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Research Article
Fertility Enhancing Effects of Combined Leaf Extracts of *Lophira lanceolata* and *Alchornea cordifolia* in Menopausal Albino Wistar Rats

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Abstract

Background and Objective: *Lophira lanceolata* and *Alchornea cordifolia* are plants used for the treatment and management of perimenopausal, menopausal and menstrual cycle disorders, with claims of high efficacies by users. This study aimed at assessing fertility hormones of menopausal rats after the administration of the herbs. **Materials and Methods:** Twenty-five female albino wistar rats divided into Groups A, C, D and E consisting of irregularly cycling menopausal rats and Group B consisting of young regularly cycling rats were used for this study. Groups A and B served as negative and positive control respectively while Groups C, D and E were the experimental groups. Extract administration to the treated groups was for 21 days and animals were sacrificed on the 22nd day (pro-oestrus phase). Blood sample was collected for hormone assay and pituitary glands were dissected out for tissue processing. **Results:** Statistical analysis of level of serum oestradiol in groups B, C and D showed a significant increase ($p<0.05$) with the combined extract group recording the highest when compared to the negative control. The value of FSH in combined extract treated group was lower but not statistically significant at $p<0.05$ when compared with the negative control. Progesterone, LH and testosterone level was not significantly different ($p<0.05$) among the groups. Cells of the pituitary gland were normal. **Conclusion:** The findings of this study demonstrate that hormone pattern for the combined extract-treated group was similar to that of positive control. Therefore, combined leaf extracts of these herbs may be useful in the management of sub-fertility associated with peri-menopause and menopause.

Key words: Hormones, menopause, *Lophira lanceolata*, *Alchornea cordifolia*, pituitary gland

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of plant extracts as a fertility enhancer and management of fertility-related issues is now on the increase because of the shifting of attention from synthetic drugs to natural plant products[1]. *Lophira lanceolata* and *Alchornea cordifolia* are plants used for the treatment and management of perimenopausal, menopausal and menstrual cycle disorders, with claims of high efficacies by users. These plants have been reported to be effective in treating infertility and other conditions[2-4]. Etuk and Muhammed[4] reported fertility-enhancing effects of aqueous stem bark extract of *L. lanceolata* in male Sprague Dawley rats, sperm count was significantly increased, but motility and morphology were not affected. Similarly, follicle-stimulating hormone (FSH) level was significantly decreased but there was no corresponding significant increase in the levels of Luteinizing Hormone (LH) and testosterone. In another study on the reproductive and toxic effects of methanol extract of *A. cordifolia* leaf in male rats, a significant increase in testicular weight, sperm count and motility and serum testosterone levels was reported at dosages lower than or equal to 400 mg kg^{-1} b.wt. But doses above 800 mg kg^{-1} b.wt., were reported to have toxic potentials[3]. Mohammed *et al.*[5] proposed antidiabetic and hypolipidemic effects of the n-butanol fraction of the leaf extract of *A. cordifolia*. Eliakim-Ikechukwu and Riman[2] reported increasing elastic recoil of the wall of the aorta and thus, may reduce blood pressure. The hypothalamus secretes gonadotropin-releasing hormone which acts on the anterior pituitary and FSH and LH are released. FSH acts on the ovary and ovarian follicles develop, LH facilitates their growth and maturation. The follicles secrete estrogen, which inhibits further release of FSH but stimulates the secretion of LH, leading to ovulation. LH stimulates the growth of corpus luteum and secretion of progesterone by the corpus luteum. Progesterone inhibits luteinizing hormone-releasing hormone (LHRH) secretion, less LH is secreted and corpus luteum atrophy. FSH secretion is no longer inhibited, much FSH is produced and the next cycle begins. The pattern of FSH secretion throughout the estrous cycle is similar to that for LH[6,7]. A plant that holds the potential to be effective in the treatment of perimenopausal symptoms or delay in onset of menopause, menstrual cycle disorder and infertility is worth studying. Regularisation of the menstrual cycle, either in the perimenopausal period or earlier in the reproductive life, improves fertility. Alleviation of the symptoms associated with perimenopause and menstrual irregularities improve women's quality of life. This study aimed at assessing the effects of combined ethanolic leaf extracts of *L. lanceolata* and *A. cordifolia* on the fertility hormone profile (FSH), (LH), estrogen, progesterone, testosterone and the pituitary gland of menopausal albino Wistar rats.

MATERIALS AND METHODS

Study area: The study was carried out in the University of Calabar, Calabar, Nigeria and the duration of the research work was from February, 2016-November, 2017.

Preparation and administration of extract: Fresh leaves of *Lophira lanceolata* and *Alchornea cordifolia* were authenticated by a botanist in the Department of Botany, University of Calabar, Calabar and voucher numbers were deposited for *Alchornea cordifolia* (BOT/UC/HERB/010) and *Lophira lanceolata* (BOT/UC/HERB/013). The ethanolic extracts were administered as daily oral doses for 3 weeks as shown in Table 1.

Animal care and experimental design: All experimental procedures were carried out following the ethics of animal handling as approved by the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, (FAREC/PA/016A30217). Twenty-five healthy female albino Wistar rats were weighed and randomly divided into 5 groups (A, B, C, D and E) of five animals each. Groups A, C, D and E consisted of aged (menopausal) rats, 24-30 months old. Group B consisted of 5 young rats (between 4-6 months old). All the rats were kept in the animal house of the College of Medicine, University of Calabar, under standard laboratory conditions (12 h light/dark cycle, temperature, humidity). Water and food (rat chow) were allowed *ad libitum*. The rats were allowed a period of acclimatization of two weeks following which the experiment commenced. Extract administration was completed on the 21st day from the commencement of administration. Sacrifice was done serially from the 22nd day, on the pro-oestrus phase of the estrous cycle of each animal, using the chloroform inhalation method. The blood sample was collected by cardiac puncture for

Table 1: Dosages and pattern of administration of plant extracts

Groups n-5	Dosage/extract of administration
A	Normal saline
B	Normal saline
C	500 mg kg^{-1} b.wt. each of *L. lanceolata* and *A. cordifolia*
D	500 mg kg^{-1} b.wt. of *L. lanceolata*
E	500 mg kg^{-1} b.wt. of *A. cordifolia*

Daily vaginal smear observation was continued throughout the duration of this experiment

hormone assay (FSH, LH, estrogen, progesterone and testosterone) using ELISA Technique. The pituitary glands were dissected out, cleaned in saline and fixed in 10% buffered formalin for tissue processing using the H and E and PAS Orange G method for microscopic viewing.

Statistical analysis: Statistical analysis was done using one-way analysis of variance ANOVA, using SPSS version 21.0.

RESULTS

Hormonal assay: From the results, the level of estrogen (estradiol) was found to be significantly lower (77.75±11.58) at $p<0.05$ in Group A (negative control) when compared to all other groups. Group B (positive control) animals had the highest level of estrogen with a mean value of 139.20±8.63 (Table 2). Among the treated groups, Group C animals that received combined extract had the highest level of estrogen (131.35±4.18). This is followed by Group E animals (*A. cordifolia* treated) with 116.00±6.20. The Group with the lowest value was Group D (*L. lanceolata* treated) with 113.25±11.12 (Table 2). Compared with Group B, Groups C and E animals had lower levels estrogen, but these differences were not significant at $p<0.05$. However, Group D animals had significantly lower estrogen levels at $p<0.05$ when compared with Group B (Table 2). There was no significant difference in the level of progesterone among the groups at $p<0.05$. Group A animals had the highest level of progesterone (40.10±2.00), this value is closely followed by values from other groups (Table 2). Similarly, for the hormones testosterone and LH, there was no significant difference observed among the groups (Table 2). The level of FSH was observed to be significantly higher in Group A (Negative control) animals with a mean value of 55.00±2.86 when compared to Positive control (42.00±2.00) at $p<0.05$. Similarly, when compared to the treatment groups, Group A animals had a significantly higher level of FSH than the *A. cordifolia* treated group (37.14±4.77) at $p<0.05$. However, when Group A is compared with Group C animals that had combined therapy (49.17±1.87) and Group D animals that were treated with *L. lanceolata* extract (47.10±6.81), no significant difference was observed in the FSH levels at $p<0.05$. Compared to the positive control (Group B), there was no significant difference in the FSH value for all treated groups ($p<0.05$). When the levels of FSH in the treated groups were compared, the only significant difference observed was between the *A. cordifolia* and the combined extract group. The combined extract treated group had a significantly higher level of FSH compared to the *A. cordifolia* treated group (Table 2). For testosterone levels, positive control group had the highest value (1.40±0.21). Negative control animals (Group A) had a value of 1.07±0.16. Though the value is lower than that of Group B (positive control), the difference is not significant at $p<0.05$. When compared with negative control, all treated groups had lower but insignificant values of testosterone (combined therapy group, 0.55±0.26; *L. lanceolata* group, 0.55±0.23; and *A. cordifolia* group, 0.92±0.36) at $p<0.05$. In comparison with the positive control group, the combined therapy and the *L. lanceolata treated* groups (Groups C and D respectively) have significantly lower values of testosterone ($p<0$-05). Group E (*A. cordifolia* treated) on the other hand, showed no significant difference when compared to the positive control (Table 2).

Histological observations: Histological study of the anterior pituitary gland using Hematoxylin and Eosin (H and E) staining method showed normal histological features in the negative control (Group A-menopausal rats) which received normal saline. The cells of the anterior pituitary gland can be seen, clustered around sinusoids. Basophils are the more round, darkly stained cells, while the larger stained cells are the acidophils and the poorly stained chromophobes (Fig. 1). The anterior pituitary gland of positive control (Group B-young rats) which received normal saline showed prominent acidophils, numerous basophils and chromophobes, as well as sinusoids (Fig. 2). No difference was observed in the features of the pituitary gland in this group of young rats when compared to the old menopausal rats of the negative control group (Fig. 2). The pituitary gland of group C rats treated with 500 mg kg^{-1} b.wt., each of combined ethanolic leaf extract of

Table 2: Hormone levels in the various groups after treatment

Groups	Estradiol (pg mL^{-1})	Progesterone (ng mL^{-1})	LH (mIU mL^{-1})	FSH (mIU mL^{-1})	Testosterone (ng mL^{-1})
A	77.75±11.58	40.10±2.00	33.78±1.38	55.00±2.86	1.07±0.16
B	139.20±8.63*	37.45±2.27	33.52±0.54	42.00±2.00*	1.40±0.21
C	131.35±4.18*	37.93±3.94	31.35±2.05	49.17±1.87[b]	0.55±0.26[a]
D	113.25±11.12*[a]	33.67±4.26	34.98±0.89	47.10±6.81	0.55±0.23[a]
E	116.00±6.20*	35.78±4.35	34.84±1.07	37.14±4.77*	0.92±0.36

Values are expressed as mean±SEM, n = 5, *Significantly different from negative control (Group A) at $p<0.05$, [a]Significantly different from positive control (Group B) at $p<0.05$, [b]Significantly different from *A. cordifolia* treated (Group E) at $p<0.05$

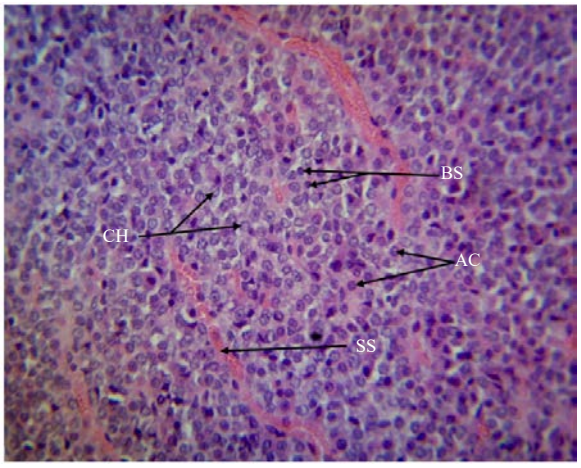

Fig. 1: Photomicrograph of the anterior pituitary gland of Group A (negative control) animals, given normal saline stained with Haematoxylin and Eosin (H and E) x400

Section of anterior pituitary gland showing the smaller, more rounded, and darker basophils (BS), the larger acidophils (AC) with pink cytoplasm and the poorly stained chromophobes (CH). Blood sinusoids (SS) are seen around cell groups

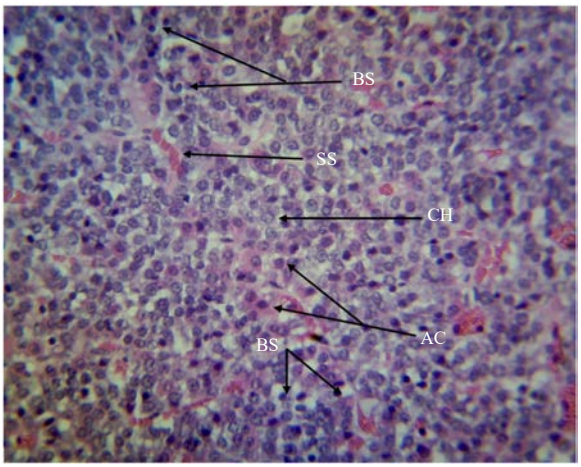

Fig. 3: Photomicrograph of anterior pituitary gland of Group C animals treated with combined extracts of Alchornea cordifolia and *Lophira lanceolata* (500 mg kg^{-1} b.wt. each). H and E (x400)

Showing normal anterior pituitary gland with acidophils (AC), basophils (BS), numerous chromophobes (CH) and sinusoids (SS) observed

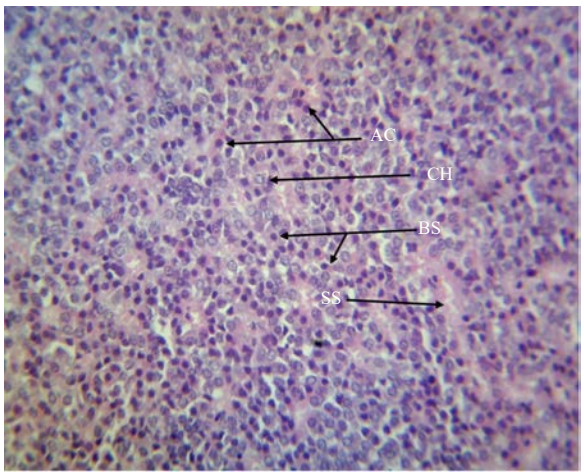

Fig. 2: Photomicrograph of anterior pituitary gland of Group B (positive control) animals given normal saline stained with H and E (x400)

Showing section of anterior pituitary densely populated with basophils (BS), acidophils (AC) and chromophobes (CH) with numerous sinusoids (SS)

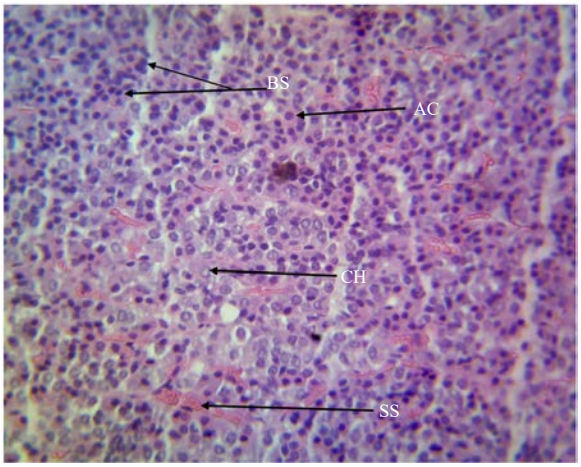

Fig. 4: Photomicrograph of the anterior pituitary gland of Group D animals treated with *Lophira lanceolata* (500 mg kg^{-1} b.wt.) stained with H and E (x400)

Normal features of anterior pituitary gland, with acidophils (AC), basophils (BS), chromophobes (CH) and sinusoids (SS) arranged around cell clusters. There are numerous cells, with basophils appearing to be more in number

A. chordifolia and *L. lanceolata* showed no abnormal features but prominently seen are basophils, acidophils and numerous chromophobes, with sinusoids around cell groups (Fig. 3). No apparent difference observed when compared to either negative control (Fig. 1) and positive control groups (Fig. 2). The anterior pituitary gland of Group D rats treated with *L. lanceolata* showed numerous basophils, acidophils

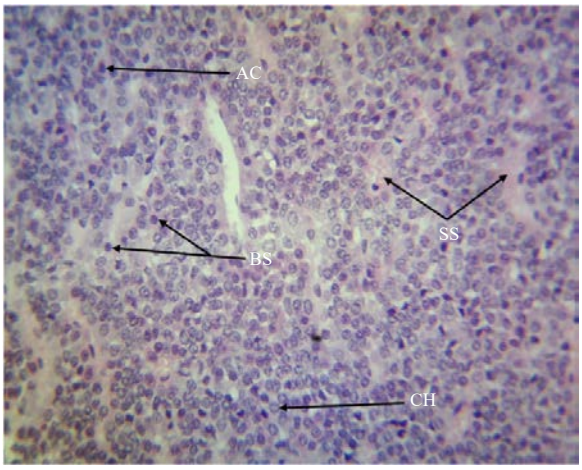

Fig. 5: Photomicrograph of the anterior pituitary gland of Group E animals treated with 500 mg kg^{-1} body weight of *Alchornea cordifolia* leaf extract. H and E (x400)
Section of anterior pituitary gland showing few acidophils (AC), basophils (BS), numerous chromophobes (CH) and sinusoids (SS)

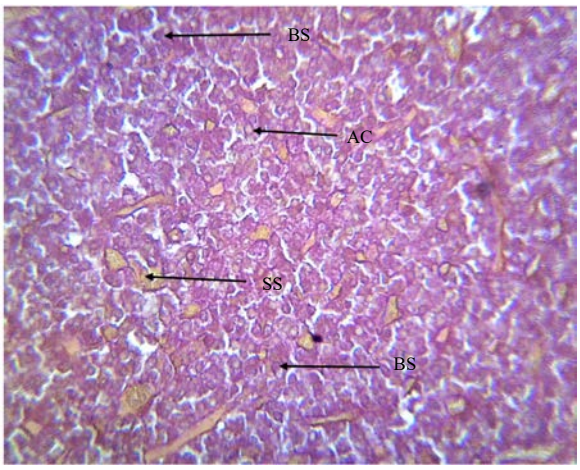

Fig. 7: Section of the anterior pituitary gland of Group B (positive control) animal stained with PAS (Periodic acid Schiff) Orange G (x400)
Anterior pituitary showing basophils (BS), acidophils (AC), scanty chromophobes (CH) and sinusoids (SS)

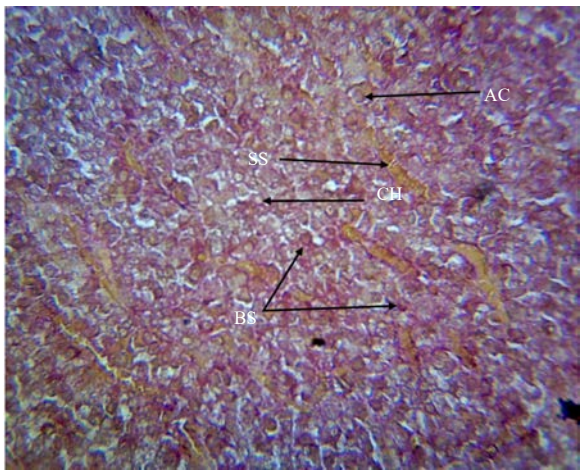

Fig. 6: Section of the anterior pituitary gland of Group A (negative control) animal stained with PAS ORANGE G (Periodic Acid Schiff Orange G) (x400)
Anterior pituitary showing larger acidophils (AC), smaller purple colored basophils (BS), chromophobes (CH) without stain and sinusoids

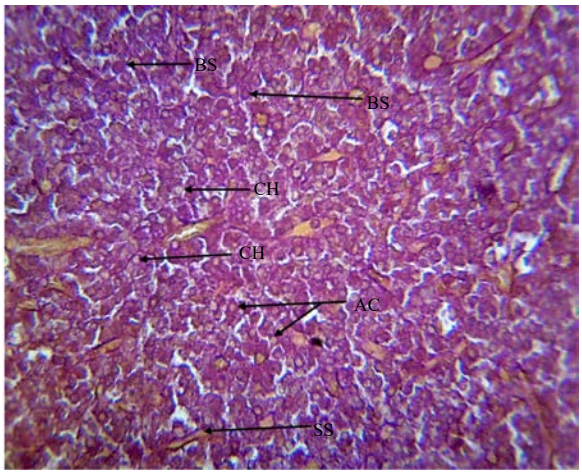

Fig. 8: Section of the anterior pituitary gland of Group C animals treated with combined extracts of *Alchornea cordifolia* and *Lophira lanceolata* (500 mg kg^{-1} b.wt. each) stained with PAS (Periodic acid Schiff) Orange G (x40)
Section of the pituitary gland showing acidophilus (AC), numerous basophils (BS), chromophobes (CH) and sinusoids (SS)

chromophobes and sinusoids. Cellular boundaries are clearly defined and no alteration in cytoarchitecture is noted (Fig. 4). In group E rats treated with *A. cordifolia*, the anterior pituitary gland showed the three cell groups prominently. No abnormal features are seen (Fig. 5). No pathology was observed.

Histochemical studies: The anterior pituitary gland of Group A animals given normal saline showed normal features with acidophils, basophils and chromophobes. The prominent sinusoids also showed in Fig. 6. Sections of the pituitary gland of Group B animals given normal saline showed prominent

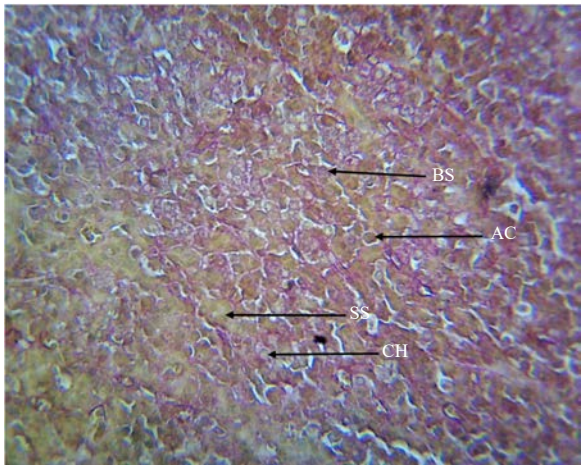

Fig. 9: Section of the anterior pituitary gland of Group D animals treated with leaf extracts of *Lophira lanceolata* (500 mg kg^{-1} b.wt.) stained with PAS (Periodic acid Schiff) Orange G (x400)
Slide shows a normal anterior pituitary gland with acidophils (AC), basophils (BS), chromophobes (CH) and sinusoids (SS)

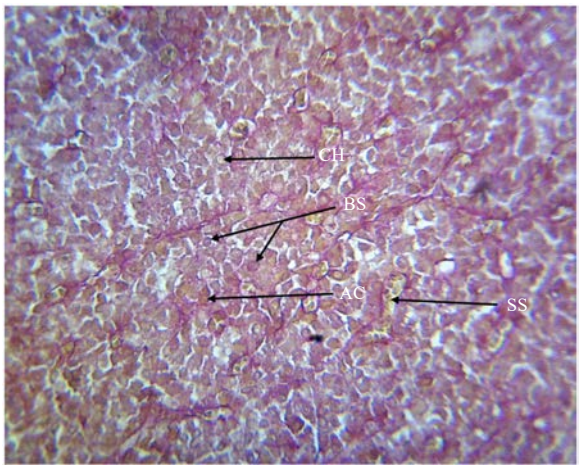

Fig. 10: Section of the anterior pituitary gland of Group E animals treated with combined extracts of *Alchornea cordifolia* (500 mg kg^{-1} b.wt.) stained with PAS (Periodic acid Schiff) Orange G (x400)
Shows normal pituitary gland histology. Acidophils (AC), basophils (BS), chromophobes (CH) and sinusoids (SS) observed

sinusoids, basophils, acidophils and chromophobes. No pathological feature was observed (Fig. 7). Sections of Section of the pituitary gland of Group C treated with combined extract of *A. cordifolia* and *L. Lanceolata* 500 mg kg^{-1} each showed normal pituitary gland histology with acidophilus, numerous basophils, chromophobes (CH) and sinusoids (Fig. 8). The section of the anterior pituitary of Group D animals treated with 500 mg kg^{-1} of *L. lanceolata* extract showed normal anterior pituitary gland with acidophils (AC), basophils (BS), chromophobes (CH) and sinusoids observed (Fig. 9). Section of the pituitary gland of Group E animals treated with 500 mg kg^{-1} *A. cordifolia* extract histology showed normal pituitary cytoarchitecture, acidophils, basophils, chromophobes and sinusoids observed (Fig. 10). No cellular distortion was observed in any of the groups.

DISCUSSION

In this study, the negative control group rats had lower serum levels of estrogen compared with the positive control group rats, a difference that is statistically significant at $p<0.05$. All the animals in groups B, C and D showed a significant increase ($p<0.05$) in the level of serum oestradiol when compared to the negative control (Table 2). Interestingly, the value of FSH was reduced in all the treatment groups, though not statistically significant in the *L. lanceolata* and combined extract-treated groups when compared with the negative control. In young regularly cycling rats, serum oestradiol level is expected to begin to rise from metoestrus through dioestrus and continues to rise significantly until the morning of pro-oestrus, reaching peak values by mid-pro-oestrus because of the growth of ovarian follicles[8]. With the increase in the serum level of oestradiol observed in the treated groups, it can be said that these plant extracts have properties that increase the serum level of the hormone. This probably resulted from the extracts facilitating the production of the hormone or inhibiting its metabolic breakdown. Possible mechanisms for these could include increasing the affinity of the receptors, altering the expression or posttranslational modification of any one of the cytochrome P450 enzymes involved in steroid biosynthesis or in the metabolism of oestradiol[9] and increasing aromatase activity[10]. Another possible explanation may be the extracts utilized a pathway outside the HPO axis for the increased oestradiol level as observed by Etuk and Mohammed[4]. The group that received combined extract showed the highest level of serum oestradiol among the treatment groups. This shows that the herbs work better in combination than when administered individually, implying that they potentiated each other's actions or worked in synergy. The phytochemicals contained in plants are said to study better in combination than in isolation[11] partly because their individual effects are summed up[12] and also because multiple targets can be acted upon at the same time[13], as rats age (peri-menopause and early menopause period) FSH[14]. The reduction in the value of FSH

though not significant by statistics, might still be able to produce biological effects, as shown by the oestradiol levels and the estrous cycle results. The group with the most striking reduction in FSH level was the *A. cordifolia* leaf extract treated, showing again that this plant has a more potent effect than *L. lanceolata*. Progesterone level was not significantly different ($p<0.05$) among the groups (Groups A-E). This is a normal finding, as the level of this hormone is not significantly affected by age in rats, especially in the morning at the pro-oestrus phase of the estrous cycle. Basal values of LH remain low through the morning of pro-oestrus, but by afternoon, a pre-ovulatory surge occurs in response to peak values of estrogen, leading to ovulation[8]. The values of testosterone were not significantly different among the groups in this study.

In this study, the serum hormone values for oestradiol, progesterone, FSH, LH and testosterone for the negative control group were observed to be in line with the pattern documented by other researchers. In middle-aged and aging rats, the normal cyclic changes in the levels of oestradiol, progesterone, LH and FSH were not observed and when compared to younger, regularly cycling rats, differences were observed[15,16]. Serum estrogen levels are either reduced or about the same when values in aging rats are compared to younger regularly cycling rats, depending on the age of the rat[15,17]. Other plant extracts have been shown to work better in combination in exerting their action[18-21]. Other studies also showed plant extracts reducing the level of estrogen. Akanksha and Anuradha[22] reported that seed powder Tephrosia purpurea caused a reduction in the levels of estrogen and progesterone in polycystic ovarian syndrome (PCOS) induced rats. Osonuga *et al*.[23] reported a dose-dependent reduction in the levels of estrogen and progesterone following administration of *Momordia charantia*, *Alstonia boonei* caused a dose-dependent and duration-dependent increase in the blood levels of estrogen and progesterone[10,24]. In this study, no pathologic feature was observed in all the groups. The cell types were clearly shown in the H and E studies (Fig. 1-5). The relative proportion of these cells defer among the groups, for example, the pituitary gland of positive control animal showed higher cellularity, with basophils appearing to be relatively more in number when compared to the other cells, Group C that received combined extract appeared to have a lot of chromophobes, while Group D had more basophils. The histological features of the pituitary gland as shown by PAS Orange G technique was normal for all the groups in this study (Fig. 6-10). The various cell types (basophils, acidophils and chromophobes) with sinusoids were shown. These findings cannot be interpreted in isolation as the level of secretory activities determines the staining characteristics of the cells. Cells that have just emptied their granules or when secretory activity is increased, take on chromophobic characteristic due to the relative abundance of secretory organelles (endoplasmic reticulum Golgi apparatus) and relative lack of granules[25]. Changes in the pituitary cells are generally secondary to altered feedback pathways to the pituitary caused by hormone imbalance elsewhere. In line with this, Abd El-Maksoud and Moustafa[26] in their study aimed at finding the age-related changes in the anterior lobe of the pituitary gland observed no significant change in all the anterior pituitary gland cells, the only exception being in the somatotrophs which showed regressive changes in young and aged female rats. This study was limited to the histological features of the pituitary cells. Immunohistochemical studies of the gonadotrophin content of gonadotropes should be assessed following the administration of these plants and phytochemical screening for the bioactive compounds involved.

CONCLUSION

The findings of this study demonstrate that hormone patterns were similar to that of positive control and no abnormal feature was noted in the histology of the pituitary gland studied. The combined extract was the most potent, gave the best results relative to the individual herbs. Therefore, a combined extract of ethanolic leaf extracts of *A. cordifolia* and *L. lanceolata* may be useful in the management of sub-fertility associated with the peri-menopause and menopause.

SIGNIFICANCE STATEMENT

This study showed synergistic effects of *Lophira lanceolata* and *Alchornea cordifolia* when used in-combination in the peri-menopausal/menopausal rat model. These effects restored normal cyclicity in irregularly cycling rats therefore may be useful in enhancing fertility during peri-menopause and menopause.

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