ORIGINAL ARTICLE

Citrullus lanatus Ethanolic Seed Extract Improved Male Sexual Behavior in Rats via Enhancement of Sexual Hormone and Hypothalamic-Pituitary-Gonadal Pathway

Atuadu Vivian Onyinye[1], Anyanwu Godson Emeka[2]*

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria, [2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria

Abstract:

Background: Current drugs used for sexual dysfunction have several limitations, necessitating the search for alternative medicine. *Aim and Objective:* The study investigated the effect of *Citrullus lanatus* ethanolic seed extract (CLESE) on the sexual behavior and reproductive hormones of adult male Wistar rats. *Material and Methods:* Twenty-four adult male Wistar rats were randomized into four groups (n=6). Group 1 received saline (1 ml/kg, p.o.) and served as normal control, groups 2 and 3 were given CLESE (500 and 1000 mg/kg, p.o.) for 14 consecutive days. Group 4 received Sildenafil Citrate (SC) (5 mg/kg, p.o.), which served as positive control drug and this was administered 4 h before sexual behavioral test only on days 7 and 14. Sexual behavioral tests were performed in a Plexiglas copulatory arena. Male sexual behavioral parameters were evaluated following one on one exposure of experimental male rats with oestrus-induced sexual seeking female rats. Thereafter, rats were sacrificed. *Results*: CLESE enhanced libido by significantly ($p < 0.05$) increasing mounting and intromission frequencies relative to normal controls. CLESE also enhanced intromission and ejaculation latencies, but decreased mounting latency and post ejaculatory interval. Serum testosterone and luteinizing hormone were also significantly ($p < 0.05$) increased. The histological findings showed improved histoarchitechture of the testes and hypothalamic sections. *Conclusion:* The findings from this study showed that CLESE enhanced sexual behavior via mechanism related to increased serum testosterone and luteinizing hormone and may be useful for men with sexual dysfunction.

Keywords: *Citrullus lanatus,* Male Sexual Dysfunction, Sexual Behavior, Impotence

Introduction:

Male sexual dysfunction is the persistent physical or emotional problems associated with sex in males [1]. The prevalence of male sexual dysfunction has been reported to increase as age advances [1]. Previous psychological studies have shown that sexual dysfunction is a common disorder. However, it is a topic that many of the patients are not willing or hesitant to share [2]. Moreover, most patients of this disease exhibit some symptoms of neurological impairments such as emotional instability, anxiety, depression and inferiority complexes [2]. The largest ever sex survey conducted between the years 2001-2002 indicated that 88% of men considered sex as being essential to their overall sense of well being [3]. However, one impeding factor currently affecting this need is the problem of sexual dysfunction characterized by decreased libido, weak erection, quick ejaculation and impotence [4]. Although the pathogenesis of sexual dysfunction appears complex and multi-faceted, altered sexual hormone such as

testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) play crucial roles in the etiology of the disorder [5]. Previous studies have shown that reduced serum testosterone, LH and FSH have been implicated in many types of sexual dysfunction and also form the basis of treatment of the disease [5, 6]. Infections, limited access to health services, socio-economic stress, stigma and cultural beliefs have been implicated in the high prevalence of sexual dysfunction in Africa [7, 8]. Perceived stigma is one of the major factors contributing to heightened neurological stress, inferiority complex and stigma in sexually dysfunctional patients [7]. However, the fear of recurrent episode of weak erection and social embarrassment has also been identified as additional factors for increased inferiority complex and stigma in these patients [8].

Current drugs used for the treatment of sexual dysfunction and impotence have several limitations such as adverse effects, relapse and ineffectiveness in some individuals [9]. It has also been reported that most patients with sexual dysfunction and impotence in developing countries do not get proper medical attention hence depend on herbal products sought from traditional medical practitioners [10]. Previous studies have shown that several medicinal plants are useful in the treatment of sexual dysfunction in traditional medicine, which required further scientific validations before clinical trials [10]. Several plant-derived chemicals have been reported to have sex enhancing activity and are used to relieve some sexual dysfunctions [11]. These plant components elevate libido, sexual potency and sexual pleasure [12]. One of such acclaimed sexual enhancing herbal plants is *Citrullus lanatus* (Water melon).

Citrullus lanatus is a climbing annual plant belonging to Cucurbitaceae plant family [13]. The plant is grown for its edible fruit known as watermelon, a special kind of berry plant. Different ethnomedicinal surveys revealed that the seed and fruit of *Citrullus lanatus* are used for treatment of hypertension, urinary diseases, renal stone [14], diarrhea, gonorrhea [15,16] and as diuretic [14]. Previous ethnopharmacological reports indicate that the fruit and seed extracts of *Citrullus lanatus* possess anti-inflammatory [18], anti-oxidant [19], anti-microbial [20], anti-prostatic hyperplasia [21], anti-ulcerogenic [22], cytoprotective [13] and anti-diabetic [23] activities. The health benefits of *C. lanatus* have been ascribed to the presence of essential phytochemical constituents such as glycosides, cucurbitacins, triterpenes, sterols, alkaloids, lycopene and quercetin [15]. Importantly, *C. lanatus* contains high concentration of citrulline, the precursor for nitric oxide synthesis, which plays important role as vasodilator [9]. Although studies have established the aphrodisiac activity of *C. lanatus* fruit and rind extracts on adult male and female wistar rats respectively[24, 25]; there is lack of documented scientific literature on the activity of seed of the plant on male sexual behavior. Hence, the present study investigated the sexual behavior and propagative hormones of male rats chronically treated with *Citrullus lanatus* Ethanolic Seed Extract (CLESE).

Materials and Methods:

Plant materials collection

Citrullus lanatus fruits were harvested from a local farm in Plateau State (Jos). The freshly collected *Citrullus lanatus* was authenticated at University of Nigeria, Nsukka, in the department of Plant Science and Biotechnology.

Plant extraction

The water melon fruits were cleaned, the seeds removed, dried and pulverized. Three hundred gram (300g) was soaked and cold macerated with ethanol (70%) with aid of a Soxhlet extractor for a period of 24 h. Using a vacuum rotary evaporator, the filtrate was concentrated to complete dryness and kept in a desiccator prior to the experiment.

Phytochemical screening

Phytochemical screening was done to qualitatively determine the phyto-constituents of the seed extract using the standard laboratory procedures of precipitation and coloration reaction as previously described to identify the secondary metabolites present [26-28].

Animal procurement

Twenty-four male (200-250g) and female (160-200g) adult Wistar rats of 12-13 weeks' old were used. They were exposed to 12 h light and dark cycle in a cage with 23 ± 2°C room temperature including free access to food (growers mash manufactured by Top Feed Nigeria Limited) and water *ad libitium*. Animals habituated in experimental home cages for a period of 14 days prior to the start of the research work. The guilds for the well-being of the experimental animals were based on the procedures of the National Institute of Health for care and use of laboratory animals.

Animal grouping and treatment protocol

Male rats were randomly distributed into 4 groups (n = 6). Group 1 received saline (1 mL/kg, p.o.) and served as normal control, groups 2 and 3 were given CLESE (500 and 1000 mg/kg, p.o.) for 14 consecutive days. However, group 4 received Sildenafil Citrate (SC)-5 mg/kg, p.o.), which served as positive control drug and this was administered 4 h before sexual behavioral test only on days 7 and 14. Sexual behavioral tests were performed in a plexiglas copulatory arena cage and videotaped. Thereafter, rats were sacrificed.

Induction of oestrus cycle

Oestrus (heat period) was artificially induced in female counterpart rats by the successive intracutaneous injection of estradiol benzoate (10 μg/kg) and progesterone (0.5 mg/kg) 48 h and 4 h respectively, before mating. Sexual behavioral assessment of the male rats, commenced after confirmation of receptivity of the female rats as previously described by Jennifer *et al*., [28]. Thereafter, male sexual behavioral assessments consisting of frequency of mount intromission frequency, ejaculation frequency, latency mount, intromission latency, ejaculation latency and post ejaculatory intervals were assessed when male and female rats (1:1) were exposed to each other.

Determination of male sexual behavior

Each male rats was placed and habituated in a plexiglass copulatory arena cage for 5 min. Thereafter, a receptive female was presented to the male and the male sexual behavioral assessments were evaluated and recorded [29, 30].

Blood collection and hormone assay

On the last day of the experiment, 1 ml of blood was collected from each of the 24 rats. The blood samples were immediately placed in a centrifuge (Centurion Scientific Ltd., UK) and centrifuged for 5 min at 12,000 revolutions per/min. The serum was quickly decanted into a test tube and stored immediately in a chest freezer (Haier Electrical Appliances Inc., Philippines) until assayed. The concentration of the serum testosterone, FSH and LH were determined shortly after using ELISA Kit according to manufacturer's protocols.

Histological study

The animals (n = 3) were anaesthetized, the brain and gonads were quickly dissected out, fixed in 10% formol saline and processed for paraffin wax embedding. They were carefully sectioned using a rotary microtome and stained in H&E and methylene blue dye for histological examination.

Statistical analysis

Data were presented as Mean ± Standard Error of the Mean (SEM). Significant difference was analyzed using one-way analysis of variance (ANOVA) and student's t-test. Statistical significant difference was taken at $p < 0.05$.

Results:

Phytochemical analysis

This revealed high presence of citrulline, lycopene and saponins, slight presence of phenols, steriods and flavanoids, trace amount of alkaloid and tannins. Cardiac glycoside and anthraquinone were not detected (Table 1).

Table 1: Qualitative Analysis of Phytochemical of CLESE

Phytochemical	CLESE
Saponin	+++
Alkaloid	+
Flavonoid	++
Tannin	+
Phenol	++
Cardiac Glycoside	-
Steroid	++
Anthraquinone	-
Citrulline	+++
Lycopene	+++

Keys: - = Not detected, ++ = Moderate concentration, + = Low concentration, +++ = High concentration

Effect of CLESE on body weight of male Wistar rats

Figure 1 showed that CLESE (500 and 1000 mg/kg, p.o.) did not produce any significant changes in body weight of animals on days 7 and 14 when compared with normal control group (Fig. 1).

Effect of CLESE on male sexual behaviors in rats

The effect of CLESE on male sexual behavior is illustrated in fig. 2.

Oral administration of CLESE (500 and 1000 mg/kg) produced marked increased mounting frequency on day 7, but higher dose of CLESE enhanced the mounting frequency on day 14 when compared with control. Oral treatment with CLESE (1000 mg/kg) also increased ($p < 0.05$) intromission frequency on days 7 and 14; whereas SC increased it only on the 7th day. Furthermore, CLESE and SC decreased the mounting and intromission latencies on days 7 and 14 in comparison with normal control groups in a significant pattern. Treatment with CLESE markedly accentuated ejaculation latency similarly to SC in comparison with the normal group. On the other hand, post ejaculation interval was significantly decreased due to administrations of the two doses of CLESE and SC on days 7 and 14 (Fig. 2).

Effect of CLESE on sexual hormones in rats

The effect of CLESE on sexual hormone concentrations is shown in Table 2.

Oral administration of CLESE (500 and 1000 mg/kg) and SC profoundly increased serum testosterone and FSH concentrations relative to normal controls. However, only CLESE (1000 mg/kg) and SC, but not CLESE (500 mg/kg) elevated LH levels when compared with normal control (Table 2).

Table 2: Effect of CLESE on Sexual Hormone Levels of Male Rats

Parameter	Normal control	CLESE (500 mg/kg)	CLESE (1000 mg/kg)	SC (5 mg/kg)
Testosterone (ng/mL)	1.3 ± 0.1	4.2 ± 0.1[a]	6.1 ± 0.7[a]	5.0 ± 0.2[a]
FSH (miu/mL)	7.7 ± 0.8	9.5 ± 5.4[a]	11.1 ± 0.7[a]	9.8 ± 1.1[a]
LH (miu/mL)	3.2 ± 1.0	4.1 ± 0.6	7.3 ± 1.8[a]	5.9 ± 0[a]

Each value represents the mean ± S.E.M of 6 animals/group. [a]p< 0.05 compared with normal control (one-way ANOVA followed by Newman-Keulspost hoc test). CLESE = Citrullus lanatus ethanolic seed extract, SC = Sildenafil citrate

Effect of CLESE on histoarchitecture of testis of rats

The effect of CLESE on histoarchitecture of testis of rats is shown in Fig 3.

The testicular sections of rats in all the groups showed normal testicular features. CLESE exhibited improved tubular size, compact seminiferous tubules and increased number of spermatozoa in the lumen of the seminiferous tubules (Fig. 3).

Effect of CLESE on histoarchitecture of hypothalamus of rats

The effect of CLESE on histoarchitecture of hypothalamus of rats is shown in Fig. 4.

Neural tissue sections of the hypothalamus of the rats in all treatment groups showed normal perikarya and neroglia cells. However, treatment with CLESE and SC significantly enhanced neurosecretory activity, as evidenced by increased perikarya and neuroglia densitormetric cell expression (Fig. 4).

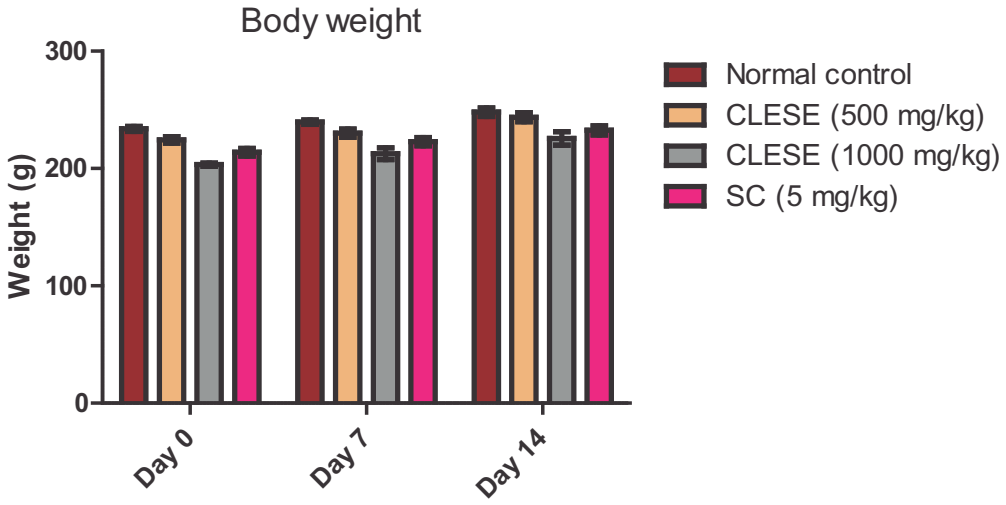

Fig. 1: Effect of CLESE on the Body Weight (g) of the Male Rats

Each value represents the mean ± S.E.M of 6 animals/group (one-way ANOVA followed by Newman-Keuls *post hoc* test). CLESE = *Citrullus lanatus* ethanolic seed extract, SC = Sildenafil citrate

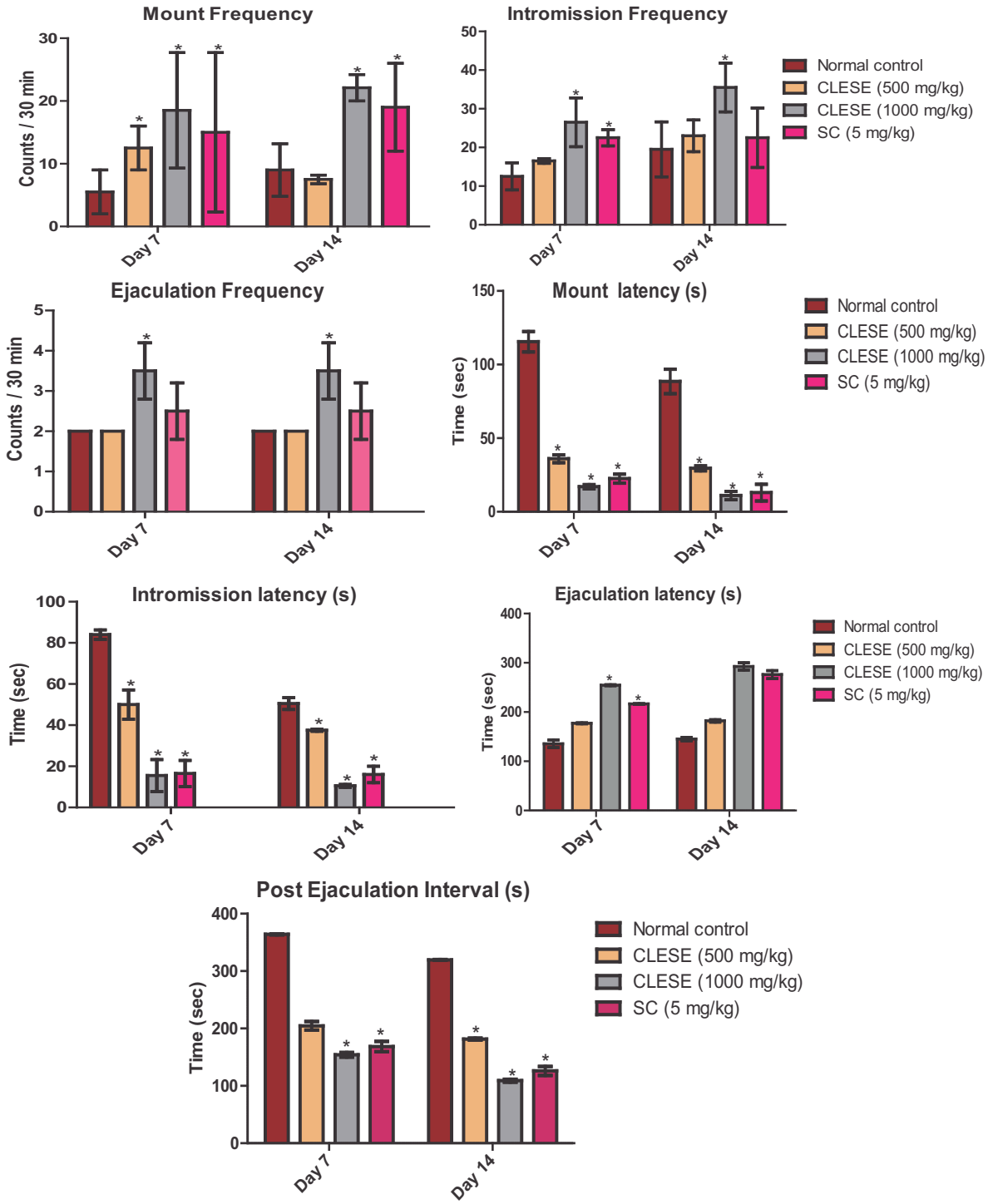

Fig. 2: Effect of CLESE on Sexual Behavior in Rats on Day 7 and 14

Each value represents the mean ± S.E.M of 6 animals/group. [a]$p < 0.05$ compared with normal control (one-way ANOVA followed by Newman-Keuls *post hoc* test). CLESE = *Citrullus lanatus* ethanolic seed extract, SC = Sildenafil citrate

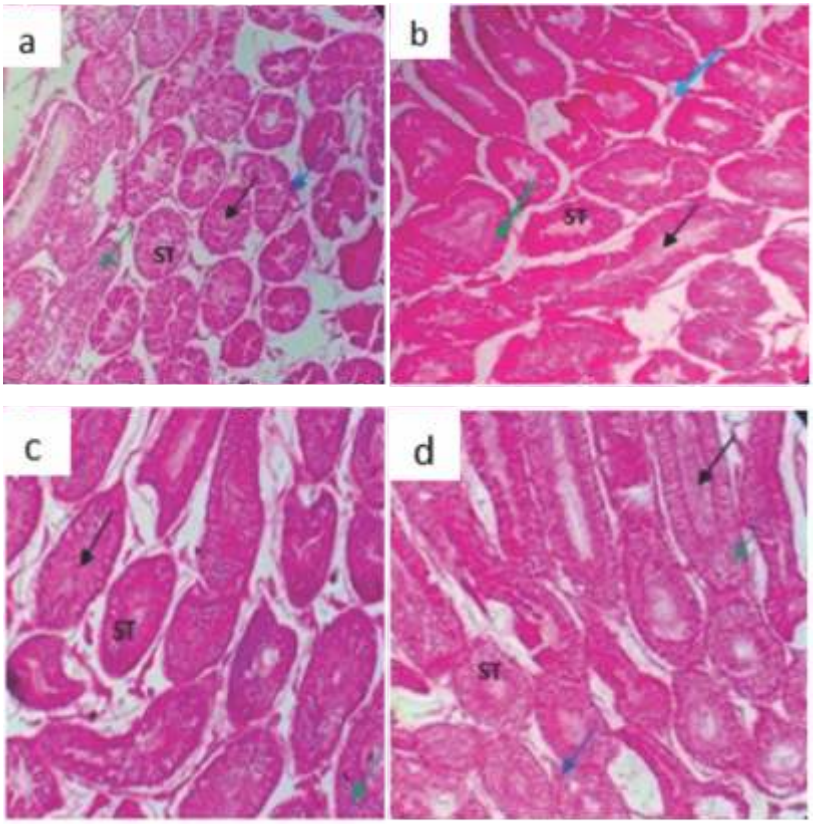

Fig. 1: Effect of CLESE on Histoarchitecture of the Testis of Male Rat

Photomicrograph of coronal section through the testis of (a) normal control, (b) CLESE (500 mg/kg), (c) CLESE (1000 mg/kg), and (d) SC (5mg/kg). (Seminiferous tubule, ST) = green arrow; Spermatogenic cells = black arrow; lumen of the tubule = blue arrow; interstitial/leydig cells). There were increased tubular size, more compact tubules and increased number of spermatozoa migrating to the lumen of the CLESE-treated groups and SC-treated rats. H&E × 100. CLESE = Citrullus lanatus ethanolic seed extract, SC = Sildenafil citrate

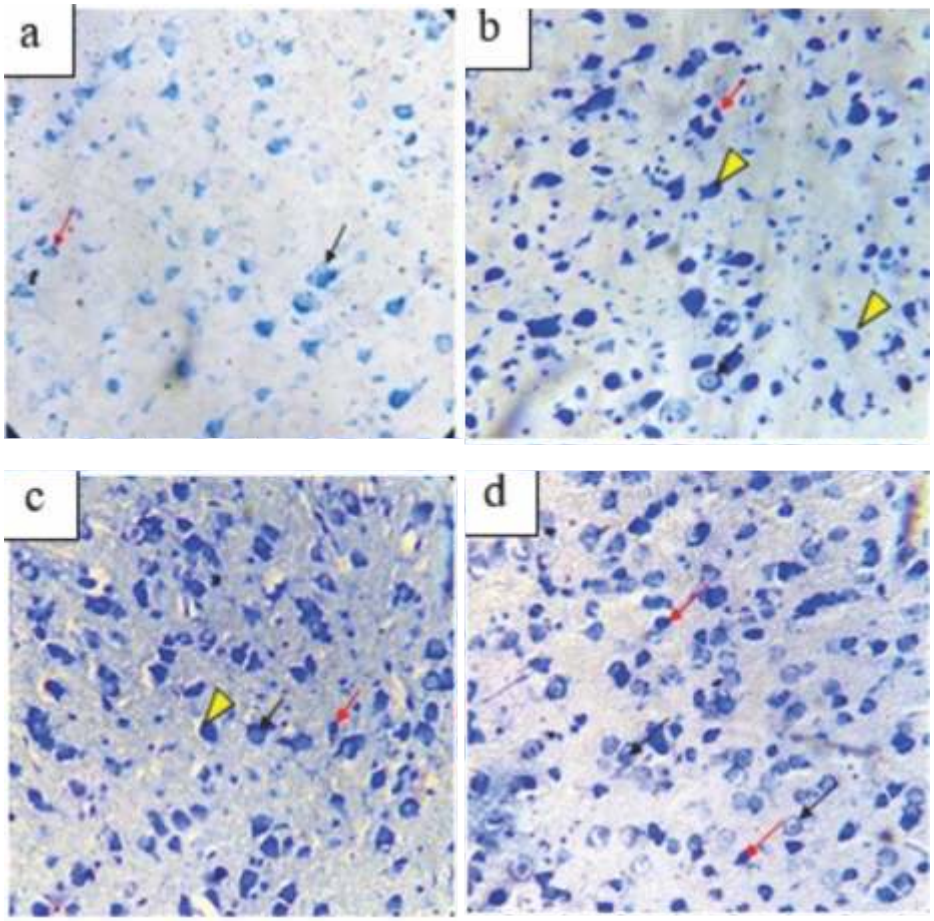

Fig. 4: Effect of CLESE on Histoarchitechture of Hypothalamus of Male Rat

Photomicrograph of coronal sections of neural tissue showing Nissl staining of perikarya and neroglia cells (red arrows) in parts of the paraventricular nucleus of the hypothalamus of (a) normal control, (b) CLESE (500 mg/kg), (c) CLESE (1000 mg/kg), and (d) SC (5mg/kg). There is an increased neurosecretory activity in CLESE (500 and 1000 mg/kg, p.o.) and SC (5mg/kg) treated rats as evidenced by increased Nissl staining densitometric intensity of the perikarya and neroglia cells however, with few hypertrophic perikarya which show prominent nucleoli and cytoplasmic vacuolation (black arrows). There are also observed hyperchromatic perikarya without cytoplasmic vacuolation (yelloy arrowheads) in both treatment groups. Methylene blue ×100. CLESE = Citrullus lanatus ethanolic seed extract, SC = Sildenafil citrate.

Discussion:

The findings from the study showed that administration of CLESE significantly enhanced sexual behavior, as evidenced by increased mounting and intromission frequencies, ejaculation latency as well as decreased mounting and intromission latencies, and decreased post ejaculation interval when compared with normal control group. Also, administration of CLESE was associated with increased serum reproductive hormones. There was a significantly increased testicular tubular size, compact seminiferous tubules and increased number of spermatozoa in the lumen of the seminiferous tubules following CLESE administration. Furthermore, neural activity of hypothalamus showed increased neurosecretory activity, as evidenced by increased perikarya and neuroglia densitormetric cell expression; however, with no noticeable increase in body weight.

The current finding from this study agrees with the finding of Munglue *et al*. [24] presenting that *Citrullus lanatus* fruit enhanced sexual activity due to the presence of aphrodisiac active compounds. This could be as a result of the presence of phyto-constituents like saponins, flavonoids, citrulline etc. Steroidal phyto-chemicals such as saponin containing high concentrations of citrulline and arginine improves sexual performance and increases fertility and thus, are highly recommended in the management of infertility conditions [31, 32]. *Citrullus lanatus* seed is known to contain high levels of precursors substrates like arginine and citrulline, both of which are responsible for the synthesis of nitric oxide, a potent vasodilator molecule [24, 33]. Nitric oxide is an endogenous molecule that acts to control the extent of turgidity of penile erection via upregulation of the guanosine monophosphate [31, 34]. Different studies have shown that a decrease in nitric oxide concentration result in erectile dysfunction [4, 34]. Thus, improvement in sexual activity at least in part focuses on increasing nitric oxide concentration [31, 34]. The finding that CLESE increases sexual behavior as indicated by increased mounting and intromission frequencies, ejaculation latency as well as decreased mounting and intromission latencies, suggest increased sexual performance characterized by prolonged coital activity. The decreased post ejaculatory interval observed in this study, which is commonly used as tool for evaluating recovering processes following first sexual encounter [35, 36] also suggests prolonged duration of coitus by the extract due to the presence of potent aphrodisiac compounds such as nitric oxide [24, 33, 37].

Earlier reports have stated that an increased testosterone level is directly proportional to increased sexual interest [9, 38, 39]. In this study, it was observed that the testosterone level was significantly increased. Indeed, the result is in accordance with the earlier investigations showing that extracts with aphrodisiac potential was found to be accompanied with increased testosterone levels [40, 41]. Remarkably, increased testosterone has been shown to exert a negative feedback mechanism to the hypothalamus through the Hypothalamic-Pituitary-Gonadal (HPG) axis [42]; thereby leading to inhibition of gonadotropin production and restoration of low concentrations of testosterone, luteinizing and follicle stimulating hormones. Herewith, we did not observe this trend

as there was a significant increase in the concentrations of reproductive hormones [42].

Mounting evidences have demonstrated the potentials of plant extracts with antioxidant activity to protect against testicular and brain damage, and improve sexual performance [4, 11]. In our present study, histological staining revealed that CLESE demonstrated testicular and brain neuronal protection, as evidenced by increased concentration of testicular cells, enhanced spermatogenesis, with increased compact and seminiferous tubular sizes. This observation is in line with earlier reports [43, 44]. The increased secretory activities in the gonad are also reflected in the increased neurosecretory activity in the hypothalamus, as mediated by the HPG pathway. Based on the testicular and neuroprotective property demonstrated by CLESE, it is suggested that the seed extract of *Citrullus lanatus* could be recommended for the management of sexual dysfunction.

Conclusion:

Our findings suggest that CLESE exerts enhancement of sexual performance activity via mechanism related to increased serum hormones as well as modulation of hypothalamic-pituitary-gonadal axis.

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[*] ***Author for Correspondence:*** *Anyanwu Godson Emeka, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria*
Email: anyanwugemeks@yahoo.comPhone: +2348036765433