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Antifertility effects of crude extracts from *Acacia nilotica* pods and *Albizia lebbeck* stem bark in female multimammate rats, *Mastomys natalensis*

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The study's main objective was to assess any pathophysiological significance of *Acacia nilotica* pods aqueous extract and *Albizia lebbeck* stem bark methanolic extract on the reproductive system of female multimammate rats *(Mastomys natalensis)*. A total of 60 sexually mature female rats were randomized into a 2 × 3 factorial experimental design for treatments (Control, *A. nilotica,* and *A. lebbeck*) and treatment duration (7 or 14 days). Control rats consumed basal feed only, whereas extract-treated rats consumed the basal feed containing 2% w/w of either of the two plant extracts. At the end of treatment duration, treated female rats were cohabited with males for 16 days and sacrificed 20 days after the first day of cohabitation. Parameters including pregnancy rates, number of fetal implantations, possible resorption sites and fetal litter size were assessed at necropsy. Further post-necropsy parameters were evaluated in ovaries including the ovarian weights, follicular and corpora lutea numbers and general histopathology. Results showed that pregnancy percentages, the number of fetal implantations and fetal litter size were significantly reduced ($P < 0.01$) in rats under the *A. nilotica* and *A. lebbeck* extract treatments relative to the control rats. The ovarian weights of rats receiving the extracts did not differ significantly from their control counterparts ($P > 0.05$). However, the number of corpora lutea of pregnancy was significantly reduced ($P < 0.001$) in ovaries of rats under extract treatments than in their control counterparts. Instead, ovaries of rats receiving the two extracts contained a larger number of degenerating follicles, signifying halted ovulatory and conception activities. The current study has demonstrated that dietary inclusion of crude extracts from *A. nilotica* pods and *A. lebbeck* stem bark can lead to decreased fertility success rates in *M. natalensis* female rats through suppression of ovulatory activities and induction of follicular atresia.

Key words: Rodent pests, medicinal plants, fertility success, reproductive system.

INTRODUCTION

Mastomys natalensis, commonly known as a multimammate rat, is a small rodent pest in the family Muridae. The rodent species is widely distributed covering almost the whole region of sub-Saharan Africa (Fichet-Calvet et al*.,* 2008). During outbreaks, the *M. natalensis* has been of great economic and public health

significance in sub-Saharan Africa. The rodent pest is highly involved in serious destruction of food crops, both in the field and in storage facilities (Mwanjabe et al., 2002), and structural damage to properties. It is also a host and a vector of some parasites and diseases, including zoonotic ones such as plague (Ziwa et al., 2013), leptospirosis (Mgode et al., 2017) and Lassa fever (Fichet-Calvet et al., 2008). *M. natalensis* is a remarkably successful pest because it can survive in a wide range of habitats and can reproduce rapidly and massively (Fichet-Calvet et al., 2008, Mulungu et al., 2013), making it a consistent threat to livelihood (Swanepoel et al., 2017) and public health (Lecompte et al., 2006) in the region.

The control of rodent populations in sub-Saharan Africa is mainly achieved using chemicals (rodenticides) (Mulungu et al., 2010). Despite being helpful in many cases of rodent management in sub-Saharan Africa, the chemical rodenticides have been with several undesirable shortfalls as well. Firstly, rodent pests reproduce rapidly and spread rapidly enabling them to overcome even the most effective control measures including the anticoagulant rodenticides (Mulungu et al., 2010). Secondly, there are reports that rodent pests are slowly becoming rodenticide resistant, risking evolving a rodenticide-resistant generation of rodents in the future (Thijssen, 1995, Phillipe et al., 2018). Also, there are many incidences where rats and mice avoid consuming familiar poisoned baits ("bait shyness"), hence reducing the efficiency of rodenticides (Saxena, 2014). Moreover, some anticoagulant rodenticides kill after inflicting too much suffering on rodents, contravening the animal welfare requirements (Watt et al., 2005). Chemical rodenticides may also easily harm or kill other untargeted organisms, commonly occurring when those poisons contaminate the environment (Thomas et al., 2011).

Thus, with all the information above, it is obvious that exploration of new techniques of rodent control that are effective, humane and less harmful to the environment is increasingly important. Among the new methods worth exploring could be those that make rodents reproduce less (contraception), to reduce their population naturally. Examples of such methods may include the uses of immuno-contraception (Massei et al., 2020), synthetic steroid hormones (Massawe et al., 2018) and plant-derived preparations (Devi et al., 2015). However, while the immune contraception technology has been merely experimental with no application in the actual field situation, the synthetic steroid hormones technology could probably be unaffordable to end users of the lower-income countries. Moreover, synthetic steroid hormones are endocrine disruptors with a high chance of harming untargeted species of organisms when they accidentally contaminate the environment (Adeel et al., 2017).

Therefore, it is increasingly important to consider exploring natural products as a potential alternative source of rodent contraceptives to the environmentally unfriendly chemical rodenticides and hormones. Medicinal herbal products could be among the potential alternative sources of contraceptives for rodent pests. Several plant species have been investigated and the antifertility actions of their products have been well revealed in some mammals (Tran and Hinds, 2013). Few examples of plant extracts with antifertility activities include the petroleum ether extract of *Polygonum hydropiper* roots, which caused significant anti-ovulatory activities when administered in female rabbits (Kapoor et al., 1974), also, the ethanolic extracts of *Calotropis gigantean* roots (Kamath et al., 2001) and *Hibiscus rosa-sinensis* flowers (Neeru and Sharma, 2008) which caused significant anti-implantation actions after those extracts were administered in females Wistar and Sprague Dawley rats respectively. Moreover, significant antispermatogenic activities were revealed in white albino rats after the crude extracts from *Plumbago zeylanica* (Parohit et al., 2008), *Baleria prionitis* roots (Gupta et al., 2000), or *Tinospora cordifolia* stem bark (Gupta and Sharma, 2003) were investigated in different respective studies in the male animals.

Other plant-derived products with some fascinating antifertility potential include the crude extracts from *Acacia nilotica* (Lampiao 2013) and *Albizia lebbeck* (Gupta et al., 2004). These two tree species are widely distributed in sub-Saharan Africa (Verma et al., 2013, 2016) with many societies using different components of the plants in traditional medicine (Koubé et al., 2016). Whether the extracts from *A. nilotica* and *A. lebbeck* could be efficacious enough in suppressing the fertility of rodent pests needs to be studied. Therefore, the current study was carried out to investigate the pathophysiological significance of the *A. nilotica* pods and *A. lebbeck* stem bark extracts on the reproductive system of female *M. natalensis*.

MATERIALS AND METHODS

Study area

The experiments were conducted at the Small Animal Research Unit (SARU) in the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture (SUA) Morogoro Region, Tanzania (6° 50' 42.66" S, 37° 39' 29.14"E). *A. 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 University Main campus. Authentication of the two tree species was carried out by a botanist from the Department of Ecosystems and

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Table 1. Experimental design and treatment allocation.

Group (n=10)	Treatment allocation	Trial duration (days)
Control (C)	10 g basal feed per rat per day	7
*A. lebbeck (*AL)	10 g basal feed containing 2% w/w *A. lebbeck* stem bark extract per rat per day	
*A. nilotica (*AN)	10 g basal feed containing 2% w/w *A. nilotica* pods extract per rat per day	
Control (C)	10 g basal feed per rat per day	14
*A. lebbeck (*AL)	10 g basal feed containing 2% w/w *A. lebbeck* stem bark extract per rat per day	
*A. nilotica (*AN)	10 g basal feed containing 2% w/w *A. nilotica* pods extract per rat per day	

Conservation, SUA.

Experimental animals and their maintenance

A total of 190 sexually mature *M. natalensis* female rats were collected from the University farms using Sherman aluminium traps. The captured rats were then kept singly in cages in a clean room with enough space and light for a two-week acclimatization period under the standard environmental conditions of 25 ± 5°C temperature, 35-60% relative humidity, and 12/12 light-dark cycle. During the period, the rats were maintained on broiler finisher pellets supplied in earthen pots to avoid any spillage. The rats had access to *adlib* drinking water provided through drinking bottles. Permission to carry out this animal trial was granted by the Tanzanian Commission for Science and Technology (COSTECH 2019-225-NA-2019-47). Guidelines for care and use of laboratory animals were followed adequately during the rat's handling and restraints.

Plant materials sampling and extraction

A total of 10 kg each of *A. lebbeck* stem barks and *A. nilotica* pods were collected in August and September of 2019 and transported in bags to the laboratory, where they were dried under the shade for several days until the plant materials were completely dry and breakable. The dried plant materials were then chopped into smaller pieces using a knife before ground into fine particles (1 mm) using an electric grinder. Extraction involved the soaking of 500 g of each plant material in 1.5 L of 70% methanol (v/v in distilled water) for *A. lebbeck* (Gupta et al*.,* 2004) and distilled water for *A. nilotica* (Lampiao, 2013). The contents were incubated for 72 h at room temperature while shaken two times daily to enhance the extraction. After 72 h of extraction, the crude extracts from *A. lebbeck* and *A. nilotica* were obtained by filtering the methanolic and aqueous extract through gauze and then cotton wool. Methanol in the *A. lebbeck* filtrate was removed using a vacuumed rotary evaporator (BUCHI ROTORVAPOR R-114, made by BÜCHI Labortechnik AG Meierseggstrasse 40, Postfach, 9230 Flawil, Switzerland) at 80°C. The resultant filtrates from both the *A. lebbeck* and *A. nilotica* were further dried to a solid mass using a water bath (Buchi water bath B-480, component of BUCHI ROTORVAPOR) at 40°C. The crude extracts were then weighed and the extraction percentages were calculated. The extraction percentage of crude extracts was 21% (21 g of extractives /100 g of raw materials) for *A. lebbeck* and 32% (32 g of extractives/100 g of raw materials for *A. nilotica*.

Preparation of basal and treated feeds

Preparation of the basal and extract-treated feeds was done according to the protocol of Massawe et al*.* (2018) with some modifications. Briefly, 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 extracts 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.

Experiment setup and treatments

A total of 190 female rats were captured and 60 of them were selected for the study. Bodyweight of 25-50 g reflecting sexual maturity in rats (Lalis et al*.,* 2006) and a sound body condition were used as inclusion and exclusion criteria for selecting suitable experimental rats. The selected rats were stratified on a bodyweight basis and randomly allocated into six groups (n = 10) in a 2 × 3 factorial experimental design, as shown in Table 1. The amount of feed supplied to each rat considered that rats could consume up to 10 g feed/100 g body weight per day (Krishnakumari et al*.,* 1979).

Feed intake

Feed intake was measured daily for each rat by subtracting the amount of feed remaining in the feeding pots from the previously supplied amount. The feed consumption index was estimated as the mean of feed intake divided by the rat's mean body weight in grams. Body weights were measured using a digital weighing balance (METTER PE 1600, made in Zurich Switzerland by METTER PE INSTRUMENTENE).

Fertility performance test

At the end of each treatment duration, female rats were paired with untreated sexually mature male rats at a ratio of 1:1 for mating. The process involved introducing female rats into male cages, and the cohabitants stayed together for 16 days while being maintained on broiler finisher pellets. Mating success was assessed daily by examining the vaginal smears at x 100 magnification on a

microscope. The presence of sperm cells in vaginal smears was an indication of mating success and was recorded as day 0 of pregnancy (Yadav et al*.,* 1999, 2009). Successful mating was noted between 3 to 5 days from the first day of cohabitation for most mated rats. Following the mating exercise, the female rats were regularly assessed for pregnancy status by palpating the lower abdomen. On day 20 from the first day of cohabitation, female rats were sedated in ether and then humanely sacrificed to assess the outcome of pregnancy based on the presence and the number of fetal implantations, fetal resorption sites and fetal litter size in the uterine horns (Hyacinth and Nwocha, 2011). Pregnancy percentages were calculated as the proportion of pregnant rats out of the successfully mated female rats (Singh and Gupta, 2016).

$$Pregnancy\ (\%) = \frac{Pregnant\ rats}{Total\ successfully\ mated\ female\ rats} \times 100$$

The fertility success rate was calculated as the percentage of pregnant rats out of the total number of cohabited female rats (Singh and Gupta, 2016).

$$Fertility\ success\ rate = \frac{Number\ of\ pregnant\ rats}{Total\ number\ of\ cohabited\ female\ rats} \times 100$$

Anti-implantation percentage = number of implants in control minus number of implants in test group divided by the number of implants in control group multiplied by 100 (Hyacinth and Nwocha, 2011)

$$Anti-implantation\ activity\ (\%) = \frac{Control\ total\ implants - Treated\ total\ implants}{Control\ total\ implants} \times 100$$

The antifertility percentages were calculated as the proportion of rats with no fetal implantations out of the total number of cohabited female rats (Hyacinth and Nwocha, 2011).

$$Antifertility\ (\%) = \frac{Non\ pregnant\ rats\ number}{Total\ number\ of\ cohabited\ rats} \times 100$$

Ovarian weight index

Following an autopsy, the ovaries of each rat were trimmed out with a surgical blade and weighed using a digital balance (METTER PE 1600, made in Zurich Switzerland by METTER PE INSTRUMENTENE). The ovarian weight index was then calculated by dividing the ovarian weight by the body weight recorded just before tissue collection (Zhao et al*.,* 2017).

Ovarian histopathology

Weighed ovaries were fixed sequentially in two fixatives; firstly, in Bouin fluids for 24 h and then in absolute ethanol until processing (Howroyd, 2005). Histological processing involved dehydrating the ovaries in graded ethanol, clearing in chloroform, embedding in paraffin wax, and serial sectioning into a thickness of 4 µm microtome sections (Zhao et al., 2017). After sectioning, three large ovarian sections, that is, 5th, 10th, and 15th sections, were selected from each ovary (Zhao et al., 2017) and stained with haematoxyline and eosin for differential follicular counts, corpora lutea counts and ovarian histopathology. To avoid double-counting only follicles with visible oocytes and nuclei were counted. Histopathological examination of the ovarian sections was done at x 100 and x 400 magnifications employing a camera (MOTICAM PRO 205A, made in German, Christian-Kremp-Straβe 11. 35578 Wetzlar, Germany) mounted bright light microscope (OLYMPUS 2467, made by Olympus Life Science Solutions, 48 Woerd Ave,Waltham, MA, 02453, United States). Histologically, follicles were classified as follows:

(i) Primordial follicle when it contained an oocyte surrounded closely by a partial or complete layer of squamous granulosa cells
(ii) Primary when it had an oocyte surrounded by zona pellucida and one to several layers of cuboidal granulosa cells without an antrum.
(iii) Secondary follicles containing an oocyte surrounded by several granulosa cells and a small fluid-filled cavity called an antrum.
(iv) Graafian follicles were the largest mature follicles with an oocyte surrounded by an enlarged antrum and a thinned stratum granulosum.
(v) Follicles were diagnosed as degenerating when they contained multiple exfoliating granulosa cells with pyknotic nuclei and network fibrous tissues.

Moreover, the corpora lutea were also identified. A corpus luteum of pregnancies was identified as a large, roughly circular yellowish-brown compact mass of lutein cells surrounded by fibrous tissues in the ovulated follicular sacs (Bowen-Shauver and Gibori, 2003).

Data analysis

IBM SPSS statistics version 20 was employed for data analysis. The normality of data sets of quantitative variables was assessed using the histogram, scatter plots and probability plots (P-P) residues. The quantitative variables were summarized as the mean and standard error of the mean. Categorical variables were analyzed as percentages. The univariate general linear model (GLM) analyzed the association of the dependent and independent variables by fitting the model for quantitative variables as dependent variables, whereas treatment groups and treatment duration were the independent variables. Initial body weight and final body weight served as covariate variables. The factorial analysis of variance (ANOVA) was used to compare the mean among the treatment groups. Levene's test assessed the homogeneity of variances of quantitative variables. Any revealed significant differences in the factorial ANOVA tests allowed for multiple comparisons among the individual groups by the post hoc Tukey test. The P-values showing the significance of independent variables were validated by assessing the involved dependent variables' multicollinearity. The Pearson Chi-squared test evaluated the impact of treatments and treatment duration on pregnancy outcomes. Values were statistically significant at P<0.05, very significant at P<0.01, and very highly significant at P<0.001.

RESULTS

Feed intake and ovarian weight index

The mean values of the rat's feed intake (g/g body weight) are presented in Figure 1. The factorial ANOVA revealed that the daily intake of test feed varied significantly (P<0.001) among the treatment groups. Rats exposed to feed containing *A. nilotica* pod extract had higher intake compared to either the rats under *A. lebbeck* stem bark extract treatments (P<0.01) or the control rats under the basal test feed (P<0.01). Furthermore, the amount of feed consumed by the *A. lebbeck* treated rats did not differ significantly from their

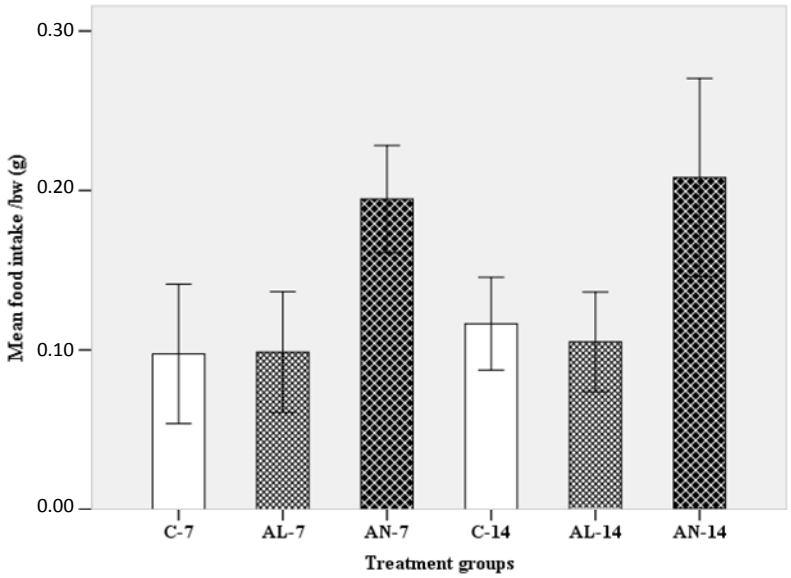

Figure 1. Effect of dietary inclusion of crude plant extracts on feed intake of *M. natalensis* female rats following treatment for 7 or 14 days. C = control groups, AL= *A. lebbeck* stem bark extract, AN = *A. nilotica* pod extract. Values are mean ± SEM

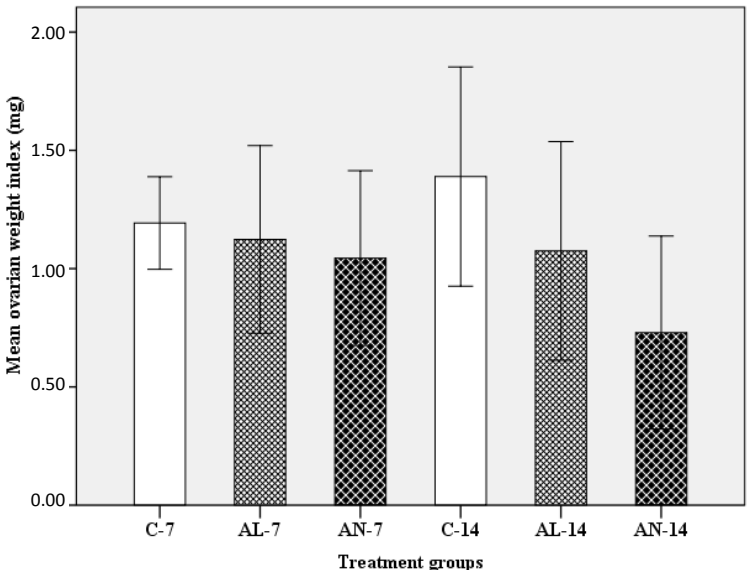

Figure 2. Effect of dietary inclusion of crude plant extracts on the mean ovarian weight index of *M. natalensis* female rats following treatment for 7 or 14 days. C = control groups, AL= *A. lebbeck* stem bark extract, AN = *A. nilotica* pod extract. Values are mean ± SEM.

control counterparts (P>0.05).

Moreover, the post-treatment ovarian weights were slightly lower (P>0.05) in the *A. nilotica* pods and *A. lebbeck* stem bark extract-treated groups of rats compared to their control counterparts at both treatment durations of 7 and 14 days (Figure 2).

Fertility performances

It was revealed at necropsy that the percentages of rats with positive pregnancy outcomes were lower in the extract-treated groups of rats than in their control counterparts (Table 2). Therefore, the calculated fertility

Table 2. Effect of dietary inclusion of crude plant extracts on the reproductive indices of *M. natalensis* female rats following treatment for 7 or 14 days. C = control groups, AL= *A. lebbeck*, AN = *A. nilotica*.

Group (n=10)	No. Female rats tested	Mating success rate N(%)	Pregnancy rate N(%)	Fetal number (Mean ±SEM)	Fertility success rate N(%)
C-7	10	9(90)	7(78)	4.3±1.00[a]	7(70)
AL-7	10	8(80)	1(13)	0.5±0.5[b]	1(10)
AN-7	10	7(70)	0(0)	0±0.00[b]	0(0)
C-14	10	10(100)	8(80)	5.6±1.04[a]	8(80)
AL-14	10	8(80)	0(0)	0±0.00[b]	0(0)
AN-14	10	8(80)	0(0)	0±0.00[b]	0(0)

Different superscripts row-wise are indicative of a statistically significant difference at P<0.05.

Table 3. Effect of dietary inclusion of crude plant extracts on the reproductive performance of *M. natalensis* female rats following treatment for 7 or 14 days. C = control groups, AL= *A. lebbeck*, AN = *A. nilotica*.

Group (n=10)	Successful mated	Rats with implantations	Number of implantations/group	Number of resorption sites/group	Anti-implantation (%)	Antifertility (%)
C-7	9	7	43	0		30
AL-7	8	1	5	0	88.89	90
AN-7	7	0	0	0	100	100
C-14	10	9	56	0		10
AL-14	8	0	0	0	100	100
AN-14	8	0	0	0	100	100

success rates were also relatively reduced in both groups of rats under extracts treatments relative to the control rats (Table 2). Also, the mean number (litter size) of fetuses recovered at necropsy differed significantly among the treatment groups (P<0.001) and was not significantly influenced by treatment duration (P>0.05). All mated female rats in the *A. nilotica* extract-treated groups had no fetuses in their uterine horns (Table 2), whereas those in the *A. lebbeck* extract-treated groups had a significantly lower (P<0.001) number of fetuses than the control group of rats.

While there were no evidence of fetal resorption sites in the uterine horns of the extracts treated and control rats (Table 3); no fetal implantation sites were observable upon necropsy among the mated female rats in the *A. nilotica* extract-treated groups (Table 3). For the *A. lebbeck* extract-treated groups, only one out of the 16 mated female rats had some fetal implantations (five in number) in their uterine horns (Table 3). Therefore, the percentages of rats without fetal implantations and the calculated antifertility rates were comparatively higher in rats receiving *A. nilotica* and *A. lebbeck* extracts than in their control counterparts (Table 3). The chi-squared test revealed further that the differences in pregnancy percentages and fertility success rate among the studied groups of rats were significantly associated with treatments ($\chi 2 = 23.125$, df = 2, P<0.001) but not with treatment duration ($\chi 2 = 0.000$, df = 1, P = 1.00).

Ovarian histopathology

The mean values of follicular and corpora lutea counts of the rats are presented in Table 4. There were no significant differences (P>0.05) in the mean counts of primordial, primary, secondary and Graafian follicles in the extracts treated ovaries relative to their control counterparts. However, the numbers of corpora lutea of pregnancies and those of degenerating follicles differed significantly among the treatment groups (P<0.001) and the difference was not associated with treatment durations (P>0.05). Likewise, the covariate variables, including initial body weight and final body weight, had no significant effects (P>0.05) on the counts of corpora lutea of pregnancies and degenerating follicles. The post hoc Tukey HSD test indicated that the ovaries of control rats had more corpora lutea of pregnancies (P<0.001) than those of the *A. lebbeck* and *A. nilotica* extracts treated rats (Table 4). Also, the ovaries of the *A. lebbeck* and *A. nilotica* extracts treated rats had more degenerating follicles (P<0.001) than those of their control counterparts (Table 4).

A detailed histopathological examination revealed corroborative results. Numerous corpora lutea of pregnancies composed of enlarged yellowish-brown lutein cells with a small spherical nucleus were abundantly scattered in the control rat's ovaries (Figure 3). On the contrary, the *A. lebbeck* and *A. nilotica* extracts treated

Table 4. Effect of dietary inclusion of crude plant extracts on ovarian follicular and corpus luteum counts of *M. natalensis* female rats following treatment for 7 or 14 days. C = control groups, AL= *A. lebbeck*, AN = *A. nilotica*. Values are Mean ± SEM.

Group N=10	Primordial follicles	Primary follicles	Secondary follicles	Graafian follicles	Corpora lutea of pregnancy	Atretic follicles
C-7	13.5±1.09^{a*}	3.1±0.71^{a}	3.2±0.42^{a}	0.3±0.21^{a}	5.6±1.20^{a}	1.3±0.40^{a}
AL-7	14.8±2.62^{a}	4.1±1.12^{a}	3±0.73^{a}	0.1±0.10^{a}	0.7±0.37^{b}	3.7±0.79^{b}
AN-7	15.2±2.25^{a}	5.1±0.86^{a}	3.7±0.58^{a}	1.1±0.48^{a}	0.6±0.40^{b}	5.2±1.11^{b}
C-14	9.2±1.41^{a}	3.6±0.60^{a}	2.6±0.64^{a}	0.4±0.22^{a}	4±0.74^{a}	1±0.30^{a}
AL-14	14.9±3.19^{a}	3±0.86^{a}	1.8±0.39^{a}	0.4±0.22^{a}	0.3±0.21^{b}	5.1±0.53^{b}
AN-14	13.1±1.19^{a}	2.8±0.77^{a}	4.4±1.05^{a}	0.9±0.41^{a}	1±0.45^{b}	4±0.96^{b}

Different superscripts row-wise are indicative of a statistically significant difference at P<0.05.

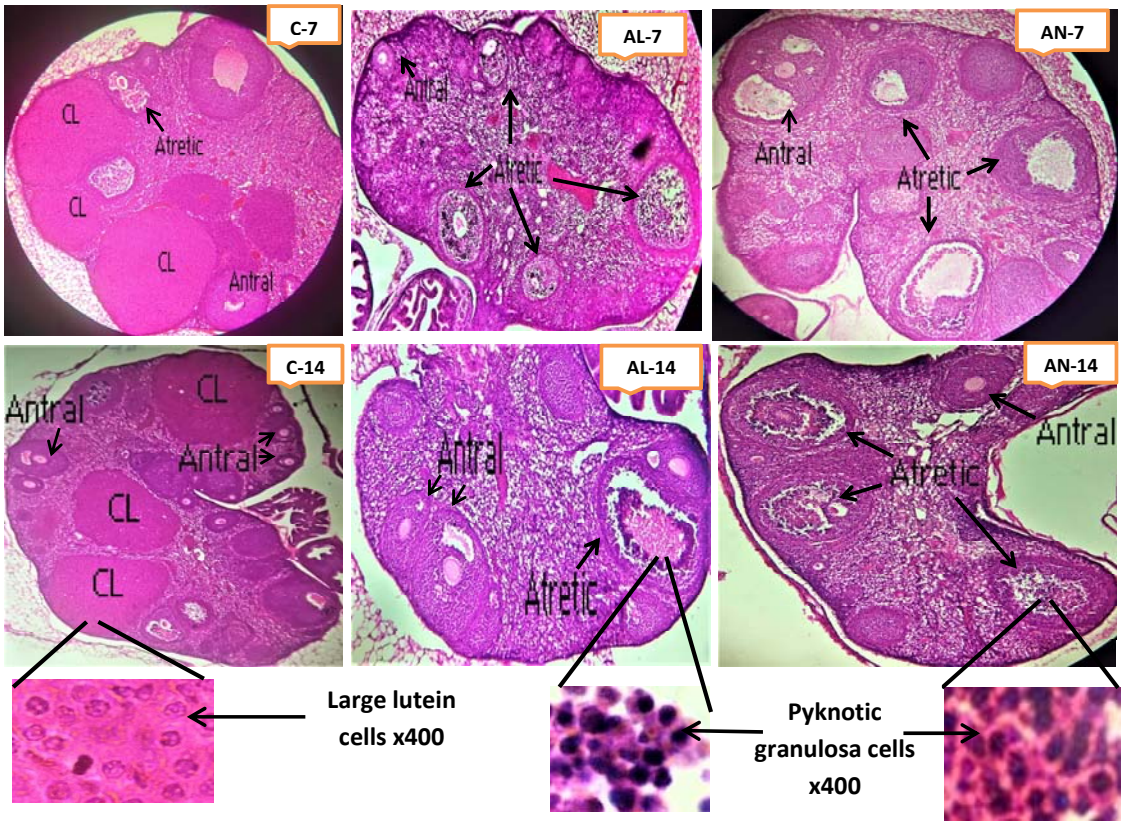

Figure 3. Histopathology of ovaries at x100 and x400 magnifications.

rats had scanty to no corpora lutea of pregnancies and the few available corpora lutea were probably those of pseudo pregnancies. However, there were numerous degenerating follicles with pyknotic granulosa cells in the ovaries of the *A. nilotica* and *A. lebbeck* extracts treated rats (Figure 3).

Upper row (7-day treatment duration): C (control); showing numerous yellowish-brown corpora lutea (CL) of pregnancies, antral follicles and few degenerating follicles. AL (*A. lebbeck*); shows numerous atretic follicles with multiple atretic follicles, no corpus luteum of pregnancies. AN (*A. nilotica*); showing numerous atretic follicles with pyknotic granulosa cells, no corpus luteum of pregnancy.

Lower low (14-days treatment): C (control); showing numerous corpora lutea (CL) of pregnancy with enlarged yellowish-brown lutein cells, developing and antral follicles. AL (*A. lebbeck*); shows multiple atretic follicles with pyknotic granulosa cells, no corpora lutea of pregnancy. AN (*A. nilotica*); showing numerous atretic

follicles with pyknotic granulosa cells, no corpus luteum of pregnancy.

DISCUSSION

In the present study, the administration of methanolic extract from *A. lebbeck* stem bark or aqueous extract from *A. nilotica* pods through feed at 2%w/w resulted into significant suppression of fertility in *M. natalensis* female rats. The study indicates that mated female rats receiving the *A. nilotica* extract had no fetal implantations in their uterine horns. Likewise, only one female rat among the 16 mated females under the *A. lebbeck* extract treatments had fetal implantations in its uterine horns. Therefore, the calculated pregnancy percentages and fertility success rate were significantly reduced in rats under the extract treatments relative to their control counterparts.

Our findings are in close agreement with those from other studies investigating similar or different plant extracts in Wistar rats. For instance, Yadav and Jain (1999 and 2009) reported a dose-dependent suppression of fertility in the female Wistar rats following treatment with *Cassia fistula* crude extracts. Hyacinth and Nwocha, (2011) revealed increased antifertility activity in the female Wistar rats treated with crude extracts of *Hymenocardia acida* stem barks. The antifertility effects of the *A. lebbeck* stem bark methanolic extract and *A. nilotica* pods aqueous extract have been reported by Gupta et al. (2005) and Lampiao (2013) in their respective studies, which involved Wistar rats. However, both these studies used the male Wistar rats and the extracts were administered orally for 60 consecutive days by gastric gavage. Thus, the current study is probably the first to indicate the antifertility actions of *A. nilotica* pods and *A. lebbeck* stem bark extracts in the female *M. natalensis* rats and that the antifertility efficacy can still be maintained when administered through feed baits. The abundance of corpora lutea of pregnancies in the ovaries of control rats was a potential sign of operational ovulatory and conception activities (Humphreys et al., 1985). On the contrary, there were none or scanty corpora lutea of pseudo pregnancies in rats receiving the *A. nilotica* and *A. lebbeck* extracts. In contrast, the extract-treated rats contained many degenerating follicles, indicating halted ovulatory and conception activities (Gadelha et al., 2014). Our results were consistent with the findings of Malashetty and Patil (2007), who used the ethanolic extracts of *Crotalaria juncea*, Solomon et al. (2010), who studied the methanolic extracts of *Rumex steudelii* roots, and Monima *et al*. (2019), who investigated the effect of *Cleome gynandra* leaf extract in female Wistar rats. All these studies revealed that follicular degeneration, interrupted oestrous cycle and halted ovulatory activities were more evident in the extracts treated than in the control groups of rats.

The effects leading to follicular degeneration and halted ovulatory activities were probably directly attributable to the toxic action of *A. nilotica* pods and *A. lebbeck* stem bark extracts in the ovaries of the extracts treated rats (Shu et al., 2015) or were indirectly linked to androgens deficiency (Ying and Greep, 1972; Islam et al., 2007). The present study did not isolate the active plant compounds contributing to the observed effects. However, studies done elsewhere indicate that the extracts of *A. nilotica* (pods) or *A. lebbeck* (stem bark) contain saponins, β-sitosterols, phytoestrogen and other plant's steroids (El-Hawary et al., 2011; Sawant et al., 2014) which have some antifertility properties (Malini and Vanithakumari, 1991, Gupta et al., 2005) among other biological activities. Forinstance, Shu et al. (2015) revealed significant anti-conceptive activities of the *Platycodon grandiflorum* saponin in female mice associated with maternal toxicity in the ovaries and uterus. In mammals, phytoestrogens and other plant steroids cause infertility by mimicking the body's estrogens (Burton and Wells, 2002). Plant-estrogens, work by binding to the same intracellular estrogenic receptors thereby triggering multiple estrogenic activities, such as uterotropic effect, sterility, or disruption of normal reproductive processes (Burton and Wells, 2002). Therefore the antifertility activities revealed in *M. natalensis* female rats in this study were very likely attributable to the toxic action of saponin and other plant steroids contained in the extract of *A. nilotica* pods and *A. lebbeck* stem bark.

Moreover, the current study indicates that the rats exposed to the test feed with *A. nilotica* pod extract recorded higher feed intake and the lowest pregnancy percentage when compared to those on *A. lebbeck* stem bark extract or the control groups on the basal diet. Uncompromised feed intake in the *A. nilotica* treated groups is an obvious added advantage to using the tested plant to control the rodents. According to Abdalla et al. (2014) report, *A. nilotica* pods are very rich in crude proteins, fat, starch, and mineral elements. Such nutritional composition might have enhanced the palatability when mixed in the feed as crude extract (Owen-smith and Cooper (1987). Lowered intake for the *A. lebbeck* stem extracts containing meal was probably due to the high content of astringent tasting tannins in the stem bark extract (El-Hawary et al*.,* 2011).

Conclusion

The present study concludes that dietary inclusion of crude extracts from *A. nilotica* pods or *A. lebbeck* stem bark can significantly reduce the fertility success rate of *M. natalensis* female rats. A reduction of ovulatory, conception, and fetal implantation activities and increased follicular atresia was revealed in female rats treated with plant extracts. It was also shown that the

extract of *A. nilotica* pods was comparatively more effective in causing the observed antifertility-related effects than the *A. lebbeck* stem bark extract.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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