



Original Research

TOXICOLOGICAL ASSESSMENT OF METHANOL HYPOCOTYL EXTRACT OF *BORASSUS AETHIOPUM* ON MALE REPRODUCTIVE SYSTEM OF WISTAR RATS

Bature F¹, Oduola T², Alhassan HM³, Adeyeye AA⁴, Dallatu MK¹

¹Department of Chemical Pathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto

²Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Federal University of Health Sciences, Ila-Orangun, Osun State.

³Department of Immunology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto

⁴Department of Theriogenology and Animal Production Department, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto

Correspondence: faridabature10@gmail.com +234 8063400345

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ABSTRACT

Aim: Hypocotyl extract of *Borassus aethiopum* is used in Northern Nigeria as aphrodisiac with no knowledge of its probable adverse effect on male reproductive system, toxic effect of medicinal plant on male reproductive organ in wistar rats have been documented, hence this study aimed at determining possible adverse effects of methanol hypocotyl extract of *Borassus aethiopum* in male Wistar rats.

Method: Nine rats were grouped into 3 were used for acute toxicity study (ATS), group 1(control), received distilled water, group 2 and 3 were given single dose of 2000 and 5000 mg/kg respectively of the extract by oral gavage respectively and observed them for signs of toxicities and mortality for 72 hours, and up to 14 days, and then sacrificed. Twenty-five rats divided into four groups were used for sub-chronic toxicity study (STS); group 1 (control) received distilled water, group 2, 3 and 4 were given oral graded doses of the extract of 500, 1000 and 1500 mg/kg respectively for 28 days, and then sacrificed. Blood samples were collected for reproductive hormonal analysis; testes and seminal vesicle were harvested for histological investigation.

Result: In ATS, the LD₅₀ of the extract is greater than 5000mg/kg, testosterone and progesterone significantly increased in group 3 than the control. In STS, there is significant increase in testosterone, luteinizing and follicle stimulating hormones; distortion of interstitial spaces of testes and epithelial lining damage of acini of seminal vesicle in the groups that received higher doses.

Conclusion: Hypocotyl extract of *Borassus aethiopum* is safe for human consumption from the LD₅₀ result, however; toxicity studies indicated that the dose and duration must be regulated to avoid untoward adverse effects.

Keywords: Toxicity, male reproductive hormones, median lethal dose, *Borassus aethiopum*

INTRODUCTION

Herbal medicine is an old form of health care to humanity and has been used in all cultures throughout history. Traditionalist methodically collected information on herbs and developed well-defined herbal pharmacopoeias [1]. Traditional medicine evolved over centuries depending on local flora, culture, and religion. Medicinal plants constitute an effective source of both traditional and modern medicine. Despite the remarkable progress in synthetic organic medicinal products in the 20th century, over 25% of prescribed medicines in developed countries are derived directly or indirectly from plants [2]. The medicinal properties of plants lie in the composition of bioactive phytochemical constituent that

produce physiological action on the human body [3]. *Borassus aethiopum* belongs to the genus *Borassus*, of the family *Arecaceae*, commonly known as African fan palm. It is a tropical plant found across Africa and used globally for medicinal and non-medicinal purposes [4]. The hypocotyl, locally called ‘*Gazari*’ is seasonally consumed either raw or boiled as food and they claim that it has aphrodisiac properties and contribute also to treatment of men sexual weakness. It is highly rich in minerals (phosphorus, magnesium, calcium, sodium, manganese, zinc, copper, iron, and silver) and other nutritional content (fat, proteins, starch, soluble sugars, vitamins, lignin, and fibers) [5]. The reproductive system represents a highly complex functional system of diverse tissues and signaling pathways that when properly functioning ensures a number of key endpoints, the most important of which are the adequate production of gametes [6].

MATERIAL AND METHODS

Animals

Thirty-five male Wistar rats of weights 150 to 170g were purchased from the animal house, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were housed in a clean metallic cage, placed in a well-ventilated room at a temperature of 26°C to 28°C, photoperiods of 12 hours’ light and 12 hours’ darkness. The rats were given on drinking water and a pellet feeds (Vital feed ®). Regular cleaning of the animal cages was ensured.

Acute Toxicity Study

Median lethal dose (LD₅₀) was performed in accordance with the protocols outlined by the Organisation for Economic Co-operation Development guidelines 423 [7]. Nine male Wistar rats were divided into 3 groups comprising 3 rats per group, with group 1 serving as control group. A single dose of 2000 and 5000mg/kg of the extract was administered by gastric gavage to the rats in groups 2 and 3 respectively using oral cannula, one animal per day starting with 2000mg/kg. The animals were then observed for signs of toxicity and mortality within the first 4 hours, 24 hours, 48 hours, 72 hours and up to 14 days. After 14 days all the rats were fasted overnight, and anaesthetized using chloroform. Blood samples were collected from the animals through cardiac puncture, into clean plain bottle and centrifuged at 4000 rpm for 5 minutes. The serum of each sample was harvested and preserved at -4°C until required for analysis. Testes, and seminal vesicle of the male wistar rats was harvested and preserved in 40% formalin.

Subchronic Toxicity Study

Sub-chronic toxicity study was carried out in accordance with OECD 407 [8] guidelines. Twenty male Wistar rats were divided into 4 groups in separate cages; each comprising 5 animals. Group 1 serve as control group and receive only normal saline. Graded doses of the hypocotyl extract of *B. aethiopum*; 500, 1000 and 1500mg/kg body weight was administered orally to the rats in groups 2, 3, and 4 respectively daily for 28 days. All the rats have free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality as described by Aniagu *et al.* [9]. After 28 days’ period, all the rats were fasted overnight, and anaesthetized using chloroform. Blood samples was collected from the animals through cardiac puncture, into clean plain bottle and centrifuged at 4000 rpm for 5 minutes. The serum obtained was preserved at -4°C until required for analysis. Testes and seminal vesicle of the Wistar rats were harvested and preserved in 10% formalin.

Sample analysis

Samples were analyzed using ACCU-BIND ® ELISA kits for Testosterone, LH, FSH, Progesterone and Prolactin following standard operating procedure.

Ethical Approval

Animal ethical approval was obtained from Faculty of Pharmaceutical science, Usmanu Danfodiyo University Sokoto.

Data Analysis

The data was analyzed using one-way analysis of variance (ANOVA) comparing the mean differences of the biochemical parameters among the groups of statistical package for social Science (SPSS) version 20.0. The result will be presented as mean \pm standard deviation (SD) and $p < 0.05$ will be considered significant.

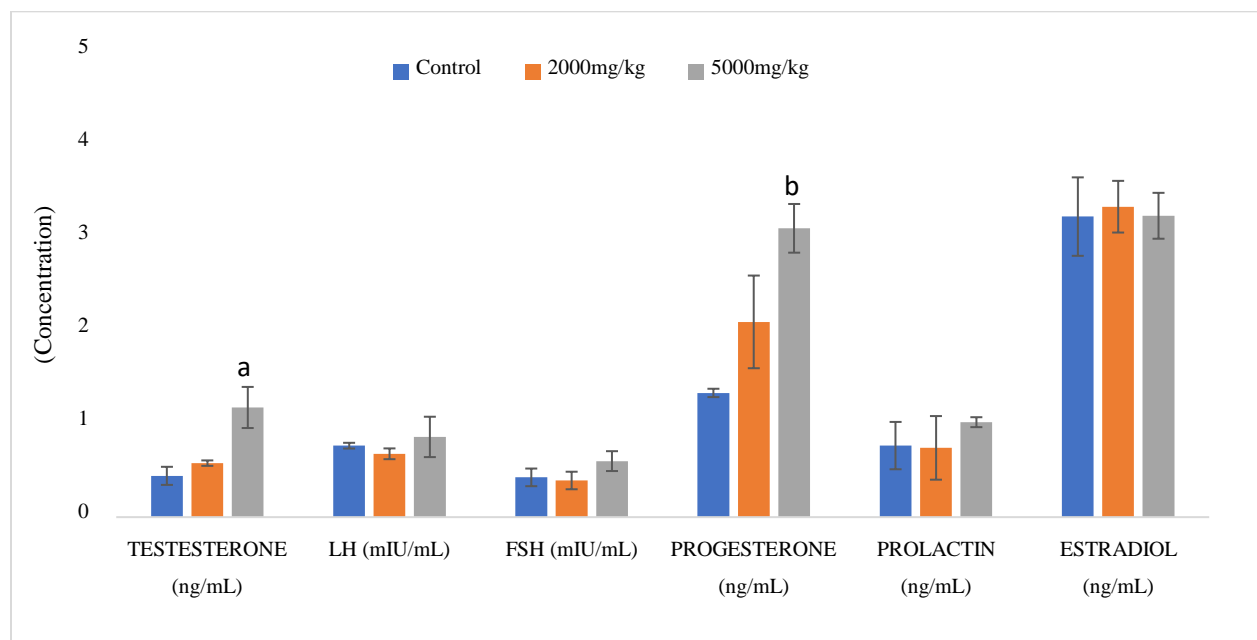
RESULT

Acute Oral Toxicity Study

Table 1.0 shows the result of acute oral toxicity (LD₅₀ determination) in male Wistar rats. There was no mortality for all the rats given 2000mg/kg and 5000mg/kg of the extract.

Table 1.0: Acute oral toxicity study (LD₅₀) of *B. aethiopum* aqueous leaf extract in male Wistar rats.

| Days | Group | Dose (mg/kg) | Observation period | Mortality |
|------|----------------------------|--------------|------------------------|-----------|
| | Control(Group 1) | | | |
| | Distilled water | | | |
| 1 | 1 st rat | 1ml | 24 hours up to 14 days | None |
| 1 | 2 nd rat | 1ml | 24 hours up to 14 days | None |
| 1 | 3 rd rat | 1ml | 24 hours up to 14 days | None |
| | 2000mg/Kg (Group2) | | | |
| 1 | 1 st rat | 2000mg/kg | 24 hours up to 14 days | None |
| 2 | 2 nd rat | 2000mg/kg | 24 hours up to 14 days | None |
| 3 | 3 rd rat | 2000mg/kg | 24 hours up to 14 days | None |
| | 5000mg/kg (Group 3) | | | |
| 4 | 1 st rat | 5000mg/kg | 24 hours up to 14 days | None |
| 5 | 2 nd rat | 5000mg/kg | 24 hours up to 14 days | None |
| 6 | 3 rd rat | 5000mg/kg | 24 hours up to 14 days | None |



Figures 1.0: Mean (+SE) concentration of reproductive hormones profile in male Wistar rats of controls and those rats exposed to graded dose of *B. aethiopum* hypocotyl extract in acute toxicity study. ^aDiffered significantly from the control group ($p=0.023$) and ^b differed significantly from the control group ($p=0.020$).

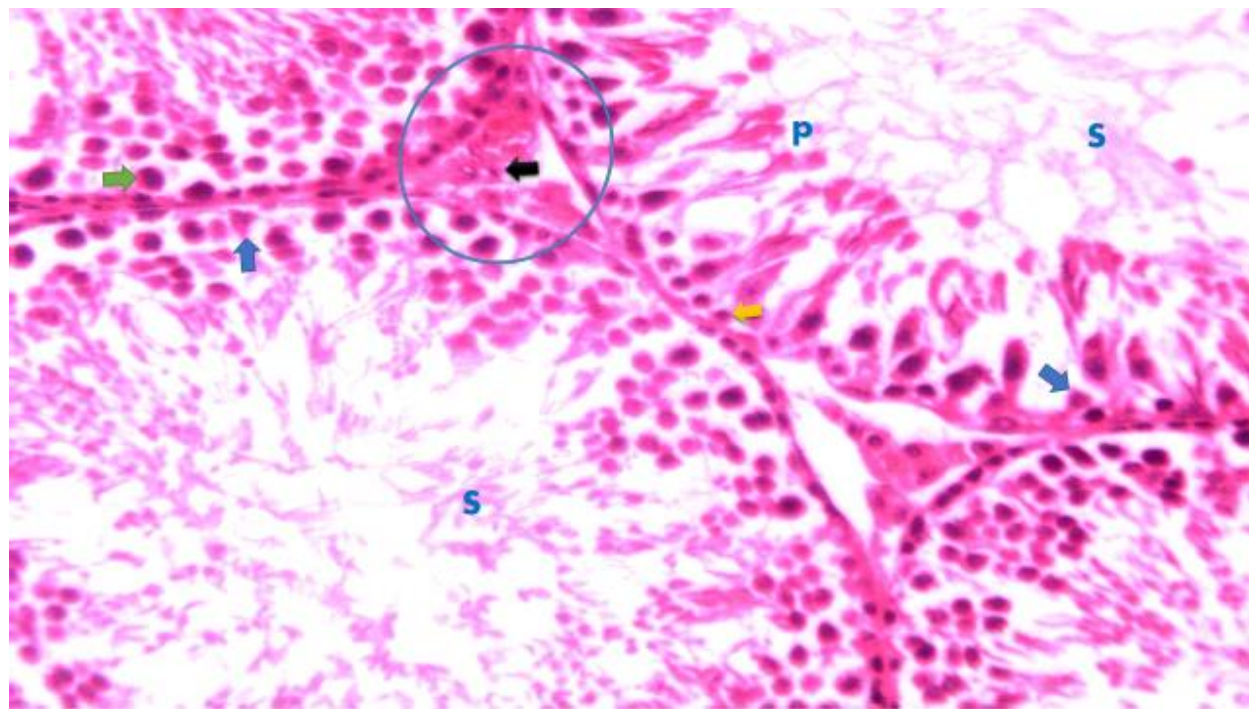


Plate 1.1: Photomicrograph section of testes of Wistar rat of group 1 acute toxicity study. The seminiferous tubules presented the normal histological features of testis showing a well-developed interstitium (circle) between regularly arranged seminiferous tubules on intact basement membrane with lumina filled with sperms. The spermatogonia were appearing as small rounded cells (yellow

arrow), primary spermatocyte with large rounded nuclei (green arrow). Early spermatids were small rounded cells with pale nuclei (P), sertoli cells had triangular and pale nuclei attached to the basement membrane (blue arrows) and late spermatids (mature sperms) (S). The interstitial cells of Leydig are within the interstitium (black arrow). H&E x250

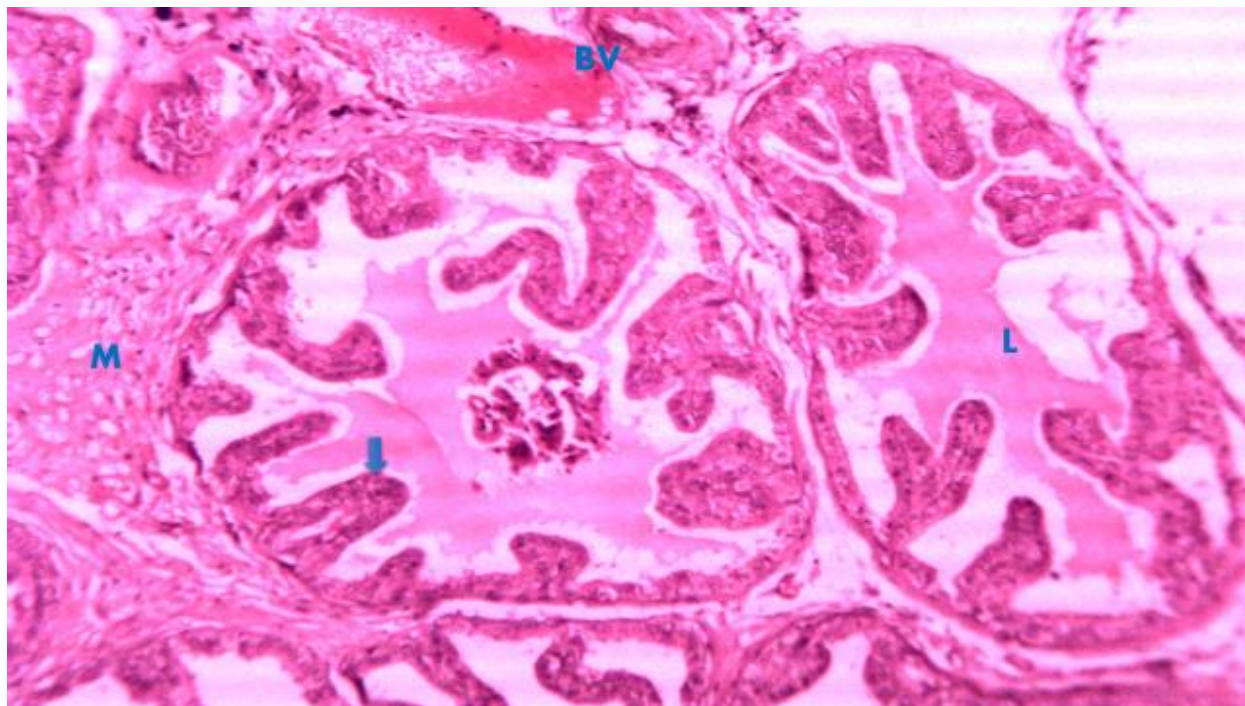


Plate 1.2: Photomicrograph section of seminal vesicle of Wistar rat of group 1 acute toxicity study, showing branched acini (blue arrow) containing smooth muscle tissue (M) in between the branches of the acini with an appearance of a dilated and congested blood vessel (BV). The lumen (L) of the vesicle contains secretions of these acini. H&E x100

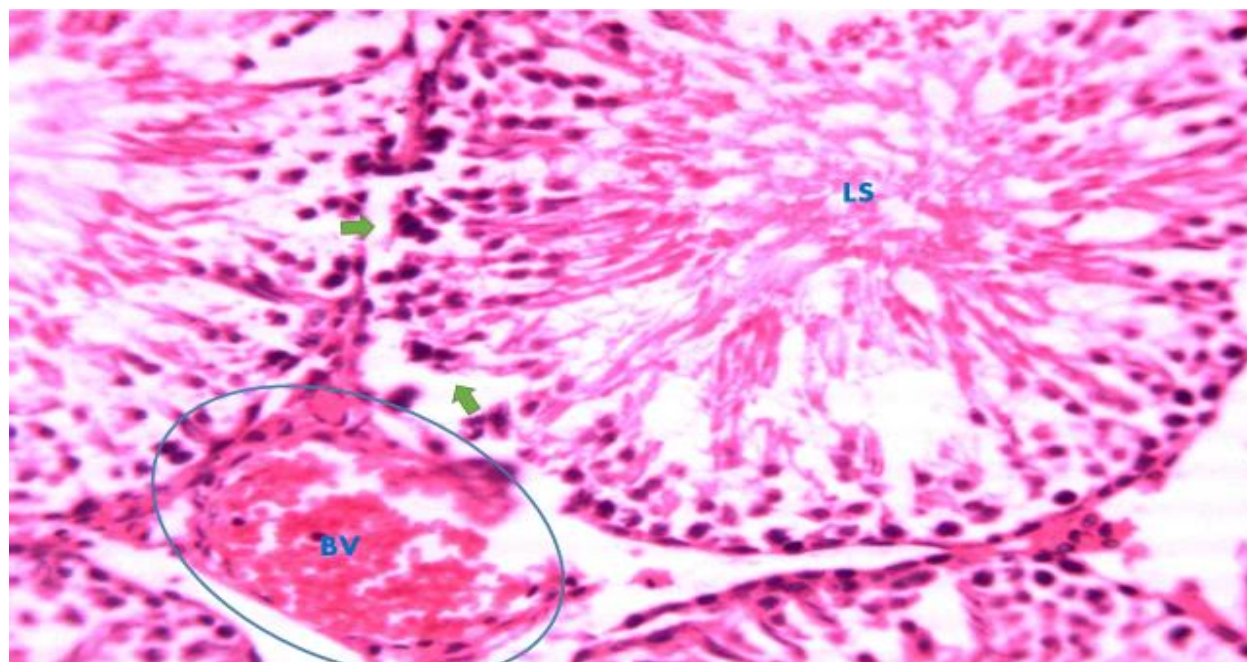


Plate 2.1: Photomicrograph section of testes of Wistar rat of group 2 acute toxicity study, showing widening of the intertubular interstitial spaces (blue circle), with dilated congested blood vessels (BV) surrounded with scanty monocellular inflammatory cells. Wide spaces (green arrow) appeared between the degenerated parts of the germinal epithelium of the basement membrane appeared degenerated with most of the late spermatids appearing intact (LS). H&E x250

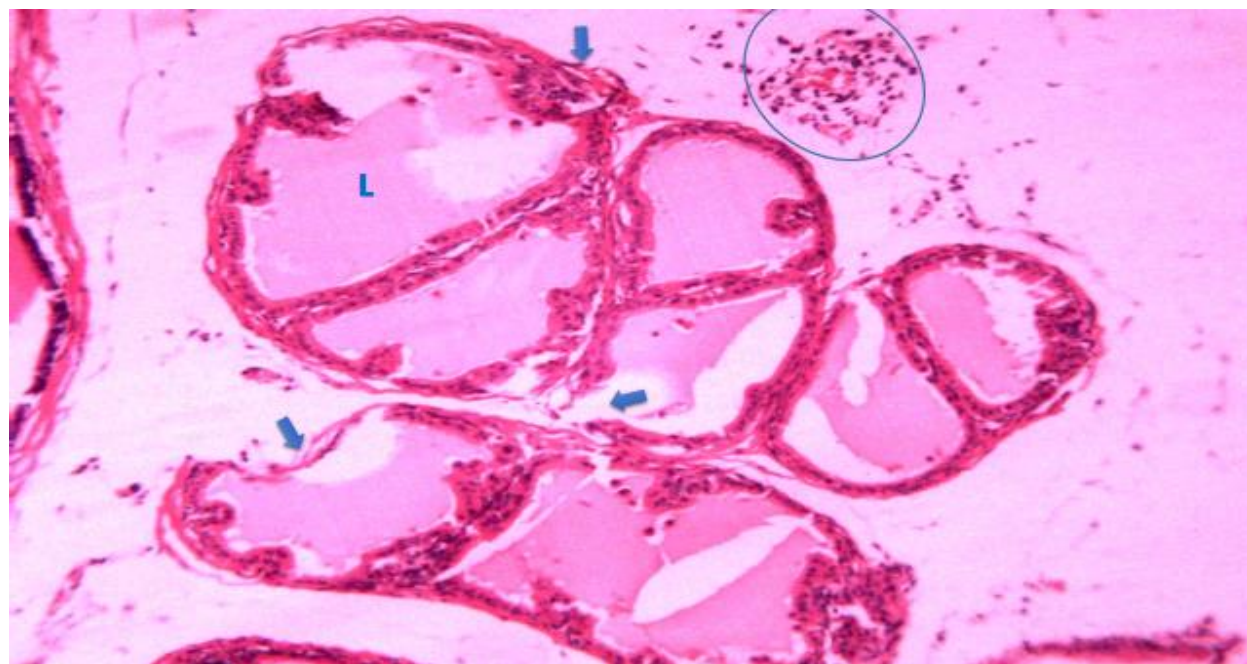


Plate 2.2: Photomicrograph section of seminal vesicle of Wistar rat of group 2 acute toxicity study, showing damaged basal and epithelial lining of the acini (blue arrow) surrounding the pink colored

lumen filled acini secretion (L). There was a notable appearance of perivascular aggregation of inflammatory mononuclear cells (circle). H&E x100

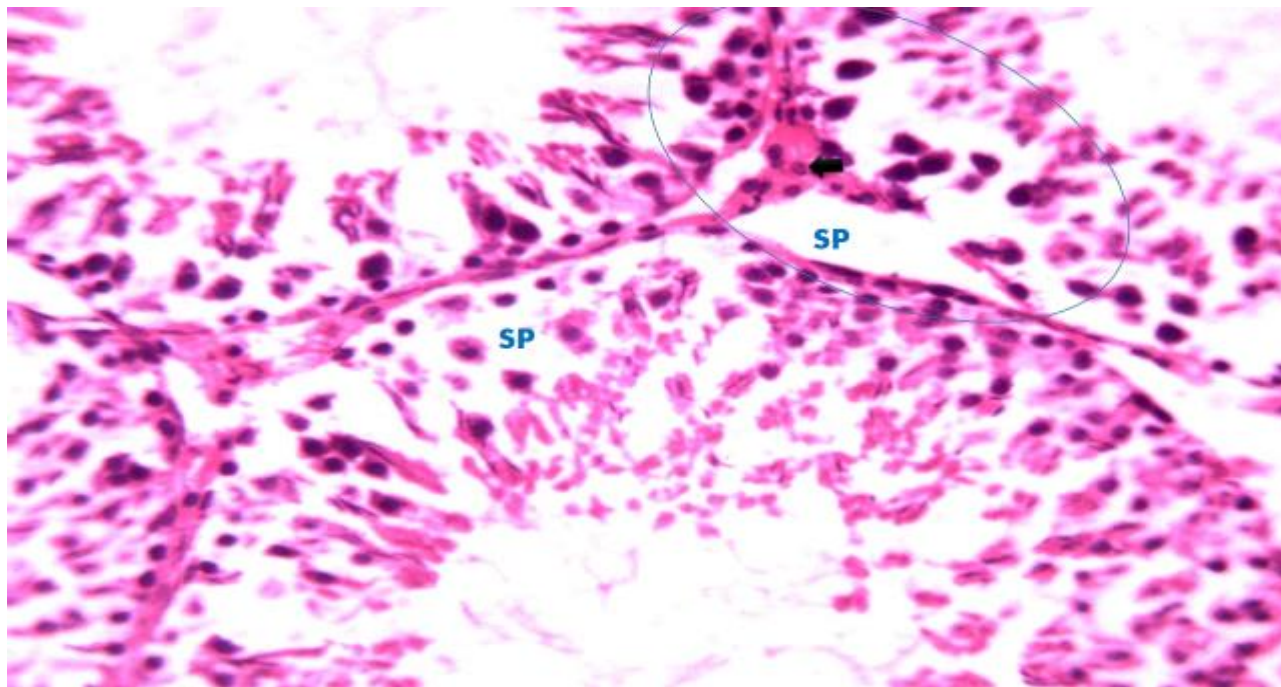


Plate 3.1: Photomicrograph section of testes of Wistar rat of group 3 acute toxicity study, showing distortion of the intertubular interstitial spaces (blue circle), with wide spaces (SP) between the degenerated germinal epithelium and distorted sperm cells. H&E x250

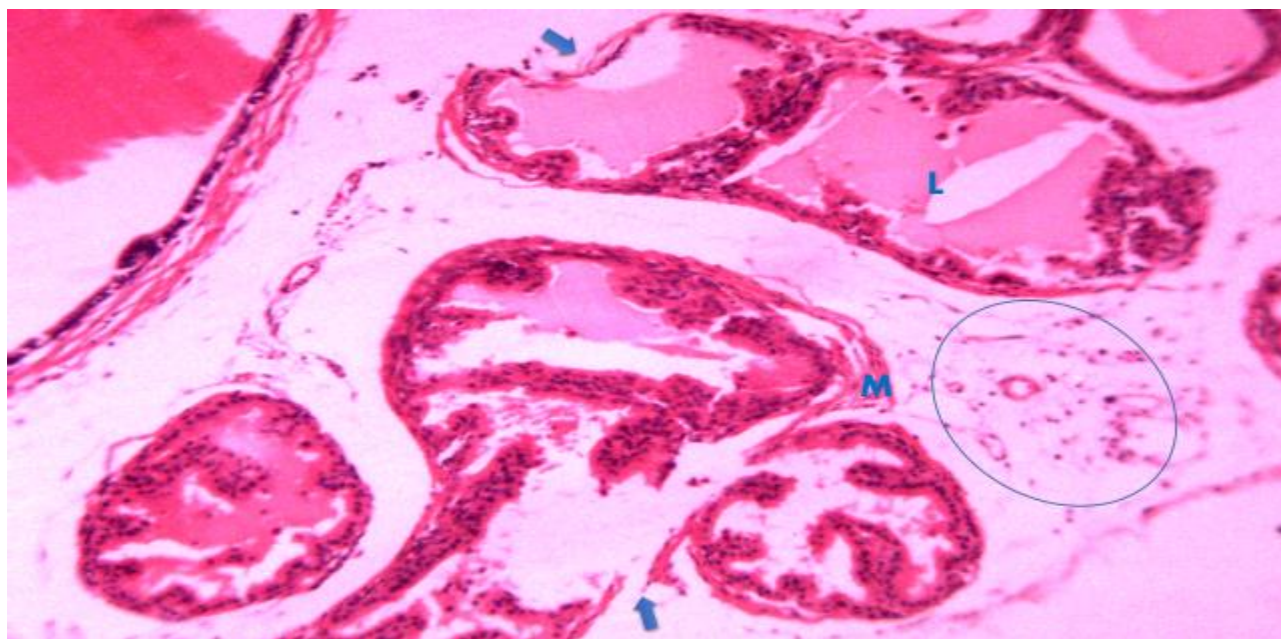
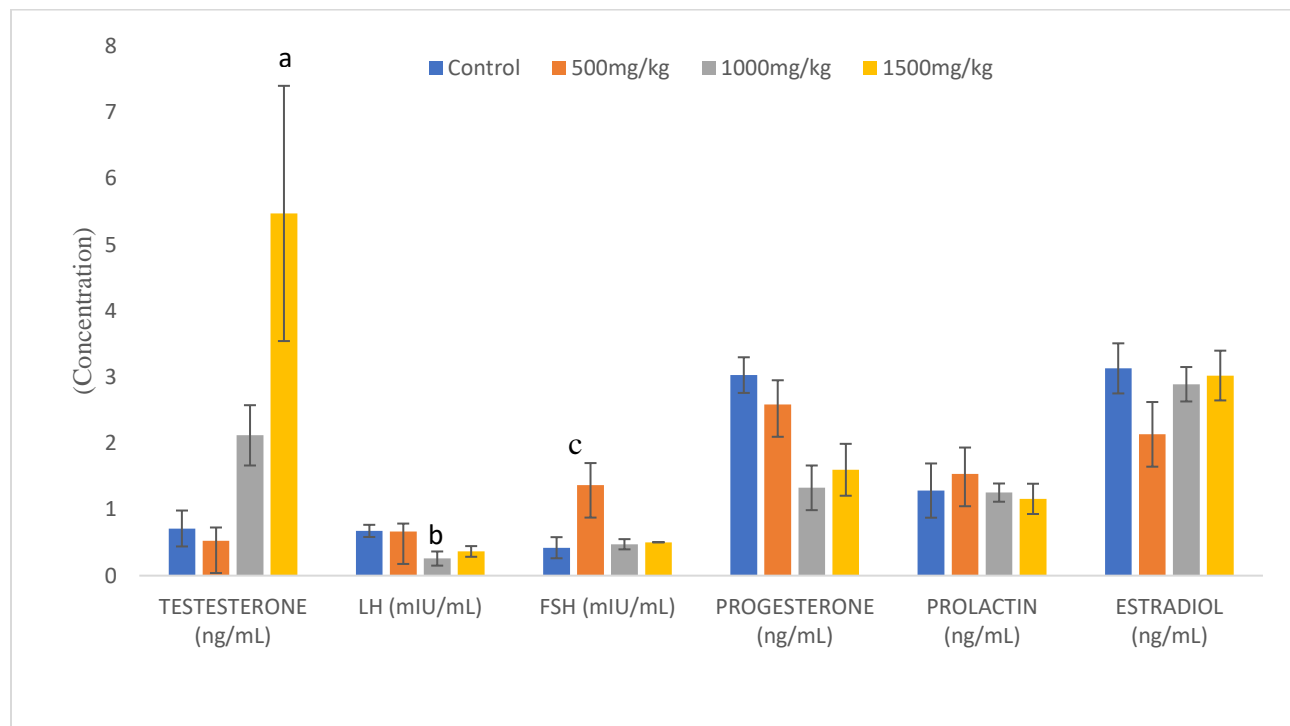


Plate 3.2: Photomicrograph section of seminal vesicle of Wistar rat of group 3 acute toxicity study, showing damaged basal and epithelial lining of the acini (blue arrow) surrounding the pink colored lumen (L). H&E x250

lumen filled acini secretion (L). There was a scanty appearance of perivascular inflammatory mononuclear cells (circle). H&E x100

Subchronic Toxicity Study



Figures 2.0: Mean (+SE) concentration of reproductive hormones profile level in male Wistar rats of controls and those rats exposed to graded doses of hypocotyl extract of *B. aethiopicum* in subchronic toxicity study. ^aDiffered significantly from groups 1 and 2 ($P=0.02$). ^bDiffered significantly from group 1 ($P=0.05$). ^c Differed significantly from groups 1 ($P=0.01$), 3 ($P=0.02$) and 4 ($P=0.03$).

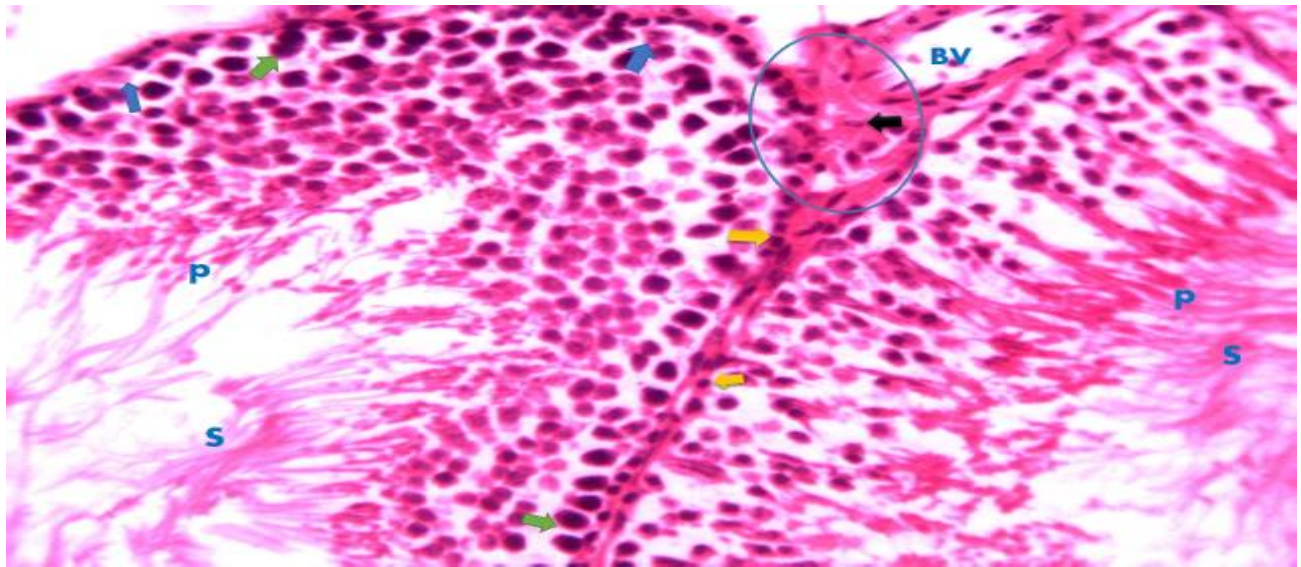


Plate 4.1: Photomicrograph section of testes of Wistar rat of group 1 subchronic toxicity study.

The seminiferous tubules presented the normal histological features of testis showing a well-developed interstitium (circle) between regularly arranged seminiferous tubules on intact basement membrane with lumina filled with sperms. The spermatogonia were appearing as small rounded cells (yellow arrow), primary spermatocyte with large rounded nuclei (green arrow). Early spermatids were small rounded cells with pale nuclei (P), sertoli cells had triangular and pale nuclei attached to the basement membrane (blue arrows) and late spermatids (mature sperms) (S). The interstitial cells of Leydig were within the interstitium (black arrow). H&E x250.

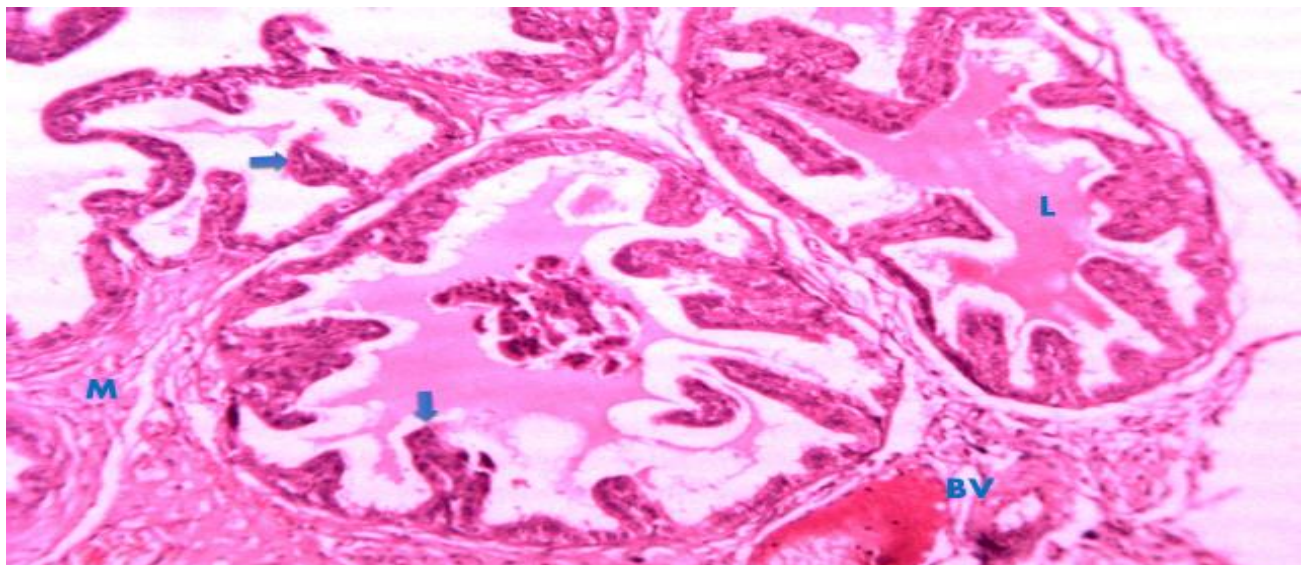


Plate 4.2: Photomicrograph section of seminal vesicle of Wistar rat of control group in subchronic toxicity study, showing branched acini (blue arrow) containing smooth muscle tissue (M) in between the branches of the acini with an appearance of a dilated and congested blood vessel (BV). The lumen (L) of the vesicle contains secretions of these acini. H&E x100

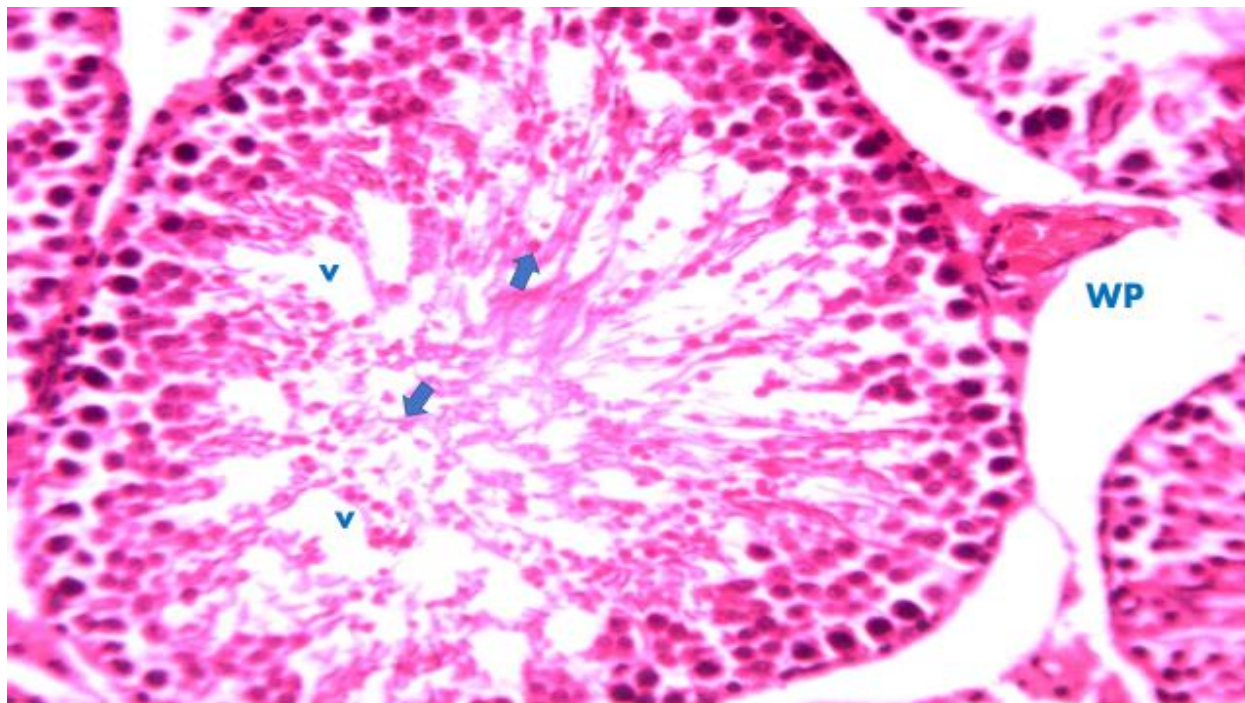


Plate 5.1: Photomicrograph section of testes of Wistar rat of group 2 subchronic toxicity study, showing wide spaces of the interstitium (WS) between seminiferous tubules, vacuolated (v) and disoriented spermatids (blue arrow). H&E x250

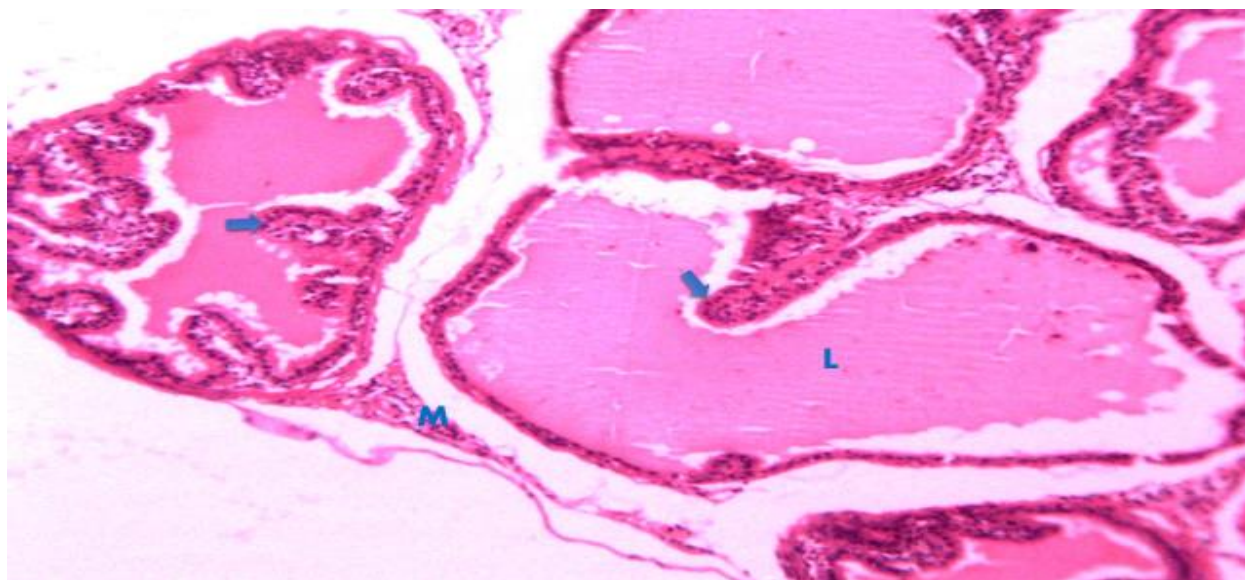


Plate 5.2: Photomicrograph section of seminal vesicle of Wistar rat of group 2 subchronic toxicity study, showing branched acini (blue arrow) containing smooth muscle tissue (M) in between the branches of the acini and pink color stained acini secretion within the lumen (L). H&E x100

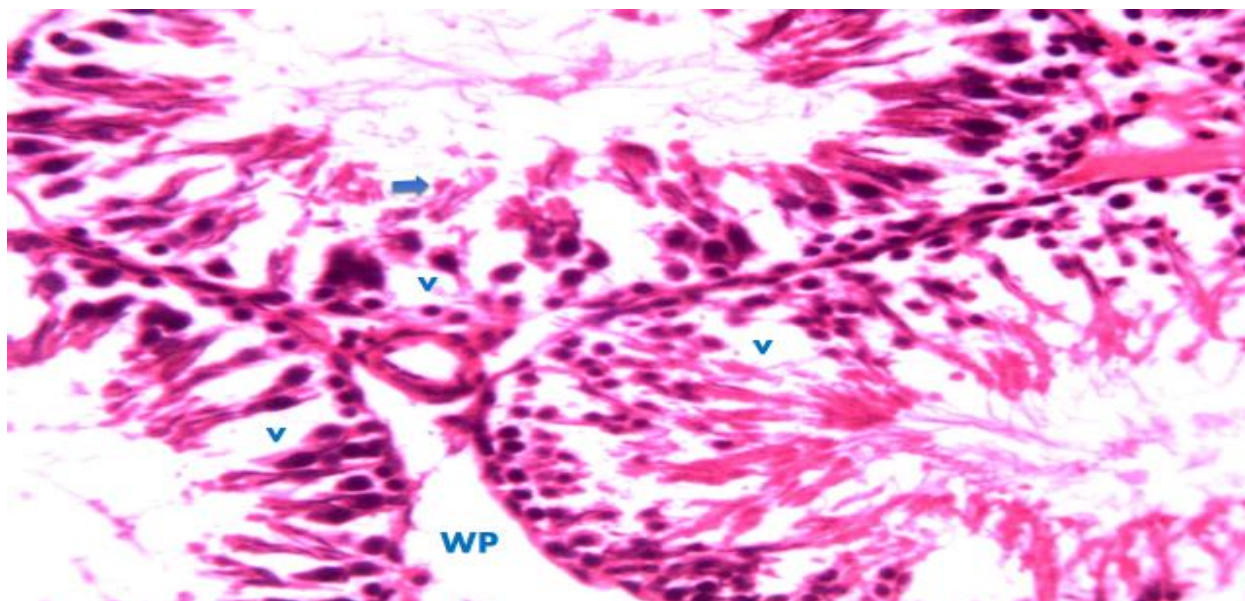


Plate 6.1: Photomicrograph section of testes of Wistar rat of group 3 subchronic toxicity study, showing wide spaces of the interstitium (WS) between seminiferous tubules, vacuolated (v) and damaged spermatids (blue arrow). H&E x250

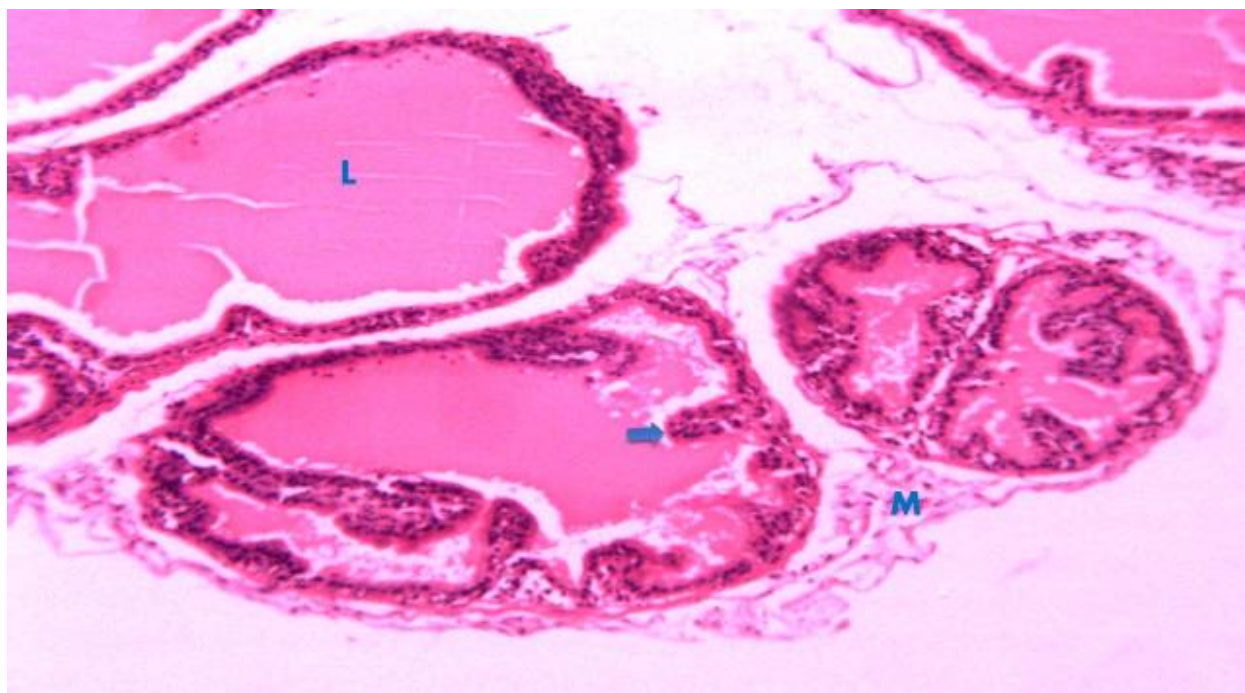


Plate 6.2: Photomicrograph section of seminal vesicle of Wistar rat of group 3 subchronic toxicity study, showing branched acini (blue arrow) containing smooth muscle tissue (M) in between the branches of the acini and pink color stained acini secretion within the lumen (L). H&E x100.

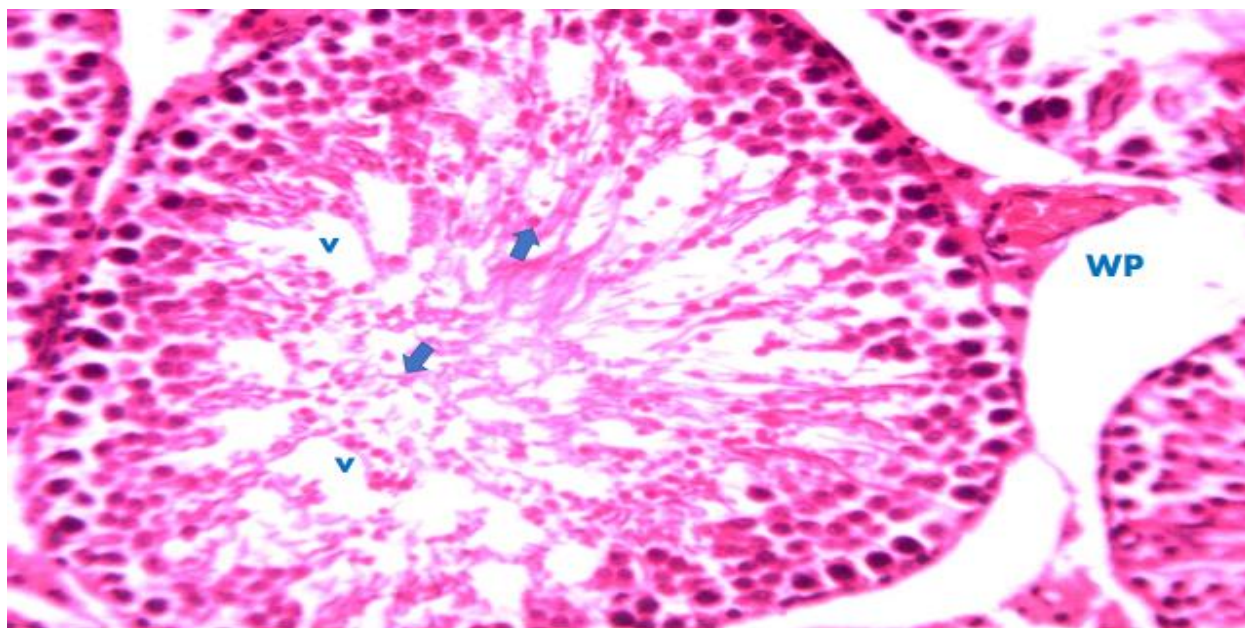


Plate 7.1: Photomicrograph section of testes of Wistar rat of group 4 subchronic toxicity study, showing wide spaces of the interstitium (WS) between seminiferous tubules, vacuolated (v) and damaged spermatids (blue arrow). H&E x250

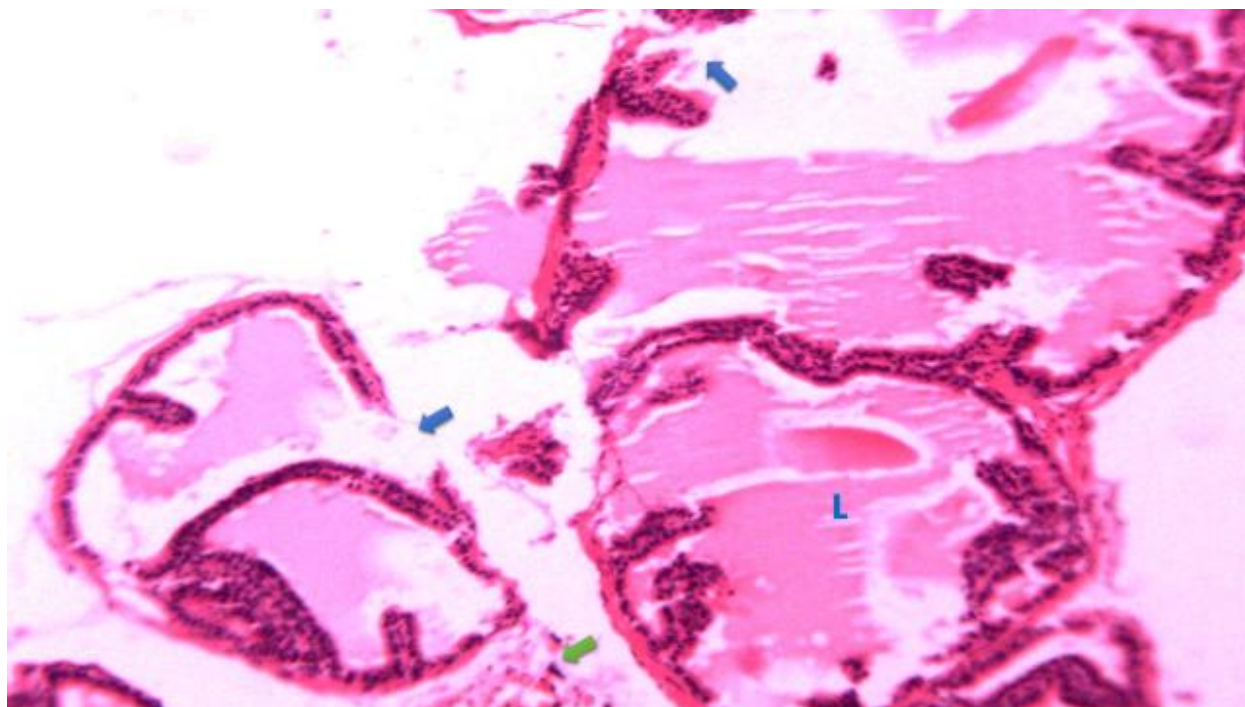


Plate 7.2: Photomicrograph section of seminal vesicle of Wistar rat of group 4 subchronic toxicity study, showing damaged basal and epithelial lining of the acini (blue arrow) surrounding the pink colored lumen filled acini secretion (L). There was a scanty appearance of perivascular inflammatory mononuclear cells (green arrow). H&E x100

Discussion

Borassus aethiopum is a common tropical palm plant with multiple uses and the hypocotyl is known for its use as food and traditional medicine. All parts of this tree such as the stem, roots, petioles, hypocotyls, leaves, final bud, resin, fruits, seeds and sap constitute richness for a rural population. Ethnobotanical survey in northern Nigeria revealed aphrodisiac properties of the hypocotyl and contribute also to the treatment of the men erectile dysfunction [10]. This is similar to the different parts of *Manniophyton fulvum* and *Flacourtia flavescens* used in treatment of sexual weaknesses [11].

This study determined the effects of methanolic hypocotyl extract of *B. aethiopum* on reproductive system in male Wistar rats. In the acute oral toxicity study, for the LD₅₀ there was no death or any sign of toxicity observed within 24 hours and up to 14 days as shown in Table 4.1. This is an indication that the LD₅₀ of methanolic hypocotyl extract is greater than 5000mg/Kg. The finding is similar with the study of Muhammad *et al.* [12] that the LD₅₀ of *B. aethiopum* hypocotyl is greater than 5000mg/Kg which makes it relatively safe. According to Globally Harmonized System of classification and labeling of chemicals, a chemical is not classified as toxic or harmful if the LD₅₀ is greater than 5000 mg/kg [13].

There is significant increase in testosterone levels (P=0.02) when compared with control group in group 3 (5000mg/Kg), which is in line with NIDA [14] that steroids can cause elevation of testosterone, and steroid is one of the phytochemical of the hypocotyl extract.

There is also significant increase of progesterone levels ($P=0.02$) when compared with control group. This finding is in agreement with the study of Chen *et al.* [15] that coumarins might cause increase in progesterone level, and coumarin is a phytochemical of the hypocotyl extract. According to Oettel and Mukhopadhyay [16], progesterone influences spermiogenesis, sperm capacitation/acrosome reaction and testosterone biosynthesis in the Leydig cells. Hence, the likely mild increase in progesterone level due to increase in testosterone level.

The photomicrograph section of testes and seminal vesicle of Wistar rat in group 3 as shown in plate 3.1 and 3.2 showed distortion of the intertubular interstitial spaces and damaged basal and epithelial lining acini respectively, this might be associated with high dose effect of the extract. However, there was no significant difference among the groups in LH, FSH, Estradiol and Prolactin ($P>0.05$) and no alteration in the histology of testicular and seminal vesicle tissues in all the groups as shown in plate 1.1, 1.2, 2.1 and 2.2.

In the subchronic toxicity study, there is significant difference in testosterone levels ($P=0.02$) of groups 2 (500mg/kg) and 4 (1500mg/kg) when compared with control group. According to Gbesso *et al.* [17] the hypocotyl phytochemicals include steroids, coumarins, saponins, tannins, mucilage, anthocyanins gallictannins, combined anthracenes O and C- heterosides. These chemicals are probably the cause of significant increases in testosterone level.

There is significant increase of LH levels in group 3 (1000mg/kg) when compared with control group. According to Mateos *et al.* [18], LH regulates the expression of the enzyme 17β -hydroxysteroid dehydrogenase that is used to convert androstenedione (produced by the testes) to testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis.

There is significant increase of FSH levels between group 2 and 1 ($P=0.01$), group 2 and 3 ($P=0.02$) and group 2 and 4 ($P=0.03$). According to Lohff *et al.* [19], FSH induces sertoli cells to secrete androgen-binding proteins (ABPs) which sustain spermatogenesis, regulated by inhibin's negative feedback mechanism on the anterior pituitary.

The photomicrograph section of testes and seminal vesicle of Wistar rat in group 4 (1500mg/Kg) as shown in plate 7.1 and 7.2 showed distortions in spermatid and damaged basal and epithelial lining acini respectively. However, there is no significant difference among the groups in estradiol and prolactin, and no alteration in the histology of testicular and seminal vesicle tissues in all the groups as shown in plate 4.1, 4.2, 5.1, 5.2, 6.1 and 6.2.

Conclusion

Borassus aethiopum hypocotyl is a nutritional plant commonly distributed in tropical and subtropical regions. From this study, the LD_{50} of the hypocotyl extract is greater than 5000mg/Kg in Wistar rats, which is an indication that it is relatively safe for human consumption. There is significant elevation of testosterone, progesterone, LH, and FSH in higher doses of the extract. However, there is distortion of interstitial spaces, spermatid and damage of acini lining in the histological architecture of testes and seminal vesicle in higher doses of the extract. Therefore, there is need to establish a dose-response relationship of the hypocotyl in order to achieve its efficacy as aphrodisiac and reduce the adverse effects on the male reproductive organs since the damages are dose dependent.

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Conflict of interest

There was no conflict of interest in any form.

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