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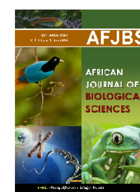

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Effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the reproductive system of male *Mastomys natalensis*: An Anti-fertility studies

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Abstract

The contraceptive potential of *Acacia nilotica* pods and *Albizzia lebbeck* stem bark methanolic extracts were evaluated in male rodent pests. Ninety (90) multimammate rats (*M. natalensis*) were randomized into a 3 × 3 factorial design for treatment groups (control, *A. lebbeck* and *A. nilotica*) (n = 10) and treatment durations (15, 30, or 60 days). Control rats consumed plain feed. Treated rats consumed feed with 2% w/w of either of the plant extract. Following treatment, male rats were mated to untreated females before they were sedated in ether and humanely sacrificed. Assessments were done on fertility success rates (number of impregnated females), weight of testes and reproductive glands, sperm cell parameters, and testes histopathology. Fertility success rate was reduced to 0% in the *A. nilotica* treated rats at all the treatment durations and in the *A. lebbeck* treated rats after 60 days of treatment. Also, the extract-treated rats revealed a significant reduction in the testes, seminal vesicles, and epididymides weights compared to the control group. Moreover, sperm cell density and the proportions of live and progressively motile spermatozoa were significantly reduced and there were numerous damaged seminiferous tubules reflected by sloughed off germ cells, thinned germinal epithelium and widened empty lumen in the extract-treated rats. Thus, treatment with *A. nilotica* or *A. lebbeck* extract in male *M. natalensis* reduced their fertility success rates through distortion of testicular structure and disruption of spermatogenesis.

Keywords: *Seminiferous tubule, Multimammate rat, Sperms parameters, Test feed, Plant extract*

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1. Introduction

The multimammate rat (*Mastomys natalensis*) is a small-sized rodent species of the Muridae family and genus *Mastomys*. The rodent species is very rampant in sub-Saharan Africa (Colangelo *et al*., 2013). The rodent pest's ability to reproduce in large numbers and spread very fast has been a significant threat to the economy and

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public health in sub-Saharan Africa (Mulungu *et al.*, 2010; and 2013). During population outbreaks, the *M. natalensis* has been competing greatly for grain crops with human beings (Mdangi *et al.*, 2013; and Mwanjabe *et al.*, 2002) besides their involvement in the transmission of some deadly zoonotic disease pathogens (Borremans *et al.*, 2015). As a result, anticoagulant rodenticides have been heavily employed to mitigate the rodent populations (Mulungu *et al.*, 2010; and 2013). However, despite being effective in rodent pest management, rodenticides are hazardous to the health of other untargeted species of animals (Brakes and Smith, 2005; and Elliott *et al.*, 2016). Also, rodent pests are increasingly becoming rodenticide resistant by avoiding consuming familiar poisoned baits ("bait shyness") (Saxena, 2014) or resisting being killed by the consumed poisons (Marquez *et al.*, 2019). Furthermore, some anticoagulant rodenticides cause too much suffering to rodents before their death, contravening the animal welfare requirements (Fisher *et al.*, 2019). Therefore, it is increasingly important to consider exploring new methods of rodent control that are both effective and less harmful to the animals and the environment. In theory, the birth control methods (contraceptives) normally used by human beings could be among the best alternative approaches for rodent fertility and population control. The philosophy/idea is, by reducing the reproductive rate of rodent pests, their population, as well as the use of rodenticides in the field will decrease. Already, several research efforts have been in line to explore the use of synthetic steroid hormones (Massawe *et al.*, 2018) and immune-contraceptives (Massei *et al.*, 2020; and Cooper and Larsen, 2006) as a means to control the fertility of rodent pests. Unfortunately, none of the approaches have been applied in the real field situation of rodent management. Medicinal plants with anti-fertility properties could probably be the best potential alternative source of contraceptives for rodent pests. There are several documented reports revealing the anti-fertility actions of various plant species in small mammals (Devi *et al.*, 2015). In male mammals, the plant's extract anti-fertility actions seem to involve disruption of the hypothalamic-pituitary-testis hormonal axis (Gupta *et al.*, 2002; and Asuquo, 2012) and induction of morphological and functional disorganization in the testes and accessory reproductive glands (Asuquo, 2012; and Lampiao, 2013).

Albizzia lebbeck and *Acacia nilotica* are among the medicinal plant species with some anti-fertility properties (Gupta *et al.*, 2006; and Lampiao, 2013). *A. lebbeck* is a legume tree of the Fabaceae family, native to Asia, Northern Australia but also available in Africa. It is a tree of 3-30 m in height and 30 m in diameter of the crown with long flat and brownish pods when dry (Orwa *et al.*, 2009; and Verma *et al.*, 2013). Studies by Gupta *et al.* (2004 and 2006) revealed a significant decrease in the weights of gonads and sex glands in rats under the *Albizzia lebbeck* pod extract treatment. It was also revealed by Gupta *et al.* (2004) that the extract of *A. lebbeck* pods brought about a significant decrease of sperm motility and density and a number of germ cells in the gonads of Wistar rats. Further evidence by Chaudhary *et al.* (2007), revealed that triterpenes isolated from *Albizzia lebbeck* (L.) Benth pods were efficacious enough to inhibit spermatogenesis after being treated orally in male Wister rats.

A. nilotica is a flowering perennial, bushy and spiny tree of the Fabaceae family found in the tropical and subtropical regions worldwide (Verma, 2016). *A. nilotica* is a popular medicinal plant due to its anti-microbial, anti-plasmodial and antioxidant activity and its use for the treatment of human immunodeficiency virus, hepatitis C virus and cancer (Ali *et al.*, 2012). In addition to that *A. nilotica* has some anti-fertility properties as revealed in the study by Lampiao (2013). However, whether the crude extracts of *A. lebbeck* stem barks and those of *A. nilotica* pods could be efficacious enough to reduce the fertility of the highly prolific rodent pests more particularly the *M. natalensis* need to be investigated. Therefore, the current study was carried out to assess the efficacies of the *A. lebbeck* (stem barks) and *A. nilotica* (pods) crude extracts on the fertility success rate, sperm cell parameters, and histopathology of gonads in the sexually matured *M. natalensis* male rats.

2. Methodology

2.1. Study area

Acacia nilotica pods were collected from Nangurukuru in Kilwa, Lindi Region in southeastern Tanzania (8° 56′0″S, 39°30′45″E), whereas stem barks of *A. lebbeck* were collected from trees around the Sokoine University of Agriculture (SUA), the main campus in Morogoro Urban, Tanzania (6° 50′42.66″S, 37°39′29.14″E). Authentication of the two trees was done by a botanist from the Department of Ecosystems and Conservation, SUA. Processing of the plants for phytochemical analysis was carried out at the Toxicology laboratory in the College of Veterinary Medicine and Biomedical Sciences (CVMBS) at SUA.

2.2. Plants collection and processing

A total of 10 kg each of *A. lebbeck* stem barks and *A. nilotica* pods, were collected in August and September and were transported in bags to the laboratory for drying under the shed until they were brittle. The plant extraction processes were done as per Mwangengwa *et al.* (2021) with some modifications to the solvent used. The dried plant materials were ground into powder form by an electric grinder. Then 500 g powdered materials of either *A. nilotica* or *A. lebbeck* were added in a conical flask containing 1.5 L of 70% methanol. After 72 h of extraction, the mixture was filtered using gauze and cotton wool. Thereafter, methanol was removed from the filtrate using a vacuumed rotor vapor. The filtrates were then concentrated to solid mass in a water bath at 46 ^{0}C.

2.3. Experimental animals

The current study employed the *Mastomys natalensis* rat species as the experimental animals. The rodent pests were collected from the SUA farms by using Sherman LFA aluminum traps. Out of the 220 collected *Mastomys natalensis* male rats, 90 of them were selected for experiments. The selected rats were housed singly in plastic cages and left to adapt to the standard conditions of 12/12 light-dark cycle, 25 ± 5*°C*, and 35%-60% relative humidity for two weeks while feeding on broiler finisher and *adlib* water. The criterion for inclusion and exclusion of the rats in the experiments was a sound body condition and the bodyweight category of 25-50 g reflecting sexual maturity (Lalis *et al.*, 2006). The Tanzanian Commission for Science and Technology (COSTECH) granted the ethical research permit (Permit No 2019-225-NA-2019-47) before the study's commencement.

2.4. Preparation of the plain and extract containing test feed

The food used to incorporate the plant crude extract was prepared according to Massawe *et al.* (2018) as modified slightly by Mwangengwa *et al.* (2021). Just briefly a stiff porridge was prepared by boiling and stirring a mixture of 10 kg of maize flour, 6 kg of roughly crushed maize, 250 g of fish meal, and 1 kg of cane sugar for 20 min. A standard pelletizer machine (KENWOOD, type MG51, designed at Hampshire, PO 9NH in UK, made in China) was employed to process a portion of the stiff porridge mixture to prepare the basal feed pellets. The remaining stiff porridge mixture was spiked with the extract of either *A. nilotica* pods or *A. lebbeck* stem barks before pelleting. Spiking was done in such a way that the treated feed contained 2% (w/w) of either of the two crude extracts. The 2% w/w was equivalent to 0.2 g (200 mg) of the extract added to 9.8 g (9,800 mg) of feed making 10 g (10,000 mg) of the feed extract mixture. The assumption was that rats may consume up to 10 g/100 g body weight per meal per day (Krishnakumari *et al.*, 1979). A dosage of 200 mg of the extract used in the current study considered the dosage range of 100 to 200 mg/ kg body weight of rats which have been used in other anti-fertility studies involving *A. lebbeck* (Gupta *et al.*, 2004; and 2006) and *A. nilotica* (Lampiao 2013).

2.5. Experiment setup and treatments

A total of 90 male rats were stratified on a bodyweight basis and randomized into a 3x3 factorial study design, as shown in Table 1. Treatment was done in parallel for 15 (to assess immediate effects), 30 (intermediate effects), and 60 days (to evaluate for potential chronic effects). The amount of test feed supplied daily for each rat was 10 g, assuming that rats consume up to 10g/100g bodyweight meals per day (Krishnakumari *et al.*, 1979).

Table 1: Experimental design. To evaluate the contraceptive potential of *A. nilotica* pods and *A. lebbeck* stem bark methanolic extracts in the male *M. natalensis*

Group (*n* = 10)	Treatment allocation	Trial duration
Control (C)	10 g of plain pellets per rat/day	15 (days)
A. lebbeck (AL)	10 g of pellets with 2% of *A. lebbeck* stem barks extract per rat/day	
A. nilotica (AN)	10 g of pellets with 2% of *A. nilotica* pods extract per rat/day	
Control (C)	10 g of plain pellets per rat/day	30 (days)
A. lebbeck (AL)	10 g of pellets with 2% of *A. lebbeck* stem barks extract per rat/day	

Table 1 (Cont.)

Group (n = 10)	Treatment allocation	Trial duration
A. nilotica (AN)	10 g of pellets with 2% of *A. nilotica* pods extract per rat/day	
Control (C)	10 g of plain pellets per rat/day	60 (days)
A. lebbeck (AL)	10 g of pellets with 2% of *A. lebbeck* stem barks extract per rat/day	
A. nilotica (AN)	10 g of pellets with 2% of *A. nilotica* pods extract per rat/day	

2.6. Feed consumption and body weight

Feed intake was measured daily for each rat by subtracting the amount of feed that remained in the pots from the previously supplied amount (10 g for each rat). Feed consumption was calculated as daily food intake (g) divided by the bodyweight (g) of each rat. Bodyweight was also recorded by a digital weighing balance and was used to calculate the weight indices of the male rat's reproductive tissues.

2.7. Assessment of fertility success rate

During fertility assessment, the untreated female rats were captured from the SUA farms. After two weeks of adaptation, those with a normal oestrous cycle, proven by examining the vaginal smears (Marcondes *et al.*, 2002), were selected for mating with the treated male rats. The mating exercise was done at the end of treatment durations by introducing female rats into the male's cages at a ratio of 1:1 for a male to a female. The period for cohabitation was 16 days targeting two possible oestrous cycles of female *M. natalensis* (Johnston and Oliff, 2010). During cohabitation, the mating pairs were maintained on the broiler finisher pellets. Successful matting was assessed by examining the vagina smears once daily early in the morning for each female rat. The presence of sperm cells in the vaginal smear was considered as day 0 of pregnancy.

Female rats from the cohabitants were maintained on the broiler finisher pellets while regularly assessed for pregnancy status by palpation of the lower abdomen. On day 20, from the first day of cohabitation, female rats were sedated in ether and then sacrificed to assess pregnancy status. Fertility successes were defined as the ability of the male to impregnate female rats (Sakpa and Popoola, 2018) and therefore, the male fertility success rate (fertility percentages) was calculated as follows:

Male fertility success rate = (number of pregnant females ÷ mated female's number) × 100

2.8. Weight indices of the reproductive tissues and glands

After the treatment durations of 15, 30, or 60 days, all the male rats were removed from the cages, sedated in ether, and then were sacrificed by opening the abdominal cavity with a pair of scissors to expose reproductive organs and glands. The testes, epididymides, prostate glands, and seminal vesicles from all rats' groups were dissected out and their weights were recorded. The values of testes, epididymides, prostate glands, and seminal vesicle weights were then divided by the bodyweight of rats recorded just before tissue collection to calculate the weight indices of the organs.

2.9. Sperm cell parameters

After 16 days of cohabitation, all the male rats were removed from the cages, sedated in ether, and were humanely sacrificed. The rat's testes' caudal epididymides were then dissected out and homogenized into 1 mL of warm (37 °C) normal saline in a small beaker using a dissection scissor to make a sperm cell suspension. For sperm cell motility assessment, one drop of a sperm suspension was placed on a slide, covered with a slip. Digital video clips showing sperm cell's motions were recorded from four different fields of microscopic slide spending 10 seconds of focus on each field using the camera-mounted microscope (Olympus BH-2 camera in motivation pro205A) at ×400 magnification (Dostal *et al.*, 1996). The status of sperm cell motions was then analyzed manually within 5minutes from the time of sperm cells collection. The proportion of progressively motile sperm cells were then calculated out of the total number of at least 200 sperm cells counted per slide (Dostal *et al.*, 1996). Sperm cell viability was determined using a staining solution composed of nigrosin and

eosin (Lucio *et al.*, 2013). One drop of the sperm cell suspension was added on a microscopic slide followed by one drop of the staining solution and mixed with a toothpick before being examined with a bright field microscope at ×200 magnification. The proportion of live sperm cells with white to the pinkish head or dead ones with the reddish head was calculated out of total sperm cells (200) counted from five different fields (Lucio *et al.*, 2013). Sperm cells count for density or concentration assessments was done as described by Uslu *et al.* (2012). A total of 10 μl of sperm suspension was diluted at 1:100 with 90 μl of a fixative/staining solution (containing 5 g sodium bicarbonate, 1 ml of 35% formalin, and 25 mg eosin in a volumetric flask and made up to a final volume of 1 L with distilled water). After vigorous shaking, 10 μl of the diluted semen was placed in a Neubauer hemocytometer to count sperm cells at 100 × magnification. Sperm cell count per mL was calculated as total count in 5 center squares × dilution factor × 10,000.

2.10. Histometric and histopathological assessments of seminiferous tubule

The testes of both the extract-treated and control rats were dissected out, weighed, and immediately fixed in Bouin fluids for 24 h and then in absolute ethanol until processing time (Howroy *et al.*, 2005). The testes were then dehydrated in graded ethanol, cleared in chloroform, embedded in paraffin wax, and sectioned into 4 μm sections for staining with haematoxyline-eosin. The specimens were then examined at ×100 and ×400 magnification to assess the integrity of seminiferous tubules. The eyepiece with calibrated micrometer was also employed to measure the lumen and germinal epithelial sizes of at least 100 seminiferous tubules for each testicular section. The measurement was done at ×100 magnification by focusing the eyepiece's micrometer on each seminiferous tubule while moving the objective straight from one end to the other at the center, lower and upper field of the testicular tissue section on a microscopic slide.

3. Data analysis

The analytical software, IBM SPSS Statistics 20, was used for data analysis. Bait intake was analyzed using repeated measure analysis. The factorial ANOVA in multivariate analysis was employed to analyze the weight indices of reproductive organs, the sizes of germinal epithelium and lumen of seminiferous tubules, and concentration of sperm cells as dependent variables while treatment groups and treatment duration served as the in-between subject factors or independent variables. Pairwise comparisons between groups used the Bonferroni test for repeated measures analysis and the Tukey test for multivariate analysis. A chi-squared test was employed to analyze the fertility success rate, and the proportion of normal morphology, progressively motile and viable spermatozoa. Pearson correlation test was employed to test the correlation between dependent variables' values against the treatment durations. All values were regarded as statistically significant at $p < 0.05$ and $p < 0.01$ and statistically highly significant at $p < 0.001$.

4. Results

4.1. Feed intake

Rats exposed to test feed with *A. lebbeck* stem barks and *A. nilotica* pods extracts for 15 and 30 days had a higher ($p < 0.05$) intake compared to their control counterpart rats which fed on the plain test feed. However, in the 60 days treated groups of rats, the intake of test feed was the same ($p > 0.05$) for rats feeding on the extract containing test feed and the rats feeding on the plain test feed (Table 2).

Table 2: Recorded intake of the plain feed and the *A. lebbeck* and *A. nilotica* extracts incorporated feed by the male Maltimammate rats (*M. natalensis*)

Treatment time (Days)	Feed intake control group (g)/g Bw,	Feed intake *A.lebbeck* group (g)/g Bw	Feed intake *A. nilotica* group (g)/g Bw
15	0.107 ± 0.011^{a}	0.139 ± 0.009^{b}	0.137 ± 0.016^{abc}
30	0.149 ± 0.013^{a}	0.163 ± 0.02^{a}	0.153 ± 0.016^{a}
60	0.136 ± 0.012^{a}	0.142 ± 0.011^{a}	0.171 ± 0.018^{a}

Note: Raw-wide, different superscripts indicate a statistically significant difference between the means at $p < 0.05$.

4.2. Weight indices of the reproductive organs

Weight indices of prostate glands of the *A. lebbeck* stem bark and *A. nilotica* pods extract-treated rats did not vary significantly ($p > 0.05$) from that of the control counterparts at all the treatment durations (Table 3).

Table 3: Effect *A. lebbeck* and *A. nilotica* extracts on the weight indices of reproductive organs in the *M. natalensis* male rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract.

Treatment duration	Treatment groups	Testis (mg/g bwt)	Prostate glands (mg/g bwt)	Seminal vesicles (mg/g bwt)	Epididymides (mg/g bwt)
15 (days)	C	13.41 ± 0.69^{a}	1.78 ± 0.57^{a}	9.72 ± 1.24^{a}	6.36 ± 1.01^{a}
	AL	8.73 ± 1.66^{b}	1.74 ± 0.24^{a}	7.27 ± 0.68^{a}	6.75 ± 0.70^{a}
	AN	10.34 ± 1.55^{abc}	2.02 ± 0.15^{a}	6.87 ± 1.44^{a}	6.61 ± 1.06^{a}
30 (days)	C	13.17 ± 1.91^{a}	1.63 ± 0.28^{a}	10.33 ± 1.18^{a}	9.11 ± 2.55^{a}
	AL	9.42 ± 1.49^{b}	2.05 ± 1.72^{a}	9.51 ± 1.13^{ab}	6.35 ± 1.58^{ab}
	AN	11.44 ± 1.88^{abc}	1.63 ± 0.21^{a}	6.67 ± 0.26^{bc}	4.94 ± 1.13^{bc}
60 (days)	C	15.31 ± 2.33^{a}	2.10 ± 0.26^{a}	14.99 ± 2.08^{a}	16.89 ± 2.69^{a}
	AL	8.31 ± 1.23^{b}	1.79 ± 0.23^{a}	9.08 ± 0.86^{b}	6.74 ± 0.79^{b}
	AN	13.41 ± 1.03^{abc}	2.35 ± 0.31^{a}	7.31 ± 1.19^{bc}	5.76 ± 0.73^{bc}

Note: Column-wide, different superscripts indicate a statistically significant difference between the means at $p < 0.05$.

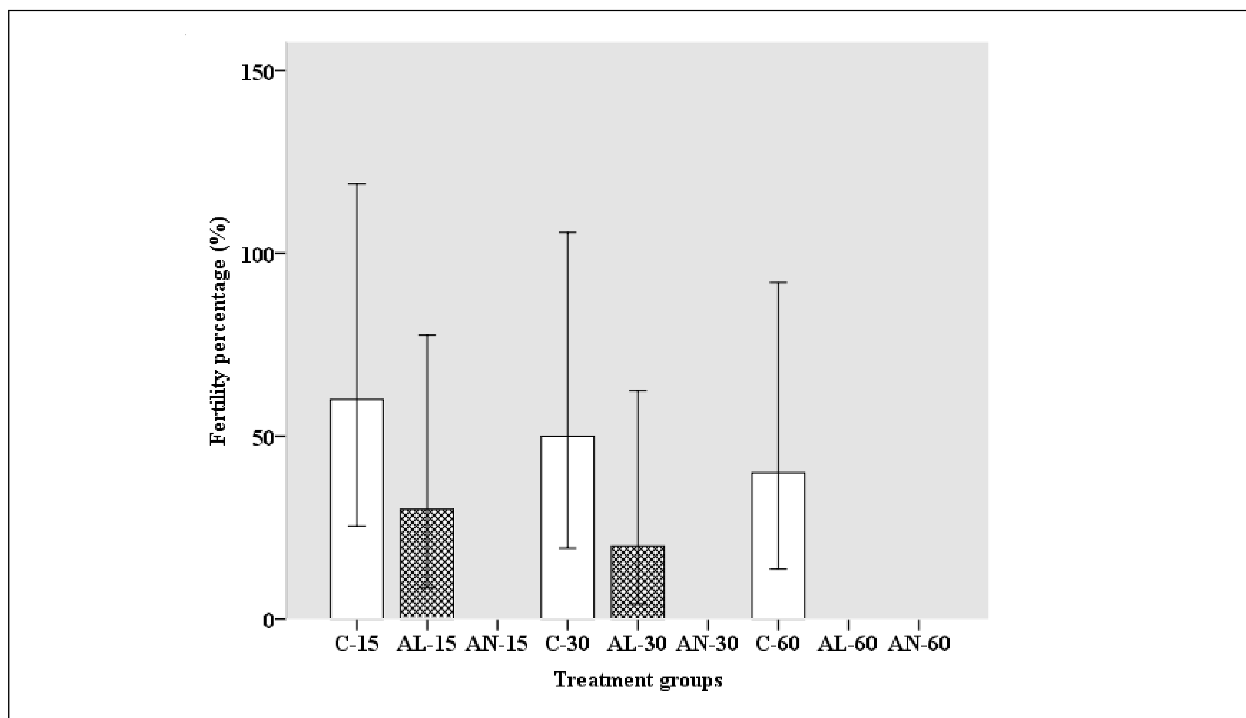

Figure 1: Effect of crude plant extracts in feed on fertility success rate (fertility percentage) following its treatment for 15, 30, or 60 days in the *M. natalensis* male rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extracts, AN = *A. nilotica* pods methanolic extracts.

However, it was revealed by the factorial ANOVA that the values of testes, seminal vesicles, and epididymides weights (mg/g body weight) differed significantly among the treatment groups ($p < 0.01$). Moreover, the weight of the testes (mg/g body weight) was significantly reduced in rats exposed to the extract of *A. lebbeck* stem bark extract and was only slightly reduced ($p > 0.05$) in rats under the *A. nilotica* pod extract treatment compared to the control (Tab 3). The results indicated further that treatments made for 15 days resulted in non-significant differences in the epididymides and seminal vesicle weights of rats in both the *A. nilotica* or *A. lebbeck* extract-treated groups relative to the control rats ($p > 0.05$). However, the treatment duration of 30 or 60 days resulted in a significant reduction of the epididymides and seminal vesicle weights ($p < 0.01$) in the *A. nilotica* pods and *A. lebbeck* extracts treated rats relative to the control. The Pearson correlation test indicated a lack of correlation between treatment duration and the resulting weights (mg/g body weight) of testes ($r = 0.27$, $p > 0.05$), prostate glands ($r = 0.239$, $p > 0.05$), seminal vesicles ($r = 0.06$, $p > 0.05$) and epididymides ($r = -0.07$, $p > 0.05$) in the *A. nilotica* pods extract-treated group of rats. Likewise, no correlation existed between treatment durations and the resulting weights (mg/g body weight) of testes ($r = -0.06$, $p > 0.05$), prostate glands ($r = -0.01$, $p > 0.05$), seminal vesicles ($r = 0.21$, $p > 0.05$) and epididymides ($r = 0.02$, $p > 0.05$), in the *A. lebbeck* stem barks extract-treated groups of rats.

4.3. Sperm cell parameters

The proportion of progressively motile sperm cells (Figure 2), viable spermatozoa (Figure 3) and the concentration or density of sperm cells (Figure 4) were decreased significantly ($p < 0.05$) in rats under the *A. nilotica* pods and *A. lebbeck* stem bark extracts treatments compared to the control groups of rats at all the treatment durations of 15, 30, and 60 days. Comparison among the extract-treated rat groups indicated that the progressively motile sperm cells were significantly more reduced ($p < 0.05$) in the *A. nilotica* pods extract-treated rats compared to rats under the *A. lebbeck* stem bark extract treatment (Figure 2). Also, the proportion of viable or live sperm cells was significantly ($p < 0.05$) more reduced in the *A. nilotica* than in the *A. lebbeck* treated at all the treatment durations except in the 60 days treated group where the proportions were comparable (Figure 3). However, the concentration or density of spermatozoa did not vary significantly ($p > 0.05$) between the *A. nilotica* and *A. lebbeck* extracts treated rats.

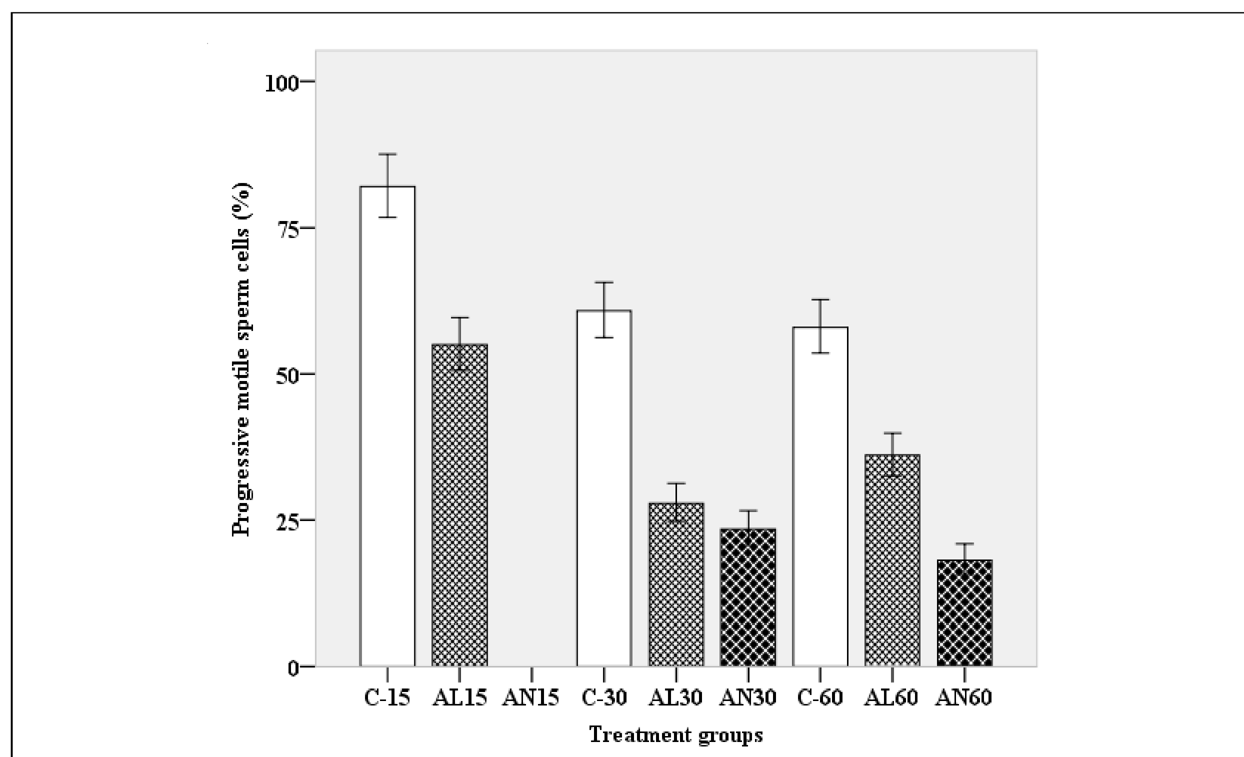

Figure 2: Effect of crude plant extract in feed on the percentages of progressively motile sperm cells following its treatment for 15, 30 or 60 days in the *M. natalensis* male rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extracts, AN = *A. nilotica* pods methanolic extracts. Pearson chi-squared test; treatment groups ($\chi^2 = 453.98$, df = 2, $p < 0.001$), treatment duration ($\chi^2 = 157.30$, df = 2, $p < 0.001$).

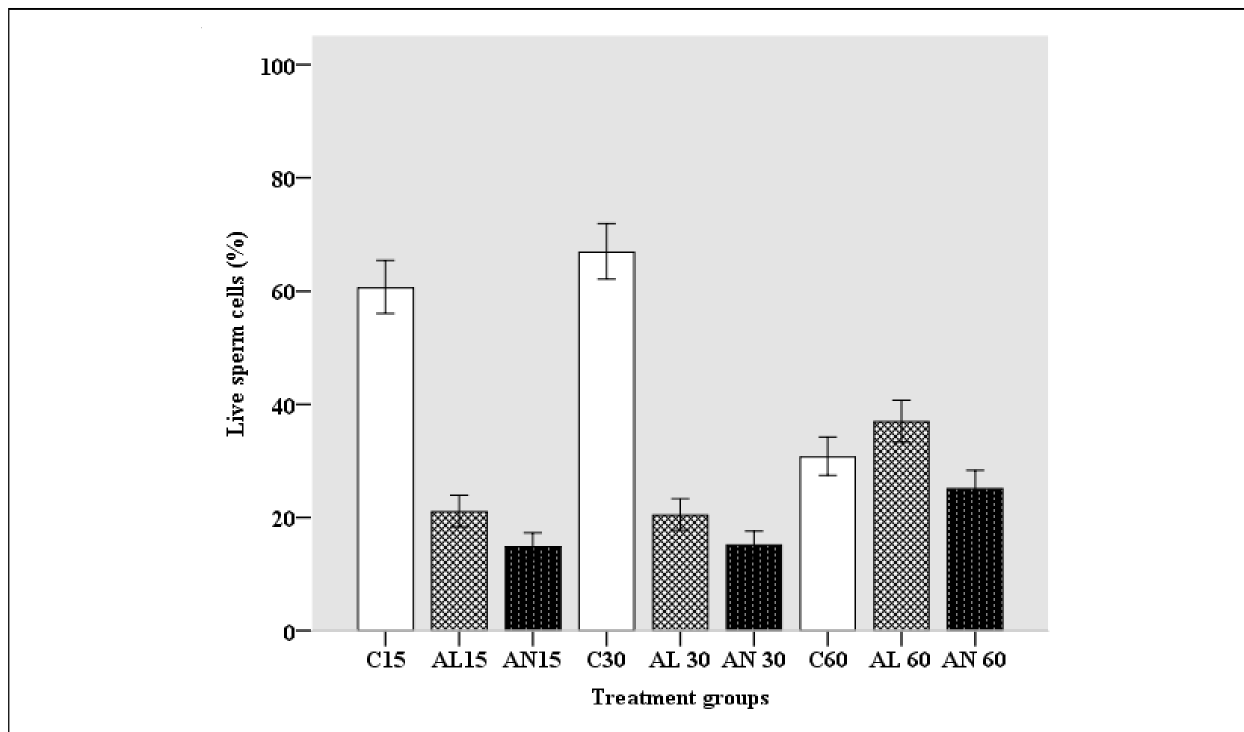

Figure 3: Effect of crude plant extract in feed on the percentage of live sperm cells following its treatment for 15, 30 or 60 days in the *M. natalensis* male rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract. Pearson chi-squared test: relation between sperm cell viability (%) against the treatment groups (χ^2 = 883.31, df = 2, $p < 0.001$) and treatment duration (χ^2 = 41.49, df = 2, $p < 0.001$)

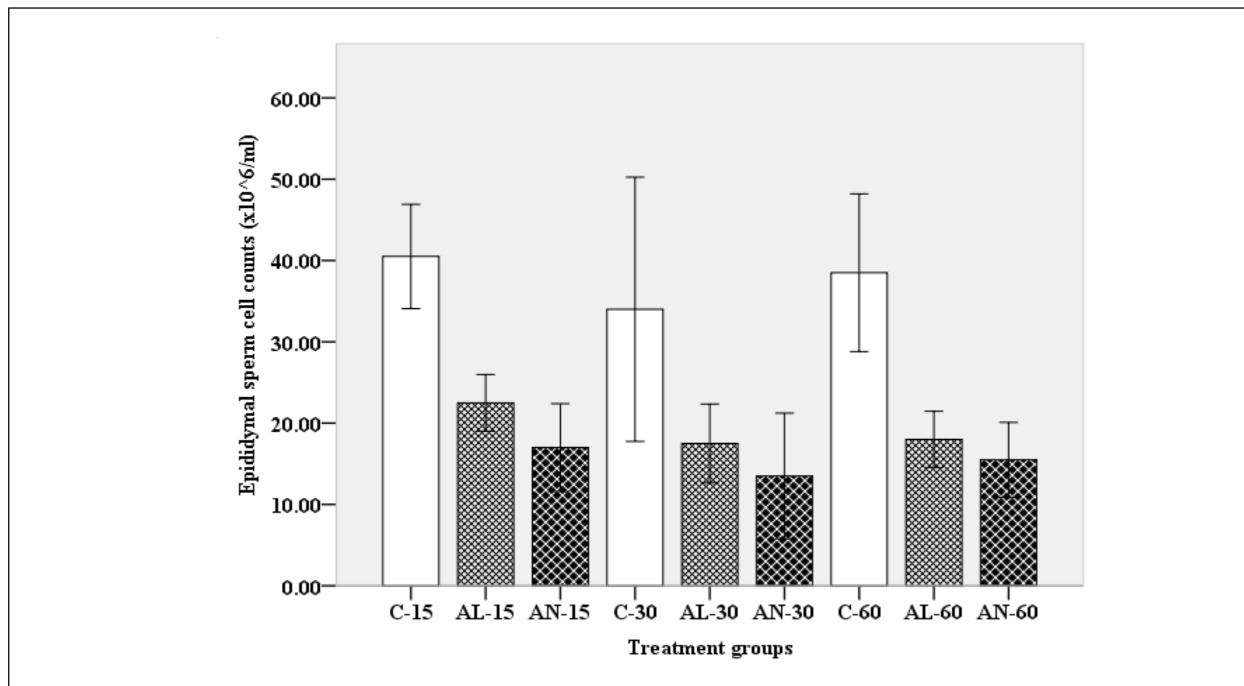

Figure 4: Effect of crude plant extract in feed on the epididymal sperm cell counts following its treatment for 15, 30, or 60 days in the *M. natalensis* male rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract

4.4. Histometric and histopathology of seminiferous tubules

The extract-treated rat testes had some damages in the seminiferous tubules reflected by thinning of the germinal epithelial layer and widening of the tubular lumen (Table 4, Figures 5a and 5b). Therefore, the sizes

Table 4: Effect of *A. lebbeck* and *A. nilotica* extract in feed on the sizes of seminiferous tubule's germinal epithelium and lumen in the male *M. natalensis*.

Treatment time	Control	*A. lebbeck*	*A. nilotica*
Germinal epithelium size (μm) of Seminiferous tubule			
15 days	66.01± 1.34[a]	47.44 ± 1.18[b]	56.01 ± 1.26[c]
30 days	63.27 ± 0.95[a]	35.51± 1.15[b]	33.27 ± 0.95[b]
60 days	64.43 ± 1.48[a]	26.84 ± 1.12[b]	33.44 ± 1.38[c]
Pearson correlation: treatment duration Vs Germinal epithelium size	r = –0.26, p < 0.01	p = 0.48 r = -0.38,	r = -0.38, p = 0.023
Lumen size (μm) of seminiferous tubule			
15 days	53.51 ± 1.57[a]	84.25 ± 1.67[b]	73.86 ± 1.79[bc]
30 days	56.46 ± 2.01[a]	94.44 ± 2.00[b]	79.56 ± 1.86[c]
60 days	52.02 ± 3.14[a]	97.89 ± 2.49[b]	86.65 ± 1.99[bc]
Pearson correlation: treatment duration Vs lumen size	r = –0.024, p < 0.01	p = 0.61 r = –0.174,	r = –0.16, p < 0.01

Note: Raw wide, different superscripts indicate a statistically significant difference between the means at $p < 0.05$.

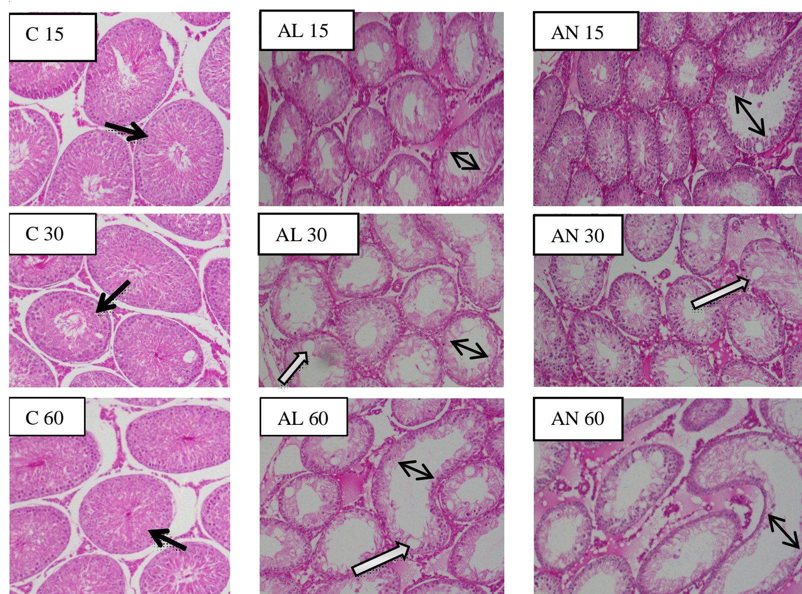

Figure 5a: Histopathology. *M. natalensis* rat's testes at x100

Note: Upper row (15 days treated) groups: C = control: normal seminiferous tubule (ST) with intact germinal epithelium, adlumen, and lumen (Single black arrows). AL= *A. lebbeck* treated group: Normal ST and ST with damaged germinal epithelium, adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (White arrow). AN = *A. nilotica* treated group: Normal ST and ST with damaged germinal epithelium, adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (White arrow).
Middle row (30 days treated) and lower row (60 days treated) groups: C = control groups: normal seminiferous tubule (ST) with intact germinal epithelium, adluminal, and lumen (Single black arrows). AL= *A. lebbeck* treated group: ST with exfoliated germinal epithelium, damaged adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (White arrow). AN = *A. nilotica* treated group: exfoliated germinal epithelium, disrupted adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (White arrow).

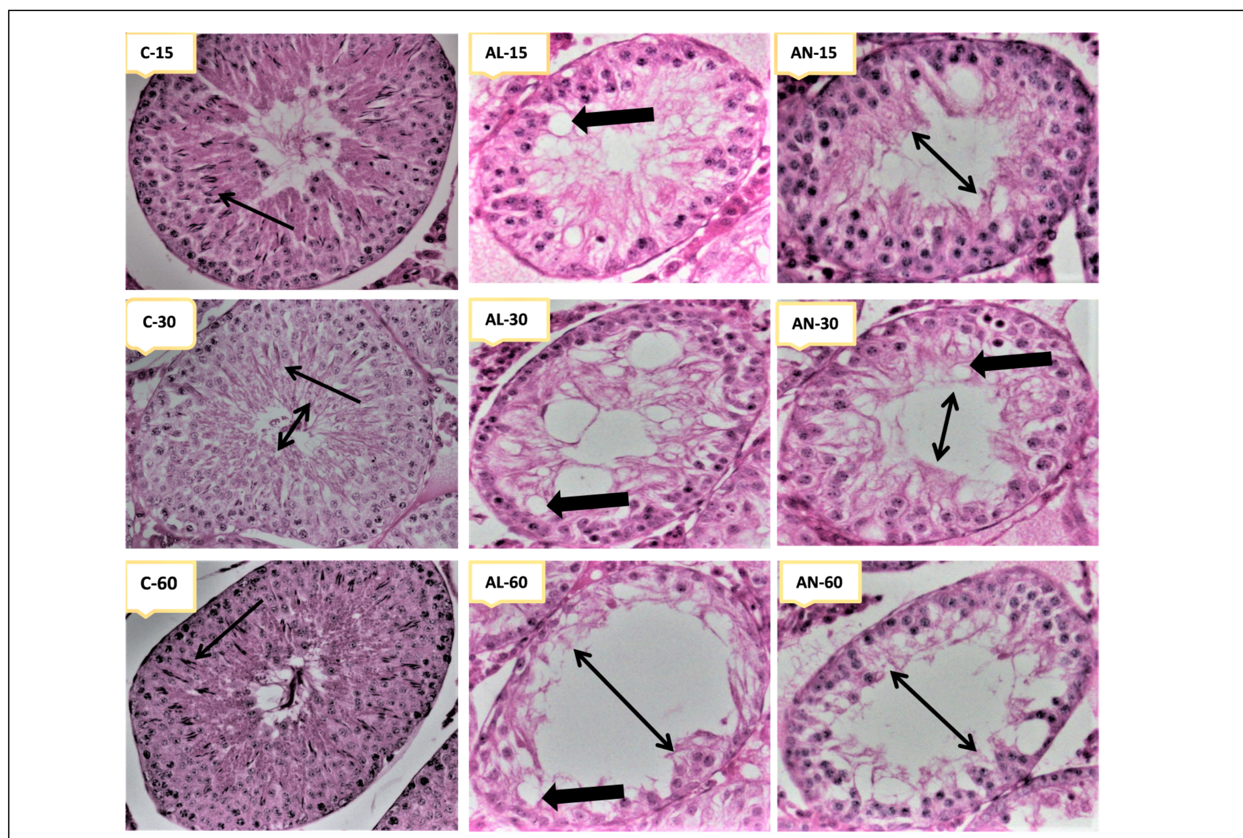

Figure 5b: Histopathology of the *M. natalensis* rat's testes at x 400

Note: Upper row (15 days treated) groups: C = control groups: normal seminiferous tubule (ST) with intact germinal epithelium, adlumen, and lumen (Single black arrows). AL= *A. lebbeck* stem bark methanolic extract treated group: a mixture of normal ST and ST with damages in germinal epithelium, adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (Thick black arrow). *A. nilotica* pods methanolic extract treated group: a mixture of normal ST and ST with damaged germinal epithelium, adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (Thick black arrow).
Middle row (30 days treated) and lower row (60 days treated) groups: C = control groups: normal seminiferous tubule (ST) with intact germinal epithelium, adlumen, and lumen (Single black arrows). *A. lebbeck* stem bark methanolic extract treated group: ST with exfoliated germinal epithelium, damaged adlumen and widened lumen (Double black arrows), intraepithelial vacuolation (Thick black arrow). AN = *A. nilotica* pods methanolic extract treated group: exfoliated germinal epithelium disrupted adlumen and widened lumen (Double black arrows) and intraepithelial vacuolation (Thick black arrow) of ST.

of the germinal epithelial layer and lumen of the seminiferous tubules differed significantly between the extract-treated rat's testes compared to those of the control ($p < 0.001$). Pearson correlation test revealed a weak correlation between the changing germinal epithelial thickness of the seminiferous tubule and treatment duration (Table 4). There was also a weak correlation between the sizes of seminiferous tubule lumen and treatment duration (Table 4). Pairwise comparisons by the Post Hock Tukey's test indicated that the thining of germinal epithelial of seminiferous tubules was more extensive in the *A. lebbeck* stem bark extract-treated rats relative to the rats under the *A. nilotica* pods extract treatments ($p < 0.001$). Detailed histopathological examination indicated further that the control rats' testes had normal seminiferous tubules with intact germinal epithelium, adlumen, and lumen components (Figure 5a and 5b). However, the *A. lebbeck* stem bark and *A. nilotica* pods extract-treated rats had damages characterized by sloughed-off spermatids and spermatocytes, intraepithelial vacuolation, destroyed adlumen and widened tubular lumen in the seminiferous tubules of their testes (Figures 5a and 5b).

5. Discussion

In the current study, the inclusion of *A. nilotica* pods or *A. lebbeck* stem bark extract in the diet (at 2% w/w) fed to *M. natalensis* male rats contributed to significant adverse effects on the structure and function of the male *M. natalensis* reproductive system. Extract treatments reduced the fertility success rate of the *A. nilottica* pods

and *A. lebbeck* stem bark extracts treated rats. The extract of *A. nilotica* pods appeared to be more effective in causing fertility suppression in the male rats than the crude extract of *A. lebbeck* stem barks. The current results agreed well with the findings of Verma *et al.* (2002), who reported a significant reduction of fertility in the Sprague-Dawley rats after treating them orally with 100mg of *Sarcostema acidum* stem barks extract for 60 days. Also, our results agree well with the results of Gupta *et al.* (2006), in a study which revealed a 100% suppression of fertility success rate in Wister rats after feeding them orally with 100 mg/kg/Bw of crude extract of *A. lebbeck* stem barks for 60 days.

The reduction of fertility success rate observed in the current study was probably related to several other anti-fertility effects which were observed in the *A. lebbeck* stem barks and *A. nilotica* pods extract treated rats. There was a reduction of testes weights in rats under the plant extract treatments most likely attributable to the damages which were visible histologically in the seminiferous tubule of their testes (Asuquo, 2012). The weight of seminal vesicles of the plant extract treated rats was also reduced in the current study and was probably attributable to androgen deficiency (Gupta *et al.* 2006). A reduction in the epididymides weights of the extract-treated rats was also evident in the current study and was probably attributable to the lowered sperm cells number in the epididymides of affected testes (Asuquo, 2012). These results were agreeable to the works by Gupta *et al.* (2006), who tested the effects of *Nyctanthes arbortristis* stem barks extract, Asuquo (2012), who investigated the effects of *Spondias mombin* crude leaf extract, and Singh and Gupta (2016) who assessed the impact of β-sitosterol from *Barleria prionitis* in the male's Wister rats. All these studies reported a significant reduction in the testes and accessories reproductive glands weights in the extract-treated male rats.

Moreover, the reduction of fertility success rate observed in the current study was most likely related to the alterations in sperm cell parameters that occurred in the plant extract treated rats. It was revealed in the current study a significant reduction in the proportional of sperm cells with progressive motility and viability also a reduction in concentration or density of spermatozoa in both the *A. nilotica* and *A. lebbeck* treated rats. These findings agreed well with the results of Lampiao (2013) who treated the extract of *A. nilotica* pods in Wister rats and those of Gupta *et al.* (2004 and 2006), who investigated in separate studies the anti-fertility effects of pods and stem bark extract of *A. lebbeck* in male Wister rats. All these authors indicated that sperm cell density, motility, and germ cell number decreased significantly in the crude extract-treated rats relative to their control counterparts.

The adverse effects on sperm cell parameters of *A. lebbeck* and *A. nilotica* treated rats were probably related to histopathological changes which occurred in the testes of the plant extract treated rats. The male *M. natalensis* under the extract treatments revealed some damages in their testicular seminiferous tubules relative to those of the control rats. The lesions in the damaged seminiferous tubules were characterized by sloughing-off of germ cells, intraepithelial vacuolation, and intra-luminal widening which could have perturbed spermatogenesis. These results agreed well with the results of Lampiao (2013), who revealed some significant damages in the male Wister rat's testes after feeding them orally with 100mg of *A. nilotica* pod extract. Our results also agree with the results reported by Gupta *et al.* (2002) and (Raji *et al.*, 2006), where their separate studies revealed some significant damages in the testes of the plant extract treated rats.

The active plant compounds which contributed to the observed effects in the reproductive organs of male *M. natalensis* in this study were not studied. However, documented reports on phytochemical analysis revealed the presence of flavonoids, tannins, saponins, steroids, terpenoids, and plant phenols in the *A. nilotica* and *A. lebbeck* crude extracts (Sawant *et al.*, 2014; and El-Ghany *et al.*, 2015) and all these compounds have been associated with some anti-fertility properties (Wocławek-Potocka *et al.*, 2013; Benhong *et al.*, 2012; Gupta *et al.*, 2005; Burton and Wells, 2002; Shu *et al.*, 2015; and Panche *et al.*, 2016).

Therefore, the revealed cellular disorganization in the seminiferous tubule epithelium of the *A. lebbeck* and *A.nilotica* extracts treated rat's testes in the current study was probably directly or indirectly attributable to the toxic action of plant's compounds disturbing the structure and function of cellular junctions in the seminiferous tubules (Cheng and Dolores, 2002).

It is well known that intact cellular organization in healthy seminiferous tubules of the testes relies on the morphological intimacy between Sertoli and germ cells (Cheng and Dolores, 2002). Several junction types including the occluding, anchoring, and communicating gap junctions play a vital role to provide a smooth morphological and functional organization between the Sertoli and germ cells for a smooth spermatogenic process (Cheng and Dolores, 2002). Therefore, anything disrupting the structure and functions of those cellular

junctions could easily lead to cellular disorganization in the seminiferous tubule leading to perturbation of spermatogenesis (Cheng and Dolores, 2002).

6. Conclusion

The current study concludes that feeding the extract of *A. nilotica* pods or *A. lebbeck* stem bark at 2%w/w contributed significantly to reducing the fertility success rates of the *M. natalensis* male rats. Extract treatment caused some significant damages in the rat's testes leading to a perturbed spermatogenic process. Moreover, the *A. nilotica* pod extract was more potent than the *A. lebbeck* stem bark extract in causing the observed adverse effects. Further studies are required to find out whether the observed anti-fertility effects are permanent or transient.

Conflicts of interest

The authors have not declared any conflict of interest.

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