WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 13, 1951-1966. Research Article ISSN 2277– 7105

FLEURYA AESTUANS PROMOTES OOGENESIS AND OVULATORY FUNCTIONS IN WISTAR RATS BY SHORTENING THE ESTRUS CYCLE.

[1]Charles CN, [2]Weleh, II (PhD) and [2] Green, IK (MBBS)

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Madonna University, PMB 48, Elele, Nigeria.

[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

[2]Department of Obstetrics and Gynecology, Faculty of Clinical Sciences, College of Health Sciences,

Article Received on
19 Sept. 2021,

Revised on 10 October 2021,
Accepted on 31 October 2021

DOI: 10.20959/wjpr202113-22180

***Corresponding Author**
Charles CN
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Madonna University, PMB 48, Elele, Nigeria.

ABSTRACT

The effects of extract of *Fleurya aestuans* on the estrus cycle and ovarian parameters of Wistar rats were investigated in this study. The study animals were divided into two phases: extract-only groups (1, 2, 3, 4) and toxicity-induced groups (A, B, C, D, E, F, and G). To determine all particulate parameters under inquiry, the study used established procedures. Histological and tissue biochemical studies were performed on the *ovary and uterus*. Treatment of the extract resulted in a significant ($P<0.05$) decrease in proestrus, estrus, metestrus and diestrus parameters, as well as improvements in female ovarian hormones and biochemical parameters. The number of primordial, primary, secondary, tertiary follicles, and corpus leutum rose significantly ($P<0.05$), but the number of atretic follicles decreased. Similarly, the treatment of the extract with *tetrahydroxyflavone* reversed the harmful effects of lead acetic acid on the ovary and uterus in the research animals. As a result, the study suggests that *Fleurya aestuans* leaves be used to stimulate ovulation and treat menstrual irregularities. Patients with ovarian and endometrial cycle dysfunctions may benefit from the dose in humans study.

KEYWORDS: *Fleurya Aestuans*, Estrus Cycle, Oogenesis, Ovulation.

INTRODUCTION

Menstrual cycle disruption is one of the most common reproductive endocrinopathies, affecting 30–40% of reproductive-age women, with 50% of them experiencing infertility - defined as the inability to conceive after one year of adequately timed unprotected intercourse.[1] Infertility in women can be caused by a problem with ovarian folliculogenesis. Premature granulosa cell maturation and differentiation has been shown to cause preantral follicular growth block, anovulation, and cyst development.[2] All of these events may have an impact on women's fertility. Infertility is routinely treated with medications such as clomiphene citrate, metformin, and gonadotropins. The goal of these treatments was to improve ovulatory activity and reduce menstrual irregularity.[3]

Clomiphene citrate has been utilized as a first-line ovulation trigger for over 40 years. The use of CC and metformin at the same time has been shown to boost ovulation and pregnancy rates. When oral ovulation-inducing medicines fail to induce ovulation in individuals, gonadotropins are utilized.[4] Surgical treatment and in vitro fertilization (IVF) are commonly used to treat infertility in people who want to start a family but haven't been successful with medication treatments.[5]

In women who are resistant to CC, laparoscopic ovarian drilling (LOD) is being examined. Following a 6-month therapy, resistance to CC is elicited in the absence of ovulation or pregnancy. IVF is designated for women who have not been able to conceive after using gonadotropins.

Treatments for anovulatory functions and menstrual irregularity may alleviate symptoms, but they are frequently linked with substantial adverse effects.[6] Furthermore, some of them (gonadotropins and surgical interventions) are expensive and time-consuming, and their use necessitates close monitoring, necessitating the development of novel active substances that are better tolerated, more efficient, and less expensive than the current pharmacological and surgical approaches. Previous research has shown that plants including Tribulus terrestris[7] and Allium fistulosum[8], are effective in restoring hormonal profiles and ovarian function in rats. These findings imply that plants with fertilizing qualities could be used to treat menstrual abnormalities as a therapy option.

For example, *Fleurya aestuans* (Urticaceae), also known as "Ile-nkita (Ohuahihara)" in Igbo and "Ofuefue (Fiyafiya)" in Yoruba, is extensively spread in tropical Africa, where its young

leaves are used in Nigerian traditional medicine to treat a variety of ailments, including diabetes, male infertility (both primary and secondary) and anemia. The extract has anti-diabetic activity in vitro, according to Green *et al.*[9] Furthermore, the leaves' aqueous and alcohol extracts were proven to boost the fertility of diabetic male Wistar rats.[9] Although possessing all these qualities, *Fleurya aestuans* has not yet been explored on an animal model of menstrual-related irregularity. The effects of *Fleurya aestuans* extract on the estrous cycle, oogenesis, sex hormones, ovarian and uterine histomorphology were studied in this regard.

MATERIALS AND METHODS

Preparation of extract

A plant taxonomist from the University of Port Harcourt's Department of Plant Science and Biotechnology gathered and verified fresh *Fleurya aestuans* leaves. The plant was given the herbarium number UPH/P/263. Extraneous materials were removed, and the leaves were mechanically pulverized after being dried at room temperature for one week. 690g of dry powdered leaves were defatted and extracted in 400ml of water-ethanol mixture (30:70) for 72 hours in an extraction jar using a sohxlet device. The extract was concentrated using a rotary evaporator to obtain the crude extract. Until it was needed, the extracted yield was maintained at 4°C in a home refrigerator.

Experimental design

For the investigation, 75 female Wistar rats were used. The rats ranged in weight from 139.3 to 177.9 grams. They were purchased from the Madonna University Animal House in Elele, Nigeria. The rats were kept in plastic cages and given commercial rat food to eat. Prior to the start of the trial, they were allowed to feed and drink as they pleased for seven days to adapt. The animals were then divided into two (2) phases based on their subjective isolation.

Phase 1

For this investigation, 40 rats were employed, divided into four groups of 10 rats each. The rat classifications are as follows:

Group 1: Control group.

Group 2: Low-dose extract (50mg/kg) group

Group 3: Medium-dose extract (75mg/kg) group

Group 4: High-dose extract (200mg/kg) group

For 60 days, the test groups were given single daily doses of hydro-ethanolic leaf extract of *Fleurya aestuans* orally

Phase 2

For this investigation, 35 rats were employed, divided into 7 groups of 5 rats each. Falana and Oyeyipo's[10] approach was used to generate ovarian toxicity using 2.25mg/kg of lead acetate. The rat classifications are as follows:

Group A: Control group

Group B: Only lead acetate (2.25mg/kg) group

Group C: Lead + Low-dose extract (50mg/kg) group

Group D: Lead + Medium-dose extract (75mg/kg) group

Group E: Lead + High-dose extract (200mg/kg) group

Group F: Lead + Tetrahydroxyflavone (100µg/kg) group

Group G: Only Tetrahydroxyflavone (100µg/kg) group

Both extract and lead acetate were administered orally for a period of 60 days.

Determination of estrous cycle

- To guarantee normal estrous cycles, vaginal smears of each rat was determined using the method of Mclean *et al.*,[11]
- Smears were collected every morning between 7 and 9 a.m. for five weeks (2 weeks before treatment and 3 weeks during treatment).
- A 1ml rubber pipette, beakers, distilled water, glass slide, and a light microscope were utilised. The animals were held in a supine position in one hand while 0.5ml of distilled water was flushed into the vaginal opening twice with the rubber pipette in the other.
- The vaginal fluid was carefully collected and put on a glass plate for microscopic inspection.[12]
- Between lavages, the rubber pipette was carefully washed to eliminate any remaining cells before being used on the next animal.
- The procedure for staining vaginal smears was adapted from Mclean et al.[11] In 100 ml of double distilled water (ddH2O), 0.1 g of crystal violet powder was added and carefully blended. The crystal violet stain (0.1 percent) was kept at room temperature in a securely sealed container until needed. Crystal violet dye (0.1 percent) was applied to one side of the cover slip with an eye dropper.
- Using a filter paper, excess fluid was drained from the borders of the slide until the stain was uniformly dispersed throughout the surface.

- The proportion of nucleated epithelial cells, cornified epithelial cells, and leukocytes was determined immediately under a light microscope (Ultra Medical MDI, Guangzhou Keyeah Optics & Electronics Instrument Co. LTD, China).
- Animals whose vaginal smears contained mainly leukocytes (60 percent) were categorized as diestrus using the Tropp and Markus[13] approach. Proestrus smears had a high percentage of nucleated epithelial cells (60%) and a low percentage of leukocytes (10%). Estrus was defined as smears with a high percentage of cornified cells (90%). Metestrus was defined as smears that comprised predominantly cornified cells (60%) with a substantial number of leukocytes (20%) and nucleated epithelial cells (20%).
- After cytological inspection, stained smears were preserved and photomicrographs were made using Honestech TVR 2.5 software and the Ultra Medical MDI microscope's integrated camera at a later date (Guangzhou Keyeah Optics & Electronics Instrument Co. LTD, China).

Preparation of Serum

The serum samples were prepared according to the procedure reported by Yakubu *et al.*[14] Blood was obtained from rats at random from each group through ocular puncture into sterile containers, spun, and stored in the fridge. Following that, the rats were sacrificed by cervical dislocation.

Hormonal Assay

Using an ELISAmicroplate (HIPO MPP-96, BIOSAN) and the protocol and procedure specified on the accubind kit from Biocode, Belgium, the serum was utilized to assay for LH, FSH, estrogen, progesterone, and prolactin levels across the various groups.

Histological Examination

Following the method described by Bancroft and Gamble[15] for fixation, tissue processing, staining, and photomicrography, samples of ovaries and uterine from all groups of rats were histologically prepared at the end of the experiment.

Statistical Analysis

The mean and standard error of the mean were used to express the findings. With Statistical Package for Social Science, version 20.0, data were analyzed using a one-way analysis of variance followed by the LSD post-hoc test to find significant differences in all parameters (SPSS, USA). Differences with p0.05 values were regarded as statistically significant.

RESULTS

Table 1: Values of estrus cycle of extract of in study animals.

Groups	Proestrus	Estrus	Metestrus	Diestrus
1	1.16±0.16	3.38±018	2.77±0.03	5.12±0.18
2	0.95±0.00	2.91±0.04	2.22±0.19[a]	2.60±0.24[a]
3	0.76±0.04	2.08±0.18[a]	0.71±0.12[a]	3.44±0.32[a]
4	0.35±0.02[a]	1.17±0.13[a]	1.77±0.16[a]	1.80±0.20[a]

KEY: Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control.

Table 2: Values of follicular parameters of extract in study animals.

Groups	Primodial follicles	Primary follicles	Secondary follicles	Tertiary follicles	Atretic follicles	Corpus leutum
1	5.22±0.11	4.12±0.25	2.78±0.12	1.51±0.22	7.45±0.00	2.80±0.37
2	4.08±0.27	6.20±0.37[a]	3.58±0.00	2.00±0.32	5.20±0.37	3.80±0.50
3	6.47±0.24	4.45±0.00	5.40±0.25[a]	2.80±0.20[a]	4.40±0.25[a]	6.20±0.58[a]
4	8.20±0.95[a]	6.80±0.37[a]	5.57±1.03[a]	3.20±0.20[a]	1.40±0.24[a]	11.40±0.87[a]

KEY: Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control.

Table 3: Values of hormones of extract in study animals.

Groups	LH (m/u/ml)	FSH (m/u/ml)	E2 (pg/ml)	PROG (pg/ml)	PRL (pg/ml)
1	0.73±0.14	0.71±0.90	44.74±0.38	23.85±0.37	12.30±0.03
2	0.98±0.09	0.23±0.06	45.40±2.07	16.10±1.44	8.10±2.00[a]
3	1.10±0.40 [a]	1.55±0.24 [a]	47.98±0.96[a]	25.20±1.67	9.78±0.06
4	2.47±0.23 [a]	1.03±0.00[a]	43.10±0.61	29.90±3.00[a]	19.30±0.01[a]

KEY: Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control.

Histological Examination

Vaginal smear, Uterus and Ovary histology of extract only groups

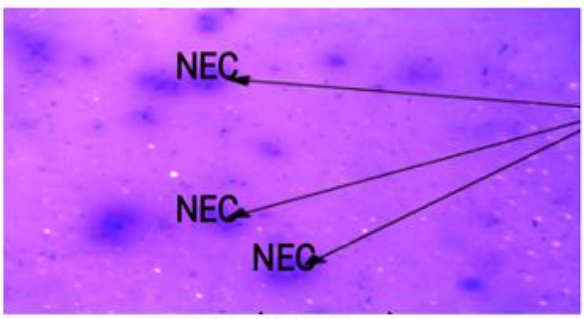

Plate 1a (CV Stain) x 600

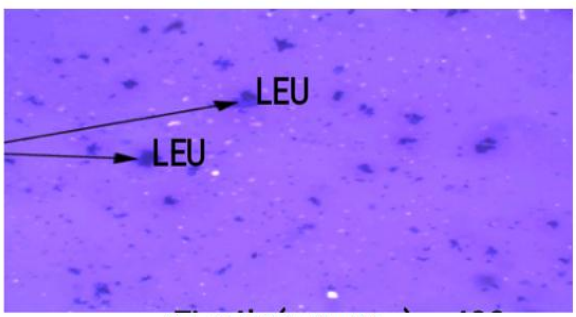

Plate 1b (CV Stain) x 600

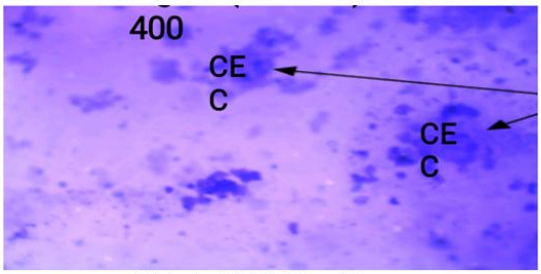

Plate 1c (CV Stain) x 600

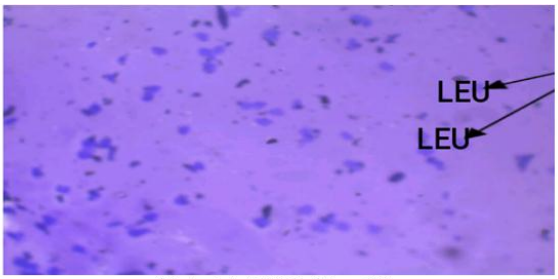

Plate 1d (CV Stain) x 600

Key: NEC= Nucleated Epithelia cells, **LEU**= Leukocytes, **CEC**= Columnar Epithelia Cells

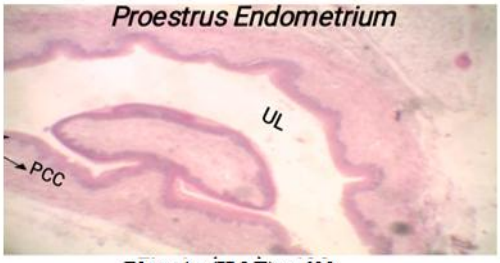

Plate 1e (H&E) x 600

Plate 1f (H&E) x 600

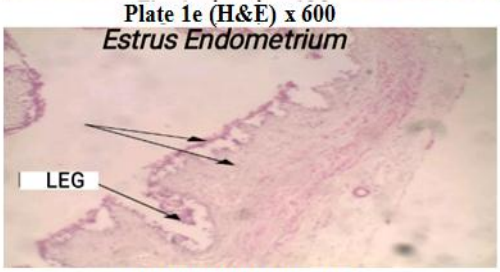

Plate 1g (H&E) x 600

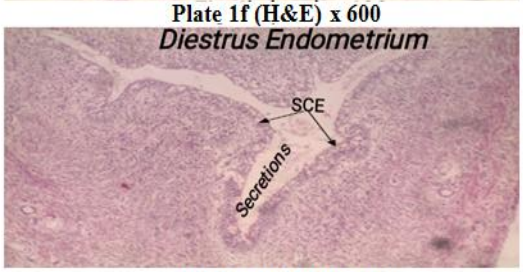

Plate 1h (H&E) x 600

Key: PCC= Prominent Columnar Cells, **UL**= Uterine Lumen, **SCE**= Simple Columnar Epithelia Cells

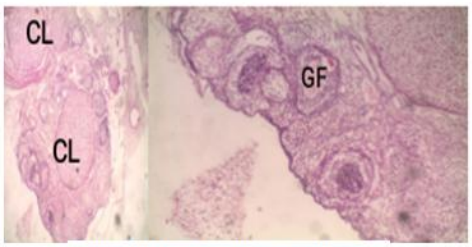

Plate 1i (H & E) x 125 & x 600

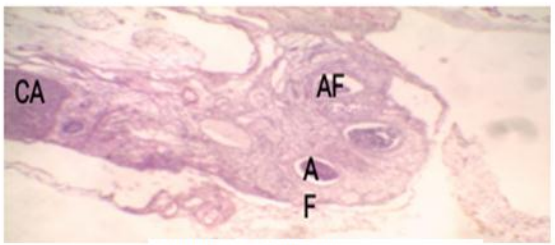

Plate 1j (H & E) x 600

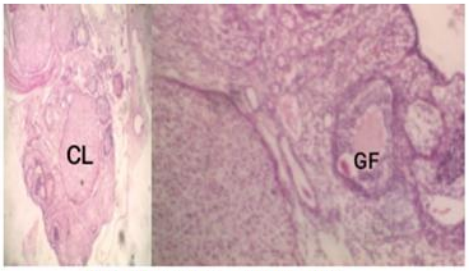

Plate 1k (H & E) x 125 & x 600

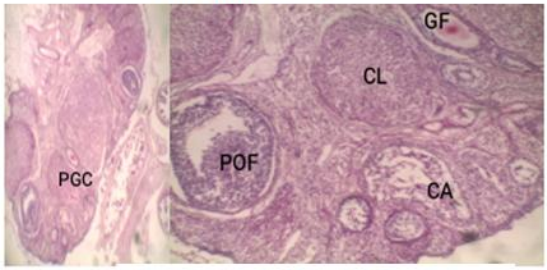

Plate 1l (H & E) x 125 & x 600

Key: **CL**= Corpus Leutum, **GF**= Growing Follicle, **AF**= Atretic F, **POF**= Post Ovulatory F, **PGC**= Primodial germ cell, **CA**= Corpus albicans

Plate 1a Shows that smears contained primarily nucleated epithelial cells *(Proestrus Stage*). Plate 1b Smears contained predominately leukocytes *(Diestrus Stage*). Plate 1c Smears contained primarily cornified cells *(Estrus Stage).* Plate 1d Smears contained predominately leukocytes *(Diestrus Stage).*

Plate 1e Proestrus endometrium lined by prominent columnar cells. Plate 1f Diestrus endometrium lined by simple columnar cells. Plate 1g. Thick estrus endometrium containing large endometrial glands. Plate 1h Diestrus endometrium lined by simple columnar cells & liminal secretions. Plate 1i to l indicated a normal architecture of the ovaries showing some growing follicles, post ovulation follicles and corpus luteum of pregnancy. This suggests an uninterrupted progression of folliculogenesis, estrus cycle and fertility properties of the extract which corroborates to the significant rise in the reproductive hormones as observed in this study.

Table 4: Values of estrus cycle of extract and tetrahydroxyflavone of lead acetate induced ovarian toxicity.

Groups	Proestrus	Estrus	Metestrus	Diestrus
A	1.19±0.20	3.56±0.00	3.03±0.04	4.59±0.24
B	3.80±0.37[a]	5.35±0.24[a]	3.79±0.20[a]	7.40±0.00[a]
C	3.25±0.35	4.25±0.12	3.33±0.00	5.57±0.23
D	2.58±0.25[b]	3.95±0.05[b]	3.80±0.11	3.55±0.77[b]
E	2.77±0.00[b]	2.00±0.32[b]	2.60±0.25[b]	3.40±0.25[b]
F	2.46±0.19[b]	3.38±0.26[b]	2.35±0.08[b]	4.00±0.71[b]
G	1.51±0.22[b]	1.40±0.25[b]	1.91±0.25[b]	4.02±0.01[b]

KEY: Values are presented as mean ± sem. n= 5.[a] = mean values are statistically significant compared to control.[b] = mean values are statistically significant to lead acetate group.

Table 5: Values of follicular parameters of extract and tetrahydroxyflavone of lead acetate induced ovarian toxicity.

Groups	Primodial follicles	Primary follicles	Secondary follicles	Tertiary follicles	Atretic follicles	Corpus leutum
A	4.02±0.476	5.00±0.84	3.40±0.51	3.40±0.40	8.80±0.37	6.00±0.32
B	1.35±0.46[a]	2.20±0.37[a]	0.87±0.08[a]	1.00±0.00[a]	15.20±0.66[a]	1.40±0.25[a]
C	2.36±0.17	4.20±0.30[b]	1.20±0.20	1.50±0.00	9.20±0.97	3.20±0.66[b]
D	2.07±0.20	4.60±0.60[b]	2.93±0.03[b]	1.99±0.00[b]	4.80±0.37[b]	3.69±0.20[b]
E	3.80±0.37[b]	7.00±0.63[b]	3.94±0.01[b]	2.62±0.00[b]	5.40±1.57[b]	3.40±0.40[b]
F	2.50±0.00	8.01±0.63[b]	3.71±0.12[b]	1.84±0.04[b]	6.60±1.08[b]	2.40±0.40
G	4.00±0.84[b]	6.40±0.25[b]	5.64±0.00[b]	2.70±0.43[b]	4.80±1.50[b]	2.40±0.25

KEY: Values are presented as mean ± sem. n= 5.a = mean values are statistically significant compared to control.[b] = mean values are statistically significant to lead acetate group.

Table 6: Values of hormones of extract and tetrahydroxylflavone on lead acetate induced ovarian toxicity.

Groups	LH (m/u/ml)	FSH (m/u/ml)	E2 (pg/ml)	PROG (ng/ml)	PRL (ng/ml)
A	1.73±0.21	0.71±0.40	44.73±7.75	23.80±0.58	12.30±0.58
B	0.30±0.18	0.11±0.60[a]	42.33±3.83	8.70±0.51[a]	7.40±0.00[a]
C	0.29±0.08	0.14±0.78	44.72±1.16	15.60±0.58[b]	10.70±0.50
D	0.57±0.30[b]	0.26±0.40[b]	46.00±0.00	20.11±0.40[b]	11.20±0.20
E	0.74±0.81[b]	0.32±1.00[b]	49.08±2.87[b]	27.90±0.25[b]	11.80±0.22
F	1.82±0.43[b]	0.62±0.60[b]	46.00±0.00	38.40±2.71[b]	12.30±2.01[b]
G	0.35±0.65	0.42±0.49[b]	42.61±2.27	21.00±3.43[b]	6.90±3.03

KEY: Values are presented as mean ± sem. n= 5.[a] = mean values are statistically significant compared to control.[b] = mean values are statistically significant to lead acetate group.

Histological Examination

Vagnal smear, Uterus and Ovary histology of extract and lead acetate groups

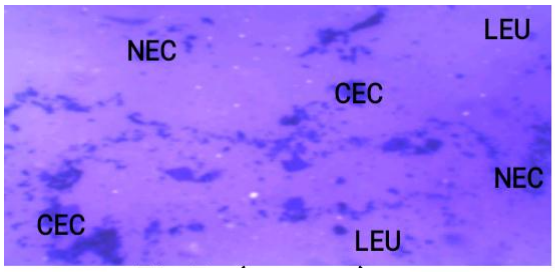

Plate 2a (CV Stain) x 600

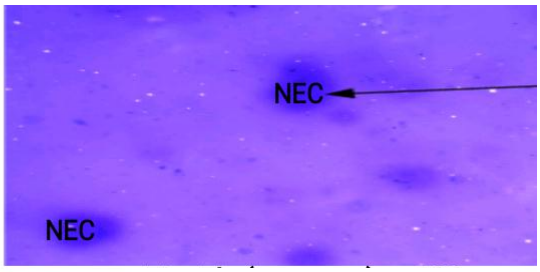

Plate 2b (CV Stain) x 600

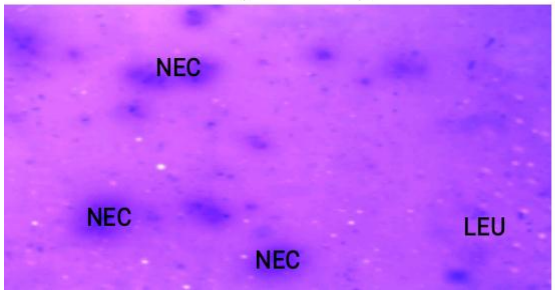

Plate 2c (CV Stain) x 600

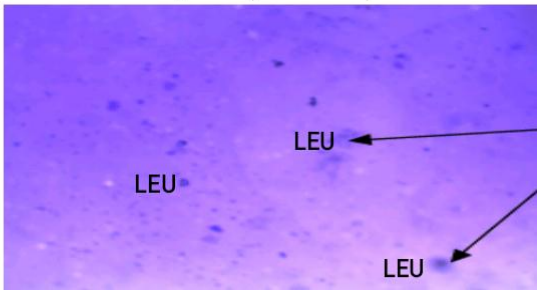

Plate 2d (CV Stain) x 600

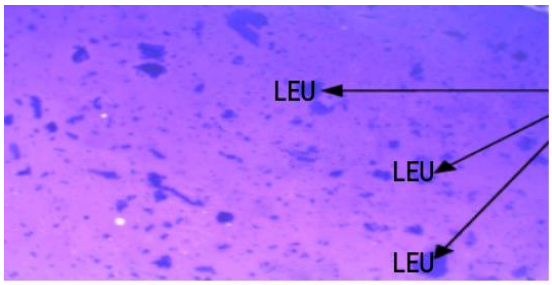

Plate 2e (CV Stain) x 600

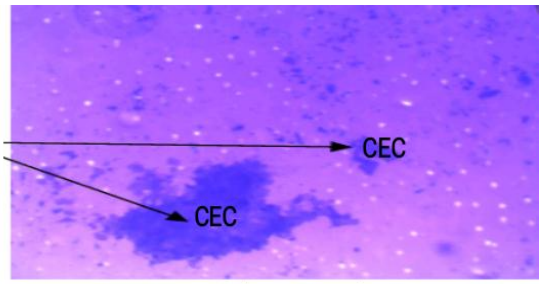

Plate 2f (CV Stain) x 600

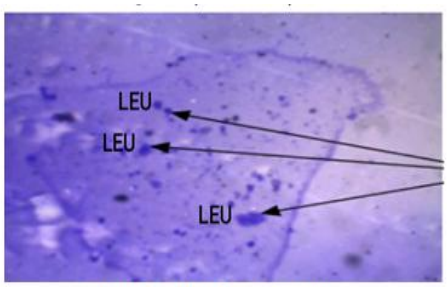

Plate 2g (CV Stain) x 600

Key: NEC= Nucleated Epithelia cells, **LEU=** Leukocytes, **CEC=** Columnar Epithelia Cells

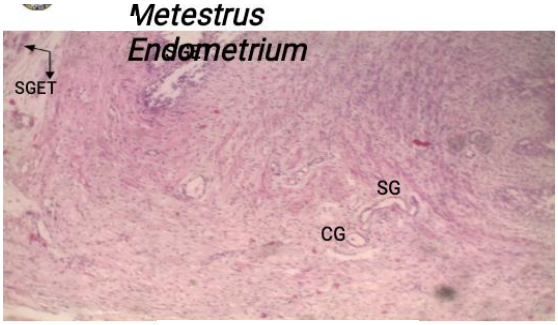

Plate 2h (H&E) x 600

Plate 2i (H&E) x 600

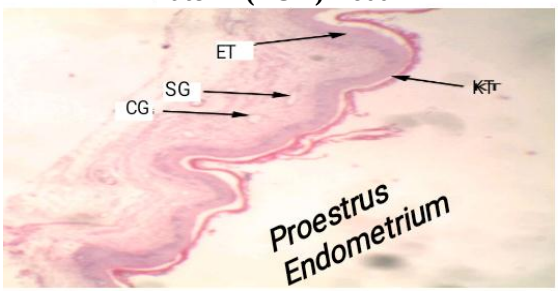

Plate 2j (H&E) x 600

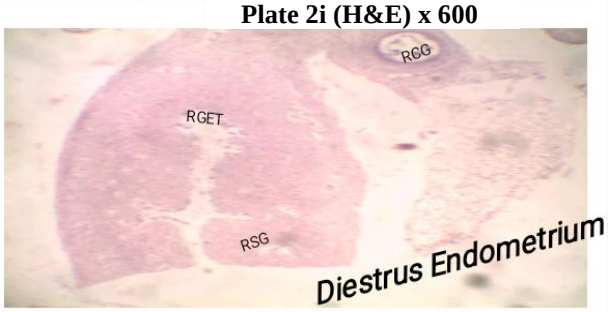

Plate 2k (H&E) x 600

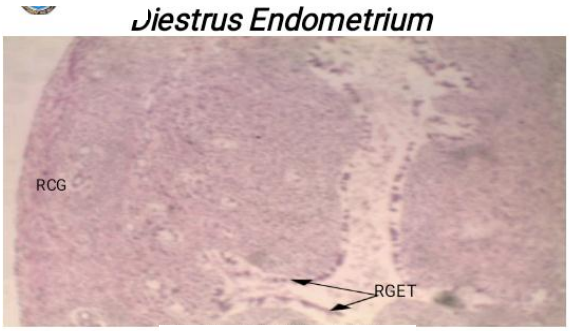

Plate 2l (H&E) x 600

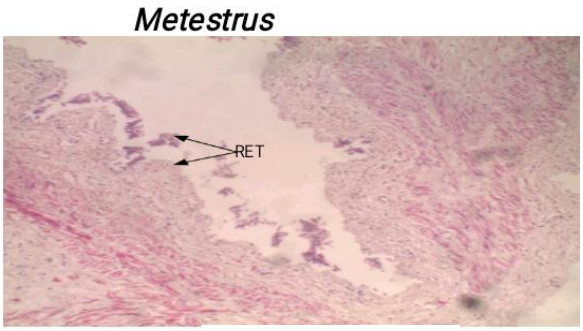

Plate 2m (H&E) x 600

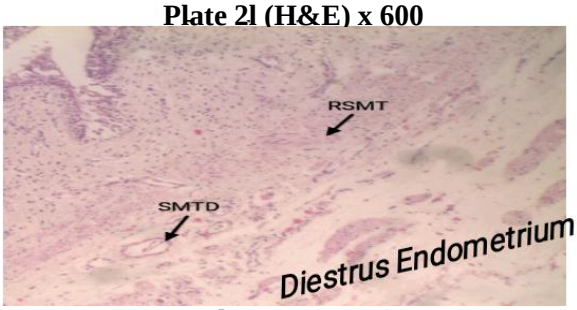

Plate 2n (H&E) x 600

Key: SGET= Shedding of glandular epth tissue into UL, **SG=** Spiral gland, **CG=** Coiled gland, **KT=** Tissue keratinization, **ETD=** Epth tissue damage, **RCG, RSG, RGET=** Recovery of coiled & spiral glands & epth tissue

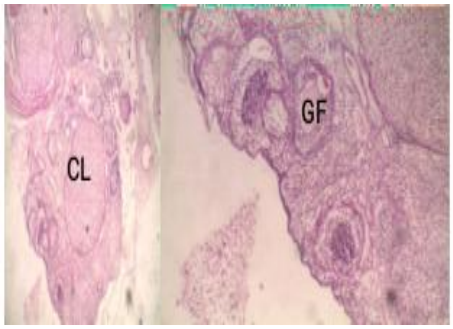

Plate 2o (H & E) x 125 & x 600

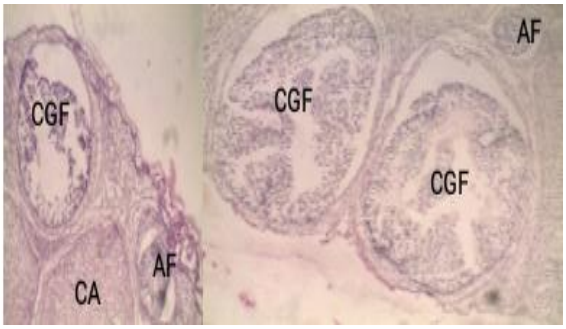

Plate 2p (H & E) x 125 & x 600

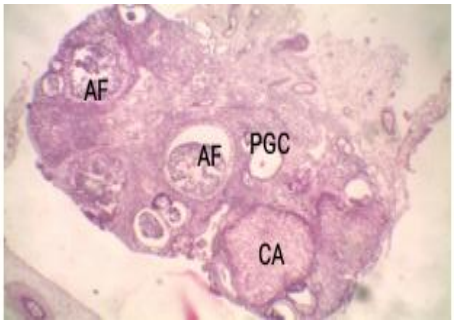

Plate 2q (H & E) x 600

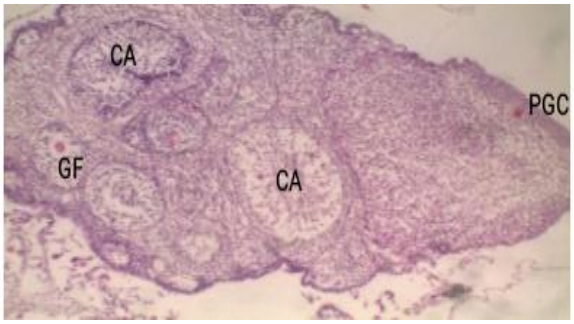

Plate 2r (H & E) x 600

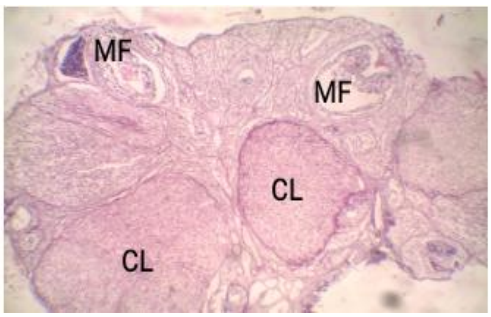

Plate 2s (H & E) x600

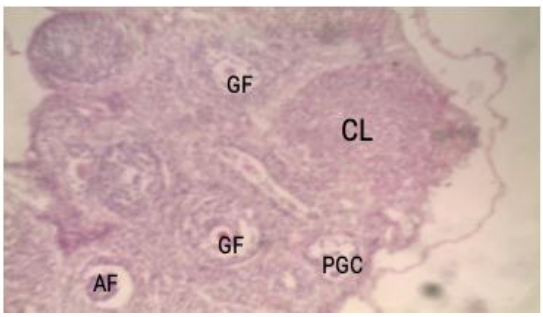

Plate 2t (H & E) x600

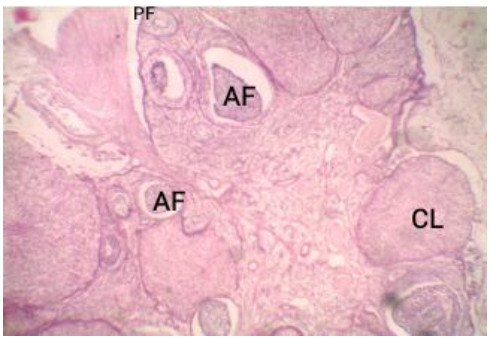

Plate 2u (H & E) x600

Key: CL= Corpus Leutum, GF= Growing Follicle, AF= Atretic F, CGF= Collapsed Graafian F, PGC= Primodial germ cell, CA= Corpus albicans

The results of the histological examination of the rat's smear, uterus & ovaries are displayed at different magnifications (x125 and x600). Plate 2a; the vaginal smears contained nucleated epithelial, cornified & leukocytes cells *(Metestrus Stage* Plate 2b-c; Smears contained nucleated epithelia cells *(Proestrus Stage*). Plate 2d smears contains mainly leukocytes *(Diestrus Stage)*. Plate 2e; Smears contained predominately leukocytes *(Diestrus Stage*). Plate 2f; Smears contained primarily cornified cells *(Metestrus Stage)*. Plate 2g; Smears contained predominately leukocytes *(Diestrus Stage)*. Plate 2h; Normal endometrium. Plate 2i; Distorted endometrium lined by epth tissue damage & fibrosis. Plate 2j-k Recovery of endometrial glands & tissues. Plate 2l-m; Recovery of endometrial glands & tissues. Plate 4.6n; indicated a normal architecture of the ovaries showing some growing follicles and corpus luteum of pregnancy. This suggests an uninterrupted progression of folliculogenesis which could be attributed to the significant rise in the reproductive hormones as observed in this study.

In Plate 2o (LA only group), numerous atretic follicles, collapsed follicles and corpus albicans were noted at various stages of development. This implies a remarkable failure of progression from primordial to graafian follicles and developmental arrest in the lead acetate treated rats. The observation corresponds with the decline in LH, FSH, E2 and progesterone hormones as well as the low sex drive demonstrated by rats in the group. In Plate 2p-t, primordial germ cells and growing follicles were sighted developing. The primordial germ cells developed to full maturity (graafian follicles) in Plate 2u of the study. This suggests that the extract instigated a gradual and progressive return in folliculogenesis and ameliorated or overturned the developmental arrest caused by lead acetate. This affirms the gradual and progressive increase in reproductive hormones and libido in the experimental groups co-treated with lead acetate and extract.

DISCUSSION

Physiologic/endocrine hormones have a significant impact on the overall function of the female reproductive system.[16] The hypothalamus regulates the cyclic changes in the ovaries and uterus through Gonadotropin-releasing hormone (GnRH).[17,18] This hormone circulates in the bloodstream and stimulates the pituitary gland to release two hormones: follicle stimulating hormone (FSH) and luteinizing hormone (LH).[19] The pituitary gland produces folicle-stimulating hormone (FSH) during the first part of the menstrual cycle. It is required for the formation of follicles in the ovaries. Environmental pollutants that disrupt

reproductive function can operate directly on reproductive organs or indirectly by displaying their impact at the hypothalamus and/or pituitary gland levels.[20]

In this study, oral treatment of rats with lead acetate extended the estrous cycle and decreased the number of follicles as compared to control rats, most likely owing to hormonal disruption or inhibition of the hypothalamo-pituitry-ovarian-axis, which are consistent with the findings of Kolawole *et al.*[21] In contrast to group 2 (LAOG), the treatment of *Fleurya aestuans* leaf extract and tetrahydoxyflavone reduced the rats' reproductive cycle and increased the number of follicles.

A single cycle with a diestrus length of 4 days or longer and/or an estrous phase of 3 days or longer was regarded uncommon, according to Lee *et al.*[18], Goldman *et al.*[12] Following the findings of this investigation, significant differences in the duration of the phases of the estrous cycle were identified in the extract treatment groups when compared to the lead acetate group. A substantial shortening of the proestrus phase, followed by estrus, metestrus, and diestrus phases, was seen in the 200mg/kg extract and 100mg/kg tetrahydroxyflavone groups. A shorter reproductive cycle indicates an early onset of ovulation and, as a result, an indication of fertility potential.

In addition, uterine histology indicated that *Fleurya aestuans* treatment resulted in a significant repair of the endometrium's injured epithelial cells, glands, and vascular structure. When compared to the lead acetate only group, the rats' uterine lumen was edematous and secretory, especially in the 200mg/kg extract and tetrahydroxyflavone groups. The uteri of the lead acetate treatment group (2.5 mg/kg B.W) were examined under a microscope and revealed damage of the cellular epithelium as well as fibrosis of the endometrial glands.

Fertility and implantation have both been linked to increased glandular epithelium. This indicates that a uterus with a high glandular epithelium may carry a growing baby to full term.

The presence of ts phytoconstutients might explain the extract's ameliorative, proliferative, and protective effects. According to the findings, *Fleurya aestuans* leaf extracts include tetrahydroxyflavone, which is capable of wiping off the reactive oxygen species (ROS) levels created in the female rats' ovaries and uterus. High amounts of oxygen radicals have been shown to extend the estrus and proestrus stages of the estrus cycle, causing reproductive

problems. The tetrahydroxyflavone component of the plant is thought to have reduced reactive oxygen species (ROS), resulting in a reduction in the harmful effects of lead acetate on animal fertility after long-term treatment. In the extract and THF treated groups, there was an increase in ovarian follicles in the histology of the ovary. In the graaffian (matured) follicles, atresia was likewise absent. In both the ovarian and uterine histology, there was no evidence of widespread vacuolization. Various plant extracts have been found to have beneficial effects on the ovarian and endometrial cycles in numerous additional studies.[22;23]

Finally, the findings of this study reveal that the hydroethanolic leaf extract of *Fleurya aestuans* had a good effect on the estrous cycle and histomorphology of the ovaries and uterus in female rats, suggesting that the leaves of *Fleurya aestuans* are beneficial to the animals' reproductive health.

REFERENCES

1. Yagmur E, Bast E, Mühlfeld AS, Koch A, Weiskirchen R, Tacke F, Neulen J. High prevalence of sticky platelet syndrome in patients with infertility and pregnancy loss. J Clin Med., 2019; 8: 1328.
2. Dou L, Zheng Y, Li L, Gui X, Chen Y, Yu M, Guo Y. The effect of cinnamon on polycystic ovary syndrome in a mouse model. Reprod Biol Endocrinol, 2018; 16: 99.
3. Yang YM, Choi EJ. Efficacy and safety of metformin or oral contraceptives, or both in polycystic ovary syndrome. Ther Clin Risk Manag, 2015; 11: 1345–53.
4. Tannys D, Ottawa A. Vancouver. Déclenchement de l'ovulation en présence du SOPK. Directive clinique de la SOGC, 2010; 242: 503–11.
5. Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev., 2012; 6: CD001122.
6. Kabiri N, Mohammad R, Seyed R. Beneficieal effects of pioglitazone and metformin in murinr model of polycystic ovaries via improvement of chemerin gene up-regulation. J Pharm Sci., 2014; 22: 39.
7. Saiyed A, Jahan N, Makbul SAA, Ansari M, Bano H, Hajera HS. Effect of combination of Withania somnifera Dunal and Tribulus terrestris Linn on letrozole induced polycystic ovarian syndrome in rats. Integr Med Res., 2016; 5: 293–300.

8. Lee YH, Yang H, Lee SR, Kwon SW, Hong EJ, Lee HW. Welsh Onion Root (*Allium fistulosum*) Restores Ovarian Functions from Letrozole Induced-Polycystic Ovary Syndrome. Nutrients., 2018; 10: 10.
9. Green I. Kinikanwo, Charles C. Nwafor and Weleh I. Iyke: Attenuation of Reproductive Dysfunctions by Hydroethanolic Leaf Extract of *Fleurya aestuans* in Diabetic Rats. *Asian Research Journal of Gynaecology and Obstetrics*, 2021; *6(2): 24-31*.
10. Falana BA, Oyeyipo IP. Selenium and Zinc Attenuate Lead-Induced Reproductive Toxicity in male Sprague-Dawley rats. *Research Journal of Medical Science*, 2012; 6(2): 66-70.
11. Mclean, A. C., Valenzuela, N., Fai, S., & Bennett, S. A. L. Performing Vaginal Lavage Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification, 2012; 4–9.
12. Goldman, J. M., Murr, A. A. S., & Cooper, R. L. The Rodent Estrous Cycle : Characterization of Vaginal Cytology and Its Utility in Toxicological Studies, 2007; 97: 84–97.
13. Tropp, J., & Markus, E. J. Effects of mild food deprivation on the estrous cycle of rats. Physiology & Behavior, 2001; 553–559.
14. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. Asian Journal of Andrology, 2005; 7(4): 399–404.
15. Bancroft, J.D. and Gamble, M. "Theory and practice of Histological Techniques"; Churchill Livingstone, Edinburgh, 2002; 16-64.
16. Okereke, C. and Onuoha, S. Effect of Ethanolic Extract of Cannabis sativa on Progesterone and Estrogen Hormones in Female Wistar Rats. *Journal of Reproductive System and Sex Disorder.*, 2015; 4: 150.
17. Chaudhari N, Dawalbhakta M, Nampoothiri L. GnRH dysregulation in polycystic ovarian syndrome (PCOS) is a manifestation of an altered neurotransmitter profile. Reprod Biol Endocrinol, 2018; 16: 37.
18. Lee YH, Yang H, Lee SR, Kwon SW, Hong EJ, Lee HW. Welsh Onion Root (Allium fistulosum) Restores Ovarian Functions from Letrozole Induced-Polycystic Ovary Syndrome. Nutrients, 2018; 10: 10.
19. Xiao, X.; Mruk, D. D.; Cheng, C. Y. "Intercellular adhesion molecules (ICAMs) and spermatogenesis". *Human Reproduction Update*, 2013.

20. Adamkovicova, M., Toman, R., Martiniakova, M., Omelka, R., Babosova, R., Krajcovicova, *et al.*. Sperm motility and morphology changes in rats exposed to cadmium and diazinon. *Reproductive Biology and Endocrinology,* 2016; 14(1): 42.
21. Kolawole T. A., Dapper D. V and Ojeka S. O. Ameliorative Effects of the Methanolic Extract of the Rind of Citrullus lanatus on Lead Acetate Induced Toxicity on Semen Parameters and Reproductive Hormones of Male Albino Wistar Rats. *European Journal of Medicinal Plants,* 2014; 4(9): 1125-1137.
22. Mvondo MA, Messongue Mbollo NS, Njamen D (2020). The ethanol extract of avocado [Persea americana Mill. (Lauraceae)] seeds reduced the hyperplastic effect of tamoxifen on uterine endometrium without changing its effect on the mammary gland. *Adv Tradit Med.*, 2020; 1–15.
23. Marie Alfrede Mvondo, Flavie Ingrid Mzemdem Tsoplfack, Charline Florence Awounfack & Dieudonné Njamen. The leaf aqueous extract of *Myrianthus arboreus*P. Beauv. (Cecropiaceae) improved letrozole-induced polycystic ovarian syndrome associated conditions and infertility in female Wistar rats *BMC Complementary Medicine and Therapies,* 2020; 20: 275.