



Oestrous Cycle and Uterine Histology of Aged Albino Wistar Rats After Combined Therapy of Leaf Extracts of *L. lanceolata* and *A. cordifolia*

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ABSTRACT

Peri-menopause, menopause and associated symptoms can be a source of distress to women, particularly as it heralds the end of the reproductive life of a woman. Oestrous cycle disorders frequently affect the quality of life of peri-menopausal women and can be indicators of serious underlying problems. This study examined the changes in the oestrous cycle and uterine histology of aged albino wistar rats. Twenty-five female albino wistar rats divided into Groups A, C, D, and E consisting of aged irregularly cycling rats and Group B consisting of young regularly cycling rats were used for this study. Extract administration of *L. lanceolata* and *A. cordifolia* to the treated groups was for 21 days and cycling of the rats was carried out during this period. The animals were sacrificed on the 22nd day (pro-oestrus phase) and the uterus was dissected out for tissue processing. The findings in this study showed oestrous cycle was regularized in most of the rats in all the treatment groups. The features of the histology of the uterus were consistent with normal expectations in the pro-oestrus phase of the cycle. In conclusion, treatment with *L. lanceolata* and *A. cordifolia* restores normal cyclicity in aged irregularly cycling rats, therefore it may be used in the management of menstrual cycle abnormalities, and sub-fertility associated with the peri-menopause, and other unpleasant symptoms associated with the perimenopause and menopause.

INTRODUCTION

Oestrous cycle of aged rats is characterized by peri-menopausal changes in the uterus, and the presence of different cell types in vaginal smears. The transition to menopause, also called the peri-menopause, is marked by menstrual irregularities and associated with distressing symptoms such as hot flushes, weight gain, night sweats, mood disorders, sexual dysfunction, headaches, and depression (Grady, 2006). Decherny *et al.*, (2013) also reported abnormal uterine bleeding, hot flushes, skin and hair changes, fatigue, mood disorders, myalgia, atrophic vaginitis, sexual dysfunction, and osteoporosis during the perimenopausal and menopausal period. Rats are polyoestrous, their cycles occur multiple times and continue throughout the year.

In rats, puberty starts between 6-8 weeks of age and the cycle begins then, typically lasting between 4-5 days, but a great majority of rats have 4 days cycle (Freeman, 1988; Goldman *et al.*, 2007, Paccola *et al.*, 2013). Peri-menopause, menopause and associated symptoms can be a source of distress to women, particularly as it heralds the end of the reproductive life of a woman. Oestrous cycle disorders frequently affect the quality of life of perimenopausal women and can be indicators of serious underlying problems, especially those who suffer from dysmenorrhoea (Karout *et al.*, 2012). Apart from health challenges, there can be consequences such as limitations on attendance at work which affects productivity at work and performance. Adebimpe *et al.*, (2016) reported that menstrual disorders constitute a challenge to a significant percentage of adolescents in their study on menstrual patterns and disorders and impact on the quality of life among university students. Similarly, a study of the menstrual pattern among female medical students showed that 12.4% of respondents present with irregular menstrual cycle patterns. Another study carried out to determine the impact of menstrual cycle disorders on health and academic activities of female undergraduates showed dysmenorrhoea to be the most prevalent menstrual disorder (64.5%), the highest health implication was dizziness ((51%) and an association was found between dysmenorrhoea and absenteeism (Olowokere *et al.*, 2014). The menstrual cycle is a physiological process, beginning during puberty, and ending at menopause. It is characterised by cyclical shedding of blood from the uterus known as menstruation and is dependent on a complex interplay of hormones released by the hypothalamus, pituitary gland, and the ovaries (HPO axis). Menstruation

stops at menopause, when the reproductive life of a woman ceases, and occurs from 45 years, with a mean age of 51 years. It can, however, occur before the age of 40 or as late as 60 years. The peri-menopause can last for four to eight years, it usually begins in the mid-late 40s, but can also begin in the late 30s. There is a sharp decline in fertility at this stage due to hormonal imbalance (Gold, 2011; Grady, 2006). Over the years, there have been several claims by traditional herbal medicine practitioners on the efficacy and potency of their herbs in treating several health conditions, including diabetes cancers, infertility, and HIV (human immunodeficiency virus) infection. World over, people are increasingly turning to phytomedicine for health issues and researches is increasing on herbal products and their usage (Ekor, 2015; Bent, 2008). The treatment offered by orthodox medicine for menstrual irregularities, peri-menopause, and infertility is mostly expensive, requiring elaborate investigations and may not be readily available in our environment. This coupled with the economic downturn has contributed to the increased patronage of herbal medical practitioners. According to a report published in the Bulletin of WHO, traditional herbal medicines are naturally occurring plant-derived substances (seeds, berries, roots, leaves, bark, or flowers) with minimal or no processing that have been used to treat illness within local or regional healing practices (Tilbert and Kaptchuk, 2008). Recently, increased attention has been given to medicinal plants and this can be attributed partially to the fact that researches are showing that quite a number of these plants do have significant medicinal values and are actually effective in the treatment of various ailments. The specific ingredients that work in particular herbs are not usually known, and herbs are usually taken in

combination for treating ailments (polyherbalism). A particular herb can be taken for several ailments and several herbs can be taken to treat a particular ailment. *A. cordifolia* is reportedly used in the treatment of gastric ulcers, cough, venereal diseases, and fertility-related problems (Adjene *et al.*, 2012); *L. lanceolata* is used for the treatment of dermatitis, muscle weakness, toothache and fertility disorders (Audu *et al.*, 2011). *A. cordifolia* and *L. lanceolata* are plants used in the treatment and management of perimenopausal, menopausal, and menstrual cycle disorders, with claims of high efficacies by users. Menstrual cycle disorders affect females of the reproductive age to varying degrees. Some experience pains that are crippling and affect their quality of life. Many women still believe that herbal medicines will delay the onset of menopause, regularise their cycles in the peri-menopausal period. Regularisation of the oestrous cycle, either in the peri-menopausal period or earlier in the reproductive life, would improve fertility. This study examined the changes in the oestrous cycle and uterine histology of aged albino Wistar rats.

MATERIAL AND METHODS

Plants of Study:

Fresh leaves of *Lophira lanceolata* and *Alchornea cordifolia* were authenticated and voucher numbers for *Alchornea cordifolia* (BOT/UC/HERB/010) and *Lophira lanceolata* (BOT/UC/HERB/013) deposited in the Department of Botany, University of Calabar, Calabar.

Animals:

Twenty-five female albino rats (Wistar strain) used for this research work consisted of two categories, 5 young rats, 4-6 months old, and 20 aged rats, between 24-30 months old. The rats were kept in the animal house

under standard laboratory conditions (daylight/dark cycle, temperature, humidity). The rats were fed on pelletized chows and water provided *ad libitum* while acclimatizing for two weeks following the commencement of the experiment. They were maintained under the guidelines and approval of the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria (FAREC/PA/016A30217).

Experimental Design:

To determine the oestrous cycle of the rats, the vaginal smear pattern method described by Marcondes *et al.*, (2002) was used. The vaginal smear of the rats was taken daily between 8 am to 9 am. Following the determination of cycling patterns of the rats by daily vaginal smear for a period of 3 weeks, the rats were divided into groups. Group A was the negative control group (consisted of 5 aged irregularly cycling rats). Group B served as a positive control group (5 young regularly cycling rats). Groups C, D, and E were the treatment or experimental groups and were made up of 5 aged irregularly cycling rats each. The extracts were administered as daily oral doses for a duration of three weeks as shown in Table 1. Daily vaginal smear observation was continued throughout the duration of this experiment. Extract administration was completed on the 21st day from the commencement of administration. Sacrifice was done serially from the 22nd day, on pro-oestrus phase of each animal, using the chloroform inhalation method. The uterus was dissected out and fixed in 10% buffered formalin for tissue processing using the H and E technique for histological analysis. Statistical analysis was done using one-way analysis of variance ANOVA, using SPSS version 21.0.

Table 1: Dosages and pattern of administration of plant extracts

Groups n-5	Dosage/ Extract of administration
A	Normal saline
B	Normal saline
C	500mg/kg body weight each of <i>L. lanceolata</i> and <i>A. cordifolia</i>
D	500mg/kg body weight of <i>L. lanceolata</i>
E	500mg/kg body weight of <i>A. cordifolia</i>

RESULTS

Morphological Observation:

Weight changes were observed in all the groups of animals (control and treated).

The final body weights in groups A (negative control), B (positive control), and D (*L. lanceolata* treated) increased from the initial or basal body weights. These increases in body weight were however not statistically significant ($p < 0.05$) in any of these groups (Table

2). For the animals treated with *A. cordifolia* (Group E) and combined extract (*A. cordifolia* + *L. lanceolata*) therapy (Group C), a reduction in weight was observed. This reduction again was statistically not significant at $p < 0.05$ (Table 2). However, the reduction in weight was only observed where *A. cordifolia* was given either alone or in combination with *L. lanceolata*.

Table 2: Initial and final body weights of animals in the various groups

Groups	Initial body weight (g)	Final body weight (g)
A (Negative control)	225.43 ± 12.03	231.75 ± 10.53
B (Positive control)	224.26 ± 14.78	238.26 ± 15.19
C (AC + LL)	284.10 ± 23.97	271.45 ± 16.70
D (LL)	261.03 ± 28.72	272.25 ± 27.95
E (AC)	249.04 ± 9.49	241.34 ± 9.31

Values are expressed as mean ± SEM, n=5

No significant difference ($p < 0.05$) between initial and final body weights in all groups

Oestrous Cycle Observations:

The animals in Group A (negative control) maintained irregular cycling throughout the duration of the experiment. One rat showed a persistent dioestrus smear pattern, while the rest either showed cycles that did not follow the normal sequence, with some cycles being very short while others were of long duration or kept in one phase for more than 4 days but less than 7 days (Tables 3 and 4). The Group B animals (positive control) maintained regular 4 days cycles throughout the duration of the research laboratory work (Tables 3 and 4). For Group C animals that received a combination of *L. lanceolata* and *A. cordifolia* leaf extract, there was a marked improvement in their cycling (Table 4). Eighty percent of the rats in this group had regular cycling, with the

normal pattern or sequence of pro-oestrus, oestrus, metoestrus, and dioestrus exhibited. Of the four rats that showed regular cycling, three (75%) had five days cycles while one (25%) had 6 days cycle. The mean cycle length was 5.25 days (Table 5). One rat, however, became persistently dioestrus, after a few pro-oestrus smear was observed. For the animals in Group D (*L. lanceolata* treated), it was observed that 80 percent of the rats showed regular cycling pattern. The mean cycle length, was 6.6 days (Table 5) and one animal (constituting 20%) also became permanently dioestrus. The rats treated with *A. cordifolia* (Group E), also showed improvement in cyclicity, with regular cycling and cycle length of 6.0 days observed in 80% of the rats during the treatment period. One of the rats

(20%) continued showing irregular cycling, keeping in dioestrus phase for

11 days consecutively, and showing distorted patterns subsequently.

Table 3: Oestrous cycle pattern of animals before extract administration

Groups	Cycling patterns	
	Regular (%)	Irregular (%)
A (Negative control)	0.0	100
B (Positive control)	100	0.0
C (AC + LL)	0.0	100
D (LL)	0.0	100
E (AC)	0.0	100

Table 4: Oestrous cycle pattern of animals during extract administration

Groups	Cycling patterns	
	Regular (%)	Irregular (%)
A (Negative control)	0.0	100
B (Positive control)	100	0.0
C (AC + LL)	80	20
D (LL)	80	20
E (AC)	80	20

Table 5 Mean oestrous cycle lengths of animals during the treatment period

Groups	Cycle lengths (days)
A (Negative control)	---
B (Positive control)	4
C (AC + LL)	5.25
D (LL)	6.6
E (AC)	6,0

Histological Observations:

Sections of the uterus of negative control group animals revealed the endometrium and part of the myometrium. The endometrium showed columnar epithelium lining a dilated uterine lumen, with small elongated endometrial glands within the endometrial stromal cells, which appeared dense. The glands are few (Figure 1a and b). Sections of the uterus of positive control rats showed dilated uterine lumen lined with columnar epithelium, numerous endometrial glands, the myometrium, and perimetrium. The uterine glands within the endometrium appear more spherical when compared with that of the negative control uterus and polymorphonuclear leukocytes were seen in the lamina propria. The stroma was densely cellular. These features

seen are that of normal uterus at the pro-oestrus phase of the oestrous cycle (Figure 2a and b). Sections of the uterus of animals treated with combined extracts of *A. cordifolia* and *L. lophira*, 500mg/kg body weight each, showed normal uterine histology, with widened uterine lumen lined with columnar epithelium, densely cellular stroma, numerous endometrial glands (spherical and elongated) were present in the endometrium. Lamina propria was infiltrated with polymorphonuclear leucocytes. The myometrium and perimetrium were clearly shown. Compared to the other groups, this group showed the widest luminal diameter (Figs. 3a and b). Sections of the uterus of Group D animals treated with *L. lanceolata* extract (500mg/kg body weight) showed widened lumen lined with

columnar epithelium, endometrial glands and polymorphonuclear leucocytes interspersed within dense stromal cells. The myometrium and perimetrium are clearly shown too. The uterine glands are fewer, compared to the combined extract treated and positive control groups (Figs. 4a and b). Sections of the uterus

of Group E animals treated with 500mg/kg body weight of *A. cordifolia* extract showed widened lumen, which is lined with low columnar epithelium, uterine glands and polymorphonuclear leucocytes within the stroma which was densely cellular. The myometrium and perimetrium were well differentiated (Figs. 5a and b).

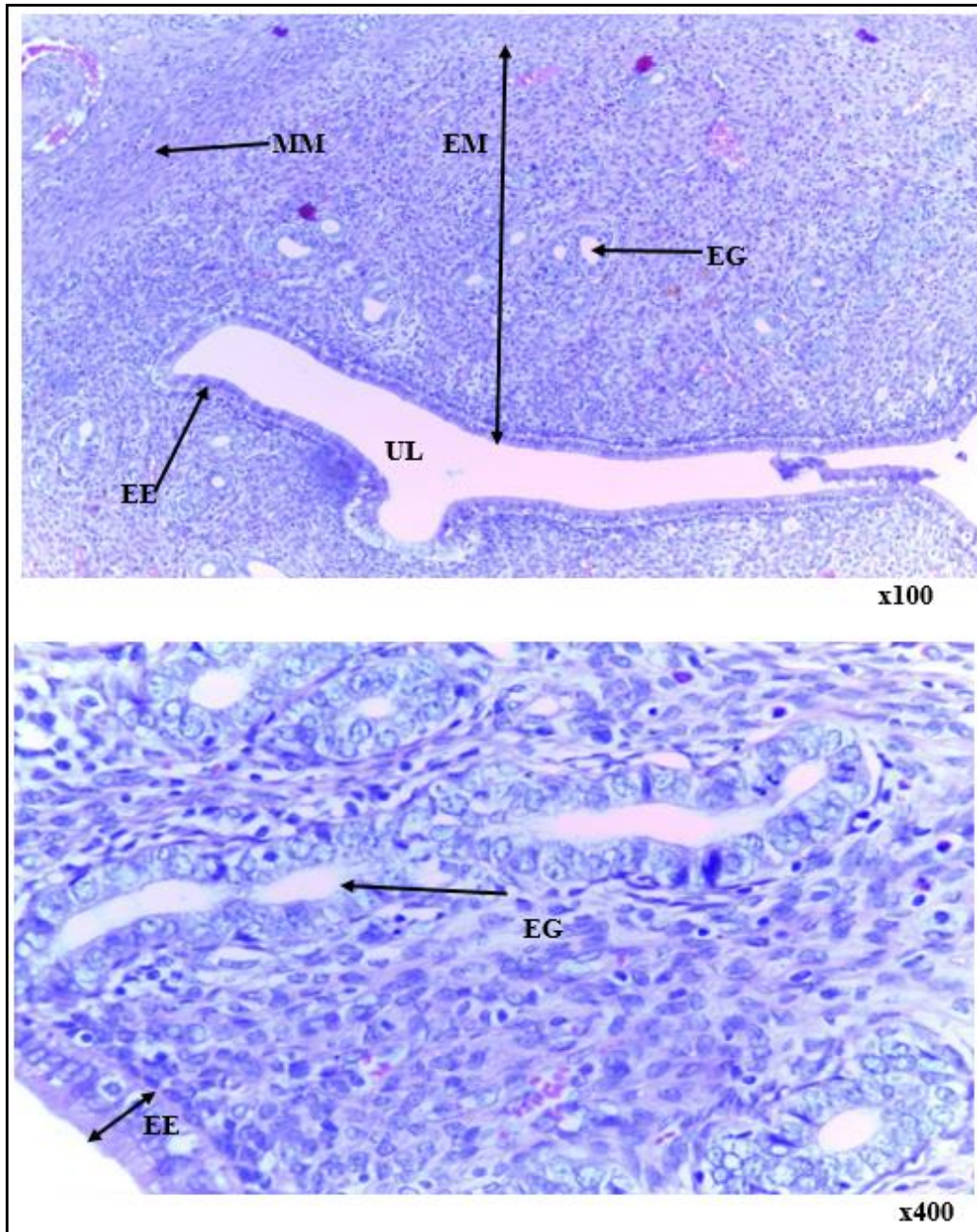


Fig 1a & b

Photomicrograph of the uterus of Group A (negative control) animals.

Uterus of a negative control rat showing the endometrium (EM) with endometrial lining epithelium (EE) lining the uterine lumen (UL), elongated endometrial glands (EG) and part of the myometrium (MM).

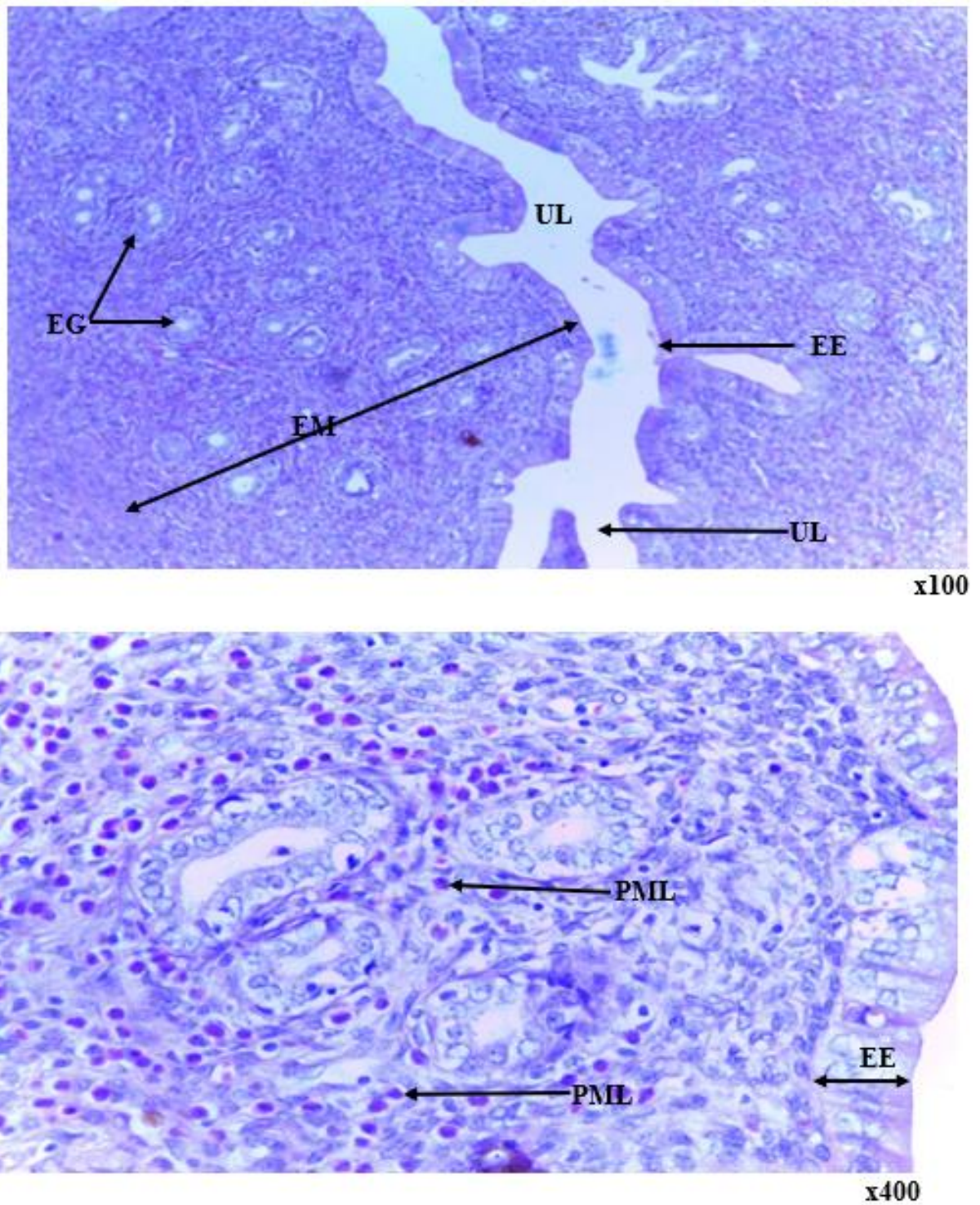


Fig 2a &b

Photomicrograph of uterus of Group B (positive control) animals given normal saline stained with H&E .

The uterus showing widened lumen (UL) lined with columnar epithelium (EE), there are numerous endometrial glands (EG) which appear slightly spherical, and polymorphonuclear leucocytes (PML) in the lamina propria.

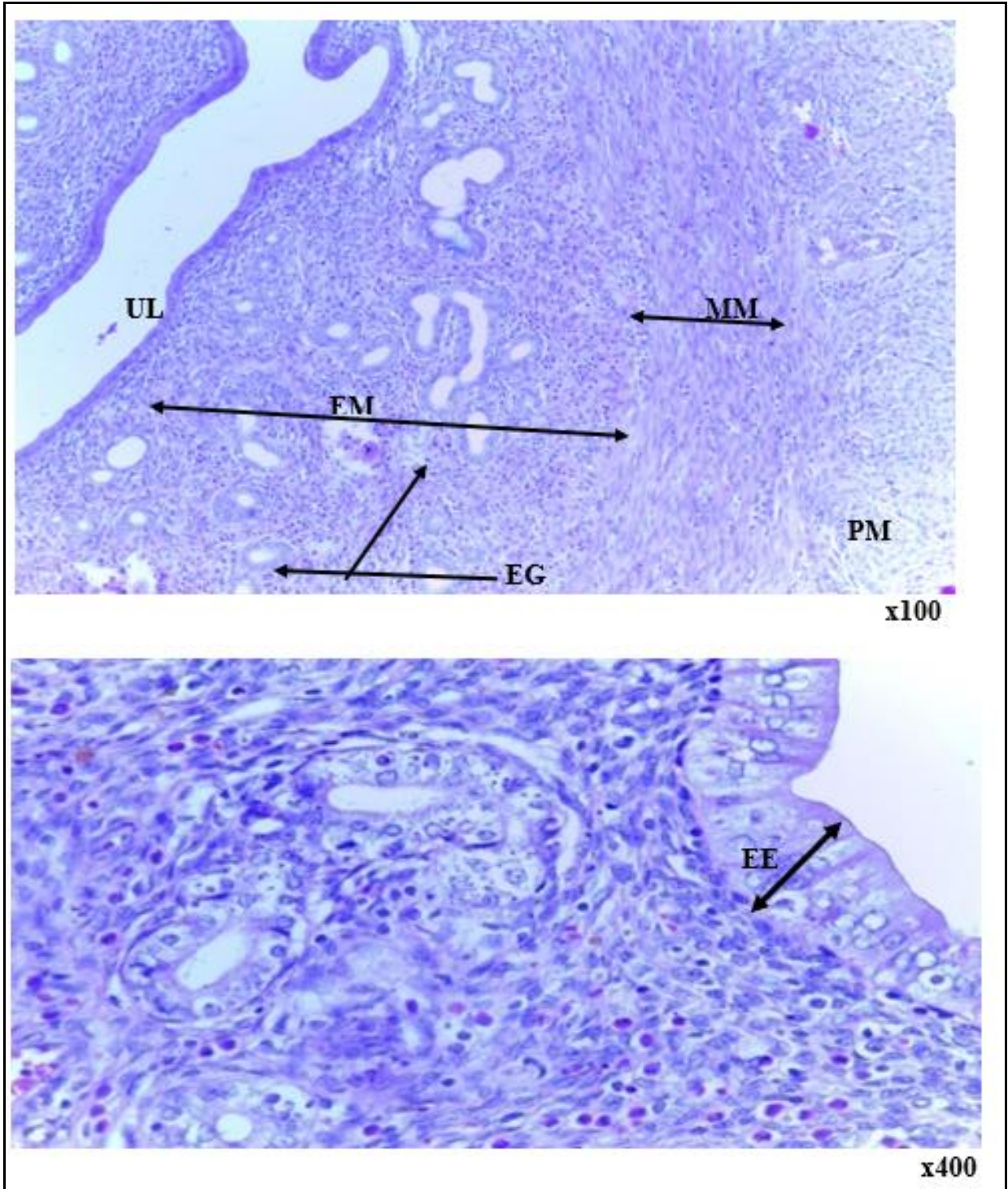


Fig 3a&b

Photomicrograph of the uterus of animals treated with combined leaf extracts of *Alchornea cordifolia* and *Lophira lanceolata* (500mg/kg body weight each) stained with H&E

Section of uterus showing well-differentiated endometrium (EM), with lining epithelium (EE) myometrium (MM) and endometrium (PM). The uterine lumen (UL) is widened, there are numerous glands in the endometrium (elongated narrow diameter and wider more spherical diameters), lamina propia is infiltrated with polymorphonuclear leucocytes.

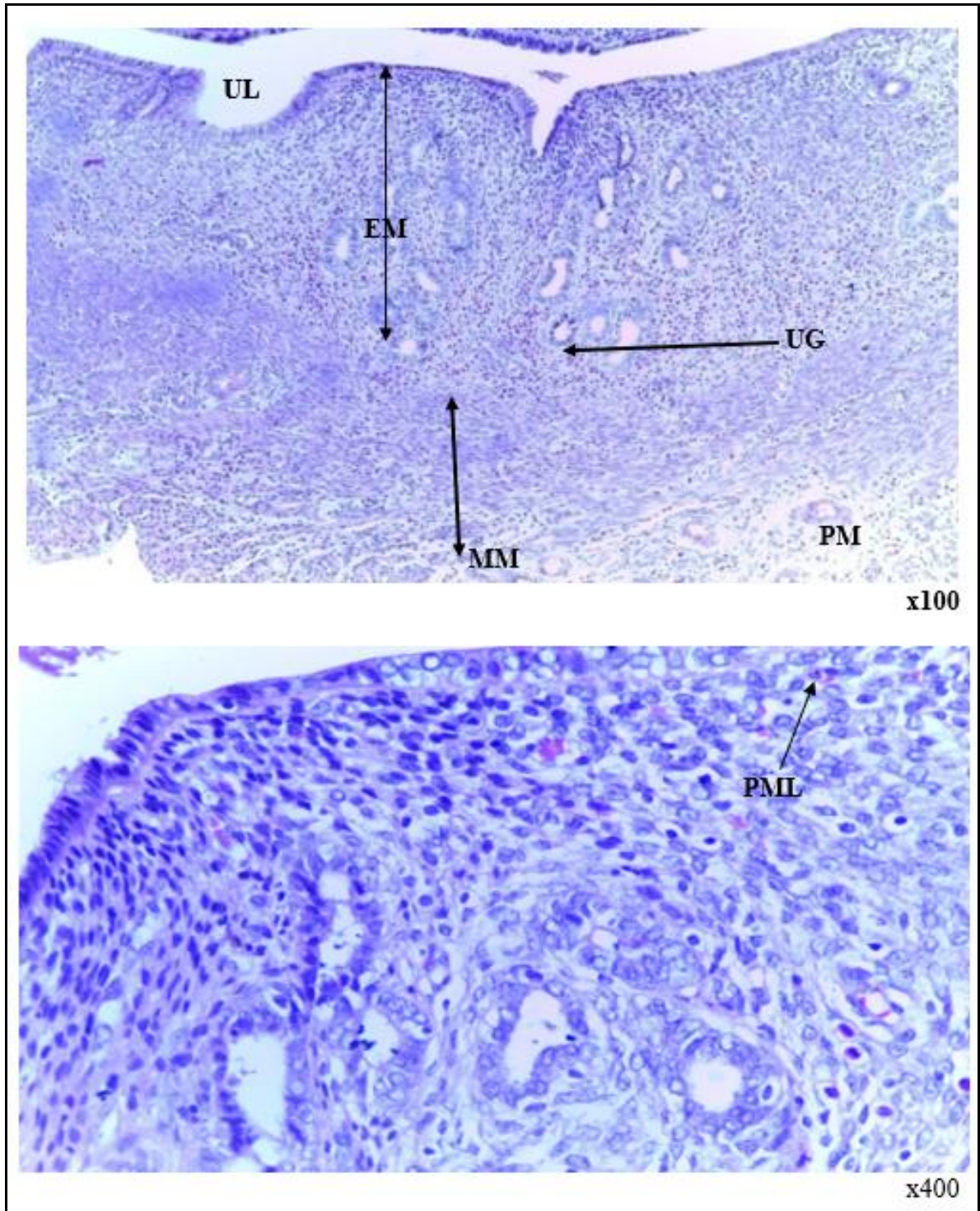


Fig 4a&b

Photomicrograph of the uterus of animals treated with leaf extract of *Lophira lanceolata* (500mg/kg body weight) stained with H&E.

Uterine section showing uterine lumen (UL), endometrium (EM), uterine glands (UG), myometrium (MM) and perimetrium (PM), and polymorphonuclear leukocytes

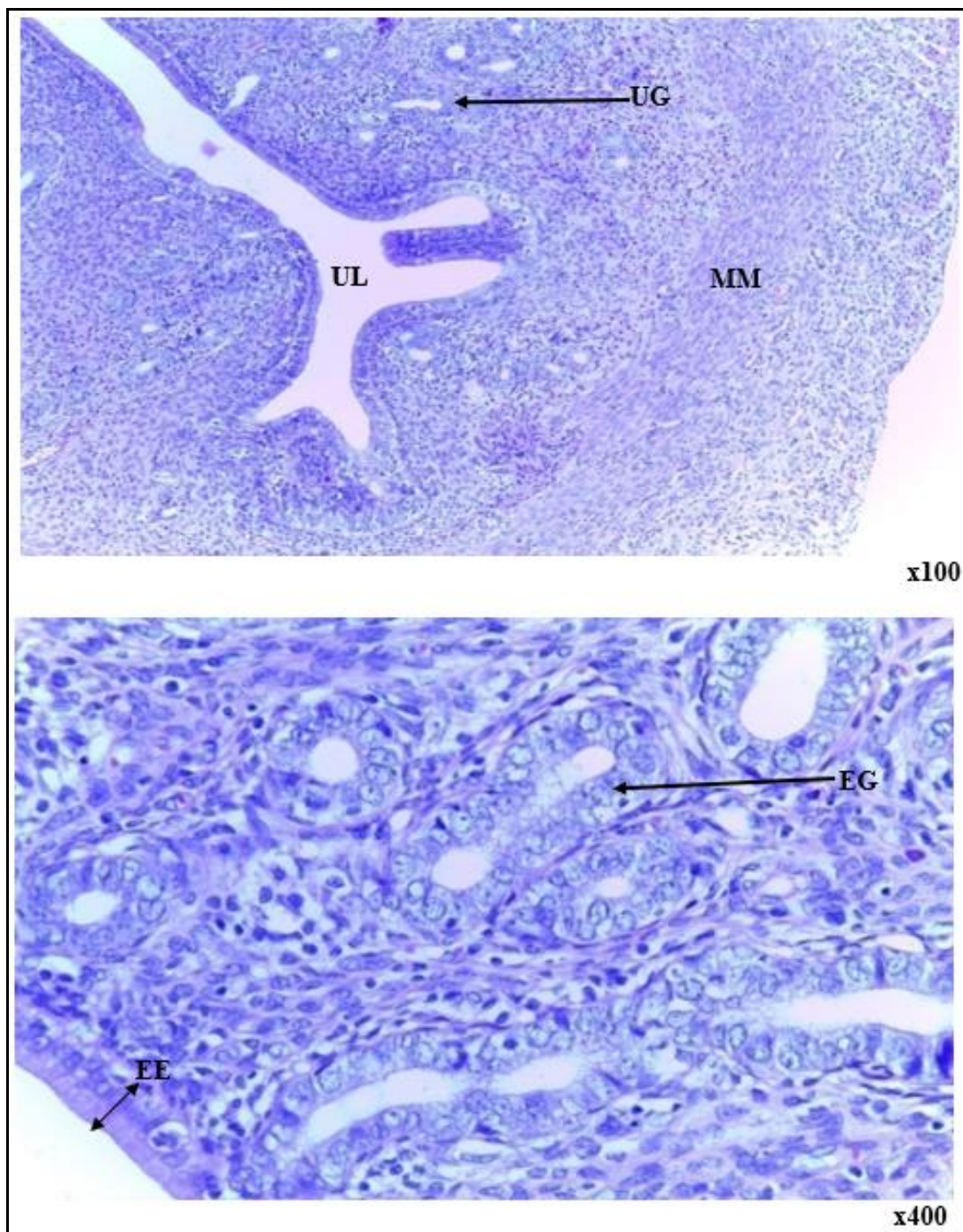


Fig 5a&b

Photomicrograph of the uterus of animals treated with leaf extract of *Alchornea cordifolia* (500mg/kg body weight) stained with H&E.

Showing uterine lumen (UL), lined with low columnar endometrial epithelium (EE), myometrium (MM), endometrial glands (EG).

DISCUSSION

An increase in body weight was observed for Group A (negative control), Group B (positive control) and Group D (*L. lanceolata* treated). This was however not significant at $p < 0.05$ when compared to the initial body weights (Table 2). This increase

can, therefore, be attributed to normal growth in these groups. In contrast to this finding, Etuk and Mohammed (2010), reported significant weight gain in rats following treatment with *L. lanceolata*. This effect was attributed to a probable increase in steroid hormones produced by the extract as

reported by them in earlier work (Etuk and Mohammed 2009). These researchers worked on adult male rats in one study and on both sexes in the second study, and the dosage administered was from 1000mg and above. Furthermore, stem bark, and not leaf extract was used. In this study, aged female rats were used, and the dosage used was 500mg/kg body weight, these factors could account for the disparity. In contrast to the above, the Group C (treated with combined plant extracts) and Group E (treated with *A. cordifolia*) group showed weight loss when final body weight was compared with the initial body weights. Since treatment with *L. lanceolata* alone resulted in weight gain, it can be safely assumed that this weight loss is attributable to *A. cordifolia* effects. Similar findings of weight loss were reported by Adjene *et al.*, (2012). This property may be important in its use for the treatment of menstrual cycle irregularities, perimenopausal and menopausal symptoms and improving fertility, because weight gain is also a feature of these states and contributes to, as well as results from hormone imbalance/deficiency in these conditions (McGrice and Porter, 2017). A 5-10% weight loss has been reported to be significant in improving hormone imbalances, and reducing the rates of spontaneous abortions and miscarriages (Lim *et al.*, 2007; Kulak and Polotsky, 2013; McGrice and Porter, 2017), and this property may account for its highly acclaimed efficacy in the management of reproductive associated problems. Ansah *et al.*, (2011), did not observe any significant change in their work on male rats.

A lot of plants used traditionally for the treatment of fertility-related problems, or for other purposes, have been shown to have effects on the rat oestrous cycle, some negative (disrupting the cycle), some positive (restoring the cycle), while others had

no effects. In this study, there was a restoration of regular cycling patterns in 80% of rats in all the treatment groups (Table 4), with a reduction in the cycle lengths to almost that found in younger rats (4-5days). The combined therapy was more effective, with the rats in this group having an average cycle length of 5.25days, followed by the *A. cordifolia* treated group with 6.0days and then the *L. lanceolata* treated group with 6.6 (Tables 5). The positive control group had an average length of 4 days, which is expected for regularly cycling young rats (4-5 days) as documented by previous researches (Maeda, 2000; Goldman *et al.*, 2007; Westwood, 2008; Paccola *et al.*, 2013). Cycle lengths for the negative control could not be quantified because of the irregularity. In aging female rats, a characteristic feature is the diminished capacity of oestradiol to induce GnRH and LH pro-oestrus surges that is critical for ovulation (Rubin, 2000). This occurs even before the loss of regular cyclicity is observed, and signals an imminent decline in reproductive function. In addition to this, the timing and amplitude of LH surge are also negatively affected by age (Downs and Wise, 2009). These age-related changes have been attributed mainly to changes at the hypothalamus (reduction in GnRH secreting neuronal activity), though decreased pituitary responsiveness to GnRH stimulation and gonadal steroid sensitisation to LHRH stimulation is also contributory (Rubin, 2000; Suckow *et al.*, 2005; Down's and Wise, 2009;). Reproductive senescence is now increasingly pointing towards a hypothalamic-pituitary dysfunction, a fact established in rodents (Gore, 2001; Rubin, 2000; Abd El-Maksoud and Moustafa, 2003) and currently proposed to be applicable to human females, and subject of ongoing researches (Down's and Urbanski, 2006; Yin and Gore, 2006; Down's and Wise, 2009). This is

because of striking similarities between middle-aged rats and middle-aged female pre- and perimenopausal women: a rise in FSH signals impending decline in reproductive function in both; the pulse and inter-pulse interval of LH release increases in both, irregular cycling lengths occurs in both; they both have normal or even elevated blood levels or serum concentration of oestradiol prior to transitioning into irregular cycling and finally, the capacity to induce GnRH/LH surges is diminished in both (Down's and Wise, 2009). Perhaps, the finding in this study implies that the plant extracts probably increased the sensitivity of the hypothalamus to circulating oestradiol, thereby reversing the age-related changes described above. It could also possibly imply that it increased the sensitivity of the pituitary to GnRH. As a result, GnRH production is increased, with a consequent increase in the level of LH, possibly leading to increased activity of aromatase and oestrogen production (Downs and Wise, 2009). It could also mean that the plant extracts have the capacity to increase the binding affinity of oestradiol and progesterone to their receptors (Skibola *et al.*, 2005). Other plant extracts have also been shown to restore regular cyclicality in rats (Ngadju *et al.*, 2013; Akanksha and Anuradha, 2014; Reyes *et al.*, 2016) while others have been reported to distort the cycle (Valsala and Karpagaganapathy, 2006; Skibola *et al.*, 2005; Ganguly *et al.*, 2007; Oyesola *et al.*, 2010; Nayanatara *et al.*, 2012; Abiodun *et al.*, 2012; Odirichukwu *et al.*, 2016). The uterus showed normal features consistent with the pro-oestrus phase of the oestrous cycle. The endometrium had medium-sized columnar epithelium cells lining dilated lumen, with minimal polymorphonuclear cell infiltration (Westwood, 2008). These features were shown in all the groups, with Group C (*A. cordifolia* treated group) having a wider lumen, with

more endometrial glands. Though the Group A rats (negative control), also had wide uterine lumen, the endometrial glands appeared fewer, and less prominent and, polymorphonuclear leucocytes were not seen. These do not indicate the pro-oestrus phase as aged females in pseudopregnancy states also show this feature (Greaves, 2012; Westwood, 2008). Therefore, interpretation of this finding should be in line with the findings in other parts of the reproductive system, as there must be synchrony in the structure and function of the different parts of the reproductive system. In other words, the individual organs may appear histologically normal, but when evaluated as a unit in terms of the phases of the oestrous cycle, they may fail to correlate (Goldman *et al.*, 2000; Li and Davis, 2007; Greeves, 2007). Amah *et al.*, (2011) reported that *Momordica charantia* had no effect on ovarian and uterine histology despite distorting the oestrous cycle. In contrast to this, Onyegeme-Okerenta and Essien, (2015), reported that *Millettia aboensis* leaf extract caused destruction of ovarian cells in a dose-dependent manner, *Aspilla africanus* disrupts was also reported to cause ovarian stromal cell degeneration, and disruption of the endometrium of the uterus (Oyesola *et al.*, 2010).

Conclusion

The findings in this study showed a non-significant weight loss in the combined extract and *A. cordifolia* treated groups, while there was insignificant weight gain in the negative control, positive control, and the *L. lanceolata* treated groups. The oestrous cycle was regularized in most of the rats in all the treatment groups. The features of the histology of the uterus were consistent with normal expectations of the pro-oestrus phase of the cycle. In conclusion, treatment with *L. lanceolata* and *A. cordifolia* restores normal cyclicality in aged irregularly cycling rats, therefore it

may be used in the management of menstrual cycle abnormalities, and sub-fertility associated with the peri-menopause, and other unpleasant symptoms associated with the perimenopause and menopause.

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