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Effects of *brassica juncea* ethanolic seed extract on the sexual behavior and reproductive organs of adult male wistar rats

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Abstract
Background: *Brassica juncea* is consumed because of its perceived numerous medicinal potentials amongst which includes aphrodisiac. This work was designed to investigate the effect of *Brassica juncea* Ethanolic Seed Extract (BJESE**)** on the sexual behavior and reproductive organs of male wistar rats. **Methodology:** Adult wistar rats were randomly assigned into four groups (n=6). Normal saline 1 mL, low and high doses (200 and 400 mg/kg body weight) of BJESE and sildenafil citrate (SC) (5mg/kg) were orally administered to groups I – IV respectively. Treatment lasted for 14days and sexual behavioral tests were conducted weekly. Thereafter, blood sample was collected through retro-orbital plexus for hormonal assay. Animals were sacrificed by cervical dislocation and the hypothalamus, testes and epididymis were carefully dissected out for histological tissue processing.
Results: BJESE administration significant decreased mount and intromission latencies and post ejaculatory interval (PEI) relative to the normal saline. Luteinizing hormone and testosterone were significant increase in both BJESE and sildenafil groups in comparison with the normal saline group. Follicle stimulating hormone was altered in the treatment groups as compared with the normal. There was slight distortion in testicular connective tissues whereas the seminiferous tubules were normal; containing germ cells in various stages of development and normal epididymal histological structures in all the groups.
Conclusion: BJESE improved sexual behavior of male wistar rats particularly in high dosage (400mg/kg).

Keywords: *Brassica juncea*, Sexual behaviour, Aphrodisiac, Male sexual dysfunction

Introduction
Male sexual dysfunction is one of the most common silent personal battles of men, oftentimes patients experience neurological symptoms like psychological/emotional imbalance, fear, anxiety, depression and complex (Fowler, 1998) [7]. In recent times there has been concerted attempt at researching on male impotency, erectile dysfunction, premature ejaculation, aphrodisiac and male infertility. So a good number of prescription drugs are available which may act as sex stimulant to enhance sexual desire in men. Albeit the use of this orthodox synthetic drugs have proven to show huge improvement in treating sexual disorders, but the side effects are equally enormous. They include irregularities in heart rate, being suicidal, mental issues, tremors and worsened condition with withdrawal of drug (Javeed *et al.*, 2011) [12]. The use of allopathic stimulant results in the expansion of blood channels in other parts of the body causing headache and dizziness. Other adverse effects are facial flushing, stomach turbulence, blurred vision and light intolerance which usually occur at higher doses (Kulkarni and Reddy 1998) [16].
Therefore, there is a growing urgent desire for aphrodisiacs of natural plant or herbal origin as against the use of synthetic compounds because of their numerous unwanted side effects (Javeed *et al.*, 2011) [12]. According to the World Health Organization (WHO) about 80% of the world's population depends primarily on folklore medicine mainly involving the use of herbs extracts (Low *et al.*, 2002) [19]. Brassica juncea is a polyphenols based consumable plant. The wide range of therapeutic uses of its seed and leave is well known to Ayurvedic health care practitioners since time immemorial (Manohar *et al.*, 2001) [20]. The medicinal potentials of various extracts and bioactive secondary metabolites of Brassica juncea leaves and seeds have been reported (Kumar and Andy, 2012; Kumar *et al.*, 2011) [17, 18]. In fact, the whole plant has been reported to cause an increased concentration of hepatic glycogen and glycogenesis, suppressing the activity of glycogen phosphorylase and gluconeogenic enzymes, resulting in reduced glycolysis and gluconeogenesis, after oral administration (10% w/w) in apparently

Health rats for 60 days (Khan *et al.,* 1995) [14], also the ancient Greeks and Romans used this seed to cure arthritis pains (Buhler and Miranda, 2000) [5]. *Brassica juncea* has been shown to calm asthma, reduce high blood pressure, revert sleep cycle to normal in women presenting this as menopause symptom, lowers the frequency of migraine attacks and to avoid heart failure in patients suffering from atherosclerosis or diabetic heart condition (Anutmidaiar *et al.,* 2009) [2]. However, in all of these researches that abound on *Brassica juncea* on one hand and the focused studies on male sexual dysfunction on the other, there is no available documented work on effects of *Brassica juncea* on male sexual behavior therefore the need for this study.

Materials and method

Plant Collection

In *Nigeria Brassica juncea* is popularly sold in churches because of its spiritual connotations so the seed for this study was bought at the Holy Ghost catholic cathedral church Enugu. The seed was identified and authenticated at the University of Nigeria herbarium with the voucher no: UNH – 358 deposited.

Plant Extraction

The Brassica juncea seeds were air dried and pulverized using a blender and the powdered samples stored in polythene bags and placed at room temperature until the time of extraction. One thousand two hundred grams (1200g) of powdered sample was weighed into an extraction bottle and 4000 ml of 70% alcohol was added, stirred and left for 72 hrs in a refrigerator at 4^{0C}. Thereafter, the solution was sieved and filtered using Whatman no 1 filter paper. The filtrates were then concentrated on water bath at 40^{0C} and stored in the refrigerator until needed (Ufelle *et al.,* 2011; Imeobong *et al.,* 2011) [31, 11].

Plant Phytochemical Analysis

The phytochemical analysis was carried out at Brain-Phosphorylation Scientific Solution Service, Ogui Road Enugu, Enugu State. Qualitative assay was done to determine the composition of the active primary and secondary metabolites of the ethanolic seed extract using the standard operating protocol as used in previous literatures (Harborne, 1984; Obdoni *et al.,* 2001) [9, 22].

Experimental Animals

Twenty-four (24) adult male rats (200 – 250) g and 24 female wistar rats (151-.218) g were procured at 10 weeks old from the animal holding of the National Veterinary Research Institute, Vom, Plateau state, Nigeria. They were kept to acclimatize for 2 weeks in standard well ventilated wire gauze cages in the animal house of Anatomy Department University of Nigeria, Enugu Campus at optimum conditions (humidity:40-50%, photoperiod: 12 h natural light and 12 h dark). The rats were given access to water and feed *ad libitum* and weighed weekely all through the experimental period. Approval for the study was obtained from the ethic committee of Faculty of Basic Medicine of University of Nigeria, and all animals were handled humanely and cared for according to the guidelines of National Institute for Health.

The rats were randomly divided into 4 groups of six rats each and the extract was administered orally for 14 days while sildenafil was given an hour before sexual behavioral test on days 7 and 14 as shown in the table below:

Table 1: Animal Grouping and Drug Administration

Groups (n=6)	Treatment	Dosage/Kg body weight
I (Negative control)	Normal Saline	1ml
II	Low Dose BJESE	200 mg
III	High Dose BJESE	400 mg
IV (Positive control)	Sildenafil citrate (SC)	5mg/kg (orally, 1hr before test).

Estrus induction in Female Rats and Monitoring of Male Sexual Behavioral Indices

Female rats were made receptive for mating by artificially bringing them on heat via intra-cutaneous injection of estradiol benzoate (10µg/kg) and progesterone (0.5 mg/kg) 48 hours and 4 hours respectively, before the test. In accordance to Jennifer *et al.,* (Jennifer *et al.,* 2012) [13], once the female receptivity has been confirmed the male Sexual behavioral assessment commenced when each male rat was placed in an observatory copulatory plexiglas cage and allowed to acclimatize for 5mins and the following parameters were assessed after male and female rats (1:1) were exposed to each other for mating in a duration of 25mins and the process was videotaped for proper assessment: Mount frequency, intromission frequency, ejaculation frequency, latency mount, intromission latency, ejaculation latency and post ejaculatory intervals were evaluated (Pfaus and Kippin, 2003; Sandroni, 2001) [24, 27].

Preparation of Serum

Twenty-four hours after the las administration, the rats were weighed for the last time and recorded in accordance to Ratnasooriya *et al.,* (Ratnasooriya *et al.,* 2000) [25]. 1ml of blood was collected by cardiac puncture from each rat into plain tubes and placed in an ice chamber. Thereafter serum was collected by centrifugation, the sera were then aspirated using Pasteur pipette into clean, dry, sample bottles and used within 12 hrs of preparation for the hormonal assay to determine the level of testosterone, FSH and LH using Elisa kit according to the manufacturer's protocol.

Rat Weights and Tissue Processing

At termination of the experiment, the animals were deeply anaesthetized with thiopental 100 mg/kg, i.p and sacrificed while the testes, epididymis and hypothalamus were harvested, rinsed in physiologic saline solution and weighed. Epididymis and hypothalamus were preserved in 10% neutral buffered formal saline while tesetes was preserved in bouins fluid. They were allowed to fix for about 72 hours and processed histologically. The reproductive organs were stained in H&E, while hypothalamus was stained in crestyl fast violet (CFV) for nissl substabces.

Statistical Analysis

The data were analyzed using one-way ANOVA and a post hoc multiple comparison was done using Fisher's least significant difference (LSD) at 95% confidence interval ($P<0.05$). Data were expressed as mean ± SD.

Results

Table 2: Qualitative Phytochemical Analysis of BJESE

S/N	Constituent	Experimental Method	BJESE
1	Carbohydrates		
A	Carbohydrates	Molisch's test	+++
B	Polysaccharides	Iodine Test	---
C	Reducing Sugar	Benedict's Test Fehling Test	---
2	Protein		
A	Protein Test	Biuret Test	---
B	Amino Acid	Ninhydrin Test	++
3	Oil	Filter paper	+++
Secondary Metabolites			
4	Saponins	Frothing Test Emulsion Test	+++
5	Tannin (Catecholic)	Ferric Chloride Test	---
6	Glycosides		+++
7	Alkaloids	Picric Acid Test Wagner's Test Dragendorff's Test	+++ +++ ++
8	Steroids		---
9	Terpenoids		---
10	Phenols		---
11	Resin		++

+ Presence in trace concentration; ++ Presence in moderately high concentration +++ Presence in very high concentration; - absent

Table 3: Effects of BJESE on Sexual Behaviour of Male Wistar Rats

Parameter	Duration	(Saline)	Low BJESE	High BJESE	SC
Mount Freq.	WK 1	5.5 ±0.7	a 9.0 ± 1.4	A 7.5 ± 2.1	20.0 ± 2.8
	WK 2	11.0 ± 0	a 12.5 ± 0.7	13.0 ± 1.4	20.5 ± 2.1
Intromission Freq.	WK 1	8.5 ± 0	10.5 ± 2.1	a y 6.0 ± 1.4	11.5± 2.1
	WK 2	2.5 ± 0.7	a X 7.0 ± 1.4	a X 6.5± 0.7	12.5 ± 2.3
Ejaculatory Freq.	WK 1	4.5 ± 0.5	2± 1	2 ± 0	2 ± 1
	WK 2	1 ± 0	1.5 ± 0.5	X Y A 3 ± 1	1.5 ± 0.5
Mount Latency	WK 1	201.5 ± 12.0	X 35.0 ± 21.2	X 29.0 ± 26.8	79.0 ± 16.9
	WK 2	428.5 ± 20.0	X 54.5 ± 10.6	A X 35.0 ± 35.4	86.5 ± 6.4
Intromission Latency	WK 1	360.0 ± 26.9	A X 86.0 ± 5.7	X 109 ± 14.1	150 ± 13.4
	WK 2	479.5 ± 41.7	A X 83.5 ± 10.6	X 112.0 ± 18.4	158.0 ± 2.8
Ejaculatory Latency	WK 1	591.5 ± 21.5	a ẍ 222.0 ± 13.4	a ẍ 211.5 ± 23.2	1134.5± 14.5
	WK 2	602.0 ± 8	a ẍ 213 ± 143.1	a ẍ 209.0 ± 21	752.0 ± 12
Post Ejaculatory Interval	WK 1	80.5 ± 2.1	100.5 ± 12.0	Ā 80.0 ± 22.6	138.5 ± 2.1
	WK 2	121.0 ± 15.5	215 ± 15.6	AY 71.0 ± 9.9	112.5 ± 95.5

Values expressed as mean ± SD, n= 6, $p < 0.05$
X and ẍ: significant when compared against negative control (enhancement and inhibitory respectively)
A and a: Significant when compared against positive control (enhancement and inhibitory respectively).
Y and y: Significant when dosages of extracts are compared (increase and reverse respectively).

Table 4: Effects of BJESE on Hormonal Level of Male Wistar Rats4

Parameter	Saline	Low BJESE	High BJESE	SC
Testosterone (ng/ml)	0.35 ± 0.07	a 0.35 ± 0.07	X Y a 7.85 ± 0.49	9.9 ± 0.56
Luteinizing Hormone(miu/ml)	2.8 ± 0.2	a 5.25 ± 0.35	X Y a 11.35±1.77	16.25±1.76
Follicle Stimulating Hormones(miu/ml)	6.35 ± 0.35	a 6.0 ± 2.26	10.1± 0.85	12.65±0.49

Values expressed as mean ± SD, n= 6, $p < 0.05$
X and ẍ: Significant when compared against negative control (enhancement and inhibitory respectively)
"A and a": Significant when compared against positive control (enhancement and inhibitory respectively).
Y and y: Significant when dosages of extracts are compared (increase and reverse respectively).

Table 5: Effects of BJESE on Weight of Male Wistar Rats

	Normal saline	Low dose BJESE	High dose BJESE	Sildenafil citrate
WEEK1	202.1 ± 1.75	234 ± 2.0	246.2 ± 3.55	215.4 ± 0.85
WEEK2	203.7 ± 2.4	237.1 ± 2.15	249.9 ± 3.1	217.63 ± 1
WEEK3	204.88 ± 2.4	239.80 ± 1.95	253.23 ± 3.9	219.0 ± 1.15
Weight change	2.34	5.8	7.03	3.6
%increase in weight	1.2	2.5	2.9	1.7
P. Value 1 p. Value 2	0.999 0.834	0.998 0.520	0.998 0.487	0.403 0.431

Values expressed as mean ± SD, n= 6, $p \leq 0.05$

There was statistically insignificant weight change difference across the groups during the course of this work.

Effect of BJESE on the Histoarchitecture of the Testes

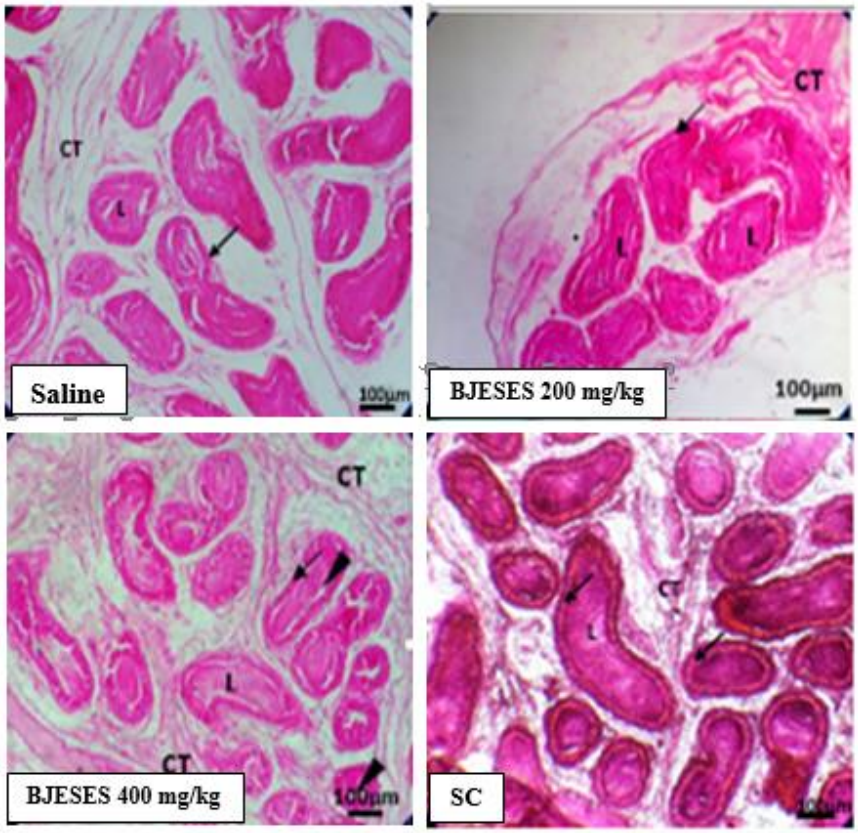

Fig 1: Photomicrograph of testes showing A) Saline, B) Low dose BJESE, C) High dose BJESE, D) SC. The seminiferous tubules (ST) are lined by different stages of sperm cells (green arrows), denser in C and they fill the lumen of the tubules (black arrows). There is slight distortion of the microanatomy of testicular tissue as evidenced by the depletion of the connective tissue interstitium (yellow arrow head) more pronounced in B and C.

Effects of BJESE on the Epididymal Histoarchitecture

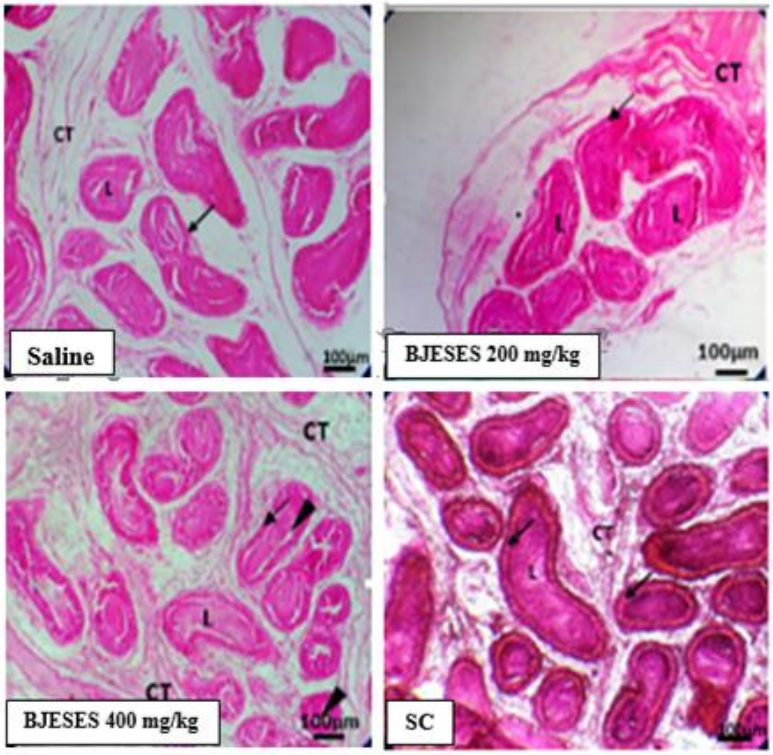

Figure 2: Photomicrograph of Epididymis showing A) Saline, B) Low dose BJESE, C) High dose BJESE, D) SC. The structures composed of epididymal tissue packed with spermatozoa in epididymal lumen, L; as well as a peripheral space between the epithelium and the luminal content (arrow heads), arrayed by pseudostratified columnar epithelium lined connective tissue CT, at varying spermatozoa densities and depletions of CT with the group C most depleted.

Effects of BJESE on the Hypothalamus of Male Wistar Rats

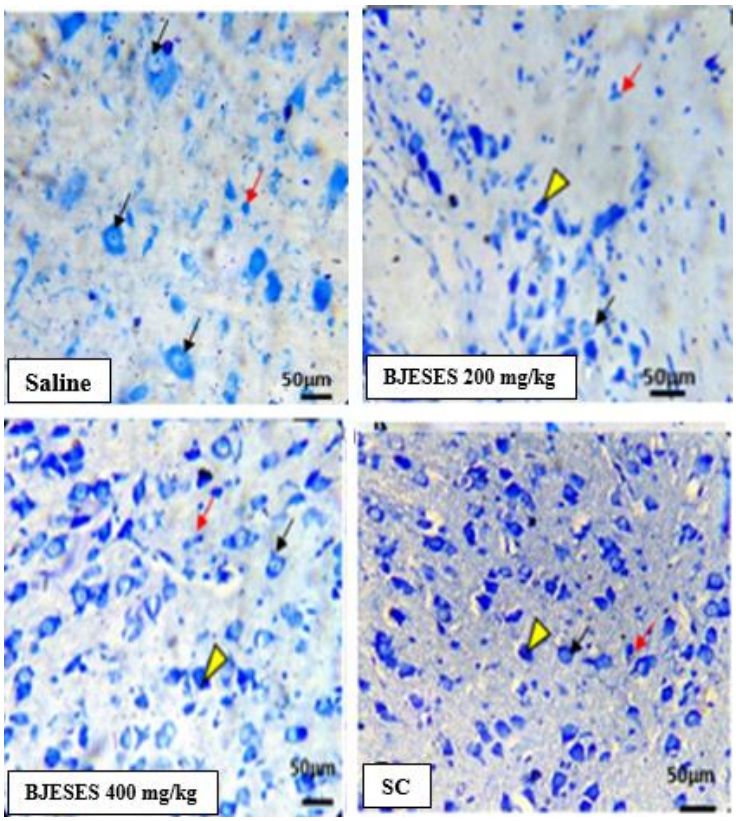

Figure 3: Photomicrograph of Epididymal showing A) Saline, B) Low dose ABJSE, C) High dose ABJSE, D) SC. Neural tissue showing nissl staining of perikarya and neuroglia cells (red arrows) in parts of the paraventricular nucleus and cytoplasmic vacuolation (black arrows). There are also observed hyperchromatic perikarya without cytoplasmic vacuolation (yellow arrowheads) in B, C and D with increased intensity of neurosecretory activities in C than B.

Discussion

At the end of this study it was observed that the BJESE significantly increased sexual behavior, as characterized by the increased mount, ejaculatory and intromission frequencies and in the same light recorded a significant decrease in mount and intromission latencies, as well as significant decrease in post ejaculation interval when compared with negative control group however the ejaculatory latency performance was low (table 2). Also, BJESE gave significant rise to the testosterone level (table 3). Equally, the tubules of the testes (fig 1) and epididymis (fig 2) increased in size due to increased number of spermatozoa and compacted seminiferous tubules with increased number of sperm cells in the lumen of the seminiferous tubules but with slight depletions at the connective tissue (fig 1). More so, the hypothalamus showed increased neurosecretory activity which was more pronounced in the high dose BJESE, by showing more intense nissl stains (fig 3); the body weights were however not significantly increased.

The results of this work concur with that of previous studies on sexual behavior using different extracts (Munglue *et al.*, 2014; Atuadu and Anyanwu, 2019; Rezvanfar., 2008; Atuadu *et al.*, 2018) [21, 3, 26, 4]. This substantial significant enhancement in aphrodisiac on most of the parameters could be linked to the phytochemical metabolites present in BJESE like Saponin, alkaloids, oil, amino acid, glycoside etc. The mechanism of action of Saponin has been shown to enhance aphrodisiac through either a conformational change it adopts after binding to hormone receptors leading to an increase in the functionality of the hormone, or directly targeting the enzymes that synthesize the hormones to improve the hormonal production (Gauthaman and Adaikan, 2008) [8]. Similarly, alkaloid acts by vasodilating the reproductive organ to enhance blood flow leading to improved coitus (Patel et al., 2011) [23], just as witnessed in this study. So this extract according to Suresh-Kumar *et al.*, (Suresh-Kumar *et al.*, 2009) [28] could act by causing alterations in the levels of neurotransmitters and regulating the action of these neurotransmitters on their target cells or by boosting androgen levels. This increment in the androgen level could probably be as a result of some adrenergic effect which is in agreement with the demonstration of adrenergic effect of aqueous and hexane extracts of *M. whitei* on chronic administration *in vivo* in rats (Watcho *et al.*, 2005) [32].

Furthermore, high level of testosterone has been linked with both moderate and significant increase in sexual behaviors. Male aphrodisiac and erection are dependent on testosterone that may act both centrally and peripherally (Suresh-Kumar *et al.*, 2009) [28]. Therefore, the significant rise in testosterone in this study explains why BJESE enhanced aphrodisiac and libido by exhibiting low exhaustion during mating sessions. This agrees with Thakur *et al.*, (Thakur *et al.*, 2009) [30], and they posited that improvement in the sexual behavioral indices of extract treated animals evidenced by enhanced penile erection and reduced hesitation time are attributable to the testosterone like effects of the extracts.

In fact, erection hemodynamics involves balance between inflow and outflow of blood within the corpus cavernosum; there is a relaxation of the smooth muscles and arterioles which allows blood supply to flow in the sinusoidal space. The increased flow of blood, compress venules between sinusoids and the tunica albuginea of the corpus cavernosum. The lack of the distension of tunica albuginea results in venous occlusion, which increases the intracavernosal pressure, generating and sustaining a full erection (Pfaus and Kippin, 2003) [24].

This discovery that BJESE increases sexual behavior as evidenced by increased intromission frequency, ejaculation frequency as well as decreased mount and intromission latencies, point to increased sexual performance characterized by quick but continuous coital activity. Meanwhile the decreased post ejaculatory interval (PEI) correlates with the observed continuity strength possessed in this study, because PEI is used as marker for measuring recovery after the first coitus (Suresh-Kumar *et al.*, 2009; Tajuddin *et al.*, 2005) [28, 29], more so this indication of sustained mating process by the extract may be due to the presence of active aphrodisiac compounds in it (Munglue *et al.*, 2014; Yakubu, 2006) [21, 33].

The ease to mount and the significant intromission also show that a plant extract has antioxidant potential to protect the testes and brain tissues, while also and improving aphrodisiac (Kovac *et al.*, 2014; Hoon *et al.*, 2017) [15, 10], and these were copiously experienced in this study. However, for the histoarchitecture examination, it revealed testicular and brain neuronal protection, indicated by accentuated testicular cells as well as spermatogenesis, with growing arrayed compact, seminiferous tubular sizes but with mild cellular depletion. This concurs with observations from previous works on effects of extracts on male reproductive organs (Amini and Kamkar, 2005; Elsaed *et al.*, 2018) [1, 6]. This gonadal step wise increase in leydig cells and spermatogenic activities are also reflective of the hypertrophic and increasing neurosecretory activity in the hypothalamus especially in the high dose BJESE which agrees with an earlier work on adrenergic modulation (Watcho *et al.*, 2005) [32].

Conclusion

This work provides preliminary evidence that sustained usage of Brassica Juncea Ethanolic Seed Extract possesses aphrodisiac activities giving some credence to its consumption as sexual stimulant and may probably act via a neurogenic mode of action to assuage hypotestosteronemia in male. But it should however be well regulated because BJESE has slight cellular depletive potentials.

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