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Original Article

Aqueous extract of *Cyperus esculentus* L. restores and boosts sexual competence in paroxetine-dysfunctioned male Wistar rats.

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ABSTRACT

Objective: To investigate Cyperus esculentus aqueous extract on paroxetine-mediated sexual dysfunction in male Wistar rats. **Methods:** Sexually dysfunctioned (SD) rats were orally treated with PowMaxM [reference drug (7.14 mg/kg)] and the extract (500 and 1000 mg/kg body weight) once daily for 10 days, and their sexual behavioural parameters were monitored and computed. Relative testes-body weight and testicular function parameters were also evaluated at the end of the treatment period. **Results:** Dim light observation on the animals revealed respective proceptive and precopulatory behaviours by the primed female animals and the extract-treated male rats. Compared with SD rats, the extract-treated groups significantly restored and improved sexual behaviour and libido as evident from the remarkably increased frequencies of mount, intromission, ejaculation and ejaculatory latency. In addition, the latencies of mount, intromission, and post-ejaculation were significantly reduced. The significantly reduced testicular activities of alkaline phosphatase, acid phosphatase, lactate dehydrogenase and the concentrations of protein, cholesterol, glycogen, testosterone, luteinizing hormone, follicle stimulating hormone and testes-body weight in the SD rats were also markedly increased following treatment with the extract. The effects elicited by the extract competed favourably with the reference drug used. The improved sexual competences exhibited by the male rats in this study are indicative of aphrodisiac attributes of the extract and could be adduced to the presence of phytonutrients as revealed by the GC-MS chromatogram. **Conclusion:** The data from this study suggest that C. esculentus is capable of restoring and boosting sexual competence and the probable mechanism is via synergistic influence of the adaptogenic bioactive principles.

KEY WORDS: Detumescence; Ethnomedicinal; GC-MS chromatogram; Orgasm; Testicular hormones; Sexual dysfunction.

INTRODUCTION

Cyperus esculentus L. (Cyperaceae), popularly called tiger nut is a tuber growing freely and consumed widely in Spain, Arabian Peninsula, east Africa and most parts of West Africa including Nigeria [1]. It is a tough erect fibrous-rooted perennial plant, 1 to 3 ft tall and reproduces by seeds and rhizomes [2]. *C. esculentus* (CE) is known by different names across the globe, but 'Aya' (Hausa), 'Imumu' (Yoruba), and 'Ofio' (Ibo) are its common nomenclatures among the three major tribes in Nigeria [3]. Its extracts are rich in phosphorus, potassium, starch, fat, sugars, protein as well as vitamins C and E [4]. The pharmacological importance of CE in the treatment of diabetes, urinary tract infections, colon cancer, dyspepsia, anaemia, diarrhea, dysentery, hypercholesterolemia and as anti-microbial agent have been well documented [5-10]. In Nigeria, CE is usually eaten raw, roasted, dried, baked or made into a refreshing beverage called 'kunnu' [11], and is one of several plants commonly used to manage and treat sexual dysfunction [12]. Globally, studies show a high prevalence and incidence of male sexual incompetence [13, 14] correlating with general dissatisfaction, age and other sexual dysfunctions. Orthodox interventions have been embraced and have provided succour through psychotherapy, vacuum devices, surgery, penile implants and drugs [15]. While the efficacy and effectiveness of these treatment options are guaranteed, affordability, sensitivity, social stigma and inherent adverse effects have limited their applications in time past [16]. Consequently, plants and plant-derived preparations with outstanding pharmacological potentials are being exploited globally as alternative therapy [17]. Little wonder, phytotherapy has continued to be treatment option for individuals seeking

to boost and enhance their sex drive [18, 19]. Though, literatures have implicated CE as sex enhancer [20-22], evidence of its much touted tendency to facilitate sexual invigoration is just receiving scientific attention. While Allouh *et al*. [23] have just recently reported the potency of its powdered formulation on the copulatory behaviour of male rats, its androgenic potential is still lacking in scientific literatures. Thus, this study aimed to provide detailed and comprehensive biochemical information on the aphrodisiac potentials of oral administration of crude aqueous extract of *C. esculentus* in paroxetine-sexually dysfunctioned male Wistar rats. The GC-MS analysis of the extract was also evaluated.

MATERIALS AND METHODS

Chemicals, drugs, reagents and assay kits

PowMaxM was a product of Beijing Kowloon Pharmaceuticals Co., Limited, Beijing, China. While paroxetine was obtained from GlaxoSmithKline, USA, estradiol benzoate and progesterone were procured from Sigma Chemical (St. Louis, USA) and Shalina Laboratories (Mumbai, India), respectively. Assay kits for protein, cholesterol, glycogen, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lactate dehydrogenase (LDH) were products of Randox Laboratories Limited (Co Antrim, United Kingdom) while those of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were obtained from Monobind Inc. (California, USA). All other reagents used were of analytical grades.

Plant collection, identification and crude extract preparation

Fresh nuts of *C. esculentus* were purchased from Oja-Oba, Ilorin, Kwara State, Nigeria and were identified by Dr AA Abdulrahaman of the department of Botany, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (no. UIH/14/21781) was deposited. The nuts were washed and oven dried at 37 ^{0}C for 48 h and thereafter pulverized into smooth powder. The pulverized sample (650 g) was suspended in 6.5 L of distilled water with regular agitation for 24 h. The solution obtained was filtered and the resulting filtrate was concentrated over water bath (40 ^{0}C) and yielded 344.11 g crude extract corresponding to 52.94% of the residue. The dried crude extract (CEAE) was kept air-tight and refrigerated before use.

Experimental animals

Healthy Wistar rats (both sex) with average weight 180±1.01 g were collected from the experimental animal facility of Kwara State University, Malete, Nigeria and kept in clean metabolic cages placed in a well-ventilated room with optimum condition (temperature 25 ± 2 °C, photoperiod; 12 h natural light and 12 h dark; humidity; 45-50%). They were acclimatized to the animal room condition for 7 days during which they had free access to feed and water *ad libitum*. The cages were cleaned daily and overall treatments were in accordance with the guidelines of National Institute of Health on the care and use of laboratory animals [24]. An approval (KSU/IECCULA/005/08/014) was granted by the Departmental Independent Ethical Committee of Kwara State University, Malete, Nigeria prior to commencement of the study.

Induction of sexual impairment in male rats

Adopting the method of Neelesh *et al*. [25], sexual incompetence was induced in the male rats. In brief, healthy sexually experienced male rats were randomized into 2 groups (X and Y) of 45 animals each. While group X served as control and received normal saline in Tween 80, rats in group Y received 10 mg/kg once daily oral dose of paroxetine (prepared in Tween 80, suspended in 0.9% saline solution) for 21 days. Exactly 30 min post last treatment on the 21st day, oestrus female was introduced into respective cages in observation for mating performance and results were recorded and compared with control group. Mating performance analyses were conducted under dim light at room temperature and evaluation of mount frequency (MF), intromission frequency (IF), ejaculation frequency (EF), mount latency (ML), intromission latency (IL), ejaculation latency (EL) and post-ejaculation latency (PEI) were monitored for 30 min post pairing period. Male rats with minimum 25% reduction in sexual behaviour were considered as sexually impaired and were recruited for subsequent study.

Induction of oestrus phase in female rats

The procedures of Tajuddin *et al*. [26] and OECD [27] were adopted in this study. Briefly, female rats were brought to oestrus by consecutive subcutaneous administration of estradiol benzoate (10 μg/100 g) and progesterone (0.5 mg/100 g) at 48 h and 6 h respectively prior to pairing. Confirmation of oestrus phase was done by vaginal smears examination.

Experimental protocol

The male rats were randomized into 5 groups of 7 animals each. Animals in group 1 (normal sexually experienced) were given sterile placebo (distilled water in Tween 80) and served as control. Group 2 comprised untreated sexually dysfunctioned rats. Animals in groups 3, 4 and 5 were sexually dysfunctioned rats treated with PowMaxM (7.14 mg/kg b.w.-reference drug), and CEAE (at doses 500 and 1000 mg/kg b.w.), respectively. All administrations (1 mL and freshly prepared daily in Tween 80) were done once daily for 10 days via oral gavaging.

Evaluation of male sexual performance

Following 24 h of last treatment (on male rats), artificially primed female rats were paired (1:1) with the males. After 20 min of proceptive and precopulatory period, the rats were observed for 20 min from another room for copulatory characteristics. Sexual behaviours including MF, IF, EF, ML, IL, EL, PEL were recorded and computed as previously done above.

Testicular homogenate preparation

After 6 h of sexual competence study, the rats were humanely sacrificed under diethyl ether euthanization and the testes were immediately but diligently isolated from the rats, cleaned and homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were centrifuged at 10000 *g* for 10 min at 4°C to obtain post-mitochondrial fractions and the resulting supernatant was stored at -20°C to ensure maximum liberation of the testicular fractions.

Determination of biochemical parameters

The testicular activities of LDH, ACP, ALP and cholesterol concentration were determined in accordance with the manufacturers' specifications in the respective manual. While the method of Lowry *et al.* [28] was adopted in the estimation of testicular protein concentration, that of glycogen was evaluated following the procedure of Kemp and Van Heijningen [29]. The levels of testosterone, FSH and LH in the testicular homogenate were determined by immunoenzymometric assay as detailed in the manufacturer's guide.

Gas chromatography-mass spectrometric (GC-MS) analysis of the extract

C. esculentus aqueous extract was subjected to GCMS analysis using an Agilent Technologies 6890 Series gas chromatograph coupled with (an Agilent) 5973 Mass Selective detector and driven by Agilent Chemstation software. A eHP-5MS capillary column was used (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250°C. The initial oven temperature was at 60 °C which was programmed to increase to 280 °C at the rate of 10 °C/min with a hold time of 4 min at each increment. Injections of 2 μL were made in the splitless mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadruple temperature 150°C, solvent delay 4 min and scan range 50-700 amu. The compounds were identified by direct comparison of the retention times and mass spectral data and fragmentation pattern with those in the National Institute of Standards and Technology (NIST) library.

Data computation and analysis

Results were expressed as mean ± standard error of mean of replicate determinations and were subjected to one way analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc., South Wacker Drive, Chicago, USA). Significant difference between the treatment means was determined at 5% confidence level using Duncan's Multiple Range Test.

RESULTS

Copulatory indices

While continuous oral administration of 10 mg/kg b.w. of paroxetine for 21 days brought about a significant ($p < 0.05$) decrease in MF, IF, EF and EL, the computed values for ML, IL and PEL increased significantly (Table 1). The

Table 1. Effect of *Cyperus esculentus* aqueous extract on mating behavioural parameters in male rats (n =7, *X* ± SEM).

Parameter	Control	Paroxetine	Paroxetine + PowMaxM /Extract (mg/kg b.w.)		
			PowMaxM	500	1000
Mount frequency	15.75±0.05[a]	7.50±0.01[b]	10.50±0.02[c]	10.75±0.02[c]	15.05±0.06[a]
Intromission frequency	12.25±0.05[a]	7.00±0.01[b]	9.25±0.03[c]	9.75±0.02[c]	11.05±0.04[d]
Ejaculatory frequency	2.75±0.02[a]	1.25±0.02[b]	1.75±0.03[c]	2.00±0.02[d]	2.75±0.02[a]
Mount latency (s)	75.35±0.15[a]	125.30±0.21[b]	105.75±0.25[c]	102.35±0.25[c]	85.85±0.16[d]
Intromission latency (s)	145.53±0.65[a]	201.65±0.71[b]	175.95±0.40[c]	179.75±0.92[c]	143.05±0.36[a]
Ejaculatory latency (s)	150.66±0.11[a]	86.35±0.21[b]	155.65±0.19[c]	160.35±0.12[c]	150.16±0.25[a]
Post-ejaculatory latency (s)	166.65±0.75[a]	189.25±0.56[b]	171.05±0.45[c]	175.31±0.39[c]	161.59±0.25[d]

Values with different superscript across the same row for each parameter are significantly ($p<0.05$) different.
[a]Not significantly ($p>0.05$) different, [b]Significantly different from normal control, [c,d]Significantly different from paroxetine-sexually dysfunctioned group.

Table 2. Effect of crude aqueous extract of *Cyperus esculentus* on the relative testes-body weight of male rats (n =7, *X* ± SEM).

Parameters	Control	Paroxetine	Paroxetine + PowMaxM /Extract (mg/kg b.w.)		
			PowMaxM	500	1000
IBW (g)	179.02±0.25	185.25±0.15	188.15±0.04	181.09±0.02	199.05±0.01
FBW (g)	197.90±0.15	179.06±0.20	208.01±0.09	196.99±0.68	215.00±0.05
TW (g)	0.95±0.01[a]	0.65±0.09[b]	1.00±0.06[a]	0.93±0.03[a]	1.09±0.03[a]
RTW (g/100g b.w)	0.48±0.02[a]	0.36±0.01[b]	0.48±0.03[a]	0.47±0.02[a]	0.51±0.01[c]

Values with different superscript across the same row for each parameter are significantly ($p<0.05$) different. IBW= Initial body weight, FBW= Final body weight, TW= Testes weight, RTW= Relative testes weight.
[a]Not significantly ($p>0.05$) different, [b]Significantly different from normal control, [c]Significantly different from paroxetine-sexually dysfunctioned group.

trends in these parameters were however dose-dependently reverted following treatment with CEAE. It is worthy of note that the effects elicited by the 500 mg/kg b.w. of the extract competed well with the reference drug (PowMaxM) employed in this study (Table 1).

Testicular parameters

Paroxetine-mediated reduction in the relative testicular weight of the animals was significantly ($p < 0.05$) increased by aqueous extract of *C. esculentus* (Table 2).

Testicular enzymes (LDH, ACP, and ALP) activities were significantly ($p < 0.05$) induced by CE aqueous extract. These inductions significantly ($p < 0.05$) reverted the paroxetine-mediated reductions in the specific activities of these enzymes and also produced effects that compared favourably with PowMaxM and the normal control (Figure 1).

Data obtained with respect to paroxetine treatment on testicular concentrations of protein, cholesterol and glycogen of male rats are shown in Table 3. The significant ($p<0.05$) reductions in the levels of these parameters facilitated by paroxetine were significantly and dose-dependently reversed in the extract-treated animals.

Table 4 showed the effect of CEAE on the testicular hormones of the animals. The significant ($p<0.05$) decreases in the levels of testosterone, FSH and LH following a 3-week continuous administration of paroxetine were not only normalized but significantly improved in the extract-treated rats especially at 1000 mg/kg b.w. dose. The effects elicited by the extract on the testicular hormonal enhancement competed well with PowMaxM.

GC-MS analysis

Crude aqueous extract of *C. esculentus* was subjected to GC-MS analysis to identify adaptogenic constituents in the extract by comparison with standard mass spectra in the NIST library. Compounds belonging to alkaloids, terpenoids and steroids groups of phytochemicals were the major identifiable constituents (Figure 2 and Table 5).

DISCUSSION

Sexual competence entails complex interactions between nervous, endocrine, and vascular systems and their normal co-ordinated activities have been closely associated with sexual rejuvenation, intercourse and vigour. Normal sexual response cycle in males comprised 5 physiologically interrelated sequences that occur in a defined consecutive pattern (libido→ erection→ ejaculation→ orgasm→ detumescence) and disruption anywhere in the entire sequence may lead to sexual dysfunction [25]. Sexual dysfunction is a common side effect of psychoactive and antidepressant medications and this was taken advantage of in the choice of paroxetine in this study. Paroxetine, a selective serotonin reuptake inhibitor, is a frequently prescribed antidepressant and decreased libido,

Table 3. Concentrations of testicular cholesterol, protein and glycogen following administration of *Cyperus esculentus* aqueous extract in paroxetine-treated rats (n =7, $X \pm$ SEM).

Treatments	Cholesterol (mmol/L)	Protein (mg/L)	Glycogen (mg/100 mg glucose)
Sterile placebo (Control)	110.80±1.02[a]	94.93±0.68[a]	76.07±0.04[a]
Paroxetine-treated	51.11±0.21[b]	39.89±0.73[b]	27.07±0.50[b]
Paroxetine + PowMaxM (7.14 mg/kg b.w.)	100.80±1.11[a]	85.65±0.73[c]	70.12±0.58[a]
Paroxetine + 500 mg/kg b.w extract	89.74 ±0.22[c]	72.30 ±0.10[d]	53.43±0.19[c]
Paroxetine + 1000 mg/kg b.w extract	103.94 ±1.52[a]	88.18 ±0.19[c]	72.85±0.40[a]

Values with different superscripts along the same column for each parameter are significantly different ($p < 0.05$).
[a]Not significantly ($p>0.05$) different, [b]Significantly different from normal control, [c,d]Significantly different from paroxetine-sexually dysfunctioned group.

Table 4. Concentrations of testicular hormones in sexually dysfunctioned rats following administration of *Cyperus esculentus* aqueous extract (n =7, $X \pm$ SEM).

Treatments	LH (mIU/ml)	Testosterone (nmol/L)	FSH (mIU/ml)
Sterile placebo (Control)	11.58±0.45[a]	16.50±0.46[a]	22.63±0.04[a]
Paroxetine-treated	7.28±0.03[b]	11.39±0.42[b]	12.97±0.06[b]
Paroxetine + PowMaxM (7.14 mg/kg b.w.)	13.86±0.45[c]	22.52±0.60[c]	25.56±0.02[c]
Paroxetine + 500 mg/kg b.w. extract	11.47 ±0.18[a]	15.93 ±0.90[a]	22.05 ±0.08[a]
Paroxetine + 1000 mg/kg b.w. extract	13.55 ±0.02[c]	20.04 ±0.97[c]	25.50 ±0.08[c]

Values with different superscripts along the same column for each parameter are significantly different ($p < 0.05$).
LH= luteinizing hormone, FSH= follicle stimulating hormone.
[a]Not significantly ($p>0.05$) different, [b]Significantly different from normal control, [c]Significantly different from paroxetine-sexually dysfunctioned group.

abolished or delayed orgasm, difficulties in maintaining an erection, and inhibitory ejaculation (which are prominent characteristics of sexual dysfuction) are common side effects of paroxetine [25]. In this study, the sexual incompetences and significantly reduced precopulatory behaviours by the paroxetine-treated male rats over the 21 days administration period may be adduced to its inhibitory effect on serotonin reuptake that consequently resulted in the observed sexual dysfunction. However, the improved precopulatory behaviours and observed advances by CEAE-treated male rats toward the females may suggest that the animals were sexually aroused and rejuvenated. The impending copulatory contacts between the extract-treated rats could be suggestive of facilitated sexual instinct and thus, lending credence to sex enhancing candidature of CEAE.

Mount and intromission frequencies are important indices of libido, vigour, and potency. While MF suggests sexual motivation, enhanced IF is reflective of coherent erection, penile orientation and the comfort by which ejaculatory reflexes are stimulated [30]. Therefore, the dose-dependent increases in MF and IF following treatment with CEAE for 10 days is indicative of improved libido [26]. Such improvement might have resulted from elevated levels of anterior pituitary hormones and testosterone, which consequently stimulated sexual competence through concerted stimulatory effect on dopamine receptors [31]. Neelesh *et al.* [25] also gave similar submission while exploring androgenic and gonadotropic activities of ethyl acetate fraction of *Allium cepa*. Furthermore, the

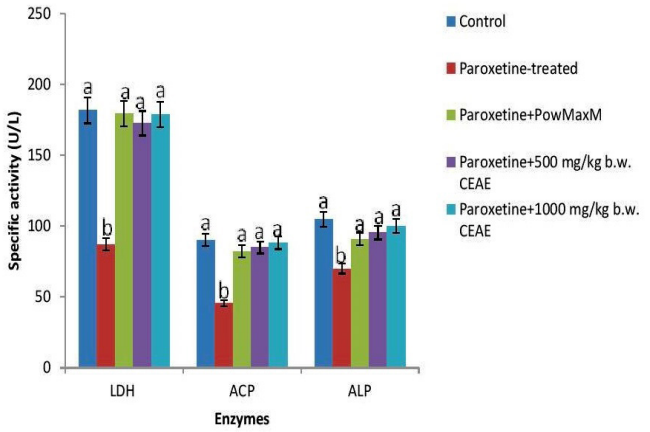

Figure 1. Effect of crude aqueous extract of *Cyperus esculentus* on the testicular activities of some enzymes in the rats (n =7, *X* ± SEM). CEAE= *C. esculentus* aqueous extract, LDH= lactate dehydrogenase, ACP= acid phosphatase, ALP= alkaline phosphatase, b.w.= body weight.
Bars carrying different superscript are significantly different (P<0.05). [a]Not significantly (p>0.05) different, [b]Significantly different from normal control.

Table 5. Adaptogenic constituents of crude aqueous extract of *Cyperus esculentus*, their phytochemical class, retention time and peak area.

S/No.	Active compound	Phytochemical class	Retention time	Peak area (%)
1	Allicin	Indole	4.40	1.11
2	Taxol	Terpenoid	6.43	0.32
3	Morphine	Alkaloid	8.17	1.36
4	Artemegenin	Terpenes	8.43	0.79
5	Aspirin	Salicylic acid	9.37	3.83
6	Codeine	Alkaloid	9.75	3.53
7	Ephedrine	Alkaloid	10.07	1.30
8	Camptothecin	Alkaloid	10.35	1.12
9	Tubocurrarine	Alkaloid	10.93	5.43
10	Digitoxigenin	Steroid	11.22	7.60
11	Gitoxigenin	Steroid	12.12	5.14
12	Digoxigenin	Steroid	12.60	8.11
13	Capscicine	Alkaloid	13.41	6.79
14	Podophyllotoxin	Alkaloid	13.90	7.20
15	Vinblastine	Alkaloid	14.55	5.24
16	Vincristine	Alkaloid	15.15	7.64
17	D-epoxyguaiene	Terpenoid	15.78	4.32
18	P-cymol	Monoterpene	16.30	7.37
19	Kobusone	Terpenoid	17.03	2.63
20	Isokobosone	Terpenoid	17.38	5.33
21	Mustakone	Terpenoid	18.43	5.02
22	Patchoulenone	Terpenoid	19.43	5.41
23	Pectin	Carbohydrate	20.38	2.05
24	Rotundiene	Alkaloid	20.78	0.87
25	Selinatriene	Terpenoid	21.38	0.49

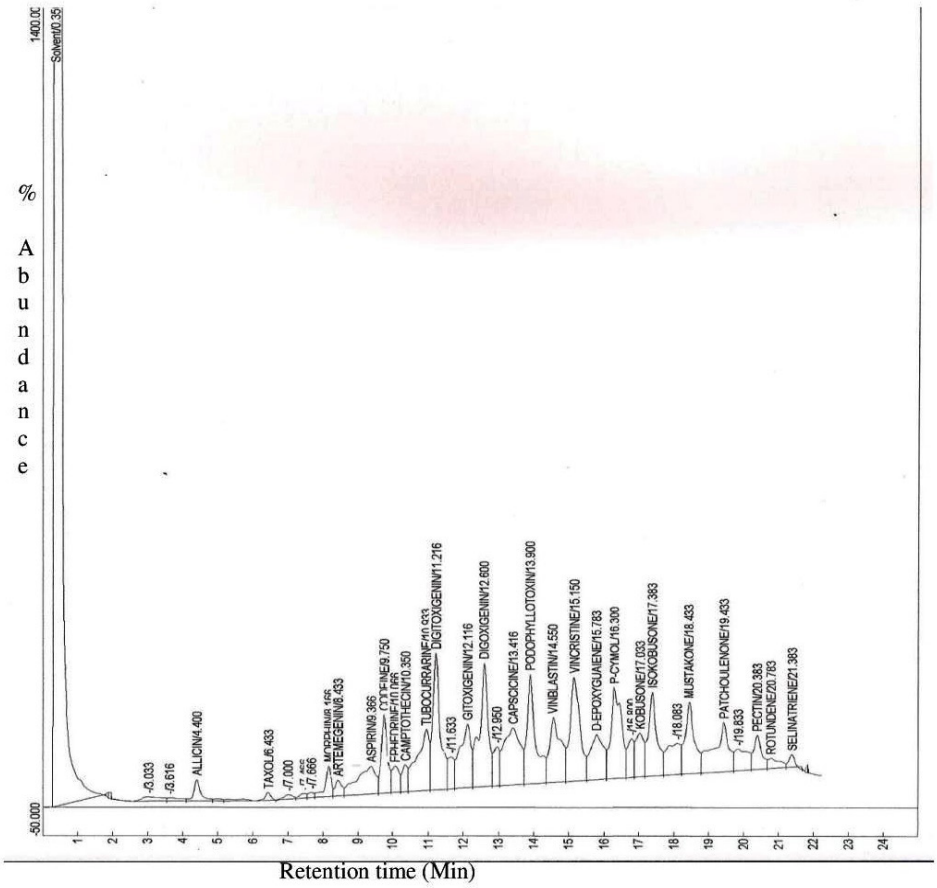

Figure 2. GC-MS chromatogram of crude aqueous extract of *Cyperus esculentus*

presence of plug in the vagina of the female rats and the genital toileting in the extract-treated male animals were indicative of ejaculation. Hence, the increase in EF by CEAE is an indication of improved aphrodisiac potential of *C. esculentus*. Unlike MF and IF whose increased values are directly linked with sexual motivation in animals, those of ML and IL are inversely related [32]. Therefore, the significantly decreased ML and IL values facilitated by CEAE treatment could be informative of invigorated sexual motivation and enhanced competence. Moreover, the reversion of short-lived EL in the paroxetine-treated rats to significantly prolonged EL following the 10 days treatment period with CEAE could indicate that the extract is composed of active principles responsible for lengthened coitus period and facilitated copulatory performance in the animals. This was further supported by the display of pelvic thrusting during intromission and ejaculation by the extract-treated rats. The PEL is an invaluable assessment tool for potency, libido and the rate of recovery from exhaustion after initial sets of mating [19]. Thus, the significantly attenuated PEL in the extract-administered animals could be linked to energy boost and less exhaustion in the initial sets of mating by the male rats. It may also be suggestive of enhanced potency and libido. Our submissions are consistent with the report of [33], where significantly reduced PEL (in the range ≤5400 s) was considered optimum for sexual satisfaction.

The use of relative organ-body weight ratio is often imperative to ascertain and clarify probable treatment-related organ weight variations in experimental animals [34]. While an increase in organ-body weight ratio may either depict inflammation or increased secretory ability of the organ, a reduction could be indicative of cellular constriction. Thus, the observed reduction in relative testes-body weight ratio in the paroxetine-treated rats may be adduced to possible testicular constriction that consequently impeded its secretory capabilities. That this was significantly increased in the extract-treated animals is not only informative of increased secretory activity of the testes but also a substantive androgenic potential of CEAE. This is closely supported by the corresponding increases in the testicular levels of protein, cholesterol, glycogen and the assayed hormones (Table 4) in this study and agrees with the submission of Olaolu *et al.* [35] that linked graded increase in testes-body weight ratio to treatment with standardized extract of *Cissampelos mucronata* in male rats.

While testicular protein has been implicated in spermatogenesis and subsequent maturation of sperm cells, the overall dependency of steroidogenesis and steady supply of energy for sperm motility are linked to testicular levels of cholesterol and glycogen, respectively [36]. Thus, the significant reduction in the concentration of testicular protein in paroxetine-treated rats may be suggestive of either

impaired spermatogenesis or defective sperm maturation process. Consequently, the observed normalization and significant improvement in its concentration in the extract-treated rats could imply enhanced spermatogenesis/ and sperm maturation. This may also be indicative of androgenic potential of CEAE within the investigation period. In addition to energy supply, glycogen reserve is germane in metabolic milieu and its cellular concentration is closely associated with steroid hormones [37]. While, the decrease in glycogen levels in paroxetine-administered animals might be a manifestation of gradual and steady depletion of energy reserve that subsequently impacted on the motility aided energy metabolism in sperm cells of the rats, the observed reversion in this trend following 10 days treatment with CEAE is an indication of its energy conservative and protective potentials on the sperm cells. Similarly, the significantly reduced testicular cholesterol concentration in animals treated with paroxetine alone may suggest altered and overall reduction in steroidogenesis particularly on the androgens. The observed dose-related extenuation of this paroxetine-mediated attenuation in the testicular cholesterol concentration by the CEAE may be attributed to its markedly improved moderation on androgen concentration through enhanced steroidogenesis [38]. These findings quite agree with earlier reported submissions [39, 40]. These authors opined that androsteroidogenic principles in plant extracts were responsible for observed improvements in testicular cholesterol concentration and offered such plants as being excellent aphrodisiac agents.

Apart from its inhibitory effect on nitric oxide synthase activity that prevents ejaculation and abolishes orgasm, paroxetine has also been reported to selectively inhibit the specific activities of ALP, ACP and LDH in the testes [41]. Testicular ALP and ACP are important enzymes involved in steroidogenesis by respectively partaking in channelizing materials for the process and maintenance of sperm physiology [42]. Hence, the reduced activities of these enzymes could be due to inhibitory role of paroxetine which might have resulted in corresponding reduction in steroidogenesis as well as overall decrease in libido and sexual competence as manifested in this study. Thus, significant induction of paroxetine-mediated reduction in specific activities of these enzymes by CEAE indicates aphrodisiac activity. This could be due to the capability of the extract to enhance the activities ALP and ACP that consequently favoured steroidogenesis and physiological engagements of sperm cells. Studies have implicated lactate rather than glucose as preferred substrate for glycolysis in primary spermatocyte and it is derived from glucose in the Sertoli cells under the influence of FSH and mechanistic action of LDH [43]. The significantly decreased testicular LDH activity in the paroxetine-treated rats may suggest possible reduction in energy metabolism. This might have obstructed spermatogenesis by preventing transformation of spermatocyte to spermatozoa. Therefore, the induced LDH activity following treatment with aqueous extract of CE connotes probable protective and androgenic potentials of the extract.

Certain metabolic hormones (testosterone, LH and FSH) have been closely associated with androgenicity and spermatogenesis [44], and treatments having modulatory influence on their concentrations could modify sexual behaviour and performance. LH and FSH, produced by anterior pituitary lobe are necessary for maintaining testosterone levels such that as their testicular concentration increases so do the testosterone [45]. Specifically, testosterone is required for the growth and development of male reproductive organs and in association with FSH, acts on the seminiferous tubules to initiate and maintain spermatogenesis [35, 46]. Similarly, LH exerts stimulatory effect on testosterone by binding on Leydig cells receptors to activate testosterone biosynthesis. In this study, paroxetine-induced reduction in testicular concentrations of these hormones in the male animals was effectively aborted, normalized and subsequently improved by the extract particularly at 1000 mg/kg b.w. dose. Researches have demonstrated sex drive and libido to be facilitated by elevated testicular levels of FSH, LH and more specifically by testosterone possibly through corresponding increases in its metabolites (Δ4-androstenedione, dihydrotestosterone and dehydroepiandrosterone). Such increases have been closely linked to androspermatogenic potentials of different plant formulations [47].

Generally, the attributes elicited by aqueous extract of CE in this study may be adduced to its steroids, alkaloids and terpenes as revealed by the GC-MS chromatogram. Steroids as one of the most diverse and widespread groups of natural compounds are probably the most important natural aphrodisiacs. In addition to being antioxidative in their mechanisms of action, they also stimulate testosterone biosynthesis via synergistic effect on LH that consequently improves sex drive, vigour and satisfaction [45]. Active involvement of alkaloids and terpenes in blood vessels vasodilation via nitric oxide production that consequently favours penile erection and stimulation of steroidogenesis in animals have also been well documented [48-51]. Therefore, it may be logical to infer that the presence of these phytonutrients in the extract could be responsible for the aphrodisiac activity elicited by the male rats in this study.

CONCLUSION

Consequent upon the available data, this study has lent scientific credence to the ethnomedicinal claim that aqueous extract of *C. esculentus* could boost androgenicity, sex drive, rejuvenation, sexual invigoration and satisfaction in males. Aphrodisiac potential elicited by the extract may be ascribed to its adaptogenic antioxidants. Complete characterization, isolation and exact mechanism of action of these aphrodisiac principles in the extract are highly encouraged. In this direction, efforts are in progress.

CONFLICT OF INTEREST

The authors have none to declare.

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