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Spermiogram and testicular morphological studies of the buck after treatment with ethanol leaf extract of *Spondias mombin*

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Abstract

Twelve sexually matured West African Dwarf bucks from were used for the experiment. Plant extraction was by cold extraction method using hexane and ethanol as solvents. All the goats had bilaterally well descended free testicles. They were kept in standard goat pen, were served water *ad libitum*, centrosema plant and ration. They were stabilised for two weeks after which pre-treatment spermiogram was done followed by 14 days of oral administration of 800mg/kg ethanol leaf extract of *Spondias mombin*. Spermiogram was repeated after treatment. Two randomly selected goats were then castrated through a midline pre-scrotal incision for morphological study and histology of the testes and epididymides. Total spermatozoa morphological abnormalities in pre-treatment of 17.1% was significantly ($p \leq 0.05$) higher than the 10% recorded for post-treatment. Curved mid-piece (1.8%) and bent tail (1.8%) constituted the highest abnormalities post-treatment while curved tail (3.5%) was highest pre-treatment. Mean values of progressive motility and percentage liveability were significantly higher ($p \leq 0.05$) in post- treatment (96.17±3.10% and 98.25±1.36% respectively) compared to pre-treatment (80.83±11.84% and 78.75±9.56% respectively). Post-treatment sperm concentration (2.50±0.32 $x10^9$ cells/ml) compared with pre-treatment (2.32±0.36 $x10^9$ cells/ml) was not significantly different. Post-treatment gross and histological features of the bucks' testes and epididymis were normal. The work revealed that *Spondias mombin* at 800mg/kgBW improved semen quality in bucks indicating its usefulness as a potential profertility agent.

Keywords: Buck, Morphological, *Spondias mombin,* Spermiogram, Testicular
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Introduction

The use of medicinal plants for optimization of litter size, good fecundity, prevention and treatment of pathological reproductive conditions have been explored (Ayoka *et al*., 2008). The use of herbal preparations in animals has been advocated, largely due to availability, affordability, and safety of use (Saleh *et al*., 2015). *Spondias mombin* is a tropical fructiferous plant with multiple medicinal use such as sedative, antiepileptic and antipsychotic effects (Taylor, 2012). Raji *et al.* (2006), studied the reproductive effects of aqueous *Spondias mombin* bark extract and reported a marked dose dependent

reduction in epididymal sperm progressive motility, sperm count, viability and a dose-dependent increase in percentage abnormal spermatozoa. However, Oloye *et al.* (2011), using teh aqueous leaves extract of *Spondias mombin*, reported that at 600mg/kg and 800mg/kg BW, the plant supported fertility in the Wistar rat. Artificial insemination remains a veritable tool for successful breed improvement in animals and good quality semen play a vital role. This work was aimed at establishing the effects of ethanol leaf extract of *Spondias mombin,* administered orally at 800mg/kgBW, on the gross testicular morphology, histology as well as semen characteristics including spermatozoal motility, concentration, percentage liveability and spermatozoal morphology.

Materials and Methods

Plant extract

Leaves of *Spondias mombin* were collected within the premises of the Federal University of Agriculture Abeokuta and authenticated at the National Centre for Genetic Resources and Biotechnology (NACGRAB), Nigeria. Extraction was done according to standard methods (Oluyemi *et al* 2007; Omotuyi *et al*., 2010). The powdered leaves (3.8kg) were soaked in hexane to reduce the fat content and air dried. The residue was treated with ethanol for 3 days and the resultant filtrate was then concentrated with the use of rota-evaporator. Dark brownish paste recovered was kept in fume hood to solidify for five days at 25°C. A yield of 62g of dried extract was obtained, from which a stock solution of 8000mg of extract in 1ml of propylene glycol was constituted. Dose was calculated using the formula:

$$\text{Dose} = \text{Weight of animal(kg)} \times \text{Dosage (mg/kg)} / \text{Concentration(mg/ml)}$$

Experimental animal management and extract administration

Twelve pubertal West African Dwarf (WAD) bucks of average age of 1.8±0.2 years and average weight of 8.8 ± 0.7 kg were used. All goats had well-formed pendulous scrotum containing bilaterally well descended free testicles. The goats, kept in a standard goat pen were fed with fresh green Centrosema leaves in the morning and feed concentrate in the evening. The animals were allowed two weeks acclimatization and water *ad libitum*. At the end of acclimatization, 800mg/kg ethanol leaf extract of *Spondias mombin* was administered to the animals orally for fourteen days using a 5ml syringe.

Spermiogram

Semen was collected before and after oral administration of the extract to the bucks using a locally fabricated electroejaculator. This was achieved by inserting a probe or electrode into the sire's rectum and stimulating nerves of the reproductive system by gradually increasing voltage in rhythmic fashion with a rheostat for a short period. Erection and ejaculation occur at 10 to 15 volts when 0.5 to 1 ampere current is passed. Semen was collected into a collection tube maintained at 37^{o}c in a water bath until it was ready for evaluation. The semen was analysed using standard procedure (Logue & Greig, 1987). Progressive spermatozoa motility was estimated by diluting the semen using 2.9% trisodium citrate solution so that individual spermatozoa can be visualized (x400 magnification). The dilution rate was 1 in 100. (Logue & Greig, 1987).

For morphology studies and percentage liveability, Nigrosin-eosin stain was used. The stain was mixed with freshly collected semen and mixed at the ratio 1(semen):10(stain). The stain had been prewarmed and the mixture was allowed to incubate at 35-37^{o}c for several minutes. A drop was then taken and smeared across a prewarmed slide and allowed to dry. Spermatozoa concentration was estimated using the improved Neubauer chamber (Deep 1/10 mm, LABART, Germany) as described by Pant & Srivastava (2003).

Gross morphological study and histology

After oral treatment with extract and semen collection, two of the animals were randomly selected for castration using the midline pre-scrotal incision method for morphological study and histology. Testes were examined for adhesion to scrotal sac, inflammation, consistency and size. Testicular and epididymal sections were sectioned and preserved in Bouin's solution. Later, histological slides were prepared from them using Schiff's reagent (PAS)

Statistical analysis

Pretreatment and post-treatment data was presented as mean ± standard deviation. Means were compared using student's *t* test and mean differences were considered significant at $p \leq 0.05$. Statistical package used was Graphpad prism (version 6.0).

Table 1: Values of morphological abnormalities before treatment with *Spondias mombin*

Buck	Tailess head	Headless tail	Rudimentary tail	Bent tail	Curved tail	Bent mid piece	Curved midpiece	Looped tail	Total	Total Cell count
A	4(1%)	16(4%)	16(4%)	18(4.5%)	10(2.5%)	4(1%)	4(1%)	12(3%)	84(21%)	400
B	6(1.5%)	8(2%)	7(1.8%)	15 (3.8%)	18(4.5%)	4(1%)	6(1.5%)	5(1.3%)	69(17.4%)	400
C	4(1%)	4(1%)	7(1.8%)	20 (5%)	15(3.8%)	5(1.3%)	5(1.3%)	10(2.5%)	70(17.7%)	400
D	4(1%)	4(1%)	12(3%)	11 (2.8%)	14(3.5%)	6(1.5%)	5(1.3%)	5(1.3%)	61(15.4%)	400
E	8(2%)	25(6.3%)	10(2.5%)	16(4%)	25(6.3%)	6(1.5%)	6(1.5%)	15(3.8%)	111(27.9%)	400
F	3(0.8%)	24(6%)	9(2.3%)	23 (5.8%)	13(3.3%)	5(1.3%)	5(1.3%)	10(2.5%)	92(23.3%)	400
G	9(2.3%)	4(1%)	7(1.8%)	11 (2.8%)	15(3.8%)	8(2%)	5(1.3%)	8(2%)	67(17.0%)	400
H	4(1%)	4(1%)	9(2.3%)	23 (5.8%)	18(4.5%)	8(2%)	0(0%)	10(2.5%)	76(19.1%)	400
I	5(1.3%)	6(1.5%)	7(1.8%)	10 (2.5%)	9(2.3%)	4(1%)	0(0%)	7(1.8%)	48(12.2%)	400
J	9(2.3%)	5(1.3%)	11(2.8%)	0 (0%)	11(2.8%)	0(0%)	0(0%)	3(0.8%)	39(10.0%)	400
K	0(0%)	14(3.5%)	1(0.3%)	4 (1%)	5(1.3%)	5(1.3%)	9(2.3%)	5(1.3%)	43(11.0%)	400
L	0(0%)	22(5.5%)	1(0.3%)	5(1.3%)	15(3.8%)	3(0.8%)	4(1%)	11(2.8%)	61(15.5%)	400
Total	56 (1.17%)	136 (2.83%)	97 (2.02%)	156 (3.25%)	168 (3.5%)	58 (1.21%)	49 (1.02%)	101 (2.1%)	821 (17.1%)	4800
Mean± SD	4.67±2.99	11.33±8.45	8.08±4. 23	13.0±7.50	14.0±5.12	4.83± 2.17	4.08±2.78	8.42±3.53	68.42 ±20.70	400±0.00

Table 2: Values of morphological abnormalities after treatment with S*pondias mombin*

Buck	Tailess head	Headless tail	Rudimentary tail	Bent tail	Curved tail	Bent mid piece	Curved mid piece	Looped tail	Total	Total cell count
A	6 (1.5%)	5 (1.3%)	1 (0.3%)	10(2.5%)	8 (2%)	8(2%)	8 (2%)	1 (0.3%)	47 (11.9%)	400
B	4(1%)	4(1%)	4(1%)	10 (2.5%)	5(1.3%)	3(0.8%)	10(2.5%)	0(0%)	40 (10.1%)	400
C	4 (1.0%)	4 (1.0%)	1 (0.3%)	7 (1.8%)	7 (1.8%)	9 (2.3%)	8 (2%)	0 (0%)	40 (10.2%)	400
D	2(0.5%)	3 (0.8%)	1 (0.3%)	10(2.5%)	5 (1.3%)	7 (1.8%)	3 (0.8%)	4(1.0%)	35 (9.0%	400
E	2 (0.5%)	4 (1%)	2(0.5%)	2(0.5%)	6 (1.5%)	3(0.8%)	8 (2%)	1 (0.3%)	28 (7.1%)	400
F	14 (3.5%)	6 (1.5%)	10 (2.5%)	10(2.5%)	7 (1.8%)	9 (2.3%)	7 (1.8%)	1 (0.3%)	64 (16.2%)	400
G	3 (0.8%)	4(1%)	1(0.3%)	7(1.8%)	7 (1.8%)	7 (1.8%)	7 (1.8%)	1 (0.3%)	37 (9.6%)	400
H	3 (0.8%)	3 (0.8%)	1 (0.3%)	10(2.5%)	7 (1.8%)	7 (1.8%)	7 (1.8%)	1 (0.3%)	39 (10.1%)	400
I	3 (0.8%)	5 (2.3%)	7 (1.8%)	1(0.3%)	0 (0%)	4(1%)	5 (2.3%)	1 (0.3%)	26 (8.8%)	400
J	3 (0.8%)	3 (0.8%)	3 (0.8%)	8 (2%)	6 (1.5%)	6 (1.5%)	6 (1.5%)	0(0%)	35 (8.9%)	400
K	5 (1.3%)	4 (1%)	4 (1%)	4(1%)	4 (1%)	3 (0.8%)	7 (1.8%)	1 (0.3%)	32 (8.2%)	400
L	3(0.8%)	2(0.5%)	11(2.8%)	7(1.8%)	11(2.8%)	5(1.3%)	10(2.5%)	7(1.8%)	56 (14.3%)	400
Total	52 (1.08%)	47 (0.98%)	46 (0.96%)	86 (1.8%)	73 (1.52%)	71 (1.48%)	86 (1.8%)	18 (0.38%)	479 (10%)	4800
Mean± SD	4.33±3.26	3.92±1.08	3.83±3.61	7.17±3.24	6.08 ±2.61	5.92±2.27	7.17±1.95	1.5±2.02	39.92±11.06	400±0.00

Table 3: Mean (±SD) semen characteristics of bucks before and after treatment with 800mg/kg crude extract of *Spondias mombin*

	Volume (ml)	Motility (%)	Concentration ($x10^9$ cells/ml)	Percentage liveability (%)
Pre-treatment	0.24±0.11	$80.83±11.84.^{x}$	2.32±0.36	$78.75±9.56^{x}$
Post-treatment	0.19±0.08	$96.17±3.10^{y}$	2.50±0.32	$98.25±1.36^{y}$

^{x,y} mean values with different superscripts within a column are significantly different (p≤0.05)

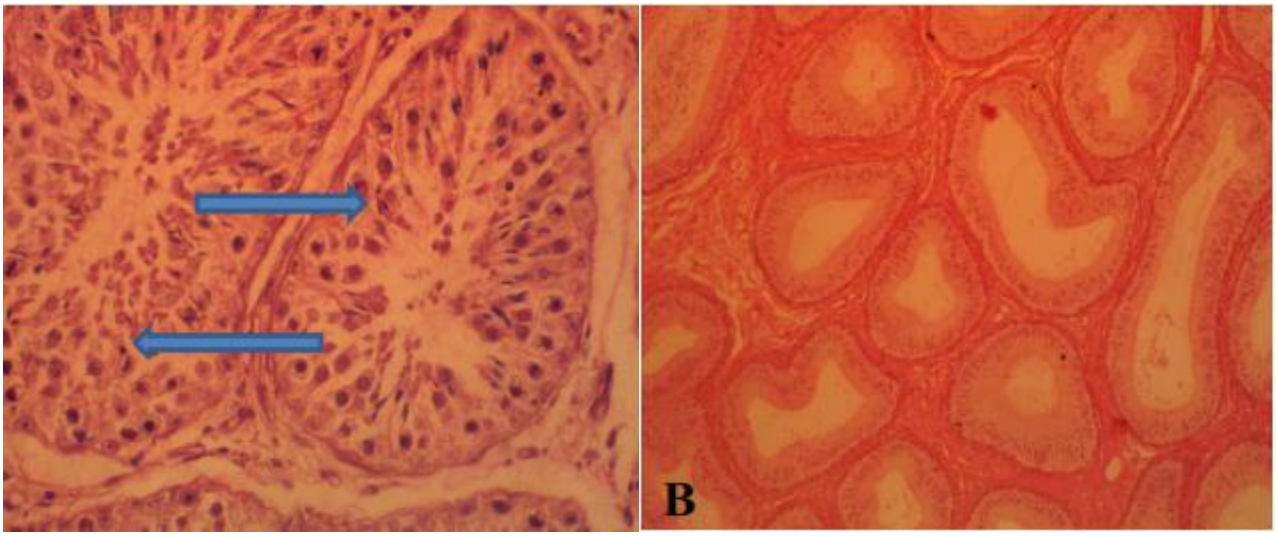

Plate 1: Normal testicular structure (A) and epididymal structure (B) of buck after treatment with 800mg/kg crude ethanol leaf extract of *Spondias mombin*. Periodic acid-Schiff (PAS) stain. (×400) Arrows show semiferous tubules

Results and Discussion

The total spermatozoa morphological abnormalities in pre-treatment was 17.1% (68.42 ± 20.70 cells) of the total sperm count (400 cells) (Table 1). This was significantly ($p<0.05$) higher than the 10% (39.92 ± 11.06) of total sperm count (400) recorded for post-treatment (Table 2). Curved tail was the highest contributor of 3.5% to abnormalities in pre-treatment while curved mid piece and bent tail gave the highest contribution of 1.8% in post-treatment (Tables 1 and 2). The mean progressive motility of 96.17 ± 3.10% and percentage liveability of 98.25 ± 1.36% in post-treatment were significantly ($p<0.05$) higher compared to pre-treatment values (progressive motility - 80.83 ± 11.84% and percentage liveability - 78.75 ± 9.56%) (Table 3). The post-treatment sperm concentration of 2.50 ± 0.32 $x10^9$ cells/ml was not significantly ($p>0.05$) different from the pre-treatment value of 2.32 ± 0.36 $x10^9$ cells/ml. However, the post-treatment semen volume of 0.19 ± 0.08ml was significantly ($p<0.05$) lower than the pre-treatment of 0.24 ± 0.11ml. Post treatment gross appearance and histological features of the bucks' testes and epididymis appeared normal (Plate 1).

The reduction in percentage spermatozoa morphological abnormalities from pre-treatment to post-treatment, which is desirable for fertility might be traced to the pro fertility quality of the crude extract. Less than 30% abnormal morphology is prerequisite for semen to be of acceptable quality as reported by Abebe (2008) or less than 20% as reported by Bitto & Egbunike (2012). Comparing these values with the submissions of Bitto & Egbunike (2012) who worked on WAD bucks in their natural state, normal spermatozoa morphology reported by the authors as 85.90 ± 0.72% was higher than 83.9% (17.1% abnormality) reported for pre-treatment and lower than 90% (10% abnormality) reported for post treatment in this work. The significantly higher post-treatment mean values of progressive motility and percentage liveability compared to pre-treatment is also a good indicator of fertility. This also compares well with the values reported by Bitto & Egbunike (2012). Also, percentage liveability reported by these workers was higher compared to pre-treatment value in this work but lower when compared with post-treatment. The normal post treatment histological features of the bucks' testes and epididymis agrees with the findings of Oloye *et al.* (2012) who worked with Wistar rats using aqueous extract of *Spondias mombin* at the same dosage of 800mg/kg used in this work.

In conclusion, the study revealed that *Spondias mombin* at 800mg/kgBW elicited profertility properties by improving semen quality in the buck. Hence the plant can be considered as profertility agent for the male animal and the specific fraction responsible should be investigated further.

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