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Research Paper

Histological Changes In The Testes Of Albino Rats Treated With*pausinystalia Yohimbe* Bark Powder (Burantashi)

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ABSTRACT:- The effect of *Pausinystalia yohimbe* bark powder (burantashi) on the histology of the testes was investigated in whole adult male albino rats administered graded concentrations of the powder in combination with feed in the order 2%, 4%, 6%, 8% and 10% for thirty days. Results obtained revealed no significant histological changes at low dose (2%) in which the histological architecture was not significantly different from that of the control with both the seminiferous tubules and interstitial cells being normal. However, with increasing doses of BBP, a spectrum of dose dependent histological changes were observed, including total loss of orderly arrangement of the germ cells, aggressive invasion of the outermost layer of the germinal epithelium by spermatids, sertoli cells dysfunction, degeneration and dissociation of germinal epithetlia with resultant outpouring of germ cells into the interstitial spaces and invasion of lumens of the seminiferous tubules by several cell types that resembled either the primary and or secondary spermatocytes, spermatogonia and few spermatids. Therefore with increasing doses of burantashi, the lumen of the seminiferous tubules became populated by immature sperm cells in the treated rats. It may be concluded that since majority of the sperm cells produced following treatment with higher doses of burantashi were immature and abnormal, the herbal substance therefore confers no advantage to couples seeking pregnancy

KEYWORDS:- Burantashi, *Pausinystalia yohimbe*, Rats, Testes, Seminiferous tubules

I. INTRODUCTION

In Nigeria, the powdered bark of the African tree *Pausinystalia yohimbe*, is popularly called burantashi; meaning penile erection, hence the preparation has been used traditionally for the treatment of various degrees of erectile dysfunction (Reepmeyer *et al*., 2007).Other medicinal values of the bark extract of the plant include management of exhaustion, chest pain, skin disorders and inflammations (en.wikipedia.org/wiki/Pausinsystalia). In our previous publication on the effects of burantashi on the male reproductive system, we reported that although burantashi caused no obvious deleterious effects on semen quality and quantity in rats; it however increased the percentage of abnormal and immature spermatozoa and may therefore impair fertility in rats and may have the same effects in man (Ogwo *et al*., 2015), even though itenhanced both testicular weights and male reproductive hormones including testosterone, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) in the treated male rats (Ogwo *et al*., 2015). In this current study, we intend to build on previous results on the effect burantashi on the male reproductive system by looking at the histological changes which occurred in the testes following 30 days treatment with various concentrations of burantashi.

II. MATERIALS AND METHODS

2.1 *Collection of plant stem and preparation of bark powder*

Pausinystalia yohimbe stem bark (burantashi) was obtained from a local herbal practitioner in Lafia, Nasarawa state and was authenticated at the department of forestry, college of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. A voucher number

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MOUAU/CVM/VPP/14/028 was assigned to the sample which was thereafter deposited at the departmental herbarium. The collected stem bark was dried under shade for 14 days and was thereafter ground into powder using an electric powered locally fabricated mill. The resulting powdered material hereafter referred to as Burantashi Bark Powder (BBP) was preserved dried for mixture with feed at various concentrations.

2.2 ***Animals***

A total of forty eight (48) adult male rats obtained from the Animal production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. The animals were housed under specific pathogen free (SPF) conditions one in a metabolic cage with 13 H/11 H light/dark schedule and were provided standard feed and water *ad libitum*. Experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as reported by Akah *et al.*, (2009). The study was carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

2.3 ***Effect of chronic consumption of BBP on testicular histology of male rats***

The forty eight adult male rats were divided into 6 groups of 8 rats each and were given diets containing varying concentrations of BBP in the order:

Group A: Normal feed and water and served as the control
Group B: 2% BBP plus feed combination and water
Group C: 4% BBP plus feed combination and water
Group D: 6% BBP plus feed combination and water
Group E: 8% BBP plus feed combination and water
Group F: 10% BBP plus feed combination and water

AT the end of thirty days of treatment, the animals were sacrificed and the testes were harvested each into a clean bottle containing boins solution and prepared for histological examination following the methods of John and Alan (1977) and Clayden, (1967). The photomicrographs of slides were observed under the microscope with magnifications of x100 and x400. Selected images were captured using a moticam 2.0 digital camera attached to a computer.

III. RESULTS

3.1 ***Testicular histology of the normal control rats***

The histological section of the testis from the control rat at x100 magnification revealed the presence of normal seminiferous tubules which are highly convoluted lined by germinal epithelium with germ cells their various stages of spermatogenesis and spermiogenesis (spermatogonia, spermatocytes, round spermatids and elongated spermatids) consistent with normal spermatogenesis. Sertoli cells which support and nourish the spermatozoa were also found. In the interstitial spaces (IS) between the tubules are the Leydig cells which form the supporting tissue. At x400, active matured functioning seminiferous tubules associated with complete spermatogenic series consisting of 3 prominent layers of spermatogenic cell generations. The outermost compose of large heterochromatic spermatogonia that are linearly arranged along the basal lamina. In the middle are about 3-4 layers of mostly euchromatic cells of both 1st and 2nd spermatocytic generations. The innermost consist of heterochromatic spindle-shaped spermatids. The basal lamina is composed of linearly arranged heterochromatic cells whose periphery is lined by some spindle shaped myofibroblasts (Plates. 1and 2)

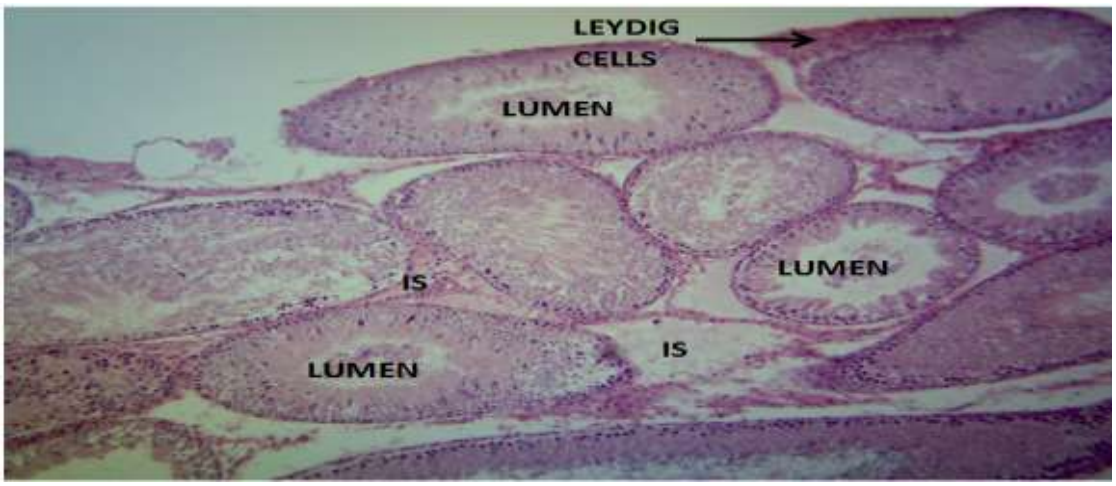

Plate 1A: Section of control testis showing normal architecture of the testis. H&E X100

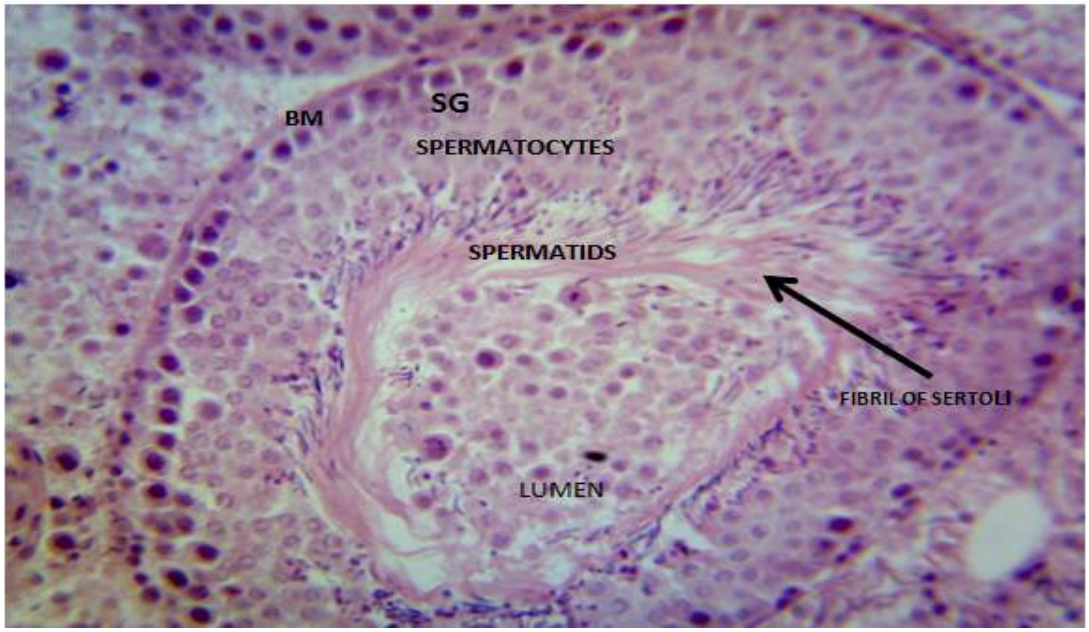

Plate 1B: Section of control testis showing the lumen of the seminiferous tubules. H&E X400

3.2*Testicular histology of rats treated with 2% BBP*

This revealed normal histo-architecture comparable to the control group with 70% of the seminiferous tubules having increased concentration of sperm cells in the center of the tubules, while 30% exhibited reduction in the number of sperm cells in the lumen of the tubules.The interstitial spaces in between the convoluted seminiferous tubules are patchy areas replaced with cavities, rich in extracellular fluid. The blood vessels and lymphatics contained therein form the peritubular plexuses in the intertubular spaces. The interstitial cells of Leydig occurred in clusters of varying sizes, sometimes in close association with blood vessels (Plate 2A). At x400 the spermatogenic series were distinguishable into spermatogonia, spermatocytes, spermatids and spermatozoa with the sertoli cells (basically columnar) resting upon the basal lamina and extending upwards through the full thickness of the epithelium to its free surface. The earliest of the germ cells, the spermatogonia, also rest upon the basal lamina, while the more advanced stages of the germ cell line are formed at the successively higher levels in the epithelium.In the center of the seminiferous tubule is a mixture of spermatogenic cell types, majority being spermatocytes and peripherally arranged spermatids. Also noted are presence of crescentic cavities between layers of spermatogonia and primary spermatocyte in contrast to the outlook of the control slide. Prominent in the picture are fibrils of sertoli almost enclosing the spermatogenic cells within the lumen of the seminiferous tubule, although less distinct in the control slide (Plate 1B).

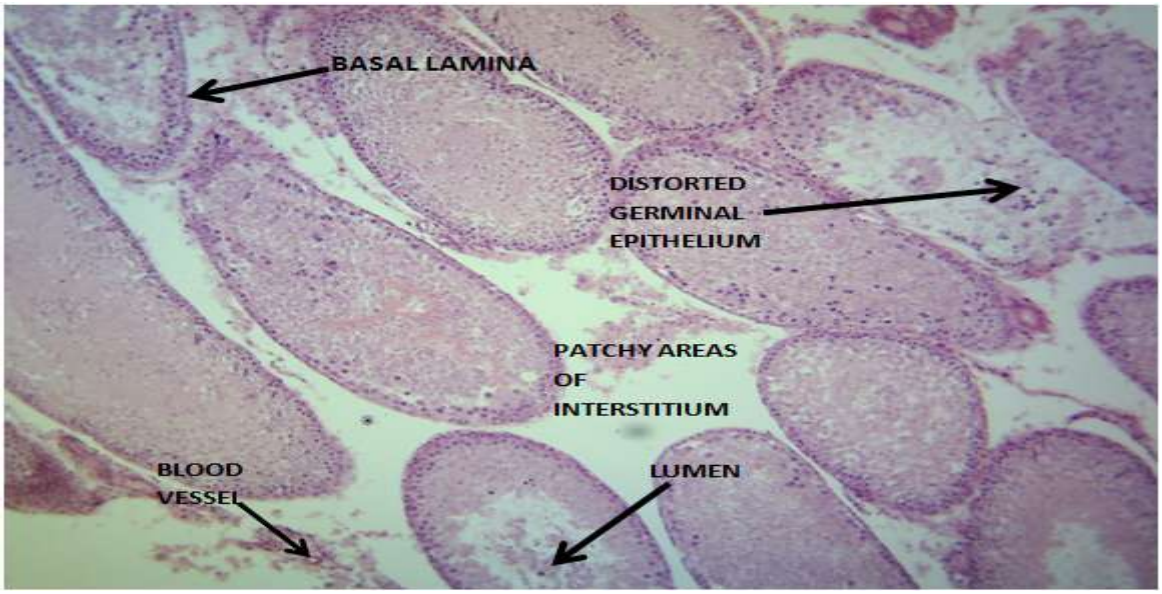

Plate 2A: Section of group B testis treated with 2% BBP. H&E X100

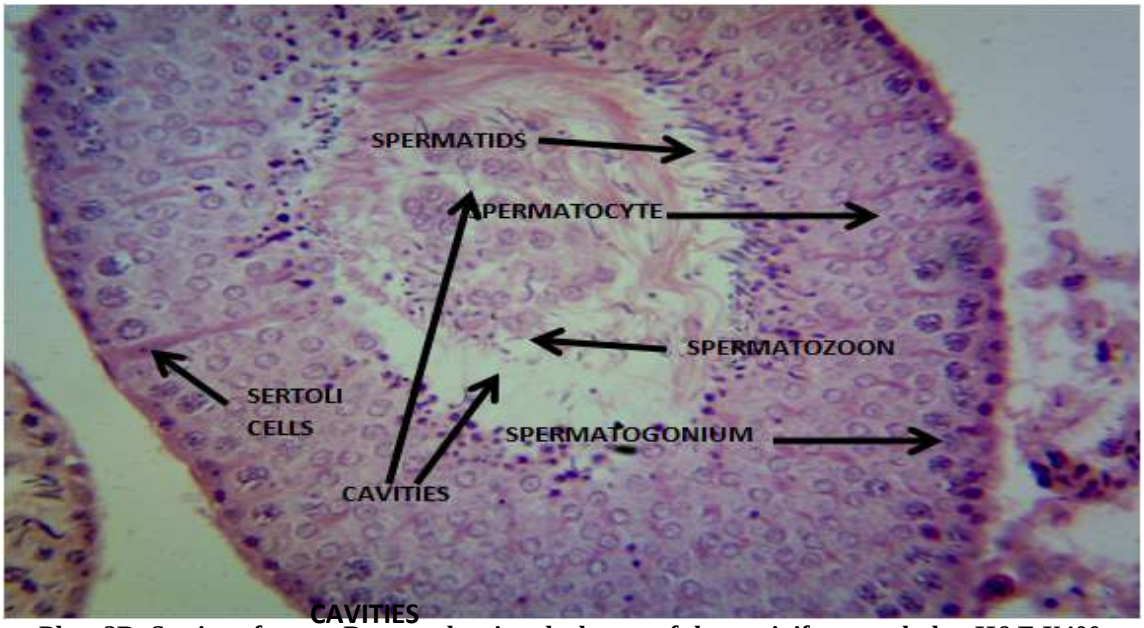

Plate 2B: Section of group B testes showing the lumen of the seminiferous tubules. H&E X400

3.3 ***Testicular histology of rats treated with 4% BBP***

Treatment with 4% BBP caused increase in the number of seminiferous tubules which were also densely populated depicting high proliferative activity. 95% of the seminiferous tubules showed complete absence of lumen due to significant increase in the number of sperm cells compared with the control. The arrow X shows high density area of interstitial cells within the interstitial space consisting of Leydig cells, small bundles of collagen fibers, macrophages, mast cells and fibroblast, in a loose areolar connective tissue rich in extracellular fluid (ECF). These noticeable features of increased cell activity are hardly observed in the control (Plate 3A). At x400 magnification, the section showed active spermatogenesis. The proliferative activity is confined to the spermatogonia and spermatocytes near the base. The continual formation of new generation of cells in this region displaces the more advanced cells to higher levels until as mature sperm; they come to border directly upon the lumen. The high activity at the lumen of the seminiferous tubule is due to increased presence of spermatids at the periphery of the lumen and multiple strands of fibrils of sertoli cells. The peripheral arrangements of the spermatids with their heads embedded in the strands of sertoli cells are poorly maintained (plate 3B).

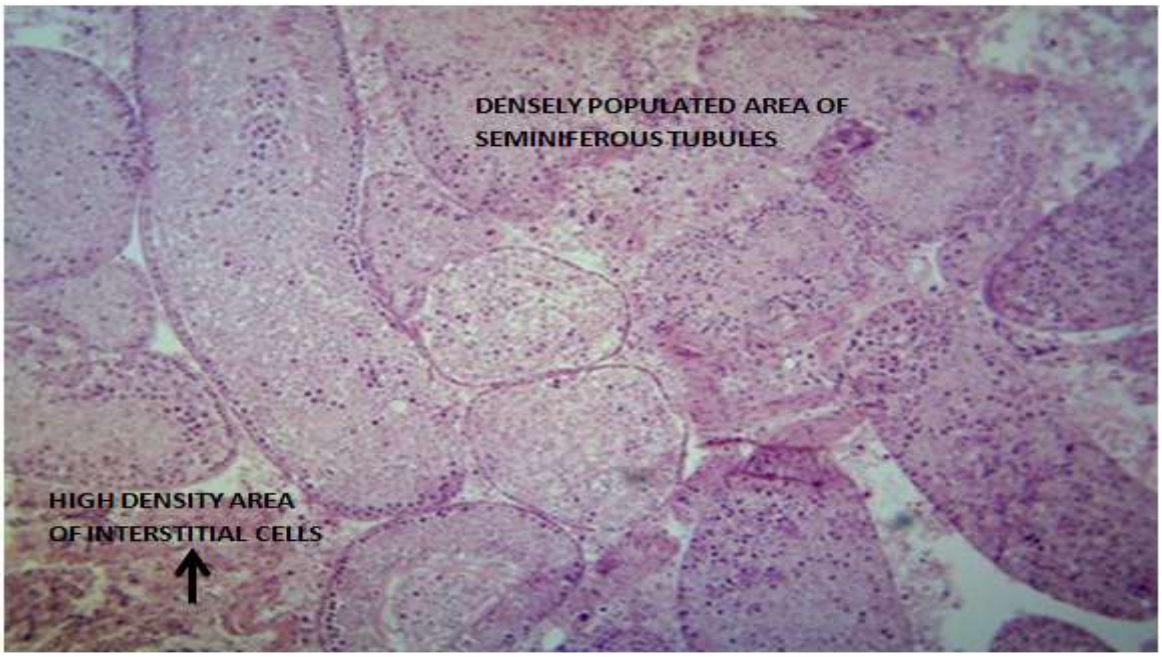

Plate 3A: Section of group C testis treated with 4% BBP. H&E X100

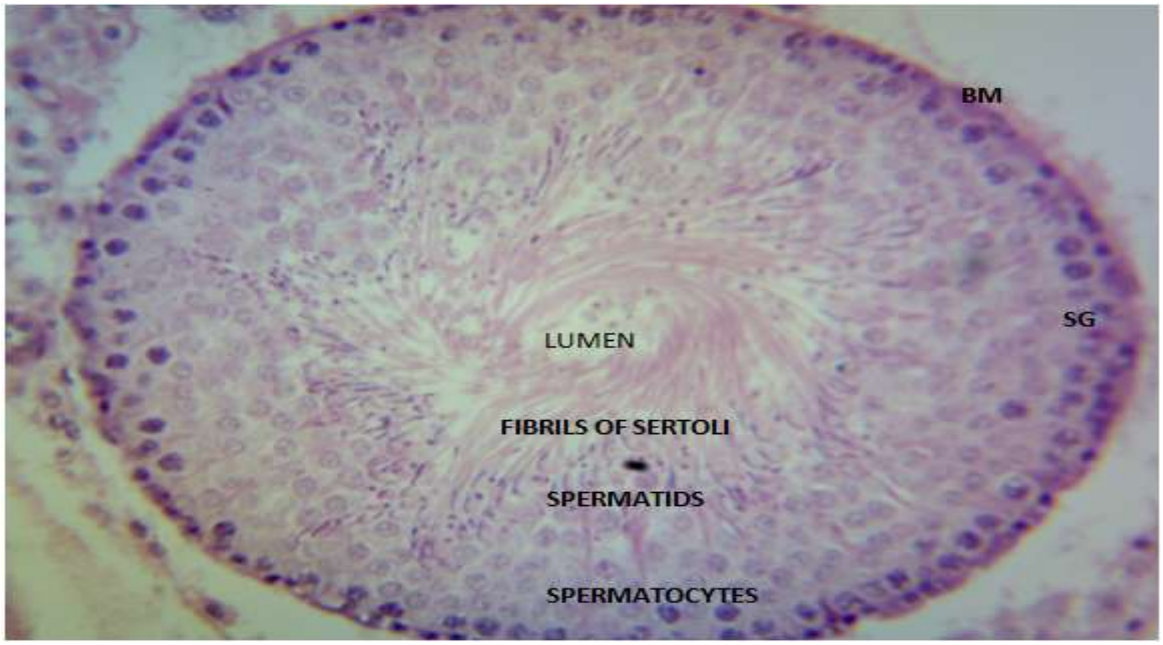

Plate 3B: Section of group C testes showing the lumen of the seminiferous tubules. H&E X400

3.4 ***Testicular histology of rats treated with 6% BBP***

High densely populated area of seminiferous tubules and compact interstitial spaces were observed with the interstitial cells of Leydig occuring in clusters of varying sizes. 95% of the seminiferous tubules appeared to have no lumen as the lumens are occluded by actively proliferating spermatogonic cells in different stages of differentiation. The compact arrangement of the intertubular spaces is evidence of high proliferative activity on the testis on 6% burantashi compare to control. At x400 total occlusion of the tubule by different spermatogenic cell types and sertoli cells in high aggressive manner were observed. Also evident is total loss of orderly arrangement of the germ cells, with aggressive invasion of the innermost layer of the germinal epithelium by spermatids when compared with the peripheral arrangement in the lumen of the seminiferous tubule in the control. The normal close association between Sertoli cells and the differentiating progeny of spermatogonia seen in the control (Plate 1B) was lost and there were early signs of derangement of the compact arrangement of the sertoli cells, with presence of few spermatogonia and spermatocytes in the lumen of the tubule.

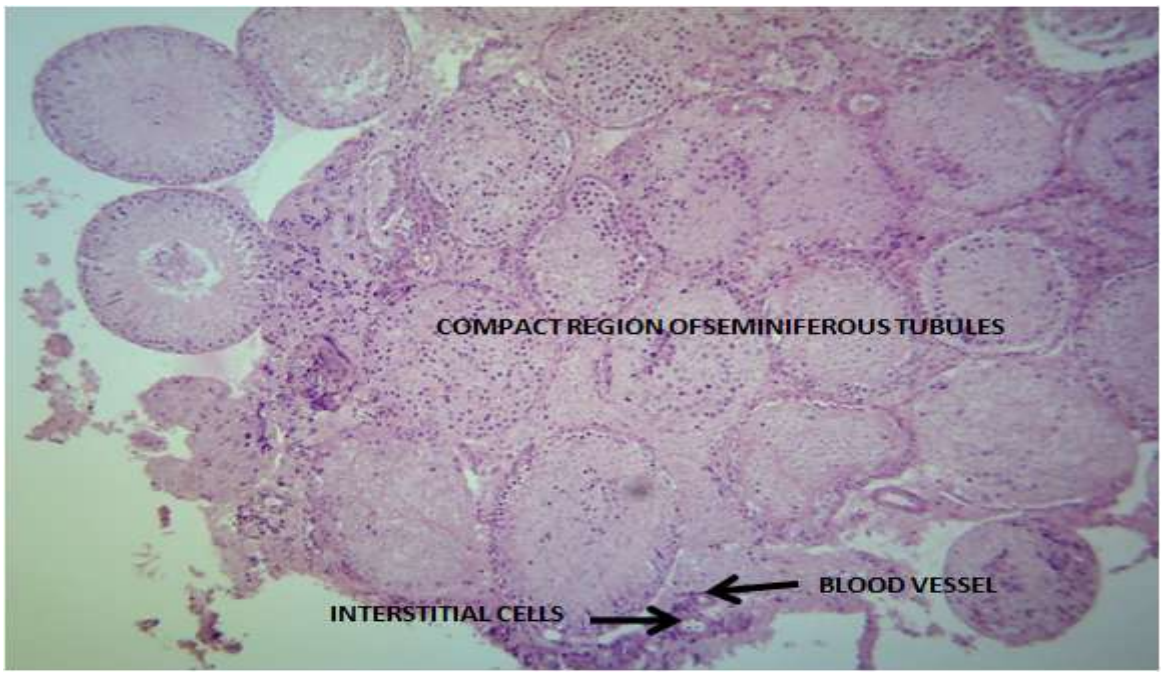

Plate 4A: Section of group D testis treated with 6% BBP. H&E X100

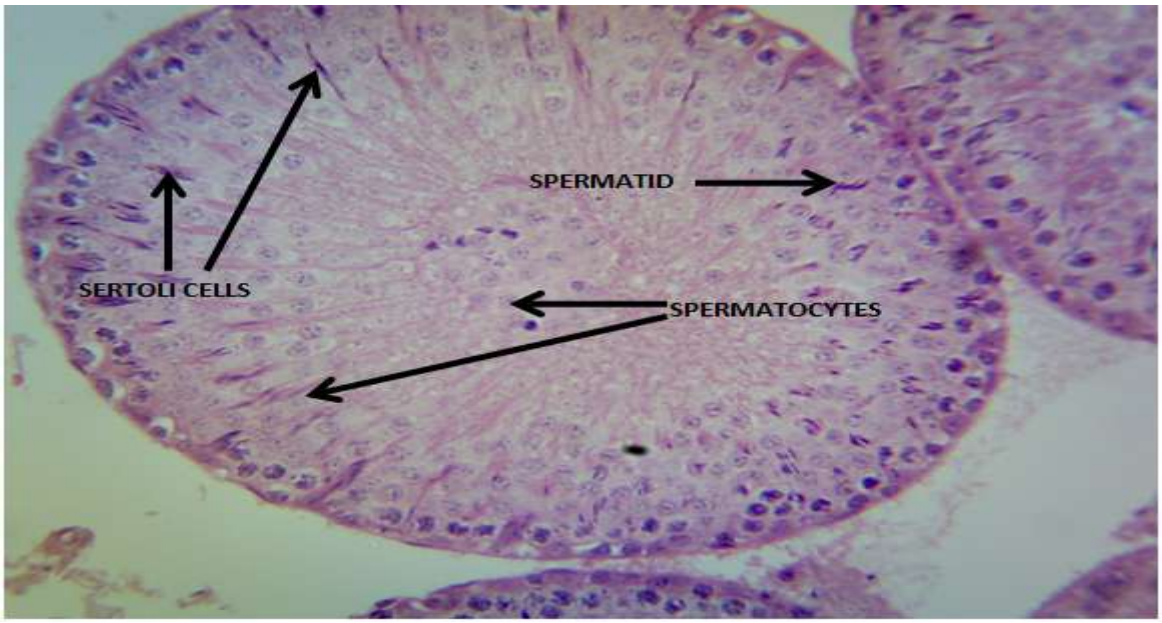

Plate 4B: Section of group D testes showing the lumen of the seminiferous tubules. H&E X400

3.5 ***Testicular histology of rats treated with 8% BBP***

Clusters of seminiferous tubules, less compactly arranged than the previous slide though still densely populated were observed and there was reappearance of intertubular cavities within the interstitial spaces as seen in the control. Some tubules marked **X**were totally degenerated and there was dissociation of germinal epithelia with resultant outpouzing of germ cells into the interstitial spaces. The histological changes range from seminiferous tubular distortion to outright destruction and degeneration of the seminiferous tubules with the boundary tissues of some seminiferous tubules becoming greatly thickened and matted together (Plate 5A). Atx400, total disorderly arrangement of spermatogenic cells within the germinal epithelium was seen with virtually no evidence of lumen due to aggressive invasion of all sorts of germinal cells into the lumen, in contrast to that of 6% BBP treatment, where the invasion was found within the germinal epithelium of their seminiferous tubules. Also the concentric layers of cells of seminiferous tubules, from spermatozoa through spermatids to spermatocytes were destroyed. The arrangement of asingle layer basement membrane with flattened polygonal cells that meet edge to edge to form a continuous epitheliod sheet surrounding the tubule as seen in the control was totally absent following this treatment (Plate 5B).

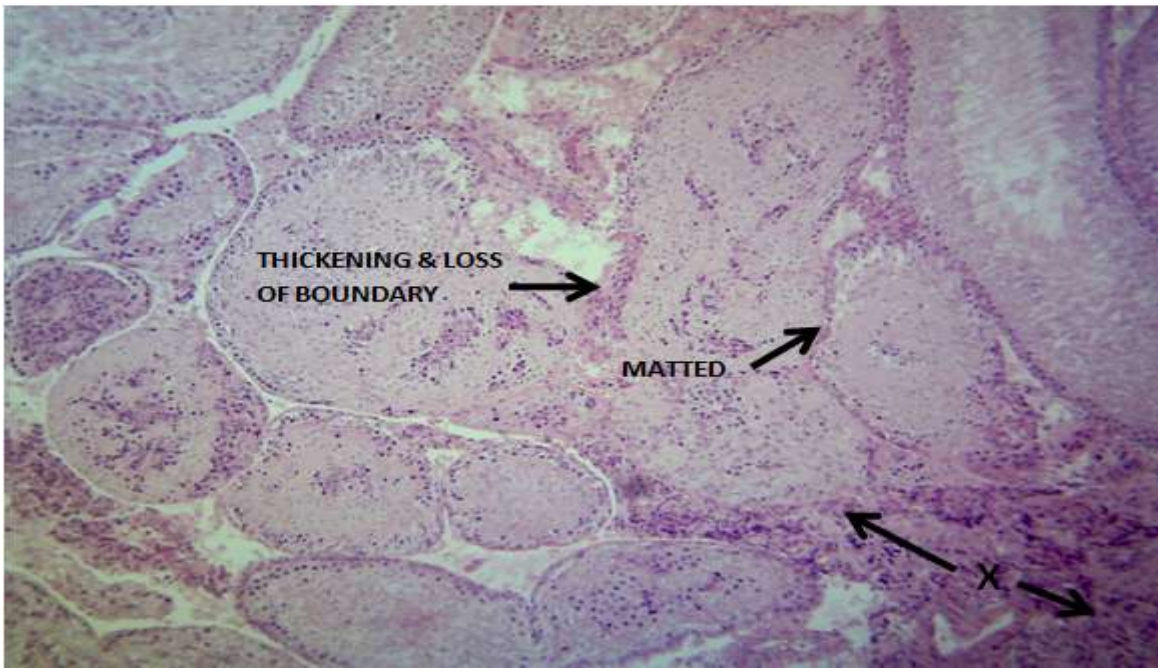

Plate 5A: Section of group E testis treated with 8% BBP. H&E X100

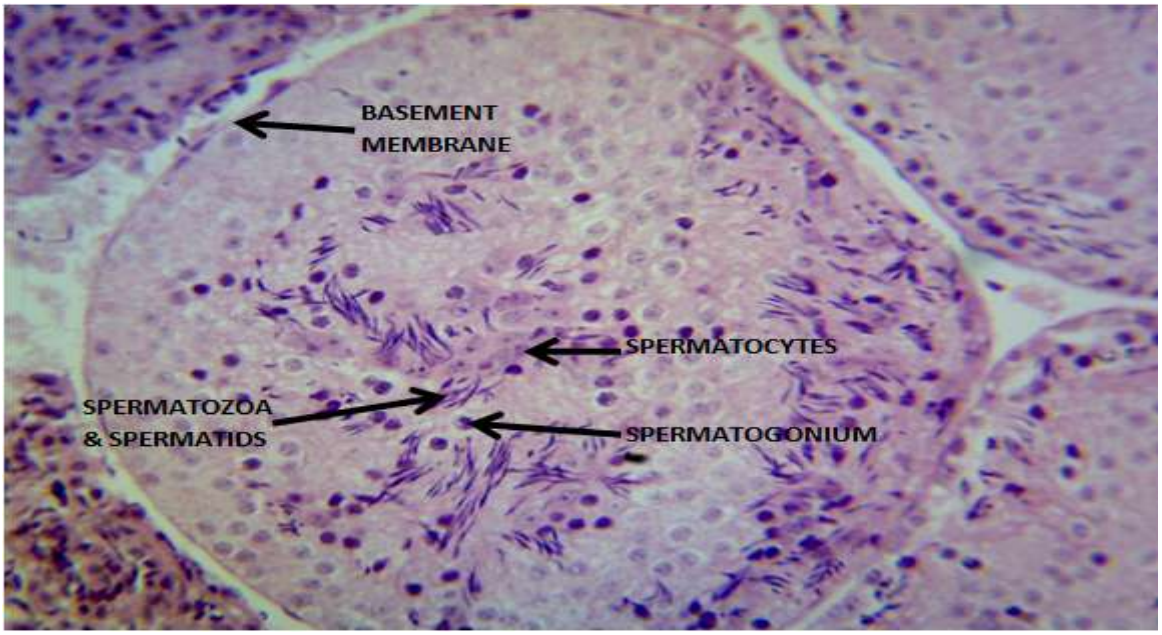

Plate 5B: Section of group E testes showing the lumen of the seminiferous tubules. H&E X400

3.6*Testicular histology of rats treated with 10% BBP*

This level of treatment caused progressive increase in number of seminiferous tubules with very high compact arrangement and denotes high proliferative activity. There was also distinct arrangement of the basal lamina within the interstitial spaces with clear cut boundaries.The obvious histopathological change here when compared to the control is the evidence of active but uncoordinated spermatogenesis with germinal cells dissociation (Plate 6A).At x400 magnification, there was disarray of the fixed population of non-proliferating supporting cells of sertoli cells and those of the non-proliferating, differentiating population of germ cells that should move slowly upward along the sides of the sertoli cells to the free surface with the lumen filled with primitive cells. Spermatocyte generation was distinctively absent in the germinal epithelia but the lumen contained several cell types that resembled either the primary or secondary spermatocytes, spermatogonia and few spermatids. In contrast to the control where there was orderly and peripheral arrangement of the spermatids along the luminal border of the tubule, here there were several spindle-shaped spermatids found in close proximity to the basal lamina in the innermost zone of the germinal epithelium (Plate 6B).

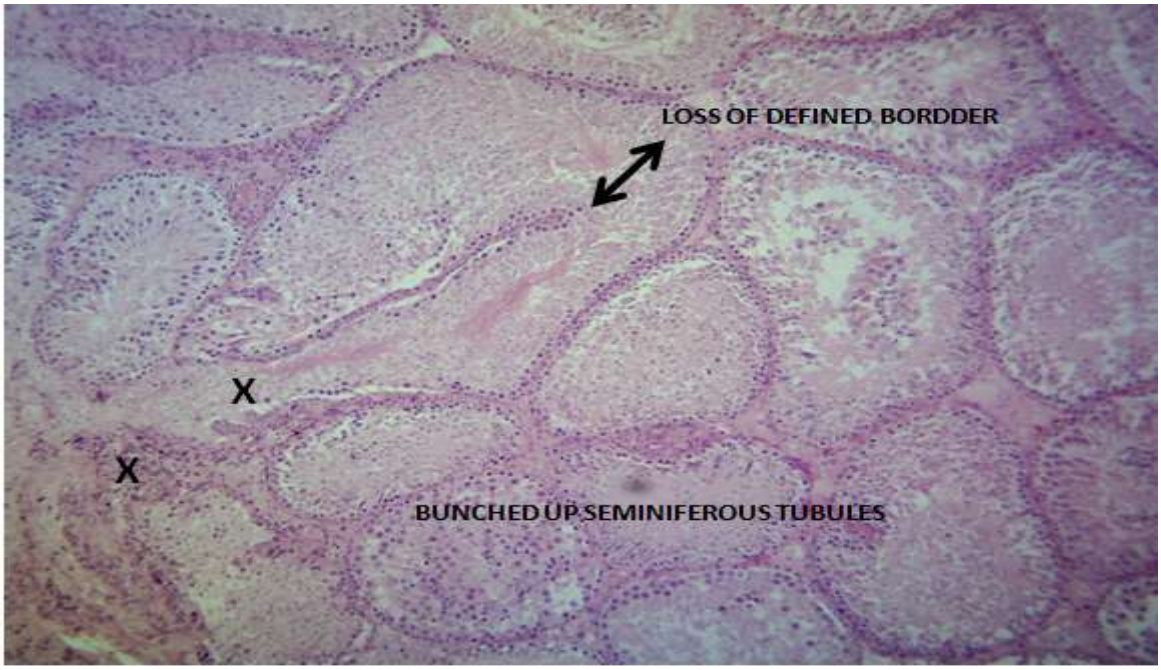

Plate 6A: Section of group F testis treated with 10% BBP. H&E X100

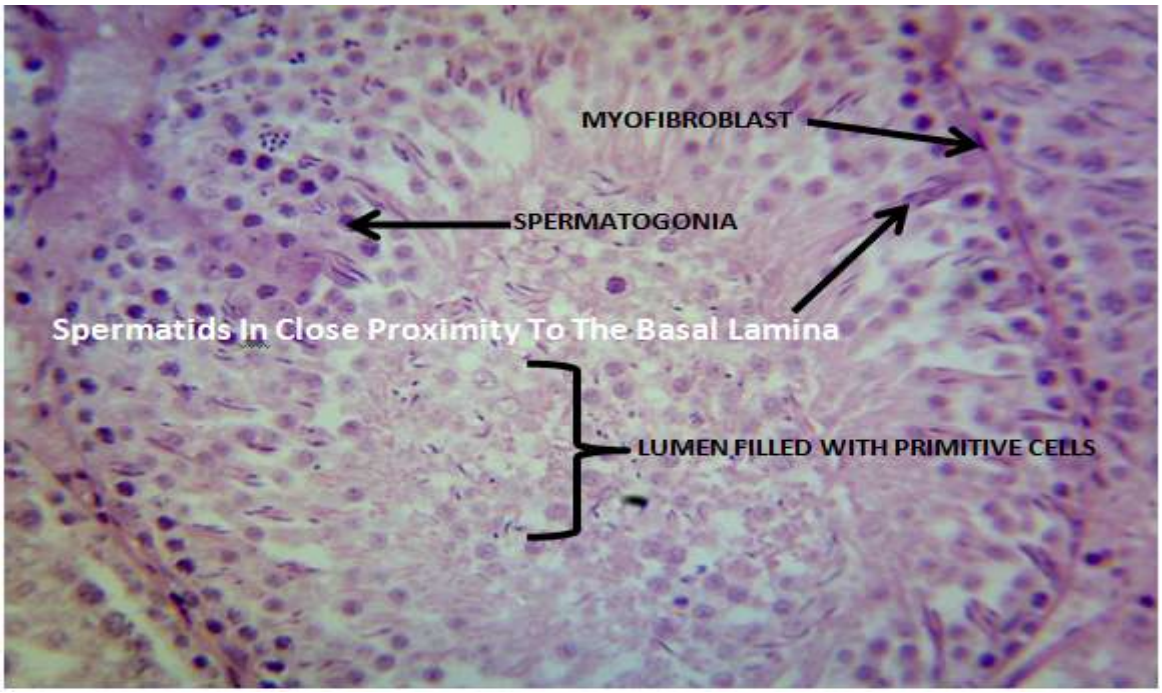

Plate 6B: Section of group F testes showing the lumen of the seminiferous tubules. H&E X400

IV. DISCUSSION

Histological findings following treatment with BBP revealed no significant effect at low doses (2%) as the group and control both the seminiferous tubules and interstitial cells had normal histo-architecture (Plates1 and 2). However, with increasing doses of BBP, a spectrum of dose dependent histological changes were observed, including total loss of orderly arrangement of the germ cells, aggressive invasion of the outermost layer of the germinal epithelium by spermatids, sertoli cells dysfunction, degeneration and dissociation of germinal epithetlia with resultant outpouring of germ cells into the interstitial spaces and invasion of lumens of the seminiferous tubules with several cell types that resembled either the primary and or secondary spermatocytes, spermatogonia and few spermatids. The implication of these results is that with increasing doses of BBP, the lumen of the seminiferous tubules became populated by immature sperm cells and may be due to high cellular activity prompted by the BBP in the experimental rats' diets. These findings agree with our earlier report that BBP treatment caused increased testicular weights, sperm counts, volume, motility and live-dead ratio. The report elucidated the fact that majority of the sperm cells produced following treatment with higher doses of BBP were immature and abnormal and therefore confersno advantage to couples seeking pregnancy (……). Fedder *et al*., (1993) had in similar study observed that the presence of immature sperm cells can be the cause of male sub-fertility in spite of normal counts.

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