



Fertility Effects of *Anacardium occidentale* (Cashew Leaves) in the West African Dwarf Bucks

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SUMMARY

Anacardium occidentale is a plant known for its vast medicinal properties and has been used in Nigeria for the treatment of several ailments. However, little is known about its effects on fertility in animals. This study was designed to assess the fertility effects of the aqueous leaf extract of *Anacardium occidentale* in the West African Dwarf breeding bucks. Sixteen West African Dwarf bucks were assigned into 2 groups A and B (n=8). Group A received a daily oral dose of 250mg/kg aqueous leaf extract of *Anacardium occidentale* for 14 days, while B served as the control. Blood and semen sample, as well as testes and epididymis were harvested from the bucks at days 7 and 14 post-treatment. Administration of aqueous leaf extract of *Anacardium occidentale* caused a significant decrease in RBC count, total protein and albumin level, semen volume and sperm motility. It also caused a reduction in the total number of morphologically abnormal sperm cells and a mild reduction in scrotal circumference, testicular and epididymal weight and diameter. It caused an increase in the size of the epididymal and testicular germinal luminal diameter and a decrease in epididymal and testicular germinal height. These suggested that its prolonged use in West African Dwarf breeding bucks may produce anti-fertility effect. Therefore, its incorporation into animal feeds must be done with caution.

Keywords: *Anacardium occidentale*, Haematology, Semen, Testes, West African Dwarf Bucks.

INTRODUCTION

Anacardium occidentale is a tree in the family of the flowering plant *Anacardiaceae*. The family contains 73 genera and about 600 species (Abulude *et al.*, 2010). *Anacardium* contains 8 species native to tropical America, of which the cashew is by far the most important economically. It is a multipurpose tree of the amazon that grows up to 15m high. It

has a thick and tortuous trunk with branches so winding that they frequently touch the ground (Abulude *et al.*, 2010). The cashew tree produces many resources and products, the bark and leaves are used for medicinal purpose. The cashew leaf and bark tea are used in Brazil and Peruvian herbal medicine. Ethanol extract of cashew leaf at a dose of 10 g/kg of basal feed improves growth, body weight, and

chicken feed efficiency of Jawa Super chickens (Setiawan *et al.*, 2018).

In Nigeria, cashew leaf is used to treat diabetes, weakness, muscular debility, urinary disorders, asthma, eczema, psoriasis, scrofula, dyspepsia, genital problems, bronchitis, cough, intestinal colic, leishmaniasis, venereal disease as well as impotence and syphilis-related skin disorder (Irare *et al.*, 2017). The leaf of cashew (*Anacardium occidentale*) contains a high concentration of Vitamin C, carotenoids, phenolic compounds, and minerals (Kozubek *et al.*, 2001). It is used as anti-ulcerogenic therapy especially the anacardic acid content which can stimulate mucus and prostaglandin production to protect the gastric and intestinal lumen (Jimmy *et al.*, 2017). Cashew leaves or bark tea is still widely used throughout the tropics as effective diarrhea and colic remedy and is considered gentle enough for children (Aiswarya *et al.*, 2011). The leaf has an antifungal effect which is due to the presence of bioactive components such as triterpenoids (Ifesan *et al.*, 2013). Its leaves and bark had also been reported for their amebicidal, antioxidant and astringent properties (Ifesan *et al.*, 2013). The Bark extract is used in the treatment of malaria (Dike *et al.*, 2012).

Earlier studies have demonstrated the vast medicinal use of *Anacardium occidentale* (Wattanathorn, 2018), however, its effects on fertility in animals have not been fully elucidated. Therefore, this study was designed to assess the fertility effects of the crude leaf extract of *Anacardium occidentale* in the West African Dwarf breeding bucks

MATERIALS AND METHODS

Experimental Animals

Sixteen West African Dwarf goats (bucks) were used for this study. The goats were kept in a standard pen of the Goat unit in the Teaching and Research Farms, University of Ibadan, Nigeria. They were fed thrice daily with concentrates, cassava

peels and wheat offal, morning, afternoon and night, respectively. The animals weighed between 15-17kg at the commencement of the study.

Plant Materials

The leaves of *Anacardium occidentale* were obtained from the Faculty of Veterinary Medicine, University of Ibadan and were identified at the herbarium of the Department of Botany, University of Ibadan with a voucher number assigned: UIH-22691.

Study Design

This study was a randomized controlled trials in which the animals were randomly assigned into 2 groups A and B (n=8). Group A received a daily oral dose of 250mg/kg aqueous leaf extract of *Anacardium occidentale* for 14 days, while B (control) served as the untreated group. Samples were collected from the bucks at days 7 and 14 post-treatment.

Blood Collection and Analysis

Blood samples were collected intravenously via the jugular vein into heparinized and non-heparinized bottles for haematology and serum chemistry, respectively (Oyeyemi and Ajani, 2014)

Semen Collection and Analysis

Semen samples were collected from the bucks using the electroejaculation method (Oyeyemi *et al.*, 1996). Sperm motility, liveability, concentration and Morphology were assayed using a modified method described by Ajani and Oyeyemi (2014).

Organs Harvest and Biometrics

Orchidectomy was performed by the open castration method. The scrotum was washed with soap, cleaned with warm water and disinfected with Clohexidine. A skin infiltration was done on the scrotum using Lidocaine to desensitize the area around the testis. A size 4 scalpel blade was used to make a skin incision from the dorsomedial aspect of the scrotum. This

was reflected laterally and the subcutaneous tissue and scrotal fascia were incised to expose the outer covering layer of the testis (tunica vaginalis). The testis was then carefully separated from the epididymis using the scalpel blade and thumb forceps (Raji and Ajala, 2015). The testis and epididymis were weighed using an electronic scale. Also, the testicular length and diameter were measured as described by Oyeyemi *et al.* (2012)

Histomorphometry of the Testes and Epididymides

Histomorphometry was done using ToupeView 3.7® Software. The diameter of the H and E sections of the testes was measured at 100X Magnification with the aid of an AmScope® camera fitted to an Olympus® microscope. The following measurements were taken: relative volume of the germinal epithelium, interstitium and lumen of the seminiferous tubules; seminiferous tubular diameter, epididymal tubular diameter, epididymal luminal diameter and epididymal epithelial height. For each parameter, ten measurements were made per section using a calibrated eye-piece micrometer (Graticules Ltd. Toubridge Kent).

Statistical Analysis

Data generated were analyzed using the Test of Homogeneity of variance, multiple comparisons and Analysis of variance (One-Way ANOVA). SPSS Version 15 for Windows (SPSS Inc, 2006) and Microsoft Excel Professional Plus (Microsoft Corporation, 2010) were used to carry out all procedures. Significance was set at ($P < 0.05$).

RESULTS

Heamatology

Haematological values presented in Table I shows that treatment caused a decrease in PCV, Hb, RBC, Platelets, WBC, Lymphocytes values at day 7 post-treatment when compared to the control. However, mean values of these parameters increased at day 14 post-treatment. These differences were significant ($P < 0.05$) for RBC and Lymphocytes only.

Conversely, the treatment caused an increase in MCH, MCV, Neutrophils, Monocytes and ESR at day 7 Post-treatment compared to the control. These values decreased at day 14 post-treatment. The differences were significant ($P < 0.05$) for MCH, MCV, Neutrophils and ESR. There were no differences in the mean values of MCHC across the groups and post-treatment days (Table 1).

TABLE I: Haematological Parameters of WAD Bucks Treated with Aqueous Extract of *Anacardium occidentale* at Days 7 and 14 Post-Treatment

PARAMETERS	CONTROL	DAY 7	DAY 14
PCV (%)	26.00±1.00 ^a	23.80±2.71 ^a	26.75±0.48 ^a
HB (%)	8.55±0.35 ^a	7.80±0.90 ^a	8.78±0.17 ^a
RBC (x 10 ¹² /L)	15.41±1.99 ^a	3.41±0.40 ^b	4.12±0.34 ^c
PLATELET (x10 ⁹ /L)	7.00±1.00 ^a	6.20±0.92 ^a	8.00±0.00 ^a
MCH (pg)	5.50±0.50 ^a	22.60±1.36 ^b	21.50±1.50 ^c
MCV (fl)	16.50±1.50 ^a	69.80±4.35 ^b	50.50±15.21 ^c
MCHC (%)	33.00±0.00 ^a	33.00±0.00 ^a	33.00±0.00 ^a
ESR	1.50±0.50 ^a	7.80±0.66 ^b	5.75±0.85 ^c
WBC (x10 ⁹ /L)	12.40±0.40 ^a	8.28±1.14 ^a	30.33±22.57 ^a
Lymph (%)	35.50±0.50 ^a	21.60±0.81 ^b	30.25±1.93 ^c
Neut (%)	63.50±0.50 ^a	77.20±0.86 ^b	68.50±1.94 ^c
Mono (%)	1.00±0.00 ^a	1.40±0.24 ^a	1.25±0.25 ^a

Values are reported as mean±SEM^{abc}: Means in the same row with different superscript differ significantly (P<0.05).

Serum Chemistry

Treatment caused a decrease in Albumin, Total Protein, Globulin, Triglycerides, AST, ALT mean values at day 7 post-treatment which later increased at day 14 post-treatment compared to the control.

These changes were significant (P<0.05) for Total Protein and Albumin (Table II). The mean values of BUN and ALP increased progressively as post-treatment days increased. The increase was significant (P<0.05) for BUN only (Table 2).

TABLE II: Serum Chemistry of WAD Bucks Treated with Aqueous Extract of *Anacardium occidentale* at Days 7 and 14 Post-Treatment

PARAMETERS	CONTROL	DAY 7	DAY 14
Total Protein (g/dl)	3.49±0.05 ^a	3.06±0.18 ^b	3.63±0.06 ^c
Albumin (g/dl)	1.17±0.03 ^a	1.06±0.02 ^b	1.23±0.03 ^c
Globulin (g/dl)	2.32±0.02 ^a	1.78±0.22 ^a	2.40±0.08 ^a
Triglycerides (g/dl)	36.00±1.00 ^a	35.00±2.41 ^a	38.00±0.82 ^a
AST (iu/L)	43.00±1.00 ^a	41.00±1.79 ^a	42.00±0.82 ^a
ALT (iu/L)	31.00±1.00 ^a	30.20±2.15 ^a	30.25±1.03 ^a
BUN (mg/dl)	1.50±0.02 ^a	2.06±0.20 ^b	2.41±0.12 ^c
ALP (iu/L)	45.50±0.50 ^a	48.40±3.08 ^a	54.25±1.03 ^a

Values are reported as mean±SEM^{abc}: Means in the same row with different superscript differ significantly P<0.05).

Biometrical Assessment of the Testes and Epididymes

The treatment caused a decrease in the Scrotal circumference (SC), left testis weight (LTW), Right testis weight (RTW), left testis diameter (LTD), Right testis diameter (RTD), Right epididymis weight (REW) , Left epididymis weight (LEW) , Body Weight(BW) at day 7 post-treatment which later increased at day 14 post-

treatment except for the BW which decreased compared with the control (Table 3). Although these differences were not significant ($P>0.05$). Also, there was an increase in the mean values of Left testis length (LTL), Right testis length (RTL) at day 7 post-treatment. These values later decreased at day 14 post treatment. The differences in value were not significant ($P>0.05$) (Table III).

TABLE III: Biometrical Assessment of the Testes and Epididymis of WAD Bucks Treated with Aqueous Extract of *Anacardium occidentale* at Days 7 and 14 Post-Treatment

PARAMETERS	CONTROL	DAY 7	DAY 14
Scrotal Circumference (cm) (SC)	18.00±1.50 ^a	14.00±1.00 ^a	16.50±0.00 ^a
Left Testis Weight (g) (LTW)	38.45±1.25 ^a	21.20±7.20 ^a	27.59±0.00 ^a
Right Testis Weight(g) (RTW)	39.05±0.35 ^a	20.55±6.65 ^a	31.50±0.00 ^a
Left Testis length (cm)(LTL)	15.18±0.78 ^a	17.15±6.05 ^a	14.77±0.00 ^a
Right testis length (cm)(RTL)	13.70±0.78 ^a	17.15±6.05 ^a	14.77±0.00 ^a
Left testis diameter (cm)(LTD)	7.40±3.10 ^a	3.35±1.15 ^a	4.00±0.00 ^a
Right testis diameter (cm)(RTD)	11.95±2.55 ^a	4.20±0.70 ^a	6.62±0.00 ^a
Right Epididymis weight (g)(REW)	8.40±0.50 ^a	4.00±0.70 ^a	6.62±0.00 ^a
Left Epididymis weight (g)(LEW)	7.80±0.10 ^a	4.65±0.85 ^a	5.60±0.00 ^a
Body weight (kg)(BW)	8.00±0.00 ^a	9.50±2.50 ^a	15.50±1.50 ^a

Values are reported as mean±SEM. ^{abc}: Means in the same row with different superscript differ significantly ($P<0.05$).

Semen Characteristics

The treatment caused a decrease ($P>0.05$) in the sperm motility and livability at day 7 post-treatment which later increased on day 14 post-treatment. However, there was a progressive significant decrease ($P<0.05$) in the semen volume across the groups at day 7 and day 14 post-treatment. The treatment increased the sperm count at day 7 post-treatment with a decrease ($P>0.05$) at day 14 post-treatment (Figures 1-4).

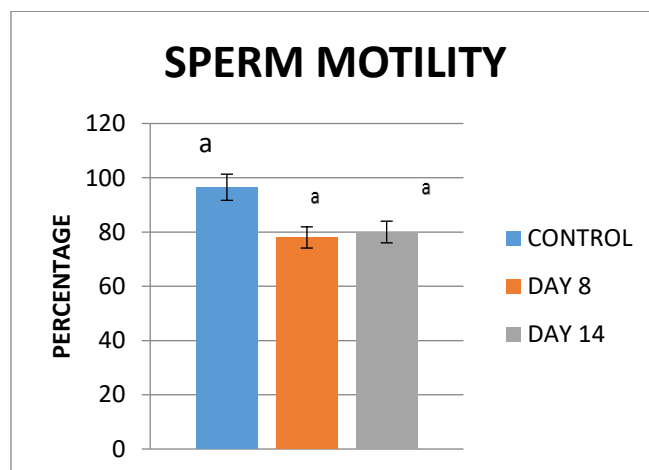


Fig 1: Comparison of percentage mean values (+SEM) sperm motility across the groups. Same superscript means there is no significant difference ($P>0.05$)

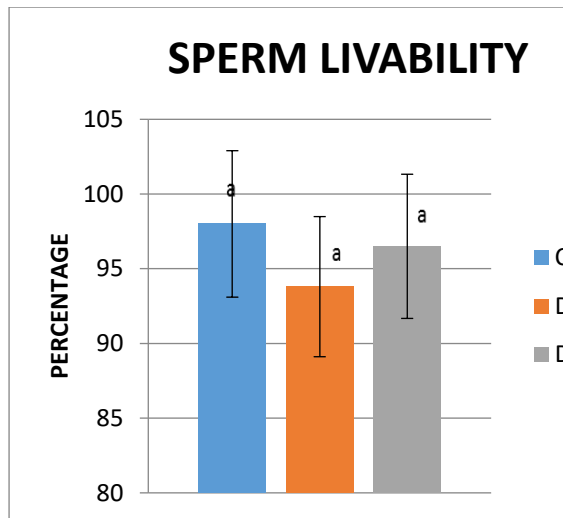


Fig 2: Comparison of percentage mean values (+SEM) of livability across the groups. Same superscript means there is no significant difference ($P>0.05$)

(+SEM) of semen volume across the groups. ^{abc}: Means in the same row with different superscript differ significantly ($P<0.05$).

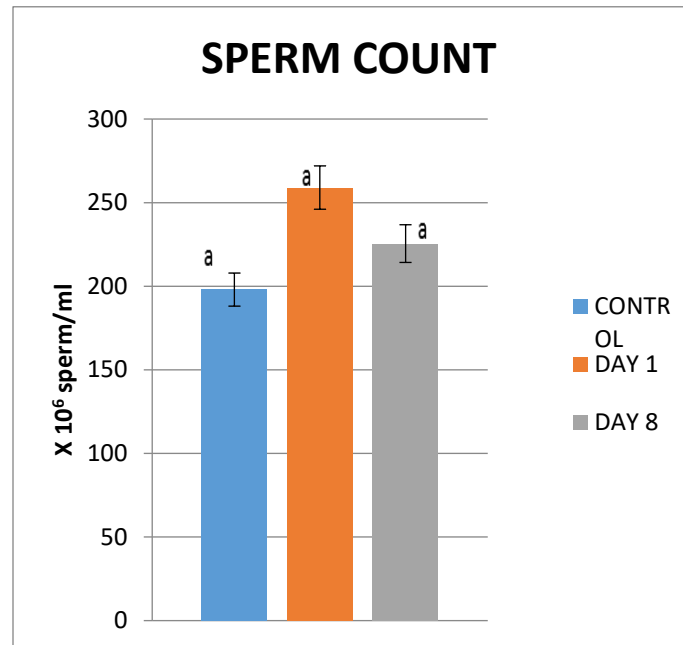


Fig 4: Comparison of percentage mean values (+SEM) of sperm count across the groups. Same superscript means there is no significant difference ($P>0.05$)

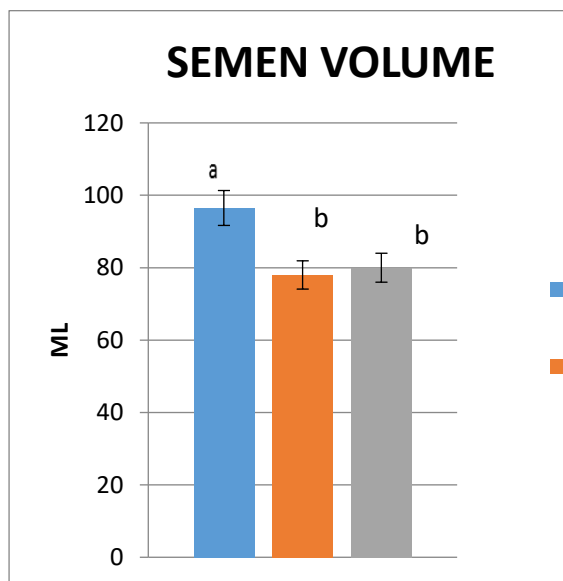


Fig 3: Comparison of percentage mean values

Sperm Morphological Abnormalities

The treatment caused a progressive decrease across the groups in the abnormal sperms with bent tails, abnormal mid-piece, tailless head (Normal head without tail) and Total abnormal sperm cells when compared with the control. The differences in value were significant ($P<0.05$) for abnormal midpiece and total abnormal sperm cells. However, the number of sperm cells with rudimentary tail and

headless tail (Normal tail without the head) increased at day 7 post-treatment and later decreased at day 14 post-treatment compared to the control. The changes were significant ($P<0.05$) for headless tail (Normal tail without head). The number of looped/curved tail and total cells decreased at day 7 post treatment and later increased at day 14 post-treatment compared to the control. The values were significant ($P<0.05$) for looped/curved tail

only. The number of coiled tail and normal cells increased at day 7 post-treatment and further increased at day 14 post-treatment

compared to the control. The values were not significant ($P > 0.05$) (Table IV).

Table IV: Sperm Morphological Abnormalities of WAD Bucks Treated with Aqueous Extract of *Anacardium occidentale* at Days 7 and 14 Post-Treatment

PARAMETERS (%)	CONTROL	DAY 7	DAY 14
Bent tail	2.38±0.00 ^a	2.07±0.14 ^a	1.80±0.27 ^a
Looped/Curved tail	3.25±0.00 ^a	0.58±0.08 ^b	0.67±0.06 ^c
Coiled tail	0.00±0.00 ^a	0.46±0.03 ^a	0.49±0.09 ^a
Abnormal midpiece	4.76±0.00 ^a	2.31±0.09 ^b	1.82±0.34 ^c
Rudimentary Tail	0.43±0.00 ^a	0.46±0.08 ^a	0.40±0.06 ^a
Headless tail	1.50±0.00 ^a	2.02±0.15 ^b	1.71±0.18 ^c
Tailless head	2.38±0.00 ^a	2.02±0.22 ^a	1.74±0.26 ^a
Total abnormal cells	14.71±0.00 ^a	9.94±0.59 ^b	8.62±1.10 ^c
Total normal cells	85.29±0.00 ^a	90.07±0.59 ^a	93.13±2.45 ^a
Total cells	462.00±0.00 ^a	345.00±24.20 ^a	372.50±29.19 ^a

Values are reported as mean±SEM

^{abc}: Means in the same row with different superscript differ significantly ($P < 0.05$).

Table 5: Histomorphometrical Assessment of the Testes and Epididymis of WAD Bucks Treated with Aqueous Extract of *Anacardium occidentale* at Days 7 and 14 Post-Treatment

PARAMETERS	CONTROL	DAY 7	DAY 14
Testicular Germinal height (µm)	677.75±31.00 ^a	485.29±33.92 ^b	463.20±19.90 ^c
Testicular Luminal Diameter (µm)	2252.77±140.68 ^a	2512.13±156.79 ^a	2018.83±151.80 ^a
Epididymal Luminal Diameter (µm)	4466.22±545.79 ^a	5258.76±570.24 ^b	3314.24±149.80 ^c
Epididymal Germinal height(µm)	715.62±48.81 ^a	381.64±27.47 ^b	338.34±16.70 ^c

Values are reported as Mean±SEM. ^{abc}: Means in the same row with different superscript differ significantly ($P < 0.05$)

Histomorphometrical Assessment

The treatment caused a significant progressive decrease ($P < 0.05$) in the testicular germinal height and epididymal germinal height across the days post-treatment compared to control. However, there was an increase in testicular luminal diameter and epididymal luminal diameter at day 7 post-treatment which later decreased at day 14 post-treatment. The differences were significant ($P < 0.05$) for epididymal luminal diameter (Table V).

DISCUSSION

Haematological analysis in this study showed that treatment of West African Dwarf Bucks (WADB) with aqueous leaf extract of *Anacardium occidentale* (Cashew Leaf aqueous extract) for 14 days

caused a decrease in PCV, Hb, RBC, Platelets, WBC, Lymphocytes values at day 7 post-treatment. There was a significant decrease in RBC and lymphocytes only. The significant decrease in RBC on day 7 could have been as a result of the Cashew leaf extract administered, this is because the prolonged use of the leaf extract of *Anacardium occidentale* which contains tannins and polyphenols with other micronutrients sometimes cause haemolysis of the red blood cells and also interfere with the iron absorption thereby influencing red blood cell production which supports the findings of Offor *et al.* (2014). The increase in blood parameters observed at day 14 post-treatment is a reflection of a

recovery process in the treated goats following possible elimination of the extract from their body. The significant decrease in lymphocytes may be attributed to physiological stress response arising from the animal's social behaviour which consists of aggressiveness and hierarchical fights. (Zapata *et al.*, 2003) noted that physiological stress response is accompanied by increase lymphopenia.

However, increased values of Neutrophils observed at day 7 on exposure to aqueous leaf extract of *Anacardium occidentale* implies that an increase in neutrophils is associated with a decrease in lymphocytes and vice versa (Lazzaro, 2001) and also an increase in MCV observed at day 7 following exposure to aqueous leaf extract of *Anacardium occidentale* agrees with (Ikhimiya *et al.*, 2007) which could have resulted from the release of immature red blood cells into circulation (Ikhimiya *et al.*, 2007). This observation was further strengthened as MCV values reduced at Day 14 post-treatment.

The mild decrease in Serum Albumin, Total Protein, Globulin, Triglycerides, AST, ALT value with a significant decrease in total protein and Albumin at day 7 post-treatment agrees with a similar study by Ogunleke *et al.*, (2014). The marked decrease in total protein and albumin in this study indicated that there were no traces of anti-nutritional factors that could diminish nutrient permeability in the gut walls (Ogunleke *et al.*, 2014).

The marked increase in BUN may be attributed to excessive tissues protein catabolism and possible elevated level of ammonia resulting from high absorption of ammonia from the rumen to the blood (Amuda, 2018).

Observations from the biometrical assessment of the testes and epididymis of WADB treated with aqueous leaf extract of *Anacardium occidentale* showed that the extract has a mild toxic effect on the testes and epididymis. The aqueous leaf extract caused a mild reduction in the scrotal circumference, testicular and

epididymal weights and diameters. Scrotal circumference, testicular and epididymal diameters in goats are direct correlate of semen volume and sperm count (Rege *et al.*, (2000). A decrease in these reproductive organs might be attributed to a possible toxic effect of the plant.

The decrease in sperm motility in the current study at day 7 post-treatment with cashew leaf extract and its subsequent increase might be attributed to a transient shock on the goat following treatment. A previous study by Mukhtar *et al.* (2011) suggests that sperm motility might be transiently affected following sudden administration of plant extracts. This is also corroborated by an earlier study by Oyeyemi *et al.* (2008) in which the spermatozoa motility of rats treated with the aqueous leaf extract of *Telfaria occidentalis* was decreased.

The decrease in sperm liveability from this study at day 7 post-treatment ($p > 0.05$) validates the findings of Oyeyemi *et al.* (2007) where there was no statistically significant difference in the percentage liveability of rats treated with the aqueous leaf extract of *Vernonia amygdalina del. Asteraeaceae.* The author opined that the decreased sperm liveability could have resulted from the initial reaction to the treatment or semen handling. The progressive significant decrease in the semen volume and fluctuations in sperm motility and liveability will compromise the fertility of the rats as these are important parameters in the determination of viability and fertility in male animals (Oyeyemi *et al.*, 2009)

Also, marked decrease in semen volume across post-treatment days could further explain the adverse effect of the extract which relates to the decreased scrotal circumference, testicular and epididymal diameters observed in this study. However, these biological reductions of the testes and epididymis might explain the high sperm count at day 7 post-treatment which later decreased at day 14 post-treatment.

Meanwhile, a reduction in the total number of morphologically abnormal sperm cells in the current study following exposure to aqueous leaf extract of *Anacardium occidentale* showed the extract is not overtly toxic on sperm cells and does not interfere with the structural integrity of spermatozoa. However, the marked increase in numbers of sperm cells with tail abnormalities might account for the low motility grading following exposure of WADB to the aqueous leaf extract of *Anacardium occidentale*.

Histomorphometrical observations of the testes and epididymis in this current study also correlate directly with the semen characteristic and the biometrical analysis. The increased testicular and epididymal lumen might account for the increased sperm count at day 7 post-treatment. However, a mild reduction of the germinal epithelial height was observed in the testes and epididymis which further confirms that aqueous leaf extract of *Anacardium occidentale* might be repro-toxic if not used with caution.

Histomorphometrical observations from this study were at variance with Jahan *et al.*, (2009) who worked on the histomorphological study to evaluate the anti-fertility effect of *Abrus precatorius L.* in adult male mice where the testicular tubular diameter reduced significantly in treated mice compared to the control. In this study, there is a negative correlation obtained among the seminiferous tubular diameter, germinal epithelial height, epididymal germinal height and Epididymal luminal diameter of the testes and epididymis among the treatment groups. This suggests that an increase in the diameter of the seminiferous tubules also yielded a corresponding decrease in the germinal epithelium of the testis which reduces the spermatogenesis but increases accommodation of more sperm cells in the lumen of the seminiferous tubule as against Olukole and Obayemi, (2010) which enhances spermatogenesis and

accommodation of more sperm cells in the lumen of the seminiferous tubule.

The negative correlation obtained between the tubular height and the luminal diameter of the epididymis in this study, suggests that a decrease in the tubular height produced a corresponding increase in the size of the lumen of the epididymis, thus allowing storage of more sperm cells which agrees with the findings of Olukole and Obayemi, (2010).

CONCLUSION

It was concluded from this study that the treatment of West African Dwarf bucks with aqueous leaf extract of *Anacardium occidentale* at 250mg/kg for 14 days resulted in a significant decrease in RBC count, total protein and albumin level, semen volume and sperm motility. It also caused a reduction in the total number of morphologically abnormal sperm cells and a mild reduction in scrotal circumference, testicular and epididymal weight and diameter. It caused an increase in the size of the epididymal and testicular germinal luminal diameter and a decrease in epididymal and testicular germinal height. These suggested that its prolonged use in West African Dwarf breeding bucks may produce an anti-fertility effect. Therefore, its incorporation into animal feeds must be done with caution.

Conflicts of Interest

The authors declare no conflict of interest.

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