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APHRODISIAC POTENTIAL OF *CALOTROPIS PROCERA* STEM AQUEOUS EXTRACT ON SOME REPRODUCTIVE MARKERS IN MALE ALBINO RATS

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Abstract

The trade in traditional aphrodisiacs on the streets of Nigeria is at all-time high. Several types are on display or advertised by vendors. One of such is *Calotropis procera.* The aphrodisiac potential of *Calotropis procera* aqueous stem extract was investigated on some reproductive markers in matured sexually active male albino rats. Phytochemical screening was performed following standard protocols, the mineral composition determination was also carried out using a standard method. A total of 50 albino rats of both sexes, (25 males and 25 females) were used for this study. The rats were grouped into five (5) with five (5) rats in each group for both sexes. The aphrodisiac potential was determined following the oral administration of graded doses (200, 300 and 400 mg/kg body weight) of *C. procera* stem aqueous extract to the male groups for 30 days. Sildenafil citrate (Viagra) and distilled water served as positive and negative controls, respectively. Sexual behavioral parameters (mounting, and intromission, frequencies as well as latencies were observed on day 1, 3 and 5. The serum follicle stimulating hormones (FSH), Luteinizing hormone (LH) and testosterone concentrations were assayed for using standard methods at the end of the study. The penile nitric oxide (NO) concentration was also determined using a standard method. Results indicated that *C. procera* stem contains; tannins, saponins, terpenoids, alkaloids, tannins, phenols glycosides, phenols and flavonoids at varying concentrations; Difference minerals were also present at varying concentrations. Sexual performance studies indicated significant ($p \leq 0.05$) dose-dependent increase in the mounting and intromission frequencies as well as delayed ejaculation latency. The result of the reproductive

markers showed significant ($p \leq 0.05$) dose-dependent increase in nitric oxide (NO), FSH and LH concentrations. The significant increase in the concentrations of LH, FSH, testosterone and penile nitric oxide with the administration of aqueous extract of *C.* procera may be responsible for the aphrodisiac activity of the extract. These observations may validate the acclaimed local use of *C. procera* stem as aphrodisiac in males.

Keywords: aphrodisiacs, erectile dysfunction Libido study, reproductive markers, hormones, phytochemicals.

Introduction

Conventional sexual intercourse and accomplishment could only happen in males if the male sexual organs (the copulatory organ, the penis) and other concerning factors to erection are working optimally well. Inability to accomplish this roles efficiently in actual fact is referred to as sexual dysfunction (Guay, *et al.,* 2003).

Male and female external genitalia play an essential role in human reproduction, and disorders of structure and function of male and female external genitalia can have profound deleterious effects on procreation.

Sexual problems are widespread and may adversely affect mood, well-being and interpersonal functioning (Laumann, et al., 1999). Erectile dysfunction is plainly seen as persistent inability to create enough penile erection sufficient for vaginal penetration and/or the failure to hold penile firmness until ejaculation. The escalating incidence of male sexual dysfunction has led an increased usage of aphrodisiacs. This has in turns; resulted to a continuous search for new and effective aphrodisiacs from various sources including medicinal plants. A few of the curative plants that have been shown to possess an aphrodisiac properties in male rats are as follows: Terminalia catappa seeds (almond fruit), Syzygium aromaticum flower bud (clove), Fadogia agrestis stem (Black aphrodisiac) (Ratnasooriya et al., 2000; Yakubu et al., 2005). This research investigates the aphrodisiac potentials of C. procera on some reproductive markers in male albino rats via the determination of follicle stimulating hormone (FSH), Luteinizing Hormone (LH) and Testosterone as well as determination of nitric oxide (NO) concentration as well as its sexual performance enhancing capacity via libido protocol study.

MATERIALS AND METHODS

Research Animals

A total of 50 mature white albino rats of both sexes, 25 male and 25 female with mean weight 180.10 g, were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Nigeria Nsukka, Enugu, Nigeria. They were kept in well-ventilated animal house under natural conditions (temperature: 28 °C – 31 °C; photoperiod: 12 hours natural light and 12 hours dark, They were fed on standard commercial animal feeds (Vital feed pellets) and water *ad libitum*. All experiments were performed on the laboratory animals in this study based on the Principles of laboratory animal care (NIH Publication, 1996).

Equipments/Instruments

The instrument used for this study includes; Electric blender (Blender/Miller III, model MS-223, Taiwan, China), Colorimeter (Jenway 6051, UK), spectrophotometer (UNICO 2150 series, USA), Atomic absorption spectrophotometer (200-A , Buck Scientific Instruments, USA) ELISA Micro Reader (EDVOTEK, SKU: EVT-088, UK) centrifuge (NEWTRY, 800-1, China) weighing balance (Vickas ltd, 80-1 C, England), Refrigerator (Thermocool, England), micropipette (Eppendorf Research, Plus. UK).

Chemicals and Reagents.

Estradiol benzoate was purchased from Sigma Chemical (St. Louis, USA) and progesterone from Shalina Laboratories (Mumbai, India). Sildenafil citrate (Viagra) was obtained from a Pharmacy outlet in Keffi town, Nasarawa State. The testosterone, luteinizing (LH) and follicle stimulating hormone (FSH) assay kit was procured from Immunometrics (London, EDVOTEK, SKU: EVT-088, UK). Randox assay kits for AST, ALT and ALP (Clinical chemistry Assay kit LC2389, LC3980) analysis were purchased from Randox Laboratory Ltd, UK.

All the chemicals were of analytical grade and were prepared with distilled water unless otherwise stated and were kept in reagent bottles for further use.

Methods

Animal Grouping

A total of 50 mature albino rats; (25 male and 25 female) were used for this research. They were divided individually in metabolic cages with cleaning of the cages done once daily. The Male rats were randomly divided into five (5) groups (A, B, C, D and E) with five (5) rats in each group and same grouping were carried out with female rats for sexual performance study.

Groups A: received 200 mg/kg body weight of the extract, group B: received 300 mg/kg of the extract, group C: received 400 mg/kg body weight of the extract, group D: received equal volume of distilled water (control group). Group E: was given the standard drug-Sildenafil citrate (100 mg/kg) of body weight. The oral administration was carried out using orogastric tube. The female counterparts were grouped as well in similar manner and administered with distilled water and fed with normal rats chows during the period of the research. They were also administered with estradiol benzoate (0.5 mg/100 g) and progesterone (0.5 mg/100 g) through subcutaneous injections to bring them to oestrus.

Collection of Plant and Authentication

The plant sample was collected from the surroundings of Nasarawa State University, Keffi Campus. The authentication was done at the Department of Forestry and Forestry Products of the Nasarawa state university where Voucher (NSU2045) specimen was deposited at their herbarium unit.

Preparation of plant extract

Calotropis procera stem aqueous extract was prepared in accordance with the procedure described by Yakubu *et al.* (2005), with little modifications. The plant stem was cut into pieces, dried under ambient temperature 25 °C to a constant weight. The dried pieces was then pulverized into powder of 75 µm mesh size. The powder was stocked in a plastic container from which 500 g was taken and extracted in distilled water for 48 hours at room temperature (27 °C). This was then filtered using filter paper (Whatman No.1 and the filtrate was concentrated in stem bath and the resulting brownish black residue was reconstituted in distilled water to give the equivalent dose of 200 mg/kg body weight (low dose), 300 mg/kg body weight (medium dose)

and 400 mg/kg body weight (high dose) was used in this study. The reconstituted aqueous extract was administered orally (oral gavage) using plastic syringes to all animals in different groups via oral intubation method.

Drug Preparation and Administration

Sildenafil citrate (Viagra®) was obtained from a Pharmacy outlet in Keffi town, Nasarawa State was used for this study. The stock solution (4 mg/mL) was prepared by dissolving 100 mg by weight of sildenafil tablet in 25 mL of distilled water. Animals in various treatment groups were dosed based on their daily body weight. The solution was constituted just before use and left over was discarded.

The Qualitative and Quantitative Phytochemical Screening of *Calotropis procera* Stem Aqueous Extract.

The procedures described by El-Olemy *et al.*, (1994) and Sofowora (1993) with some modifications were used for the qualitative screening of alkaloid, tannin, glycoside, saponin, and phenolic, flavonoid and triterpene and steroid.

Determination of Mineral Elements Composition.

The mineral composition of the *Calotropis procera* stem aqueous extract was carried out using atomic absorption spectrophotometer (AAS) method AOAC (2005). .

The ash solution of the plant root sample was prepared by weighing 5 g of the powdered plant sample. It was ashed at 103 °C in muffle furnace for 5 h, and the residue dissolved in 100 ml of deionized water. Standard solutions of the minerals (Sodium, Magnesium, Potassium, Calcium, Manganese, Iron, Zinc, lead and cadmium) to be analyzed were prepared. The atomic absorption spectrophotometer (model 200-A, Buck Scientific) was set with power on for ten minutes to stabilize. The standard minerals solutions were injected to calibrate the AAS using acetylene gas at specific wavelengths. Aliquots of ash solution were injected and the concentrations obtained from the standard curve.

Sexual Behaviour Testing Protocol

Sexual behaviour testing Protocol was carried out according to the method described by Gauthaman *et al.* (2002). A total of 25 sexually active male rats were housed individually in metabolic cages of dimensions 33.0 cm × 20.5 cm × 19.0 cm, with cleaning of the cages done once daily. The rats were randomly divided into five groups (A, B, C, D and E) of 5 animals each. Rats in groups A, B, and C were administered with the plant extract once daily at 24 h intervals at the dose of 200 mg/kg, 300 mg/kg and 400 mg/kg body weight respectively, Group D served as the control, which received appropriate volume of the vehicle (distilled water) in a similar manner while the rats in group E received 100 mg/kg body weight of Sildenafil citrate base dissolved in distilled water daily for days, through feeding tube. Each rat from each of the groups was monitored for sexual behaviour after 1, 3 and 5 daily doses respectively.

Twenty (25) female rats will be brought to oestrus by the sequential administration of estradiol benzoate (0.5 mg/100 g) and progesterone (0.5 mg/100 ml) through subcutaneous injections, 48 hours and 4 hours respectively prior to pairing. Sexual behaviour studies was monitored in a separate room for 2 hours following the administration and were given 20 minutes adaptation period, after which a primed female was placed in the same cage with the male (1:1) on days 1, 3 and 5, the following sexual behaviors; mounting frequency (MF), intromission frequency (IF), ejaculatory frequency, mounting latency and, intromission, and ejaculatory latencies were monitored:

Hormone Assay

Preparation of Serum

The procedure described by Yakubu *et al.,* (2003), was used to prepare the serum.

The same set of animals for sexual behaviour parameters were be used for the hormonal assays; the animals were sacrificed 24 hours after the extract dosing. Under ether anesthesia, the blood samples were collected through ocular puncture using capillary tubes. The rats were made to bleed into clean, dry corked centrifuge tubes which was left at room temperature for 10 minutes. After that, the tubes were centrifuged at 4000 rpm for 15 minutes using Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, England).

The sera were thereafter collected using Pasteur pipettes into clean, dry, sample bottles and was stored frozen overnight before being used for the testosterone assay.

Testosterone Assay

The blood samples collected were centrifuged at 4000 rpm for 15 minutes using Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, England) to obtain the serum sample which was analysed for testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) using enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc., USA) (Ekaluo *et al*., 2010).

The method described by Tietz (1995) was used to determine the serum testosterone concentration. The serum testosterone concentration was quantitatively determined using the direct human serum testosterone enzyme immunoassay kit as outlined in the manufacturer's protocol. The determination was based on the principle of direct assay of a limited (competitive) type following the general antibody-antigen reaction based on enzyme linked immunoabsorbent assay using Serozyme IÔ Serono (Diagnostics, Freiburg, Germany). The serum testosterone concentration was interpolated from a standard calibration curve

Determination of Follicle Stimulating Hormone (FSH)

The Follicle Stimulating Hormone (FSH) concentration was determined based on the principle of solid phase enzyme-linked immune-absorbent assay similar to that described by Uotiola *et al.,* (1981). The assay system utilized a mouse monoclonal anti-FSH antibody for solid phase (microliter wells) immobilization and another monoclonal anti-FSH antibody in the antibody enzyme (horseradish peroxidase)-conjugated solution. Sixty microliters of standard, test and control will be pipetted into appropriate wells after which 100 µl of enzyme conjugated reagent was taken and added into each of the well, and then mixed thoroughly for 30 seconds and was incubated at room temperature for 45 minutes. The incubation mixture was removed by flickering the plate contents into a waste container. The microliter was rinsed five times with distilled water after which the wells was shaken sharply onto absorbent paper to remove all residual water droplets. Hundred microliters of Tetramethylbenzidine (TMB) reagent was pipetted into each well and gently mixed for 5seconds. This was incubated in the dark for 20 minutes. A 100 µl of the stop solution was added to each well to stop the reaction. This was observed for 30 seconds for colour development from blue to yellow. The absorbance at 450 nanometer was read with microtitre plate reader within 15 minutes. The absorbance of the test

sample was correlated with that of the standard curve to give the concentration of the FSH in the serum.

Test procedure for luteinizing hormone (LH)

The concentration of Luteinizing Hormone (LH) in the serum was determined based on a solid phase enzyme-linked immune absorbent assay as described by Uotila *et al*., (1981). The assay system utilized a mouse monoclonal anti-LH antibody for solid phase (microliter wells) immobilization and another monoclonal anti-LH antibody in the antibody enzyme (horseradish peroxidase)-conjugated solution. Sixty microliters of standard, test and control was pipetted into appropriate wells after which 100µl of enzyme conjugated reagent was taken and add into each of the well, and then mixed thoroughly for 30secs and was then be incubated at room temperature for 45 min. The incubation mixture was removed by flickering plate contents into a waste container. The microliter was rinsed five times with distilled water after which the wells was shaken sharply onto absorbent paper to remove all residual water droplets. Hundred microliters of tetramethylbenzidine (TMB) reagent was pipetted into each well and gently mixed for 5 seconds. This was then incubated in the dark for 20 minutes. 100 µl of the stop solution was added to each well to stop the reaction. This was observed for 30 seconds for colour development from blue to yellow. The absorbance at 450 nm was read with microtitre plate reader within 15 min. The absorbance of the test sample will be correlated with that of the standard curve to give the concentration of the LH in the serum.

Measurement of Nitric Oxide Level (NO)

Nitric oxide content in penile tissue homogenate was estimated in a medium containing 400 milliliters of 2% vanadium chloride (VCl_3) in 5% HCl, 200 ml of 0.1% N-(l-naphthyl) ethylenediaminedihydrochloride, 200 ml of 2% sulphanilamide (in 5% HCl). After incubating at 37 °C for 60 minutes, nitrite levels, which correspond to an estimative level of NO, were determined at 540 nm, based on the reduction of nitrate to nitrite by VCl_3 (Miranda *et al.*, 2001). Penile nitrite level was expressed as micromole of NO per gram of tissue.

Statistical Analysis

All results were analyzed and presented as mean ± SD and analysis was done using Statistical Package for the Social Sciences (SPSS) Version 23. One-way analysis of variance (ANOVA)

was used. Differences in mean was considered to be statistically significant at ($p \leq 0.05$) confidence level.

Results

Qualitative and quantitative phytochemical Composition of aqueous stem extract of *Calotropis procera*

The results of qualitative phytochemical of aqueous extract of *Calotropis procera* stem are presented in table 1 and 2. The results showed the presence of tannin, alkaloids, flavonoids, steroids glycosides, saponins, terpenoids and phenol at varying concentrations. The quantitative phytochemical screening of the aqueous stem extract of *Calotropis procera* showed the concentrations of alkaloid, tannin, flavonoid, saponin, steroids glycosides, phenol HCN and terpinoid in the extract.

Table 1: Qualitative Phytochemical Composition of *Calotropis procera* stem aqueous extract.

Phytochemical	Remark
Alkaloids	+++
Saponins	+
Tannins	+++
Flavonoids	+++
HCN	+
Steroids	+
Terpenoid	++
Phenol	+++
Glycosides	++

Keys; + means present, ++ means Abundant while +++ means Very Abundant

Table 2: Quantitative Phytochemical Composition of *Calotropis procera* stem aqueous extract

Phytochemicals constituents	Concentrations (mg/ 100 g)
Alkaloids	610.39 ± 6.62^{c}
Saponins	0.30 ± 0.01^{a}
Tannins	912.78 ± 3.47^{d}
Flavonoids	1232.80 ± 3.56^{e}
HCN	0.20 ± 0.01^{a}
Steroids	0.86 ± 0.03^{a}
Terpenoid	330.21 ± 2.95^{b}
Phenol	688.17 ± 7.63^{c}
Glycosides	460.86 ± 2.95^{b}

Results are expressed in Means ± SD (n= 3)
Mean values with different superscripts down the column are considered significantly different at ($P< 0.05$).

Mineral Element Composition of *Calotropis procera* Stem Aqueous Extract

The results in of mineral element contents in *Calotropis procera* stem aqueous extract as presented in Table 3. The results showed varied concentrations of Mg, Ca, Na, K, Zn, Cu, P, Cd, and Fe in the extract with Cu and K having the lowest and highest concentration respectively, in the extract as seen in the Table 3 below.

Table 3 Mineral composition of *Calotropis procera* stem aqueous extract.

Minerals	Concentrations (mg/ 100 g)
Magnesium	15.86 ± 1.70^{d}
Calcium	6.85 ± 0.17^{c}
Sodium	18.30 ± 0.62^{d}
Potassium	38.74 ± 1.50^{e}
Zinc	9.50 ± 0.01^{c}
Copper	1.86 ± 0.01^{a}
Phosphorus	3.2 ± 1.31^{b}
Cadmium	2.25 ± 0.11^{a}
Iron	2.03 ± 0.01^{a}

Results are expressed in Means ± SD (n= 3)
Mean values with different superscripts down the column are considered significantly different at ($p< 0.05$).

Effect of aqueous extract of *Calotropis procera* stem on sexual performance in male albino rats at day 1, 3 and 5 of treatment.

The result on the chat below showed the effect of the oral administration of aqueous extract of *C. procera* stem at day 1, 3 and 5 on the sexual performance of the male albino rats paired with receptive females. It was observed that at day 1 the mounting frequencies of treated groups A, B, C and E respectively were significantly ($p\leq 0.05$) higher when compared with the negative control (group D), however, comparing the result in the extract treatment and positive control (group E), the result also showed significant increase in positive control group compared to extract treatment group and negative control respectively, the same trend of result was obtained with the intromission. The result, also showed dose dependent increase on the latencies among the treated when compared with control groups However, the result of the ejaculatory latency showed a significant ($P\leq 0.05$) delayed time among the treated groups A, B, C and E, when compared with group D (untreated).

Also the graph chat showed the effect of the oral administration of aqueous extract of *Calotropis procera* stem at day 3 on the sexual performance of the male albino rats paired with receptive females to have same trends of result but more proactive.

At day 5 of oral administration of aqueous extract of *Calotropis procera* stem on the sexual performance of the male albino rats paired with receptive females. The result showed that the mounting frequencies of treated groups (A, B, C and E); were significantly ($P \leq 0.05$) higher when compared with control groups (group D). Similarly, the result showed that in the treatment groups, there was a delayed time of ejaculation when compared with control groups. The results of performance latencies showed a significant decrease among the treatment groups (A, B, C and E) when compared with negative control group (Group D).

Table 4. **Effect of aqueous extract of *Calotropis procera* stem on sexual performance in male albino rats at Day 1 of treatment**.

Groups	MF (NO)	IF (NO)	ML (Sec.)	IL (Sec.)	EL (Sec.)
A	13.02 ± 1.33^{a}	8.78 ± 0.83^{b}	24.30 ± 1.0^{c}	27.90 ± 1.93^{c}	164.40 ± 8.91^{b}
B	16.34 ± 2.46^{b}	11.74 ± 0.50^{b}	15.67 ± 0.76^{b}	21.64 ± 2.37^{b}	173.60 ± 5.50^{c}
C	20.60 ± 2.59^{c}	14.71 ± 0.48^{c}	12.34 ± 0.47^{a}	13.08 ± 0.18^{a}	178.20 ± 7.33^{c}
D	11.23 ± 0.37^{a}	7.12 ± 1.15^{a}	29.33 ± 1.28^{d}	30.42 ± 0.72^{d}	159.60 ± 3.46^{a}
E	25.60 ± 3.59^{d}	18.50 ± 2.50^{d}	10.60 ± 0.59^{a}	18.40 ± 1.29^{b}	180.60 ± 9.59^{d}

Keys: Group A: 1 ml of 200 mg/kg extract administration, Group B: 1 ml of 300 mg/kg extract administration, Group C: 1 ml of 400 mg/kg extract administration, Group D: Distilled water and Group E: 1 ml of 100 mg/Kg Sildenafil Citrate. MF= Mounting Frequency, IF= Intromission Frequency, ML= Mounting Latency, IL= Intromission Latency and EL= Ejaculation Latency. No = number, and Sec, = seconds.

Results are expressed in Means ± SD (n =5). Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Table 5 Effect of aqueous extract of *Calotropis procera* stem on sexual performance in male albino rats at day 3 of treatment.

Groups	MF (NO)	IF (N)	ML (Sec.)	IL (Sec)	EL (Sec.)
A	15.62 ± 1.04^{b}	9.40 ± 0.89^{a}	24.30 ± 1.0^{c}	27.90 ± 1.93^{b}	184.00 ± 1.30^{b}
B	20.66 ± 0.85^{c}	13.00 ± 2.12^{b}	15.67 ± 0.76^{b}	21.64 ± 2.37^{b}	187.20 ± 1.30^{b}
C	22.74 ± 2.67^{d}	15.78 ± 1.32^{b}	12.34 ± 0.47^{a}	13.08 ± 0.18^{a}	187.20 ± 1.30^{b}
D	11.66 ± 1.49^{a}	8.40 ± 1.52^{a}	29.33 ± 1.28^{d}	30.42 ± 0.72^{c}	191.60 ± 0.89^{c}
E	24.54 ± 2.87^{e}	22.74 ± 2.87^{c}	12.43 ± 0.67^{a}	12.44 ± 1.67^{a}	178.74 ± 2.67^{a}

Keys: Group A: 1 ml of 200 mg/kg extract administration, Group B: 1 ml of 300 mg/kg extract administration, Group C: 1 ml of 400 mg/kg extract administration, Group D: Distilled water and Group E: 1 ml of 100 mg/Kg Sildenafil Citrate.
MF= Mounting Frequency, IF= Intromission Frequency, ML= Mounting Latency, IL= Intromission Latency and EL= Ejaculation Latency. No = number, and Sec, = seconds.
Results are expressed in Means ± SD (n =5)

Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Table 6: Effect of Aqueous Extract of *Calotropis procera* Stem on Sexual Performance in Male Albino Rats at day 5 treatment.

Group	MF (No)	IF (No)	ML (Sec.)	IL (sec.)	EL (sec)
A	22.20±1.30^{b}	16.80±1.48^{b}	26.82±0.81^{c}	24.20± 2.17^{c}	162.60±9.89^{c}
B	30.84±0.85^{c}	23.40±1.14^{c}	18.20±0.84^{b}	18.40 ± 1.14^{b}	154.60±8.59^{b}
C	32.14±1.40^{c}	22.60±6.12^{c}	12.60±1.52^{a}	13.80 ± 2.49^{a}	144.80±6.65^{a}
D	17.36±1.12^{a}	13.60±1.67^{a}	37.00±1.22^{d}	46.80 ±10.94^{d}	181.60±3.54^{c}
E	38.14±1.85^{d}	27.54±2.85^{d}	10.84±0.85^{a}	12.30 ±1.65^{a}	154.60±8.59^{b}

Keys: Group A: 1 ml of 200 mg/kg extract administration, Group B: 1 ml of 300 mg/kg extract administration, Group C: 1 ml of 400 mg/kg extract administration, Group D: Distilled water and Group E: 1 ml of 100 mg/Kg Sildenafil Citrate.
MF= Mounting Frequency, IF= Intromission Frequency, ML= Mounting Latency, IL= Intromission Latency and EL= Ejaculation Latency. No = number, Sec. Seconds.
Results are expressed in Means ± SD (n = 5).
Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Effect of *Calotropis procera* stem aqueous extract on some Reproductive Markers

Table 7, shows the effect of aqueous extract *C. procera* stem on some reproductive markers. The results showed that the concentration of serum luteinizing hormone (LH) and in the extract treatment groups and positive control (sildenafil citrate) to be significantly ($p > 0.05$) higher (A,B, C and E), respectively when compared to negative control group (D) treated with distilled water also, the concentrations of serum follicle stimulating hormone (FSH)) in the treatment groups and positive control (A, B, C and E) respectively, were observed to be significantly (($p \leq 0.05$) higher when compared to negative control (group D). Similarly, there was significant ($p \leq 0.05$) increase on testosterone serum levels (of the treatment groups and positive control groups (A, B, C and E) respectively, higher when compared to control group (D).

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Table 7: Effect of *Calotropis procera* stem aqueous extract on some Reproductive Markers

Group	LH (mg/ml)	FSH (mg/ml)	TESTOSTERONE (mg/ml)
A	2.38 ± 0.12^{b}	3.38 ± 0.24^{b}	2.15 ± 0.21^{a}
B	2.39 ± 0.24^{b}	3.52 ± 0.19^{b}	2.23 ± 0.36^{a}
C	2.72 ± 0.19^{b}	3.53 ± 0.37^{b}	2.52 ± 0.17^{a}
D	1.93 ± 0.53^{a}	2.82 ± 0.29^{a}	2.01 ± 0.17^{a}
E	2.98 ± 0.32^{b}	3.98 ± 0.32^{b}	2.88 ± 0.32^{b}

Keys; LH: Luteinizing Hormone, FSH: Follicle Stimulating hormone

Group A: received 1 ml of 200 mg/kg body weight of extract, group B: received 1 ml of 300 mg/kg body weight of extract, group C: received 1 ml of 400 mg/kg body of extract, group D: received equal volume of distilled water and group E: received 1 ml of 100 mg/Kg Sildenafil Citrate (control group).

Results are expressed in Means ± SD (n = 5)

Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Effect of *Calotropis procera* Stem Extract on Penile Nitric oxide level

The effect of *Calotropis procera* aqueous stem extract on the nitric oxide levels of penile tissue homogenate is presented in table 8. The result showed that the concentration of nitric oxide in the treatment groups and positive control were significantly (($p \leq 0.05$) higher than the negative control group (group D), with treated positive control group that received the standard drug having highest concentration followed by the highest dose (400 mg/kg) extract treated group and negative control group having the lowest concentration. The result showed that the increase in nitric oxide concentrations of extract treated groups when compared with control groups were dose-dependent.

Table 8: Effect of *Calotropis procera* Stem Extract on Nitric Oxide (NO) of Rats

Group	Nitric Oxide (mMol/g)
A	35.23 ± 2.66^{a}
B	41.33 ± 4.46^{b}
C	54.37 ± 3.21^{c}
D	32.11 ± 1.85^{a}
E	58.57 ± 2.21^{c}

Group A: received 1 ml of 200 mg/kg body weight of extract, group B: received 1 ml of 300 mg/kg body weight of extract, group C: received 1 ml of 400 mg/kg body of extract, group D: received equal volume of distilled water and group E: received 1 ml of 100 mg/Kg Sildenafil Citrate (control group).

Results are expressed in Means ± SD (n = 5)

Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Discussion

The background for confirming any medicinal plant as having the potential to stimulate and enhance sexual performance was reported by Ratnasooriya and Dharmarsiri (2000). In their opinion they stated that medicinal plant with a tendency to stimulate and enhance sexual behavior should produce a statistically significant increase in mount frequency and intromission frequency and also significant reduction in mount latency and intromission, since these indices are indicators of sexual arousability, motivation, and vigor. Various parts of *C. procera* has been reported to be used in Nigeria and many other countries for the treatment of varieties of diseases, such as muscular spasm, joint pain, constipation, skin diseases and etc. (Mossa *et al*., 1991). The results of the present study indicated that the aqueous extract of stem of *C. procera* has aphrodisiac potential since its ingestion improved the sexual performance of the rats. Also, *C. procera* extract significantly increased serum luteinizing hormones (LH) and follicle stimulating hormones (FSH) with increased testosterone levels that are statistically significant in a dose dependent manner. There was also dose dependent significant ($P \leq 0.05$) increase in penile tissue Nitric Oxides concentration on the treatment group when compared with the control. These findings indicated that C. *procera* is a potential medicinal drug for increasing sexual performances and management of erectile dysfunction in sexually experienced rats. Several studies reported that aphrodisiac plants are good alternatives for the improvement of of sexual behavior (Guoha *et al.,* 2009), probably due to their efficacy and availability. The The mounting latency and intromission latency are considered as indicators of sexual motivation motivation Yakubu and Afolanyan, (2009). The significant reduction figure 1 in these parameters parameters observed in the rats treated with *C. procera* aqueous stem extracts imply improvement of sexual motivation and sexual appetite which further justifies the folkloric use of use of this plant as sexual booster. Moreover, the increase of ejaculation latency after treatment treatment with the plant extracts indicates the persistence of sexual drive Yakubu and Afolanyan, Afolanyan, (2009). On the other hand, mounting frequency and intromission frequency are the are the indicators of vigor, libido, and potency. The increase in the mounting frequency indicates indicates sexual motivation while elevated intromission frequency reflects the efficiency of of erection. These findings agree with earlier report by Ratnasooriya and Dharmasiri (2000), (2000), Yakubu and Afolayan, (2008); Yakubu and Akanji (2011); Gbankoto *et al*. (2015) on the (2015) on the significant changes in mounting and intromission latencies. Also, the prolonged

prolonged ejaculatory latency (EL) by the aqueous stem extract of *C. procera* is a strong indication that the sexual function of the male rats was enhanced (prolonged duration of coitus) coitus) suggesting an aphrodisiac activity. These findings which is in line with the work of on reproductive health by Fouche *et al*., 2015, the aphrodisiac property of *C. procera* could be attributed to the various active components present in this plant. The phytochemical analysis of *C. procera* revealed the presence of saponin, terpenoid, alkaloid, tannin, phenol glycoside, phenol and flavonoid. It has been reported that flavonoids facilitate male sexual behavior by boosting testosterone production and/or preventing its metabolic degradation (Yakubu and Akanji, 2011).The presence of alkaloid, flavonoid, sterol and saponin in this plant may account for its use as aphrodisiac. Phytochemicals have been reported to enhance erection and prolong ejaculatory latency in male albino rats (Yakubu *et al*., 2005).

Terpenoids have an implication in the triggering of penile erection as well as in the improvement of the sexual performances (Kim and Christianson, 2004). Kim *et al*., (2012), demonstrated that saponin facilitated relaxation of the corpus cavernosum muscles by stimulating the L-arginine/nitric oxide pathway.

These bioactive components could have an effect on the central nervous system by activating neurotransmitters or the periphery by stimulating the release of nitric oxide. Micronutrients play essential roles in metabolism and serves as co-factors and co-enzymes for enzymatic reactions. As shown in Table 3 the plant part is very rich in magnesium a co-factor for many biochemical reactions in the body which include synthesis of sex hormones such as androgens, estrogens and neurotransmitters from the brain that modulate sex drive such as dopamine and norepinephrine. The concentration of potassium in stem sample of *Calotropis procera* was significantly high. Potassium is important as the major cation of the intracellular fluid and helps to maintain the electrode potential, regulate the aldosterone concentration and consequently the permeability of the cell membrane. (Kaplan, 1991; Robert et al., 2003). The level of calcium in *Calotropis procera* is low. Calcium performs two categories of physiological functions; one category involves provision of the structural integrity of the skeleton. The second category depends on the depends on the calcium ion in cellular and intracellular fluids. Calcium ion serves as the coupling coupling factor linking excitation and contraction in skeletal and cardiac muscles. Intracellular Intracellular calcium ion is also required in control of key enzymes regulating intermediary

intermediary metabolism and so may play a role in providing energy for contraction (Breslau, (Breslau, 1991). Calcium also play role in the permeability and excitability of plasma membrane. membrane. It has been implicated as an important coupling factor in neurotransmitter release, release, exocrine secretion (for example, amylase) and endocrine secretion, such as the case of case of insulin. Zinc in the plant apart from boosting the immune system is also required for the for the production of testosterone (the male sexual hormone) and help to ward off male infertility. Deficiency in any of these micronutrients could lead to muscle weakness and general general fatigue (Davis, 1998; Yakubu *et al*., 2005).

Conclusion

This study suggested that *C. procera* stem aqueous extract possesses aphrodisiac potential particularly at higher dose of 400 mg/kg of the extract, which showed the highest aphrodisiac effects on mounting frequency, intromission frequency, mounting latency, intromission latency and ejaculation latency,. The extract has a functional capacity to increased FSH, LH, Testosterone and Nitric oxide concentrations which are possible mechanisms of action for its aphrodisiac property and validated its use as an aphrodisiac by local herbalists.

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