

Original Research Article

FERTILITY EVALUATION OF ADULT FEMALE WISTAR RATS ADMINISTERED WITH ETHANOLIC EXTRACT OF *Pausinystalia yohimbe* STEM BARK

ABSTRACT

Pausinystalia yohimbe (*P. yohimbe*) stem bark powder is commonly used as seasoning for barbecue beef in Nigeria and some West African countries. This study evaluated the effect of ingesting ethanol extract of *P. yohimbe* stem bark on some hormones and tissues linked with fertility in female Wistar rats. A total of fifteen adult female Wistar rats weighing between 140 - 160g were used for this study after an initial Acute Toxicity test was done to establish a safe dose range of the extract. The animals were randomly divided into three groups of five rats per group. Group 1 served as control and received normal saline while Groups 2 and 3 received 400 mg/kg and 800 mg/kg body weight (bw) of ethanol extract of *P. yohimbe* stem bark respectively, via oral gavage, for 21 days. At the end of the treatment period, the rats were weighed, sacrificed and the blood, ovary and uterus samples collected for determination of reproductive hormones (follicle-stimulating hormone, estrogen, and progesterone) and histological examination using standard methods. The results showed that LD₅₀ of the ethanol extract for the female Wistar rats was 3807.89 mg/kg bw. There were significant ($p < 0.05$) increases in body weight, estrogen and follicle-stimulating hormone (FSH) of the treated groups compared with the control. Histological examination also showed degenerative changes in the uterus of the rats in groups 2 and 3, with no alterations in the ovary when compared to control. These results suggest that continuous consumption of ethanol extract of *P. yohimbe* stem bark may alter the systemic concentration of estrogen and follicle-stimulating hormone as well as morphology of the uterus which may lead to reduced reproductive function and female infertility.

KEYWORDS: *Pausinystalia yohimbe*, Reproductive Hormones, Fertility, Uterus, and ovary

INTRODUCTION

Fertility is a significant aspect of human life that is not just a perception associated with miracle and mystery but has direct conceivable effect on reproduction and the future [1]. Though the present population growth rate is increasing on daily basis, it is projected that about 78 million people every year have reproductive issues relating to infertility, thus contributing significantly to global health problem worldwide [1]. Earlier a World Bank study had discovered that poor reproductive health is the main cause of death and incapacity, especially among women of 15 – 49 years in developing countries [2].

The normal physiological principle guiding female reproductive efficiency involves interplay between the hypothalamus that secretes gonadotropin-releasing hormone (GnRH) in a pulsatile manner and the pituitary gland which is stimulated by the hypothalamus via the hypothalamic-pituitary-adrenal axis to regularly control the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In addition, the ovary has both methodical enzymatic system and steroidogenesis for producing sex steroid hormones such as estrogen and progesterone alongside a functional uterus that responds to these hormones [3].

There is increasing use of plant-based spices and herbs in developing countries due to the beneficial effects of plants and herbal preparations [4-10]. *Pausinystalia yohimbe* (*P. yohimbe*), whose stem bark is commonly used as seasoning and added to barbecue meat (“suya”) in Nigeria, is a common evergreen tree that belongs to the family of Rubiaceae. This plant is known to be found amongst the South, West, and Central African Region in the main forest and jungle of Cameroun, Nigeria, Gabon, and Equatorial Guinea [11]. The plant is also known as “Burantashi” amongst the local people of Northern Nigeria.

P. yohimbe contain yohimbine, an alkaloid that has been widely used for sexual erectile dysfunctions. Ojatula[12] reported that *P. yohimbe* exhibited remarkable increase in sexual orientation, libido, and spermatogenic activity which are some of the indices that determine the ability of a male to produce viable spermatozoa. Its antifertility effect on male animals has also been extensively evaluated by other workers. Ogwoet *al.*[13] reported that prolonged use of *P. yohimbe* caused deleterious effects on the testes, accessory glands, sperm motility, and sperm concentration which can lead to male infertility. Louis *et al.* [14] also reported that as *P. yohimbe* improves erectile dysfunction, its continuous use may lead to reproductive tissue destruction, low sperm counts, and poor sperm motility. Lei *et al.* [15] also examined the severe acute intoxication with yohimbine in four simultaneous poisoning cases and concluded that yohimbine could cause deleterious effects on reproductive organs and hematological parameters on consumption. It has also been reported that *P. yohimbe* is traditionally used as a spice and for the treatment of exhaustion, chest pain, skin disorder, and inflammation [11,16]

Despite these findings, there is paucity of reports of its effect on fertility indices in females. Consequently, this study evaluated the effects of ethanol extract of *P. yohimbe* stem bark on some sex hormones and tissues linked with fertility in female Wistar rats.

MATERIALS AND METHOD

Collection and Identification of Plant Material

The *P. yohimbe* stem bark was obtained from local herb dealers in Lafia, Nasarawa State, Nigeria and brought to the Biochemistry Research Laboratory of the Department of Biochemistry, Rivers State University. The plant material was taxonomically identified and authenticated at the Department of Plant Science and Biotechnology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. The stem bark was chopped into pieces and dried

under shade for 14 days. The dried stem bark was crushed in a mortar and thereafter ground into powder using an electrically powered locally fabricated mill.

Preparation of plant extract

Two hundred and fifty grams of the powdered material was macerated in 1.5 liters of ethanol for 48 hours and was filtered to obtain the extract in solution as filtrate. The filtrate was thereafter concentrated and evaporated to dryness in a hot air oven at 40°C to obtain a dark green crude extract that weighed 38.0g, representing a percentage yield of 15.20%. The dried extract was thereafter preserved in the refrigerator until needed. When needed, the extract was dissolved in normal saline (0.90% NaCl) at a concentration of 1g/ml, and the different concentrations were prepared from the stock.

Acute toxicity evaluation

For the acute toxicity evaluation, the new Lorke's method used by Orieko *et al.* [17] was adopted with slight modification. Two stages were involved in the test. In the first stage, 9 Wistar rats were assigned to 3 groups (A, B and C) of 3 rats each and were treated with 10.0, 100.0, and 1000.0 mg/kg of the extract respectively. The animals were thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours.

With zero mortality recorded, the study proceeded to the second stage which also involved the use of 9 rats assigned to 3 groups (A, B and C) and administered with single oral doses of 1600.0, 2900.0 and 5000.0 mg/kg respectively. The rats were again monitored for toxicity signs and deaths within 24 hours. The highest dose (5000 mg/kg) used was repeated on another set of 3 rats to confirm the earlier observations.

Acute toxicity values were calculated using Lorke's formula:

$$LD_{50} = \sqrt{A \times B}$$

Where;

A = Maximum dose that produced no mortality and

B = Minimum dose that killed all animals in a group.

Experimental animals

A total of 15 adult female Wistar rats weighing 140 -160g, obtained from the animal house of the Department of Pharmacology, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt were used. The rats were assigned to 3 groups of five rats each in separate cages and allowed to acclimatize within a period of 14 days. They were housed under specific pathogen-free (SPF) conditions and were provided standard feed ("Vital feed, Nigeria") and water *ad libitum*, but starved for 12 hours before the commencement of the experiment.

Ethical considerations

All animal experiments in this study were carried out in compliance with guidelines for the Care and Use of Laboratory Animals [18], as well as the U.K Animals (Scientific Procedure) Act, 1986 and associated guidelines including EU Directives 2010/63/EU for animal experiments. Also, ethical approval for use of animals for experiments was sought and obtained from the Ethical Committee of Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Experimental design

List 1 :The various groups were treated according to the following order:

Group	Identification	Treatment
Group 1	Normal Control	2ml normal saline
Group 2	Experimental Group 1	400mg/kg body weight (bw) of ethanolic extract of <i>P.yohimbe</i> stem bark
Group 2	Experimental Group 2	800 mg/kg bw of ethanolic extract of <i>P.yohimbe</i> stem bark

Groups 2 and 3 received repeated doses of the respective ethanolic extract of *P.yohimbe* stem bark via oral gavages once daily for 21 days. The doses of *P.yohimbe* stem bark ethanol extract given were extrapolated from the result of acute toxicity test.

Biochemical analysis

At the end of the 21 days of administration of the extract, the rats were weighed, sacrificed by cervical dislocation, and blood was collected using ocular puncture into plain bottles. The collected blood samples were allowed to clot and then centrifuged at 3000 rpm for 20 minutes using Table Centrifuge (Surgifield, SM80-2, England). The serum samples obtained were then transferred into clean sterile sample bottles and stored at 20°C until analyzed. The serum was used to assay for Follicle Stimulating Hormone (FSH), Estrogen, and Progesterone levels across the various groups using ELISA microplate (HIPO MPP-96, BIOSAN) following the protocol and procedure provided on the accubind kit from Biocode, Belgium.

Histological Examination

The ovaries and uterus of the rats were excised, placed in sample holders containing 10% normal saline, and then taken to the laboratory for a histopathology examination using standard methods [11].

Statistical analysis

All data obtained were subjected to statistical analysis and values expressed as mean \pm standard error of the mean (SEM). To determine whether there was a statistically significant difference among the experiment groups, the one-way analysis of variance (ANOVA) was used, followed by a post hoc Turkey test. A p-value of less than 0.05 was considered statistically significant. All statistical analysis was performed using GraphPad Prism 9.1.0 software.

RESULTS

Percentage yield of extract

Two hundred and fifty grams (250.0g) of the dry *P. yohimbe* stem bark powder yielded a dry solid extract that weighed 38.0 g representing a percentage yield of 15.20%. The extract was brown, sticky and oily paste in consistency.

Acute toxicity study

No death was recorded in the first stage of the study following administration of 10, 100 and 1000 mg/kg bw oral doses of the extract to the female rats. All the rats retained their physical activities and showed no signs of toxicity (Table 1a). However, in the second phase of the study when 1600, 2900 and 5000 mg/kg bw of the extract were administered to separate set of animals, mortalities were observed. While zero mortality was observed in groups 1 and 2 administered with 1600 and 2900 mg/kg bw, one hundred percent mortality was observed in Group 3 administered with 5000.0 mg/kg bw (Table 1b). A repeat of 5000.0 mg/kg bw oral dose on a separate set of 3 rats during the confirmatory test phase also produced 100% mortality. Using Lorke's formula, Acute Toxicity (LD_{50}) value of the ethanol extract for the female Wistar rats was calculated to be 3807.89 mg/kg body weight.

Table 1a: Phase 1 acute toxicity evaluation of ethanolic extract of *P. yohimbe* stem bark

Group	Dose (mg/kg bw)	Mortality rate	Observation
1	10.0	0/3	Rats were active and physically balanced
2	100.0	0/3	Rats were active and physically balanced

3	1000.0	0/3	Rats were active and physically balanced
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Table 1b: Phase 2 acute toxicity evaluation of ethanolic extract of *P. yohimbestem* bark

Group	Dose (mg/kg bw)	Mortality rate	Observation
1	1600.0	0/3	Rats were active and physically balanced
2	2900.0	0/3	Rats were calm and physically inactive for a moment but regained physical activities within 24 hours.
3	5000.0	3/3	One hundred percent mortality recorded

LD₅₀ = 3807.89 mg/kg body weight

Body weight

Results of body weight changes following administration of ethanolic extract of *P. yohimbestem* bark is showed in Figure 1. There was significant ($p < 0.05$) increase in the percentage body weight of the albino rats in groups 2 and 3 that received 400 mg/kg and 800 mg/kg body weight of the extract respectively when compared with the control. However, this increase in body weight was not concentration dependent.

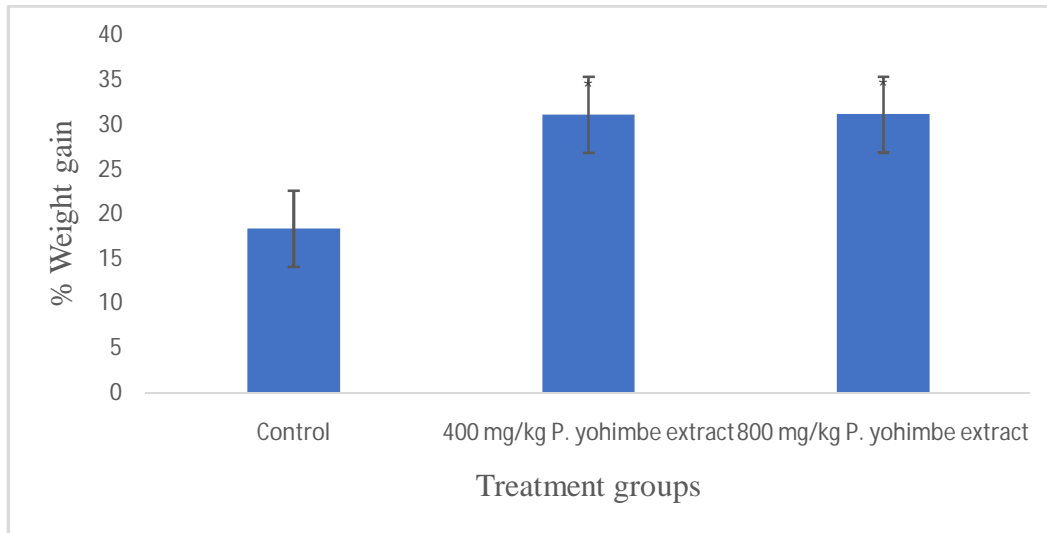


Figure 1: Body weight of adult female albino rats treated with *P. yohimbestem* bark

Values are expressed as Mean \pm Standard error of mean (SEM). Bars with superscript* represents significant difference when the treatment groups are compared with control at $p < 0.05$.

Serum Follicle Stimulating Hormone, Estrogen and Progesterone level

The results of serum Follicle Stimulating Hormone (FSH) level following administration of *P. yohimbestem* bark ethanol extract is shown in Figure 2. There was significant ($p < 0.05$) **higher (increase) in the** serum FSH in Groups 2 and 3 that received 400.0 mg/kg ad 800.0 mg/kg body weight extract respectively when compared with the control. **(the biochemical analysis as mentioned in the methodology was done once at the end of the 21 days , so there is just higher levels not increase in the levels)**

Figure 3 shows the serum estrogen level of female Wister rats treated with the ethanol extract. There was significant **higher (increase)** ($p < 0.05$) **in the** serum estrogen level in groups 2 and 3 that received 400 mg/kg and 800 mg/kg body weight of the extract respectively when compared with the control.

Result of the serum progesterone level after treatment with ethanolic extract of *P. yohimbestem* bark is as shown in Table 2. There was no significant ($p < 0.05$) alteration in serum progesterone level among all the groups tested.

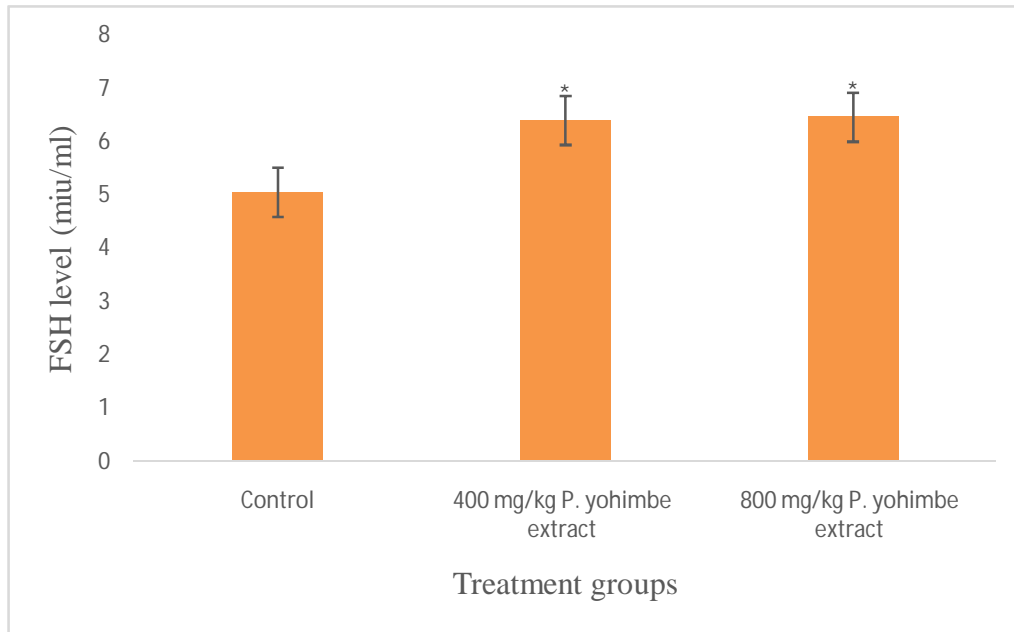


Figure 2: Serum Follicle Stimulating Hormone levels of adult female Wistar rats treatment with *P. yohimbestem* bark Extract

Values are expressed as Mean \pm Standard error of mean (SEM). Bars bearing superscript* represents significant difference when the treated groups are compared with control at $p < 0.05$.

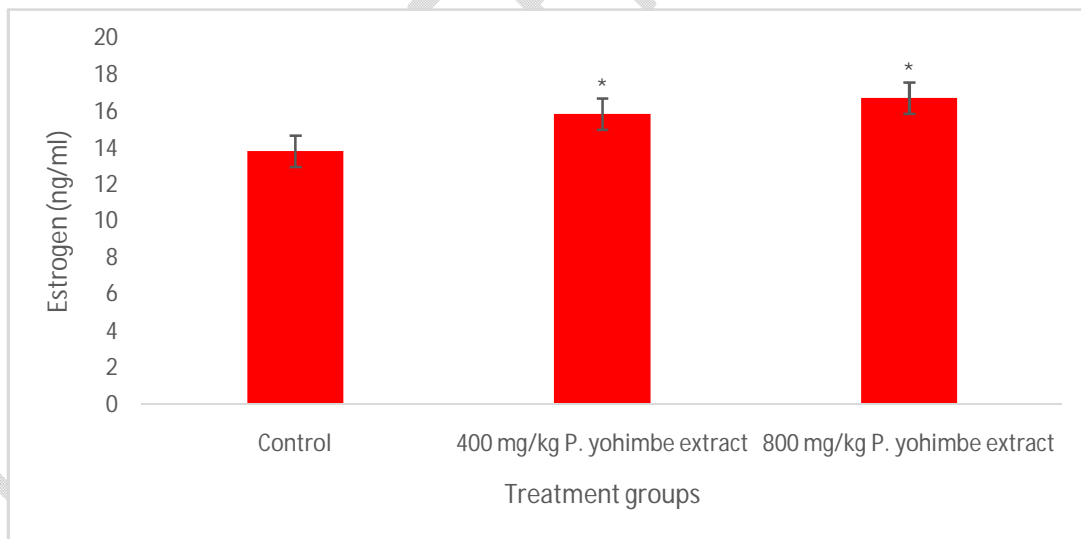


Figure 3: Serum estrogen levels of adult female Wistar rats treated with *P. yohimbestem* bark extract.

Values are expressed as Mean \pm Standard error of mean (SEM). Bars bearing superscript* represents significant difference when the treated groups are compared with control at $p < 0.05$.

Table 2: Serum progesterone levels adult female Wistar rats treated with *P. yohimbestem* bark extract

Group	Treatment	Progesterone (ng/ml)
1	Control	8.56 ± 0.25 ^a
2	400mg/kg body weightethanolicextract of <i>P. yohimbestem</i> bark	8.68 ± 0.07 ^a
3	800 mg/kg body weightethanolicextract of <i>P. yohimbestem</i> bark	8.39 ± 0.24 ^a

Values are expressed as Mean ± Standard error of mean (SEM). Values with the same superscript on the same column are not significantly different at p<0.05

"a" refers to.....??

Effects of ethanolic extract of *P. yohimbe* stem bark on the uterus and ovary Histology

The photomicrographs of the uterus, showing sections in estrus of the cycle, of female Wistar rats administered with ethanolic extract of *P. yohimbe* stem bark are shown in Figure 4. The uterus of treated rats (Figure 4A2 and 4A3) showed extensive apoptosis of both luminal and glandular epithelia (green arrow) with marked infiltration of the connective tissue stroma by eosinophils. The epithelial lining is crowded and tall (white arrow).

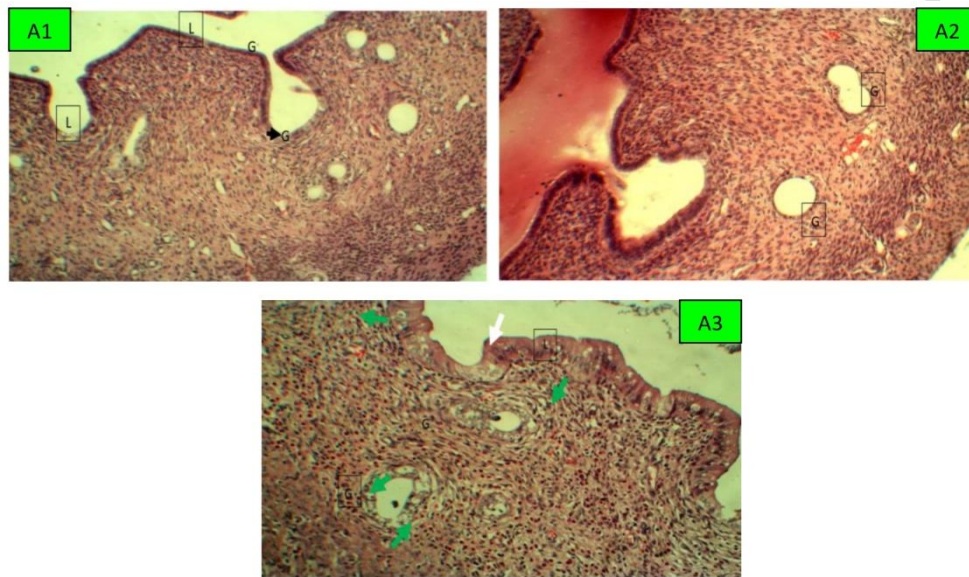


Figure 4: (A1 – A3) Photomicrograph of the uterus of female Wistar rats. A1: Group 1 (Control); A2: Group 2 animals treated with 400.0mg/kg ethanolic extract of *P. yohimbe* stem bark; A3: Group 3 animals treated with 800.0 mg/kg ethanolic extract of *P. yohimbe* stem bark. The epithelial lining of the lumina and glands are low columnar and distinct cells (Black arrow), Lumen (L); endometrial glands (G). Magnification: H&Ex200

Figure 5 shows the histomicrograph of the uterus of female Wistar rats exposed to ethanolic extract of *P. yohimbestem* bark. All the sections of the ovary presented appear to be in their estrus or metestrus stage of their cycles and showed normal histomorphology.

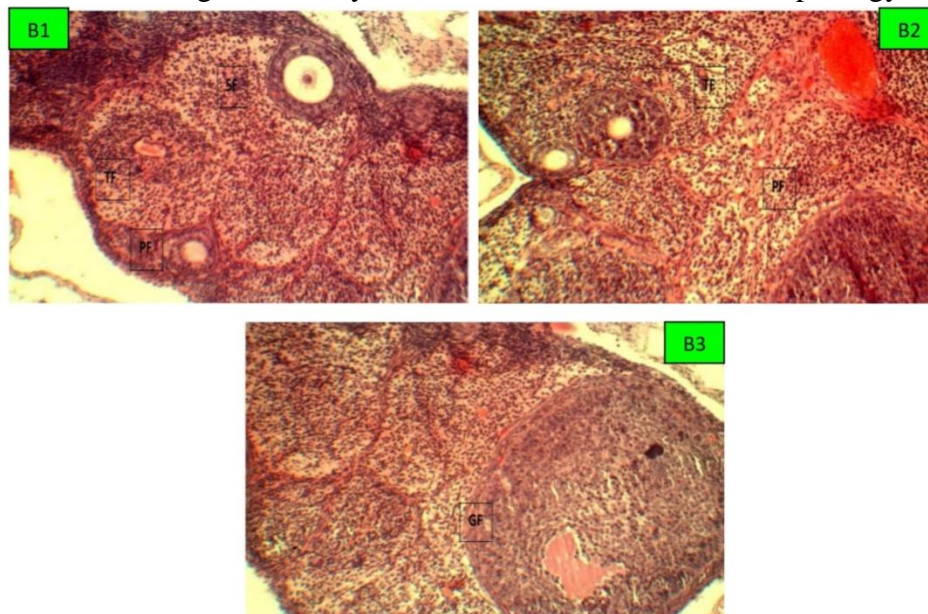


Figure 5:(B1 – B3)Photomicrograph of the ovary of female Wistar rats. B1: Group 1 (Control); B2: Group 2 animals treated with 400.0mg/kg ethanolic extract of *P. yohimbestem* bark; Group 3 animals treated with 800.0 mg/kg ethanolic extract of *P. yohimbestem* bark. Primordial follicle (PF); Secondary follicle (SF); Tertiary follicle (TF); Graafian follicle (GF);Magnification: H&E x200

DISCUSSION

The Acute Oral Toxicity value (LD_{50}) of ethanolic extract of *P. yohimbestem* bark for the female Wistar rats in this study was 3807.89mg/kg body weight. This result is higher than that of Okolo and Egu [19] who reported the LD_{50} value of 2000.0mg/kg body weight for methanolic extract of *P. yohimbe* stem bark in Wistar rats. Ijioma *et al.* [11] also reported a lower LD_{50} of 1050.0 mg/kg in mice. The difference in LD_{50} may be attributable to variations in the animal species used for the study [20], or the extraction solvent, route of administration, and inter-laboratory variations [21]. The result obtained from this present study indicates a relatively higher margin of safety for the ethanolic extract of *P. yohimbestem* bark.

Clarke and Clarke [22] had earlier reported that oral acute toxicity of a substance above 1000.0 mg/kg is relatively safe. Also, the extract was well tolerated at low to moderate doses and toxic only at a very high dose of 5000.0 mg/kg body weight where it recorded one hundred percent mortality. This conclusion agrees with the OECD guideline for acute toxicity studies. OECD [18] stated that death is the expected endpoint of acute toxicity, and the absence of death within a population treated with a dose range at which death is expected indicates tolerance or lack of acute toxicity. The toxicity of this *P. yohimbe* stem bark alcohol extract at a higher dose as

obtained in this study may be attributed to its unique phytochemical compositions. Although further work is required to properly reveal the nature and mechanism of action of these phytochemicals [23]. Studies have shown that the main alkaloid in *P. yohimbe* stem bark extract is yohimbine which is implicated as a major cause of toxicity [24].

There was significant ($p < 0.05$) increase in body weight (Figure 1) and serum estrogen (Figure 3) of the rats in the groups treated with graded doses of the extract. Increased estrogen level promotes the storage of fat for healthy reproductive years which leads to weight gain [25]. The results obtained in this study agree with this assertion.

Administration of the ethanolic extract of *P. yohimbe* stem bark for 21 days significantly ($p < 0.05$) increased the level of serum Follicle Stimulating Hormone (FSH) and serum estrogen (Figures 2 and 3) with no significant ($p > 0.05$) alteration in the serum level of progesterone in all test groups when compared with the control group (Table 2). This result agrees with the work of Ajonuma *et al.* [26], who reported an increase in FSH level in adult female Sprague Dawley (SD) rats that received 300.0 mg/kg body weights of aqueous extracts of *P. yohimbe* for 6 weeks when compared to the control. Our results suggest that the ethanolic extract of *P. yohimbe* stem bark may stimulate the secretion of FSH by affecting hypothalamic/pituitary axis. However, the results do not have dose-dependent effects on progesterone, a critical reproductive hormone, in adult female Wistar rats.

The increasing serum estrogen levels observed following administration of ethanolic extract of *P. yohimbe* stem bark in the female Wistar rats may be due to induced de novo estrogen synthesis and/or presence of some phytoestrogens in the extract. According to Amah-Tariah *et al.* [27] phytoestrogens are plant-derived natural compounds similar to estrogens in terms of structural and functional performance and therefore have estrogenic effects. This estrogenic property either activates the estrogen receptors or increases estrogen synthesis by improving FSH concentrations [25,28]. Phytoestrogens have been reported to influence the hypothalamic-pituitary-gonadal axis as well as the external genitalia [27]. Ingestion of phytoestrogens in plants may also have some stimulatory role in the release of enzymes such as aromatase, and 17- α hydroxylase which are necessary for estrogen production, thus, increasing the secretion of gonadotropins (FSH), and release of estrogens [27].

There was no significant ($p > 0.05$) alteration in progesterone levels in the tested groups compared to control (Table 2). This result agrees with Ajonuma *et al.* [26] who also reported that there was no significant difference in progesterone level in adult female Sprague Dawley (SD) rats that received 300.0 mg/kg body weights of aqueous extracts of *P. yohimbe* for 6 weeks when compared to the control. This suggests that progesterone does not act as an anti-estrogen in this circumstance and that this hormone, particularly in low doses, may have a stimulating influence in facilitating effect on the hypothalamic-pituitary system. Reproductive systems are regulated by hormones, and hormonal disorders such as inadequate or excessive production of certain hormones are the most common causes of infertility.

The result of histological examination shows remarkable alteration in tissue morphology of the uterus in treated rats compared to control. The uteri of the treated groups were in the late

metestrus stage of their cycle and the epithelial lining of the Lumina and glands are low columnar and distinct cells. This revealed an alteration in the duration of the phases of the estrous cycle in treatment groups when compared with that of the control.

Endometrial hyperplasia is a state of excessive proliferation of the cells of the endometrium or inner lining of the uterus [29]. Most cases of endometrial hyperplasia are a consequence of high levels of estrogens, combined with insufficient levels of the progesterone-like hormones which ordinarily counteract estrogens' proliferative effects on this tissue [3,30]. Endometrial hyperplasia is a major risk factor for the progress or even co-existence of endometrial cancer [31,32]. Estrogen stimulates the uterus thereby changing the uterine milieu and creating non-receptive conditions [33,34].

There was no observable pathologic change in the ovary histology of all test groups; the ovary showed normal cortical stroma containing ovarian follicles in various stages of development. The observed ovaries appear to be in the estrus or metestrus stage of their cycles

Continuous ingestion of ethanolic extract of *P. yohimbe* stem bark could ultimately result in alterations in the functional integrity of the uterine cycle as well as other hormone interaction and secretion. A similar result has been reported in female Sprague Dawley rats [26] and in male albino rats [13-15]. Further investigation is required to ascertain the duration of the phases of the estrous cycle and the number of matured follicles in the ovary of treated groups.

CONCLUSION

P. yohimbe stem bark powder, commonly known as "Burantashi", has been used as spice for barbecue beef and sexual stimulant. The result of this study shows that chronic ingestion of ethanolic extract of *P. yohimbe* stem bark by female Wistar rats significantly increased serum estrogen and follicle stimulation hormone levels as well as body weight. However, serum progesterone levels were not significantly altered. Histological examination of the uterus also revealed extensive apoptosis of both luminal and glandular epithelia with marked infiltration of the connective tissue stroma by eosinophils in the treated groups compared to control. Histology of the ovaries showed intact cells with normal histomorphology and no significant alterations.

Chronic consumption of *P. yohimbe* stem bark powder by females could result in hormonal imbalance, impairment of the internal reproductive organ function and infertility. Hence, careful use of such herb should be encouraged especially when managing couples with reproductive challenges.

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