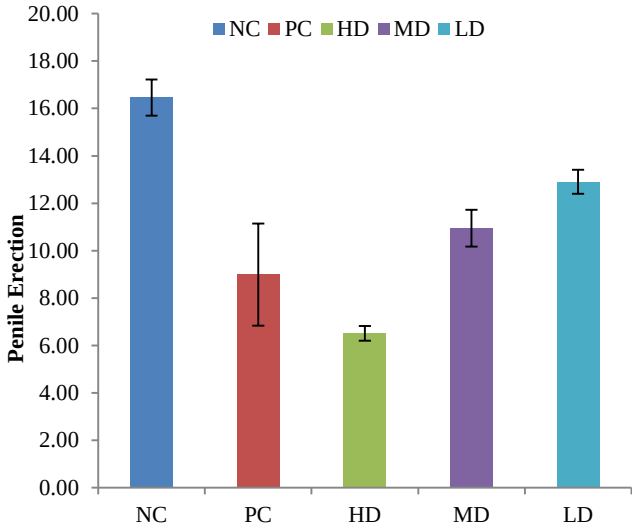

Figure 8: The effect of the extract on the Penile Erection. Where HD = 400 mg/kg, MD = 200 mg/kg, LD = 100 mg/kg

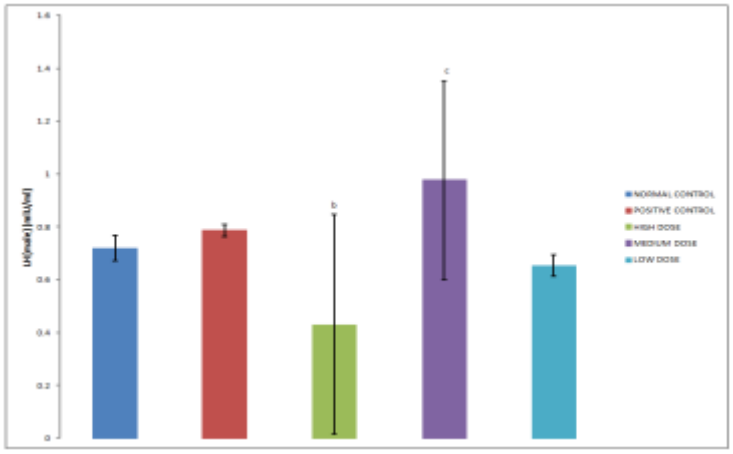

Figure 9: The Effect of *Eremomastax Speciosa* Leaf Extract on Luteinizing Hormone. Where High Dose = 400 mg/kg, medium dose = 200 mg/kg, Low dose = 100 mg/kg

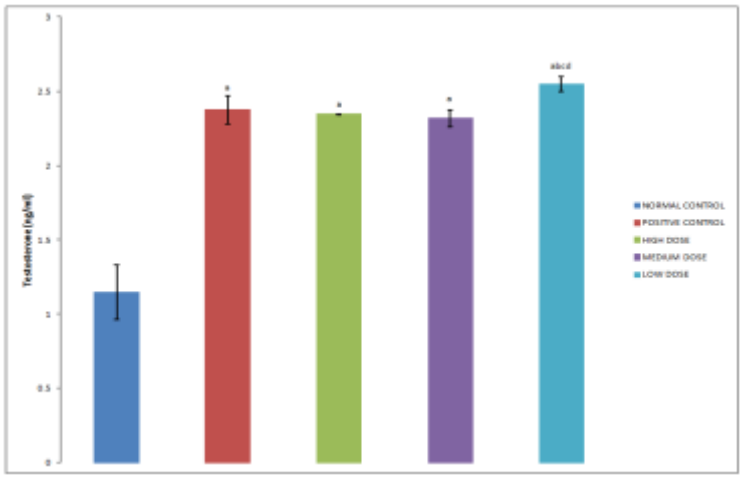

Figure 10: The Effect of *Eremomastax speciosa* Leaf Extract on Testosterone. Where High Dose = 400 mg/kg, medium dose = 200 mg/kg, Low dose = 100 mg/kg

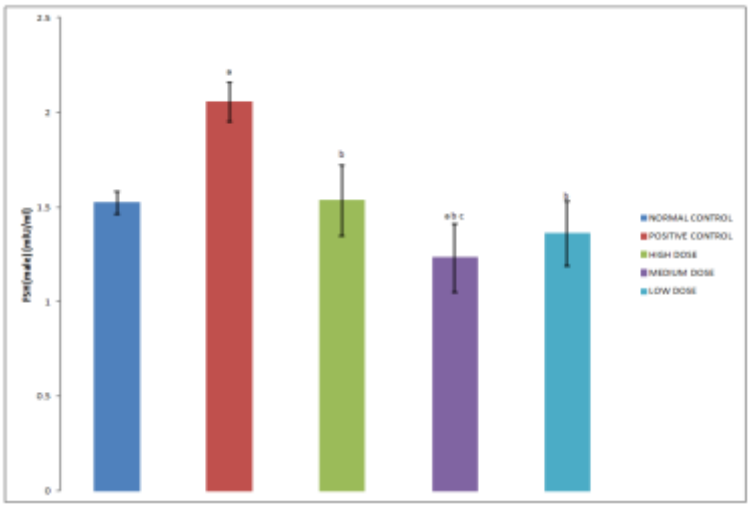

Figure 11: The Effect of *Eremomastax speciosa* Leaf Extract on Follicle Stimulating Hormone. Where High Dose = 400 mg/kg, medium dose = 200 mg/kg, Low dose = 100 mg/kg.

Conclusion

The results showed that the ethanol leaf extract of *Eremomastax speciosa* may improve sexual behaviour in male rats. It was also observed that the extract caused an increase in serum testosterone level thereby suggesting an aphrodisiac activity. The effects of this extract may be attributed to its phytochemical constituents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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