Medicine & Clinical Science

Original Article

Effects of a Stem-Bark Aqueous Extract of *Massularia accuminata* (Rubiaceae) on the Sexual Activity of Hyperlipidemia-Induced Sexually Impaired Albino Male Rats

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- Received Date: 10 May 2026
- Accepted Date: 20 May 2026
- Publication Date: 28 May 2026

Keywords
hyperlipidemia; sexual impairment; M. accuminata; antioxidant; androgen/ neurotransmitter; phytocompounds.

Abstract

The stem-bark of Massularia accuminata (RUBIACEAE) is used in Eyumojock Sub-division of Cameroon to improve on strength and energy during sexual activity. Its aphrodisiac properties have earlier been reported. The traditional meal of this same people called "nfoh or eru" comprises about 15%palm oil which is likely to provoke hyperlipidemia and subsequently sexual impairment. The present study was aimed at inducing hyperlipidemia in young male rats and evaluating the pro-sexual effects of the aqueous extract of the stem-bark of this plant. Nine-weeks feeding of 6 weeks old male albino rats with a high lipid diet (HLD) (15% Palm oil) resulted in hyperlipidemia, compared to their control counterparts. Subsequent evaluation of their sexual activity also revealed a significant decrease in sexual desire, disorders of ejaculation, as well as a drop in sexual potentiality. They were partitioned into 5 groups of 7 rats each and treated as follows: Group 1(Control 1): normal diet only; Group 2 (Control 2): HLD only; Group 3(Control 3): high lipid diet + distilled water (SI+DW); Group 4(Experimental 1): high lipid diet +Viagra (SI+Viagra); Group 5(Experimental 2): high lipid diet + M. a 100 (SI+Ma100); Group 6 (Experimental 3): high lipid diet +Ma 500 (SI+Ma500). Animals treated with the aqueous extract of the stem-bark of M. accuminata showed improved sexual activity and decreased hyperlipidemia. The aqueous extract of the stem-bark of M. accumunata has the potential to remedy hyperlipidemia-induced male sexual deficiencies either through antioxidant mechanisms or androgen/neurotransmitter syntheses thanks to its phyotocompounds including sterols, polyterpenoids, flavonoids, saponins, tannins and alkaloids.

Introduction

Since time immemorial, men and women have employed all means to develop, preserve or regain their own sexual capacities and to stimulate and satisfy their partner's desire. This is because in human life, sexual relationships remain the most important of all human, social and biological relationships [1]. However, some men still face the problem of repeatedly being unable to perform a sexual function effectively or have a disorder that interferes with their full sexual response cycle [2]. This pathology is referred to as male sexual dysfunction (MSD) and could come from various origins such as personal life styles (chronic alcohol abuse, cigarette smoking), androgenic deficiency, ageing, psychological disorders, side effects of some psychiatric medications like antidepressants, as well as chronic medical conditions like diabetes and hyperlipidemia (Dyslipidemia) [3,1]. MSD could take different forms, such as disorders of desire (libido); erectile dysfunction (ED); disorders of ejaculation marked by delayed ejaculation and recurrent ejaculation with minimum sexual stimulation that occur before, during, or shortly after the penetration; and increase libido (sexual desire and arousal), sexual potentiality (effectiveness of erection) and sexual pleasure or orgasm [4] Dyslipidemia, characterized by elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and/or decreased high-density lipoprotein cholesterol (HDL-C), contributes to atherosclerotic plaque formation in vascular beds, including the penile arteries. This impairs vasodilation and penile perfusion, leading to erectile dysfunction. Several studies have reported a high prevalence of dyslipidemia among patients with ED, especially in those with underlying metabolic disorders like type 2 diabetes mellitus (T2DM) [5]. Hypercholesterolemia has been associated with ED by impairing endothelium-dependent relaxation; through the oxidation of LDL, the

citation: Egbe B. Besong, Nchegang Benjamin, Gilbert A, Kamanyi A. Effects of a Stem-Bark Aqueous Extract of Massularia accuminata (Rubiaceae) on the Sexual Activity of Hyperlipidemia-Induced Sexually Impaired Albino Male Rats. Med Clin Sci. 2026;8(2):61

major causative cholesterol of the impaired relaxation response; and via a chain reaction that produces superoxide radicals and functional impairment of endothelial nitric oxide synthase (eNOS), which is responsible for the functional impairment in the early stages of hypercholesterolemia [6]. Studies have also shown that arterial stenosis and occlusion caused by hyperlipidemia could be attributed to the advanced-stage mechanism of ED induced by hyperlipidemia. Hyperlipidemia may damage man's erectile function at an early stage by affecting the endothelial cells and smooth muscles of the penis and the peripheral nerves for penile erection [7]. Several options exist in treating erectile dysfunction. One and the most commonly used is pharmacological method using the phosphodiesterase-5 inhibitors. However, promising emerging pathways and mechanisms for ED treatment are rising to clinical prominence, including the Rho-kinase and JAK-STAT pathways, nitric oxide and dopamine neurotransmitters, and calcium-regulated ion channels. These alternative targets offer potential for managing ED, particularly in cases where comorbid conditions like cardiovascular disease and diabetes are present [8]. Another treatment option is the non-pharmacological interventions (NPIs) which include electrical stimulation (ES), exercise, low-intensity extracorporeal shockwave therapy (Li-ESWT), acupuncture-based therapies (ABT) and hyperbaric oxygen therapy (HOT) [9]. These two approaches and others have the disadvantages of being invasive, very costly and thus, inaccessible and unaffordable for the majority of the populations within Sub-Saharan Africa [10], including Cameroon. In addition, these substances are non-tolerable to most sufferers and the secondary effects they induce are not easily endurable. These reasons and more motivate these populations to prefer the traditional medicine using local plants or plant parts [1]. Apart from dietary therapy and drug therapy aiming at hyperlipidemia, the traditional Chinese medicine therapy and gene therapy are two promising approaches to the treatment of ED caused by hyperlipidemia [7].

The use of herbal medicine to treat ailments in Africa is quite old and today, herbal medicine has become a global alternative and complementary treatment to most ailments globally. This has resulted in an increase in interest on research on plant-derived medicine. The stem-bark of *Massularia accuminata* (RUBIACEAE) is traditionally used in Eyumojock Sub-division, Manyu Division, South-West Region of Cameroon to improve strength and energy during sexual activity. It is an area bordered by the Crossed-Rivers State of the Federal Republic of Nigeria and where there is high consumption of palm oil in a traditional meal called "eru" in which palm oil constitutes about 15% of its 1 kilogram. Also, no data exists on the prevalence of MSD within this locality; meanwhile, sex-enhancing properties of *Massularia accuminata* have been demonstrated by Yakubu et al. [11], thus, confirming its folk use by the population of the Eyumojock Sub-Division. The present study was therefore aimed at inducing hyperlipidemia (dyslipidemia) in young male rats and evaluating the pro-sexual effects of the aqueous extract of the stem-bark of this plant on this animal model.

Materials and Methods

Chemicals and/or Products

Diazepam (Farbe Firma PVT.LTD, INDIA), Ketamine (Rotex Medica, Tritau, GERMANY), penicillin G and sildenafil citrate (Viagra) (Pfizer Inc, USA); estradiol and progesterone (Sigma Chemicals, USA); chloroform (Sigma-Aldrich / Merck KGaA, Germany) Total Cholesterol, Triglycerides and HDL-Cholesterol assay kits (US Biological, Salem, Massachusetts, USA) and NaCl (Bell Pharma Pvt Ltd: Mahim, Mumbai, India) which were all purchased and handled under recommended conditions until used.

Plant material

Fresh stems of Massularia accuminata were harvested from the tropical rainforest of Mamfe, South-West Region of Cameroon, under the guide of a local traditional healer and poacher who confirmed the plant's identity based on its local vernacular name. A full branch and an attached flower of the plant were carefully preserved in a local newspaper and taken to the National Herbarium Yaounde for authentication, where a voucher of the specimen is found. Meanwhile, the stem-bark was carefully removed, chopped into smaller pieces, air-dried under shade for about one month and ground using an electric grinder. Two hundred and fifty grams (250g) of the powder were introduced into 3000ml of distilled water and kept for 72 hours, while occasionally agitating mechanically. This was followed by filtration first using the Laboratory test sieve (ENDOCOTTS LTD, ENGLAND) with 38µm aperture and then Whatman Paper No1 before evaporating the filtrate in an oven (DHG-9023A, China) at 45°-50°C to obtain 43.12g of a black dry residue, giving a 17.25% yield of extraction.

Part of the extract was submitted to the Laboratory for Plant and Organic Chemistry of the Department of Chemistry, University of Buea for phytochemical screening. Meanwhile, administrative doses were determined following directives from traditional healers and screening tests.

Animals

Raising of Animals

Animals used were rats of the Wistar Strain of either sex raised in the Animal house of the Animal Biology and Conservation Department of the Faculty of Science, University of Buea (Cameroon) under standard conditions of temperature, humidity and light (12H cycle). They were given free access to water and laboratory diet. International guidelines for the use of laboratory animals in biochemical research as recommended by the University of Buea council of Animal health (UBAC, 1985) were respected.

Preparing estrous females

A total of 30 females obtained from the breed of the Animal House of the Department of Animal Biology and Conservation, Faculty of Science of the University of Buea, were starved for 24 hours and prepared for surgical operation. The process of ovariectomy and induction of estrus was done following the technics and procedure described in our previous studies [12,1,13]

Induction of hyperlipidemia (Dyslipidemia) and sexual impairment

Induction of hyperlipidemia

Young male albino rats were weaned at 21 days old and kept in cages separate from females. At the age of 6 weeks, they were partitioned into 2 dietary groups: 1 group called A of about 100 rats was subjected to a high-lipid diet (HLD) (High calorific diet, 1365Kcal) comprised of 15% palm oil and other nutrients, and the other group called B of about 20 rats was subjected to a standard Laboratory diet (Table 1). The number of calories were determined by multiplying the grams of macronutrients by their energy value (4 kcal/g for protein/carbs, 9 kcal/g for fat/lipid and 7 Kcal/g for alcohol). They were fed with this diet for a period of 9 weeks.

***Table 1**: Composition of a high-lipid diet (HLD) and a standard laboratory diet*

NUTRIENT	STANDARD DIET (g)	HIGH-LIPID DIET (g)
Corn flour	668.70	562.15
Fish flour	102.70	84.95
Roasted soy-bean flour	208.50	180.10
Palm oil	1.10	150.00***
Vitamin	1.10	1.10
Salt	10.30	10.30
Bone flour	10.30	10.30
TOTAL	**1000.00**	**1000.00**
TOTAL CALORIES (Kcals)	**4,972**	**5,559.3**

At the end of the 9th week (105th day) of feeding with either diet, about 20 HLD rats and 10 normal diet rats were randomly selected, sacrificed and their blood collected into anticoagulant-free test-tubes. It was kept for about 12 hours after which the supernatant was collected and put into new clean test-tubes. It was then centrifuged for 15 minutes at 2500 rpm. The serum triglycerides, LDL-Cholesterol and Total cholesterol levels were assayed using enzymatic kits and standardized reagents. In each case, a blank solution was prepared to help calibrate or standardize the spectrophotometer. The procedure for the assay of the various serum lipid components was as described in the manual and summarized below.

(i) Total cholesterol assay

❖ The blank

This was prepared by pipetting 1000µl of the reagent into a test-tube to which 10µl of distilled water (DW) were added subsequently.

❖ The standard

One thousand microliters (1000µl) of the reagent were introduced into a test-tube, followed by the addition of 10µl of the standard.

❖ The sample

Using the micropipette, 1000µl of the reagent were put into a test-tube and 10µl of serum added.

- The contents of each test-tube were mixed and incubated at 37oC for 10 minutes.
- To determine the Optical Density (OD) of each entity, each mixture was transferred into a 1cm thick assay bath which was then placed in a spectrophotometer and the OD read at 500nm, within 60 minutes.
- This enabled us measure the absorbance of the standard and serum against the reagent blank.
- Using their respective absorbance, the concentration of total cholesterol in the standard and the serum (sample) were obtained using the relationship:

$$\text{Total Cholesterol}(\text{mg/dl}) = \frac{\text{ÄA Sample}}{\text{ÄA Std/Cal}} \times \text{Concentration of Std/Cal}(\text{mg/dl})$$

Where, ΔA Sample= Absorbance (OD) of Sample

ΔA Std/Cal= Absorbance (OD) of Standard/Calculated

(ii) Triglycerides assay

❖ The blank

This was prepared by pipetting 1000µl of the reagent into a test-tube to which 10µl of distilled water (DW) were added subsequently.

❖ The standard

One thousand microliters (1000µl) of the reagent were introduced into a test-tube, followed by the addition of 10µl of the standard.

❖ The sample

- Using the micropipette, 1000µl of the reagent were put into a test-tube and 10µl of serum added.
- The contents of each test-tube were mixed and incubated at 37°C for 10 minutes.
- Like with total cholesterol, each mixture was transferred into a 1cm thick assay bath which was then placed in a spectrophotometer and the OD read at 500nm, within 60 minutes.
- This enabled us measure the absorbance of the standard and serum against the reagent blank.
- Also, using their respective absorbance, the concentration of triglycerides in the standard and the serum (sample) were obtained using the relationship:

$$\text{Triglycerides}(\text{mg/dl}) = \frac{\Delta\text{A Sample}}{\Delta\text{A Std/Cal}} \times \text{Concentration of Std/Cal}(\text{mg/dl})$$

Where, ΔA Sample= Absorbance (OD) of Sample

ΔA Std/Cal= Absorbance (OD) of Standard/Calculated

(iii) High-Density Lipoprotein-Cholesterol (HDL-Cholesterol) assay

This comprised 2 phases: precipitation and cholesterol analyses

(a) Precipitation

❖ ***Standard***

Macro: 500µl of the standard were put into a test-tube and 1000µl of undiluted precipitant added

Semimicro: This comprised of 200µl of the standard plus 500µl of diluted precipitant (diluted 4:1with DW)

❖ ***Sample***

Macro: 500µl of serum and 1000µl of undiluted precipitant were respectively put in a test-tube

Semimicro: Prepared by pipetting 200µl of serum and 500µl of diluted precipitant (diluted 4:1 with DW), respectively.

The content of each test-tube were hen mixed and allowed for 10 minutes at 25oC

They were then centrifuged for 10 minutes at 4000rpm. After centrifugation, the clear supernatant of each test-tube was separated from the precipitate within 60 minutes.

(b) Cholesterol determination

❖ The blank: It was comprised of 1000µl of blank only, pipetted into a test-tube

❖ The standard: Here, 1000µl of reagent and 100µl of supernatant standard were respectively put into a test-tube

❖ The sample: It was made up of 1000µl of reagent and 100µl of supernatant serum put into a test-tube

- The contents of each tes-tube were mixed and incubated for 10 minutes at 37oC.
- The OD (absorbance) of each entity was then read at 500nm within 60 minutes.
- With the help of their respective absorbance, the concentration of HDLs in both the standard and the serum were determined using the relationship:

$$\text{HDL}(\text{mg/dl}) = \frac{\Delta\text{A Sample}}{\Delta\text{A Std/Cal}} \times \text{Concentration of Std/Cal}(\text{mg/dl})$$

(iv) Determination of Low-Density Lipoprotein (LDL)-Cholesterol concentration

The concentration of LDL was calculated using the Friedewald formula:

$$\text{LDL}\,(\text{mg}/\text{dl}) = \text{TotalCholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL}$$

Sexual impairment (SI)/Erectile dysfunction (ED) screening.

To determine whether the animals were sexually impaired, each HLD male was paired with an estrous female, from day 106 to day 110 of feeding. The sexual activity of the male was evaluated daily following the procedure described in our previous studies [12,1,13] while feeding with the HLD continued. Compared to the control group, animals were considered sexually impaired (SI) when they showed minimum (25%) reduction in sexual activity based on the criteria used in one of our previous study [1].

Partitioning and treatment of animals

On day 107, males that showed low libido, poor sexual arousal, poor sexual vigor and poor sexual performance obtained from the preceding section were then grouped into 5 groups of 7 rats each to constitute groups 2,3,4,5 and 6 of the experimental design; while animals of group 1 comprised the NLD males. Each group was treated as follow:

Group 1(Control 1): normal diet only

Group 2 (Control 2): HLD only

Group 3(Control 3): high lipid diet + distilled water (SI+DW)

Group 4(Control 4): high lipid diet +Viagra (SI+Viagra)

Group 5(Experimental1): high lipid diet + M. a 100 (SI+Ma100)

Group 6 (Experimental 2): high lipid diet +Ma 500 (SI+Ma500)

Substances were administered orally using the oropharyngeal cannula daily and at about 18 hour Cameroon time.

Evaluation of sexual activity

Sexual activities were evaluated on days 107, 114, 121 and 128 of treatment with either substance while the same methods used in screening males for ED and evaluating sexual behavior in other rat models, described in our previous studies were repeated here [14,15,12,1,13]. Briefly, each normal lipid and high lipid fed male was paired separately to a receptive female, 30 minutes after receiving either substance. Observations were conducted in the dark phase (as from 20:00 Cameroon time) of the light-dark cycle under dim light and very quiet conditions. Each test session was considered ended when mount latency (ML) and post-ejaculatory interval (PEI) were 20 minutes. The following performance parameters were assessed: mount, intromission, penile licking and ejaculation, from which the following indices were determined or calculated:

1. **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 or libido of the male. It is often expressed or measured in seconds.
2. **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.
3. **Intromission latency:** 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.
4. **Intromission frequency (IF):** this represents the number of intromissions preceding an ejaculation. Like intromission frequency, it measures the degree of erection.
5. **Ejaculation latency (EL):** it is the time interval from the introduction of the female to the first ejaculation. It ranges from a few seconds to minutes and determines the efficiency of copulation in rats.
6. **Postejaculatory interval (PEI):** it is the time interval between an ejaculation and the next first mount. In rats, it generally ranges from 6 to 10 minutes. It measures the refractory period that comes after ejaculation.
7. **Mean intromission interval (MII) or inter-copulatory efficiency (ICE):** it was calculated as ejaculation latency divided by intromission frequency. It measures both the degree of erection and the efficiency of copulation.
8. **Percentage ejaculation (PE):** Calculated using the formula:

$$\text{PE} = \frac{\text{Number of rats that ejaculate}}{\text{Number of rats in the group}} \times 100$$

Plasma lipid concentrations of triglycerides, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and total cholesterol were assessed using standardized kits and following the procedure described by the manufacturer

Animal termination

Female animals were terminated under humane conditions as prescribed by UBIACUC, while males were terminated through overdose of anesthesia followed by cervical dislocation.

Statistical Analyses

Values were expressed as Mean±SEM. Mean values were calculated for each animal and quantitative comparison between groups established from those means. Oneway Analysis of Variance (ANOVA) followed by Duncan test was done using SPSS for windows version 20.0. Significant levels were tested at $P<0.01$, $P<0.05$ or $P<0.001$.

Results

Effects of the high lipid diet (HLD) on the serum lipid profile of albino male rats

Subjection of 6 weeks old male albino rats to a 15% palm oil rich diet (HLD) for 9 weeks resulted in hypercholesterolemia, hypertriglyceridemia, a significant ($p<0.01$) increase in HDL-cholesterol and a significant ($p<0.05$) decrease in LDL-cholesterol in their serum, compared to their counterparts fed on a standard laboratory diet (Table 2).

Table 2: *Serum concentrations of TC, Triglycerides, HDL-Cholesterol and LDL-Cholesterol recorded in rats fed on a HLD for 9 weeks.*

Serum lipid (mg/dl)	Normal diet	HLD
Total cholesterol (TC)	85.48±09.56	180.41±14.27**
Triglycerides	74.20±08.51	144.62±11.77**
HDL-Cholesterol	23.12±05.11	63.22±07.55**
LDL-Cholesterol	48.34±07.31	18.78±03.77**

Values are Mean± SEM; HLD: high lipid diet; HDL: high density lipoprotein; LDL: low density lipoprotein; **=p<0.01, compared to normal diet animals

Effects of a HLD on the sexual activity of albino male rats

Prolonged exposure of normal albino male rats to a HLD resulted in significant ($p<0.01$) increase in mount, intromission and ejaculatory latencies, mean intromission and post ejaculatory intervals and significant ($p<0.05$) decreases in mount frequency, intromission frequency, percentage ejaculation and penile licking (Tables 3 and 4).

Effects of the aqueous extract of the stem-bark of *M. accuminata* on the serum lipid profile of HLD-Fed (SI) albino male rats

Subjection of HLD-fed (SI) albino male rats to a 21-day treatment with the aqueous extract of the stem-bark of *M. accuminata* resulted in a significant ($p<0.01$) decrease in serum concentrations of HDL-Cholesterol, Total Cholesterol and Triglycerides and a significant increase in LDL-Cholesterol in extract-treated animals, compared to all control groups, with the 500 mg/kg dose appearing more efficient (Table 5).

Effects of the aqueous extract of the stem-bark of *M. accuminata* on the sexual activity of HLD-fed (SI) albino male rats.

Effects on mount latency (ML) and mount frequency (MF)

Following the prolonged feeding of young albino male rats with a HLD for 9 weeks, there was a significant ($p<0.05$) increase in their ML values and a significant decrease in their MF values. As shown in Tables 6 and 7, treatment of these HLD (SI) male rats with the aqueous extract of the stem-bark of *M. accuminata*, provoked a significant ($p<0.05$) decrease in mount latency (ML) and a significant ($p<0.001$) increase in mount frequency (MF) in animals that received either dose, from day 1 through day 21 compared to those that did not receive any substance (control 2) and distilled water-treated (control 3) animals which recorded a non-significant ($p<0.05$) increase in this parameter. Extract-treated animals recorded effects similar to those treated with the standard drug (Viagra) and produced 3 ejaculations, compared to 2 ejaculations recorded in control 2 and control 3 animals (Table 7).

Table 3: *Mount latency (ML)(s), intromission latency (IL)(s), mount frequency (MF) and intromission frequency (IF) values recorded in albino male rats subjected to 9 weeks feeding with a HLD.*

Treatment	Parameter			
	ML (s)	IL (s)	MF	IF
Normal diet	124.33±18.17	133.63±17.30	38.65±03.17	27.35±04.08
HLD	194.63±23.37**	293.43±24.37**	10.55±01.51*	05.22±00.85*

Values are Mean± SEM; HLD: high lipid diet; ML: Mount latency (s); IL: intromission latency (s); MF: mount frequency; IF: intromission frequency; *=$p<0.05$, compared to normal diet animals
**=$p<0.01$, compared to normal diet animals

Table 4: *Ejaculation latency (EL)(s), post ejaculatory interval(PEI)(s), percentage ejaculation (PE) (%), mean intromission interval (MII)(s) and penile licking (PL) values recorded in albino male rats subjected to 9 weeks feeding with a HLD.*

Treatment	Parameter				
	EL (s)	PEI (s)	PE (%)	MII (s)	PL
Normal diet	847.61±27.70	462.71±21.55	81.71±07.91	42.91±03.64	10.13±01.23
HLD	1159.71±45.28**	647.88±33.45**	27.30±01.80**	203.32±17.39**	02.74±0.11**

Values are Mean± SEM; HLD: high lipid diet; EL: Ejaculation latency (s); PEI: post ejaculatory interval(s); PE: percentage ejaculation (PE) (%); MII: mean intromission interval (MII)(s); PL: penile licking
*=$p<0.05$, compared to normal diet animals; **=$p<0.01$, compared to normal diet animals

Table 5: *Serum lipid profile of HLD-Fed (SI) albino male rats treated with the aqueous extract of the stem-bark of M. accuminata.*

Treatment/Group (n=7)	Serum lipid profile (mg/dl)			
	HDL-Cholesterol	LDL-Cholesterol	Total Cholesterol	Triglycerides
Control 1: Normal diet only	48.34±04.31	20.12±05.11	85.48±09.56	74.20±08.51
Control 2:HLD (SI) only	16.31±03.38	57.18±07.28	176.41±11.37	145.45±10.27
Control 3: SI+DW	16.78±03.77	56.66±07.55	174.21±11.27	146.33±10.36
EXP 1: SI+ Viagra	18.55±03.17	54.22±07.35	175.64±11.30	144.23±10.40
EXP 2: SI+ Ma100	40.39±07.80**	27.78±03.96**	142.23±10.40**	115.62±10.58**
EXP 3: SI+ Ma500	37.16±07.43**	30.14±03.52**	132.41±10.54**	109.84±11.17**

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired
**=$p<0.01$, compared to control 3 animals

Table 6: *Mount latency (ML) values recorded in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata.*

Treatment/Group (n=7)	Mount latency (ML)(s)			
	Day 1	Day 7	Day 14	Day 21
Control 1: Normal diet only	120.63±21.24ab	122.44±17.31ab	118.11±12.56ab	123.25±18.11ab
Control 2:HLD (SI) only	180.63±21.24ac	182.44±17.31ac	178.11±12.56ac	183.25±18.11ac
Control 3: SI+DW	179.77±16.22ac	183.71±18.36ac	185.66±21,42ac	184.88±14.22ac
EXP 1: SI+ Viagra	155.68±14.44d***	135.55±13.63e***	110.15±09.75f***	84.28±06.32g***
EXP 2: SI+ Ma100	160.33±09.28d***	150.17±13.33h***	120.44±08.71f***	106.28±07.22i***
EXP 3: SI+ Ma500	150.77±12.11d***	140.16±13.23d***	115.26±08.65j***	90.47±06.41k***

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; on the same row, values accompanied by the same letter are not significantly different; on the same column, values accompanied by the same letter are not significantly different; ***=p<0.001 compared to control 3.

Table 7: *Mount frequency (MF) values recorded in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata.*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Mount frequency (MF)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	40.40±03.14	30.50±02.31	24.33±03.20	17.30±01.79
	Control 2:HLD (SI) only	10.00±01.51	11.16±01.25	12.35±01.34	13.76±01.11
	Control 3: SI+DW	11.26±01.76	11.82±01.52	11.91±01.36	12.84±01.32
	EXP 1: SI+ Viagra	30.26±03.45^{a*}	37.42±04.66^{b*}	35.62±03.96^{b*}	42.33±03.67^{c*}
	EXP 2: SI+ Ma100	19.76±02.28^{b*}	25.61±03.73^{c*}	28.15±02.69^{c*}	34.66±03.77^{d*}
	EXP 3: SI+ Ma500	24.63±03.18^{c*}	28.36±03.44^{c*}	32.11±04.40^{c*}	38.37±02.93^{d*}
S2	Control 1: Normal diet only	37.70±03.74	28.21±02.33	26.24±03.27	19.43±01.46
	Control 2:HLD only	11.10±01.31	11.45±01.15	12.65±01.27	12.80±01.17
	Control 3: SI+DW	11.35±01.09	11.68±01.22	11.75±01.20	11.91±01.22
	EXP 1: SI+ Viagra	35.31±03.65^{a*}	39.42±03.56^{a*}	39.92±03.36^{a*}	44.23±03.27^{a*}
	EXP 2: SI+ Ma100	23.82±02.57^{b*}	26.37±02.77^{b*}	31.22±02.83^{b*}	34.88±03.52^{b*}
	EXP 3: SI+ Ma500	28.33±03.24^{b*}	32.26±03.84^{b*}	35.56±03.96^{b*}	39.47±02.75^{b*}
S3	Control 1: Normal diet only	25.32±02.24	27.13±02.64	24.17±03.60	17.48±01.72
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	35.81±03.24^{a*}	40.82±03.75^{a*}	40.97±03.43^{a*}	43.38±03.87^{a*}
	EXP 2: SI+ Ma100	24.79±02.66^{b*}	28.40±02.53^{b*}	30.58±02.25^{b*}	32.63±03.39^{b*}
	EXP 3: SI+ Ma500	30.42±03.34^{b*}	35.32±03.24^{c*}	38.38±03.74^{c*}	40.57±02.82^{c*}

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; S: ejaculatory series

Within each ejaculatory series: on the same row, values accompanied by the same letter are not significantly different; on the same column, values accompanied by the same letter are not significantly different; *=p<0.05 compared to control 3.

Table 8. *Intromission latency (IL) (s) values in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Treatment/Group (n=7)	Intromission latency (IL)(s)			
	Day 1	Day 7	Day 14	Day 21
Control 1: Normal diet only	130.63±18.20	132.44±15.25	128.11±11.26	130.32±16.55
Control 2:HLD (SI) only	287.43±19.24	301.46±19.31	306.51±19.56	315.68±18.27
Control 3: SI+DW	289.77±16.62a**	303.51±18.46b**	305.77±22.42b**	314.63±17.22b**
EXP 1: SI+ Viagra	165.68±13.14b**	145.53±10.33c**	115.17±09.55d**	90.68±06.53e**
EXP 2: SI+ Ma100	180.36±14.28c**	160.13±13.43d**	130.46±08.22e**	110.28±07.08f**
EXP 3: SI+ Ma500	160.74±12.13d**	148.26±11.23e**	120.55±08.37f**	94.58±06.71h**

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia acccuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; on the same row, values accompanied by the same letter are not significantly different; on the same column, values accompanied by the same letter are not significantly different; **=p<0.01 compared to control 3.

Effects on intromission latency (IL) and intromission frequency (IF)

Like with mount, HLD-fed young male rats recorded significantly ($p<0.05$) high ML and low IF values, compared to those fed on a standard laboratory diet for the same duration. However, administration of the aqueous extract of the stem-back of *M. accuminata* in these HDL-induced sexually impaired male rats led to a significant ($p<0.05$) decrease in IL and a significant increase in IF in a dose-dependent manner, compared to animals that did not receive any substance and those treated with distilled water (control 2 and control 3) (Tables 8 and 9). The extract induced significant ($p<0.05$ and $p<0.01$) effects that were similar to those of the standard drug (Viagra), compared to the control 3 animals.

Effects on ejaculation latency (EL) and post-ejaculatory interval (PEI)

Prolonged feeding of young male albino rats with a HLD resulted in a significant ($p<0.01$) increase in EL and PEI compared to the normal diet animals. According to Tables 10 and 11, subsequent treatment of these HLD-induced sexually impaired animals with the aqueous extract of the stem-bark of *M. aacumuinata* caused a significant ($p<0.05$) decrease in EL and PEI values both within each ejaculatory series and from day 1 through day 21of the treatment, compared to those that did not receive any substance and those treated with distilled water (control 2 and control3) where EL values did not significantly ($p<0.01$) change with the duration of treatment (Table 10).

Table 9. *Intromission frequency (IF) values in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Intromission frequency (IF)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	25.35±03.18	20.52±02.11	15.18±01.17	08.42±01.63
	Control 2:HLD (SI) only	06.10±00.55	06.37±00.67	07.35±00.78	07.47±00.84
	Control 3: SI+DW	06.26±00.76	06.59±00.52	06.91±00.56	07.25±00.32
	EXP 1: SI+ Viagra	20.33±02.45^{a*}	27.16±02.71^{b*}	30.47±02.77	37.17±02.54
	EXP 2: SI+ Ma100	14.48±00.28^{b*}	17.23±03.73^{bc*}	22.23±01.69	27.38±03.47
	EXP 3: SI+ Ma500	19.38±00.78^{c*}	22.65±00.87^{c*}	26.13±01.22	32.69±02.54
S2	Control 1: Normal diet only	20.50±02.47	21.17±02.40	16.34±01.51	10.71±01.38
	Control 2:HLD only	06.33±00.47	06.45±00.85	06.65±00.97	06.80±00.87
	Control 3: SI+DW	06.35±00.59	06.68±00.52	06.75±00.35	06.91±00.86
	EXP 1: SI+ Viagra	28.21±02.65^{a*}	32.27±02.54^{a*}	33.04±02.37^{a*}	38.23±02.97^{a*}
	EXP 2: SI+ Ma100	18.42±02.19^{b*}	20.44±02.74^{b*}	23.19±02.13^{b*}	29.55±02.31^{a*}
	EXP 3: SI+ Ma500	24.63±02.60^{b*}	26.96±02.54^{b*}	29.56±02.66^{b*}	36.70±02.75
S3	Control 1: Normal diet only	18.64±02.18	19.39±01.64	17.38±01.60	10.77±00.88
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	30.64±02.43^{a*}	34.73±02.63^{a*}	34.95±02.41^{a*}	38.40±03.57^{a*}
	EXP 2: SI+ Ma100	18.58±01.54^{b*}	22.69±01.73^{b*}	24.80±02.31^{b*}	30.74±02.29^{a*}
	EXP 3: SI+ Ma500	27.42±02.17^{b*}	30.32±02.44^{b*}	30.41±02.16^{b*}	36.43±02.67^{b*}

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: Massularia accuminata 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; S: series; SI: sexually impaired; S: ejaculatory series.
Within each ejaculatory series: on the same row, values accompanied by the same letter are not significantly different; on the same column, values accompanied by the same letter are not significantly different; *=$p<0.05$ compared to control 3.

Table 10. *Ejaculation latency (EL) (s) values recorded in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Ejaculation latency(EL)(s)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	859.66±20.70	856.74±20.67	850.23±22.37	846.33±20.40
	Control 2:HLD (SI) only	1199.71±22.66	1197.31±22.77	1196.33±22.51	1193.26±22.16
	Control 3: SI+DW	1197.38±22.43	1196.24±22.54	1196.17±22.80	1193.51±22.30
	EXP 1: SI+ Viagra	1110.45±20.60^{a**}	910.47±22.93^{b**}	813.46±18.87^{c**}	680.23±19.16^{d**}
	EXP 2: SI+ Ma100	1160.22±20.83^{b**}	970.21±22.85^{c**}	835.64±19.74^{d**}	743.28±19.67^{e**}
	EXP 3: SI+ Ma500	1140.19±20.19^{c**}	950.63±22.80^{d**}	825.72±18.28^{e**}	720.35±19.80^{f**}

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Ejaculation latency(EL)(s)			
		Day 1	Day 7	Day 14	Day 21
S2	Control 1: Normal diet only	849.28±19.78	845.44±20.77	852.17±20.37	846.65±21.35
	Control 2:HLD only	1194.15±22.41	1194.44±22.83	1195.28±22.51	1192.60±22.25
	Control 3: SI+DW	1195.17±22.80	1196.34±22.70	1196.47±22.07	1194.71±22.37
	EXP 1: SI+ Viagra	1100.37±20.52^{a**}	900.42±21.63^{b**}	820.46±19.34^{c**}	675.77±19.21^{d**}
	EXP 2: SI+ Ma100	1140.55±20.09^{b**}	940.71±21.65^{c**}	830.32±19.36^{d**}	700.31±19.82^{e**}
	EXP 3: SI+ Ma500	1115.19±20.45^{c**}	920.63±20.48^{d**}	820.60±18.62^{e**}	690.52±19.27^{f**}
S3	Control 1: Normal diet only	848.65±22.33	845.28±22.63	840.77±22.51	833.56±22.37
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	1101.36±20.15^{a**}	937.71±21.47^{b**}	831.18±19.43^{c**}	681.85±18.52^{d**}
	EXP 2: SI+ Ma100	1135.40±20.59^{b**}	933.40±21.24^{bc**}	827.60±19.71^{d**}	701.31±19.73^{e**}
	EXP 3: SI+ Ma500	1110.39±20.66^{c**}	915.63±20.25^{d**}	815.76±18.20^{e**}	685.18±19.67^{f**}

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; S: ejaculatory series.
Within each ejaculatory series: on the same row, values accompanied by the same letter are not significantly different; on the same column, values accompanied by the same letter are not significantly different; **=p<0.01 compared to control 3.

Table 11: *Post ejaculatory interval (PEI) (s) values in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Post ejaculatory interval (PEI) (s)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	459.66±21.70	456.74±21.67	452.23±21.37	443.33±21.40
	Control 2:HLD only	547.46±21.45	540.81±21.53	550.23±20.77	546.33±20.48
	Control 3: SI+DW	552.73±21.45	550.74±21.47	545.36±20.32	548.18±20.95
	EXP 1: SI+ Viagra	497.78±18.70^{a**}	438.62±18.67^{b**}	405.16±18.37^{c**}	385.14±18.40^{d**}
	EXP 2: SI+ Ma100	487.37±18.82^{b**}	458.55±18.22^{c**}	418.82±18.63^{d**}	390.26±18.41^{e**}
	EXP 3: SI+ Ma500	477.50±18.70^{c**}	455.77±18.49^{cd**}	410.28±18.64^{de**}	375.17±18.20^{f**}
S2	Control 1: Normal diet only	444.28±21.36	395.81±18.23	412.23±20.37	433.58±18.42
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	440.78±18.27^{a**}	410.62±18.36^{b**}	380.16±18.66^{c**}	370.14±18.87^{d**}
	EXP 2: SI+ Ma100	460.37±18.82^{b**}	438.57±18.33^{c**}	408.82±18.97^{d**}	390.88±18.74^{e**}
	EXP 3: SI+ Ma500	450.47±18.51^{c**}	425.77±18.12^{d**}	402.47±18.64^{e**}	380.17±18.53^{f**}
S3	Control 1: Normal diet only	18.64±02.18	19.39±01.64	17.38±01.60	10.77±00.88
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	30.64±02.43^{a*}	34.73±02.63^{a*}	34.95±02.41^{a*}	38.40±03.57^{a*}
	EXP 2: SI+ Ma100	18.58±01.54^{b*}	22.69±01.73^{b*}	24.80±02.31^{b*}	30.74±02.29^{a*}
	EXP 3: SI+ Ma500	27.42±02.17^{b*}	30.32±02.44^{b*}	30.41±02.16^{b*}	36.43±02.67^{b*}

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; S: ejaculatory series.
Within each ejaculatory series: on the same row, values accompanied by the same letter are not significantly different; on the same column, values accompanied by the same letter are not significantly different; **=p<0.01 compared to control 3.

Effects on percentage ejaculation (PE)

Like with other parameters presented earlier, feeding of 6 weeks old male albino rats with a HLD for a period of 9 weeks resulted in a decrease in the number of animals that ejaculated as demonstrated by low PE values recorded in those animals, compared to the normal diet animals. In a similar manner, when these HLD-induced sexually impaired rats were treated with either dose of the aqueous extract of *M. accuminata*, they recorded increase in PE values compared to the control 2 and control 3 animals, though in a non-significant ($p<0.05$) manner (Table 12).

Effects on mean intromission interval (MII) (s) or inter-copulatory efficiency

Mean intromission interval (MII) increased significantly in 6 weeks old male albino rats, following their exposure to a HLD for nine weeks, compared to their counterparts fed on a standard laboratory diet. It can be seen in Table 13 that MII values recorded in these animals after treatment with the aqueous extract of *M. accuminata* decreased significantly ($p<0.01$) within each ejaculatory series and with the duration

Table 12: *Percentage ejaculation (PE) (%) values recorded in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Percentage ejaculation (PE) (%)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	85.71±07.17	85.71±07.17	85.71±07.17	100.00±0.00
	Control 2:HLD only	28.57±01.55	28.57±01.55	42.86±01.78	42.86±01.78
	Control 3: SI+DW	28.57±01.55	28.57±01.55	42.86±01.78	42.86±01.78
	EXP 1: SI+ Viagra	$57.14±05.45^{a*}$	$71.43±06.71^{b*}$	$85.71±08.77^{c*}$	$100.00±0.00^{d*}$
	EXP 2: SI+ Ma100	$42.86±02.28^{b*}$	$57.14±02.73^{c*}$	$71.43±06.58^{d*}$	$85.71±08.49^{e*}$
	EXP 3: SI+ Ma500	$57.14±02.78^{a*}$	$57.14±05.83^{a*}$	$71.43±06.22^{b*}$	$100.00±0.00^{c*}$
S2	Control 1: Normal diet only	85.71±07.17	85.71±07.17	100.00±0.00	100.00±0.00
	Control 2:HLD only	26.33±01.83	26.45±01.85	36.71±01.97	36.80±01.87
	Control 3: SI+DW	26.35±00.59	26.68±00.52	36.75±01.35	37.21±01.86
	EXP 1: SI+ Viagra	$71.43±02.65^{a*}$	$71.43±01.54^{a*}$	$85.71±07.17^{b*}$	$85.71±07.17^{b*}$
	EXP 2: SI+ Ma100	$42.86±02.28^{b*}$	$57.14±02.73^{c*}$	$71.43±06.58^{d*}$	$85.71±08.49^{bc*}$
	EXP 3: SI+ Ma500	$57.14±05.45^{c*}$	$57.14±05.45^{c*}$	$85.71±07.17^{dc*}$	$85.71±08.49^{dc*}$
S3	Control 1: Normal diet only	85.64±02.36	79.45±01.80	97.38±03.60	100.00±0.00
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	$57.14±05.45^{a*}$	$71.43±06.71^{b*}$	$85.71±08.77^{c*}$	$100.00±0.00^{d*}$
	EXP 2: SI+ Ma100	$42.86±02.28^{b*}$	$57.14±02.73^{c*}$	$71.43±06.58^{d*}$	$85.71±08.49^{e*}$
	EXP 3: SI+ Ma500	$57.14±02.78^{a*}$	$57.14±05.83^{a*}$	$71.43±06.22^{cd*}$	$100.00±0.00^{d*}$

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; S: ejaculatory series.

Within each ejaculatory series: on the same row, values accompanied by the same letter a not significantly different; on the same column, values accompanied by the same letter are not significantly different; *=$p<0.05$ compared to control 3.

Table 13: *mean intromission interval (MII) (s) values recorded in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	mean intromission interval (MII) (s)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	33.91±02.11	41.75±02.76	55.99±02.21	100.51±07.22
	Control 2:HLD only	199.83±11.46	187.91±11.39	162.72±11.24	159.74±11.38
	Control 3: SI+DW	199.50±11.40	181.49±11.33	173.11±11.17	164.62±11.62
	EXP 1: SI+ Viagra	$55.50±02.45^{a**}$	$33.52±02.42^{b**}$	$26.70±02.78^{c**}$	$18.30±02.17^{d**}$
	EXP 2: SI+ Ma100	$82.85±04.93^{b**}$	$56.31±02.77^{c**}$	$37.58±02.81^{d**}$	$27.15±02.08^{e**}$
	EXP 3: SI+ Ma500	$57.00±02.66^{c**}$	$41.97±02.53^{d**}$	$31.60±02.78^{e**}$	$22.06±02.92^{f**}$
S2	Control 1: Normal diet only	41.43±02.74	39.94±02.27	52.15±02.35	35.05±03/44
	Control 2:HLD only	188.63±11.23	185.18±11.39	179.74±11.28	175.38±11.39
	Control 3: SI+DW	188.19±11.39	179.09±11.22	177.25±11.56	172.90±11.32
	EXP 1: SI+ Viagra	$38.99±02.11^{a**}$	$27.90±02.14^{b**}$	$24.82±02.69^{b**}$	$17.68±02.38^{c**}$
	EXP 2: SI+ Ma100	$61.89±03.21^{b**}$	$46.02±02.88^{c**}$	$35.81±02.95^{d**}$	$23.74±02.45^{e**}$
	EXP 3: SI+ Ma500	$45.27±02.43^{c**}$	$35.62±02.66^{d**}$	$27.76±02.01^{e**}$	$18.82±02.03^{f**}$

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	mean intromission interval (MII) (s)			
		Day 1	Day 7	Day 14	Day 21
S3	Control 1: Normal diet only	45.49±02.34	43.59±02.25	46.61±02.81	77.40±03.76
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	35.95±02.11^{a**}	27.00±02.83^{b**}	23.78±02.16^{b**}	17.76±02.07^{c**}
	EXP 2: SI+ Ma100	61.11±02.97^{b**}	41.14±02.77^{c**}	33.37±02.54^{d**}	22.81±02.66^{cc**}
	EXP 3: SI+ Ma500	40.50±02.89^{c**}	30.20±02.78^{d**}	26.83±02.06^{e**}	18.81±01.99^{cc**}

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massulatia accuminata* 500mg/kg b.w; SI: sexually impaired; S: ejaculatory series.
Within each ejaculatory series: on the same row, values accompanied by the same letter a not significantly different; on the same column, values accompanied by the same letter are not significantly different; **=p<0.01 compared to control 3.

Table 14: *Penile licking (PL) values recorded in HLD (SI) male rats treated with the aqueous extract of the stem-bark of M. acccuminata*

Ejaculatory series(S)	TREATMENT (GROUP) (n=7)	Penile licking (PL)			
		Day 1	Day 7	Day 14	Day 21
S1	Control 1: Normal diet only	08.00±0.98	07.25±0.87	08.19±0.88	09.56±0.08
	Control 2:HLD only	02.88±0.09	02.98±0.09	02.67±0.37	03.48±0.28
	Control 3: SI+DW	02.76±0.11	02.83±0.11	02.96±0.44	03.35±0.43
	EXP 1: SI+ Viagra	04.62±0.88	07.78±0.13	09.00±01.22	11.54±01.63
	EXP 2: SI+ Ma100	04.31±0.28	05.22±0.98	06.15±0.27	08.67±0.55
	EXP 3: SI+ Ma500	03.11±0.75	07.23±0.79	09.17±0.88	10.36±01.21
S2	Control 1: Normal diet only	06.75±0.11	07.45±0.19	08.02±0.72	08.78±0.66
	Control 2:HLD only	02. 95±0.23	02.98±0.77	02.85±0.34	02.98±0.87
	Control 3: SI+DW	02.76±0.24	02.83±0.71	02.96±0.78	03.23±0.54
	EXP 1: SI+ Viagra	04.83±0.65	06.92±0.13	09.23±01.16	11.97±01.39
	EXP 2: SI+ Ma100	04.55±0.35	05.22±0.77	06.65±0.27	08.93±0.72
	EXP 3: SI+ Ma500	04.65±0.75	07.76±0.79	09.28±0.64	09.36±01.41
S3	Control 1: Normal diet only	06.92±0.47	07.45±0.19	08.27±0.93	08.98±0.73
	Control 2:HLD only	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	Control 3: SI+DW	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	EXP 1: SI+ Viagra	05.09±0.65	06.92±0.31	07.23±01.27	09.97±01.47
	EXP 2: SI+ Ma100	04.55±0.35	05.52±0.34	06.70±0.63	08.98±0.47
	EXP 3: SI+ Ma500	04.85±0.75	06.76±0.44	08.28±0.64	09.58±01.63

Values are Mean±SEM; DW: distilled water; EXP: experimental; HLD: high lipid diet; Ma100: *Massularia accuminata* 100 mg/kg b.w; Ma500: *Massularia accuminata* 500mg/kg b.w; SI: sexually impaired; S: ejaculatory series

of treatment.

Effects on Penile licking (PL)

Prolonged exposure of 6 weeks old male albino rats to a HLD for 9 weeks equally resulted in a non-significant ($p<0.05$) decrease in PL values recorded in them, compared to those recorded in animals fed with a standard laboratory diet. Their subsequent treatment with the aqueous extract of *M. accuminata* stem-bark resulted in a non-significant increase in PL values, compared to control 2 and control 3 animals (Table 14).

Discussion

Nine-week feeding of 6 weeks old albino male rats with a high lipid diet (HLD) (15% Palm oil) resulted in hyperlipidemia (dyslipidemia) as demonstrated by the hypercholesterolemia, hypertriglyceridemia, significant increase in LDL-cholesterol and a significant decrease in HDL-cholesterol recorded from the assay of the lipid profile in their serum, compared to their counterparts fed on a standard laboratory diet. Subsequent evaluation of their sexual activity when paired with estrous females also revealed a significant decrease in sexual desire (libido) translated by high ML and IL values, an important degree of erectile dysfunction (ED) marked by significantly low IF, PL and higher MII values; disorders of ejaculation noticed from high ejaculation latency values and low percentage ejaculation values, as well as a drop in sexual potentiality (effectiveness of erection) seen in low IF values, compared to the animals fed with the normal diet. These findings demonstrate that the HLD induced sexual impairment in our animals.

The qualitative phytochemical screening of our extract revealed the presence of compounds such as sterols, polyterpenoids, flavonoids, saponins, tannins and alkaloids, which have each been reported to be involved in the different phases/aspects of the male sexual cycle consisting of repeated series of mounts and intromission, accompanied by penile licking and culminating

with ejaculation, followed by a refractory period called post ejaculatory interval [16,15], in different animal models.

Our study investigated the actions of *M. accuminata* stem-bark aqueous extract on disorders of sexual desire. The extract induced a significant decrease in mount latency (ML) and a significant increase in mount frequency (MF) in animals that received either dose, from day 1 through day 21 compared to those that did not receive any substance (control 2) and distilled water-treated (control 3) animals which recorded a non-significant increase in this parameter. These 2 parameters are indicators of sexual motivation since only a sexually excited or motivated male rat will mount on a receptive female, and the shorter the ML (and higher MF values), the higher the level of sexual excitement. This shows that there was a reduction in the hesitation time of males towards receptive females and increased penile erection. These pro-erectile properties of the extract could be due to the actions of Polyterpenoids revealed in our extract. These bioactive principles probably act by inducing changes in the levels of neurotransmitters, modulating the actions of these neurotransmitters on their target cells or by increasing androgen levels [17]. Similarly, in a dose-dependent manner, both doses induced a significant increase in mount (MF) and intromission frequencies (IF). These increases are of physiological interest since these sexual performance parameters constitute important criteria for the determination of libido or sexual desire and penile erection [18]. While the number of mount (MF) reflects sexual motivation, increase in the number of intromission (IF) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated [14].

Also, we investigated the actions of the aqueous extract of the stem-bark of *M. accuminata* on disorders of erection or erectile dysfunction (ED) induced by hyperlipidemia in albino male rats. It should be recalled that the plant M. accuminara is widely known amongst the people of Eyumojock Sub-Division for its sexual potency and its aphrodisiac properties have earlier been reported by Yacubu et al [11]. The traditional meal of this same people called "nfoh or eru" comprises about 15%palm oil, which would likely induce hyperlipidemia in them. As stated earlier, dyslipidemia, characterized by elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and/or decreased high-density lipoprotein cholesterol (HDL-C), contributes to atherosclerotic plaque formation in vascular beds, including the penile arteries. This impairs vasodilation and penile perfusion, leading to erectile dysfunction. Hypercholesterolemia has also been associated with ED by impairing endothelium-dependent relaxation; through the oxidation of LDL, the major causative cholesterol of the impaired relaxation response; and via a chain of reaction that produces superoxide radicals and functional impairment of endothelial nitric oxide synthase (eNOS), which is responsible for the functional impairment in the early stages of hypercholesterolemia [19]. Furthermore, studies have revealed that arterial stenosis and occlusion caused by hyperlipidemia could be attributed to the advanced-stage mechanism of ED induced by hyperlipidemia. Again, hyperlipidemia may damage man's erectile function at an early stage by affecting the endothelial cells and smooth muscles of the penis and the peripheral nerves responsible for penile erection [7].

ED can be attributed to oxidative stress caused by an imbalance between excessive free radicals and antioxidant defenses in the penile cavernous tissues. The presence of an increased number of free radicals contributes to oxidative damage, inflammation, and impairment of nitric oxide signaling pathways, ultimately affecting erectile function. Addressing oxidative stress and maintaining a balance between free radicals and antioxidants is crucial in managing and preventing ED. Phenols and flavonoids have been widely recognized for their potent antioxidant activities [20]. These compounds have the ability to scavenge free radicals and reduce oxidative stress [21,22], which is known to contribute to the development of erectile dysfunction (ED). By reducing oxidative stress, phenols and flavonoids may help protect the cavernous tissues and maintain normal erectile function [23,24]. Intromission translates effective erection which is sexual potency and that was significantly reduced in HLD-fed animals as seen in the IF values recorded within the first ejaculatory series on day 1 of treatment. Like mount, intromission is androgen-dependent and triggered by nitric oxide (NO) [25,26,27]. It has been established by Escrig *et al.* [28, Jesse, *et al.*] that high serum cholesterol levels impair androgen function and reduce nitric oxide synthase (NOS) through the action of calveolins [26]. These impaired androgen function and reduced NOS activity could therefore be responsible for the low IF values recorded in HLD-fed rats on day 1. Nevertheless, IF values increased dose-dependently in animals treated with either dose of the *M. accuminata* from day 1 to day 21which suggests that the plant extract could exert its action independently of endothelins, a function that could be attributed to saponins which have been reported to also possess antioxidant properties [29], even though the antioxidant properties of the extract were not evaluated in this study. However, the extract provoked a significant decrease in serum concentrations of LDL-Cholesterol, Total Cholesterol and Triglycerides as well as a significant increase in HDL-Cholesterol, compared to all control groups, with the 500 mg/kg dose appearing more efficient, which are actions related to antioxidant potentials of some medicinal plants. Both doses of the plant-stem-bark aqueous extract induced an increase in PL and a decrease in MII (ICE) throughout the treatment period compared to the distilled water-treated group, actions that were similar to the Viagra-treated group. PL and MII (ICE) are also important indices for evaluating the effect of drug administration on erectile function [30]. This further shows that the aqueous extract of the stem-bark of *M. ccuminata* increases potency. The increased penile erection noticed with both doses suggest the involvement of NO in the intervention [31].

In our study, we equally assessed the actions of the *M. accuminata* stem-bark aqueous extract on the ejaculatory disorders induced by hyperlipidemia in albino male rats. We noticed that both doses of the plant extract reduced ejaculation latency and showed a decreased PEI, effects that were similar to those of Viagra. PEI is the time interval between one ejaculation (ejaculatory series) and the first mount of the next ejaculatory series. It is a measure of the strength available for sexual activity and together with the number of ejaculatory series, translates endurance during sexual activity. It is an important parameter for evaluating the effect of administered extract on erectile function. PEI is regarded as an indicator of potency, libido and potential to recover from exhaustion after the first ejaculatory series. This aspect of sexual activity was seriously affected in HLD animals as can be seen in those treated with DW which gave only 2 ejaculatory series/day throughout the treatment period. The decrease in post-ejaculatory interval (PEI) observed in male rats indicates an increase in libido, potency and a faster recovery rate from exhaustion during sexual intercourse. A shorter PEI suggests enhanced sexual vigor and a higher likelihood of

engaging in subsequent sexual activity [32]. A great reduction in PEI was observed in rats treated with the *M. accuminata* extract like in those administered with sildenafil citrate. This indicates that these treatments resulted in shorter recovery times between ejaculations, reflecting better penile erections and improved copulation. Decreased PEI could be as a result of enhanced potency and libido, and/or reduced exhaustion in the first ejaculatory series This data clearly supports the role of the stem-bark of *M. accuminata* in enhancing male sexual function and thus confirms its folk use by men to ensure endurance during sexual activity [33]. The ability of the plant extract to provide endurance during male sexual activity could again be attributed to the presence of phenols [34]. Percentage ejaculation(PE), which shows the number of animals that ejaculate per ejaculatory series, in addition to EL, are indicative of the pro-ejaculatory effects of a product. In the control 2 and control 3 animals, PE was not up to 50% throughout the treatment period compared to those that received either the plant extract or the standard drug. Amongst the compounds found in our extract are polyterpenoids, a compound belonging to the terpens family. This family of compounds has been reported to possess pro-ejaculatory activities in rats [35,36,].

Conclusions

Hyperlipidemia can be induced by prolonged intake of a high lipid diet and in turn, leads to erectile and ejaculatory deficiencies. The stem-bark aqueous extract of *M. accuminata* has the potential to remedy hyperlipidemia-induced male sexual deficiencies either through antioxidant mechanisms, androgen or neurotransmitter syntheses thanks to its phyocompounds including sterols, polyterpenoids, flavonoids, saponins, tannins and alkaloids. However, there is need for another study to investigate the antioxidant properties of this plant extract to better understand the mechanism used in its actions against hyperlipidemia-induced sexual impairment.

Acknowledgements

The authors are grateful to the administration of the University of Buea and the Faculty of Science in particular for the Laboratory space and other materials provided to them in the course of this study. They also acknowledge the assistance provided by Mr Mbaabe Felix of the Faculty of Science Teaching Laboratories which enabled them to realize this study..

Conflicts of Interest

The authors declare no conflicts of interest related to this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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