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# Pausinylstalia yohimbe Affects Reproductive Hormones, Estrus Cycle and Folliculogenesis in Adult Female Rats

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**Abstract**
*Pausinystalia yohimbe* (*P. yohimbe)* has long been used by West African tribes, as an aphrodisiac among men and for the treatment of erectile dysfunctions in the sub-Saharan region of Africa. More recently, women use it to enhance arousal and libido while infertile women use it to improve chances of conceiving. However, there are no studies on the effects of *P. yohimbe* on female reproduction. Therefore, this study examined its effects on reproductive hormones and organs, estrus cycle and folliculogenesis in adult female rats.Eighteen adult female Sprague-Dawley (SD) rats were randomly allocated into three groups. Group A was the control while groups B and C served as the treatment groups. Group A received 0.5ml normal saline daily while groups B and C received 150mg/kg and 300mg/kg body weights of aqueous extracts of *P. yohimbe* respectively via oral gavages for 6 weeks. Vaginal smear of the rats were taken daily for 4 weeks after the first week of drug administration to determine their estrous cycles. After six weeks, rats were sacrificed and sera obtained from the rats were assayed for sex steroid hormones; Estradiol (E2) and Progesterone and gonadotropic hormones; Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Ovaries and uteri were removed for histological processing and antral follicle counting.Estrous cycle was irregular in groups B and C. Serum hormonal levels of E2, Progesterone, FSH and LH for the test groups differed from those of the controls were not significant. Follicles including corpus lutea were significantly decreased and tissue destruction as well as necrosis were observed in some areas of the ovaries and uteri of groups B and C.*P. yohimbe* despite its usefulness in enhancing arousal and libido in females, it affects reproductive hormones, estrous cycle and folliculogenesis. Its prolonged use may lead to reduced reproductive functions and negatively affect fertility.
**Keywords**: Estrus cycle, Folliculogenesis, *Pausinylstalia yohimbe*, Rats, Reproductive hormones

## 1.0 Introduction

*Pausinystalia yohimbe* (*P. yohimbe)* was first discovered and used by tribes in West Africa, where it grows in the wild, mostly in the Atlantic evergreen forest from South-east Nigeria to Congo (Guay *et al*, 2002, Adeniyi *et al*., 2007). *P. yohimbe* is native to the West African Cameroun, Congo, Democratic Republic of Congo, Equatorial Guinea, Gabon and Nigeria (Oliver Beyer, 1989, Sunderland *et al*., 1997, Onwa *et al.,* 2009). The West African tribes still use *P. yohimbe* for its powerful aphrodisiac effects. It has a more recent history of use as aphrodisiac and hallucinogen (Okonkwo, 2012, Sahelian, 2016). Aphrodisiac can arouse or enhances sex drive, libido or pleasure. Most aphrodisiacs also enhanced sensory awareness contributes to sexual arousal and pleasure (Yakubu *et al* 2007).

Due to the effective aphrodisiac actions of *P. yohimbe*, it is widely used for the treatment of erectile dysfunctions in the sub-Saharan region of Africa (Aliyu and Alkali, 2006, Sunderland *et al*., 1997, Eweka *et al*., 2010, Etiani, 2012). Recently in many West African cities, it is also commonly used to spice food especially barbecued meat and sauce. Interestingly, women now use it more to enhance arousal and libido while infertile women use it to improve chances of conceiving. Recent studies from our laboratory show that *P.* yohimbe affects the male accessory glands, testes, sperm concentration and motility in rats (Ajonuma et al. 2017 a, b).

Although *P.* yohimbe has long been used for the treatment of erectile dysfunctions and widely as food additive, its effects on female fertility are not well understood. There are no studies on the effects of *P. yohimbe* on female reproduction. Therefore, this study examined its effects on reproductive hormones and organs, estrus cycle and folliculogenesis in adult female rats.

## 2.0 Materials and Methods

### 2.1 *Animals*

Eighteen adult female Sprague Dawley (SD) rats were obtained from Animal House Unit of Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos State, Nigeria. The rats were fed with standard rat chow and clean water ad libitum. The animals were kept under standard laboratory conditions of 12 hours light and 12 hours darkness, proper humidity and temperature. They were allowed to acclimatize for 2 weeks prior to experiments. All procedures in this study conformed to the guidelines for research involving animals as recommended by the Lagos State University College of Medicine Animal House Unit on the care and use of

laboratory animals.

**2.2** ***Preparation of P. yohimbe extract***

This has been described previously (Etiani JC, 2012, Ajonuma et al. 2017 a, b). In brief, dried stems of *P.yohimbe* purchased locally from a herbal merchant at Alabarago, Ojo, Lagos, Nigeria and confirmed by several others as authentic. *P.yohimbe* stems were pounded and then blended in an electric blender to a fine smooth powder. 150g of powdered *P.yohimbe* was boiled in 3litres of distilled water in a beaker on a hot plate with a magnetic stirrer for 90mins at a temperature of 100°C, after which the boiled extract was allowed to cool, filtered and placed in oven at about 100°C to dry. The oven dried extract was weighed indicating that 150g of powdered *P.yohimbe* yield 29.76g dried *P.yohimbe* crude extract.

**2.3** ***Experimental Design***

The rats were randomly allotted into 3 groups of 6 each. Group A was the control group while groups B and C served as the test groups. Group A received 0.5ml of normal saline daily while groups B and C received orally 150mg/kg and 300mg/kg body weights of aqueous extracts of *P. yohimbe* respectively for 6 weeks via oral gavages. Dose for each animal = weight (kg) x dose/concentration (Ajonuma et al. 2017 a, b). The concentration was 49.6mg/ml and it was then administered to each of the animals according to their weights following this formula.

Estrus cycles of rats were monitored daily from the second week of drug administration for 4 weeks. After 6 weeks of administration, the rats were anaesthetized using ketamine HCL, and were dissected open; blood samples were collected via cardiac puncture and were stored in plain sample bottles for hormonal assay. Uteri and ovaries obtained from the rats were weighed and fixed in 10% buffered formalin for histological processing.

**2.4** ***Serum Preparation and Hormonal analysis***

The collected blood samples were allowed to clot and then centrifuged (Surgifield, SM80-2, England) for 20mins at 3000 rpm. The serum samples were then transferred into clean sterile sample bottles and stored at -20ºC until analyzed. The serum samples were assayed for Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2) and Progesterone using AccuBind test kits and the enzyme linked immunosorbent assay (ELISA) technique. Test kits used were made by MonoBind Inc, Lake Forest, CA, USA.

This has previously been described (Ajonuma et al. 2017 a, b). In brief, reagents and the serum were brought to room temperature (25 ºC). 25µl of serum sample was placed into micro plate wells using a pipette. 50µl of enzyme reagent was added to the wells. Then the micro plates were swirled gently for 30 seconds to enable mixing and covered to incubate for 90 minutes at room temperature. The contents of the micro wells were discarded by decanting with absorbent paper. 350µl of wash buffer was added and washing was repeated for three times. 100µl of substrate solution was added to the wells and incubated at room temperature for 20 minutes. 50µl of stop solution was added to each well and was gently mixed for 20 seconds. The solution was read within 30 minutes, and each well was read at 450nm using a reference wavelength of 630nm optical density on a STAT Fax 4700 ELISA Microplate reader (Stat Fax by Awareness Technologies, USA).

**2.5** ***Estrus cycle Determination***

This was done as previously described (Fellicio et al, 1988; Shannon et al, 2012). Cotton tipped swab wetted with distilled water was carefully inserted into the vagina of the restrained rats. The swab was gently turned and rolled 360$^{o}$ against the vaginal wall and then removed. Cells on the swab were transferred to a dry glass slides by rolling the swab on the slides. The slides were air dried and fixed by dipping into a mixture of cold 50% vv of acetone and methanol. The slides were allowed to dry and then dipped into stain A and stain B for 30secs (stain A and stain B is Eosin and methylene blue respectively) each. The slides were then inserted into distilled water to wash out excess stain and allowed to dry. Each slide was observed under microscope to check the estrous cycle stages of the rats (Fellicio et al, 1988; Shannon et al, 2012).

**2.6** ***Histology and antral follicle count***

This was done as previously described (Ajonuma et al., 2005). In brief, all organs obtained from the rats (uteri and ovaries) were dissected free of fatty tissues and fixed in 10% buffered formalin overnight. Tissue samples were dehydrated in graded ethanol and embedded in paraffin wax and processed with KD-TS6A tissue processor. Sections of 5µm thick were cut using a Shandon Finesse Manual Rotary Microtome, model 325**,** Thermoscientific, and dried onto microscope slides (Fisher Scientific, Pittsburgh, PA, USA). For hematoxylin and eosin (H&E) staining, slides were dewaxed in xylene and dehydrated in graded alcohol and stained for light microscopy. Observation was performed under a Novel Optic Binocular microscope; model NLCD-307 and for antral follicle count.

### 2.7 *Statistical Analysis*

Data are presented as mean and standard error of mean (SEM). Analysis of variance (ANOVA) was use for data analysis and differences between groups were compared using Tukey comparison test. A p-value of $\leq 0.05$ was considered significant. Statistical analyses were carried out using Graph Pad Prisms software version 7, Graph Pad Prisms Inc. San Diego, CA, USA.

## 3.0 Results

### 3.1 *Relative organ wet weights*

Relative organ wet weights of the uterus in both groups B and C were decreased from those of the controls. In the ovaries, the weights of group C were decreased but not in group B (Table 1). However, these were not statistically significant (Figure 1).

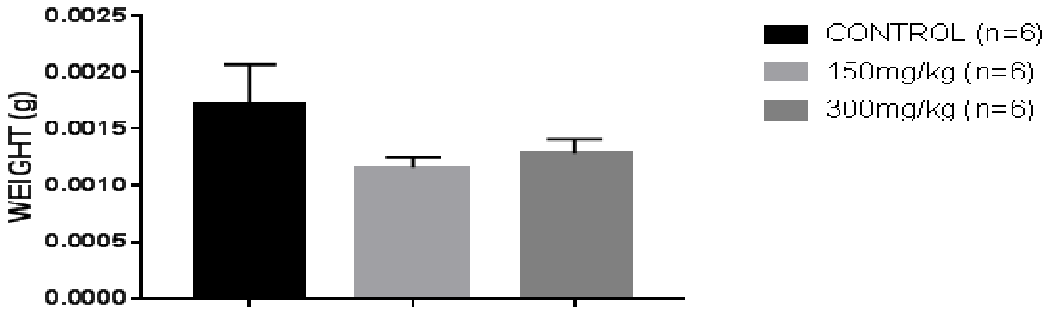
**Table 1: Effect of P. yohime on Relative organ weights**

| GROUPS | OW (g) | UW (g) | ROW (g) | RUW (g) |
|---|---|---|---|---|
| GROUP A | (0.09567±0.01682) | (0.3113±0.06625) | (0.0004875±0.0001021) | (0.001712±0.0003514) |
| GROUP B | (0.1427±0.03654) | (0.2183±0.03076) | (0.000799±0.0002255) | (0.001153±9.599e-005) |
| GROUP C | (0.1053±0.0274) | (0.286±0.03564) | (0.00045±8.859e-005) | (0.001283±0.0001299) |

Group A (control), Group B (150mg/kg bw), Group C (300mg/kg bw), P>0.05 when compared to the control, OW= ovarian weight, UW= uterine weight, ROW= relative weight of ovaries, RUW= relative weight of uterus, n = 6.

**Figure 1: Relative organ wet weights**

**A**




**B**

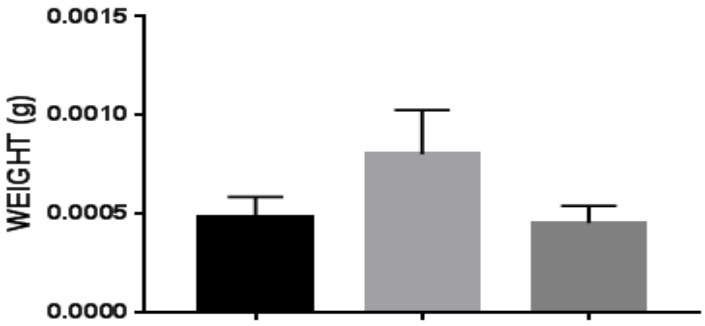



**Figure 1: Effect of *P. yohimbe* on Relative organ wet weights**

Effect of *P. yohimbe* on Relative organ wet weights after 6 weeks treatment. Group A Control, Group B 150mg/kg and Group C-300mg/kg showing differences in both treatment groups; when compared to the control GroupA. **A,** uterus and **B,** ovary; n=number of rats. *P>0.05*

### 3.2 *Effect of P.yohimbe on Serum hormones*

As shown in table 2, serum estradiol level between the test groups; group B (31.361±7.855) and group C (59.52±12.04) were different compared to group A (44.91±7.642). It was elevated in Group C and decreased in group B. Serum progesterone levels in the test groups; group B (33.65±6.462) and group C (15.27±5.388)

compare to control group A (20.7±2.941) were different too. Group B was elevated while group C seem to be similar to the control group.

Serum FSH levels were similar in groups; B (0.25±0.06708) and control group A (0.2±0.04282) with slight increase in group C (0.30±0.06325). Serum LH level of the test groups; group B (0.8±0.04472) and group C (0.7333±0.1476) were decreased compare to control group A (1.517±0.4909). However, these differences were not statistically significant (Figure 2).

**Table 2: Effect of P. yohimbe on Estradiol, Progesterone, FSH and LH,**

| GROUPS | ESTRADIOL (pg/ml) | PROGESTERONE (pg/ml) | FSH (mIU/ml) | LH (mIU/ml) |
|---|---|---|---|---|
| GROUP A | (44.91±7.642) | (20.7±2.941) | (0.200±0.04282) | (1.517±0.4909) |
| GROUP B | (31.36±7.855) | (33.65±6.462) | (0.250±0.06708) | (0.800±0.04472) |
| GROUP C | (59.52±12.04) | (15.27±5.388) | (0.300±0.06325) | (0.7337±0.1476) |

Group A (control), Group B (150mg/kg bw), Group C (300mg/kg bw), P>0.05, FSH - Follicle Stimulating hormone, LH - Luteinizing hormone, n = 6.

**Figure 2: Effect of *P. yohimbe* on Serum hormones**

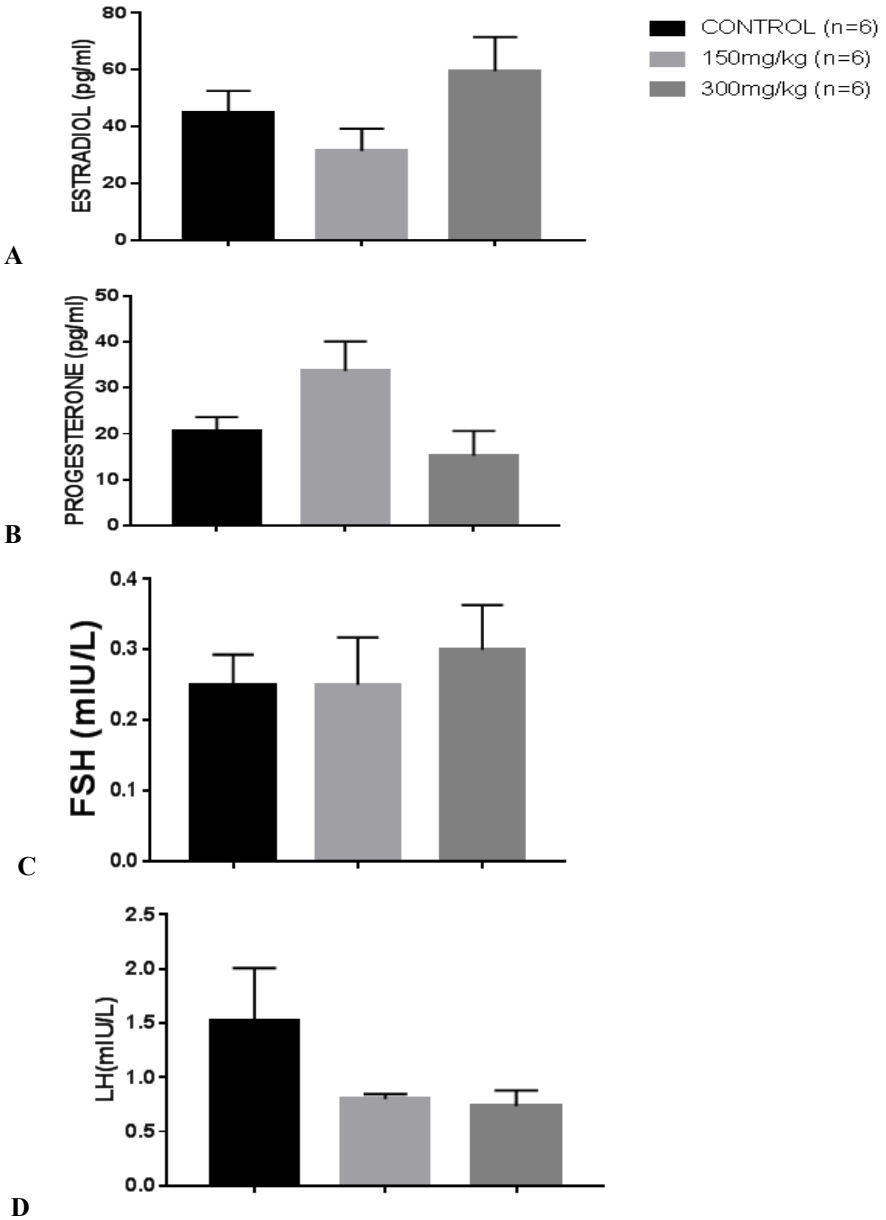



**Figure 2: Effect of *P. yohimbe* on Serum hormones**

Effect of *P. yohimbe* of different doses (Group B-150mg/kg, Group C-300mg/kg, and Group A-Control after 6 weeks treatment on Serum hormones showing differences in both treatment groups; Group A (control), Group B (150mg/kg bw), Group C (300mg/kg bw), *P>0.05*, FSH - Follicle Stimulating hormone, LH - Luteinizing hormone, n = 6.

### 3.3 *Estrus cycle Determination*

The rate of progression from proestrus and estrus stages in the test groups (groups B and C) was delayed when compared to the control group. Similarly, groups B and C progression in metesetrus stage was delayed when compared to the control group. In diestrus stage, it was quicker in group B and a difference was observed in group C.

### 3.4 *Histology of the uteri, ovaries and antral follicle count*

The uteri of groups B and C are unremarkable and look much like the controls except few areas of tissue distortion and necrosis (figure 3). The Follicles including corpus lutea in the ovaries of groups B and C were significantly decreased and degenerative changes were observed in some areas. The controls show many follicles at different stages of development (Figure 4).

**Figure 3: Effect of *P. yohimbe* on the Uterus**

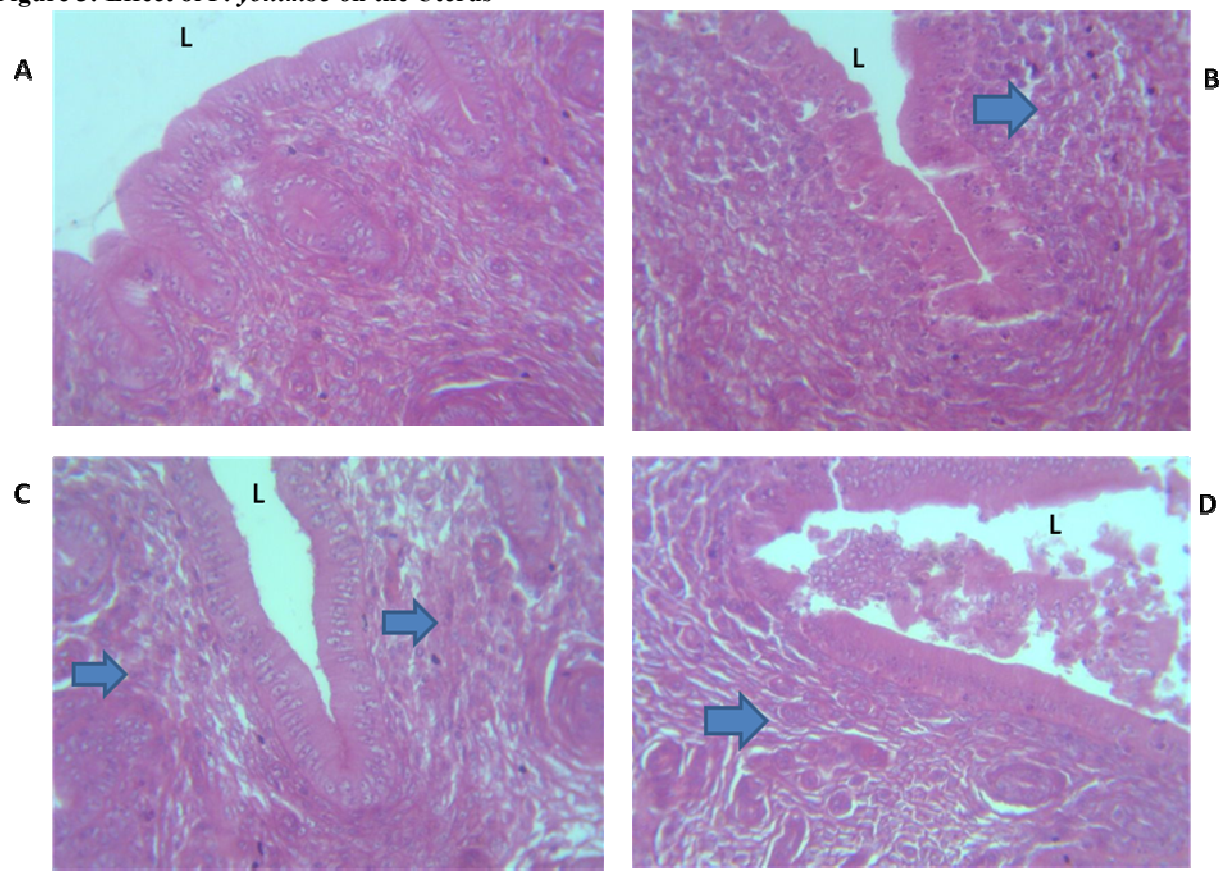



**Figure 3: Effect of *P. yohimbe* on the Uterus**

Photomicrograph of the uterus showing the effects of *P. yohimbe* different doses after 6 weeks treatment. A, Group A-Control uterus showing normal uterine cell architecture. B, Group B-150mg/kg, and C, Group C-300mg/kg, showing areas of tissue distortion and necrosis (thick arrows ) D, also areas of tissue necrosis with epithelial damage and debris in the endometrial. L = lumen, Magnifications 10X

**Figure 4: Effect of *P. yohimbe* on the Ovaries**

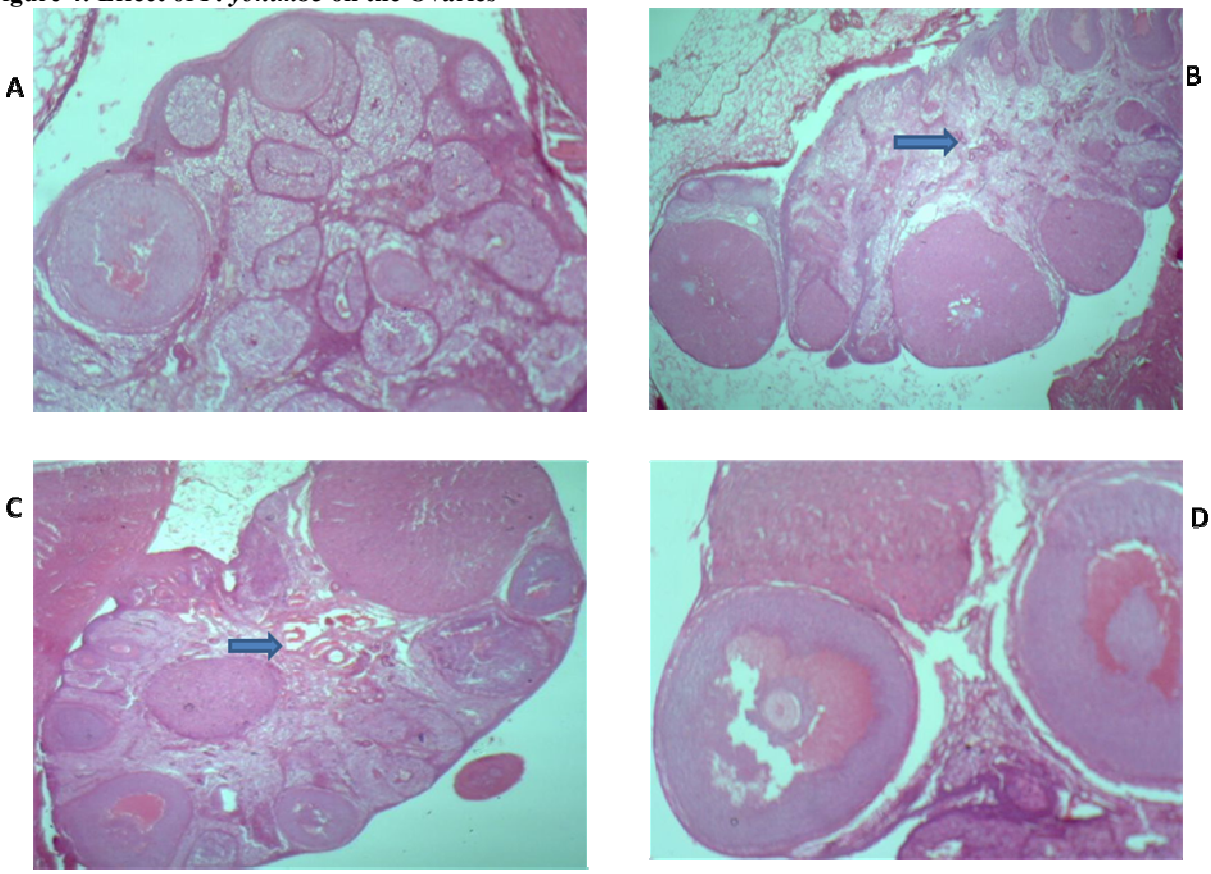



**Figure 4: Effect of *P. yohimbe* on ovaries**

Photomicrograph of the ovaries showing A, control group A and *P. yohimbe* treated rats B and C for 6 weeks treatment. Control group A shows the normal architecture of the ovaries with varying sizes of follicles in different developmental stages. B and C. shows the effect of *P. yohimbe* of different doses (Group B-150mg/kg, Group C-300mg/kg, shows areas of tissue necrosis of the ovaries (thin arrow heads) D. Photomicrograph of mature follicles in the control Ovaries. Magnifications 10X.

**Figure 5: Effect of *P. yohimbe* on antral follicles**

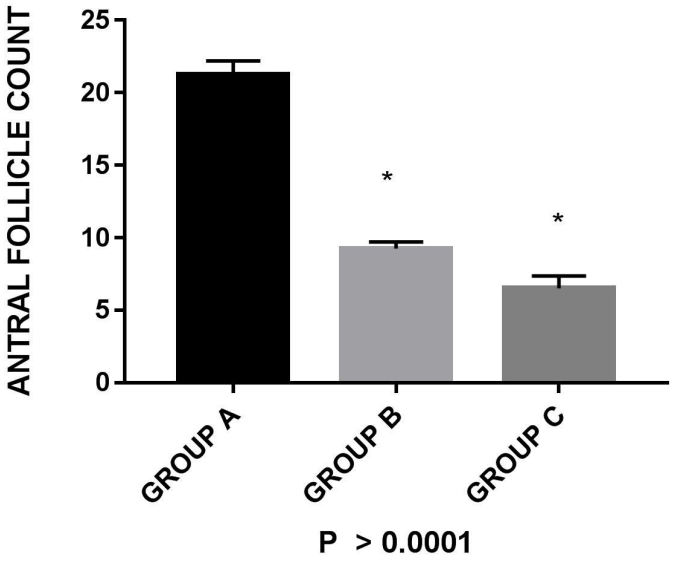



**Figure 5: Figure 4: Effect of *P. yohimbe* on Antrtal follicles**

Effect of *P. yohimbe* different doses (Group B-150mg/kg, Group C-300mg/kg, and Group A-Control) after 6 weeks treatment on ovarian antral follicles. P. yohimbe treated rats groups B and C had significantly fewer

follicles compared to control group. n =number of rats, *P<0.0001.*

**4.0 Discussion**

*P. yohimbe* has long been used by West African tribes, as an aphrodisiac among men and for the treatment of erectile dysfunctions in the sub-Saharan region of Africa (Oliver Beyer, 1989, Sunderland *et al*., 1997, Onwa *et al.,* 2009). Recently, it has been incorporated in barbecue meat, sauce and used widely. Women also use it to enhance arousal and libido while infertile women use it to improve chances of conceiving. Some studies have looked at the effects of *P. yohimbe* on male reproduction (Ajonuma et al. 2017 a, b), however, the present study to the best of our knowledge is the first on the effects of *P. yohimbe* on female reproduction.

Similar to the findings in male, relative organ wet weights differences were not significant as well as those of reproductive hormones (Ajonuma et al. 2017 a, b). It is possible that the reduced weight in the uteri of test group seen may be due to tissue distortion and necrosis. However, the reason for the elevated estardiol in group C (300mg/kg bw) and progesterone in group B (150mg/kg bw) is not exactly known at the moment. This varying difference in reproductive hormones between test groups and control may indicate destruction in female hormonal regulation as well as degenerative changes observed in some areas of the ovaries.

Increase in FSH levels may indicate an increase in folliculogenesis (Vegetti and Alagna, 2006). However, the level of FSH secretion in test group B (150mg/kg) is equal to that of the control group which may indicate (150mg/kg) and a slight increase in test group C compared to that of the control which indicate that *P.yohimbe* has no effect on FSH secretion. LH optimizes final follicular development and allows ovulation (The European Recombinant Human LH Study Group, 1998; Gordon et al., 2001; Filicori et al., 2003). Decrease in secretion of LH seen in test groups compared to controls in this study may suggest disturbed folliculogenesis and ovulatory processes which might affect fertility.

Tissue distortion and necrosis seen in the uteri of test groups may lead to decreased glandular epithelial cells, transepithelial transport and decreased uterine secretions need for early embryo development and implantation.

In summary, *P. yohimbe* affects reproductive hormones, estrous cycle and folliculogenesis in adult female rats. Therefore, its prolonged use may lead to reduced fertility. Molecular and cellular mechanisms involved as well as its effect on early embryo development and implantation needs to be evaluated.

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