

Original Research Article

EFFECTS OF AQUEOUS SEED EXTRACTS OF *SPHENOSTYLIS STENOCARPA* ON THE REPRODUCTIVE INDICES OF MALE RATS

Comment [BN1]: The article is quite interesting.

ABSTRACT

Effects of aqueous seed extracts of *Sphenostylisstenocarpa* on the reproductive indices of male rats were investigated. A total of 144 adult rats were used for the experiment, and were divided into 4 (A – D) and replicated thrice. Group A served as the normal control, while Groups B, C and D received three graded doses (800mg/kg, 1200mg/kg and 1600mg/kg) of the extracts respectively by oral intubation. The gonad characteristics, sperm parameters and hormonal analyses of the male rats were determined using standard procedures. These were ascertained prior to the commencement of treatment, and on weekly basis. Data were analyzed statistically using *SPSS* and *R* software at 95% confidence interval. An overall dose and time dependent significant differences in the mean weekly gonad characteristics of the male rats in the treatment groups when compared with the control. There was a significant reduction ($p < 0.05$) in the body weights of the male rats, but a significant increase ($P < 0.05$) in the testes weights, gonad somatic index, sperm count and sperm motility in the rats. The gonadal hormone testosterone, responded to the plant extracts, while follicle-stimulating and luteinizing hormones were largely undetected. There were significant increases in the testosterone levels of all the treated rats. Conclusively, aqueous seed extracts of *Sphenostylisstenocarpa* seems to possess ability to enhance reproductive health in male rats.

Comment [BN2]: Please check the spelling. This may be "thrice"

Keywords: Seed extract, *Sphenostylisstenocarpa*, Albino rats, Reproductive indices, Male

1. INTRODUCTION

Plants and their associated products have been used since ancient times all over the world for treatment of various human ailments [1]. They have played an important role in world health and have been well known for their biological activity. The African yam bean (*Sphenostylisstenocarpa*) belongs to the family papilionaceae. The seed may be boiled and eaten with local seasoning, or converted to paste for the production of a type of "moimoi" [2]. The African yam bean, *Sphenostylisstenocarpa* is mainly used as food but can be fed to animals. *Sphenostylisstenocarpa* is native to tropical west and central Africa and is cultivated in southern and eastern Africa. According to Ejereet *al.* [3], seed extract of *S. stenocarpa* possesses antiobesity properties, and in addition, is hypolipidaemic, hypoglycemic and hepatoprotective. Processes such as heating, soaking or fermenting can be used to decrease anti-nutritional factors and improve the nutritional value of *Sphenostylisstenocarpa* products and its by-products [4]. The crop is used extensively in various dietary preparations; it can supplement the protein requirements of many families throughout the year [5]. The administration of aqueous seed extract of *Sphenostylisstenocarpa* showed a significant decrease in the values of plasma urea and creatinine levels, the decrease on the urea and creatinine levels may be contributing to the

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reduction in high blood pressure in the individuals taking *S. stenocarpa* tea extract, as reported by Okoye and Esiobise [6]. There is dearth of published work on the reproductive potential of African yam bean. The present work investigated the effect of aqueous seed extracts of *Sphenostylisstenocarpa* on reproductive indices of male rats.

2. MATERIALS AND METHODS

2.1 Procurement of *Sphenostylisstenocarpa*

The seeds of *Sphenostylisstenocarpa* was purchased from NkwoIbagwa Market in Nsukka. Identity was authenticated at the Taxonomy Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.2 Procurement and Management of Experimental Animals

A total of one hundred and forty four(144) male rats aged between 6 – 9 months and weighing 80 – 90g were used. The entire animal models were purchased from the Genetics and Animal Breeding Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were fed *ad libitum* with 30% crude protein (Grand Brand) commercial feed. The rats were allowed to acclimatize for a week under standard photoperiodic condition 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.

2.3 Preparation of Aqueous Extract

Two kilogram (2kg) of the dry seed of *Sphenostylisstenocarpa* was pulverized with commercial blower. One thousand five hundred grams (1500g) of the powdered product was put into conical flask to which 1500ml of water was added. The mixture was allowed to stand for 24 hours and then filtered using Whatman No 1 filter paper. The percentage yield was calculated by dividing the weight of concentrated extract by the weight of dried-grinded seed and multiplying by 140. The extract was then concentrated using a rotary evaporator at low temperature (30°C- 40°C). The concentrated extract was then used to prepare a stock solution of 1,600mg/kg with Tween 80. Thereafter, graded doses to be used for the experiment were calculated based on the body weight of the rat. This was kept in a refrigerator for phytochemical analysis and bioassay. Experimentation was carried out after determination of lethal dose (LD₅₀) of the aqueous extract according to the method of Lorke [7].

2.4 Experimental Design

The experiment lasted for a period of 91 days. The 144 rats were broadly divided into 4 groups (A – D) and were replicated three times. Group A served as the normal control and received normal growers mash diet and distilled water. Group B, C and D received diet, distilled water and three graded doses (800mg/kg, 1200mg/kg and 1600mg/kg) of aqueous seed extracts of *Sphenostylisstenocarpa* by oral intubation. The three treatment doses were established after the LD₅₀ determination. The animals were fed once daily while their water was changed anytime in the day when the need arises. After acclimatization of the experimental rats, treatment with graded doses of extracts commenced. The parameters of the male rats studied include body weight, testes weight, gonad somatic index, sperm morphology, sperm count and sperm motility.

These were determined before the commencement of 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 various hormonal analyses before commencement of treatment (week 0) and subsequently on a weekly basis. The blood samples were used to ascertain the serum levels of testosterone (TL) of the rats.

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2.5 Statistical Analysis

Data was analyzed using R version 3.6.3 (R Core Team, 2020) and the Statistical Packages for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, New York, USA). Preliminary data explorations to decide suitable analytical approaches (whether parametric or non-parametric) were done using Kolmogorov-Smirnov and Shapiro-Wilk tests and graphical tools (e.g. normality plots, histograms, and the *gally* multiple plots). Turkey HSD was used for *post-hoc* test. Level of significance for all tests was set at $p < 0.05$.

3 RESULTS

3.1 Effect of Aqueous Seed Extract of *Sphenostylisstenocarpa* on the Body Weights of Male Rats

Table 1 shows the effects of *Sphenostylisstenocarpa* aqueous seed extract on the body weights (BWs) of male albino rats. There was an overall dose-dependent significant difference ($p < 0.05$) in the mean weekly BWs of the treated rats when compared to the control group. Results from the dose-dependent analysis showed that, BWs of the rats in the treatment groups were significantly lower ($p < 0.05$) than the BWs of the rats in the control group in all the weeks. Based on duration, there were minimal fluctuations in the mean weekly BWs of the rats in the treatment groups. In the rats administered 800mg/kg of the extract, there were significant decreases ($p < 0.05$) in weeks 1, 2, 5, 7, 8, 9, 10, 12 whereas in weeks 3, 4, 6 and 1, there were significant ($p < 0.05$) increases in the BWs when compared to week 0. Furthermore, in the rats administered 1200mg/kg, there was a significant decrease of the BWs in all the weeks except week 2 with a significant increase ($p < 0.05$) when compared to week 0. In 1600mg/kg, significant decreases ($P < 0.05$) of their BWs in all weeks when compared to week 0 were recorded.

Table 1: Weekly Effects of different concentrations of aqueous seed extract of *S. stenocarpa* on the body weight of male albino rats

Conc. (mg/kg)	Duration (Weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	84.50± 1.45 ^{d2}	94.00± 1.11 ^{a9}	81.13± 1.66 ^{c1}	93.36± 2.74 ^{a8}	98.36± 1.92 ^{a13}	90.90± 1.01 ^{a4}	93.03± 1.15 ^{a7}	96.05± 0.05 ^{a10}	97.95± 1.75 ^{a11}	90.80± 0.10 ^{a3}	98.20± 1.00 ^{a12}	91.05± 1.50 ^{b5}	92.40± 5.10 ^{a6}
800	90.66± 7.57 ^{a9}	79.56± 1.25 ^{b2}	84.06± 1.53 ^{b5}	90.96± 3.85 ^{b10}	94.56± 4.38 ^{b13}	82.43± 1.59 ^{b3}	91.26± 0.73 ^{b12}	89.03± 1.30 ^{b8}	83.25± 0.95 ^{b4}	85.50± 1.30 ^{b6}	85.80± 2.40 ^{b7}	91.80± 1.53 ^{a11}	72.00± 0.40 ^{b1}
1200	86.90± 5.64 ^{b12}	32.10± 2.95 ^{c2}	90.20± 4.50 ^{a13}	36.10± 5.27 ^{c3}	38.20± 2.88 ^{d1}	74.07± 3.35 ^{d9}	84.33± 8.10 ^{c10}	84.83± 3.96 ^{c11}	64.05± 3.45 ^{c8}	43.30± 13.00 ^{e5}	43.43± 2.49 ^{c6}	58.55± 1.85 ^{c7}	40.45± 9.95 ^{c4}
1600	84.53± 5.22 ^{c13}	38.83± 2.46 ^{d4}	32.28± 6.37 ^{d2}	36.66± 4.45 ^{d1}	32.80± 1.90 ^{c3}	80.06± 1.44 ^{c12}	78.43± 0.66 ^{d10}	79.36± 0.56 ^{d11}	61.00± 10.40 ^{d9}	31.45± 3.05 ^{d6}	31.40± 2.26 ^{d5}	32.85± 12.65 ^{d7}	35.95± 7.45 ^{d8}

Comment [BN5]: The superscripts mentioned in the numbers are not linked to any of the context. Recommended to provide the denotations of these superscripts in all the tables throughout the article.

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

3.2 Effects of Aqueous Seed Extracts of *Sphenostylisstenocarpa* on the Testes Weight (TW) (g) of Male Albino Rats

Table 2 shows the effects of aqueous seed extract of *Sphenostylisstenocarpa* on the testes weight of male albino rats. There was an overall dose-dependent significant difference ($p < 0.05$) in the mean weekly testes weights (TWs) of the treated rats when compared to the control group. The dose-dependent analysis showed that in all weeks, the TWs of rats in the treatment groups were significantly higher ($p < 0.05$) except in week 0, where the TWs of rats in the 1200mg/kg treatment group were significantly lower ($p < 0.05$) when compared to control. The time dependent analysis showed that minimal fluctuations occurred in the mean weekly TWs of the rats in the treatment groups. In the control group, there were significant decreases ($p < 0.05$) in the TWs of rats throughout the weeks except in week 3 where the TWs of the rats were significantly higher ($P < 0.05$) when compared to week 0 (baseline). However, the TWs of rats in the treatment groups increased significantly ($p < 0.05$) except in the 1600mg/kg treatment group, where the TW of the rat decreased significantly ($p < 0.05$) in week 6 when compared to week 0.

Table 2: Weekly effects of different concentrations of aqueous seed extract of *S. stenocarpa* on the testes weight of male albino rats

Conc. (mg/kg)	Duration (Weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	5.13± 0.40 ^{c12}	4.53± 0.51 ^{d10}	3.43± 0.20 ^{d2}	5.63± 0.51 ^{d13}	3.40± 0.43 ^{d1}	3.63± 0.15 ^{d5}	3.60± 0.26 ^{c4}	3.70± 0.20 ^{d6}	4.40± 0.20 ^{d8}	3.55± 0.05 ^{d3}	4.50± 0.20 ^{d9}	4.15± 0.05 ^{d7}	4.70± 0.20 ^{d11}
800	5.34± 0.05 ^{a1}	5.93± 0.37 ^{c3}	6.50± 0.62 ^{b6}	7.66± 2.08 ^{b10}	7.60± 0.20 ^{b8}	6.36± 0.55 ^{c4}	5.86± 0.20 ^{a2}	7.60± 0.20 ^{a9}	6.45± 0.05 ^{a5}	7.25± 0.05 ^{b7}	7.95± 0.05 ^{a11}	7.50± 0.10 ^{c8}	8.80± 0.10 ^{c12}
1200	4.70± 0.45 ^{d1}	7.26± 0.70 ^{a9}	5.83± 0.32 ^{c5}	6.33± 0.70 ^{c7}	7.66± 0.25 ^{a9}	8.40± 0.80 ^{b11}	4.70± 0.20 ^{b2}	5.40± 0.34 ^{c4}	5.25± 0.15 ^{c3}	5.95± 0.05 ^{c6}	7.10± 0.17 ^{c8}	8.40± 0.10 ^{a11}	9.50± 0.10 ^{a12}
1600	5.30± 0.79 ^{b2}	6.00± 0.79 ^{b5}	8.46± 0.85 ^{a10}	8.60± 0.52 ^{a11}	7.43± 0.15 ^{c7}	8.66± 0.35 ^{a12}	4.70± 0.26 ^{b1}	5.46± 0.37 ^{b3}	5.60± 0.10 ^{b4}	7.35± 0.95 ^{a6}	7.66± 0.70 ^{b8}	7.75± 0.15 ^{b9}	9.35± 0.05 ^{b13}

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

3.3 Effects of Aqueous Seed Extract of *Sphenostylisstenocarpa* on the Gonad Somatic Index (GSI) of Male Albino Rats

Table 3 shows the effects of aqueous seed extract of *Sphenostylisstenocarpa* on the gonad somatic index (GSI) of male albino rats. There was an overall dose-dependent significant difference ($p < 0.05$) in the mean weekly GSI of the treated rats when compared to the control group. The dose-dependent analysis showed that in week 0, GSI of the rats in the treatment groups were significantly lower ($p < 0.05$) than the GSI of the rats in the control. However, the GSI of rats in the treatment groups were significantly higher ($p < 0.05$) in all other weeks when compared to control. There were minimal fluctuations in the mean weekly GSI of the rats in the treatment groups with respect to duration. In the control group, there were significant decreases in the GSI of the rats throughout the weeks when compared to week 0. However, in the rats administered 800mg/kg and 1600mg/kg of the extract, there were significant increases ($p < 0.05$) in the GSI in all the weeks except week 10 and 6 respectively where the GSI of the rats, significantly decreased when compared to week 0. Similarly, in the 1200mg/kg treatment group, there were significant increases ($p < 0.05$) of the GSI of the rats in all the weeks when compared to week 0.

Table 3: Weekly effects of different concentrations of aqueous seed extract of *S. stenocarpa* on the gonad somatic index of male albino rats

Conc. (mg/kg)	Duration (Weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	5.52± 0.14 ^{b13}	4.78± 0.06 ^{d8}	4.64± 0.09 ^{d6}	5.31± 0.13 ^{d12}	4.19± 0.16 ^{d1}	4.58± 0.10 ^{d5}	4.40± 0.20 ^{d2}	4.44± 0.10 ^{d3}	4.75± 0.06 ^{d7}	4.48± 0.02 ^{d4}	4.79± 0.75 ^{d10}	4.79± 0.04 ^{d9}	5.06± 0.10 ^{d11}
800	5.04± 0.47 ^{c2}	6.18± 0.01 ^{c7}	6.13± 0.34 ^{b6}	6.31± 0.12 ^{c9}	6.57± 0.10 ^{c11}	6.06± 0.03 ^{c5}	5.66± 0.05 ^{a3}	6.73± 0.09 ^{a12}	6.25± 0.06 ^{b8}	6.05± 0.23 ^{c4}	5.01± 0.16 ^{c1}	6.52± 0.00 ^{c10}	7.18± 0.10 ^{c13}
1200	4.98± 0.35 ^{d1}	9.70± 0.47 ^{a11}	5.76± 0.22 ^{c4}	8.08± 0.08 ^{b8}	10.87± 0.16 ^{a13}	7.52± 0.38 ^{a6}	5.19± 0.09 ^{c2}	5.60± 0.14 ^{c3}	6.25± 0.04 ^{c5}	7.77± 0.67 ^{b7}	8.49± 0.40 ^{b10}	8.44± 0.03 ^{b9}	10.35± 0.65 ^{b12}
1600	5.66± 0.32 ^{a2}	8.45± 0.73 ^{b6}	10.90± 0.25 ^{a12}	11.78± 0.84 ^{a13}	10.07± 0.11 ^{b10}	7.52± 0.16 ^{b5}	5.49± 0.07 ^{b1}	5.93± 0.15 ^{b3}	6.60± 0.36 ^{a4}	9.42± 0.50 ^{a8}	9.41± 0.18 ^{a7}	9.67± 0.80 ^{a9}	10.59± 0.62 ^{a11}

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

3.4 Effects of Aqueous Seed Extracts of *Sphenostylisstenocarpa* on the Sperm Motility of Male Albino Rats

Table 4 shows the effects of aqueous seed extract of *Sphenostylisstenocarpa* on the sperm motility (SM) of male albino rats. There was an overall dose-dependent significant difference ($p < 0.05$) in the mean weekly SM of the treated rats when compared to the control group. The dose-dependent analysis showed that in week 0 and 7, SM of rats in the treatment groups were significantly lower ($p < 0.05$) than the SM of the control whereas in weeks 1, 2, 3, 4, 5, 6, 8, 9, 10, 11 and 12, the SM of the rats in all the treatment groups were significantly higher ($p < 0.05$) when compared to control. The time dependent analysis showed that, there were minimal fluctuations in the mean weekly SM of the rats in the treatment groups. Rats in the control group had significant decreases ($p < 0.05$) in the SM throughout the weeks except in week 8 where SM increased significantly ($p < 0.05$) when compared to week 0. However, in the treatment groups, there were significant increases ($p < 0.05$) in the SM of the rats in all the weeks when compared to week 0.

Table 4: Weekly effects of different concentrations of aqueous 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± 2.51 ^{a12}	21.67± 2.51 ^{d7}	20.00± 1.00 ^{d3}	21.00± 3.60 ^{d5}	22.67± 2.30 ^{d10}	21.33± 1.52 ^{d6}	22.00± 2.00 ^{d9}	21.67± 3.21 ^{d8}	33.00± 2.00 ^{a13}	20.00± 6.55 ^{d4}	17.33± 2.51 ^{d1}	18.33± 2.88 ^{d2}	23.67± 2.30 ^{d11}
800	22.00± 2.64 ^{c1}	55.33± 1.52 ^{b2}	66.67± 1.52 ^{b3}	71.00± 1.00 ^{b4}	77.67± 1.52 ^{a6}	77.00± 2.64 ^{a5}	82.00± 1.00 ^{a7}	77.00± 2.64 ^{a5}	85.00± 1.00 ^{a9}	86.00± 2.00 ^{b10}	84.00± 2.64 ^{a8}	87.67± 2.51 ^{a11}	90.33± 3.05 ^{c12}
1200	20.67± 1.15 ^{d1}	62.00± 2.64 ^{a7}	53.00± 2.64 ^{c2}	62.33± 2.08 ^{c8}	76.00± 3.46 ^{b9}	60.33± 1.52 ^{c6}	54.67± 1.52 ^{b3}	59.00± 2.64 ^{b5}	57.33± 3.05 ^{a4}	81.33± 1.52 ^{c11}	80.00± 1.00 ^{b10}	85.33± 2.08 ^{b12}	96.33± 1.52 ^{a13}
1600	22.67± 1.52 ^{b1}	51.67± 1.52 ^{c2}	72.00± 2.64 ^{a7}	77.67± 1.52 ^{a10}	75.67± 1.52 ^{c8}	66.33± 2.08 ^{b6}	52.67± 1.52 ^{c3}	53.67± 3.21 ^{c4}	56.00± 2.64 ^{a5}	88.33± 2.08 ^{a12}	77.00± 1.00 ^{c9}	81.33± 2.51 ^{c11}	94.00± 2.00 ^{b13}

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

3.5 Effects of Aqueous Seed Extracts of *Sphenostylisstenocarpa* on the Sperm Count of Male Albino Rats

Table 5 shows the effects of aqueous seed extract of *Sphenostylisstenocarpa* on the sperm count (SC) of male albino rats. There was an overall dose-dependent significant difference ($p < 0.05$) in the mean weekly SC of the treated rats when compared to the control group. The dose-dependent analysis showed that in all weeks, the SC of rats in the treatment groups were significantly higher ($p < 0.05$) when compared to control. Based on duration, there were minimal fluctuations in the mean weekly SC of the rats in the treatment groups. Rats in the control had significant decreases in the SC throughout the weeks except in week 9 where SC of the rat increased significantly ($p < 0.05$) when compared to week 0. In all the treatment groups, however, there were significant increases ($p < 0.05$) in the SC of the rats in all the weeks when compared to week 0.

Table 5: Weekly effects of different concentrations of aqueous seed extract of *S. stenocarpa* on the sperm count of male albino rats

Conc. (mg/kg)	Duration (Weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	30.67± 3.05 ^{d11}	22.67± 2.51 ^{d2}	30.00± 1.00 ^{d10}	26.33± 7.02 ^{d5}	28.67± 4.16 ^{d8}	29.33± 1.52 ^{d9}	28.67± 1.52 ^{d8}	25.00± 4.00 ^{d4}	28.00± 1.00 ^{d7}	31.00± 7.00 ^{c12}	23.00± 1.00 ^{d3}	22.00± 3.00 ^{d1}	27.00± 1.00 ^{c6}
800	36.33± 2.08 ^{a1}	65.67± 1.52 ^{b2}	79.67± 2.08 ^{b3}	81.33± 1.52 ^{b4}	87.00± 1.00 ^{c6}	87.67± 1.52 ^{a7}	95.33± 1.15 ^{a11}	85.00± 2.64 ^{a5}	94.67± 0.57 ^{a10}	92.00± 1.00 ^{a9}	92.00± 1.00 ^{a9}	88.67± 1.52 ^{c8}	98.00± 1.00 ^{b12}
1200	35.33± 1.52 ^{c1}	73.00± 2.64 ^{a5}	62.67± 2.51 ^{c3}	73.00± 2.64 ^{c5}	89.00± 1.00 ^{a8}	68.33± 2.08 ^{c4}	61.00± 1.00 ^{c2}	61.00± 1.73 ^{c2}	63.00± 4.00 ^{c4}	85.00± 4.58 ^{b7}	84.00± 4.00 ^{c6}	94.00± 1.00 ^{a9}	99.67± 2.08 ^{a10}
1600	36.33± 3.05 ^{b1}	61.67± 1.52 ^{c2}	83.67± 3.21 ^{a6}	87.33± 1.52 ^{a7}	88.67± 1.52 ^{b8}	73.33± 1.52 ^{b5}	63.00± 1.00 ^{b3}	61.33± 3.78 ^{b2}	65.00± 1.73 ^{b4}	92.00± 3.00 ^{a11}	90.33± 1.52 ^{b10}	89.00± 1.00 ^{b9}	98.00± 1.00 ^{b12}

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

3.6 Effects of Aqueous Seed Extract of *Sphenostylisstenocarpa* on the Testosterone Level (TL) of Male Albino Rats

Table 6 shows the effects of aqueous seed extract of *Sphenostylisstenocarpa* on the testosterone levels (TLs) of male albino rats. There was an overall dose-dependent significant difference ($p < 0.05$) in the mean weekly TLs of the treated rats when compared to the control group. The dose-dependent analyses showed that in week 0, the TLs of the male rats were significantly lower ($p < 0.05$) in all treatment groups when compared to the control group. However, the TLs of rats in the treatment groups were significantly higher ($p < 0.05$) in all other weeks, throughout the duration of the study when compared with the TLs of rats in the control group. The time dependent analysis showed that, there were minimal fluctuations in the mean weekly TLs of the rats in the treatment groups. TLs of rats in the control group, decreased significantly ($p < 0.05$) throughout the weeks except in week 1 with a significant increase ($p < 0.05$) when compared to week 0. However, in the treatment groups, there were significant increases ($p < 0.05$) in the TLs of the rats in all the weeks when compared to week 0.

Table 6: Weekly effects of different concentrations of aqueous seed extract of *S. stenocarpa* on the testosterone of male albino rats

Conc. (mg/kg)	Duration (Weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.00	0.57± 0.01 ^{a11}	0.60± 0.41 ^{d12}	0.43± 0.05 ^{d6}	0.46± 0.01 ^{d8}	0.57± 0.06 ^{d10}	0.46± 0.04 ^{d9}	0.38± 0.05 ^{d4}	0.44± 0.03 ^{d7}	0.39± 0.03 ^{d5}	0.35± 0.03 ^{d2}	0.38± 0.04 ^{d3}	0.38± 0.04 ^{d3}	0.35± 0.02 ^{d1}
800	0.54± 0.02 ^{c1}	0.66± 0.02 ^{c2}	0.87± 0.03 ^{b4}	2.24± 0.39 ^{b10}	3.93± 0.01 ^{c11}	0.83± 0.11 ^{c3}	1.00± 0.08 ^{c8}	0.88± 0.03 ^{c4}	0.92± 0.01 ^{c6}	0.95± 0.01 ^{c7}	1.00± 0.08 ^{c8}	0.87± 0.07 ^{c5}	1.00± 0.08 ^{c8}
1200	0.51± 0.02 ^{d1}	1.43± 0.43 ^{b6}	0.66± 0.07 ^{e2}	0.84± 0.04 ^{c4}	5.09± 0.75 ^{a11}	0.79± 0.10 ^{b3}	1.10± 0.10 ^{b5}	1.83± 0.25 ^{a8}	1.55± 0.25 ^{b7}	4.75± 0.45 ^{b10}	4.40± 0.20 ^{b9}	6.75± 0.15 ^{b12}	7.27± 0.27 ^{b13}
1600	0.54± 0.05 ^{b1}	1.84± 0.12 ^{a5}	4.25± 0.44 ^{a9}	4.08± 0.16 ^{a7}	4.62± 0.15 ^{b8}	0.90± 0.20 ^{a2}	1.40± 0.20 ^{a4}	1.03± 0.20 ^{b3}	1.95± 0.15 ^{a6}	5.76± 0.35 ^{a10}	5.86± 0.20 ^{a11}	6.95± 0.25 ^{a12}	9.15± 0.75 ^{a13}

Values as mean ± standard deviation. Values with different small letter alphabet superscript down a column for each solvent were significantly different between concentrations ($p < 0.05$), while different numeric superscripts across a row were significantly different for durations ($p < 0.0$)

4. DISCUSSION

Several bioactive compounds of plant origin have been shown to have the capacity to interfere with the reproductive cycle [8]. In the male reproductive system, reduced sperm count, sperm motility, and reduced testosterone levels are considered standard criteria for the characterization of toxic agents that may cause fertility problems in the treated subjects [9, 10].

The evaluation of body weight during treatment with a particular substance provides information on the general health of the animals. Body weight of male rats changed in relation to the extract concentration and duration of the treatment. The extracts were associated with a decline in the weight of the rats especially at the two higher extract concentrations (1200 and 1600 mg/kg). The decline in weight was especially more obvious in weeks 1–4 and weeks 9–12. This reduction of body mass or a decrease in weight gain could be traceable to varied responses to anorexia or systemic treatment-induced toxicity as previously documented [11, 12].

Male reproduction encompasses the production of viable sperm, their delivery into the female reproductive tract, fertilization of the female oocytes, and production of normal offspring [13, 14]. Successful male fertility requires adequate sperm count and sperm motility. There is also intratesticular endocrine, autocrine, and paracrine regulation. In this study, the testes and gonad characteristics, namely sperm count, testes weight, gonad somatic index and sperm motility were affected in a manner that depended on the extract concentration and duration of treatment. The extracts caused increase in the testicular weights, sperm count and sperm motility. The absence of reduction in mass of the reproductive organs reinforces the idea that the extract does not have contraceptive action in rats, since reductions of masses in testis of rats are indicators of impaired fertility or contraceptive activity. The research is similar to the findings of [15, 16]. The increase in sperm number of the treatment group is due to increased production of testosterone. This is similar to the work by Al-Sa'aidi *et al.* [17]. Testosterone is responsible for maturation of spermatozoa [18]. Sperm count was often used as a measure of sperm production, testicular function and/or male fertility. Low sperm count and high percentage of abnormal spermatozoa each have been associated with reduced fertility. This confirms the findings of [19]. The significant increase in sperm number recorded in this study reveals the potential of the plant to cure male fertility problems, especially those related to hormonal levels, sperm count, and sperm viability. Thus, the increase in sperm count and mobility shows that treatment with *S. stenocarpa* improves and enhances the fertilizing capacity of semen. However, it is noteworthy that administration of some plant extracts may adversely affect sperm motility, sperm count, sperm viability, reproductive hormones and fertility generally in rats [20, 21].

Testosterone plays an important role in maintaining spermatogenesis, accessory sex organs and secondary sexual characters in male rats [22]. In male albino rats administered the *Sphenostylis stenocarpa* extracts, the changes noticed in the gonadal hormone were related to the extract concentration and duration of administration. The hormone testosterone responded to the plant extracts. Testosterone concentration changed significantly in relation to extract concentration and treatment duration. There were significant increases in the testosterone levels of all the treated rats. Our work corroborates earlier report that aqueous extract of *Carpolobia lutea* increased testosterone levels and this may be adduced to the induction of hormone synthesis by the Leydig cells, as these cells are the main source of testosterone [23]. Testosterone is

Comment [BN6]: In the discussion, no tables have been referred in the context. Recommended to provide the respective table number which will be easy to compare with the table and the texts.

synthesized in the Leydig cells via several important enzymes, carrier proteins or receptors from cholesterol synthesized *denovo* [24]. Hormones are believed to play a key role in the etiology of prostate cancer [25]. No alterations in the sperm count, motility, and serum testosterone levels in this study suggested the normal functioning of Leydig cells.

5. CONCLUSION

In conclusion, the data in this work points to the fertility-enhancing ability of the aqueous extract of *S. stenocarpa*. The gonad characteristics, sperm parameters and hormonal indices of male rats were not affected, showing a normal functioning of the reproductive system and its related endocrine system.

Comment [BN7]: Limitation of the study to be mentioned which leads to further research.

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Comment [BN8]: To be verified by the authors once to ensure the formatting as per the journal guidelines.

UNDER PEER REVIEW

