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Adverse Effects of Prolonged Use of Pausinystalia yohimbe on Sperm and Reproductive Organs in Rats

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Abstract
Pausinystalia yohimbe (*P. yohimbe)* was first discovered and used by tribes in West Africa, where it grows wild mostly in the Atlantic evergreen forest throughout West Africa from South-east Nigeria to Congo. *P. yohimbe* bark extract is commonly used as an aphrodisiac among men and for the treatment of erectile dysfunctions. More recently, due to infertility more men have resorted to using *P. yohimbe* to improve chances of having baby. However, there are no studies on the effects of *P. yohimbe* on sperm motility and movement characteristics. This study examined its effects on reproductive organs, sperm production and motility in rats.Fifteen male Sprague Dawley (SD) rats were divided into 3 groups of 5 rats. Group A was the control 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 4 weeks. Serum samples obtained from the rats were assayed for reproductive hormones (Testosterone, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Estradiol (E2)). Testes, Seminal Vesicles and the Prostates were removed for histology, while sperm count, motility and vitality were done using sperm from the caudal epididymis and vas deference.Mean percentage change in body weights of groups B and C were significant when compared to control group A. There was significant reduction in sperm motility and concentration in the test groups, but none in sperm vitality. Serum hormonal levels of Testosterone, FSH, LH and E2 were not significantly different from the control. However, there was a significant reduction in testosterone / E2 ratio in the test groups. Degenerative changes were observed in the testes, prostate and seminal vesicles.Prolonged use of *P.yohimbe* adversely affects the testes, male accessory glands, sperm concentration and motility. This may lead to reduced reproductive functions and male infertility.
Keywords: *P. yohimbe,* Accessory glands, Testis, Sperm motility, Sperm concentration

1.0 Introduction

Pausinystalia yohimbe (*P. yohimbe)* was first discovered and used by tribes in West Africa, where it grows wild. It occurs mostly in the Atlantic evergreen forest widespread throughout West Africa but more from South-east Nigeria to Congo (Guay *et al.,* 2001, Guay *et al*, 2002, Adeniyi *et al*., 2007). *P. yohimbe* is native to Cameroun, Congo, Democratic Republic of Congo, Equatorial Guinea, Gabon and Nigeria (Oliver Beyer, 1989, Sunderland *et al*., 1997, Onwa *et al.,* 2009). These tribes still use and praise *P. yohimbe* for its powerful and aphrodisiac effects. They considered it a treatment of fevers, leprosy and coughs. It has also been used to dilate pupil, for heart disease, and as a local anaesthetic. 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).
Yohimbe works by blocking alpha – 2-adrenoreceptors to increase blood flow. There are a number of feedback mechanisms that prevents the release of norepinephrine (NE), one of the body's lipolytic hormones (Galitzky *et al.,* 1988; Kucio *et al*., 1991).

Due to the effective aphrodisiac actions, it is widely used for the treatment of erectile dysfunctions in the sub-Saharan region of Africa (Sunderland *et al*., 1997, Eweka *et al*., 2010, Etiani, 2012). Decoctions of stem and root bark as well as whole roots are usually taken for the treatment of erectile dysfunctions (Aliyu and Alkali, 2006). Recently in many West African cities, it is also commonly used to spice food especially barbecued meat.

Although *P.* yohimbe has long been used for the treatment of erectile dysfunctions and widely as food additive, its effect on male fertility is not well understood and has not been studied to any reasonable extent. Therefore, this study investigated the effects of *P. yohimbe* on reproductive hormones, testes, male accessory glands and sperm in the rat.

2.0 Materials and Methods

2.1 Animals

Adult *Sprague Dawley* (SD) male rats used in this study were obtained from Babcock University, Ilishan, Ogun State and then housed in the Animal House Unit, Lagos State University College of Medicine (LASUCOM) under standard laboratory conditions of 12 hours light and 12 hours darkness, proper humidity and temperature. They were fed with standard rodent's chow and clean water *ad libitum.* This study was done in strict compliance

with the regulations of the use of animals in experiments of Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.

2.2 Preparation of *P.* yohimbe extract

P. yohimbe stem was obtained from a local herb merchant in Lagos, Nigeria. It was chopped into pieces, crushed in a mortar and blended into fine powder using an electric blender. It was then sieved with a standard mesh. Crude extraction has been previously described (Bordbar *et al*., 2013; Etiani, 2012). In brief, the dried powder was weighed and 50g dissolved in 1Liter of distilled water in a large beaker, and heated on a hot plate with magnetic stirrer for 90 minutes at 100°C. Subsequently, it was allowed to cool for 30 minutes and filtered. The resulting filtrate was concentrated in an electric oven at 60°C until a solid residue was obtained.

2.3 Experimental Design

The SD rats were randomly divided into 3 groups. Group A (control group) had 5 rats and received 0.5mls. of normal saline, while groups B and C (test groups) had 6 rats each and received 150mg/kg and 300mg/kg body weights of the aqueous *P. yohimbe* extract respectively via oral gavages daily for 4weeks. The concentration to be given was determined as previously reported by Bordbar *et al*., 2013 and Etiani, 2012.

After 4 weeks of administration, the rats were anaesthetized using Ketamine hydrochloride, and were dissected open. Blood samples were collected via direct cardiac puncture and the blood samples were stored in plain sample bottles. The following reproductive organs (testes, seminal vesicles, prostate) obtained were weighed and fixed in 10% buffered formalin for histology and epididymis for sperm analysis.

2.4 Serum Preparation and Hormonal analysis

The collected blood samples were allowed to clot and then placed in a table top centrifuges (Surgifield, SM80-2, England) and were spurn for 20mins at 3000 rpm. The separated serum samples were then aliquot into clean sterile sample bottles and stored at -20ºC until analyzed. The serum samples were assayed for Testosterone, Luteinizing Hormone (LH), Estradiol (E2), and Follicle Stimulating Hormone (FSH) using the enzyme linked immunosorbent assay (ELISA) technique. Test kit used was made by JD Biotec Corp. Taiwan R.O.C for Testosterone, LH and FSH while E2 was done using Accu-Bind Elisa Microwells.

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 Sperm analyses

2.5.1 Sperm collection and motility analysis

The epididymis obtained from the rats was dissected free of fatty tissues. Caudal epididymis and vas deferens were minced into a sample bottle and mixed with 50µl of sperm washing medium supplemented with HEPES (Ajonuma et al, 2002) to enable the sperm cells swim out and the temperature was maintained at 37 ^{o}C. For motility assessment, 10µl of sperm washing medium was obtained using sterilized pipettes and then placed on glass microscopic slides and then observed under the microscope (Olympus, XSZ-107BN, Japan) at × 400 magnifications, and at least 100 spermatozoa in a total of 5 fields were evaluated.

A simple system for grading motility by WHO (2010) was used to distinguish spermatozoa with progressive or non-progressive motility from those that are immotile. Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed. Non-progressive motility (NP): all other patterns of motility with an absence of progression, the flagella force hardly displacing the head, or when only the flagella beat can be observed. Immotility (IM): no movement. Assessment of only progressive motility (PR) was regarded to be motile and was scored as the percentage motile sperms (WHO, 2010).

2.5.2 Sperm Concentration

This parameter was checked using the improved neubauer haemocytometer. It was used with a special thick cover-slip. The dilution of spermatozoa and sperm diluents was 1+9 (1:20), 50µl of the sperms mixed with sperm washing medium and 950µl of sperm diluents. Assessment was done by counting of sperm in 5 of the calibrated boxes seen under the microscope as instructed by WHO, (2010).

2.5.3 Sperm Vitality

Sperm vitality was assessed as previously described WHO, (2010). Vitality test using eosin alone was done. The

spermatozoa mixed with the sperm washing medium were kept at a temperature of 37 ºC. 5µl of sperm in sperm washing medium was mixed thoroughly with equal amount of eosin dye on a microscopic slide, then covered with a 22mm × 22mm cover-slip and left for 30 seconds. The slide was observed under a simple light microscope at ×400 magnification using Olympus, XSZ-107BN, Japan. Spermatozoa having white heads were scored and considered to be live and those that stain dark pink to red on the head are considered dead (WHO 2010).

2.6 Tissue Preparation for Light microscopy

This was done as previously described (Ajonuma et al., 2005). In brief, all collected organs (testis, seminal vesicle and prostate) were dissected free of fatty tissues and fixed in 10% formalin overnight. Tissue samples were dehydrated in graded ethanol and embedded in paraffin wax and processed with KD-TS6A tissue processor. Sections 5 mm 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.

2.7 Statistical Analysis

Results were expressed as mean and standard error of mean (SEM). Statistical analyses were carried out using Analysis of variance (ANOVA). Comparisons and differences between groups were analyzed using Tukey post hoc test. $P \leq 0.05$ (two-tailed) was considered statistically significant. Analyses were carried out on Graph Pad Prisms Version 7 (Graph Pad, Inc., San Diego, CA, USA).

3.0 RESULTS

3.1.1 Effect of *P. yohimbe* on body weights of rats

The mean body weights of the rats are shown in Table 1. The mean percentage change in body weights in groups A, B and C are 18.39±1.71%, 7.066±2.66%, 3.84±1.03%, respectively. The percentage change in weight of both group B (P value=0.0024) and C (P value=0.0003) are significantly low when compared to the control group A.

Table 1: Effect of *P. yohimbe* on body weight of the treated rats

TREATMENT GROUPS	BASELINE WEIGHT (g)	AFTER 4 WEEKS (g)	PERCENTAGE CHANGE OF WEIGHT (%)
GROUP A (Control) N=5	204.4±8.35	250.2±6.58	18.39±1.71
GROUP B (150mg/kg) N=5	220.6±12.95	237.0±9.86	7.06±2.66*
GROUP C (300mg/kg) N=5	251.0±20.54	250.8±20.38	3.84±1.03*

Effect of *P. yohimbe* on body weight after 4 weeks treatment. groups B and C are significantly different from the control (group A). N=number of rats. * P< 0.05.

3.1.2. Effect of *p. yohimbe* on the Reproductive Organs

Relative Testicular Weight

In table 2, group B (P value=0.321) and group C (P value=0.415) showed no significant difference when compared to the Control (group A).

Relative Prostate Weight

In table 2, group B (P value=0.246) and group C (P value=0.7105) showed no significant difference when compared to the control (group A)

Relative Seminal Vesicle Weight

In table 2, group B (P value=0.0124) is significantly reduced (P<0.05) when compared to the control, while group C (P value=0.0903) showed no significant difference (P>0.05) when compared to the control group A

Table 2: Effect of *P. yohimbe* on Relative Organ Weight

GROUPS	TW (g)	RTW (g)	PW (g)	RPW (g)	SV (g)	RSVW (g)
GROUP A (Control) (n=5)	2.788 ±0.088	0.011 ±0.0002	0.342 ±0.026	0.0014 ±0.00014	1.128 ±0.043	0.0045 ±0.00023
GROUP B (150mg/kg) (n=5)	2.426 ±0.336	0.01 ±0.0015	0.258 ±0.0396	0.0011 ±0.00017	0.458 ±0.0380	0.0019* ±0.00016
GROUP C (300mg/kg) (n=5)	2.84 ±0.20	0.011 ±0.00057	0.394 ±0.0317	0.0015 ±0.000026	0.676 ±1.80	0.0028 ±0.0009

*p<0.05 when compared to the control. TW= Testicular Weight, n=number of rats, RTW= Relative Testicular Weight, PW= Prostate Weight, RPW= Relative Prostate Weight, SVW= Seminal Vesicle Weight, RSV= Relative Seminal Vesicle Weight.

3.2 Effect of *P. yohimbe* on sperm parameters: Sperm Motility, Concentration and Vitality

3.2.1 Sperm motility

In sperm motility, both group B (150mg/kg) and group C (300mg/kg) were significantly reduced in a dose-dependent manner when compared to the control as shown in Figure 1. Sperm head to head agglutination was present in in the two treatment groups.

3.2.2 Sperm concentration

In sperm concentration, both group B (P value=-0.0002) and group C (P value=0.0001) are significantly reduced (a dose-dependent decrease) (P<0.05) when compared to the control group A as shown in figure 2.

3.2.3 Sperm vitality

In sperm vitality, both group B (P value=0.07) and group C (P value=0.186) showed no significant difference (P>0.05) when compared to the control group A as shown in figure 3.

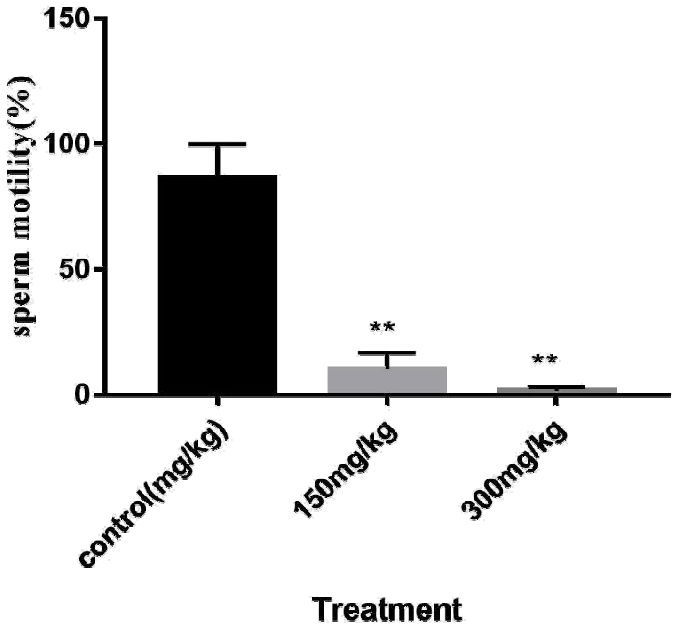
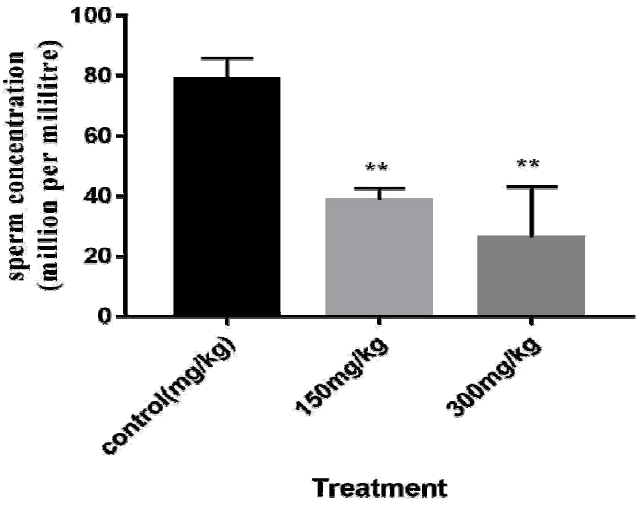


Figure 1: Effect of *P. yohimbe* on sperm motility

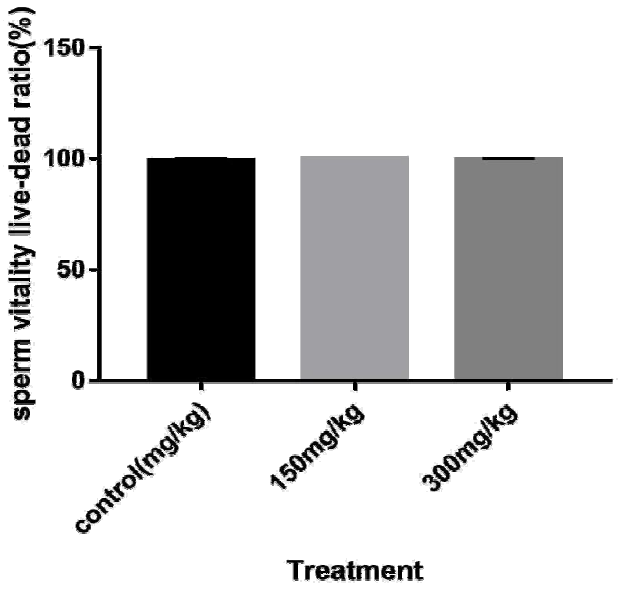
Effect of *P. yohimbe* on sperm motility after 4 weeks treatment. Group A Control (n=5), (Group B 150mg/kg (n=5), Group C-300mg/kg (n=5) showing significant decrease in both treatment groups; Group B (10±3.03%), Group C (1.6±0.678%), when compared to the control Group A (86.6±5.92%). n=number of rats. ***P<0.05*

Figure 2: Effect of *P. yohimbe* of on sperm concentration



Effect of *P. yohimbe* of different doses (group B-150mg/kg, n=5, group C-300mg/kg, n=5) group A-Control (n=5) for 4 weeks treatment on sperm concentration (million/ml) showing significant decrease in both treatment groups; group B(38.6±1.80million/ml), group C (26.2±7.57million/ml), when compared to the control group A (78.8±3.169). n= number of rats. **P<0.05)*

Figure 3: Effect of *P. yohimbe* on sperm vitality



Effect of *P. yohimbe* of different doses (group B-150mg/kg, n=5, group C-300mg/kg, n=5) group A-Control (n=5) for 4 weeks treatment on sperm vitality showing no significant difference in both treatment groups; group B (100±0%), group C (99.8±0.2%), when compared to the control group A (99.2±0.37%), n=number of rats.

3.3 Effect of *P. yohimbe* on Reproductive Hormones

3.3.1 Effect of *P. yohimbe* on Serum Follicle Stimulating Hormone (FSH) level

There is no significant difference between treatment groups; group B (P value=0.134). Group C (P value=0.896) and when compared to the control, group A for FSH. For Serum LH level, there is no significant difference between the treatment groups; group B (P value = 0.373), group C (P value=0.581) and when compared to the control too.

Serum Testosterone level had no significant difference when each of the treatment groups; group B (P value=0.481), group C (P value=0.0972) was compared to the control, group A However, serum E2 level had significant increase in group B (P value=0.0002) when compared to the control, group A, but no significant difference in group C (P value=0.99) when compared to the control group A (Table 3).

3.3.2 Effect of *P. yohimbe* on Testosterone-Estradiol ratio (T/E2)

There is significant decrease (P<0.05) in each of the treatment groups; group B (P value=0.022), group C (P value=0.025) when compared to the control, group A, as shown in Figure 4.

Table 3: Effect of *P. yohimbe* on Follicle Stimulating hormone, Luteinizing Hormone, Estradiol and Testosterone.

GROUPS	FSH (mIU/mL)	LH (mIU/mL)	ESTRADIOL (pg/ml)	TESTOSTERONE (pg/ml)
GROUP A (Control)(n=5)	2.02±0.33	0.99±0.08	25.22±2.32	69.47±29.11
GROUP B (150mg/kg)(n=5)	2.76±0.19	1.33±0.25	402±82.66*	36.19±24.79
GROUP C (300mg/kg)(n=5)	2.17±0.26	1.24±0.18	24.22±0.76	3.168±0.46

**p<0.05*

FSH=Follicle stimulating hormone, LH= Luteinizing hormone, n=number of rats

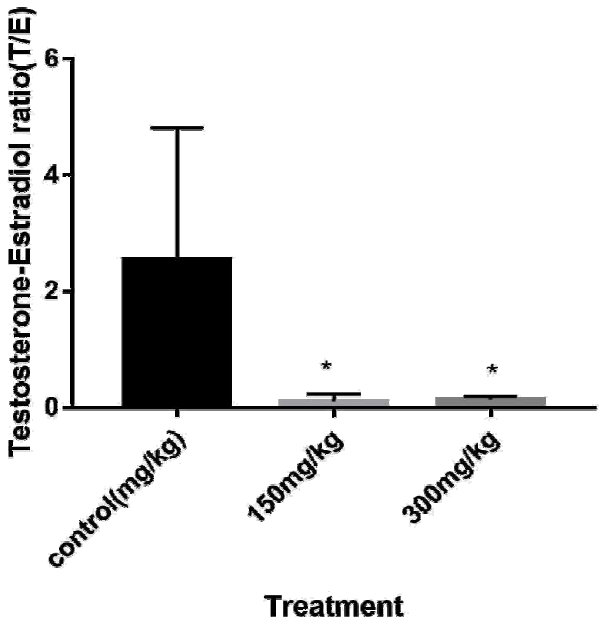


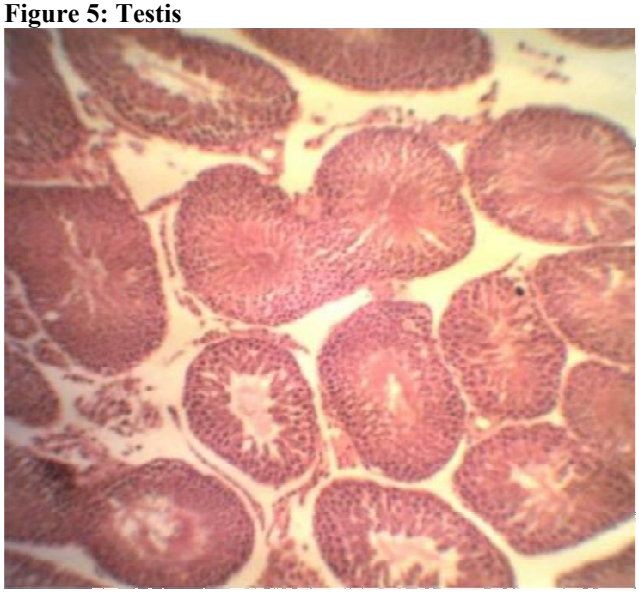
Figure 4: Effect of *P. yohimbe* on Testosterone-Estradiol (T/E) ratio

Effect of *P. yohimbe* of different doses (group B-150mg/kg, n=5 group C-300mg/kg, n=5) group A-Control, n=5, for 4 weeks treatment on Testosterone-Estradiol ratio (T/E) showing significant decrease in both treatment groups; group B (0.09±0.06), group C (0.13±0.022), when compared to the control group A (2.54±1.018). n=number of rats. **P<0.05.*

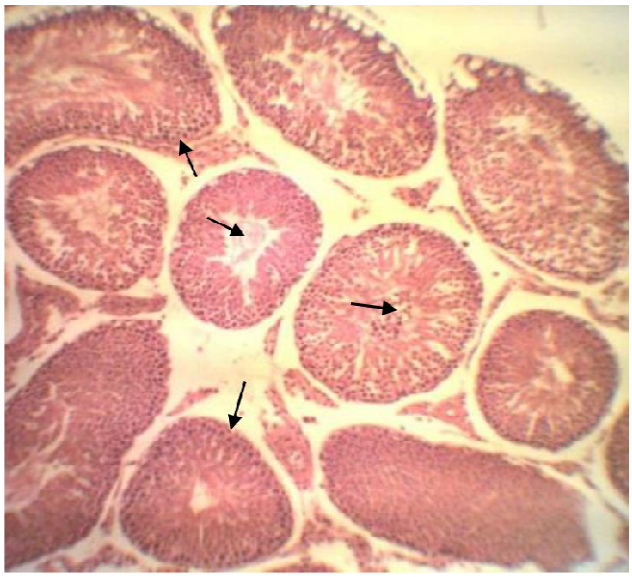
3.4. Light microscopy

The control (group A) displayed normal epithelia architecture while test groups showed various degrees of abnormality.

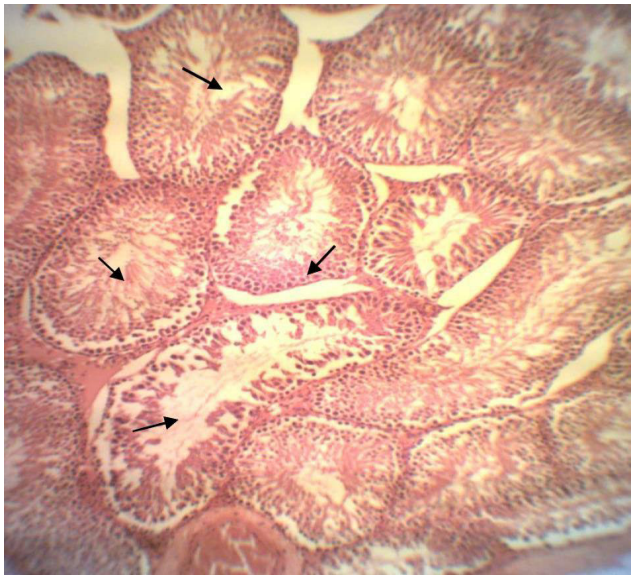
Figure 5: Testis



A (Control)



B (150mg/kg)

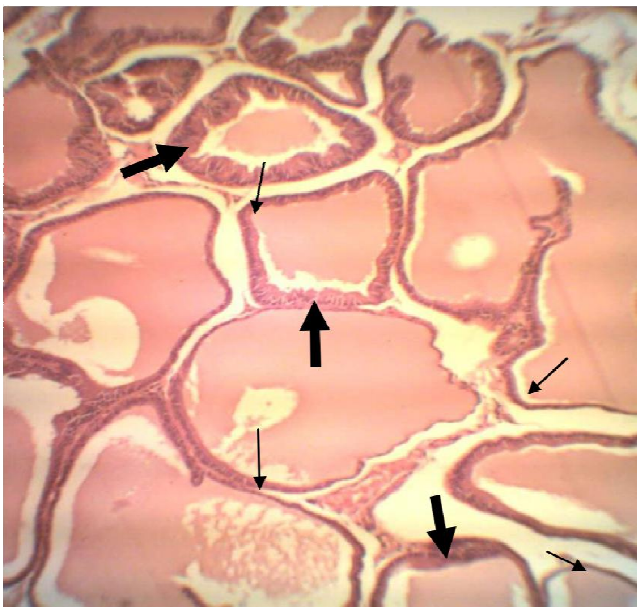


C (300mg/kg)

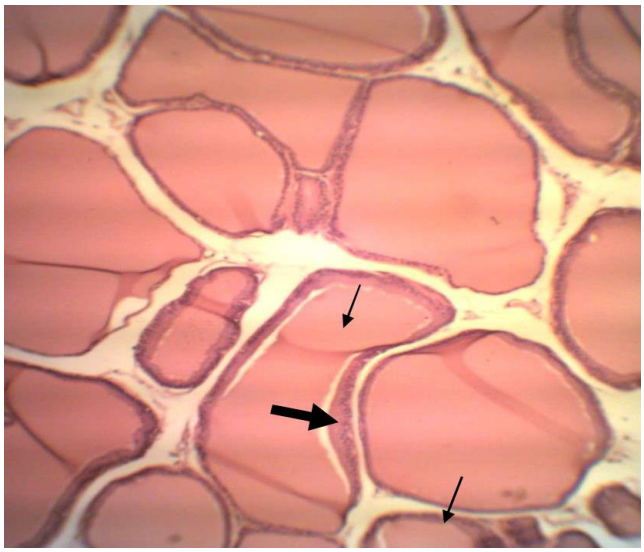
Photomicrograph of the testes showing control and P. yohimbe treated rats: A (control) shows the normal architecture of the testes while B and C (150mg/kg bodyweight and 300mg/kg bodyweight). *P. yohimbe* shows the testes are undergoing degenerative changes. White arrows (detachment of basement membrane), white pigmented arrows (loss of cellular architecture and decreased spermatozoa in the lumen of seminiferous tubules) (Black Arrows in B=loss of cellular architecture, Black Arrows in C= increased interstitial space). Magnifications 10X.

Figure 6: Prostate

Group A (Control)



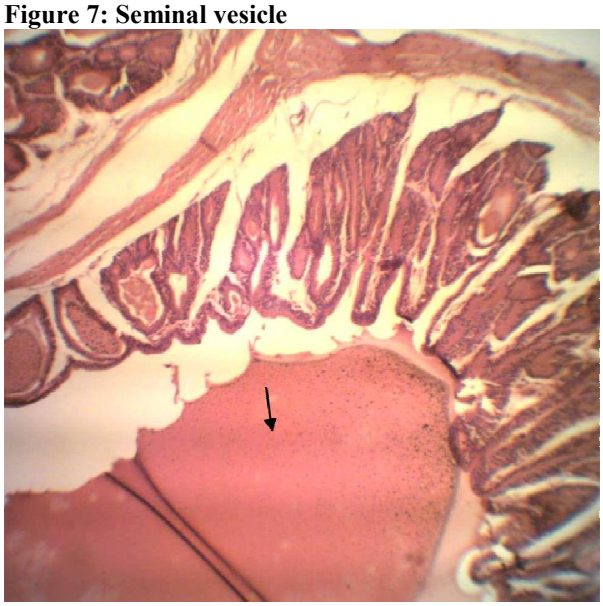
Group B (150mg/kg)



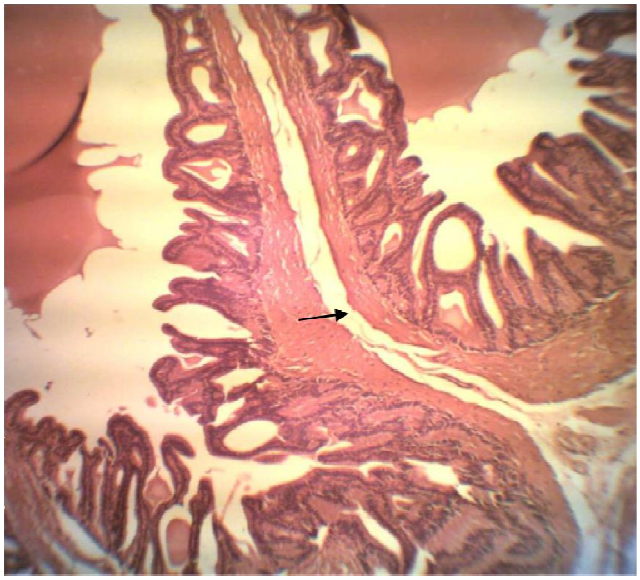
Group C (300mg/kg)

Photomicrograph of the prostate in control and *P. yohimbe* treated rats: A (control) shows the normal architecture of the prostate, while B and C (150mg/kg bodyweight and 300mg/kg bodyweight *P. yohimbe* treatment groups) shows thinning of the glandular epithelium (Grey Arrows), degeneration of glandular epithelium (white Pigmented Arrows), pseudostratification (White Arrows) and increased interstitial space in between the glands (Black Arrows). Magnifications 10X.

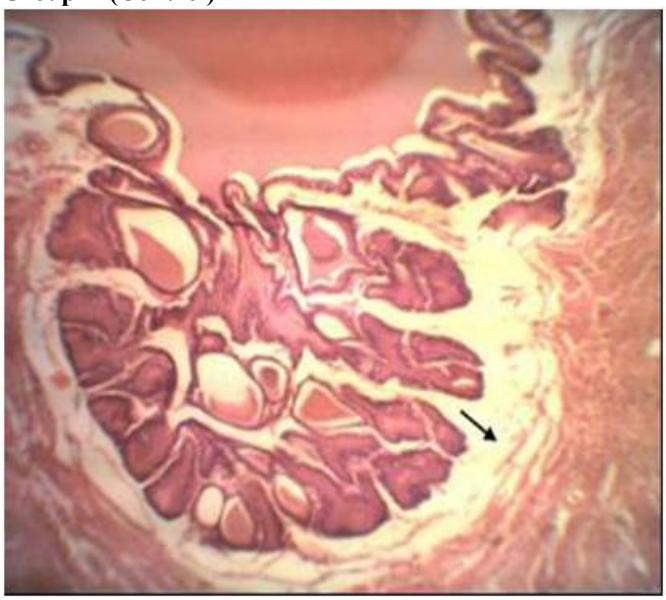
Figure 7: Seminal vesicle



Group A (Control)



Group B (150mg/kg)



Group C (300mg/kg)

Photomicrograph of the seminal vesicle in control and *P. yohimbe* treated rats: A (control) shows the normal architecture of the seminal vesicle while B and C (150mg/kg bodyweight and 300mg/kg bodyweight of *P. yohimbe* treated groups) shows loss of glandular layers (Grey Arrows) increased interstitial space (White Arrows) and thinning of the epithelial layer (Black Arrows). Magnifications 10X.

Discussion

Although a few studies (Galitzky *et al*., 1998; Galitzky *et al*., 1991, Berlan *et al*., 1991, Ogwo *et al*., 2015) have tried to look at the effects of *P. yohimbe* on rats, our study is the only one that consistently delivered the daily dose of *P. yohimbe* through oral gavages. This ensured that the rats received correct daily doses. Our results showed that *P. yohimbe* decreased the net body weight in a dose-dependent manner. The change in weight of both treatment groups was significantly reduced when compared to controls. Weight loss observed in this study is similar to what we observed in another study using Sweet *P. yohimbe* (Ajonuma et al., Unpublished data) and in agreement with a previous study saying that *P. yohimbe* has lipolytic effect (Sax, 1991). This is contrary to Galitzky *et al*., 1990, which explains that *P. yohimbe* had no effect on body weight, body fat and cholesterol at low levels. However, the weight profile of the 300mg/kg test group supports the observation that *P. yohimbe* aids lipolysis and fat depletion by (Galitzky *et al*., 1998; Berlan *et al*., 1991). These results contradict the findings of Ogwo *et al*., (2015) that reported significant weight gain across test groups. We did not also observe significant changes in the relative organ / body weight ratios as reported by Ogwo *et al*. that observed increase in the

relative testicular weights following *P. yohimbe* administration (Ogwo *et al*., 2015).

The observed reduction in sperm motility for both dose 150mgkg and 300mg/kg suggests *P. yohimbe* causes low sperm motility when compared to the control in a dose-dependent manner. Reduced percentage of motile sperm in both groups will greatly affect fertility as suggested. Head to head agglutination of sperm was also observed in both treatment groups. Agglutination may indicate the presence of anti sperm antibodies and this may have adverse effect on fertility. Sperm agglutination also is a major source of decreased sperm motility (WHO, 2010).

Reduction in sperm concentration another factor that determines male fertility in this study by *P. yohimbe* is suggestive of adverse effects on spermatogenesis. The molecular mechanism involved needs to be elucidated. Interestingly, vitality test result showed no significant difference when compared to control group, indicating that the spermatozoa are alive despite their inability to move.

Although serum testosterone, FSH, LH levels were not significantly elevated, we observed a significant increase in Estradiol in group B (150mg/kg) when compared to the control but no significant difference in group C (300mg/kg) when compared to the control. The reason for the increase in Estradiol in group B (150mg/kg b.w) rather than group C (300mg/kg b.w) is unknown. Testosterone-Estradiol ratio showed a significant decrease in both treatment groups; group B and group C when compared to the control. A low testosterone-estradiol (T/E2) ratio may lead to decreased reproductive functions and cause male infertility.

The effect of *P. yohimbe* on other sperm functions tests and its effect on spermatogenesis remains to be evaluated.

Considering the contribution of accessory glands including seminal vesicles to fertility (Gonzales, 2001), we looked at *P. yohimbe* effects on the accessory glands. *P. yohimbe* showed adverse effects on the testis, prostate and seminal vesicles. Varying degrees of tissue destruction for both test groups of the epithelial cells of the seminiferous tubules may prevent spermatogenesis leading to significant decrease in sperm concentration. Destruction of glandular epithelial cells as seen in this study may course decreased secretion leading to decreased semen volume. Although we did not examine the histology of the epidydims in this study, it is highly possible that *P. yohimbe* would have induced abnormality in the epididymis too. Therefore, its prolonged use warrants caution considering its safety and effectiveness.

In summary, prolonged use of *P. yohimbe* cause deleterious effects on testes, accessory glands, sperm motility and sperm concentration which can lead to male infertility. As *P. yohimbe* improves erectile dysfunction, its continuous use may lead to reproductive tissues destruction, very low sperm counts and poor sperm motility. Molecular and cellular mechanisms involved as well as its effect on sperm functions needs to be elucidated.

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