Trop J Nat Prod Res, October 2021; 5(10):1852-1858 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Aphrodisiac Effect of Methanol Hypocotyls Extracts of *Borassus aethiopum Mart.* in Male Wistar Rats

Mansur A. Ramalan*, Adamu B. Shuaibu, Umar S. Abdussalam, Abdullahi H. Yaro

Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria

ARTICLE INFO

Article history:
Received 19 March 2021
Revised 15 June 2021
Accepted 23 October 2021
Published online 02 November 2021

ABSTRACT

Borassus aethiopum is a tropical plant found in most parts of West Africa. The fruits and hypocotyl are used in traditional herbal medicine for the treatment of various diseases (malaria, bronchitis, skin infections and infertility) and nutritional purposes. In many parts of Nigeria especially in Northern Nigeria, it is used as an aphrodisiac agent in males. This study evaluated the aphrodisiac effect of the methanol extract of the hypocotyl of *Borassus aethiopum* in male Wistar rats. Adult male Wistar rats were divided into five groups and administered orally with the plant extract at doses of 250, 500 and 1000 mg/kg body weights. Sildenafil citrate (5 mg/kg) and distilled water (1 mL/kg) were administered orally, and served as positive and negative controls, respectively. The acute toxicological evaluation of the plant extract was based on behavioural changes and mortality. Sexual behavioural parameters (mounting and intromission frequencies, and ejaculatory latencies) were observed on days 1, 7 and 28. Serum testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and gonadotrophin releasing hormones (GnRH) were measured. Acute toxicity studies of the plant extract did not show any behavioural changes in the animals and there was no mortality. The extract significantly increased mounting and intromission frequencies ($p<0.05$) and decreased mounting and intromission latencies in a dose-dependent manner on days 1, 7 and 28 ($p<0.05$). It also prolonged ejaculatory latency. The extract was also noted to increase GnRH, serum testosterone and gonadotrophins. The results suggest that the methanol hypocotyl extract of *Borassus aethiopum* possess aphrodisiac properties.

***Keywords*:** Aphrodisiac, *Borassus aethiopum*, Male sexual behaviour, Testosterone, Traditional medicine.

Introduction

The term sexual dysfunction describes a range of functional disorders of the reproductive system.[1] It encompasses clinical syndromes that impair sexual functioning such as sexual aversion, sexual desire disorders, sexual arousal disorders, sexual pain and vaginismus (in females), erectile dysfunction and premature ejaculation.[2,3] From ancient times, man has device ways and methods to improve his sexual desire and performance. This has led to the emergence of substances known as aphrodisiacs, which are drugs, scents, food or devices that can increase sexual drive, function or libido.[3-5] Aphrodisiacs exert these effects by altering the levels of the neurotransmitters or specific sex hormones such as testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[5-7]
Globally, sexual dysfunction has been estimated to affect about 30% of men[8] and 43% of women.[9] Studies in Nigeria reported the prevalence of sexual dysfunction as 57.4% in men above 35 years of age,[10] 38.5% and 63.9%[11] in men aged between 31- 40 and 61-70 years respectively and 84% among women attending antenatal clinic at Aminu Kano Teaching Hospital in Kano.[12]
Currently, the management of sexual dysfunction includes; the use of various drugs, cognitive behavioural therapy, vacuum devices, surgical procedures and penile implants.[13]
However, due to the unwanted sides effects like cardiovascular adverse effects (palpitations, tremors and headaches),[13] skin irritation, [13] dermatitis, [13] and bleeding,[13] there is therefore the need to identify newer products with the therapeutic efficacy that are equally safe to be utilized in managing the problem of sexual dysfunction. Additionally, the costs and the efficacies of some of the treatment modalities are debatable, there is, therefore, the demand for new drugs that can be used in the management of sexual dysfunction.[5] Plants are a very rich, but untapped potential source of vegetable drugs that can be useful aphrodisiac agents. [5] However, only a few of these plants have been subjected to detailed scientific investigation.[4-6] Plants with reported aphrodisiac properties include *Carica papaya*,[14] *Garcinia kola*,[14] *Cajanus caja*,[15] *Cyperus esculentus*,[15] *Phoenix dactylifera*[15]and *Cocos nucifera*.[15]
Borassus aethiopum Mart. is a tropical plant species that is widely grown in many parts of Africa especially in the West African region of Nigeria, Togo, Niger, Ghana and Benin republic. It is also commonly known as the African Fan Palm or African Palmyra Palm because of its huge fan-shaped leaves.[16,17] The plant belongs to the Order Arecales, and the family: Arecaceae.[18] In Nigeria, different ethnic groups identify this plant by different names, it is called *Giginya* in Hausa,[19] *Agbon Oludu* in Yoruba,[19] *Ubiri* in Igbo[19] and *Kemelutu* in Kanuri.[19] The leaves and roots of the plant are used for the treatment of headache,[20] impetigo and other skin diseases,[21] epilepsy,[22] stomach parasites, bronchitis, sore throats and asthma,[22] aphrodisiac,[23] treatment of fungal infections[24] and wounds.[25]
Several studies have established its anti-inflammatory activity,[25,26] analgesic, antioxidant effects,[25] anti-malarial, antibacterial activity,[25,26] hypolipidemic[16] and antidiabetic activity.[28]
Borassus aethiopum shoot known as *Muruci*[19] in Hausa has been known to be utilized as an aphrodisiac in many parts of Northern

*Corresponding author. E mail: mmramalan@gmail.com
Tel: +2348036783737

Citation: Ramalan MA, Shuaibu AB, Abdussalam US, Yaro AH. Aphrodisiac Effect of Methanol Hypocotyls Extracts of *Borassus aethiopum Mart.* in Male Wistar Rats. Trop J Nat Prod Res. 2021; 5(10):1852-1858. doi.org/10.26538/tjnpr/v5i10.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Nigeria[19,29] and some parts of West Africa.[23] A previous study has shown that extract of hypocotyl *B. aethiopum* has androgenic properties in rabbits.[19] Adeyina *et al*[30] reported that it can increase fertility in chickens.

Scientific information on the aphrodisiac activity of the hypocotyl of *B. aethiopum* is scanty. This study evaluated the effect of the extract of the hypocotyl of *B. aethiopum* on the sexual function of male Wistar rats.

Materials and Methods

Drugs and assay kits

Sildenafil citrate, ethinylestradiol and progesterone were obtained from Pfizer Pharmaceutical (USA), Novo Nordisk Pharmaceutical (Denmark) and Astra Zeneca Pharmaceutical (UK) respectively. Methanol used for the experiment was supplied by Sigma Aldrich Chemical, USA. The ELISA assay kit for Gonadotropin-releasing more (GnRH), Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone (Finebiotech), were obtained locally from Alpha Laboratories Ltd, Lagos, Nigeria.

Collection and authentication of plant material

The fresh plant was collected between July and August 2019 from Kumbotso town in Kumbotso Local Government Area of Kano State. The plant was identified and authenticated by Dr Yusuf Nuhu of the Department of Plant Sciences, Bayero University, Kano by comparing with the previous specimen (voucher specimen number BUKHAN 276) already deposited at the Herbarium of the Department of Plant Sciences, Bayero University, Kano. A certificate was issued.

Preparation of the extract

The fresh hypocotyl of *B. aethiopum* was detached from the seed after harvesting them from the soil washed and rinsed in clean water. They were air-dried in the laboratory at room temperature for 1 week. The plant material was pulverized with the aid of an electric blender. The powdered plant material (300 g) was extracted with 3 L of 70% methanol by cold maceration for 7 days with occasional stirring to ensure maximum extraction. The resultant mixture was filtered with Whatman No 1 filter paper, to remove the mac and the solvent was allowed to evaporate to dryness over a water bath at 50°C.

The percentage yield of the methanol extract was determined using the formula;

$$\% \text{ Yield} = \frac{\text{weight of extract}}{\text{weight of extract/weight of powdered sample}} \text{x}100$$

Phytochemical screening of methanol hypocotyl extract of B. aethiopum

The phytochemical composition of the extract was determined using the method described by Trease and Evans.[31]

Determination of LD_{50} of the extract

The method of Lorke[34] was used to determine the median lethal dose (LD_{50}). This method is divided into two phases. In phase 1, nine animals were divided into 3 groups of three animals each and treated with the methanol extract of the plant at doses of 10, 100 and 1000 mg/kg body weight. Signs of acute toxicity and death were observed for 24 hours. A second phase of the experiment was carried out, where three (3) fresh animals divided into 3 groups of one rat each were administered with three more doses (1600, 2900 and 5000 mg/kg) of the extract and observed for abnormal behaviour and death. The LD_{50} was determined as the highest dose for which the animals survived.[34] The experiment was carried out for two routes of administration (oral and intraperitoneal) and using two animal species (rats and mice).

Animal groupings and extract administration

Experimental animals

Adult male and female Wistar rats (10-12 weeks of age), weighing 120-180 g were used for the study. They were obtained from the Animal House, Department of Pharmacology and Therapeutics, Bayero University Kano. The rats were maintained in a well-ventilated room, fed standard rodent chow (Vital feed) and permitted free access to water. All experimental animals were kept in clean cages under standard environmental conditions and allowed to acclimatize to the laboratory environment for two weeks before commencement of the experiment. Experimental and animal handling was carried out according to the guide for the care and use of laboratory animals (NIH, 2010) and guidelines of the institutional ethical committee.

The drugs and extract were administered daily using the metal gavaging cannula. The female rats were prepared and made receptive by the sequential administration of ethyl estradiol (100 µg/animal) and progesterone (1 mg/animal), dissolved in olive oil through subcutaneous injections, 48 h and 6 h respectively, before pairing.[4,5]

Ethical approval

Ethical approval was obtained from the College of Health Sciences of the Bayero University, Kano (reference number BUK/CHS/REC/120).

Experimental design

The male rats were randomized into 5 groups of 10 rats each namely; Group I was given distilled water (1 mL/Kg) while groups II, III and IV were given 250, 500 and 1000 mg/kg body weight respectively, of the extract. Group V was given the standard drug - Sildenafil citrate (5 mg/kg). The extract was administered orally at a dose of (250, 500 and 1000 mg/kg), corresponding to (5, 10 and 20%) of the LD_{50}. The dose of Sildenafil (5 mg/kg) body weight was based on a previous study.[5] After days 1, 7 and 28, five rats were isolated from each group, weighed and tested for their sexual behaviour with a receptive female rat that has been artificially brought to oestrous (female animals only allow mating during the oestrous cycle).[32]

Animal sexual behavioural studies

The sexual behavioural studies of the male rats were conducted between 19.00 and 03.00 h under a faint light in the laboratory. The receptive female rats were introduced to the male rats, 30 min after administration of the extract at the respective doses to the male rats in a transparent plastic cage. The female rats were paired with the male rats in all the various doses including controls in the ratio 1:1 (1 female to 1 male). The test was terminated at any point when a male rat fails to show sexual interest. The sexual training for each male was done for 15 min per animal until sexual behaviour was achieved, at the notice of the sexual behaviour the males were exposed to receptive females. All sexual behaviour tests were observed for 30 mins. The observation for mating behaviours commenced after 10 min of placing the paired animals in the cage and was recorded with the aid of a Sony video camera (Model no. DCR-SX20E 1724470) coupled on a tripod stand. Any female animal that does not show receptivity was replaced by another artificially 'heated' female. The females were artificially brought to the oestrous cycle by administration of ethyl estradiol (100 µg/animal) and progesterone (1 mg/animal), dissolved in olive oil through subcutaneous injections, 48 h and 6 h respectively before to pairing.[4,5] The occurrence of events and phases of mating after the video recording were analyzed and the frequencies and phases determined.

Serum preparation

Under chloroform anaesthesia, the animals were euthanized, blood was collected via cardiac puncture using a 5 mL syringe and needle per animal into the appropriately labelled clean sample bottles, lithium heparin (to collect plasma) and non-coagulant (plain), to collect serum. The samples were stored and analyzed in batches.

Estimation of serum hormones

The serum hormones (Testosterone, LH, FSH, and GnRH) concentrations were measured by enzyme-linked immunoassay, using the Rat Testosterone ELISA Kit (Elab Science, Houston Texas, USA) for LH, FSH GnRH and Testosterone) according to the manufacturer's instructions.[33]

Testosterone assay procedure
The serum concentrations of testosterone were determined using the competitive inhibition enzyme immunoassay (ELISA) technique. The microtiter plate provided in the ELISA kit has been pre-coated with goat-anti-rabbit testosterone antibody. Briefly, 10 µL of the Standards or samples were added to each of the 96 wells with 100 µL of testosterone-HRP conjugate reagent (blue colour). Another 50 µL of rabbit anti- testosterone reagent were separately dispensed into each well. The resulting solution was thoroughly mixed for 30 seconds and incubated at 37°C for 90 minutes. The microwells were rinsed and flicked 5 times with distilled water (to remove the unbound testosterone peroxidase conjugate) before dispensing 100 µL of TMB reagent into each well. The resulting solution was mixed gently for 5 seconds. This was later incubated at room temperature for another 20 minutes for the blue colour to develop. The colour development was stopped with the addition of 100 µL of Stop Solution (1 N HCl) to each well and when gently mixed, the colour changed from blue to yellow. The absorbance was read within 15 mins at 450 nm with a microtiter well reader. The intensity of the colour formed was proportional to the amount of enzyme present and was inversely proportional to the amount of unlabeled testosterone in the sample.[33]

Serum LH assay Procedure
Solid-phase enzyme-linked immunosorbent assay (ELISA) was used to measure the concentration of Luteinizing Hormone (LH) in the serum of the rats. The assay system utilized a mouse monoclonal anti-LH antibody for solid-phase microtiter wells) immobilization and another monoclonal anti-LH antibody in the antibody-enzyme (horseradish peroxidase)-conjugated solution. Briefly, sixty microliters of standard, test and control were pipetted into appropriate wells after which 100µl of enzyme-conjugated reagent was taken and then added into each of the wells, and then mixed thoroughly for 30secs and then incubated at room temperature for 45 min. The incubation mixture was removed by washing the content with an ELISA washer. A hundred microliters tetramethylbenzidine (TMB) reagent was pipetted and added into each well, gently mixed for 5 secs and incubated in the dark for 20 mins. Finally, 100 µl of the stop solution was added to each well to stop the reaction. The microplate was then observed for 30 secs for colour development from blue to yellow. The absorbance at 450 nm was read with a microtiter plate reader within 15 min. The absorbance of the test sample was correlated with that of the standard curve to give the concentration of the LH in the serum.[33]

Serum Follicle Stimulating (FSH) Hormone Assay Procedure
The Follicle Stimulating Hormone (FSH) concentration of the rats was measured using solid-phase enzyme-linked immunosorbent assay (ELISA). The principle of the assay requires a mouse monoclonal anti-FSH antibody for solid phase (microtiter) immobilization and horseradish peroxidase-conjugated solution (another monoclonal anti-FSH antibody) in the antibody-enzymes system. Briefly, 60 µL of standard, test and control solutions were pipetted into appropriate wells after which 100 µL of enzyme-conjugated reagent was added into each well and mixed thoroughly for 30 secs. The resulting mixture was incubated at room temperature for 45 min. After the incubation, the mixture was removed by flickering plate contents into a waste container. The microplate was washed with the aid of an ELISA washer. A hundred microliters of TMB reagent was pipetted into each well and gently mixed for 5 secs. This was then incubated in the dark for 20 mins. To stop the reaction, 100 µL of the stop solution was added to each well and observed for 30 secs for colour development from blue to yellow. The absorbance at 450 nm was read with a microtiter plate reader within 15 min. The absorbance of the test sample was correlated with that of the standard curve to give the concentration of the FSH in the rat serum.[33]

Serum Gonadotrophin Releasing Hormone (GnRH) Assay Procedure
Serum Gonadotrophin Releasing Hormone (GnRH) assay was measured using the double-sandwich ELISA technique. The pre-coated antibody is Rat GnRH monoclonal antibody and the detecting antibody is polyclonal antibody biotin labelled. Briefly, the sample is added into ELISA plate wells, then biotinylated Rat GnRH antibody liquid was added to each well (100 µL for each) and allowed to incubate in the dark at 37℃ for 60 min. The resulting mixture was washed out the enzyme-conjugated-conjugate liquid to each well except blank wells (100 µL for each) and incubated in the dark at 37℃ for 30 mins. The ELISA microplate was then washed five times and 100 µL Colour Reagent liquid was added into an individual well (also into a blank well) and allowed to incubate in the dark at 37℃. The reaction was stopped by the addition of a stop solution consisting of 0.16M sulfuric acid.
The concentration of GnRH in the samples was then determined by comparing the O.D. of the samples to the standard curve.[33]

Statistical analysis
Data analysis was carried out using Statistical Package for Social Sciences (SPSS)software (IBM, Version 22.0) (SPSS Inc., Chicago, USA) and data obtained were expressed as Mean ± Standard Error of Mean. The differences between means were analysed using One-way of variance (ANOVA) followed by repeated measure ANOVA. The probability values of ≤ 0.05 were considered statistically significant.

Results and Discussion

Phytochemical constituents of the extract
The phytochemical constituents identified in the methanol hypocotyl extract of *B. aethiopum* include flavonoids, tannins, phenols, saponins, terpenoids, steroids, glycosides and alkaloids while anthraquinones were absent (Table 1).

Acute toxicological study
An acute toxicological study of the extract showed that it was safe up to the maximum dose of 5000 mg/kg. No signs or symptoms of toxicity were observed (Table 2).

The percentage yield of extract
The extraction of 300g of the powdered hypocotyl plant material of *B. aethiopum* yielded 75 g of extract, the percentage yield was thus calculated to be 25% $^{w}/_{w}$.

Effect of B. aethiopum Methanol Extract Hypocotyls on Physical Sexual Behavior of Male Rats
The mounting frequency (MF), intromission frequency (IF), and ejaculation frequencies (EF) were increased on days 7 and 28 as the dose of the extract increases (Table 3 and 4). The MF was significantly increased ($p < 0.05$) at 500 mg/kg and 1000 mg/kg on day 7 and 500 mg/kg on day 28 of the extract administration compared to the negative control. The Intromission frequency was significantly increased ($p < 0.05$) at 500 mg/kg on day 7, 250 mg/kg and 1000 mg/kg on day 28 of extract administration when compared to the negative control. Ejaculatory frequency was similarly significantly increased ($p < 0.05$) at 250 and 500 mg/kg on day 7 and 500 mg/kg on day 28 when compared to the negative control (Tables 3 and 4).
Administration of the methanol extract of *B. aethiopum* extract led to a decrease in the mounting latency of male rats at doses of 500, 1000 mg/kg on days 7, 250 and 1000 mg/kg on day 28. Intromission and Ejaculatory latencies were similarly decreased. This decrease becomes more significant ($p < 0.05$) at doses of 250, and 500 mg/kg on day 7 and 250, 500 and 1000 mg/kg on day 28 of extract administration when compared to the negative control (Tables 3 and 4).

Effect of the Methanol Hypocotyl Extract of B. aethiopum on Hormones levels of male rats
The administration of the methanol extract of *B. aethiopum* significantly ($p < 0.05$) increased the serum Testosterone of the male Wistar rats at 250 and 1000 mg/kg on days 7, 250 and 1000 mg/kg on day 28 when compared to the negative control (Tables 5 and 6).
The serum concentrations of GnRH have significant increased in all extract-treated groups, ($p<0.001$) at 250 and 500 mg/kg on days 7 and 1000 mg/kg on day 28 of administration (Table 5 and 6).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The levels of LH were significantly ($p<0.05$) increased at the dose of 1000 mg/kg on both days 7 and 28 compared to the negative control (Tables 5 and 6).
Serum levels of FSH were demonstrated to have increased significantly ($p<0.05$) at all the doses given (250, 500 and 1000 mg/kg respectively) on days 7 and 28 compared to the negative control (Tables 5 and 6).

Table 1: Phytochemical Constituents Present in the Methanol Hypocotyls Extract of *Borassus aethiopum*

Constituent	Inference
Tannins	+
Alkaloid	+
Flavonoids	+
Glycosides	+
Lipid	+
Steroids	+
Proteins	+
Phenols	+
Saponins	+
Anthraquinones	-

Key: + = Present; - = Absent

Table 2: Median lethal doses of the Methanol Hypocotyls Extract of *Borassus aethiopum* in mice and rats

Species	Route of Administration	LD_{50} values (mg/kg)
Mice	Intraperitoneal	>5000
Mice	Oral	>5000
Rats	Intraperitoneal	>5000
Rats	Oral	>5000

The phytochemical analysis of the extract of *B.* aethiopum revealed the presence of tannins, alkaloids, phenols, flavonoids, glycosides, steroids and saponins. This is similar to the findings in previous studies.[23,28] Among these phytochemicals identified, alkaloids, flavonoids, glycosides and saponins have been reported to play important roles in male sexual function. They help in decreasing oxidative stress of testicular tissues by replenishing antioxidants levels and scavenging free radicals leading to the optimal synthesis of sex hormones and sexual function.[14,35] Zamble *et al*[36] postulated that alkaloids dilate blood vessels, which causes an increase in blood flow to the penile organ and enhances the engorgement of the penis for optimal sexual performance and pleasure.[33] Flavonoids have been described as phenolic substances with the capacity of suppressing reactive oxygen species (ROS) formation, either by inhibition of enzymes or chelating trace elements involved in free radical production, scavenging ROS, and upregulating antioxidant defences.[37] Glycosides are known to specifically inhibit plasma membrane Na-K ATPase activity which promotes the influx of extracellular calcium ions, the ultimate trigger for neurotransmitter as well as hormone release,[38] while tannins can stimulate the synthesis of sex hormones.[39,40] The reported aphrodisiac activity of saponins in plants has been attributed to the membrane permeability and stimulation of the release of luteinizing hormone (LH), thereby promoting the release of testosterone.[41,42] Phenols have been reported to be responsible for the aphrodisiac effect of *Carpolobia*[40] and clove plants.[42] Based on the aforementioned, it can be speculated that *B. aethiopum* could possess some beneficial effects on male reproductive function based on the presence of some of these phytochemical constituents.
Mounting and intromission frequencies are important indicators of libido. Mounting frequency shows the increase in sexual desire and the increase in intromission frequency reflects the activity of penile erection, penile introduction, and the responses which trigger ejaculation.[43] The copulatory behaviour of rodents is usually marked by higher frequencies (mounting, intromission, and ejaculation) and shorter latencies (mounting, intromission, and ejaculation).[41] The ability of the extract to increase the mount, intromission and ejaculation frequencies could imply that the extract enhances libido and potency; and improves sexual performance in the rats. It is an indication that the male rats were aroused. It also reflects enhanced performance, motivation and vigour.[42,43] These findings agree with reports from the previous studies.[4,5,41,45] The significant reduction in mounting, intromission and ejaculatory latencies observed in this study as a result of the effect of the extract is a strong indication that the sexual function of the male rats was enhanced, thereby suggesting an aphrodisiac activity.[4,5,41,45]

Table 3: Effect of the Methanol Hypocotyl Extract of *Borassus aethiopum* on Sexual Behavior of male rats (day 7)

Treatment Group	Parameter					
	MF	IF	EF	ML	IL	EL
Distilled Water	3.2±1.16	2.8±0.86	0.6±0.24	145.2±20.93	158±1.16	163.4±18.57
Sildenafil (5 mg/Kg)	8.6±0.81*	6±1.16	3.6±0.51*	58±12.92	44±1.16*	90.4±5.41*
BA250 mg/kg	4.8±0.37	4.6±0.98	3.2±0.56*	73.0±14.09	62.0±1.16*	84.0±10.85*
BA500 mg/kg	7.0±1.12*	7.3±0.45*	2.8±0.58*	68.0±11.01*	100.0±1.16	94.8±6.99*
BA1000 mg/kg	8.4±1.29*	6.0±1.36	2.2±0.73	40±1.16*	96.3±40.76	124.5±18.02

Values are expressed as mean ± SEM (n = 5), *significant at $p<0.05$ compared with control after one-way ANOVA and Dunnet *post hoc* analysis. MF = mount frequency; IF = intromission frequency; EF = Ejaculation frequency; ML = mount latency; IL = intromission latency; EL = ejaculation latency; BA = Borassus aethiopum

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Effect of the Methanol Hypocotyls Extract of *Borassus aethiopum* on Sexual Behavior of male rats (day 28)

Treatment Group	Parameter					
	MF	**IF**	**EF**	**ML**	**IL**	**EL**
Distilled Water	3.6±1.29	3±1.00	0.8±0.37	136±19.03	152±24.48	170.2±19.1
Sildenafil (5mg/Kg)	7.2±0.73*	8.1±1.16*	3.8±0.58	43±18.16	56±8.89*	86.8±7.00*
BA250mg/Kg	4.8±1.35	6.3±1.58*	3±0.51	66±21.01*	61±8.86*	115.8±19.83
BA500mg/Kg	8±1.06*	4.3±0.58	3±0.71*	78±16.30	49±4.47*	96.2±7.56
BA1000mg/Kg	7±1.36	6.8±1.58*	2.4±1.40	51±8.91*	78±6.97	91.6±9.83*

Values are expressed as mean ± SEM (n = 5), *significant at p<0.05 compared with control after one-way ANOVA and Dunnet *post hoc* analysis. Where MF = mount frequency; IF = intromission frequency; EF = Ejaculation frequency; ML = mount latency; IL = intromission latency; EL = ejaculation latency; BA = *Borassus aethiopum*

Androgens (testosterone and dihydrotestosterone (DHT)) secreted by the gonads (testes) have been known to enhance sexual function by direct and indirect effects on the reproductive organs.[47] It has been earlier reported that androgens are important modulators of male sexual behaviour including erection and libido. These androgens may act both at the central and peripheral nervous system levels. Testosterone administration in animals deficient in the hormone has been reported to enhance sexual function, increase libido, and improves the intensity of orgasm and ejaculation.[30]

The significant effect of the extract on the increased serum testosterone levels may be due to either the direct effect of the phytochemicals on the secretion of the testosterone or locally due to the up-regulation of Leydig cells.[36] Saponins have been found to stimulate the Leydig cells of the testes to directly increase the testosterone production system[48]. In a previous study, the extract of the hypocotyl of *B. aethiopum* has been shown to have androgenic properties.[18]

Gonadotropin-releasing hormone (GnRH), is known to induce spermatogenesis and sexual behaviour through its stimulatory action on the Hypothalamic – Pituitary – Gonadal (H-P-G) axis. Phytochemicals present in the extract could have been responsible for the increased serum concentration of GnRH demonstrated in the current study. Alkaloids have been shown to cause increases in the serum GnRH levels of rabbits.[49] Increases in the behavioural activities of the rats may also lead to enhancement in GnRH secretion. In mammals, following coitus, GnRH release is increased in multiple folds.[50] Pulsatile GnRH therapy in men with idiopathic hypogonadotropic hypogonadism has been very successful in inducing androgen production and spermatogenesis with normalization of the LH-Leydig cell-testosterone axis, and the FSH-Sertoli cell axis[51]. Follicle-stimulating hormone (FSH) is essential for gonadal development and maturation at puberty as well as gamete production.[52] It is a known regulator of testicular development and it also increases spermatogenesis and steroidogenesis.[53]

In addition to its role in follicular development, the FSH prevents apoptosis in spermatogonia and spermatocytes; thus, increasing viability.[50,51] The extract might cause an increase in the levels of the FSH in the rats directly via stimulation of the FSH secreting cells in the anterior pituitary or indirectly through the hypothalamus by stimulating the secretion of GnRH, which could, in turn, have stimulated, the secretion of FSH via the positive feedback loop. It is noteworthy that the secretion of FSH is regulated by the gonadotropic releasing hormone (GnRH) secreted by the hypothalamus.[36]

Luteinizing hormone promotes male reproductive functions by stimulating testosterone production from Leydig cells in the testis[45] and could be one of the reasons for the increase in testosterone in extract-treated animals. The mechanisms for increased LH levels may be via central influences to increase LH production or locally via the stimulation of the LH secretory neurons in the anterior pituitary gland.[43] Another pathway that the extract could have exerted its effect is to increase the secretion of GnRH in the hypothalamus thereby leading to an increase in the LH through positive feedback mechanisms. Centrally, the GnRH stimulates the production of the gonadotrophs (LH and FSH) by upregulating the LH and FSH secreting neurons in the anterior pituitary.[54] Previously, it has been reported in the literature that GnRH is the most potent stimulus for the secretion of the gonadotrophs (LH and FSH).[50]

Table 5: Effect of the Methanol Hypocotyls Extract of *Borassus aethiopum* on Hormonal Profile of male rats (day 7)

Hormone	Treatment groups				
	Distilled Water	Sildenafil (5 mg/kg)	BA250 mg/kg	BA500 mg/kg	BA1000 mg/kg
T(ng/mL)	6.40±1.08	8.40±0.69	9.80±.049*	9.40±0.51	10.80±0.20*
FSH(Miu/mL)	48.40±5.23	60.40±4.52	81.80±6.34*	77.60±6.55*	80.00±2.51*
LH Miu/mL)	72.20±9.16	90.60±15.99	90.60±4.382	87.40±8.65	92.80±5.74*
GnRH(pg/mL)	743.40±9.16	972±25.03*	989.60±27.59**	1000.80±24.22**	928±32.64*

Values are expressed as mean ± SEM (n = 5), *significant at p<0.05 compared with control after one-way ANOVA and Dunnet *Post hoc* analysis. Where BA = *Borassus aethiopum*; FSH = Follicle Stimulating Hormone; GnRH = Gonadotropin Releasing Hormone; LH = Luteinizing Hormone; T = Testosterone

Table 6: Effect of the Methanol Hypocotyls Extract of *Borassus aethiopum* on Hormonal Profile of male rats (day 28)

Hormone	Treatment groups				
	Distilled Water	Sildenafil (5mg/kg)	BA250mg/Kg	BA500mg/Kg	BA1000mg/Kg
T(ng/mL)	5.60±0.75	8.40±0.51	9.20±.058*	8.60±0.51	9.60±0.51*
FSH(Miu/mL)	31.00±2.41	46.00±1.70*	55.40±2.77*	64.00±3.54*	55.20±3.89*
LH (Miu/mL)	55.60±1.47	67.20±4.13	76.00±6.11	73.40±7.56	77.20±3.32*
GnRH(pg/mL)	578.80±31.87	679.20±63.17	896.00±17.30**	811.40±43.60*	907±53.28**

Values are expressed as mean ± SEM (n = 5), *significant at $p<0.05$ compared with control after one-way ANOVA and Dunnet *Post hoc* analysis. Where BA = Borassus aethiopum; FSH=Follicle Stimulating Hormone; GnRH=Gonadotropin Releasing Hormone; LH=Luteinizing Hormone; T= Testosterone

Conclusion

The findings revealed that the methanol hypocotyl extract of *Borassus aethiopum* possesses aphrodisiac activity in rats. This supports its ethnomedicinal use as an aphrodisiac agent in many parts of Northern Nigeria and some parts of West Africa.

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.

Acknowledgements

We appreciate the technical assistance of the staff of the Pharmacology laboratory, Bayero University Kano.

References

1. Gonsalves L, Cottler-Casanova S, Vantreeck K, Say L. Results of A World Health Organization Scoping of Sexual Dysfunction-Related Guidelines: What Exists and What Is Needed. J Sex Med. 2020; 17(12):2518-2521.
2. Mccabe MP, Sharlip ID, Lewis RW, Atalla E, Balon R, Fisher AD, Segraves RT. Incidence and Prevalence of Sexual Dysfunction In Women And Men: A Consensus Statement From The Fourth International Consultation On Sexual Medicine 2015. J Sex Med. 2016; 13(2):144-152.
3. Chen L, Shi GR, Huang DD, Li Y, Ma CC, Shi M, Su BX, Shi GJ. Male Sexual Dysfunction: A Review of Literature On Its Pathological Mechanisms, Potential Risk Factors, And Herbal Drug Intervention. Biomed Pharmacother. 2019; 112(1):108585.
4. Singh R, Ali A, Jeyabalan G, Semwal A. An Overview Of The Current Methodologies Used For The Evaluation Of Aphrodisiac Agents. J A Dis. 2005; 2(2):85-81.
5. Erhabor JO And Idu MD. Aphrodisiac Potentials Of The Ethanol Extract Of *Aloe Barbadensis Mill*. Root In Male Wistar Rats. Bmc Compl Altern Med. 2017: 17(1):1-10.
6. Yakubu, MT, Akanji MA, Oladiji AT. Aphrodisiac Potentials Of The Aqueous Extract Of *Fadogia Agrestis* (Schweinf. Ex Hiern) Stem In Male Albino Rats. A J Androl. 2005: 7(4):399-404.
7. Ajao AA, Sibiya NP, Moteetee AN. Sexual Prowess from Nature: A Systematic Review Of Medicinal Plants Used As Aphrodisiacs And Sexual Dysfunction In Sub-Saharan Africa. S Afr J Bot. 2018: 122(1):342-359.
8. Lotti F And Maggi M. Sexual Dysfunction And Male Infertility. Nat Rev Urol. 2018: 15(5):287-307.
9. Oindi FM, Murage A, Lema V, Mukaindo AM. Association of Female Sexual Dysfunction and Fertility: A Cross-Sectional Study. Fertil Res Pract. 2019; 5(12):1-11.
10. Idung A, Abasiubong F, Ukott I, Udoh S, Unadike B. Prevalence And Risk Factors Of Erectile Dysfunction In Niger Delta Region, Nigeria. Afr H Sci. 2012: 12(2):160-165.
11. Oyelade BO, Jemilohun AC, Aderibigbe SA. Prevalence Of Erectile Dysfunction And Possible Risk Factors Among Men Of South-Western Nigeria: A Population-Based Study. Pan Afr Med J. 2016; 8(24):124.
12. Abdullahi HM, Abdurrahman A, Ahmed ZD, Tukur J. Female Sexual Dysfunction Among Women Attending The Family Planning Clinic At Aminu Kano Teaching Hospital: A Cross-Sectional Survey. Nig J Basic Clin Sci. 2019; 16(1):32-37.
13. Lue TF. Erectile Dysfunction. N Engl J Med. 2000; 342(24):1802-1813.
14. Bayero B. Pharmacological Effects of Methanolic Extract Of *Garcinia Kola (Heckel)* Seed on The Reproductive Profiles of Some Male Experimental Animals. University Of Jos. Unpublished Phd Thesis. 2015.
15. Idakwoji PA, Uzuazokaro MA, Nweje PC, Anyalogbu EA, Okafor SC. Aphrodisiac And Fertility-Enhancing Actions Of 'Kunu Aya' (A Beverage Blend Developed From *Cyperus Esculentus, Phoenix Dactylifera* And *Cocos nucifera*) Via Testosterone-Boosting, Anxiolysis and Inhibition of Key Enzymes Associated with The Erectile Process. W J Pharm Res. 2018: 7(1):139-164.
16. Peprah M. The Anti-Lipidemic Effect of Borassus Aethiopum "Oman Kube" on Individuals With Cardiovascular Disease At The 37 Military Hospital In Accra, Ghana. Kwame Nkrumah University of Science And Technology, Kumasi, Ghana. 2017 Unpublished Msc Dissertation.
17. Salako KV, Moreira F, Gbedomon RC, Tovissode F, Assogbadjo AE, Kakai RLG. Traditional Knowledge and Cultural Importance of *Borassus Aethiopum* Mart. In Benin: Interacting Effects of Socio-Demographic Attributes And Multi-Scale Abundance. J Ethnobiol Ethnomed. 2018; 14(1):36.
18. Zongo O, Nikiéma NW, Kaboré S, Hama C, Sawadogo A, Zoungrana BA, Nikiéma F, Traoré Y, Savadogo A. Evaluation of Nutritional Composition of *Borassus Aethiopum* Mart. Hypocotyl (*Koboula*) Consumed In Burkina Faso. A J Food Sci Technol. 2019; 7(6):215-222.
19. Akinniyi JA, Waziri M, Usman HS. Assessment of The Anabolic Effect of Androgens of The Edible Portion of The Shoot of Giginya Plant (*Borassus Aethiopum* Mart). J Sci Res. 2010; 2(2):362-368.
20. Ziblim IA, Timothy KA, Deo-Anyi EJ. Exploitation And Use of Medicinal Plants, Northern Region, Ghana. J Med Plants Res. 2013; 7(27):1984-1993.

21. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanohoun E, Ahyi Mra, Ekpere J, Tackie AN. Traditional Medicine And Pharmacopoeia: Contribution To The Revision Of Ethnobotanical And Floristic Studies In Ghana. Organization Of African Unity/Scientific, Technical And Research Commission. 2000. 286 P.
22. Tchacondo T, Karou SD, Agban A, Bako M, Batawila K, Bawa Ml, Gbeassor M, De Souza C. Medicinal Plants Use In Central Togo (Africa) With An Emphasis On The Timing. Pharm Res. 2012; 4(2):92-103.
23. Gbesso F, Adjatin A, Dansi AA. Aphrodisiac Properties of Hypocotyls Extracts Of *Borassus Aethiopum* Mart (Arecaceae) Collected In Central Of Benin Republic. Int J Curr Microbiol Appl Sci. 2016: 5(3): 802–814.
24. Salako V, Kégbé A, Chadaré FJ, Amagnide A, Gbedomon R, Agbangla C, Kakai RG. Potential for The Domestication Of *Borassus Aethiopum* Mart., A Wild Multipurpose Palm Species In Sub-Saharan Africa. Genet Resour Crop Evol. 2019; 6(5): 1129–1144.
25. Vincent A, Esumaba SA, Jacob K, Nadratu MB, Fidelis Mk. Development and Evaluation of African Palmyra Palm (*Borassus Aethiopum*) Fruit Flour-Wheat Composite Flour Noodles. Con Food Agric. 2020; 6(1):174-176.
26. Sakande E, Kabre M, Lompo E, Pale JB, Nikiema OG, Nacoulma M, Guissou IP. Anti-inflammatory and Antioxidants Activities of a Fraction L1 of Male Inflorescences of *Borassus Aethiopum* Mart (Arecacaeae). A J Biochem Mol Biol, 2013; 3(1): 101–109.
27. Sarkodie AJ, Squire AS, Kretchy AI, Bekoe OE, Domorozo YF, Ahiagbe MJ, Emmanuella A, Dominic EA, Issac AK, Maxwell S, Vida AK, Seigfrewid AO, Duncan Jl, Phillip D, Benoit NB, Nyarko K. *Borassus Aethiopum*, A Potential Medicinal Source Of Antioxidants, Anti-Inflammatory And Antimicrobial Agents. Herb Med. 2015; 2(1):1-7.
28. Nwachi UE. Effects Of Borassus Aethiopium Mesocarp Extracts In Alloxan-Induced Diabetic Rats [University Of Nigeria Nsukka]. Unpublished Phd Thesis. 2012 Http://Www.Unn.Edu.Ng/Publications/Files/Images/Uche Phd Project _Last Final_.Pdf
29. Sabo S, Otimenyin S, Uguru M, Bukar B. Ethnobotanical Survey Of Medicinal Plants Used As Aphrodisiacs In Bauchi Local Government Area. J Compl Altern Med Res. 2018; 4(4):1–13.
30. Adeyina OA, Akanbi AS, Okukpe KM, Alli OI, Ogbah OI, Ajao BH. Effects of *Borassus Aethiopum* Extracts on Semen Characteristics, Fertility and Hatchability in Chicken. J Agric Res D Bel. 2017:62(4):385-393.
31. Trease GE and Evans WC. Pharmacognosy. (15th Ed). London: Saunders Publishers; 2002. Pp. 42-44. 221-229, 246–249, 304–306, 331–332, 391-393
32. Ajayi AF and Akhigbe RE. Staging of The Oestrous Cycle and Induction of Estrus in Experimental Rodents: An Update. Fertil Res Pract. 2020; 14:6(5):1-15.
33. Taylor AE, Keevil B, Huhtaniemi I. Mass Spectrometry and Immunoassay: How to Measure Steroid Hormones Today and Tomorrow. Eur J Endocrinol. 2015; 12:173(2):1-12.
34. Lorke DA. A New Approach To Practical Acute Toxicity Testing. Arch Toxicol. 1983; 54(4): 275–287.
35. Khojasteh SB, Khameneh RJ, Houresfsnd M, Yaldagard E. A Review On Medicinal Plants Used For The Improvement of Spermatogenesis. Biol Med. 2016; 16: 8(4):292-299.
36. Zamblé A, Sahpaz S, Bounet C, Bailleul F. Effect Of *Microdesmis Keayana* Roots On The Sexual Behaviour Of Male Rats. Phytomed. 2008; 15(8):625–629.
37. Pietta PG. Flavonoids as Antioxidants. J Nat Prod. 2000: 63(7): 1035-1042.
38. Tan CM And Powis DA. Cardiac Glycosides, Calcium And The Release Of Neurotransmitter From Peripheral Noradrenergic Nerves. In *Naunyn-Schmiedebergs* Arch Pharm. 1985; 329(1):1-8.
39. Manzoor F, Nisa MU, Hussain HA, Ahmad N, Umbreen H. Effect of Different Levels of Hydrolysable Tannin Intake On the Reproductive Hormones and Serum Biochemical Indices In Healthy Female Rats. Sci Rep. 2020; 10(1):20600.
40. Nwidu LL, Nwafor PA, Vilegas W. The Aphrodisiac Herb *Carpolobia*: A Biopharmacological And Phytochemical Review. Pharmacogn Rev. 2015; 9(18):132-139.
41. Aslam M And Sial AA. Effect Of Hydroalcoholic Extract Of *Cydonia Oblonga Miller* (Quince) On Sexual Behaviour Of Wistar Rats. Adv Pharmacol Sci. 2014; 6(1): 1-6.
42. Tajuddin AS, Latif A, Qasmi IA. Effect Of 50% Ethanol Of *Syzygium Aromaticum* (L) Merr. And Perry. (Clove) On The Sexual Behaviour Of Normal Male Rats. Bmc Compl Altern Med. 2004; 5(4): 17–24.
43. Ahmed H and Aslam M. Evaluation Of The Aphrodisiac Activity Of Ethanol Extract Of *Ganoderma Lucidum* In Male Wistar Rats. Clin Phytosci. 2018; 4(26): 86-87.
44. Mayra LR, Enrique H, Marisela H, Manuel A, Trilce M, Carolina S, Sotelo T, Guevara MA. Annual Changes in The Copulatory Behaviour Of Male Rats Maintained Under Constant Laboratory Conditions. Chronobiol Int. 2020; 37(2): 209-217.
45. Liu ZW, Jiang N, Tao X, Wang XP, Liu XM, Xiao SY. Assessment of Sexual Behavior Of Male Mice. J Vis Exp. 2020; 3(157):25-32.
46. Itoga A, Zha X, Nagase K, Aoki Y, Ito H, Yokoyama O. Correcting Imbalance of Sex Hormones by A Phosphodiesterase 5 Inhibitor Improves Copulatory Dysfunction in Male Rats with Type 2 Diabetes. Bmj Open Diab Res Care. 2020; 8(1):E001111.
47. Fouche G, Afolayan J, Wintola OA, Khorombi TE, Senabe J. Effect of The Aqueous Extract of the Aerial Parts of *Monsonia Angustifolia* E. Mey. Ex A. Rich., On The Sexual Behaviour of Male Wistar Rats. Bmc Compl Altern Med. 2015: 2(15):343.
48. Davey RA And Grossman M. Androgen Receptor Structure, Function And Biology: From Bench To Bedside. Clin Biochem Rev. 2016; 37(1):3-15.
49. Adebayo EM. Effects of Oral Administration of a Decoction On Serum Levels of Luteinizing Hormone, Follicle Stimulating Hormone, Progesterone and Estradiol In Female Dutch-White Rabbits. Res J Med Plants. 2015;9(3): 141-145.
50. Ratto MH, Berland M, Silva ME, Adams GP. New Insights into The Role Of B-Ngf In The Ovulation Mechanism Of Induced Ovulating Species. Reprod. 2019; 157(5):R199-R207.
51. Pitteloud N, Hayes FJ, Dwyer A, Boepple PA, Lee H, Crowley WF. Predictors of Outcome of Long-Term Gnrh Therapy in Men with Idiopathic Hypogonadotropic Hypogonadism. J Clin Endo and Met. 2002; 87(9):4128-4136.
52. Omotuyi IO, Ologundudu A, Onwubiko VO, Wogu A. *Hibiscus Sabdariffa* Linn. Anthocyanins Alter Circulating Reproductive Hormones In Rabbits. J Diab Endocrinol. 2011; (1): 36-45.
53. Cesare C, Simona M, Cinzia S, Elisa C, Daria N, Elena M, Gabriele B, Alessandro Dal B, Giuseppe B, Thierry D, Claudio D, Giulia C. Effect of N-3 Dietary Source On Rabbit Male Reproduction. Oxid Med Cell Longev. 2019; 16(1):32-35.
54. Haghmorad D, Mahmoudi MB, Haghighi P, Alidadiani P, Shahvazian E, Tavasolian P, Hosseini M, Mahmoudi M. Improvement of Fertility Parameters with Tribulus Terrestris and Anacyclus Pyrethrum Treatment in Male Rats. Int Braz J Urol. 2019; 45(5):1043-1054.