Journal of Science and Practice of Pharmacy
December 2015; 2 (1): 55-63 Available at http://www.jsppharm.org
ISSN: 2449-0458 (print); 2449-0466 (electronic)

Original Research Article

Preliminary endocrinological, histological and haematological investigation of *Alchornea laxiflora* (Euphorbiaceae) leaf extract effects on the ovary, uterus and cervix of mouse models

Enitome E Bafor[1*], Stacy E Eyohan[1], Osemelomen Omoruyi[1], Uloma B Elvis-Offiah[2], Buniyamin Ayinde[3], Gerald I Eze[4], Isibhakhomhen V Okosun[1], Philip A Obarisiagbon[1], Osamwonyi Igbinuwen[5] and Kadiri P Braimoh[6]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria; [2]Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Nigeria; [3]Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria; [4]Department of Anatomy, School of Basic Medical Sciences, University of Benin, Nigeria; [5]Haematology Department, University of Benin Teaching Hospital, Benin City, Nigeria; [6]Histopathology Department, University of Benin Teaching Hospital, Benin City, Nigeria
Nigeria.

*For correspondence: *Email:* enitome.bafor@uniben.edu

Abstract

Purpose: The decoction from the leaves of *Alchornealaxiflora* (AL) is often used by women in Edo state, Nigeria to prevent preterm birth (miscarriage). This study was therefore undertaken to investigate the effect of the extract on the female reproductive structures.
Methods: The methanol leaf extract of **AL** (100 mg/kg and 1000 mg/kg p.o.) was administered for 6 days at 10 a.m. daily to non-pregnant female mice using 0.2 ml of 10 % tween 80 as negative control and progesterone (10 mg/kg s.c.) as positive control. The weights of the animals were taken before administration, daily during administration and after **AL** administration.
Results: The findings reveal that the lower dose of **AL** (100 mg/kg) showed progesterone-like effects on the ovaries, uterus and cervical glands causing increased number and development of ovarian follicles and proliferation of the uterine endometrial glands while the high dose resulted in extrusion of the ovum from the Graafian follicle and resulted in glandular hyperplasia and atrophy of the uterine stroma.
Conclusion: **AL** has been shown to exert progesterone-like effects at low dose and this may account for its use by traditional healers in Nigeria for maintaining pregnancy.

Keywords: miscarriage, preterm birth, progesterone, mice

Indexing: Index Copernicus, African Index Medicus

Introduction

In traditional African communities, herbal plants are primary sources of medicines and are useful in the treatment of various diseases[1]. About 80% of the global population still depends on herbal drugs for their primary health needs and choice of medicinal plants is usually not based on a random selection but rather on knowledge passed from generation to generation. In addition, medicinal plants are cheap and readily available and this has increased dependence on such alternatives to orthodox medicine [1].

While several health issues require attention, preterm birth remains a problem especially in developing societies. Preterm birth (PTB)often referred to by some as miscarriage, is considered one of the significant causes of maternal and neonatal morbidity and mortality[2]. Low- and middle-income countries are significantly affected by PTB and have been reported to show a higher incidence of PTB [3]. *Alchornealaxiflora* (Benth) Pax and Hoffman, of the

Euphorbiaceae family is one of the plant remedies used in South-South Nigeria for the prevention and management of PTB (personal communication with traditional healer Mr. Eyohan, Benin City, Edo State Nigeria).It is called "uwenriotan" by Edo natives, as "Ububo" in Igbo language and as "Ijan" or 'Opoto' by Yoruba tribe of Nigeria. It is a broad-leaved shrub that sheds its leaves annually and grows to a height of about 6–10m high. It grows naturallyin Nigeria, in DR Congo, throughout East Africa and in Zimbabwe as well [4].The leaves from *A.laxiflora*are collected fresh, preferably at dawn and dusk. The leaves are then washed and squeezed into a glass full of water. The resulting decoction is filtered and mixed with some quantity of 'calabash chalk' or 'calabash clay' popularly called 'eko' in Nigeria and taken immediately. It is to be taken twice a day (morning and evening) for a period of six (6) months after conception has taken place (personal communication with traditional healer Mr. Eyohan).While *A. laxiflora* is popularly used for several ailments. There has been no report on its effect on the female reproductive system. Owing to the urgent need for drugs or therapies to prevent PTB in order to reduce the global incidence of maternal and neonatal mortality and morbidity [5], this study posits itself in the current goal of the United Nations in contributing to the improvement of women's health.

This study is therefore designed to investigate the effect of the plant extract on several female reproductive organs in order to provide an insight and possible justification for its use as an 'anti-miscarriage' agent or as a tocolytic agent. This is necessary as it will provide information into possible pathways through which the plant exerts its effect on reproductive function at hormonal, tissue and cellular level.

Methods

Preparation of the plant material

Fresh whole shrubs of *A.laxiflora* (**AL**) were collected from Egor, Benin city, Edo state, Nigeria in the month of April. It was initially identified by Dr H. A. Akinnibosun of the Department of Plant Biology and Biotechnology and authenticated by Prof. B.A Ayinde, of the Department of Pharmacognosy, both of the University of Benin, Benin City. The plant was given a voucher specimen number of UBHe0286. The leaves were cleaned and then shade-dried for two weeks to a constant weight. The dried leaves were powdered using the milling machine at the Department of Pharmacognosy, University of Benin, Nigeria.

Extraction of the plant material

The powdered material (855.24 g) was transferred into a Soxhlet apparatus and was subjected to exhaustive extraction using methanol as a solvent. The extracts obtained were concentrated by means of a rotary evaporator set at 40°C, and the resulting concentrate further dried in an oven set at 40°C.The resulting extract weighed 122.3 g (% yield of 14.3 w/w). The extract was transferred into a pre-weighed sample container and stored in the refrigerator (4 °C) till needed.

Animals

Twenty (20) healthy non-pregnant female mice (18-20 g) were purchased from the laboratory animal unit of the Department of Pharmacology and Toxicology, University of Benin, Edo state, Nigeria where they were housed during the period of the experiment. All animals were maintained as much as possible under standard approved conditions and were given access to food and water *ad libitum*. Cleanliness of the housing environment was maintained daily. The animals were equally handled according to standard guidelines for use of laboratory animals in experiments [6] and as approved by the Ethical Committee Faculty of Pharmacy, University of Benin, Nigeria (approval number: EC/FP/016/06).

Experimental protocols

Non-pregnant female mice were randomly divided into groups and assigned group numbers of 1 – 4. Group 1 served as the negative control group and received 0.2 ml of 10 % tween 80 (the vehicle for the extracts) orally with orogastric tube; Group 2 animals received **AL** (100 mg/kg p.o.); Group 3 received **AL**(1000 mg/kg p.o.);and group 4 served as the positive control group and received progesterone (10 mg/kg s.c.).

All animals of the different groups were treated for a period of 6 days at 10 a.m. daily. The animals were food fasted 3 h prior to treatment and 1 h after treatment daily. The animals were monitored after treatment or 3 h for changes in normal activity. After 3 h monitoring the animals were returned to their housing unit. The weights of the animals were also collected daily before and during treatment and on the morning of euthanasia.

Blood Sample and Tissue Collection

On the 7^{th} day, the animals were anaesthetized with urethane (10 mg/kg i.p.).Blood samples (about 1.5–2 ml) were collected via cardiac puncture into lithium heparin sample bottles for hormonal and haematological analyses. The uterine horns, ovaries and cervices were also carefully collected and placed

in appropriately labelled sample bottles containing 10% formalin for histological analysis.

Measurement of reproductive hormones

The hormonal assay was done with the aid of an automated qualitative test {Minividas Analyzer, (VIDAS Kit), France} on serum or plasma (lithium heparin), using the Enzyme Linked Fluorescent Assay (ELFA) technique as described [7-8].

The reaction medium was first cycled in and cycled out of the SPR several times. The process was then repeated with samples which had been previously transferred into a well containing alkaline phosphatase-labelled anti-follicle stimulating hormone, and anti-luteinizing hormone; as well as anti-oestradiol and anti-progesterone antibodies (conjugate). The cycling of the sample/conjugate mixtures were necessary to increase the reaction speed. The antigen was then bound to the antibodies coated on the SPR and then to the conjugate which had a "sandwich-like" formation. At this stage some components remained unbound and a substrate, 4-methyl-umbelliferyl phosphate was formed. The unbound components were removed during washing steps and at the final detection step the substrate was cycled in and out of the SPR as well. The substrate was hydrolyzed by a catalytic enzyme into a fluorescent product called 4-methyl-umbelliferone which was measured at 450 nm. The intensity of the fluorescence was observed to be proportional to the concentration of the antigen present in the sample.

The assay procedure described was implemented for all the hormones to be analyzed however, the reaction and testing time varied. The assay for follicle stimulating hormone (FSH)and luteinizing hormone (LH), was completed within approximately 40 min; progesterone assay was completed within 45 min while oestrogen was completed within 1 h. The results from the assay were automatically calculated by the analyzer with the aid of calibration curves using a 4-parameter logistics model as earlier described. The results were then printed out and analyzed.

Tissue histological analyses

Uterine horns, cervices and ovaries were fixed in 10% formalin solution and cut into short segments using the paraffin technique as described [9-10]. Briefly, sections of 5 µm thicknesses were cut and stained using routine hematoxylin and eosin method. All organs were observed and measured on hematoxylin and eosin stained slides, and 3 randomly chosen areas of the sections were measured per slide. The fixed tissue sections were processed for histopathological examination. The tissue sections were washed in tap water for 30 min, and later dehydrated in graded concentration of ethanol, then passed through two changes of equal volumes of chloroform; xylene mixture and cleared in two changes of pure xylene. The sections were impregnated in two changes of molten paraffin wax at 60 °C to remove the clearing agents, and embedded in the molten paraffin enblocked in a mould. The blocks were allowed to solidify. Solid blocks of tissues in paraffin wax were sectioned to the required thickness of 15µm, using microtome (Behr Manning Troy, N.Y). The embedded specimens were cut into thin paraffin ribbons and smeared on the slide and stained with haematoxylin (Sigma, U.S.A) and eosin (Sigma, USA) following a standard staining procedure. The prepared slides and processed specimens on the slides were examined with an Olympus optical microscope (Germany). Photomicrographs of the changes were made with a Samsung digital camera SV 120,12 Mega pixels attached to the microscope and connected to a computer by a USB cord [11-12]

Data analysis

All data were represented as the mean ±standard error of mean (S.E.M.). The one-way analysis of variance (one-way ANOVA) with Dunnett's post hoc testing for group differences was employed. Differences were considered to be significant with *p*-values ≤ 0.05.

Results

Effect of AL on hormonal levels

The result obtained in Table 1 showed that **AL** (1000 mg/kg) reduced significantly the levels of luteinizing hormone (LH) and progesterone (**p< 0.01 and*p < 0.05 respectively), whereas at 100 mg/kg, a significant increase ($^{\#}$ p < 0.05) in the levels of LH in the plasma was observed and a concurrent decrease in progesterone levels (* p < 0.05) when compared with the control.

Effect on hematological indices

Overall, **AL**(1000 mg/kg) induced slight increases in the values of the indices while **AL** (100 mg/kg) produced slight decreases in the values which were similar to that produced by progesterone compared to control. However, these were all statistically insignificant compared with the control.

Effect of extract on the cellular structure of ovaries

AL (100 mg/kg) was observed to increase the number and development of the follicles while at 1000 mg/kg an extrusion of the ovum from the Graafian follicle was observed compared to the control (Figure 1). Progesterone displayed a similar effect to the low dose of **AL** causing an increase in the number and development of follicles (Figure 1).

Table 1: Effect of AL and progesterone on hormonal levels after 6 days of administration

Hormones	Control	AL (100 mg/kg)	AL (1000 mg/kg)	Progesterone (10 mg/kg)
FSH	3.14 ± 0.58	3.04 ± 0.49	2.83 ± 0.46	2.53 ± 0.61
LH	2.98 ± 0.70	3.52 ± 1.08$^{\#}$	1.83 ± 0.5**	2.75 ± 0.66
PROG	1.46 ± 0.22	0.74 ± 0.14**	0.88 ± 0.26*	1.3 ± 0.29
OEST	25.14 ± 2.58	25.98 ± 3.53	23.08 ± 2.53	22.33 ± 3.35

Values are expressed as mean ± SEM (*n = 5 animals),* $^{*}p<0.05$; $^{**}p<0.01$was considered to be significantly decreased compared with the control using one-way ANOVA; $^{\#}p<0.05$was considered to be significantly increased compared with the control using one-way ANOVA.*FSH, follicle stimulating hormone ;LH, luteinizing hormone; PROG, progesterone; OEST, oestrogen.*

Table 2: Effect of AL on haematological parameters after 6 days administration

Parameters	Control (Water)	AL (100 mg/kg p.o.)	AL (1000 mg/kg p.o.)	Progesterone (10 mg/kg s.c.)
HCT	41.33 ±1.76	39.61 ± 0.52	42.00 ± 0.95	39.33 ±1.76
Hgb (g/dl)	13.33 ± 0.56	13.11 ± 0.20	19.82 ± 6.05	13.22 ± 0.42
MCV (fl)	43.98 ± 2.19	40.62 ± 1.51	44.84 ± 1.83	40.2 ± 0.76
MCH (pg/cell)	31.25 ±0.85	31.32 ± 0.34	31.44 ± 1.74	30.94 ± 1.25
MCHC (g/dl)	41.08 ±1.00	39.36 ± 0.43	40.34 ± 0.80	38.63 ± 1.73
NEUT(x 10^3/µl)	46.33 ± 3.18	40.4 ± 1.94	41.82 ± 2.69	28.00 ± 9.02
LY (%)	58.75 ± 13.9	52.6 ± 2.54	60.42 ± 10.44	65.00 ± 12.66
PLT (x 10^3/µl)	108.75 ± 2.39	105.33 ± 3.71	112.00 ± 7.37	108.75 ± 2.39

Values are expressed as mean ± SEM (n = 4 - 5); control group received 0.2 ml of 10 % tween 80 (the vehicle for the extracts) orally. **WBC**, white blood cells; **LY**, lymphocytes; **Hgb**, haemoglobin; **HCT**, haematocrit; **MCV**, mean cell volume; **MCH**, mean cell haemoglobin; **MCHC**, mean cell haemoglobin concentration; **PLT**, platelets

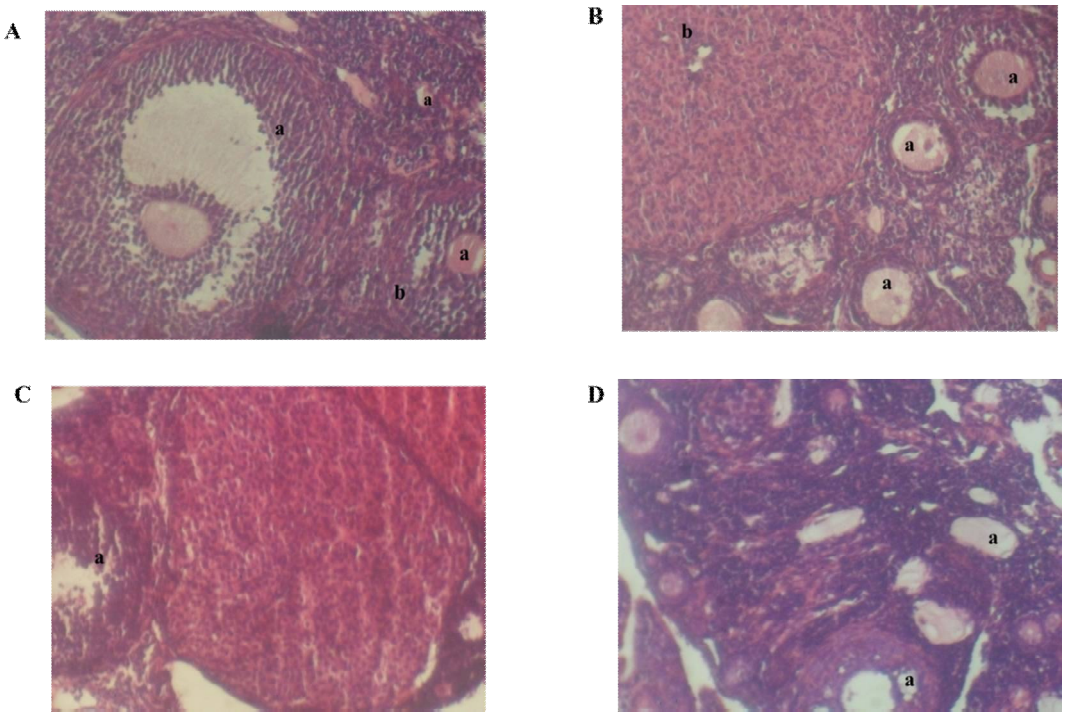

Figure 1: Mouse ovary H & E x 100: Representative images of haematoxylin and eosin staining of ovarian tissue from mice after 6 days of AL treatment. A) Control (10% Tween 80), a = follicles at the different stages of development and b = stroma; (B) AL (100 mg/kg), a = follicles at early stages of development and b = corpus luteum, (C) AL (1000 mg/kg), a = enucleated Graafian follicles; (D) Progesterone (10 mg/kg), a = follicles at early stages of development.

Effect of extract on the cellular structure of the uterus

AL (100 mg/kg), was observed to induce proliferation of the uterine endometrial glands compared to the control while at the higher dose of 1000 mg/kg glandular hyperplasia and atrophy of the uterine stroma was observed (Figure 2). Administration of progesterone however resulted in uterine glandular proliferation and oedema of the submucous layer which again was similar to the **AL** at 100 mg/kg (Figure 2).

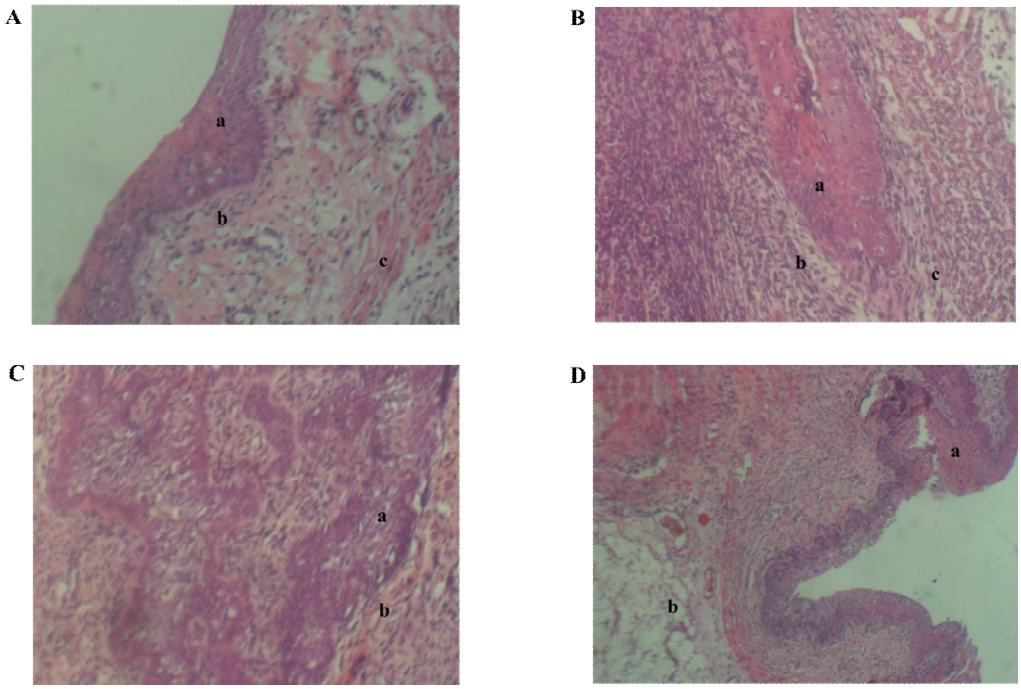

Figure 3: Mouse cervix H & E x 100: Representative images of haematoxylin and eosin staining of cervical tissue from mice after 6 days of AL treatment (A) Control (10% Tween 80), a = ectocervix, b = submucosa and c = muscular layer; (B) AL (100 mg/kg), a = mild thickening of the ectocervix, b = subepithelial oedema and c = stromal hypertrophy; (C) AL (1000 mg/kg), a = papillary projection of the thick ectocervix into the submucosa and b = thickening of the submucosa; (D) Progesterone (10 mg/kg), a = thickened and mild folding of the epithelial lining and b = subepithelial oedema.

Effect of the extract on the cellular structure of the cervix

At low dose (100 mg/kg), **AL** induced mild thickening of the ectocervix, oedema and atrophy of the subepithelium and stroma respectively. At 1000 mg/kg **AL**, papillary projection of the ectocervix into the submucosa was observed as well as thickening of the submucosa (Figure 3). Progesterone caused mild thickening of the epithelial lining and oedema of the subepithelium.

Discussion

Female reproductive organs undergo numerous physiological and biochemical changes that depend on the ovarian steroid hormones. The physiological level of ovarian steroid hormones is associated not only with the initiation, but also in the developmental process of the reproductive system [9-10].

Based on the traditional use of the plant in the management of miscarriages, it was therefore hypothesized that **AL** possibly possesses anti-estrogenic activities or a progesterone-like effect such as is similar to the effect of progesterone on the female reproductive system. This was based on the knowledge that the uterus in rodents and humans undergoes cyclical changes of growth and degeneration. In both species, oestrogens produced from the developing follicles stimulate endometrial growth, and progesterone is responsible for converting the oestrogen-primed endometrium into a receptive state[15]. The endometrium is the site of implantation and pregnancy. Preparation for this important biological event relies primarily on progesterone, which takes the oestrogen-primed endometrium toward a state of receptivity. It is the balance between oestrogen and progesterone that maintains the endometrium in a state of health and provides the synchronous timing necessary for a successful implantation to occur[15].Progesterone, the natural hormone produced by the corpus luteum and other steroid-secreting glands, is endowed with anti-oestrogenic action and has a fundamental role in the initiation and maintenance of pregnancy and in the regulation of gonadotropin secretion[16]. Intraperitoneal administration of progesterone results in circulating levels of progesterone within the lower third of those found in the luteal phase of the human menstrual cycle. Those levels were shown to delay the recovery of fertility in nursing women without adverse effects to the mother or the infant and has placed the use of progesterone as an effective and safe contraceptive method[15]. It can therefore be extrapolated that drugs with progesterone-like effects may prove useful as tocolytic agents.

In the current study, **AL** at both 100 and 1000 mg/kg decreased the levels of progesterone with the decrease observed on administration of 100 mg/kg being more significant. However, no significant effect on oestrogen levels were observed at both doses. It is however important to note that **AL** (1000 mg/kg) increased significantly the circulating levels of LH while **AL** (100 mg/kg) decreased the circulating levels of LH. The blockade of ovulatory LH surge by drugs occur due to the drug's interference with the response of the maturing follicles to gonadotrophic signals [12-13]. Some of these compounds are able to effect this activity via a central opioid receptor stimulation which has been reported to inhibit ovulation [14–16].Of the many factors involved in the primary events of pregnancy, progesterone receptor (PR) and oestrogen receptor (ER), and their cognate ligands, undoubtedly play central roles in this biological process[17-18].It is therefore suggested that **AL** at low doses may inhibit ovulation in the non-pregnant uterus via its effect on LH and may contribute to its tocolytic effect.

A look at how the extracts interact with hematological indices can also point to possible similarities with the sex hormones' activities. The decrease in HCT observed in the presence of the extract is a condition that can promote fluid retention and is usually due to an increase in plasma volume and subsequently haemodilution and can be due to hormonal changes [24]. A total increase in lymphocyte count occurs in pregnancy and this plays a central role in the maternal adaptation to pregnancy. Overall, hormonal changes particularly due to an increase in progesterone results in a decrease in platelet count, PCV, and Hb in pregnancy [24]. This overall decrease in haematological indices was observed in the presence of the low dose of **AL** and progesterone in this study and suggests similarities in the activities of the low dose **AL** and progesterone.

On the ovarian structure, **AL** (100 mg/kg) was observed to increase the number and development of the follicles while at 1000 mg/kg an extrusion of the ovum from the Graafian follicle was observed. Progesterone displayed a similar effect to the low dose of **AL** where an increase in the number and development of follicles was observed. During proestrous (proliferative phase in humans, cycle days 6 – 14) follicles develop and start to produce oestrogens that stimulate endometrial growth. During oestrous (peri-ovulatory period in humans, cycle days 13– 15) ovarian follicles mature[25].It had been reported that the epithelial surface of the ovary, which is derived from the same epithelial lining of the peritoneal cavity and other Müllerian structures, undergoes rapid increase during 24 h after ovulation, and that the epithelial invaginations which form clefts and inclusion cysts within the ovarian stroma are also most pronounced immediately after ovulation and is due to a rising level of progesterone [26].This may contribute to the effect of **AL** (100 mg/kg) on proliferation of ovarian cells as observed in this study. Casagrande et al. [27]associated this increased proliferation of ovarian cells with anovulation that occurs in the presence of progesterone and so it can be inferred that **AL** (100 mg/kg) may possess progesterone-like activities. Cramer and Welch noted that the ovarian epithelium repeatedly in vaginates all through life to form clefts and inclusion cysts and suggested that, in the presence of excessive gonadotropin (follicle-stimulating hormone [FSH] or luteinizing hormone [LH]),ovarian stroma stimulation and the resulting stimulation by oestrogen or oestrogen precursors, significant proliferation and transformation occurs in the epithelium[28].However, pregnancy acts to suppress ovulation due to progesterone and may contribute to lower basal as well as peak gonadotropin stimulation [28].

On the uterine structure, **AL** (100 mg/kg), was observed to induce proliferation of the uterine endometrial glands compared to the control while at the higher dose of 1000 mg/kg glandular hyperplasia and atrophy of the uterine stroma was observed. Administration of progesterone however resulted in uterine glandular proliferation and oedema of the submucous layer which again was similar to the effect of **AL** at 100 mg/kg. The endometrium consists of two layers: the basalis, the layer from which it regenerates after menstrual shedding, and the outer functionalis. Each layer has two major components, the epithelium, that is present as either glandular elements or superficial epithelium, and the mesenchymal component of stromal cells[29]. During a normal menstrual cycle, the human endometrium undergoes a synchronized sequence of proliferation, differentiation and degeneration due to variation in the concentrations of steroid hormone. Oestrogens induce proliferation of the epithelial and stromal elements of the endometrium during the pre-ovulatory proliferative phase and, post-ovulation, while progesterone is involved in glandular differentiation and glycogenesis among others[30].The concentrations and contents of progesterone receptors (PR) increases in both compartments of the epithelial and stromal layers during the proliferative phase and remains high during the early secretory phase. In mid- to late secretory phase, a decline in PR expression is observed and this is more pronounced in glandular cells than the stromal cells[26-27]. That PR is maintained throughout the secretory phase of the menstrual cycle suggests constitutive PR expression and implies the continued need for progesterone to support further growth and development in this tissue [26-27].It also suggests a mitogenic and constitutive PR expressionon basalis epithelial glands needed for tissue regeneration during menstrual reconstruction. The predominance of PR type A observed in these

glands implicates the involvement of this PR isoform with progesterone-mediated proliferation of the basalis [29].From the foregoing, it would appear that the role of progesterone in the regulation of uterine growth is evolving. A recent study also showed that oestrogen by itself is not a mitogen for uterine growth [33]. It has been reported that the action of progesterone via progesterone receptors (PR) increases tissue volume through cell proliferation and accumulation of the extracellular matrix (ECM) [33].It therefore implies that oestrogen lacks mitogenic effect but it is essential for the growth and maintenance of uterine tissues and it sensitizes uterine cells to progesterone by inducing PR [33]. It is a well-known fact that progesterone is the hormone of pregnancy and unequivocally required in all mammals for maternal support of conceptus (embryo/fetus and associated membranes) survival and development. It has also been reported that the endometrial lumenal (LE) and glandular epithelia (GE) of a number of species exhibit a loss of PR expression prior to the stages of uterine receptivity and implantation [34].Therefore, the actions of progesterone on endometrial epithelia during most of gestation appear to be mediated by the endometrial stroma that remains PR-positive throughout pregnancy. Administration of progesterone or progesterone-like drugs can therefore be used to create such a state in a non-pregnant uterus, creating an unattractive environment for implantation and consequently infertility. Stromal cells produce several growth factors, that have receptors expressed specifically in the endometrial epithelia [34]. However, these factors have been reported to be progesterone-responsive and assist in mediating epithelial-mesenchymal interactions necessary for support of pregnancy. Studies on the uterine gland of knockout ewes showed that uterine glands and their secretions which are supported by progesterone are required for peri-implantation conceptus survival and growth. Stimulation of GE proliferation and production of secretory proteins, during mid-pregnancy all act to produce GH that can also act on a progestinized uterus to stimulate GE hypertrophy and increased glandular function and by inference increased secretory function [34]. From the foregoing, progesterone is indeed involved in the glandular and secretory activities of the myometrium and may explain the effect of **AL** (100 mg/kg) and progesterone on the endometrial glands observed in this study.

The cervix is the lower portion of the uterus and consists of two major structures: the ectocervix and the endocervix. Throughout the menstrual cycle, changes in the size, texture and location of the cervix occurs. Prior to ovulation, oestrogen levels rise resulting in an enlarged and softened cervix which also aids dilatation of the external os promoting easier sperm access to the uterus. However at the end of ovulation, progesterone causes the cervix to harden, close, and secrete a thick milk-like mucus which functions as a plug, and helps prevent entry of bacteria and/or sperm to the uterus[30-31].These functional changes in cervix also occurs in parturition mainly in the subepithelium and the muscular region. The changes in the connective tissue of the cervical subepithelium is useful in cervical ripening [37].In most mammals, coordination between cervix and uterus at term is controlled partially by a decrease in circulating progesterone (progesterone withdrawal) [38].It has been reported that collagen remodelling involved in cervical ripening is temporally and spatially associated with a decrease in PR. Before parturition, the muscular region of the cervix also present a decreased expression of PR. Collagen remodelling in the uterine cervix is evidence of the cervical softening that allows its dilatation and a normal delivery [39]. During pregnancy, it is important that the cervix must be firm and remain closed to hold the foetus till term, a role played by progesterone.The mild thickening of the ectocervix, and the oedema of the subepithelium and stroma respectively observed in this study on administration of **AL** (100 mg/kg) appears similar to the effect of progesterone on the cervix. At 1000 mg/kg, **AL** was also observed to cause a mild thickening of the epithelial lining and oedema of the subepithelium.

Conclusion

In conclusion, the methanol leaf extract **AL** has been shown in this study to exert possibly progesterone-like effects at a low dose and oestrogen-like effects at a high dose. The effect observed at the low dose may account for its use by traditional healers in maintaining pregnancy. The mechanisms for its effect are currently unknown but it is suggested that the extract may possibly interact with progesterone receptors. Further studies are however advised to investigate this knowledge-based hypotheses further. This study also proposes a potential plant source for the investigation of new oral contraceptive agents and also a source for plant-based tocolytic agents for the rural community in contribution to the goals of sustainable development in Nigeria.

Declarations

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

References

1. S. Tiwari, "Plants: A rich source of herbal medicine," *J. Nat. Prod.*, vol. 1, pp. 27–35, 2008.
2. R. Cirillo, E. G. Tos, P. Page, M. Missotten, A. Quattropani, A. Scheer, M. K. Schwarz, and A. Chollet, "Arrest of preterm labor in rat and mouse by an oral and selective nonprostanoid antagonist of the prostaglandin F2α receptor (FP)," *Am. J. Obstet. Gynecol.*, vol. 197, 2007.
3. L. E. Simmons, C. E. Rubens, G. L. Darmstadt, and M. G. Gravett, "Preventing Preterm Birth and Neonatal Mortality: Exploring the Epidemiology, Causes, and Interventions," *Seminars in Perinatology*, vol. 34. pp. 408–415, 2010.
4. H. M. Burkill, *The Useful Plants ofWest Tropical Africa. Volume 2: Families E-I*, 2nd ed. Richmond, UK: Royal Botanical Gardens, Kew, 1994.
5. J. E. Lawn, K. Kerber, C. Enweronu-Laryea, and S. Cousens, "3.6 Million Neonatal Deaths-What Is Progressing and What Is Not?," *Seminars in Perinatology*, vol. 34. pp. 371–386, 2010.
6. Committee, "Guide for the Care and Use of Laboratory Animals: Eighth Edition," in *Guide for the Care and Use of Laboratory Animals*, 2011, p. 118.
7. C. Miles, LE, Hales, "Labelled Antibodies and Immunological Assay Systems," *Nat.*, vol. 219, no. 5150, pp. 186–189, 1968.
8. R. M. Lequin, "Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA)," *Clin. Chem.*, vol. 51, no. 12, pp. 2415–2418, 2005.
9. A. H. Fischer, K. a Jacobson, J. Rose, and R. Zeller, "Hematoxylin and Eosin (H & E) staining," *CSH Protoc.*, vol. 2008, no. 4, p. pdb.prot4986, 2005.
10. M. a Ruegg and S. Meinen, "Histopathology in Hematoxylin & Eosin stained muscle sections," *TREAT-NMD Neuromuscul. Netw.*, no. Id, pp. 1–9, 2012.
11. S. Bradbury, *Peacock's Elementary Microtechniques*, 4th Editio. London: Arnold, 1974.
12. S. Bradbury, P. J. Evennet, H. Hasselmann, and H. Piller, *Dictionary of Light Microscopy Royal Microscopical Society*. Oxford: Oxford University Press, 1989.
13. G. Rasier, J. Toppari, A. S. Parent, and J. P. Bourguignon, "Female sexual maturation and reproduction after prepubertal exposure to estrogens and endocrine disrupting chemicals: A review of rodent and human data," *Mol. Cell. Endocrinol.*, vol. 254–255, pp. 187–201, 2006.
14. D. Wilhelm, S. Palmer, and P. Koopman, "Sex determination and gonadal development in mammals.," *Physiol. Rev.*, vol. 87, no. 1, pp. 1–28, 2007.
15. H. B. Croxatto and S. D??az, "The place of progesterone in human contraception," *J. Steroid Biochem.*, vol. 27, no. 4–6, pp. 991–994, 1987.
16. J. Szekeres-Bartho, M. Halasz, and T. Palkovics, "Progesterone in pregnancy; receptor-ligand interaction and signaling pathways," *J. Reprod. Immunol.*, vol. 83, no. 1–2, pp. 60–64, 2009.
17. J. M. Goldman, R. L. Cooper, S. C. Laws, G. L. Rehnberg, T. L. Edwards, W. Keith McElroy, and J. F. Hein, "Chlordimeform-induced alterations in endocrine regulation within the male rat reproductive system," *Toxicol. Appl. Pharmacol.*, vol. 104, no. 1, pp. 25–35, 1990.
18. J. M. Goldman, A. S. Murr, A. R. Buckalew, and R. L. Cooper, "Suppression of the steroid-primed luteinizing hormone surge in the female rat by sodium dimethyldithiocarbamate: Relationship to hypothalamic catecholamines and GnRH neuronal activation," *Toxicol. Sci.*, vol. 104, no. 1, pp. 107–112, 2008.
19. W. R. Butler, "Inhibition of ovulation in the postpartum cow and the lactating sow," in *Livestock Production Science*, 2005, vol. 98, no. 1–2, pp. 5–12.
20. C. H. SAWYER, J. E. MARKEE, and W. H. HOLINSHEAD, "Inhibition of ovulation in the rabbit by the adrenergic-blocking agent dibenamine.," *Endocrinology*, vol. 41, no. 5, pp. 395–402, 1947.
21. C. G. Smith, "Reproductive toxicity: hypothalamic-pituitary mechanisms," *Am. J. Ind. Med.*, vol. 4, no. 1–2, pp. 107–112, 1983.
22. D. D. Carson, I. Bagchi, S. K. Dey, A. C. Enders, A. T. Fazleabas, B. A. Lessey, and K. Yoshinaga, "Embryo Implantation," *Dev. Biol.*, vol. 223, no. 2, pp. 217–237, 2000.
23. S. K. Dey, H. Lim, S. K. Das, J. Reese, B. C. Paria, T. Daikoku, and H. Wang, "Molecular cues to implantation," *Endocrine Reviews*, vol. 25, no. 3. pp. 341–373, 2004.
24. and O. L. E. Patrick Chukwuyenum Ichipi-Ifukor, "Changes in Haematological Indices in Normal Pregnancy," *Physiol. J.*, vol. 2013, no. 1, p. 4, 2013.
25. P. G. Groothuis, H. H. N. M. Dassen, A. Romano, and C. Punyadeera, "Estrogen and the endometrium: Lessons learned from gene expression profiling in rodents and human," *Human Reproduction Update*, vol. 13, no. 4. pp. 405–417, 2007.
26. M. F. Fathalla, "INCESSANT OVULATION-A FACTOR IN OVARIAN NEOPLASIA ?," *The Lancet*, vol. 298, no. 7716. p. 163, 1971.
27. J. T. Casagrande, M. C. Pike, R. K. Ross, E. W.

Louie, S. Roy, and B. E. Henderson, "'INCESSANT OVULATION' AND OVARIAN CANCER," *Lancet*, vol. 314, no. 8135, pp. 170–173, 1979.

28. D. W. Cramer and W. R. Welch, "Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis.," *J. Natl. Cancer Inst.*, vol. 71, no. 4, pp. 717–721, 1983.
29. P. A. Mote, R. L. Balleine, E. M. McGowan, and C. L. Clarke, "Heterogeneity of progesterone receptors A and B expression in human endometrial glands and stroma," *Hum Reprod*, vol. 15 Suppl 3, pp. 48–56, 2000.
30. J. D. Graham, S. D. Roman, E. McGowan, R. L. Sutherland, and C. L. Clarke, "Preferential stimulation of human progesterone receptor B expression by estrogen in T-47D human breast cancer cells.," *J. Biol. Chem.*, vol. 270, no. 51, pp. 30693–700, 1995.
31. C. Bergeron, A. Ferenczy, D. O. Toft, and G. Shyamala, "Immunocytochemical study of progesterone receptors in hyperplastic and neoplastic endometrial tissues," *Cancer Res.*, vol. 48, no. 21, pp. 6132–6136, 1988.
32. B. A. Lessey, A. P. Killam, D. A. Metzger, A. F. Haney, G. L. Greene, and K. S. McCarty, "Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle.," *J. Clin. Endocrinol. Metab.*, vol. 67, no. 2, pp. 334–40, 1988.
33. H. Ishikawa, K. Ishi, V. Ann Serna, R. Kakazu, S. E. Bulun, and T. Kurita, "Progesterone is essential for maintenance and growth of uterine leiomyoma," *Endocrinology*, vol. 151, no. 6, pp. 2433–2442, 2010.
34. T. E. Spencer and F. W. Bazer, "Biology of progesterone action during pregnancy recognition and maintenance of pregnancy.," *Front. Biosci.*, vol. 7, pp. d1879–d1898, 2002.
35. F. Martyn, F. M. McAuliffe, and M. Wingfield, "The role of the cervix in fertility: is it time for a reappraisal?," *Hum. Reprod.*, vol. 29, no. 10, pp. 2092–8, 2014.
36. K. M. Myers, H. Feltovich, E. Mazza, J. Vink, M. Bajka, R. J. Wapner, T. J. Hall, and M. House, "The mechanical role of the cervix in pregnancy," *J. Biomech.*, vol. 48, no. 9, pp. 1511–1523, 2015.
37. G. B. Huszar and P. W. Michael, "Relationship between myometrial and cervical functions in pregnancy and labor," *Semin. Perinatol.*, vol. 15, no. 2, 1991.
38. J. R. G. Challis and S. J. Lye, "Parturition," in *The Physiology of Reproduction*, E. Knobil and J. D. Neill, Eds. New York: Raven press, 1994, pp. 985–1031.
39. E. H. Luque, J. G. Ramos, H. a Rodriguez, and M. M. Muñoz de Toro, "Dissociation in the control of cervical eosinophilic infiltration and collagenolysis at the end of pregnancy or after pseudopregnancy in ovariectomized steroid-treated rats.," *Biol. Reprod.*, vol. 55, no. 6, pp. 1206–12, 1996.