AMELIORATIVE EFFECTS OF FLEURYA AESTUANS LEAVES ON MATING BEHAVIOURS AND REPRODUCTIVE HORMONES OF LEAD ACETATE INDUCED OVARIAN TOXICITY

[1]Charles CN, [2]Amah-Tariah FS [2], Adienbo OM & [2]Dapper DV
[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**
Corresponding Author's E-mail: nwaforcharles3@gmail.com

ABSTRACT

Fleurya aestuans (FA) is one of the most commonly botanic plants with therapeutic values in Nigeria. Nonetheless, there have neither been studies to explain the contribution of *Fleurya aestuans* on mating behaviors and sex hormones-related female ovarian toxicity nor the possible remedial potential of Kaempferol on ovariantoxicity in females exposed to heavy metals. This study investigated the ameliorative property of *Fleurya aestuans* on gonadotoxic effects of lead acetate (LA) in female rats. Fifty five female rats and eleven male rats were assigned into eleven groups of six animals each (female to male ratio, 5:1), such that the rats in groups 1, 2, 3 and 4 received orally 1ml of distilled water, 50mg/kg, 75mg/kg and 200mg/kg of hydroethanolic extract of *Fleurya aestuans* leaves, respectively, while the rats in groups A, B, C, D, E, F and G received orally 1ml of distilled water, 2.25mg/kg of Lead acetate, 50mg/kg of FA plus LA, 75mg/kg of FA plus LA, 200mg/kg of FA plus LA, 100mg/kg of Kaempferol plus LA and 100mg/kg of Kaempferol respectively. The female mating behaviour parameters were monitored on day 30 of the experiment. Luteinizing hormone, follicle stimulating hormone, estradiol, progesterone and prolactin were determined. Hydroethanolic extract of *Fleurya aestuans* leaves significantly ($p<0.05$) increased darting frequency, hopping frequency, lordosis frequency, licking behavior, FSH, LH, estradiol, progesterone and prolactin respectively. However, it caused a decline in the darting, hopping and lordosis latencies significantly ($p<0.05$). All these effects were significantly enhanced ($p<0.05$) when combined with kaempferol. The study revealed that administration of lead acetate decreased sexual behaviour parameters and reproductive hormones which were ameliorated by extract of *Fleurya aestuans* leaves and kaempferol.

Keywords: *Fleurya aestuans*, kaempferol, mating behavior, reproductive hormones.

INTRODUCTION

Diminished sexual drive is a repetitive sexual issue in ladies. Its frequency emphatically relies upon the age of the responders, hormonal disequilibrium and cerebral glitches controlling libido. Different social, mental, clinical reasons and the utilization of specific prescriptions and introduction to substantial metals can likewise prompt its turn of events (Kingsberg and Rezaee, 2013). Flibanserin, a blended serotonin receptor 1A agonist and receptor 2B antagonists, was the principal drug for the

condition affirmed by the National Agency for Food and Drug Administration and Control (NAFDAC). Flibanserin was demonstrated to be efficacious in the behavioral tests of female rodents and marmosets and various clinical preliminaries or trials with ladies. Adjustment of the cerebral monoamine balance by flibanserin upgrades solicitations - a marker of mating motivation in the female rodents, though consummatory sexual behavior isn't influenced.

The activity of flibanserin is by all accounts intervened by the mesolimbic dopaminergic pathway and hypothalamic structures associated with the integration of sexual clues identified with sexual motivation (Gelez et al., 2013). Different substances, including off-labeled utilization of hormones and natural plants advances female sexual receptivity in the rodent.

In the current investigation, we describe a novel therapeutic plant - *Fleurya aestuans*, which may have a strong capacity to advance mating behaviors in female rodents when conveyed orally.

Individuals of Iwofe village, Benin and Ilorin accepts that *Fleurya aestuans* leaves have fertility action. *Fleurya aestuans leaves* are typically devoured by these individuals to achieve pregnancy and for appropriate development of the baby (Gill 1992). This examination was conducted based on how the people of Iwofe, Benin and Ilorin Village devoured *Fleurya aestuans* leaves. Emphirical information shows that people in the aforementioned villages devoured *Fleurya aestuans* leaves by boiling in water or absorbing in liquor, consequently this research was conducted utilizing hydroethanol as solvent, by reason of polarity similar with water and alcohol.

Past research inspected the antioxidant and antifungal action of *Fleurya aestuans* in different dosages. It demonstrated that *Fleurya aestuans* extract has antioxidant and antifungal activity (Okereke *et al.,* 2014).

A few herbalists or botanists have persistently announced that the leaves of *Fleurya aestuans* can be utilized both as aphrodisiac and fertility enhancer agent; in any case, this has no logical or scientific premise. Thusly, the current investigation targets looking at the remedial value of *Fleurya aestuans* leaves on sexual drive and sex hormones in female humans utilizing Wistar rodents as models.

Materials and method

Preparation of extract

Fresh leaves of *Fleurya aestuans* (West Indian wood nettle) were gathered from the botanic homestead of University of Port-Harcourt, Nigeria and scientifically defined by the herbarium, Department of Plant Sciences, Faculty of Sciences, University of Port-Harcourt, Choba, Nigeria. The

leaves were altogether washed and dried at room temperature. The fine quality of dried leaves was kept in dry plastic container until use for extract processes. The technique of Al-Attar and Abu Zeid (2013) was utilized to prepare the extract with slight changes.

Animal model

Fifty five (55) female rodents and eleven (11) male rodents were utilized to lead the study. The rats weighed between 172.4 – 199.0g. They were purchased from the animal house, Madonna University Elele, Nigeria. The animals were housed in plastic cages and placed on commercial rat feed. They were permitted to feed and water ad libitum for seven days to adjust prior to initiation of study. Then the animals were subjectively isolated into two (2) stages;

Stage 1: Effects of extract on mating behaviors and sex hormones of female wistar rodents.

20 female rats and 4 male rats were used for this study and were divided into 4 groups of 6 rats each. The rat's groupings are as follows:

Group 1: Control group

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

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

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

The experimental groups were orally treated with single every day dosages of hydro-ethanolic leaf extract of *Fleurya aestuans* for 30 back to back days.

Stage 2: Effects of extract and kaempferol on mating behaviours and sex hormones of lead acetate induced ovarian toxicity.

35 female rodents and 7 male rodents were used for this study and partitioned into 7 groups of 6 rats each. Ovarian toxicity was induced with 2.25mg/kg of lead acetate using the method of Falana and Oyeyipo, (2012). The rat groupings are as follows:

Group A: Control group

Group B: Lead acetate group (2.25mg/kg)

Group C: Lead + 50mg/kg extract.

Group D: Lead + 75mg/kg extract

Group E: Lead + 200mg/kg extract

Group F: 2.25mg/kg Lead + 100µg/kg Kaempferol

Group G: Kaempferol group (100µg/kg)

Both extract and lead acetate were administered orally for a period of 30days

Mating behavior test

The procedure described by Giuliano *et al.,* (1999) was adopted for the assessment of mating behaviour in the female rats. The mating behaviour was carried out on every one of the female rats in all the groups by placing each in an opened plastic cage with the male in that group and this was done between 6:00am and 8:00am local time. The female animals were falsely brought into estrus (heat) phase (as the female rodents permit mating just during the estrus phase) by administering suspension of ethinyl estradiol orally at the dose of 100 mg/kg 48 h prior to the pairing and subcutaneous administration of progesterone at the dose of 1 mg/kg 6h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control and test animals. The most receptive females were selected for the study.

The following female sexual behavioral parameters were monitored on day 30:

Darting Latency: A short run that is portrayed by abrupt stoppage and opening of the posterior parts to the male rodent.

Darting Frequency: The number of darting/runs during the period of observation,

Hopping Latency: A short jump with stiff legs followed by immobility and a presenting behavior

Hopping Frequency: The number of hops during the period of observation

Lordosis Latency: The time taken to exhibit posture that allows mounting by the male and

Lordosis Frequency: The number of lordosis postures during the period of observation.

Licking Behavior: The number of times the female rat lick its private part to show sign of sexual arousal.

The experimental protocol was approved by Ethical Committee of the University of Port harcourt, Choba, Rivers State, Nigeria, University of Port harcourt, Choba, Nigeria.

Preparation of Serum

The procedure described by Yakubu *et al.,* (2005) was adopted for the preparation of the serum samples. The female rats were sacrificed under anaesthesia and blood was collected by cardiac puncturing into sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×g for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, England). The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -4 ℃ and used within 12 hours of preparation.

Hormonal Assay

The serum hormone concentrations of LH, FSH, estrogen, progesterone and prolactin were quantified according to the instruction provided by assay kit manufacturers, using microplate immunoenzymometric (ELISA) assays. The serum hormone concentrations were then interpolated from their respective calibration curves. The analyzer was calibrated and validated for use with rat sera.

Statistical Analysis

Results were expressed as the mean ± standard error of mean. Data were analyzed using a one-way analysis of variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with Students Package for Social Science, version 20.0 (SPSS , USA). Differences with values of $p<0.05$ were considered statistically significant.

Results

Table1:Values of female proceptive copulatory parameters following 30 days treatment of extract

Groups	Darting Lat	Darting Freq	Hopping Lat	Hopping Freq
1	102±1.00	7.20±0.00	68.00±0.00	4.00±0.11
2	98±0.20	6.00±0.00	63.00±0.00	5.10±0.00
3	93±0.00[a]	12.28±0.00 [a]	59.30±0.01 [a]	8.02±0.00 [a]
4	87±0.00 [a]	10.30±0.00	60.10±0.00 [a]	8.80±0.21 [a]

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

TABLE 2: Values of female receptive parameters following 30 days treatment of extract.

GROUPS	Licking Behavior	Lordosis Latency	Lordosis Frequency
1	1.51±0.20	115±2.00	3.00±0.00
2	1.60±0.00	119±0.00	3.50±0.22
3	3.60±0.11 [a]	108±0.00 [a]	2.70±0.05
4	2.90±1.00	101±0.03 [a]	4.02±0.00 [a]

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

TABLE 3: Values of female reproductive hormones following treatment of hydro-ethanolic leaf extract of *Fleurya aestuans* in wistar rats.

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

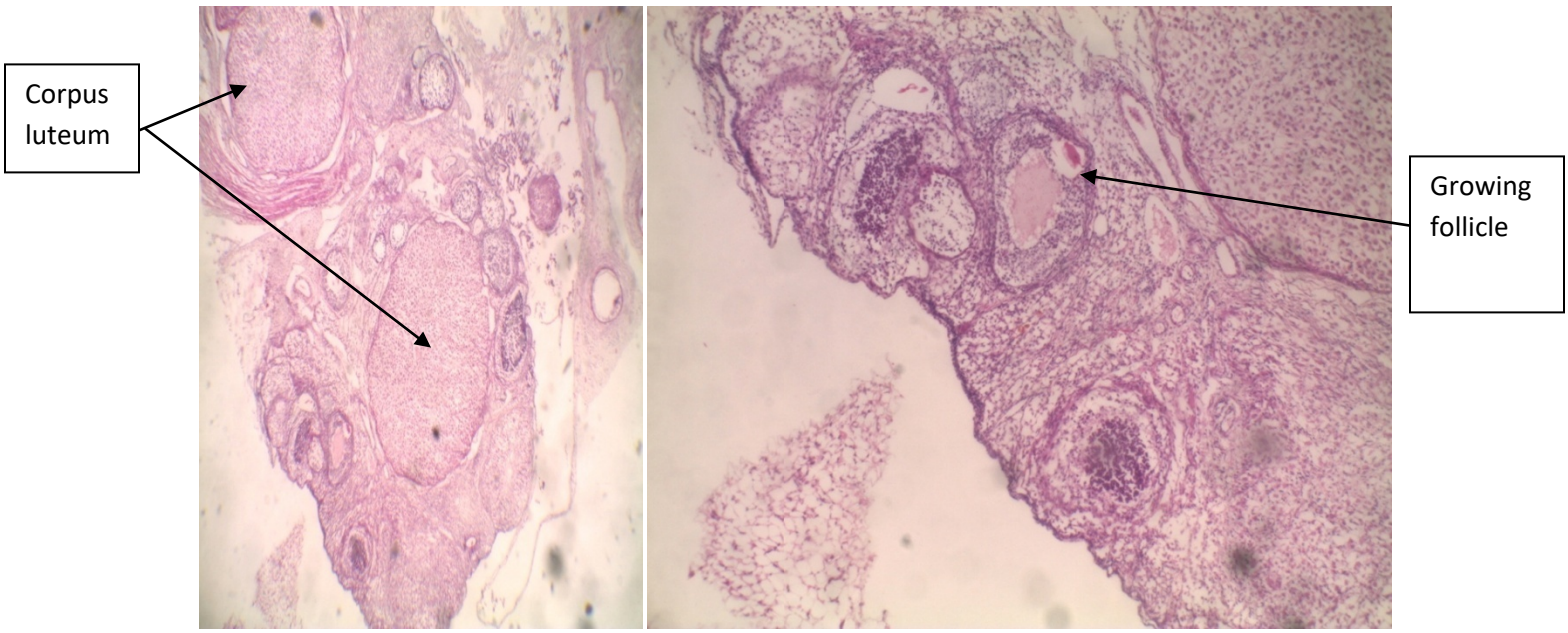

PLATE 1. Photomicrograph of transverse section of the ovary of rats in Group 1 (Control) at x125 and x600 Mag., shows growing follicle and corpus luteum of pregnancy

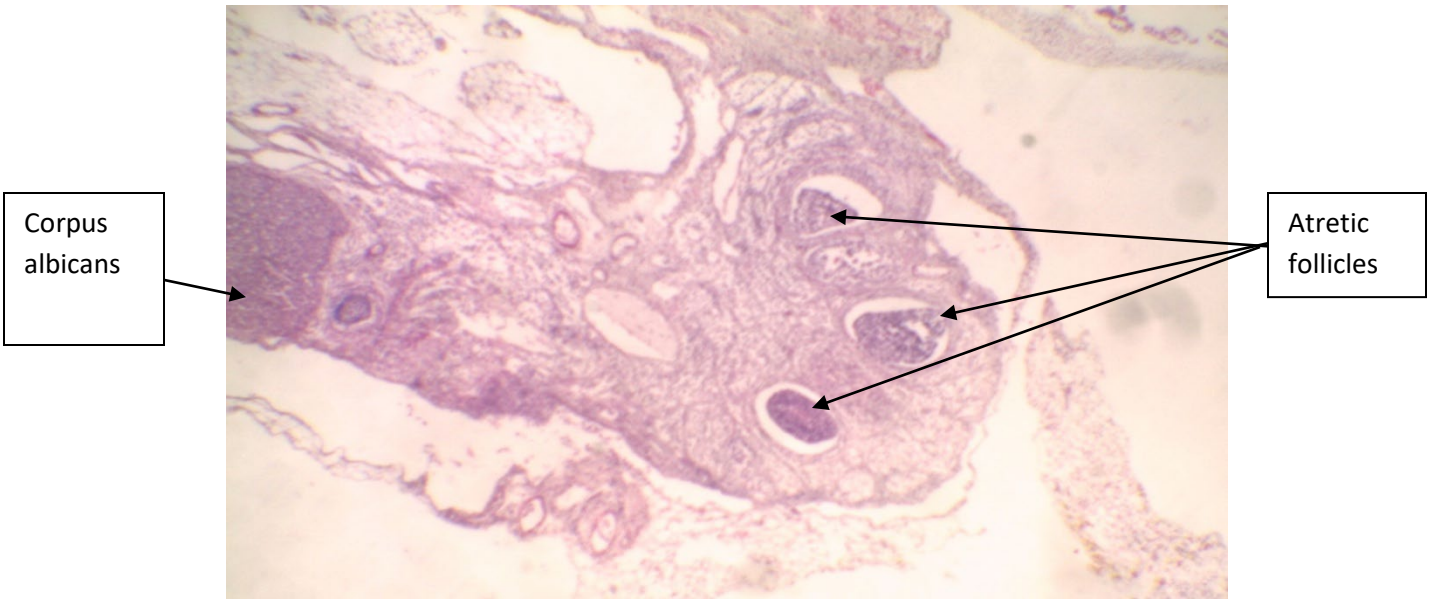

PLATE 2. Photomicrograph of transverse section of the ovary of rats in Group 2 (FA50mg/kg) at x600 Mag., shows atretic follicles and corpus albicans

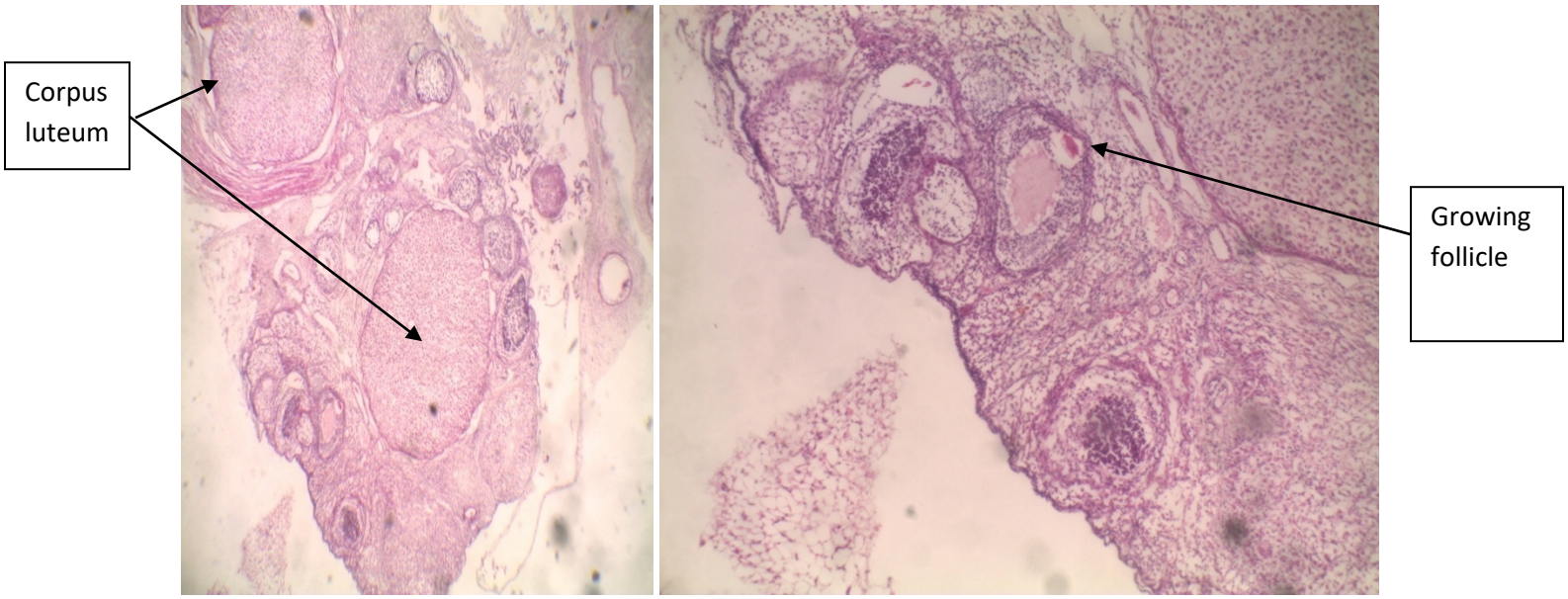

PLATE 3. Photomicrograph of transverse section of the ovary of rats in Group 3 (FA75mg/kg) at x125 and x600 Mag., shows growing follicle and corpus luteum of pregnancy

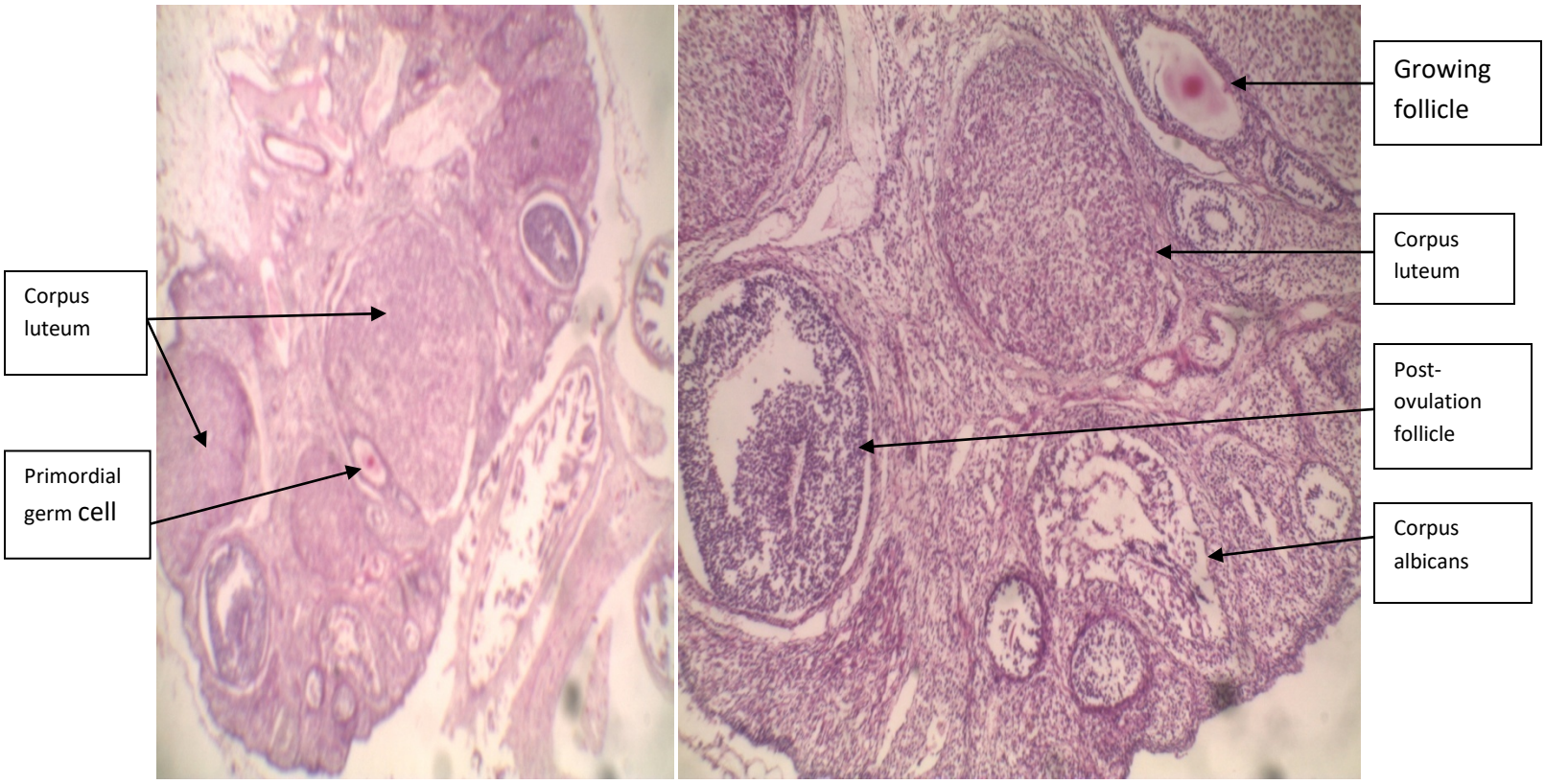

Plate 4. Photomicrograph of transverse section of the ovary of rats in Group 4 (FA 200) at x125 and x600 Mag., shows Graaffian follicles at different stages of development.

TABLE 4: Values of proceptive parameters following treatment of extract and kaempferol on lead acetate induced ovarian toxicity.

Groups	Darting Latency	Darting Frequency	Hopping Latency	Hopping Frequency
A	99.30±0.21	9.00±0.32	42.00±0.25	13.00±0.58
B	114.00±0.18 [a]	4.02±0.30[a]	56.05±3.00[a]	6.03±0.51[a]
C	107.86±0.08	4.58±0.00	48.72±1.00	6.55±0.58
D	111.70±0.30	6.90±0.00	51.00±0.00	10.00±0.40 [b]
E	97.40±0.81[b]	8.00±1.00 [b]	44.08±2.07 [b]	9.40±0.25
F	101.42±0.43 [b]	6.33±0.20	50.00±0.00	12.00±2.71 [b]
G	105.56±0.65 [b]	7.04±0.03 [b]	43.00±0.03 [b]	9.80±3.43

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 receptive parameters following treatment of extract and kaempferol on lead acetate induced ovarian toxicity.

Groups	Licking Behavior	Lordosis Latency	Lordosis Frequency
A	6.30±0.21	8.81±0.40	101.73±7.75
B	2.49±0.18[a]	3.84±0.60[a]	88.33±3.83[a]
C	3.86±0.08	5.01±0.78	82.72±1.16
D	3.70±0.30	5.90±0.40	78.00±0.00
E	7.40±0.81 [b]	7.32±1.00 [b]	99.08±2.87 [b]
F	6.42±0.43 [b]	7.14±0.60 [b]	91.00±0.00
G	8.56±0.65 [b]	10.68±0.49 [b]	104.61±2.27 [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 6: Values of reproductive hormones following treatment of extract and kaempferol 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[a]	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

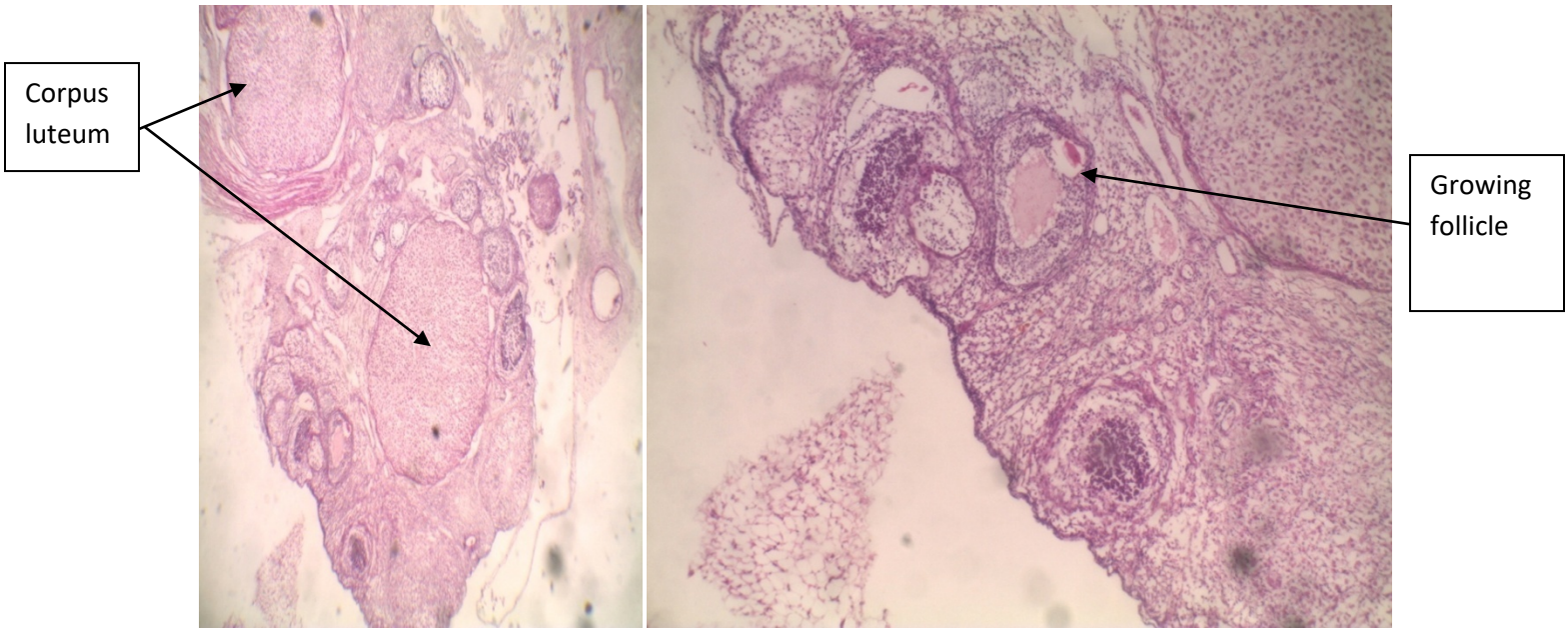

Plate A. Photomicrograph of transverse section of the ovary of rats in Group A (Control) at x125 and x600 Mag., shows growing follicle and corpus luteum of pregnancy

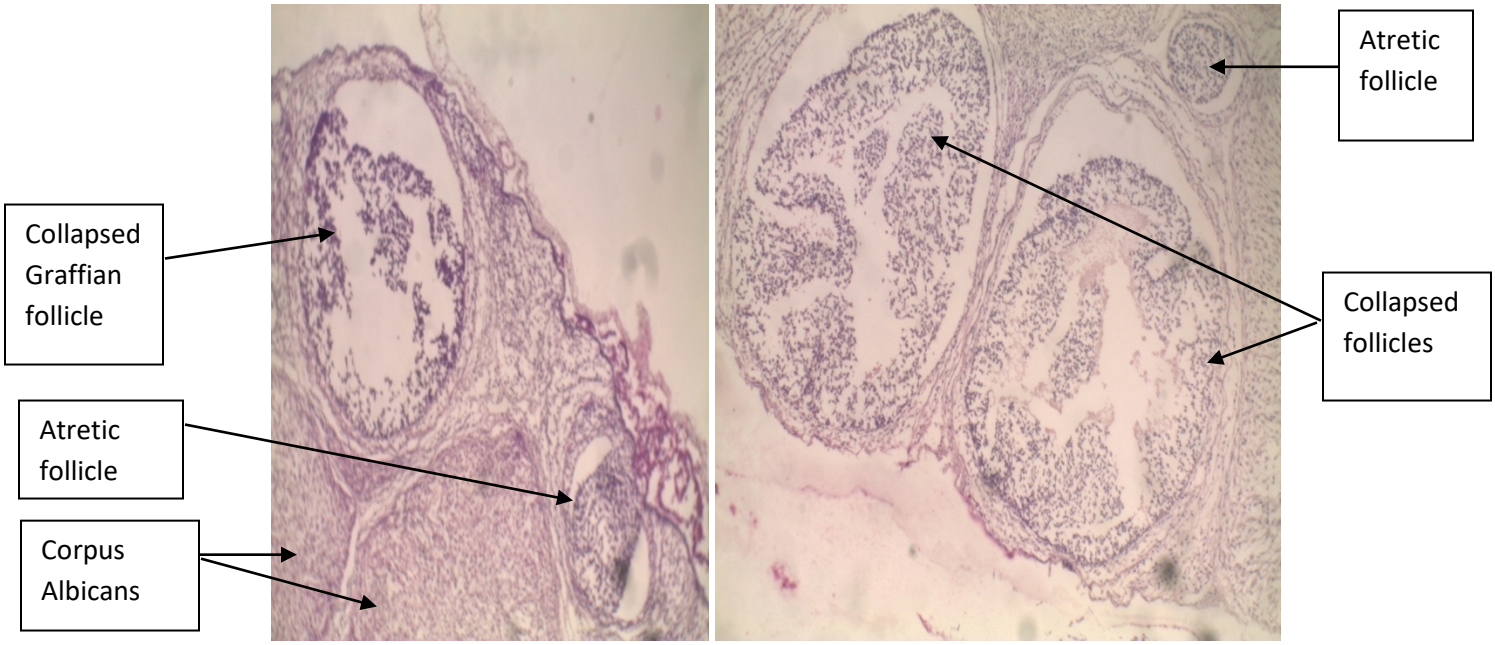

Plate B. Photomicrographs of transverse section of the ovary in Group 2 (Lead acetate rats) at x125 and x600 Mag., shows atretic follicles, collapsed follicles and corpus albicans at various stages of development

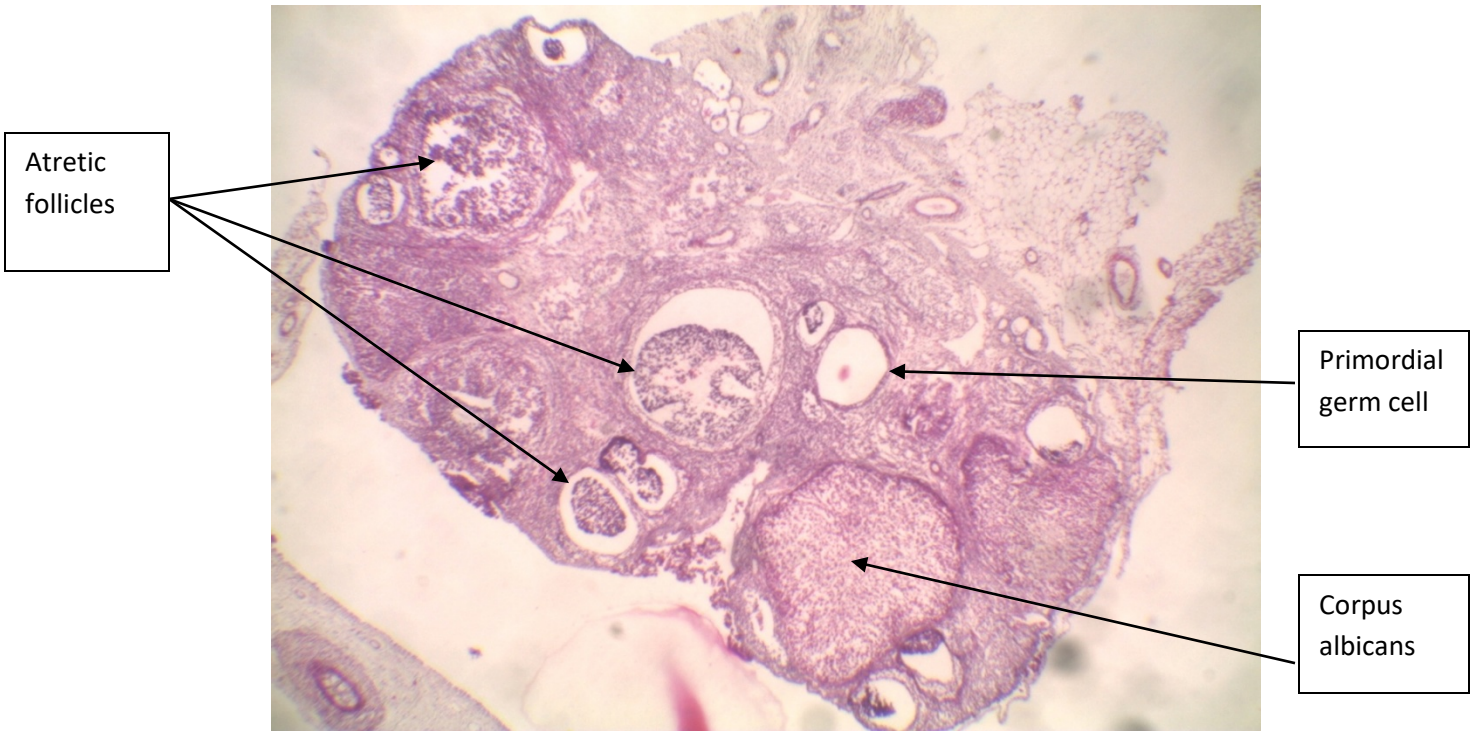

Plate C. Photomicrograph of transverse section of the ovary of rats in Group C at x600 Mag., shows atretic follicles and corpus albicans. A primordial germ cell is seen developing.

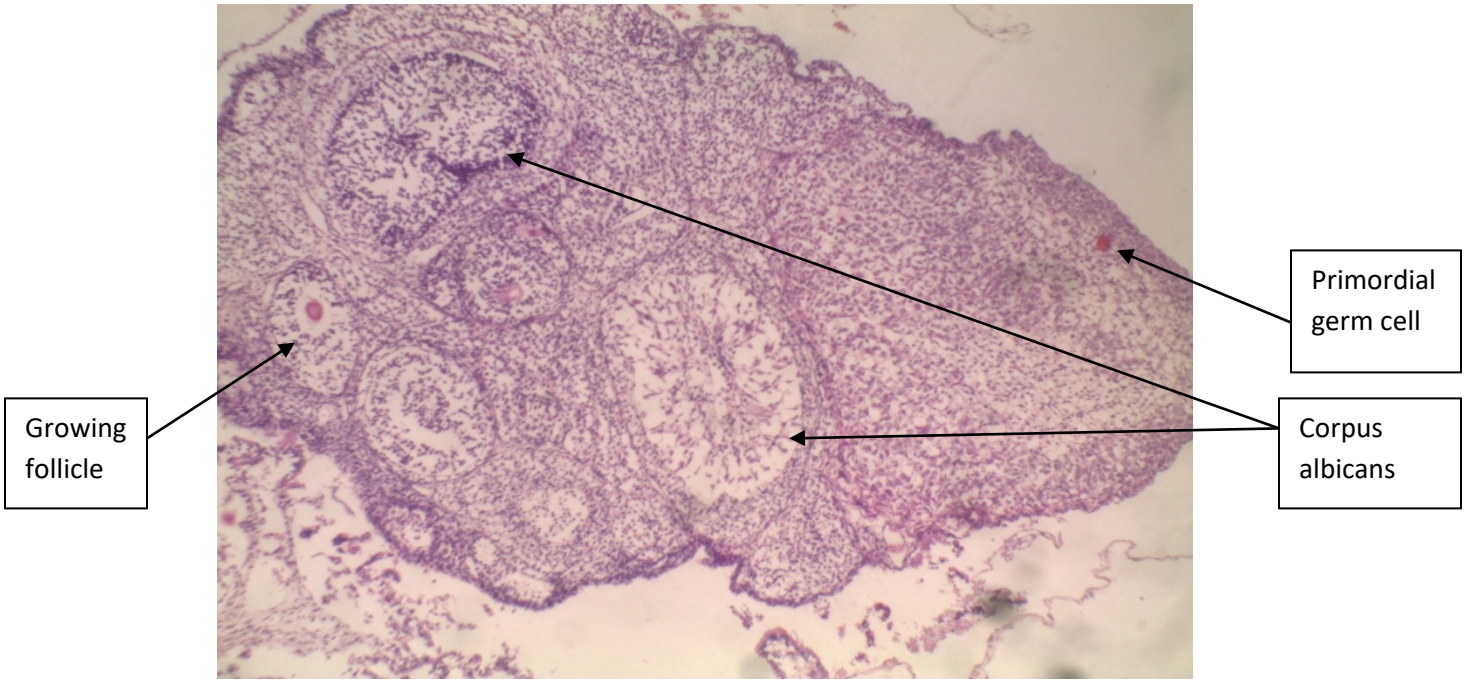

Plate D: Photomicrograph of transverse section of the ovary of rats in Group D at x600 Mag., shows primordial germ cell, growing follicles and corpus albicans.

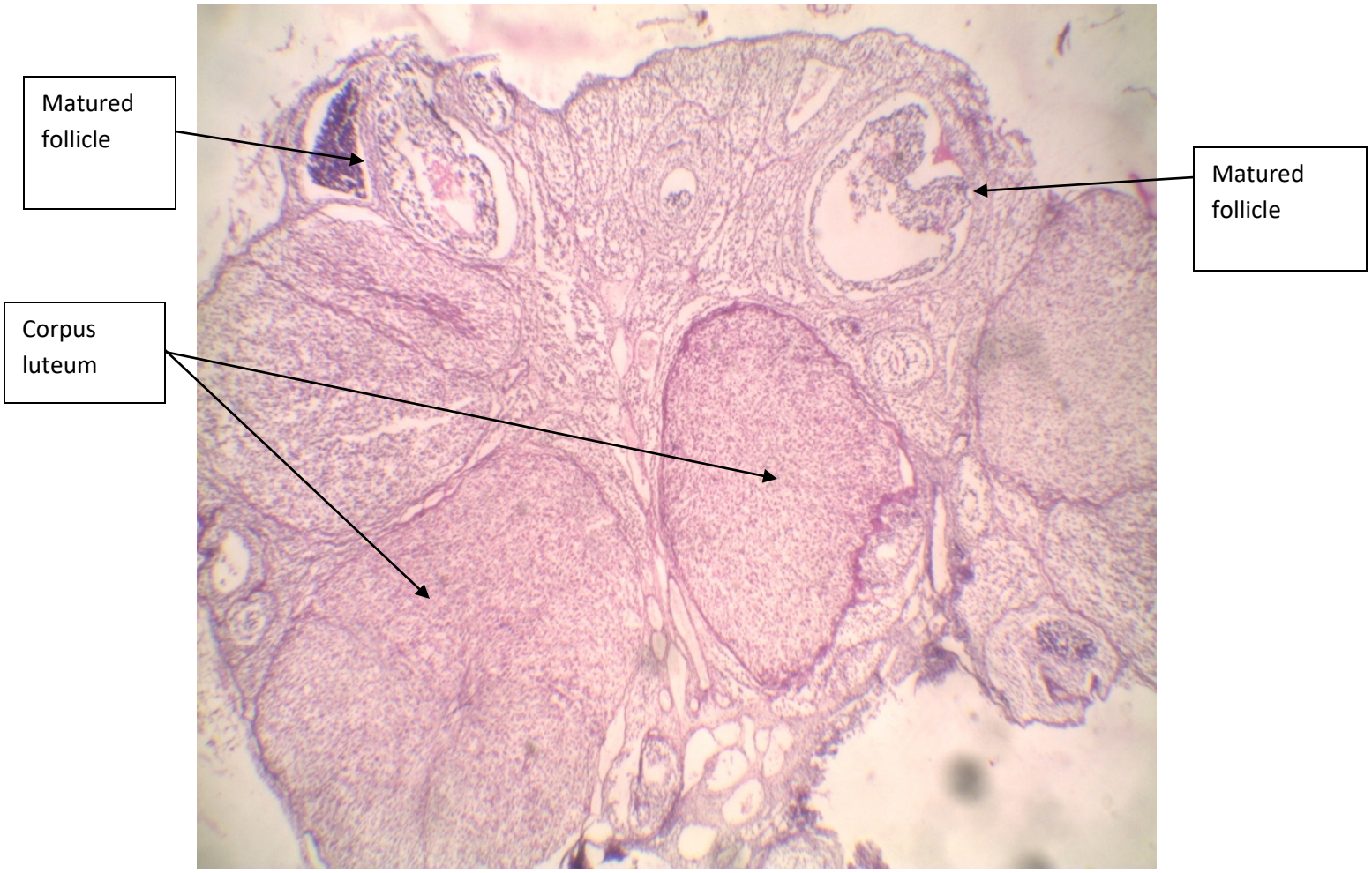

Plate E. Photomicrograph of transverse section of the ovary of rats in Group E at x600 Mag., shows reversal of the collapsed matured follicle at preovulatory phase and corpus luteum.

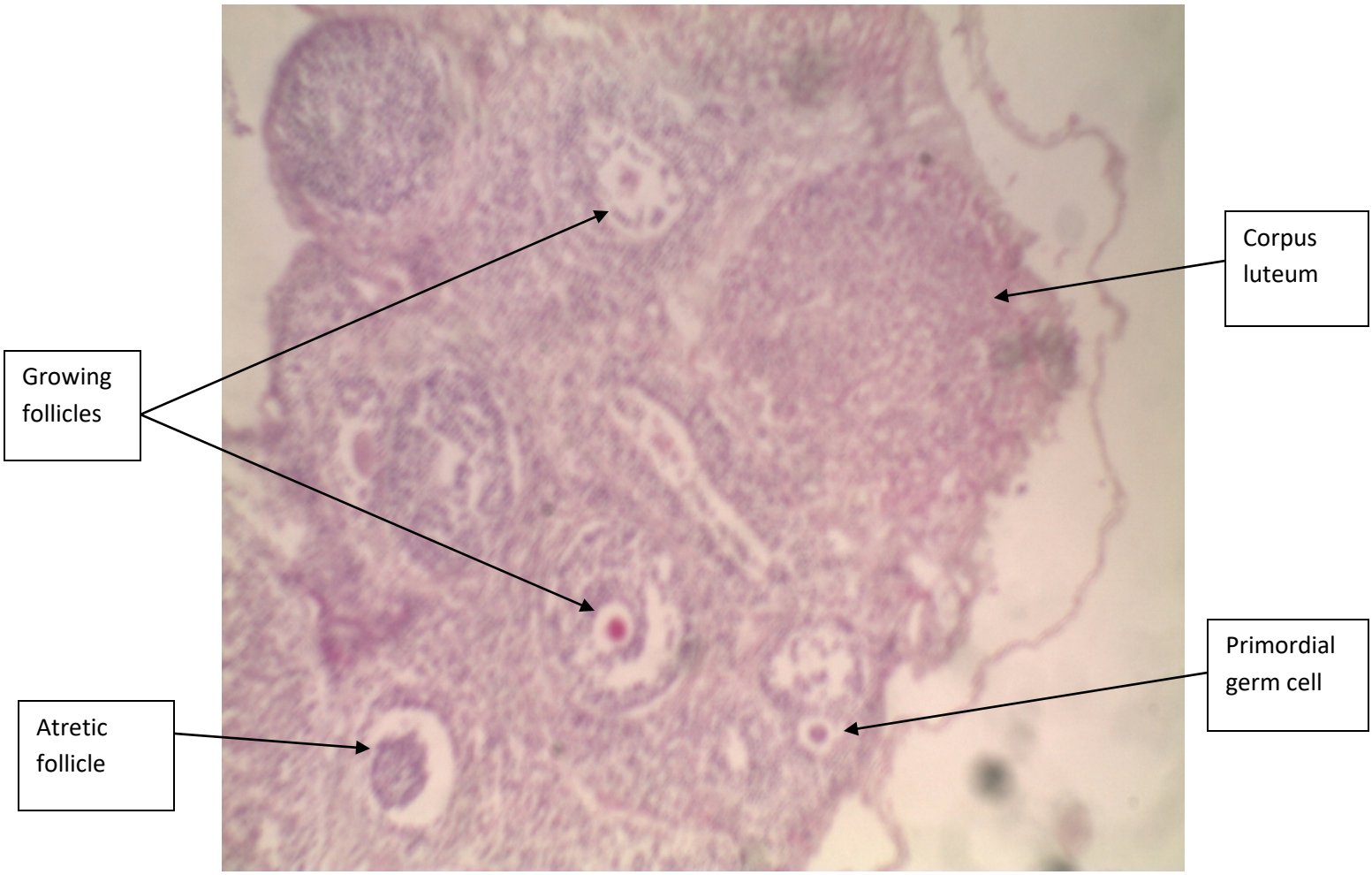

Plate F. Photomicrograph of transverse section of the ovary of rats in Group F at x600 Mag., shows normal primordial and growing follicles, corpus luteum and some atretic follicles.

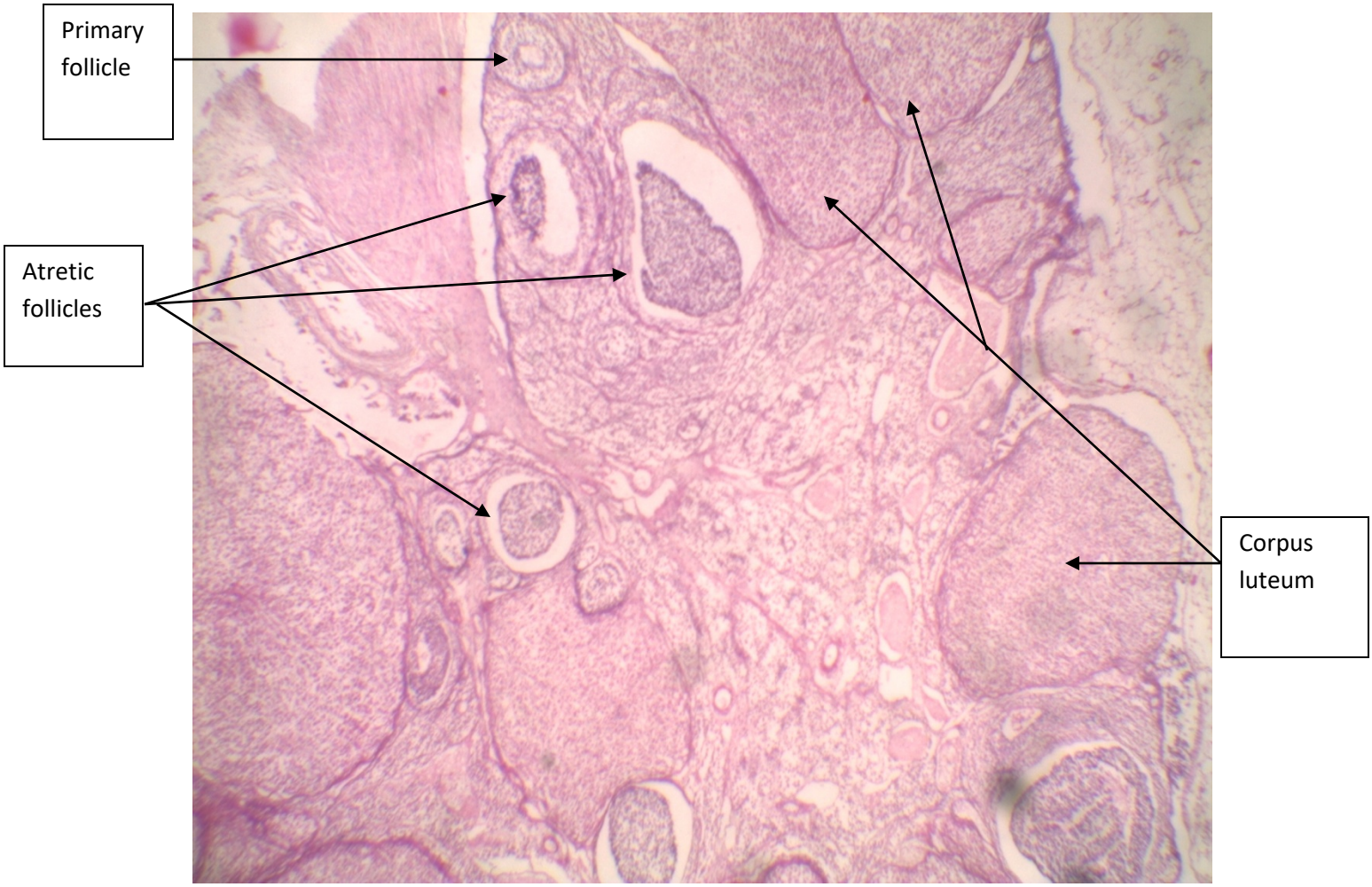

Plate G. Photomicrograph of transverse section of the ovary of rats in Group G at x 600 Mag., shows primary follicle, atretic follicles and corpus luteum.

DISCUSSION

Remedial or therapeutic plants have been recorded to have phytochemicals, for instance, alkaloids, flavonoids, phenolics and saponins which may upgrade aphrodisiac activity, although, some may improve the degree of conceptive hormones, others may upgrade the activity of these sex hormones in the body (Modelska and Milián, 2004). The leaves of *Fleurya aestuans* extract has been recorded to have restorative active ingredients, for instance, tetrahydroxyflavone (kaempferol), 1-methyl-4-cyclohex-1-ene (limonene), flavan-3,3,4,5,7-pentol (catechin), narigenins, anthocyanins, phenols and flavonoids in enormous amount, henceforth making it advantageous in the management of persistent infections (Okereke *et al.,* 2014).

Results from the current examination uncovered that the administration of lead acetic acid caused a remarkable decrease in ($p<0.05$) DF, HF, LF, LB, LH, FSH, E2, PRL and Progesterone. Nonetheless, DL, HL and LL increased essentially ($p<0.05$) in the lead acetate-treated female rats compared with other groups. This could be credited to the reactive oxygen species (ROS) produced by lead acetic acid in the cerebral tissues and ovarian tissues. Wang *et al*. (2017) analyzed that Pb exposure decreases reproductive hormones and elevates risk of still births which corresponds with the findings of the present study.

In facilitation to this, when lead acetic acid was given in combination with *Fleurya aestuans* leaves and Kaempferol, it improved the low sexual drive in the female rodents, proposing a stimulatory viability on aphrodisiac activity and the hypothalamic-pituitary-ovarian axis (Yakubu *et al.,* 2016).

This might be because of remarkable amount of alkaloids, flavonoids, phenolic and saponin present in the leaves of *Fleurya aestuans* extract (hydroethanolic) which has been documented to possess high libido potential (Da Silva *et al.,* 2012).

The decline in the conceptive hormonal levels observed in the lead acetate-treated group might be connected to the hindrance of hormone synthesis by the granulosa cells of the developing follicles in the ovary which might be because of diminished FSH level, which diminished the emission of the hormone. A past report has archived that concealment or suppression of estrogen receptors in the ventromedial nucleus of the nerve center in female rodents could likewise be utilized to clarify diminished proceptivity and receptivity in female rodents (Olivier et al., 2011).

Accordingly, the decrease in these sexual behavioral indices by lead acetic acid treated group might be because of the conceivable decrease in the estradiol content.

Normally, female conceptive capacities rely upon the discharge of LH and FSH by the pituitary gland mediated by hypothalamic gonadotropin-releasing hormone (GnRH). In females, LH invigorates the theca cells of the ovaries to discharge progesterone while FSH instigates the granulosa cells of the developing follicles to deliver estradiol. Subsequently, the diminished degrees of LH, FSH and progesterone observed in the lead acetic acid treated group might be credited to an inhibitory impact on the hypothalamic-pituitary-ovarian-axis which was enhanced when co-treated with *Fleurya aestuans* and kaempferol.

The results of the current study corroborates with the histological examination of the rat's ovaries displayed at different magnifications (x125 and x600).

Plates 1 to 4 and Plate A, 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 B (LA 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 plates C to G, primordial germ cells and growing follicles were sighted developing. The primordial germ cells developed to full maturity (graafian follicles) in Plate E 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.

Taking everything into account, this investigation has uncovered that lead acetic acid diminished female mating behaviors which could be invigorated by the creation of oxygen free radicals and hormonal interference. Nonetheless, these impacts might be improved by *Fleurya aestuans* leaves dissolved in water and alcohol (hydroethanol).

Since the utilization of remedial/therapeutic plants is becoming popular around the world in light of its clinical and medical advantages, the utilization of *Fleurya aestuans* as palliatives is suggested for females with low sex drive and hormonal disequilibrium.

REFERENCES

Al-Attar, A.M., Abu Zeid, I.M., 2013. Effect of tea (Camellia sinensis) and olive (Olea europaea L.) leaves extracts on male mice exposed to diazinon. *Biomedical Research International.* 1–6

Da Silva, C. V., Borges, F. M. and Velozo, E. S. (2012). Phytochemistry of some Brazilian plants with aphrodisiac activity. In: Rao V. Phytochemicals—a global perspective of their role in nutrition and health. Croatia: InTech: 953–978.

Dasofunjo K; Asuk A.A; Ezugwu H.C; Nwodo O.F.C.; Olatunji T.L. 2013. Aphrodisiac Effect of Ethanol Extract of Piliostigma thonningii Leaf on Male Albino Wistar Rats. *Journal of Applied Pharmaceutical Science* Vol. 3 (10), pp. 130-135

Essiett, U., A., Edet, N., I., Bala, D., N. (2011): Phytochemical and physiochemical analysis of the leaves of Laportea-aestuan (Linn) chew and laporteaaestuan (schumach)chew (male and female); *Asian Journal of plant science and Research* 1:35-42

Falana BA, Oyeyipo IP (2012). Selenium and Zinc Attenuate Lead-Induced Reproductive Toxicity in male Sprague-Dawley rats. *Research Journal of Medical Science*. 6(2):66-70.

Gelez H, Greggain-Mohr J, Pfaus JG (2013). Flibanserin treatment increases appetitive sexual motivation in the female rat. *Journal of Sex Medicine*;10:1231-1239.

Kingsberg SA, Rezaee RL (2013). Hypoactive sexual desire in women. Menopause;20:1284-1300.

Modelska, K., Milián, M. (2004). Treatment of female sexual dysfunction in postmenopausal women: what is the evidence? Rev Gynaecological Practice. 4(2): 121–132.

Okereke Stanley, C., Elekwa, I., Chukwudoruo Chieme, S. (2014): Preliminary Phytochemical Screening and Gas Chromatographic FID Evaluation of Fleurya Aestuans Leaf Extracts. *International Journal of Current Biochemistry Research* 2(3) 37 43.

Olivier B., Chan J. S., Snoeren E. M., Olivier J. D., Veening J. G., Vinkers C. H., Waldinger M. D. and Oosting R. S. (2011). Differences in sexual behavior in male and female rodents: role of serotonin. Current Top Behavioural Neuroscience., 8: 15–36.

Yakubu MT, Akanji MA, Oladiji AT. (2005)a. Aphrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. Asian Journal of Andrology. 7(4): 399–404.

Yakubu, M. T. and Olutoye, A. F. (2016)b. Aphrodisiac activity of aqueous extract of Anthonotha macrophylla P. Beauv. leaves in female Wistar rats. *Journal of Integrated Medicine*., 14(5): 400–408.