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EFFECT OF AQUEOUS EXTRACT OF CALOTROPIS PROCERA (ROOSTER TREE) STEM ON SOME FERTILITY HORMONES AND BIOCHEMICAL PARAMETERS IN MALE ALBINO RATS

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ABSTRACT

For ages plants have been used for various purposes, one of which is its use for treatment of varieties of illness/diseases such reproductive dysfunctions. One of such medicinal gifted plant of nature is Calotropis procera (Rooster Tree). In this research, the effect of Calotropis procera aqueous stem extract on some fertility hormones and biochemical parameters were assessed in male albino rats. A total of 25 male albino rats, were used for this study. The rats were grouped into five (5) with five (5) rats in each group. The fertility improving capability and its effects on some biochemical markers were determined using standard procedures, the effect of the continuous administration on liver and kidney function markers were also investigated following standard protocol after the oral administration of graded doses (100, 200 and 300 mg/kg) of C. procera stem aqueous extract and distilled water and sidnafil serving as negative and positive controls respectively, The result of the fertility markers showed significant ($p<0.05$) dose-dependent increase in FSH and LH concentrations. Results on the liver function marker showed a non-significant ($p<0.05$) increase in ALT, AST and ALP and the results of the kidney function parameters were observed to be significantly lower in the treated groups when compared group not administered with extract. The significant increase in the concentrations of Luteinizing hormones, follicle stimulating hormones and testosterone with the administrated of aqueous extract of C. procera may be responsible for the aphrodisiac activity of the extract. The results of this research may validate the folkloric use of C. procera stem in management of reproductive dysfunction in males.

Keywords: Fertility, Erectile Dysfunction, Libido Study, Biochemical Markers, Hormones, Medicinal Plants.

I. INTRODUCTION

Sexual Dysfunction

For most couples, procreating is a natural part of life that involves either special planning or intervention (Trussell, 2013). Unfortunately, when trying to conceive, 15% to 25% of the couples struggle and, consequently, seek medical advice on how to improve their chances of fertilization and successful pregnancy (WHO, 2010). An estimated six percent of adult males are thought to be infertile. Infertility is defined by most authorities as the inability to achieve a pregnancy after one year of unprotected regular intercourse (Purvis and Christiansen, 1992). Sexual dysfunction in men refers to repeated inability to achieve normal sexual intercourse. It can also be viewed as disorders that interfere with a full sexual response cycle. These disorders make it difficult for a person to enjoy or to have sexual intercourse. While, sexual dysfunction rarely threatens physical health, it can take a heavy psychological toll, bringing on depression, anxiety, and debilitating feelings of inadequacy. Unfortunately, it is a problem often neglected by the health care team who strive more with the technical and more medically manageable aspects of the patients illness. Sexual dysfunction is more prevalent in males than in females and thus, it is conventional to focus more on male sexual difficulties. It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problems (Guay et al., 2003). Generally, a prevalence of about 10% occurs across all ages. Since sexual dysfunction is an inevitable process of aging, the prevalence is over 50% in men between 50 and 70 years of age (Rendell et al., 1999). As men age, the absolute number of Leydig cells decreases by about 40%, and the vigor of pulsatile luteinizing hormone release is dampened. In association with these events, free testosterone level also declines by approximately 1.2% per year. These have contributed in no small measure to prevalence of sexual dysfunction in the aged (Guya et al., 2003). Male sexual dysfunction (MSD) could be caused by different factors. These include: psychological disorders (performance anxiety, strained relationship,

depression, stress, guilt and fear of sexual failure); androgen deficiencies (testosterone deficiency, hyperprolactinemia); chronic medical conditions (diabetes, hypertension, vascular insufficiency: atherosclerosis and venous leakage; penile disease (priapism, phimosis, smooth muscle dysfunction); pelvic surgery (to correct arterial or inflow disorder); neurological disorders (Parkinson's disease, stroke, cerebral trauma, Alzheimer's disease, spinal cord or nerve injury); drugs side effects (anti-hypertensive, central agents, psychiatric medications, antiulcer, antidepressants, and anti-androgens); life style (chronic alcohol abuse, cigarette smoking); aging (decrease in hormonal level with age); and systemic diseases (cardiac, hepatic, renal pulmonary, cancer, metabolic, post-organ transplant) (Feldman et al., 1994; Kandeel et al., 2001; Guay et al., 2003).

Classification of Male Sex Disorders

Sex disorders of the male are classified into disorders of sexual function, sexual orientation, and sexual behaviour. In general, several factors must work in harmony to maintain normal sexual function. Such factors include neural activity, vascular events, intracavernosal nitric oxide system and androgens (Guay et al., 2003). Thus, malfunctioning of at least one of these could lead to sexual dysfunction of any kind.

Disorders of desire

Problems of desire could involve either a deficient or compulsive desire for sexual performance.

Dysfunctions that can occur during the desire phase include:

1. Hypoactive sexual desire (HSD)

This is simply seen as recurrently deficient (or absent) sexual fantasy and desire for sexual activity leading to marked distress or interpersonal difficulty. It results in a complete or almost complete lack of desire to have any type of sexual relation (APA, 1994).

2. Compulsive sexual Behaviours (CSBs)

Constitute a wide range of complex sexual behaviour that have strikingly repetitive, compelling, or driven qualities. They usually manifest as obsessive-compulsive sexuality (e.g. excessive masturbation and promiscuity), excessive sex-seeking in association with affective disorders (e.g. major depression or mood disorders), addictive sexuality (e.g. attachment to another person, object, or sensation for sexual gratification to the exclusion of everything else), and sexual impulsivity (failure to resist an impulse or temptation for sexual behaviour that is harmful to self or others such as exhibitionism, rape, or child molestation) (Kaplan, 1996).

Erectile dysfunction (ED)

This is a problem with sexual arousal. ED is the difficulty in achieving or maintaining an erection sufficient for sexual activity or penetration, at least 50% of the time, for a period of six months. Erectile dysfunction is also defined as the persistent inability to obtain and maintain an erection sufficient for naturally satisfactory intercourse. It results in significant psychological, social and physical morbidity, and annihilates the essence of masculinity (Laumann and Kandeel et al., (2001). Many people believe that impotence is a disorder associated with modern civilization. However, preoccupation with potency has been present through the ages (Shah, 2002). The term "impotence" is derived from the Latin word impotentia, which when literally translated means lack of power. It has traditionally been used to signify the inability of the male to attain and maintain erection of the penis sufficiently long to permit satisfactory sexual intercourse. This term, together with its pejorative implications, has often generated confusion in both clinical and basic science investigations; so it was recently replaced by the more precise term "erectile dysfunction" (NIH, 1993). Erectile dysfunction is adversely affected by diabetes mellitus, antihypertensive, antipsychotic, antidepressant therapeutic drugs. Organic causes of ED include hypogonadism, hyperprolactinaemia, and neurological disorders (Sharma et al., 2012). For years, psychological factors were implicated as the main cause of impotence, but during the last decade there has been an important change in the management of sexual dysfunction. This revolution was essentially due to the improved understanding of erectile physiology and to the development of new and effective medical therapies. Even though the loss of erectile function is not an inevitable consequence of normal aging, it becomes more frequent with age (Sharma et al., 2012).

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Disorders of ejaculation

There exists a spectrum of disorders of ejaculation ranging from mild premature to severely retard or absent ejaculation. Normally, by age 17 or 18 years, 75% of men are able to control their ejaculation. Premature ejaculation is the most common male sexual dysfunction (Sharma et al., 2012). Several surveys among different populations estimate its prevalence at 29%, with a range between 1% and 75% depending on the population and criteria used to define the condition. The diagnostic criteria for premature ejaculation as follows (Sharma et al., 2012):

1.) Persistent or recurrent ejaculation with minimum sexual stimulation that occurs before, upon, or shortly after penetration and before the person wishes it.

ii.) Marked distress or interpersonal difficulty

iii.) The condition does not arise as a direct effect of substance abuse, i.e., opiate withdrawal (Sharma et al., 2012).

Premature ejaculation and sexual desire disorders were the frequent reported problems in young adult males with adverse familial relationship. Premature ejaculation was also found to be associated with anxiety. Several classifications for premature ejaculation have been reported. In one, premature ejaculation was classified into primary and secondary disorders. Primary premature ejaculation describes persons who, since the beginning of sexual experience, have never been able to control the ejaculatory function, whereas secondary premature ejaculation describes individuals who develop the condition after years of satisfactory sexual activity (Fouad et al., 2001). Painful ejaculation has been reported as a side effect of tricyclic antidepressants in at least two patients. Psychogenic post ejaculatory pain syndrome (PEPS) is a rare sexual disorder of male dyspareunia that was first described in 1979 as a persistent and recurrent pain in the genital organs during ejaculation or immediately afterward (Fouad et al., 2001). Ejaculatory pain in the testicular region may result from epididymal congestion after vasectomy or from duct obstruction and/or infection, testicular torsion, mass lesion, or prostatitis. In some cases, specific etiological factors other than psychological stress cannot be identified (Courtois and Betts et al., 1994; Fouad et al., 2001).

Disorders of orgasm

Male orgasmic disorder is defined as a persistent or recurrent delay in or absence of orgasm after a normal sexual excitement phase during sexual activity (APA, 1994). The most common causes of orgasmic disorders include:

1.) Drugs like (selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, substance abuse)

Ii.) CNS disease: (multiple sclerosis, Parkinsons, Huntingtons chorea, lumbar sympathectomy)

iii.) Psychogenic: (performance anxiety, conditioning factors, fear of impregnation, hypoactive sexual desire) (Fouad, 2001).

Failure of Detumescence

It is a prolonged erection usually lasting for between 4 hour and more. It is painful and always unaccompanied by sexual desire despite the fact that it is often preceded by usual sexual stimuli. The condition is self-perpetuating and is characterized by diminished perfusion of the corporeal bodies. When chronically present, corporeal fibrosis and erectile dysfunction occur. At least two classifications of priapism have been described. The first is etiologically based and classifies the condition into primary (idiopathic) and secondary priapism. The second classification is physiologically based and depends on measurement of penile blood gases and pressures. It classifies priapism into low-blood flow (ischemic) and high-blood flow (non-ischemic) conditions. In the majority of ischemic priapism cases, erection probably starts with a normal or high-blood flow state, particularly in cases induced with intra-penile drug (Betts et al., and 1994; Fouad et al., 2001).

Management of Male Sexual Dysfunction

As more and more information is gained on the mechanism underlying penile erection, more drugs are being developed to treat erectile dysfunction. The most promising of these synthetic medications are the phosphodiesterase inhibitors which are effective in treating both natural and psychological impotence (Hackett et al., 2018).

Some Drugs used to Manage Male Sexual Dysfunction

As information is progressively gained on how the relaxation process takes place in the penis, more drugs are being developed to treat erectile dysfunction. The phosphodiesterase type 5 (PDE5) inhibitors are new drugs which affect local regulation of erectile function by potentiating the effects of nitric oxide (NO). The first developed and consequently the most famous is sildenafil, the active principle of the oral pill Viagra®. This active compound is effective in treating both organic and psychological impotence (Boolell et al., 1996).

II. MATERIALS AND METHODS

Research Animals

A total of 25 mature albino rats with mean weight 180 – 200 g, were obtained from the Small Animal Holding Unit of the Department of Animal science, 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 with free access to water All experiments were performed on the laboratory animals in this study based on the Principles of laboratory animal care.

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.

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 25 mature albino rats; were used for this research. They were randomly divided into five (5) groups (A, B, C, D and E) with five (5) rats in each group.

Groups A: received 100 mg/kg body weight of the extract, group B: received 200 mg/kg of the extract, group C: received 300 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.

Collection of Plant and Authentication

The plant sample was collected from the surroundings of Nasarawa State University, Keffi Campus. The identification/authentication was done at the Department of Forestry and Forestry Products of the Nasarawa state university.

Preparation of plant extract

Calotropis procera stem aqueous extract was prepared in accordance with the procedure described by Yakubu et al., and Akanji. (2005), with little modifications. The plant stem was pulverized into powder of 75μm mesh size after drying and stocked in an airtight container from which 800 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 100 mg/kg body weight (low dose), 200 mg/kg body weight (medium dose) and 300 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.

Hormone Assay

Preparation of Serum

Standard guideline as described by Yakubu et al., (2003), was used to prepare the serum.

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. 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 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 immune-absorbent 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). 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 min. 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 nm 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). 100 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

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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.

Assessment of some liver function parameters

Determination of the Aspartate Aminotransferase (AST) Activity

The activity of aspartate aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit used. Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazine with 2, 4-dinitrophenylhydrazine.

Procedure

The serum samples (0.1 ml) pipetted into the samples test tubes only and 0.1 ml of distilled water was pipette into the blank test tube. The, 0.5 ml of reagent one (R_1) containing phosphate buffer, L aspartate and beta-oxoglutarate was pipetted into both the blank and serum sample test tubes respectively. The entire reaction medium was well mixed and incubated for 30 minutes in a water bath at 37^0C. Immediately after incubation, 0.5 ml of reagent two (R_2) containing 2,4-dinitrophenylhydrazine was added to the blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25 ^{0}C. Finally, 5.0 ml of 0.4M sodium hydroxide solutions was added to both the blank and serum sample test tubes, respectively and mixed thoroughly. The absorbance was read at a wavelength of 546 nm after 5 minutes.

Determination of the alanine aminotransferase (ALT) activity

The activities of alanine aminotransferase was assayed by the method of Reitman and

Procedure

The serum sample (0.1 ml) was pipetted into the samples test tube and 0.1 ml of distilled water pipette into blank test tube, 500 µl of the ALT substrate buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate (R_1) were added. The entire reaction media were well mixed and incubated for 30 minutes in a water bath at 37 ^{0}C and pH 7.4. Immediately after incubation, 0.5 ml of reagent two (R_2) containing 2, 4-dinitrophenylhydrazine was added to the blank and sample test tubes. These were thoroughly mixed and allowed to stand for exactly 20 minutes at 25 ^{0}C. Finally, 0.5 ml of sodium hydroxide solution was added to both the blank and serum samples test tubes respectively and mixed thoroughly. The absorbance were read at wavelength of 546 nm after 5 minutes.

Determination of the alkaline phosphatase (ALP) activity

Principle

The activities of alkaline phosphatase was assayed by the method of Babson et al (1966), as outlined in Randox kit, used. The alkaline phosphatase act upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure

Into three test tubes labeled test, serum, standard and blank were added 50 µl of serum, 50 µl of standard and 50 µl distilled water, respectively. Then, 0.05 ml (50 µl) of alkaline phsophatase substrate into labeled test tubes for 3 minutes mix gently and incubate for exactly 10 minutes at 37 ^{0}C. After that, alkaline phosphatase color developer (2.5 ml) was added at timed intervals and mix well. Measure the absorbance at 630 nm

Alkaline phosphatase = Absorbance of sample x value of standard
Absorbance of standard

Statistical Analysis

All results obtained from this research were analyzed and presented as mean ± SD and analysis was done using Statistical Package for the Social Sciences (SPSS) Version 26. One-way analysis of variance (ANOVA) was used. Differences in mean was considered to be statistically significant at ($p \leq 0.05$).

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III. RESULTS

Preliminary phytochemical screening of aqueous stem extract of Calotropis procera (Rooster Tree)

The results of both qualitative and quantitative phytochemical of aqueous extract of Calotropis procera stem are presented in table 1 & 2 respectively. The results showed the presence of abundance phytochemical at varying concentrations.

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 contents of Calotropis procera stem aqueous extract

Phytochemicals constituents	Concentrations (%)
Alkaloids	14.38 ± 1.02^{d}
Saponins	0.01 ± 0.01^{a}
Tannins	21.55 ± 1.47^{e}
Flavonoids	21.10 ± 1.56^{e}
HCN	0.00 ± 0.01^{a}
Steroids	0.02 ± 0.03^{a}
Terpenoid	7.79 ± 0.65^{b}
Phenol	16.24 ± 2.13^{d}
Glycosides	10.88 ± 0.95^{c}

Results are expressed in Means ± SD (n= 3)

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

Table 3: Effect of Calotropis procera stem aqueous extract on some male fertility hormones

Group	LH (mg/ml)	FSH (mg/ml)	TESTOSTERONE (mg/ml)
A	2.72 ± 0.19^{b}	3.53 ± 0.37^{b}	2.52 ± 0.17^{a}
B	2.38 ± 0.12^{b}	3.38 ± 0.24^{b}	2.23 ± 0.36^{a}
C	2.39 ± 0.24^{b}	3.52 ± 0.19^{b}	2.15 ± 0.21^{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}

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

Group A: received 1 ml of 100 mg/kg body weight of extract, group B: received 1 ml of 200 mg/kg body weight of extract, group C: received 1 ml of 300 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 ($p < 0.05$).

Table 4: Effect of Calotropis procera stem extract on some Liver function parameters in albino Rats

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
A	26.20 ± 1.64^{a}	43.00 ± 7.31^{b}	42.31 ± 1.37^{b}
B	25.40 ± 3.44^{a}	41.60 ± 4.04^{b}	41.53 ± 0.93^{b}
C	26.80 ± 1.48^{b}	45.40 ± 5.90^{b}	40.35 ± 1.39^{b}
D	25.80 ± 64^{a}	36.80 ± 5.54^{a}	38.53 ± 4.11^{a}
E	30.10 ± 1.48^{c}	50.80 ± 7.48^{c}	45.50 ± 5.48^{c}

Group A: given 1 ml of 100 mg/kg extract administration, Group B: given 1 ml of 200 mg/kg extract administration, Group C: given 1 ml of 300 mg/kg extract administration, Group D: given Distilled water and Group E: given 1 ml of 100 mg/Kg Sildenafil Citrate.

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

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

IV. DISCUSSION

Many scholars has affirmed that medicinal plants enhances sexual performance. Ratnasooriya and Dharmarsiri (2000) stated that medicinal plant with a tendency to stimulate and enhance sexual behavior should produce a statistically significant increase indicators of sexual arousability, motivation, and vigor and as well induce secretion of reproductive hormones. 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 fertlity potential since intake significantly increased serum luteinizing hormones (LH) and follicle stimulating hormones (FSH) with increased testosterone levels that are significant in a dose dependent manner 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 fertility enhancing plants are good alternatives for the improvement of sexual behavior (Guoha et al., 2009), probably due to their efficacy and availability. The fertility enhancing property of C. procera stem could be attributed to the various active components present in this plant. The phytochemical analysis of C. procera revealed the presence of varying concentration of phytochemical such as flavonoid. It has been reported that flavonoids, alkaloid and saponin 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 sexual/reproductive enhancing capacity. Phytochemicals have been reported to enhance erection and prolong ejaculatory latency in male albino rats (Yakubu et al., 2005).

Effects Liver function parameters of the C. procera aqueous stem extract was evaluated by assaying some liver biomarker of rats in each of the groups. From the results obtained they were dose-dependent significant ($p < 0.05$) decrease in ALT, AST and ALP activities in the extract treatment groups; (A, B and C) respectively, when compared to their activities in the positive control group (group E) (with standard drug). However, compared to negative control groups (administered with distilled water), there was no significant ($p<0.05$) increase in ALT activity, but AST and ALP activities were significantly increase (($p<0.05$). This suggests that the extract had no deleterious effect on the liver health on the extract treated groups compared with effect posed by the standard drug, such as sildenafil citrate (Ozougwu, 2017).

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V. CONCLUSION

This study suggested that C. procera stem aqueous extract had the highest fertility enhancing potential particularly at lowest dose of 100 mg/kg of the extract, which showed the highest increase on FSH, LH and Testosterone concentrations. A marker for reproductive or sexual effectiveness or function and proves its use as sexual enhancer and reproductive dysfunction herbal remedy by local users.

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