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Ascrobic Acid Reduced Pausinytalia Yohimbe Induced Sperm And Reproductive Organs Toxicity In Male Wister Rats

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Abstract

Pausinystalia yohimbe (*P. yohimbe*) is aphrodisiac used in the treatment of organic, psychogenic and substance induced erectile dysfunctions, impotence and other male sexual disorders. It increases blood flow to erectile tissues and exerts its erotogenic effects through a central action and peripheral autonomic nervous system effects. However it is has been demonstrated that *P.yohimbe* chronic use induces toxicity in sperm and reproductive organs. This study was carried out to determine the role of Ascorbic acid on reduction of the effects of *P.yohimbe* induced toxicity on sperm and male reproductive organs. Twenty adult male rats were randomly divided into 4 groups (A, B, C and D) of five rats each. Group A control, received 0.5mls of normal saline, Group B received 5% Ascorbic acid, Group C received 300mg/kg body weight (bw) of *P.yohimbe,* and Group D received 300mg/kg bw of *P.yohimbe* and 5% Ascorbic acid respectively daily via orall gavage for six weeks. Reproductive organs, testes, prostrate, epididymis and seminal vesicles obtained from the rats were weighed and sections were used for Heamatoxylin and Eosin (H&E) staining. The epididymal sperm obtained was used to evaluate sperm concentration and motility. *P.yohimbe* alone caused degenerative changes in the testes, epididymis, seminal vesicles and prostate of the rats and this was ameliorated with the co-treatment of ascorbic acid. Sperm motility and concentration were also improved by ascorbic acid. We concluded that ascorbic acid reduced *P.yohimbe* induced toxicity in sperm and male reproductive organs of Wistar rats. It may be that the mechanism of *P. yohimbe* induced sperm and reproductive tissues destruction is via oxidative stress.

Keywords: Ascorbic acid, Pausinytalia Yohimbe, Reproductive toxicity, Sperm, Wister rats, oxidative stress

Introduction

Medicinal plants have been used as a source of healing over several years and it has gained acceptance and widespread use in every culture of the world (Hoareau and Dasilva 1999, Hassan 2012). Medicine plants have been used to treat different kinds of disease conditions including hypertension, diabetes and infertility. Different tribes, cultures all over the world have also used medicine plants as a source of aphrodisiac. An aphrodisiac has been described as an agent (food or drug) that arouse sexual desire (Singh et *al.* 2013). Several plants have been associated with arousal of sexual desire (Malviya et *al.* 2011). *P.yohimbe* is a plant that had been used in folklore medicine by tribes in Nigeria as aphrodisiac (Yakubu et *al.* 2007). Despite its beneficial effects as an aphrodisiac, we had demonstrated that *P. yohimbe* had adverse effects on the male reproductive system as evidenced by its effects in causing hormonal and structural changes in

the male reproductive system (Ajonuma *et al*. 2017a, Ajonuma *et al* 2017b) We further demonstrated that in female rats, it causes degenerative changes in both the uterus and ovaries (Ajonuma *et al.* 2018). The common pathway for reproductive dysfunction is oxidative stress.

Ascorbic acid is a water-soluble vitamin that plays essential role in physiological protection of organs as a powerful antioxidant (Saygin et al. 2018). Ascorbic acid as an antioxidant had been used to restore reproductive functions and anomaly in reproductive systems in various species (Saygin et *al*. 2018; Laili et *al*.2015). This study evaluated the ameliorative effects of Ascorbic acid on P. *yohimbe* induced reproductive toxicity in male Wistar rats.

Materials and Methods

Preparation of P. *yohimbe* Extract

The extract was prepared as earlier described (Ajonuma et al 2017a; Ajonuma et al 2017b; Ajonuma *et al* 2018). In brief, P. *yohimbe* obtained from a local herbal practitioner was crushed with a mortar and pestle and then pulverized into fine powder using an electric blender. 150g of the powder of P.*yohimbe* was dissolved in 600mls of distilled water in a large beaker and heated at 100^0C for 60 minutes. The extract was allowed to cool and filtered with a clean white handkerchief into a medium-sized beaker. The resulting filtrate in the beaker was dried and concentrated in an electric oven at 60^0C until hard solid residue was obtained.

Ascrobic Acid

Ascrobic acid was purchased from KUNIMED Pharmachem Limited; Nigeria was prepared by dissolving 5g in 100mls of distilled water.

Animals

Twenty male Wistar rats weighing 250-300g were used for the study and rats were obtained from Lagos State University College of Medicine Animal House, Ikeja, Lagos State, Nigeria. The rats were fed with standard rat chow obtained from Agege Livestock Feed Mills, Agege, Lagos, Nigeria and water *ad libitium*. The animals were housed in an environment of 12 hours dark and 12 hours light cycle, adequate relative humidity and at room temperature. They were allowed to acclimatize for 2 weeks before commencement of experiments. The study was carried out in LASUCOM Animal House. Ethics Approval was obtained from LASUCOM Animal Research Ethics Committee. The study was in compliance with international guiding principles for research involving animals and the LASUCOM Animal House guidelines for care and use of animals.

Experimental Design

The experimental animals were randomly allotted into 4 groups of 5 rats each. Group A was the control group while groups B and C and D served as the test groups. Group A received 0.5ml of normal saline daily while group B received 5% Ascorbic acid, groups C and D received 300mg/kg body weights (bw) of aqueous extracts of *P. yohimbe* and D received additional 5% Ascorbic acid respectively for 6 weeks.

After 6 weeks of administration, the rats were anaesthetized using ketamine HCL, and were dissected open; blood samples were collected via cardiac puncture. The separated serum samples were stored in plain sample bottles at -20^{o}C for hormonal assay. Testes, epididymis, prostrate glands and seminal vesicles were removed from each rat, weighed and stored in 10% buffered formalin for histological processing.

Evaluation of Epididymal Sperm

Caudal epididymis gently minced in 1ml of normal saline (0.9% Nacl) to allow the sperm to swim out and this was incubated at 37^{o}C. The sperm suspension was examined within five minutes after their isolation from epididymis. The sperm was used for sperm count, sperm motility and vitality.

Sperm concentration

Microscopic sperm concentration was done according to WHO lab. Manual (WHO.2010).the sperm number was determined using the Neubaeur improved haemocytometer chamber. A dilution ratio of 1:20 from each well-mixed sample was prepared by diluting 50µl of epidymal spermatozoa that was suspended in the washing medium of 950µl of sperm diluent and 10µl of the mixture was then pipetted on each chamber (upper and lower chamber) of improved Neubaeur haemocytometer chamber and a cover slip was placed on it. This was counted on a light microscope (Olympus Germany XS2-107BN) using ×40 objectives. To count the number of cells,

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four chambers were read, and two chambers from the grid was randomly selected (one upper and lower chamber).

Sperm motility

Using sterilized pipettes the mixture 10µl of epidymal spermatozoa was dropped onto a glass slide covered with 22×22mm cover slip to give a depth of about 20µm and examined under a light microscope (Olympus, Germany XS2-107BN) at a ×40 objective.

Heamatoxylin and Eosin (H & E) tissue staining

This was carried out on previously fixed in 10% formalin as described by Akpantah *et al*., (2003) and Ajonuma et al. (2017a, 2017b) with modifications. In brief, fixed tissue is transferred to the mould containing paraffin wax and the wax was blown on the surface until a thin film of wax solidified. The mould containing the tissue was transferred to a container of cold water. It remained submerged until the wax hardens. The paraffin block was trimmed and placed on ice for 1 hour and the block was fixed in position on the microtome and sections were cut at 5µm. The thin section is floated with 20% alcohol in a warm bath at 40°C. The thin section was picked and dried on hot plate at 75°C. The section was taken into water and stained in Haematoxylin for 10 minutes. The stained section was rinsed in water and differentiated in 1% acid – alcohol. This was rinsed in water for 1 minute and counter stained with 1% Eosin for 1 minute. The Excess stain was washed, and the section was dehydrated in ascending order with 70%, 90%, 100% alcohol for 15 seconds each, the section was cleared in Xylene and mount in dihydroxy phthalate xylol (DPX). The stained section was evaluated using a light microscope (Olympus) and images of the sections were captured using attached camera.

Statistical Analysis

Data are presented as mean and standard error of mean (SEM). Analysis of variance was used for data analyses and differences between groups were compared using Turkey comparison test. A p-value of ≤ 0.05 was considered significant. Statistical analyses were carried out using Graph Pad Prism Software Version 8.0.2, Graph Pad Inc., San Diego, CA, USA.

Results

Effect of Ascorbic acid on Sperm Motility and Concentration of male Wistar rats treated with *P.yohimbe*

There was statistical significance difference between group A (control) and group C and between group A and D, when compared with the group A (fig. 1). There was also statistical significance difference between group A and group C and between group A and D, and none with group B when compared with the control group A (fig. 2).

Effect of Ascorbic acid on the H&E stained section of testes, epididymis, seminal vesicle and prostate of male rats treated with *P.yohimbe*

The testis of the P.yohimbe only treated group demonstrated degenerative and toxic changes and this changes include degeneration of the leydic cells and, sertoli cells , there was also deformation of the cells walls, the spermatogonia, the spermatocytes, the round spermatids and elongated spermatids and this changes were ameliorated in the group given both ascorbic acid and P.yohimbe (fig. 3).

The epididymis of the P.yohimbe only treated group there was a degeneration of the sterociliated columnar epithelium, loose connective tissue and the deformation of the sperm cells in the spermatozoa in the lumen and this changes were ameliorated in the group given both ascorbic acid and P.yohimbe (fig. 4).

In seminal vesicle of the P.yohimbe only treated group, there was a degeneration of the cell wall and the lumen and this was ameliorated in the group given both ascorbic acid and P.yohimbe (fig. 5).

In the prostate of the P.yohimbe only treated group, there was a degeneration of the acinus cells and the basal cell wall this was ameliorated in the group given both ascorbic acid and P.yohimbe (fig. 6).

Discussion

This study evaluates the effect of ascorbic acid on *P.yohimbe* induced reproductive toxicity. We had earlier demonstrated that various types of *P. yohimbe* caused reproductive toxicity in male rats (Ajonuma et al., 2017a; Ajonuma *et al.,* 2017b). In this study there were no significant changes in the testicular weights in the various groups. However the testis in *P.yohimbe only* treated group demonstrated degenerative and toxic changes and this changes

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include degeneration of the leydic cells and, sertoli cells , there was also deformation of the cells walls, the spermatogonia, the spermatocytes, the round spermatids and elongated spermatids and the findings aligns with our earlier report (Ajonuma et al., 2017a; Ajonuma *et al.,* 2017b).This toxic changes were however ameliorated by ascorbic acid as seen in the group given both ascorbic acid and *P.yohimbe.* This effect of ascorbic acid is seen in other studies were ascorbic acid have been demonstrated to ameliorate testicular toxicity in various condition cimetidine-induced testicular toxicity (Aprioku *et al.,* 2014), hyperglycemia induced testicular toxicity (Fernandes *et al.,* 2011), lead testcular toxicity (Ayinde *et al.,* 2012), and Monosodium glutamate-induced testicular toxicity (El Kotb *et al.,* 2020). The restoration of testicular structure implies the testis may have the capacity to perform it main function of hormone and sperm production (Sembuligam and Sembuligam 2012).

There was no significant change in the weight of the epididymis of control and test groups, however there was degenerative and toxic changes in the epididymis of *P.yohimbe* only group and this changes include degeneration of the sterociliated columnar epithelium, loose connective tissue and the deformation of the sperm cells in the spermatozoa in the lumen and this in line with our earlier findings (Ajonuma et al., 2017a; Ajonuma *et al.,* 2017b). Ascorbic acid however ameliorated this abnormality. The restoration of epididymal structure is very important because it plays a vital role in reproductive function. It is involved in transportation, concentration, maturation, and storage of spermatozoa and these functions are important for male fertility and impairment of any of these processes may adversely affect male reproduction (Turner, 2008).

There was no significant change in weight of seminal vesicles in control and test group but *P.yohimbe* only group had degenerative changes in the seminal vesicle there was a degeneration of the cell wall and the lumen in the group C (P.yohimbe) and this agrees with our previous work (Ajonuma et al., 2017a; Ajonuma et al., 2017b). These changes were reversed in the group both ascorbic acid and *P.yohimbe*.

These abnormalities imply the critical role of the seminal vesicles will not be served. Seminal vesicular fluid helps in semen coagulation. Semenogelin, a 52 KD protein, present in this fluid helps to perform this function. Semen coagulation does not occur in individuals with dysfunctional seminal vesicles (Lundwall and Olsson, 2001; Mandal and Bhattacharyya, 1985). The fluid also plays important role in producing semen consistency. Semen with high viscosity is associated with infertility and this usually seen in subjects with hypo functioning seminal vesicle. Several components of this fluid have sperm motility enhancing capacity and this include potassium, bicarbonate, and magnesium (Gonzales, 2001). The seminal vesicle fluid also helps in antioxidant activities, suppression of immunologic response in female reproductive tract, stabilizing sperm chromatin contents and sperm autophagy (Aumuller and Riva, 1992; Gonzales *et al.,* 2001).

There was no significant change in the prostate weight but there were degenerative changes in the prostate in *P.yohimbe* treated group and this agrees with our earlier work. This degenerative changes were absent in rats give both ascorbic acid and *P.yohimbe*. The prostate is important because its secretions contain zinc, citrate, glucose, kallikrein and spermine that are important in the process of ejaculation and regulation of proteins that activate sperm maturation, semen liquefaction and sperm motility (Gilany *et al.,* 2015; Verze *et al.,* 2016). The gland also secretes prostasomes that play important role in sperm motility, capacitation, acrosome reaction, immunological modulation, antioxidant, and antibacterial activities (Burden *et al.,* 2006).

Although there was no statistical significant difference serum FSH between the control and the test groups, it appears *P.yohimbe* alone suppressed FSH secretion and this aligned with our initial finding (Ajonuma et al 2017b). Ascorbic acid however caused improvement in the secretion of this hormone as demonstrated in the group administered both ascorbic acid and *P.yohimbe*. This finding is in line with the effect of ascorbic acid in stimuating FSH production (Ayinde et al. 2012). FSH play a significant role in spermatogenesis and it deficiency may contribute to abnormal spermatogenesis seen in rats treated with *P.yohimbe* alone.

Testosterone was not statistically significant across the groups but it appear to be slightly elevated in the

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group treated with *P.yohimbe* alone and this is line with our previous report (Ajonuma et al 2017b) and this may account for the use of *P. yohimbe* as aphrodisiac.

Estradiol was elevated in the group treated with *P.yohimbe* alone and this was suppressed by ascorbic acid co-administration. This suppression of estradiol is beneficial because elevated circulating oestradiol causes infertility because it impairs sperm production (Balasinor et al., 2010; Leavy et al., 2017; Lubbert et al., 1992).

P.yohimbe caused oxidative stress in epididymal sperm as evidenced by conversion of conversion of NBT to formazan when spermatozoa from rats treated with *P.yohimbe* were incubated with NBT (data not included). This oxidative was ameliorated by co-adminstration of ascorbic acid with *P.yohimbe*. Ascorbic acid is an antioxidant and this effect has been demonstrated previous studies (El kotb et al 2012).

P.yohimbe alone caused significant reduction in sperm motility and this we had earlier reported (Ajonuma et al 2017a; Ajonuma et al 2017b). Ascorbic acid administration significantly improved *P.yohimbe* induced impaired sperm motility. The impaired sperm motility may be caused elevated reactive oxygen species (ROS) caused by ROS. Sperm motility is reduced by ROS because ROS causes peroxidation of the membrane lipids of the spermatozoa reducing their flexibility and inhibiting their motility mechanism (Dutta *et al.* 2019; Lenzi *et al.*, 1993; Sabeti *et al.*, 2016). ROS may also cause axonemal and mitochondrial destruction resulting in the reduction of sperm movement (Aitken 1987; Peris *et al.*, 2007). The ability of ascorbic acid to mitigate this effect is in its antioxidant properties. *P.yohimbe* caused reduced sperm concentration and this effect was ameliorated by the administration of ascorbic acid. Several studies have demonstrated that ascorbic acid supports testicular spermatogenesis (Ayinde *et al* 2012).

In summary, ascorbic acid reduced *P.yohimbe* induced toxicity in sperm and male reproductive organs of Wistar rats. It may be that the mechanism of *P. yohimbe* induced sperm and reproductive tissues destruction is via oxidative stress. Further studies involving all ROS parameters and DNA damage are needed.

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Table and Figures

Table 1. Effect of Ascorbic acid on sperm motility and concentration of rats treated with P.yohimbe

Sperm parameter	Group A	Group B	Group C	Group D
Sperm Motility	49±1.0	42±1.2*	20±2.2*	33±3.7*
Sperm Concentration	185±9.6	161±13	45±3.9*	75±3*

Ascorbic acid improved sperm motility and concentration of rats treated with P.yohimbe. Group A control, B - Ascorbic acid, C - P.yohimbe and D - P.yohimbe + Ascorbic acid. *p<0.05, n=5.

Figure 1: Ascorbic acid improved sperm motility of rats treated with P.yohimbe. Group A control, B - Ascorbic acid, C - P.yohimbe and D - P.yohimbe + Ascorbic acid. *p<0.05, n=5

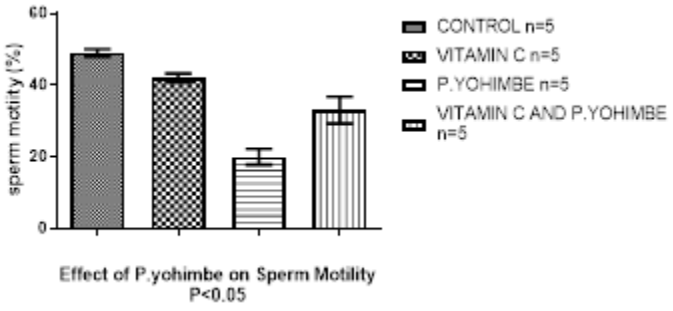

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Figure 2: Ascorbic acid improved sperm concentration of rats treated with P.yohimbe. Group A control, B - Ascorbic acid, C - P.yohimbe and D - P.yohimbe + Ascorbic acid. ***p<0.05, n=5.

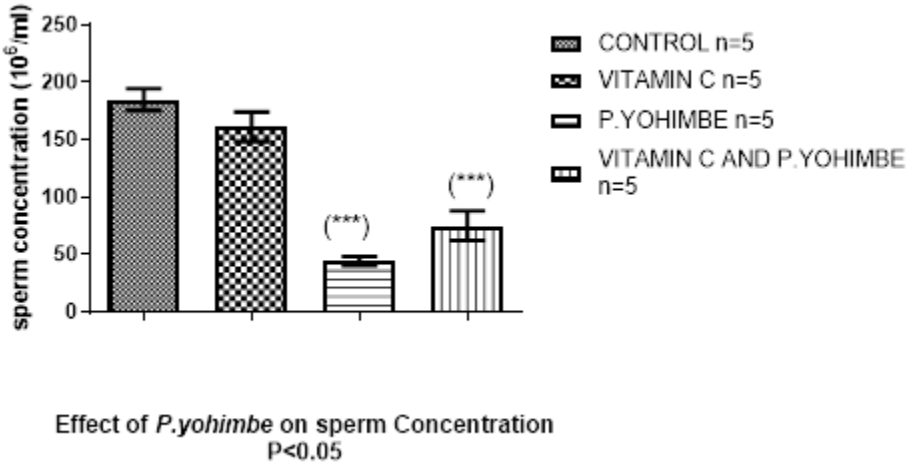

Figure 3: Photomicrograph of H&E sections of the testes of rats from group A, B,C and D. There was a degeneration of the leydic cells and, sertoli cells , there was also deformation of the cells walls, the spermatogonia, the spermatocytes, the round spermatids and elongated spermatids in the group C (P.yohimbe) and some regeneration in group D (P.yohimbe with Ascorbic acid) which indicates the reduction of P.yohimbe induced toxicity by vitamin C. Magnification X 10. A= Control B= Ascorbic acid C= P.yohimbe, D= Ascorbic acid and P.yohimbe SL= Spermatozoa in lumen, LC = Leidig cells.

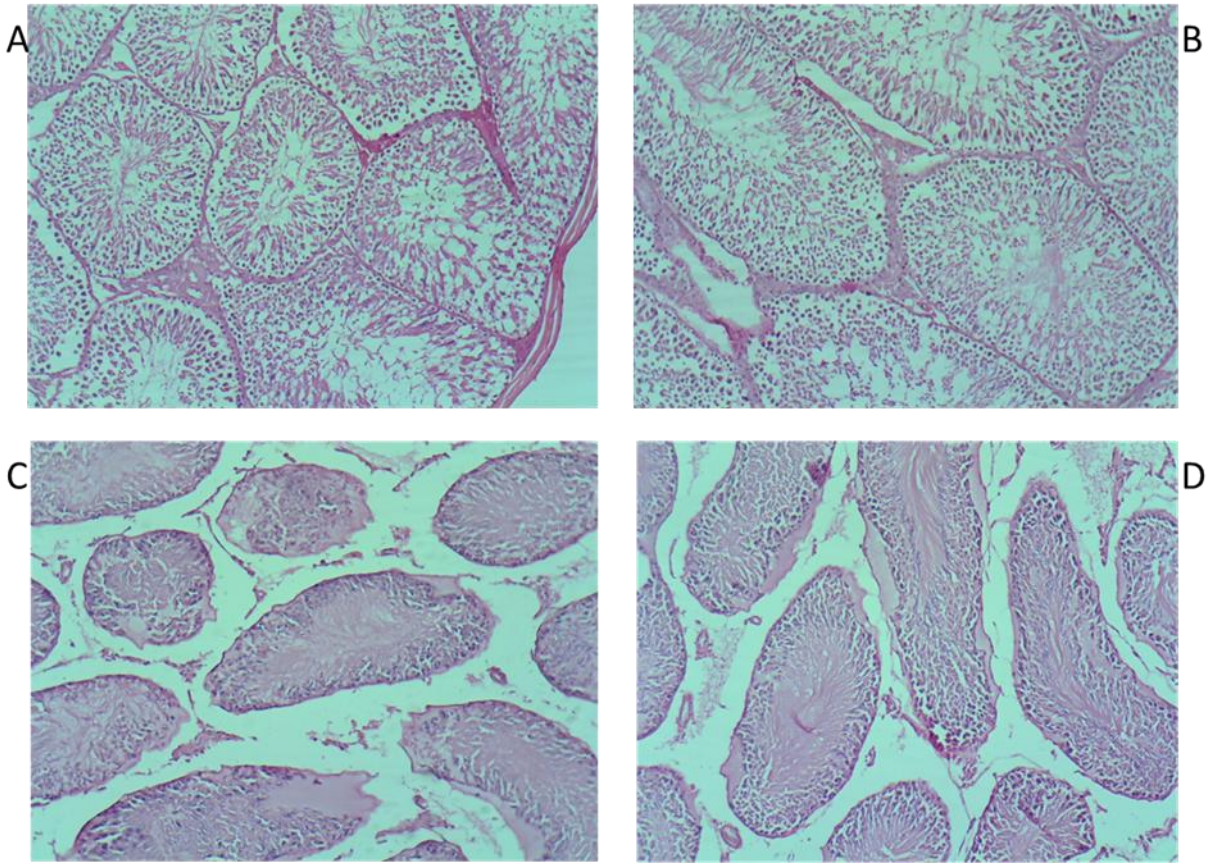

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Figure 4: Photomicrograph of H&E sections of the epididymis of rats from group A, B,C and D. In the group C (P.yohimbe) there was a degeneration of the sterociliated columnar epithelium, loos of connective tissues and the deformation of the sperm cells in the spermatozoa in the lumen. Regeneration of cells in group D (P.yohimbe with Ascorbic acid).Note the increased sperm density in the epididymal lumen. Magnification X 10. A= Control B= Ascorbic acid C= P.yohimbe, D= Ascorbic acid and P.yohimbe SL= Spermatozoa in lumen E= Stereociliated simple columnar epithelium.

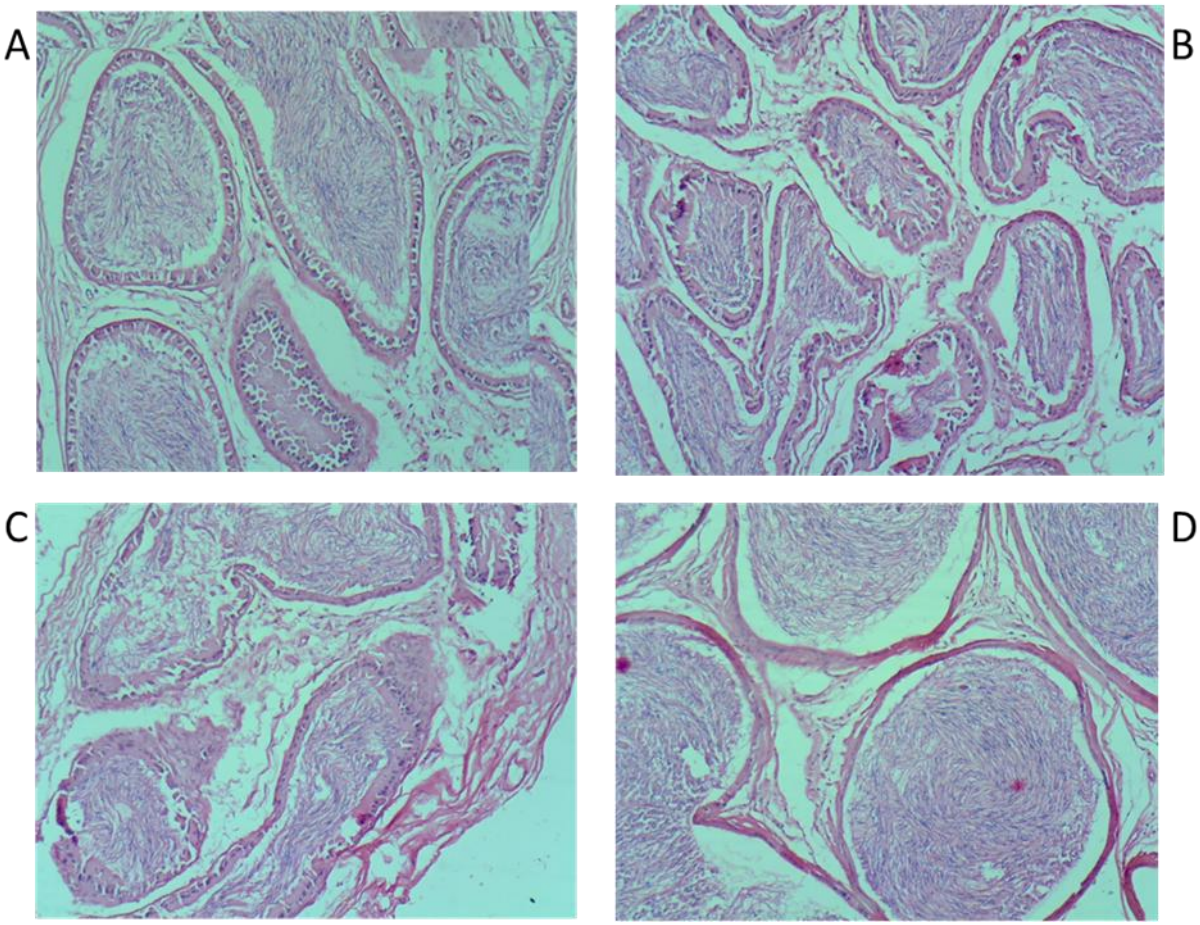

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Figure 5: Photomicrograph of H&E stained section of the testes seminal vesicles of rats from group A, B,C and D. In the seminal vesicle there was a degeneration of cell wall and lumen in group C (P.yohimbe) and regeneration in group D (P.yohimbe with Ascorbic acid). Magnification X 10. A= Control B= Ascorbic acid C= P.yohimbe, D= Ascorbic acid and P.yohimbe L= Lumen M= Muscular layer.

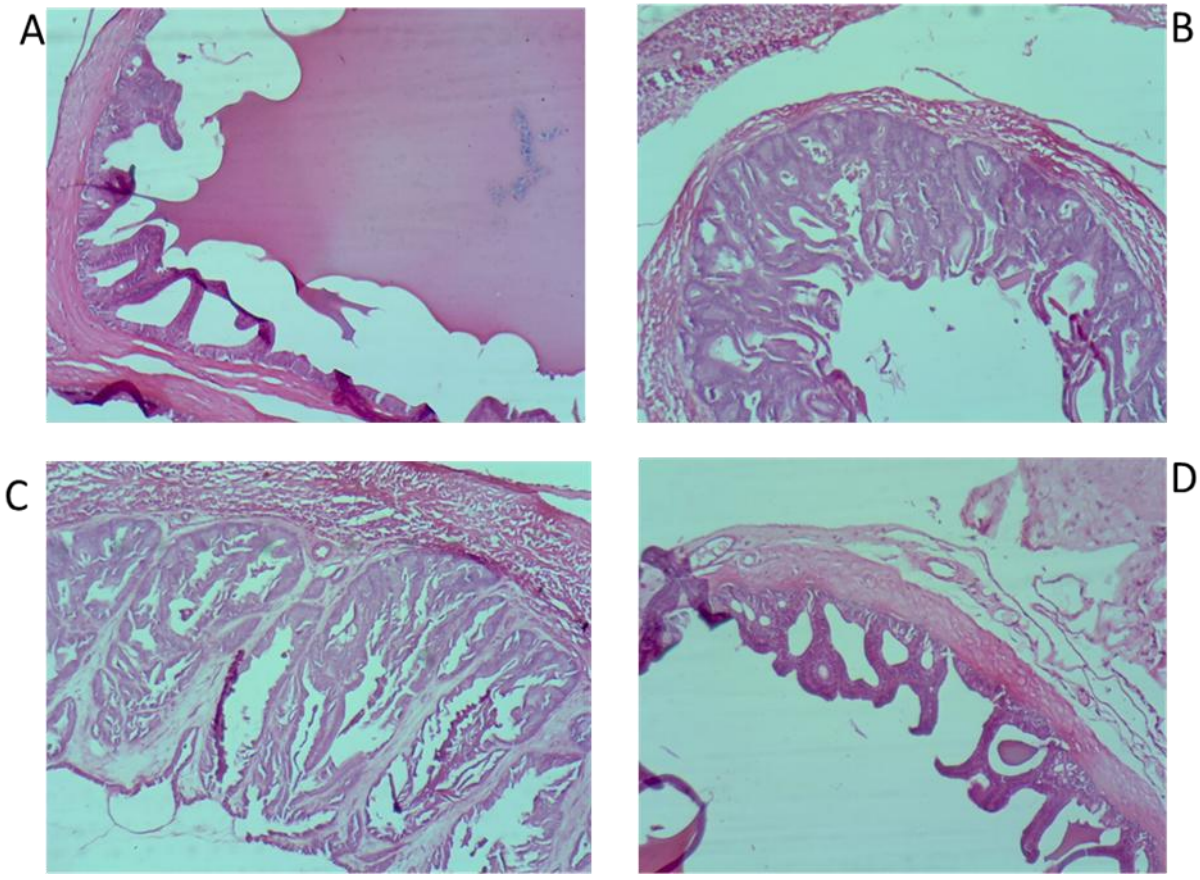

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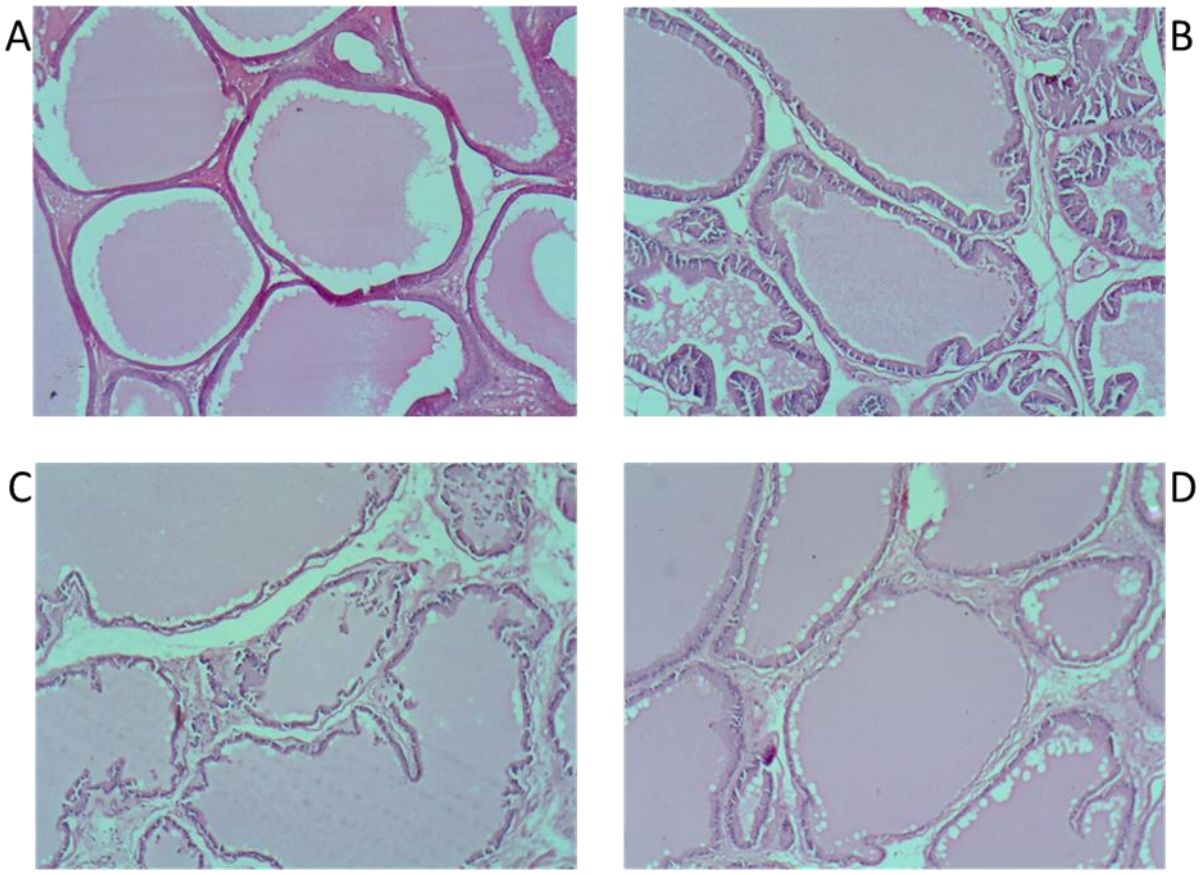
Figure 6: Photomicrograph of H&E stained sections of prostate of rats from group A, B, C and D. In group C (P.yohimbe), there was a degeneration of the acinus cells and basal cell wall. Regeneration of the cell wall in group D (P.yohimbe with Ascorbic acid). Magnification X 10. A= Control B= Ascorbic acid C= P.yohimbe, D= Ascorbic acid and P.yohimbe, AC= Acinar cells BC= Basal cells



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