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Original Article

Reproductive-Enhancing Potentials of Methanol Extracts of *Sphenostylis stenocarpa* Seeds in Male Wistar Rats

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Abstract

Background and Aim: Medicinal plant extracts are still commonly used in folk medicine in developing countries. The seed of African yam bean (*Sphenostylis stenocarpa*) is a very expensive food legume in Nigeria. Previous studies on *S. stenocarpa* plant have proven it to be of an outstanding medical significance. Hence, this study investigated the reproductive-enhancing potentials of methanol extracts of *Sphenostylis stenocarpa* seeds in male Wistar rats.

Materials and Methods: A total of 144 adult rats were used for the experiment. They were divided into 4 groups (A – D) and replicated trice. Group A served as the normal control, while groups B, C and D received three graded doses (800, 1200 and 1600 mg/kg) of the extracts respectively by oral intubation. The rats' gonad characteristics, sperm parameters and hormonal analyses were determined weekly using standard procedures starting from week 0 (day 1) to week 12. The data were analyzed statistically using ANOVA.

Results: The mean weekly gonad characteristics of the male rats in the treatment groups showed overall dose and duration-dependent significant differences compared with the control. The body weights of the male rats significantly reduced ($p<0.05$), whereas the testes weights, gonad somatic index, sperm count and sperm motility of the rats significantly increased ($p<0.05$). Testosterone responded to the plant extracts. The testosterone levels of all the treated rats significantly increased.

Conclusion: The methanol seed extracts of *S. stenocarpa* demonstrated an overall potency to enhance the reproduction in the Wistar rats.

Keywords: Methanol, *Sphenostylis stenocarpa*, Male rats, Reproductive indices

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Introduction

Since poverty and illiteracy hinder the availability and accessibility of traditional medical services, the use of plant extracts in folk medicine is still prevalent in underdeveloped nations around the world (1). According to Burns, plants constitute the source of 30% of all current medications (2). For its edible seeds, the African yam bean (*Sphenostylis stenocarpa*) is mostly farmed in Southern Nigeria. It is known as Okpochundu, Ijiriji or Azama in Southern Nigeria (3). Its seeds can be roasted and enjoyed as snack with palm

kernel (4, 5). Due to its high level of crude protein content, its seeds are very expensive dietary legumes in Southern Nigeria (6). Several studies conducted on *S. stenocarpa* have shown the plant to be of an outstanding medical significance (7-10). It has been reported that this plant can significantly reduce the blood glucose level. However, the methanolic seed extract of *S. stenocarpa* does not affect the hypoglycemic level (7). The methanolic extract of *S. stenocarpa* seed demonstrated anti-anemic activity, confirming the beneficial use of the plant seeds in the control and treatment of anemia (8). The antifungal and antioxidant activities of the plant extract have also been reported (9, 10). Despite all these wide arrays of medicinal values exhibited by *S. stenocarpa*, there is not much information about its reproductive potential (11). Hence, the present research investigated the effect of methanol seed extracts of *Sphenostylis stenocarpa* on reproductive indices of male Wistar rats.

Materials and Methods

Procurement of *Sphenostylis Stenocarpa*

The seeds of *Sphenostylis stenocarpa* were commercially procured from Nkwo Ibagwa Market in Nsukka. The seeds were identified (12) and authenticated at the Taxonomy Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, where the voucher specimen (PSBH/SSs/2019/008) was deposited in the departmental herbarium.

Procurement and Management of Experimental Animals

A total of 144 male rats aged between 3–4 months and weighing 30–40g were used. The animal models were procured from the Genetics and Animal Breeding Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were fed with feed (Vital Feeds® with 18.0 % crude protein and 2800 kcal/kg metabolizable energy) and water ad libitum. Weekly weights were recorded accordingly. All the processes and procedures in handling the rats were in compliance with the guidelines of the National Research Council (13). Ethical approval (UNN/BIOL/ZEB/PHD/61/009) was granted by the Research Ethics Committee of the University of Nigeria. The rats were allowed to acclimatize for a week under standard photoperiodic conditions in clean cages in the Animal Breeding Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were allowed free access to food and water.

Preparation of the Methanol Extract

Sphenostylis stenocarpa dry seeds weighing two kilograms (2 kg) were cleaned, free of any local pests, and processed into a fine powder using a commercial blender. In a conical flask, 1500 g of the processed powdered product was placed, and 1500 ml of concentrated methanol was then added. After being left to stand for 24 hours, the mixture was filtered through Whatman No. 1 filter paper. The weight of concentrated extract was divided by the weight of dried, ground seed, and then multiplied by 140 to determine the percentage yield. A rotary evaporator was then used to concentrate the extract at low temperature (between 30 – 40^{o}C). Subsequently, a stock solution containing 1,600 mg/kg of Tween 80 was made using the concentrated extract. Then, graded doses to be used for the experiment were calculated based on the body weight of the rats. This was preserved in a refrigerator for phytochemical analysis and bioassay. Experimentation was carried out after determination of lethal dose (LD50) of the methanol extract according to the Lorke's method (14).

Toxicity

Lorke's method was employed for the acute toxicity test (14). Thirty albino rats were used in the present study. The test was comprised of two phases. In the first phase, the rats were divided into three groups of five rats. They were administered 10, 100 and 1000 mg/kg body weight, respectively. In the second stage, 1600, 2900 and 5000 mg/kg body weight of the extract were administered to the rats. The extracts were administered by oral intubation. The methanol extract of *S. stenocarpa* seeds was not toxic at above 5000 mg/kg.

Experimental Design

The experiment lasted for a period of 91 days. The entire 144 rats were broadly divided in a completely randomized design of four treatment groups (A – D) each replicated three times. Group A served as the standard control and was given distilled water and typical growers mash diet. Groups B, C and D received a diet, distilled water and three graded doses (800, 1200 and 1600 mg/kg) of the methanol seed extracts of *S. stenocarpa* by oral intubation for 12 weeks. The three

treatment doses were established after the LD50 determination as 6.25 times reduction of the LD50 and subsequently increased by the addition of 400mg/kg per body weight of the rats. The animals were fed and watered al libitum and the cages were regularly cleaned. After acclimatization of the experimental rats, treatment with graded doses of extracts commenced. Reproductive parameters studied in male rats included body weight, testes weight, gonadosomatic index, sperm morphology, sperm count and sperm motility (15). These parameters were specified before the beginning of the treatment (week 0) and subsequently on weekly basis (7 days' interval) by harvesting the testes for gonad characteristics and measuring the body weight. Similarly, blood samples were obtained from the orbital sinus of each rat for the hormonal analysis before the beginning of the treatment (week 0) and then on a weekly basis. The blood samples were used to ascertain the serum levels of testosterone (TL) using Heywood's method (16).

Statistical Analysis

The collected data were analyzed using analysis of variance (ANOVA). Preliminary data explorations to decide suitable analytical approaches (whether parametric or non-parametric) were carried out using Kolmogorov-Smirnov. Turkey HSD was used for post-hoc test. Level of significance for all the tests was set at $p<0.05$. Significant means were accepted at $p<0.05$ and presented as mean ± standard error of mean. All the analyses were performed using Statistical Packages for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, USA).

Results and Discussion

Effects of the Methanol Seed Extract of *Sphenostylis stenocarpa* on the Body Weight of Male Albino Rats

Effects of the methanol seed extract of *S. stenocarpa* on the body weights (BWs) of the male albino rats indicated an overall dose-dependent remarkable distinction ($p<0.05$) in the mean weekly BWs of the treated rats when compared with the control group (Table 1). The dose-dependent analysis revealed that in week 0, the BWs of the treatment groups were significantly higher ($p<0.05$) than the BWs of the control. In weeks 1, 4, 8, 9, 10 and 12, the BWs of the rats in the treatment groups were significantly lower ($p<0.05$) compared with the control group. However, in weeks 2, 3, 5, 6, and 11, the BWs of the rats administered 1200 and 1600 mg/kg were significantly lower ($p<0.05$), and those administered 800mg/kg of the extract were significantly higher ($p<0.05$) than the control group. Similarly, minimal fluctuations were observed in the mean weekly BW of the rats in the treatment groups with reference to the duration of treatment compared with that of week 0. A significant increase was observed in the BW throughout the weeks in the control group. BW of the rats in week 1 was significantly lower ($p<0.05$) compared with week 0. Moreover, whereas there was a significant increase in BW ($p<0.05$) of all the rats administered the 800mg/kg in all the weeks with the exception of weeks 1 and 10 which were significantly lower, there was no significant difference ($p>0.05$) in that of week 2 compared with week 0. In 1200 mg/kg treatment groups, there was a significant decrease at all weeks ($p<0.05$) except weeks 6 and 7 with a significant increase ($p<0.05$) compared with week 0. However, there was a significant decrease ($p<0.05$) in all the weeks compared with week 0 in the body weights of the rats administered the 1600 mg/kg of the extract.

Effects of the Methanol Seed Extracts of *Sphenostylis stenocarpa* on the Testes Weight of Male Albino Rats

Effects of the methanol seed extract of *S. stenocarpa* on the testes weights (TWs) of the male albino rats indicated that there was an overall dose-dependent noticeable distinction ($P<0.05$) in the mean weekly TWs of the treated rats compared with the control group (Table 2). The dose-dependent analysis revealed that in week 0 and 1, the TWs of the rats in the treatment groups were significantly lower ($p<0.05$) than the TWs of the rats in the control group; whereas in weeks 2, 7, 8, 9, 10, 11 and 12, the TWs of the rats in the treatment groups were significantly higher ($p<0.05$) compared with the control group. In week 3 and 5, the TWs of rats in the treatment groups were significantly lower ($p<0.05$) except in the 800 mg/kg treatment group where the TWs increased significantly ($p<0.05$) compared with the control group. In weeks 4 and 6, the TWs of the rats were significantly higher ($p<0.05$) in the treatment groups except in the rats administered 1600mg/kg of the extract where the TWs of the rats were significantly lower ($p<0.05$) compared with the

Table 1. Weekly Effects of Different Concentrations of the Methanol Seed Extract of *S. stenocarpa* on the Body Weight (g) of Male Albino Rats

Conc. (mg/kg)	Duration (Weeks)												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0.00	184.5±	194.0±	181.1±	193.3±	198.3±	190.9±	193.0±	196.0±	197.9±	190.8±	198.2±	191.0±	192.4±
	1.45^{d2}	1.11^{a9}	1.66^{b1}	2.74^{b8}	1.92^{a13}	1.01^{b4}	1.15^{b7}	0.05^{b10}	1.75^{a11}	0.10^{a3}	1.00^{a12}	1.50^{b5}	5.10^{a6}
800	187.3±	186.03±	187.33±	197.46±	192.56±	191.06±	198.16±	196.86±	190.10±	189.30±	186.80±	192.65±	191.25±
	2.56^{b3}	2.56^{b1}	2.56^{a3}	2.56^{a11}	2.56^{b8}	2.56^{a6}	2.56^{a12}	2.56^{a10}	2.56^{b5}	2.56^{b4}	2.56^{b2}	2.56^{a9}	$2 .561^{b7}$
1200	186.21±	139.33±	129.55±	178.33±	159.16±	155.56±	187.80±	186.43±	169.85±	156.00±	160.43±	168.55±	132.70±
	2.56^{c11}	2.56^{d3}	2.56^{d1}	2.56co	2.56^{c6}	2.56^{c4}	2.56^{d13}	2.56^{c12}	2.56^{d9}	2.56^{c5}	2.56^{c7}	2.56^{c8}	2.56^{c2}
1600	191.96±	148.26±	131.67±	173.33±	136.96±	155.16±	187.96±	186.00±	179.00±	138.05±	133.30±	132.80±	130.65±
	2.56^{a13}	2.560	2.56^{c2}	2.56^{d9}	2.56^{d5}	2.56^{d8}	2.56^{c12}	2.56^{d11}	2.56^{c10}	2.56^{d6}	2.56^{d4}	2.56^{d3}	2.56^{d1}

Values as mean ± standard error. Values with different small letters superscript down a column for each solvent were significantly different between the concentrations ($p<0.05$), while different numeric superscripts across a row were significantly different for durations ($p<0.05$).

Table 2. Weekly Effects of Different Concentrations of the Methanol Seed Extract of *S. stenocarpa* on the Testes Weight of Male Albino Rats

Conc.	Duration (Weeks)												
(mg/kg)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0.00	5.13±	4.53±	3.43±	5.63±	3.40±	3.63±	3.60±	3.70±	4.40±	3.55±	3.50±	3.15±	3.70±
	0.40^{a12}	0.51^{a10}	0.20^{d2}	0.51^{b13}	0.43^{d1}	0.15^{b5}	0.26^{c4}	0.20^{d8}	0.20^{d8}	0.05^{d3}	0.20^{d9}	0.05^{d7}	0.20^{d11}
800	5.10±	5.66±	5.73±	5.83±	6.83±	6.63±	5.36±	7.63±	7.20±	7.75±	4.85±	7.25±	7.60±
	1.30^{b2}	0.30^{b4}	0.40^{a5}	0.15^{a7}	0.30^{a8}	0.35^{a6}	0.15^{a3}	0.15^{a12}	0.10^{a9}	1.15^{a13}	0.05^{c1}	0.15^{b10}	0.10^{a11}
1200	4.90±	5.80±	5.03±	4.08±	4.03±	2.87±	3.63±	5.03±	6.10±	5.90±	5.43±	7.20±	7.25±
	0.606	0.20^{d9}	0.660	0.550	0.37^{b3}	0.35^{d1}	0.15^{b2}	0.516	0.30^{b11}	0.10^{b10}	0.20^{a8}	0.20^{d12}	0.15^{d13}
1600	5.03±	5.10±	5.63±	4.86±	2.86±	2.96±	3.47±	5.06±	5.40±	5.50±	5.40±	8.10±	7.45±
	0.30^{c5}	0.430	0.45^{b11}	0.5564	0.45^{d1}	0.30^{c2}	0.65^{d3}	0.35^{b6}	0.20^{c8}	0.10cio	0.40^{b9}	0.10^{a13}	0.15^{b12}

Values as mean ± standard error. Values with different small letters superscript down a column for each solvent were significantly different between the concentrations ($p<0.05$), while different numeric superscripts across a row were significantly different for durations ($p<0.05$).

control group. The results of the time-dependent analysis revealed that the rats in the treatment groups' mean weekly TWs fluctuated only slightly over time. In the control group, there were significant decreases ($p<0.05$) in the TWs of the rats throughout the weeks except in week 3 where the TWs were significantly higher ($p<0.05$) compared with week 0. In the rats administered 800 mg/kg of the extract, there were significant increases in the TWs ($p<0.05$) in all the weeks except week 10 where the TW of the rat decreased significantly compared with week 0. Similarly, in the rats administered 1200 and 1600 mg/kg of the extract, there was a significant increase of the TWs of the rats in all the weeks except in weeks 3, 4, 5 and 6 with a significant decrease ($p<0.05$) compared with week.

Effects of the Methanol Seed Extract of *Sphenostylis stenocarpa* on the Gonadosomatic Index of Male Albino Rats

Effects of the methanol seed extract of *S. stenocarpa* on the gonadosomatic index (GSI) of the male albino rats showed that there was an overall dose-dependent significant difference ($p<0.05$) in the mean weekly GSI of the treated rats compared with the control group (Table 3). The dose-dependent analysis revealed that in week 0, the GSI of the treatment group rats was significantly ($p<0.05$) lower than the GSI of the rats in the control group. With the exception of week 3, when the GSI of rats given the doses of 1200 and 1600 mg/kg of the extract was compared with the control, the GSI of the rats in the treatment groups was significantly higher ($p<0.05$) in all other weeks. The mean weekly GSI of the rats in the treatment groups fluctuated very little based on time. In the control group, there were significant decreases ($p<0.05$) in the GSI of the rats throughout the weeks compared with week 0; whereas in the rats treated with 800 mg/kg of the extract, there were significant increases ($p<0.05$) in GSI at all weeks except weeks 6 and 10 where the GSI of the rats significantly decreased ($p<0.05$) compared with week 0. Similarly, in the rats treated with 1200 and 1600 mg/kg of the extract, there were significant increases ($p<0.05$) in GSI at all weeks except weeks 3, 5 and 6 where the GSI of the rats significantly decreased ($p<0.05$) compared with week.

Effects of the Methanol Seed Extracts of *Sphenostylis stenocarpa* on the Sperm Motility of Male Albino Rats

Effects of the methanol seed extract of *S. stenocarpa* on the sperm motility (SM) of the male albino rats showed that there was an overall dose-dependent significant difference ($p<0.05$) in the mean weekly SM of the treated rats compared with the control group (Table 4). The dose-dependent analysis showed that in weeks 0 to 12, the SM of rats in the treatment group was significantly lower ($p<0.05$) than the SM of rats in the control. Based on duration, there were minimal fluctuations in the mean weekly SM of the rats in the treatment groups. SM of the rats in the control group significantly decreased ($p<0.05$) throughout the weeks except in week 8 with a significant increase ($p<0.05$) compared with week 0. However, the SM of the rats in the treatment groups increased significantly ($p<0.05$) in all weeks compared with week.

Effects of the Methanol Seed Extract of *Sphenostylis stenocarpa* on the Sperm Count of Male Albino Rats

Effects of the methanol seed extract of *S. stenocarpa* on the sperm count (SC) of male albino rats indicated that there was an overall dose-dependent significant difference ($p<0.05$) in the mean weekly SC of the treated rats compared with the control group (Table 5). The dose-dependent analysis revealed that in all weeks, the SC of the rats in the treatment groups were significantly higher ($p<0.05$) compared with the control group except in week 2 where there was no significant difference ($p>0.05$) in the SC of the rats administered 800 mg/kg of the extract compared with the control group, and where SC of the rats treated with 1200 and 1600 mg/kg of the extract were significantly lower ($p<0.05$) compared with the control group. There were minimal fluctuations in the mean weekly SC of the rats in the treatment groups with reference to duration. Significant decreases ($p<0.05$) were observed in the SC throughout the weeks except at week 9 in the rats in the control group where SC of the rats increased significantly compared with week. However, SC of the rats in the treatment groups significantly decreased ($p<0.05$) in all the weeks compared with week.

Effects of the Methanol Seed Extract of *Sphenostylis stenocarpa* on the Testosterone Levels of Male Albino Rats

Effects of the methanol seed extract of *Sphenostylis stenocarpa* on the testosterone levels (TLs) of male albino rats indicated that there was an overall dose-

Table 3. Weekly Effects of Different Concentrations of the Methanol Seed Extract of *S. stenocarpa* on the Gonad Somatic Index of Male Albino Rats

Conc.	Duration (Weeks)												
(mg/kg)	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	5.52±	4.78±	4.64±	5.31±	4.19±	4.58±	4.40±	4.44±	4.75±	4.48±	4.79±	4.79±	5.06±
	0.14^{a13}	0.06^{d8}	0.09^{d6}	0.13^{b12}	0.16^{d1}	0.10^{d5}	0.20^{d2}	0.10^{d3}	0.06^{d7}	0.02^{d4}	0.75^{d10}	0.04^{d9}	0.10^{d11}
800	5.43±	5.66±	5.84±	6.05±	6.16±	6.05±	5.18±	6.37±	6.41±	5.91±	5.27±	6.36±	6.59±
	0.45^{c3}	0.03^{c4}	0.27^{c6}	0.12^{a8}	0.13^{a9}	0.09^{a7}	0.05^{a1}	0.15^{a11}	0.03^{a12}	0.36^{c5}	0.03^{c2}	0.09^{c10}	0.03^{d13}
1200	5.46±	7.68±	7.58±	5.14±	5.71±	5.08±	4.51±	5.52±	6.54±	6.99±	6.49±	7.26±	9.25±
	0.44^{b4}	0.24^{a12}	0.31^{b11}	0.27^{c3}	0.19^{c6}	0.38^{c2}	0.171	0.04^{c5}	0.18^{b8}	0.30^{b9}	0.10^{b7}	0.39^{b10}	0.38^{b13}
1600	5.27±	6.85±	7.90±	5.12±	5.77±	5.25±	4.53±	5.60±	5.81±	7.63±	7.81±	9.87±	9.57±
	0.100	0.23^{b8}	0.24^{a10}	0.23^{d2}	0.48^{b6}	0.07^{b3}	0.27^{b1}	0.27^{b5}	0.16^{c7}	0.49^{a9}	0.44^{a11}	0.28^{a13}	0.61^{a12}

Values as mean ± standard error. Values with different small letters superscript down a column for each solvent were significantly different between the concentrations ($p<0.05$), while different numeric superscripts across a row were significantly different for durations ($p<0.05$).

Table 4. Weekly Effects of Different Concentrations of the Methanol Seed Extract of *S. stenocarpa* on the Sperm Motility of Male Albino Rats

Conc. (mg/kg)	Duration (Weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	23.67±	21.67±	20.00±	21.00±	22.67±	21.33±	22.00±	21.67±	33.00±	20.00±	17.33±	18.33±	23.67±
	2.51^{d12}	2.51^{d7}	1.00^{d3}	3.606	2.30^{d10}	1.52^{d6}	2.00^{d9}	3.21^{d8}	2.00^{d13}	6.55^{d4}	2.51^{d1}	2.88^{d2}	2.30^{d11}
800	24.33±	59.00±	55.00±	72.67±	73.00±	68.33±	82.67±	83.00±	81.33±	85.00±	81.33±	83.00±	85.33±
	1.52^{b1}	1.00^{a3}	1.00^{a2}	1.52^{a5}	2.64^{a6}	2.08^{a4}	1.52^{a9}	2.64^{a10}	2.08^{a7}	2.64^{a11}	3.05^{a8}	$2.64^{a1}8$	2.08^{a12}
1200	24.00±	42.67±	44.33±	43.33±	35.00±	42.33±	54.67±	57.33±	57.67±	73.67±	74.67±	74.67±	80.33±
	4.58^{d1}	8.08^{c4}	6.02^{c6}	1.63^{b5}	2.64^{b2}	8.62^{b3}	1.52137	5.50^{b8}	3.51^{b9}	6.11^{b10}	4.93^{b11}	5.13^{b12}	1.52^{d13}
1600	28.00±	51.67±	51.67±	35.00±	30.00±	40.33±	52.67±	54.00±	56.00±	62.00±	73.00±	84.00±	81.33±
	3.60^{a1}	1.52^{b5}	1.521)5	2.64^{c3}	3.00^{c2}	5.500	1.526	3.60a	5.56^{c8}	2.64^{c9}	2.64ci0	2.64^{d12}	3.78^{b11}

Values as mean ± standard error. Values with different small letters superscript down a column for each solvent were significantly different between the concentrations ($p<0.05$), while different numeric superscripts across a row were significantly different for durations ($p<0.05$).

Table 5. Weekly Effects of Different Concentrations of the Methanol Seed Extract of S. stenocarpa on the Sperm Count of Male Albino Rats

Conc.	Duration (Weeks)												
(mg/kg)	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	30.67±	22.67±	30.00±	26.33±	28.67±	29.33±	28.67±	25.00±	28.00±	31.00±	23.00±	22.00±	27.00±
	3.05^{d12}	2.51^{d2}	1.00^{a11}	7.02^{d5}	4.16^{d9}	1.52^{d10}	1.52^{d8}	4.00^{d4}	1.00^{d7}	7.00^{d13}	1.00^{d3}	3.00^{d1}	1.00^{d6}
800	37.00±	68.00±	65.00±	82.67±	83.67±	77.67±	92.00±	91.67±	92.00±	96.00±	92.33±	93.00±	94.33±
	4.35^{a1}	1.00^{a3}	1.00^{a2}	1.52^{a5}	1.52^{a6}	2.51^{a4}	1.00^{a1}8	1.52^{a7}	1.00^{a8}	2.00^{a12}	1.52^{a9}	1.00abo	3.21^{a11}
1200	37.33±	48.00±	52.67±	47.67±	45.67±	47.67±	55.67±	61.33±	68.00±	83.00±	82.00±	84.00±	90.67±
	1.52^{b1}	2.641)5	3.216	2.08^{b3}	1.52^{b2}	2.51^{c4}	5.50^{c7}	6.421)8	3.60^{b9}	6.00^{b11}	5.29^{b10}	5.00^{c12}	2.08^{d13}
1600	33.00±	39.67±	53.00±	46.33±	42.00±	52.00±	56.67±	58.00±	60.00±	69.00±	79.00±	92.00±	91.60±
	1.00^{c1}	4.16^{c2}	2.64 ^{b6}	2.08^{c4}	1.73^{c3}	2.64^{b5}	7.50^{b7}	2.64^{c8}	3.60^{c9}	1.00^{c10}	3.00^{c11}	2.00^{b13}	3.60^{b12}

Values as mean ± standard error. Values with different small letters superscript down a column for each solvent were significantly different between the concentrations ($p<0.05$), while different numeric superscripts across a row were significantly different for durations ($p<0.05$).

Table 6. Weekly Effects of Different Concentrations of the Methanol Seed Extract of S. stenocarpa on the Testosterone of Male Albino Rats

Conc.	Duration (Weeks)												
(mg/kg)	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	0.57±	0.60±	0.43±	0.46±	0.57±	0.46±	0.38±	0.44±	0.39±	0.35±	0.38±	0.38±	0.35±
	0.01^{b11}	0.41^{d12}	0.05^{d6}	0.01^{d8}	0.06^{d10}	0.04^{d9}	0.0564	0.05^{d7}	0.03^{d5}	0.03^{d2}	0.04^{d3}	0.04^{d3}	0.02^{d1}
800	0.55±	0.70±	0.64±	0.74±	0.84±	0.76±	0.83±	0.78±	0.73±	0.73±	0.74±	0.83±	0.87±
	0.03^{d1}	0.02^{c3}	0.05^{c2}	0.02^{b6}	0.05^{c11}	0.01^{b7}	0.05^{a10}	0.02^{b8}	0.01^{c4}	0.01^{c4}	0.06^{c5}	0.01^{c9}	0.10^{d12}
1200	0.60±	2.57±	2.21±	0.74±	0.97±	1.06±	0.51±	0.65±	1.19±	3.05±	1.65±	3.15±	3.81±
	0.08^{a2}	0.25^{a10}	0.28^{b9}	0.08^{a4}	0.11^{b5}	0.15^{a6}	0.02^{c1}	0.05^{c3}	0.21^{b7}	0.05^{a11}	0.15^{b8}	0.45^{b12}	0.31^{b13}
1600	0.56±	2.34±	2.58±	0.73±	1.09±	0.70±	0.63±	0.87±	1.40±	1.15±	2.46±	3.90±	9.15±
	0.05^{c1}	0.16^{b9}	0.16^{a11}	0.04^{c4}	0.14^{a6}	0.10^{c3}	0.02^{b2}	0.25^{a5}	0.20^{a8}	0.35^{b7}	0.25^{a2}0	0.30^{a2}2	0.75^{a2}3

Values as mean ± standard error. Values with different small letters superscript down a column for each solvent were significantly different between the concentrations ($p<0.05$), while different numeric superscripts across a row were significantly different for durations ($p<0.05$).

dependent significant difference ($p<0.05$) in the mean weekly TLs of the treated rats compared with the control group (Table 6). The dose-dependent analysis demonstrated that in week 0 the rats' TLs were significantly lower ($p<0.05$) in all the treatment groups than in the control group, with exception of the rats given 1200 mg/kg of the extract. In this group, the rats' TLs were significantly higher ($p<0.05$). However, the TLs of the rats in the treatment groups were significantly higher ($p<0.05$) in all the weeks compared with the control group. Similarly, there were minimal fluctuations in the mean weekly TLs of the rats in the treatment groups with reference to the duration of the study. Significant decreases ($p<0.05$) were observed in the TL throughout the weeks except in week 1 in the rats in the control group, and there was a significant increase ($p<0.05$) compared with week 0. However, significant increases ($p<0.05$) were observed in the rats in the treatment groups in the TLs in all the weeks except in week 6 of the rats administered 1200 mg/kg of the extract where the TLs decreased significantly ($p<0.05$) compared with week. Numerous active compounds found in plants are possibly responsible for their various therapeutic characteristics utilized in folk medicine. Nevertheless, these naturally occurring compounds may have adverse impacts on the development or normal functioning of the reproductive system (17). The present study showed that the methanolic leaf extract of *S. stenocarpa* could enhance the fertility of male Wistar rats.

In the present study, the extract was associated with a decline in the weight of the rats in a dose-dependent and duration-dependent manner. This was more obvious in the first and last months of the study. Our findings are consistent with the findings of Gupta *et al*., where the methanol stem extract of *Dendrophthoe falcata* decreased the body weights of male albino rats (18).

It has been shown that indicators of fertility include sperm count and normal testicular histology (19, 20). In this study, the testes and gonad characteristics, i.e. sperm count, testes weight, gonadosomatic index and sperm motility were affected in a manner that depended on the extract concentration and duration of treatment. Each of the parameters was significantly affected by extract concentration and duration of treatment. The extract caused increases in the testicular weights, sperm count and sperm motility. An adequate sperm count and sperm motility are essential for successful male fertility. The findings of this study corroborate the report of Ekere *et a*l., where the methanol extract of *Dracaena arborea* in albino rats caused dose-dependent significant increases in the testes weights, gonad somatic index, sperm count and sperm motility (21). The gradual increase in the mean testicular weight of the treated rats is possibly because of the increased activity in their testes. This might also result in increased testosterone secretions (22). According to Mylchreest *et al*., elevated serum testosterone levels in rats was associated with an increase in the epididymal sperm number (23). Meanwhile, Oyeyemi and Okediran (24) reported that high-quality food with a high protein content could improve semen quality. Mutwedu *et al*. (25) also recently confirmed this finding. The potential of the *S. stenocarpa* methanol extract to increase sperm motility and counts, as it was found in this study, is of high significance because these parameters affect sperm cells' capacity for fertilization. In the male albino rats administered the *S. stenocarpa* extracts, testosterone responded to the plant extracts. There were significant increases in the testosterone levels of all the treated rats. The findings of this study are consistent with the report of Gamal *et al*., where ethanol extracts of *Emex spinosa, Leptadenia pyrotechnica, Haloxylon salicornicum* and *Ochradenus baccatus* significantly increased the serum levels of testosterone in treated rats (26).

Conclusion

The findings of this study proved that the methanol seed extract of *S. stenocarpa* might yield similar or related results of reproductive enhancement in humans. Gonad characteristics, sperm parameters, and hormonal indices increased following the administration of the extract, and lent credence to the fact that *S. stenocarpa* seed extract could have a fertility-enhancing ability.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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None

References

1. Raji Y, Fadare OO, Adisa R A, Salami SA. Comprehensive assessment of the effect of *Sphenocentrum jollyanum* root extract on male reproductive activity in albino rats. Reprod. Med. Biol. 2006;5(4):283–292. DOI: 10.1111%2Fj.1447-0578.2006.00154.x
2. Burns MM. Alternative medicine: herbal preparation. Clin. Pediatr. Emerg. Med. 2000;1(3):186– 190. DOI: 10.1016/S1522-8401(00)90026-0
3. Ekpo AS. Changes in amino acid composition of African yam bean (*Sphenostylis stenocarpas*) and African locust beans (*Parkia filicoida*) on cooking. Pak. J. Nutr. 2006;5(12):254–256. DOI: 10.3923/pjn.2006.254.256
4. Onyeike EN, Ayalogu EO, Uzogara SG. Influence of heat processing of African yam bean seed (*Sphenostylis stenocarpa*) flour on the growth and organ weights of rats. Plant Food. Hum. Nutr. 1995;48(2):85–93. DOI: 10.1007/BF01088303
5. George TT, Obilana AO, Oyeyinka SA. The prospects of African yam bean: past and future importance. Heliyon, 2020;6(11):e05458. DOI: 10.1016/j.heliyon.2020.e05458
6. Asoiro FU, Ani AO. Determination of some physical properties of African yam beans. Pac. J. Sci. Technol. 2011;12(1):374–380.
7. Ubaka CM, Ukwe CV. Antidiabetic effect of the methanolic seed extract of *Sphenostylis stenocarpa* (Hoechst ex. A. Rich. Harms) in rats. J. Pharm. Res. 2010;3(9):2192–2194.
8. Okonkwo C, Njoku U, Obioma T, Mbah A, Mary J. Anti-anaemic effect of methanol seed extract of *Sphenostylis stenocarpa* (African yam bean) in Wistar albino rats. Afr. J. Pharm. Pharmacol. 2013;7(45):2907–2913. DOI: 10.5897/AJPP12.1388
9. Onyeke CCI, Ugwuoke KI. Effects of botanical extracts on the mycelial growth of seed borne fungi of the African yam bean, *Sphenostylis stenocarpa* (Hochst exa. Rich) Harms. Nig. J. Biotech. 2011;22:1–7.
10. Uchegbu NN, Amulu NF. Effect of germination on proximate, available phenol and flavonoid content, and antioxidant activities of African yam bean (*Sphenostylis stenocarpa*). Int. J. Nutr. Food Eng. 2015;9(1):106–109. DOI: 10.5281/zenodo.1099750
11. Ogbu PN, Ezeanyika LUS, Ogugua VNE, Ogbu M, Aloke C, Onyeji GN. *Sphenostylis stenocarpa* seed extract attenuates dyslipidemia in testosterone propionate-induced benign prostatic hyperplasia in rats. Pak. J. Biol. Sci. 2021;24(1):151–157. DOI: 10.3923/pjbs.2021.151.157
12. ITIS. *Sphenostylis stenocarpa* (Hochst. ex A. Rich.) Harms. ITIS Report, 2021. https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=506266#null
13. NRC. Guide for the Care and Use of Laboratory Animals. 8th Edition, National Research Council (NRC), National Academies Press, Washington DC, USA, 2010.
14. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983;54(4):275–287. DOI: 10.1007/bf01234480
15. Idris BM, Dikko AAU, Yarube IU, Salim MA, Saleh MIA, Alhassan AW. Disturbances in calcium and zinc homeostasis during testicular damage induced by *Citrus aurantifolia* juice in Wistar rats. Nig. J. Physiol. Sci. 2018;33(2):201–209.
16. Heywood LH. Testosterone levels in the male laboratory rat: variation under experimental conditions. Int. J. Androl. 1980;3(16):519–529.
17. Abdel-Magied EM, Abdel-Rahman HA, Harraz FM. The effect of aqueous extract of *Cynomorium coccineum* and *Withania somnifera* on testicular development in immature Wistar rats. J. Ethnopharmacol. 2001;75(1):1–4. DOI: 10.1016/s0378-8741(00)00348-2
18. Gupta RS, Kachhawa JBS, Sharma A. Effect of methanolic extract of *Dendrophthoe falcata* stem on reproductive function of male albino rats. J. Herb. Pharmacother. 2009;7(2):1–13. DOI: 10.1300/j157v07n02_01
19. Al-Sa'aidi JAA, Al-Khuzai ALD, Al-Zobaydi NFH. Effect of alcoholic extract of Nigella sativa on fertility in male rats. Iraqi J. Vet. Sci. 2009;23(Suppl. II):123–128.
20. Etuk EU, Muhammad AA. Fertility enhancing effects of aqueous stem bark extract of Lophira lanceolate in male Spargue Dawley rats. Int. J. Plant Physiol. Biochem. 2009;1(1): 1–4.
21. Ekere SO, Okoye CN, Udoumoh AF. Fertility enhancing effects of methanolic leaf extract of Dracaena arborea in albino rats (*Rattus norvegicus*). Czech J. Anim. Sci. 2013;58(11):520–524. DOI: 10.17221/7047-CJAS
22. O'Keane JC, Brien TG, Hooper AC, Graham A. Testicular activity in mice selected for increased body weight. Andrologia. 1986;18(2):190–195. DOI: 10.1111/j.1439- 0272.1986.tb01760.x
23. Mylchreest E, Sar M, Wallace D, Foster P. Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats. Reprod. Toxicol. 2002;16(1):19–28. DOI: 10.1016/s0890-6238(01)00201-5
24. Oyeyemi MO, Okediran BS. Testicular parameters and sperm morphology of chinchilla rabbits fed with different planes of soy meal. International Journal of Morphology, 2007;25(1):139 – 144.
25. Mutwedu VB, Ayagirwe RB, Bacigale SB, Mwema LM, Butseme S, Kashosi T, et al. Effect of dietary inclusion of small quantities of *Mucuna pruriens* seed meal on sexual behavior, semen characteristics, and biochemical parameters in rabbit bucks (*Oryctolagus cuniculus*). Trop. Anim. Health Prod. 2019;51:1195–1202.
26. Gamal A, Soliman I, Abd M, Amani S, Awaad SI, Hasan Y. Effect of *Emex spinosa*, *Leptadenia pyrotechnica*, *Haloxylon salicornicum* and *Ochradenus baccatus* extracts on the reproductive organs of adult male rats. Pharm. Biol. 2012;50(1):105–112. DOI: 10.3109/13880209.201.