Original Research Article

Haematological Profile and Some Reproductive Indices of Male Albino Rats Treated with Ethanolic Stem Bark Extract of *Picralima nitida*

ABSTRACT

Aims: To evaluate the effects of graded doses of ethanolic stem bark extract of *Picralima nitida* on haematological profile, some serum reproductive hormones, sperm reserves and testicular histomorphology of male albino rats..
Study design: Thirty male albino rats, weighing between 120 and 200 grams were randomly assigned into six groups of five rats each. Group A received 0.5 ml of distilled water (normal control). Groups B, C and D received 100, 200 and 400 mg/kg BW of the extract respectively. Group E received 2.5 mg/kg BW of $CdCl_2$ (negative control), while Group F received 5 mg/kg BW of Sildenafil (positive control); orally every other day for six weeks.
Place and duration of study: Department of Veterinary Physiology and Pharmacology (Laboratory Animal Unit), University of Nigeria, Nsukka, between June and September, 2015.
Methodology: At the end of the experiment, blood samples were collected for haematology and serology, whereas testicular sections were collected for histopathology following the standards procedures. Data obtained were analyzed by One-way ANOVA.
Result: The result showed that all doses of the extract increased neutrophil count, but decreased *($P < 0.05$)* monocyte and RBC counts; 200 and 400 mg/kg BW of the extract decreased *($P < 0.05$)* PCV values; and only 400 mg/kg of the extract reduced *($P < 0.05$)* HB values. 200 and 400 mg/kg BW of the extract, and 2.5 mg/kg BW of $CdCl_2$ decreased *($P < 0.05$)* serum testosterone and sperm counts. The normal control, 100 mg/kg BW of the extract and 5 mg/kg BW of Sildenafil had normal testicular histoarchitecture; whereas 200 and 400 mg/kg BW of the extract, and 2.5 mg/kg BW of $CdCl_2$ had slight, moderate and severe degeneration of the germinal epithelium and interstitium, with depletion of sperm cells in the lumen of the seminiferous tubules respectively.
Conclusion: This study revealed that treatment with ethanolic stem bark extract of *Picralima nitida* above 100 mg/kg BW adversely affected haematological and reproductive parameters in male albino rats.

Key words: Picralima nitida, Haematology, Fertility, Male Rats

1. INTRODUCTION

Infertility is a widespread health challenge [1], faced by approximately 48.5 million couples worldwide [2, 3]. Male infertility or impotence in particular is of global population health concern in recent times. Statistics has shown that not less than 30 million men worldwide are infertile with the highest rates in Africa and Eastern Europe. More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or combination of both [3]. Routinely, testicular and epididymal sperm reserves, histomorphologic features and serum hormonal assay are among the parameters reported as basis for the assessment of fertility in males [4, 5]. The management options available for the treatment of infertility in males include the use of drugs, both synthetic and natural, as well as various surgical procedures [6].

Medicinal plants have a long folkloric reputation in the management of infertility and improvement of fertility in both males and females. This is due to the fertility-enhancing properties and aphrodisiac qualities inherent in some medicinal plants [7, 8].The use of plant extracts as fertility enhancer in both man and animals is now on the increase because of the shifting of attention from synthetic drugs to natural plant products [9]. Plant that were once considered of insignificant are now being investigated, evaluated and developed into cheaper therapeutic preparations with little or no side effects [10]. A review of ethno-medicinal plants employed in the treatment of male infertility reported 84 plant extracts from which 21 active ingredients have been isolated [11].

Picralima nitida (Stapf), being a representative candidate of the family Apocynaceae, is a rain forest hairless shrub or tree of *3-10 m* high and 60 cm in diameter, with white latex in all parts. Its distribution is confined to Africa, occurring in African forest region, from Ivory Coast to Zaire and Uganda. *Picralima nitida* (Stapf) is known as limeme (Congo), Eban, Obero (Gabon), Erin (Yoruba), Osuigwe (Igbo), Bamborutuk, Eban (Cameroon) [12]. Phytochemical screening of the freshly prepared extracts of *Picralima nitida* revealed the presence of alkaloids, flavonoids, saponins, tannins and glycosides [13]. The stem bark, fruit and seeds of *Picralima nitida* also known as "Akuamma" contain as major compounds, the indole alkaloids akuammine, akuammicine, akuammidine, akuammiline, akuammigine, alstonine, pseudo-akuammigine, picraline and picracine [14].

Picralima nitida has diverse applications in West African traditional medicine [15]. Different parts of the plant, such as the bark, fruit, seed, and leaves have been employed for therapeutic purposes. In ethno-medicine, the bark is used to prepare remedies for male sexual impotence. On the other hand, the fruits are used for the management of painful menstruation and disorder of the gastrointestinal tract [12, 16]. Pharmacological investigations have proved that this plant also possesses anti-plasmodial [17], anti-diarrheic [18, 19], anti-diabetic [20], analgesic [21], opioid [22], antimicrobial [23, 24], anti-inflammatory [21, 25], anti-pyretic [26], trypanocidal [27], anti-Shigellosis [28], as well as anti-leishmanial [29] potencies. Despite the vast ethnomedical and scientific reports on the therapeutic efficacy of this plant, detailed toxicological studies and research observations associated this plant with some level of toxicity especially in higher doses and in immune-compromised animal subjects [14, 30, 31].

Even though the stem bark of *Picralima nitida* has folkloric claim of enhancement of male fertility, it is noteworthy that ethno-medicinal products are sometimes exaggerated or based on trial and error. Again, in addition to the curative ability of some plants, they may harbour some toxic principles that negatively impact the anatomy and physiology of the animal subject. Finally, notwithstanding the extensive therapeutic use of *Picralima nitida*, there is limited information on the blood picture of experimental animals subjected to treatment with different extracts made from this plant. This research therefore was designed to evaluate the effect of ethanolic stem bark extract of *Picralima nitida* on the haematological profile, and the reproductive physiology of male albino rats with respect to some serum reproductive hormones, sperm counts and testicular histoarchitecture.

2. MATERIAL AND METHODS

2.1 Plant collection, Identification and Extraction

Fresh sample of the stem bark of *Picralima nitida* were collected from Okija, Ihiala Local Government Area of Anambra State by a local herbalist who planted the tree in his compound. Botanical identification was performed with the aid of the freshly collected stem bark and leaves by a Taxonomist at the International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka, where a voucher number INTERCEDD/32 has already been designated for *Picralima nitida* (Stapf). The fresh barks of *Picralima nitida* were dried under shade and subsequently ground into a fine powder using an electric grinding machine. Cold extraction of the powdered stem bark was performed using 30% ethanol with intermittent shaking at intervals of 2 hours for 72 hours. The extract was filtered using Wattman number 1 filter paper and concentrated to dryness.

The percentage yield of the extract was determined as follows $\frac{w^1}{w} \times \frac{100}{1}$

Where w^1 = weight of concentrated extract

w = weight of dry pulverized stem bark

The extract was preserved at 4°C throughout the experiment.

2.2 Experimental Animals

A total of thirty male albino rats, 13-15 weeks of age, weighing between 120-200 grams were used for this study. The animals were procured from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were housed in clean metal cages at room temperature of 27-32°C and kept at Laboratory Animal Unit of Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary

Medicine, University of Nigeria, Nsukka. Prior to the commencement of the experiments, the animals were acclimatized for a period of 2 weeks. All animals were fed *ad libitum* on a standard commercial grower feed with 15 % crude protein (Top feeds®, Eastern Premier feed mills Ltd) and clean drinking water. The animals were maintained under a cycle of 12 hours light and 12 hours of darkness daily throughout the period of experiment. The standard institutional and national guidelines for the care and use of laboratory animals for experimental purposes were strictly adhered to [32]. The study was approved by an institutional review and ethics committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

2.3 Experimental Design

The thirty male rats were assigned into six groups of five rats each and orally treated every other day for forty-two days (6 weeks) as follows:
Group A rats were given 0.5 ml of distilled water (normal control). Rats in Groups B, C and D were treated with 100 mg/kg body weight (BW), 200 mg/kg BW and 400 mg/kg BW of the ethanolic stem bark extract of *Picralima nitida* dissolved in distilled water respectively. Group 5 rats were treated with 2.5 mg/kg BW of cadmium chloride (negative control), while Group 6 rats were treated with 5 mg/kg BW of sildenafil citrate (positive control).

2.4 Haematology

Blood samples were collected from the animals using orbital techniques for haematology. The blood samples were collected with the aid of capillary tubes from the retro bulbar plexus of the median canthus of the eyes of the rats into EDTA-contained plastic bijoux bottles for haematology. The blood samples were analyzed for the Packed Cell Volume (PCV), Erythrocyte count (RBC), Haemoglobin concentration (HB), Total White Blood Cells count (WBC) and Differential White Blood Cells count

2.4.1 Determination of Packed Cell Volume (PCV)

Micro haematocrit method [33]
Procedure:
The capillary tubes were filled with the blood samples up to three quarter level, via capillary action. One end of the tubes was sealed using plasticine and placed in a Micro haematocrit centrifuge. These were centrifuged at 11,000 revolutions per minute for 5 minutes using a micro haematocrit centrifuge. The PCV was then read as a percentage, using the Micro haematocrit reader [34].

2.4.2 Erythrocytes Count

Haemocytometer method [33]
Procedure:
This was done using the standard method described by Coles [34]. 0.02ml of blood sample drawn with a micropipette was mixed with 4ml of erythrocyte diluting fluid in a test tube. A drop of the mixture was used to charge the Neubauer chamber. Then the erythrocytes were counted under the microscope with x40 objective, using the tally counter. Erythrocytes were counted in the five squares, four at the edges and the centre square. A factor of 10,000 was used to multiply the number obtained from the count, giving the absolute count of erythrocytes per microliter of blood.

2.4.3 Determination of Haemoglobin Concentration

Cyanomethaemoglobin method [35].
Procedure:
The haemoglobin concentration of the blood samples was determined by the Cyanomethaemoglobin method [35]. 5 ml of Drabkin's haemoglobin reagent was added to a clean test tube. 0.02ml of the blood sample was then added to the reagent and mixed properly. The mixture was allowed to react for 20 minutes, and the absorbance was read at 540nm wavelength against a reagent blank on a

spectrophotometer. Standards were also prepared as above and also read at 540nm. The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with a calibration factor derived from the absorbance and concentration of the mean of the standards.

2.4.4 Total Leukocyte Count (TLC)

Haemocytometer method [33]
Procedure:
This was also determined using the standard method of Coles [34]. 0.02ml of the blood sample was drawn with the micropipette and diluted with 0.4ml of white blood cell diluting fluid in a test tube. The Neubauer chamber was then charged with a drop of the mixture and viewed with x10 objective of the microscope. White Blood Cells (Leukocytes) were counted in the 4 squares at the edges of the chamber using the tally counter. The number of leukocytes obtained from the counting was multiplied by a factor 0f 50 giving the total number of leukocytes per microliter of blood.

2.4.5 Differential Leukocyte Count (Differential White Blood Cell Count)

Leishmann Technique [33]
Procedure:
The blood sample was shaken gently and a drop of blood was placed on a clean grease-free slide. The drop of blood was carefully smeared on the slide using a cover slip to make a thin smear. The smear was air-dried and thereafter stained by the Leishmann technique using the Leishmann stain. The stained slides were later examined with an immersion objective using a light microscope. 200 cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. Results for each type of white blood cell was expressed as a percentage of the total count and converted to the absolute value per microliter of blood.

2.5 REPRODUCTIVE INDICES

2.5.1 Serum Hormonal assay

Blood samples collected from the medial canthus of the eye for serum hormonal assay were drained into plain plastic bijoux bottles devoid of EDTA and allowed to clot. This was subsequently centrifuged at 3,000 revolutions per minute for 10 minutes. The supernatant was carefully aspirated and used for serology. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured by radioimmunoassay (RIA) using special kits (Radim, Italy) as described in the instructions provided with the kits.

2.5.2 Determination of Testicular Weight

The rats were anaesthetized and the testicles surgically harvested and cut free of fat and extraneous tissues. A digital weighing scale (Metler, Switzerland) was used to determine the testicular weights by placing each testicle on the weighing pan and allowed to stabilize for the accurate reading to be taken.

2.5.3 Determination of Testicular and Cauda Epididymal Sperm Reserve

This was done using the method described by Amann [36]. Under mild anaesthesia, the testicles were harvested. The tail (cauda) of the epididymis or the testis as the case may be was crushed to express the sperm cells. This was washed with 10 ml of normal saline and 0.1ml of the suspension was further diluted with 0.9 ml of white blood cells diluting fluid in a test tube. A drop of the mixture was then used to charge the Neubauer chamber and viewed under the microscope. The numbers of sperm cells were multiplied as shown below to obtain the total number of cells in 10ml of normal saline:
Length of Neubauer chamber – 0.05 mm
Width of Neubauer chamber – 0.05 mm
Depth of Neubauer chamber – 0.1 mm
Area of Neubauer chamber = length x width= 0.05 x 0.05= 0.0025 mm^2

Volume of Neubauer chamber = area x depth= 0.0025 mm^2 x 0.1mm= 0.00025 mm^3 (ml)
Number of squares counted = 64
Total number of cells in Naubauer chamber = 169
64 squares = Y cells
169 squares = (169 ÷ 64) x Y) cells = Z cells
0.00025ml = Z cells
0.1ml = (0.1 ml ÷ 0.00025ml) x Z cells = K cells
10ml = (10 ml ÷ 0.1 ml) x K cells (expressed as $x10^6$)
Where Y, Z, K are imaginary numbers expected from the count and deductions respectively.

2.5.4 Evaluation of Mass activity or Grade of motility

The cauda epididymis was punctured with a pin and the expressed fluid mixed with normal saline. A drop of the specimen was placed on a clean slide, using a pipette with a rubber bulb, and covered with a cover slip. This was then examined under the microscope. The grade of motility or mass activity was then categorized as follows: no movement (0), sluggish (1+), medium (2+), active (3+), rapid (4+) [37].

2.5.5 Determination of Percentage Motility

The cauda epididymis was punctured with a pin and the expressed fluid mixed with normal saline. A drop of the dilute specimen was placed on a clean slide, using a pipette with a rubber bulb, and covered with a cover slip. This was then examined under the microscope using a limited field ocular to determine the number of motile sperm per 100 sperm seen. Two counters were used, one for a total of 100 sperm, the other for non-motile sperm only. The count was repeated and the average of the two counts taken [37].

2.5.6 Differential Sperm cell count

A modified Papanicolaou technic was used to stain the specimen. Smears of fluid expressed from the puncture of the cauda epididymis were made and dropped wet into the alcohol-acetic acid fixation solution. 100 stained sperm were differentiated, using the oil-immersion objective (X100) and percentage were taken for the normal, immature and teratoid forms [37].

2.5.7 Histopathology

Immediate collection of the testicles was made following mild anaesthesia. Tissue sections of about 3–5 mm thick were fixed for about 12 h in Bouin's fixative (75 ml saturated aqueous of picric acid, 25 ml of formalin (40 % formaldehyde and few drops of acetic acid). At the expiration of the 12 h, the tissues were successively dehydrated in ascending grades of alcohol (70, 80, 90 and 100 %) at the interval of 90 min each. Thereafter, the tissues were cleared in xylene twice for 90 min each. After clearing, the tissues were transferred into the infiltrating chambers I and II, containing molten paraffin wax, for 90 min. After this stage, the tissues were embedded in a fresh molten paraffin wax in order to form a hard block. The blocks were then mounted on a microtome and sections of about 5–6 µm thick obtained. The tissue sections were mounted on glass slides coated with 20 % albumin and kept on a dryer at the temperature of 45 ℃. The tissues were deparaffinised twice usi ng xylene for 5 min each. Following this, the tissues were rehydrated by rinsing in descending grades of alcohol (100, 100, 95, 95, 80 and 70 %) and water for 10 min. Finally, the tissue sections were stained with haematoxylin and eosin (H&E) and cover slips placed on the dry, ready-to-view slides [38].

2.6 Statistical Analysis

The computer software, Statistical Package for Social Sciences (SPSS) version 16 for Windows was used for the statistical analyses. The data obtained were analyzed using one way Analysis of Variance (ANOVA) and the means separated using Duncan's new multiple range test. The results were presented as the mean with the standard error of the mean (SEM). Differences in the means were considered significant at probability values less than 5 % *($P < 0.05$)*.

3. RESULTS AND DISCUSSION

Herbs have been used since time immemorial to treat many different ailments. One of the ailments commonly managed by the aid of medicinal plants is infertility. In both rural and urban settlements, reports abound on the evidence of enhanced fertility and correction of fertility problems following the use of herbs in male animals and humans alike [39]. Stem bark of *Picralima nitida* is one of such plants widely employed in the treatment of male sexual impotence, especially in the rural or traditional medicine [12]. Besides, it cannot be overemphasized that the circulatory system, particularly blood and its components, to a great extent influence the physiology of every tissue or cell of the animal. In this present study, effects of ethanolic stem bark of *Picralima nitida* on haematological profile and reproductive indices of male albino rats were evaluated.

3.1 Plant extraction

The ethanolic stem bark extract of *Picralima nitida* was brown in colour. A dark brown solid was obtained after evaporation to dryness and concentration with percentage yield of 7.13%.

3.2 Haematological profile

The result of the effect of ethanolic stem bark of *P. nitida* on haematology is presented in Table 1. The result showed that treatment with different doses of the extract did not $(P > 0.05)$ alter the total WBC count when compared to the control group and rats treated with 5 mg/kg BW of sildenafil citrate. Furthermore, it was observed that treatment with 2.5 mg/kg BW of cadmium chloride caused a further reduction $(P < 0.05)$ in the mean erythrocyte count, HB concentration and total WBC count. This implies that stem bark extract of *Picralima nitida* administration did not affect the overall status of the immune system of the treated rats. Studies on the seed extract of this plant recorded a decrease in the total WBC counts [40, 41]. However, it was observed that the rats given 2.5 mg/kg BW of cadmium chloride had a significantly fewer WBC compared to the rest of the experimental groups. This could be attributed to the cytotoxic property of this compound [42]. Congruent with our finding, sub chronic treatment with cadmium has been reported to cause leucopenia in wistar rats [43, 44]. Ghiasi et al [45] also observed a significant decrease, in comparison with the control group, in the total leukocyte count and leucocytic index in cadmium-treated fish. Similar observations were also made in mice treated with lead [46, 47].

All doses of the extract used in this study significantly $(P < 0.05)$ decreased the mean RBC count when compared to the control group and rats treated with 5 mg/kg BW of sildenafil citrate. This could be attributed in part to the anti-proliferative activity of this extract [15], hence negatively affecting the proliferation of the erythroblasts in the bone marrow. It could also be as a result of imbalance in the rate of production and destruction of blood corpuscles (erythropoiesis) as a result of administration of this extract. Sunmonu *et al* however reported no significant difference in the erythrocyte count rats treated with the aqueous extract of *P. nitida* seed when compared to the control group [48]. The erythrocyte count of rats administered cadmium chloride was the lowest. This was likely due to suppression of hematopoietic tissues by cadmium, being a heavy metal. This idea is supported by the study of Gill and Epple [49] that attributed anaemia to impaired erythropoiesis caused by a direct effect of metals on hematopoietic centres (kidney, spleen). 200 mg/kg BW and 400 mg/kg BW of the extract together with 2.5 mg/kg BW of cadmium chloride significantly $(P < 0.05)$ reduced the PCV values when compared to the control group, groups treated with 100 mg/kg BW of the extract and 5 mg/kg BW of sildenafil citrate. The mean PCV values of the rats treated with 100 mg/kg BW of the extract was statistically comparable $(P > 0.05)$ to those of the control and 5 mg/kg BW of sildenafil citrate. Only 400 mg/kg BW caused a significant $(P < 0.05)$ reduction in the HB values. The mean PCV values of the rats treated with 100 mg/kg BW of the extract which was statistically comparable to those of the control and 5 mg/kg BW of sildenafil citrate reveals the relative safety of this extract at lower doses. Meanwhile, the haemoglobin concentration did not vary from that of the control and the sildenafil treated groups. This suggests that haemoglobin synthesis was not affected by treatment with ethanolic stem bark of *Picralima nitida*, and that the anaemia caused by this extract is not hypochromic in nature, except at a higher dose of 400 mg/kg BW. This could

also imply that either the incorporation of haemoglobin into the red blood cells or the morphology and osmotic fragility of the red blood cells were altered at the administration of the high dose (400 mg/kg body weight) of the extract. Again, Sunmonu *et al.,* [48] who used of the aqueous seed extract of *P. nitida* reported no significant effect on haemoglobin concentration of Wistar rats. The disparity observed may be as a result of difference in the phytochemical composition in different parts of this plant or the difference in the extraction solvent utilized.

Table 1: Mean values of PCV, RBC count, Hb concentration and Total WBC count

Groups	PCV (%)	RBC (x 10^6)	Hb conc. (g/dl)	WBC (x 10^3)
A (Control)	40.00 ±0.89^a	10.36 ±0.46^a	14.68 ±0.04^a	10.87 ±0.80^a
B (100 mg/kg Extract)	38.20±1.87^a	9.38 ±0.70^b	14.68 ±0.02^a	11.24 ±0.40^a
C (200 mg/kg Extract)	31.20±1.14^b	9.53 ±0.70^b	14.68 ±0.04^a	10.91 ±0.60^a
D (400mg/kg Extract)	32.00±1.86^b	8.71 ±1.10^b	13.85 ±0.01^b	11.02 ±0.50^a
E (2.5mg/kg Cad)	33.00±1.64^b	5.61 ±0.86^c	11.51 ±0.04^c	9.20 ±0.40^b
F (5mg/kg Sildenafil)	42.20 ±2.63^a	10.20±0.62^a	14.85 ±0.05^a	11.46 ±0.60^a

^{abc} =Different superscripts in a column indicate significant difference in the means of the groups *(P < 0.05)*.

The result of the differential leukocyte count is presented in Table 2. The result showed that there was no significant difference *(P > 0.05)* in the mean basophil counts across all the study groups. Again, the treatment doses of ethanolic stem bark extract of *Picralima nitida* used in this experiment did not cause any significant *(P > 0.05)* variation in the mean counts of eosinophils and lymphocytes, but significantly increased *(P < 0.05)* the neutrophil counts and conversely decreased *(P < 0.05)* the monocyte count when compared to the control group and rats treated with 5 mg/kg BW of sildenafil citrate. Treatment with 2.5 mg/kg BW of cadmium chloride significantly *(P < 0.05)* increased the mean eosinophil count and caused a further increase *(P < 0.05)* in the neutrophil count, while decreasing *(P < 0.05)* the lymphocytes, when compared to the rest of the groups. It is noteworthy that treatment with 2.5 mg/kg body weight of cadmium chloride significantly *(P < 0.05)* increased the mean neutrophil count when compared with rats administered 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW of *P. nitida* extract. The mean monocyte counts of cadmium-treated and Sildenafil-treated rats were statistically the same *(P > 0.05)*. The neutrophilia and monocytopenia observed with ethanolic stem bark extract in this research has been previously reported when rats were treated with seed extract of *Picralima nitida*. It has been suggested that it could be traced to usual inflammatory process or immune response accompanying injection of substances considered foreign by the animal's body, especially as toxic components are suspected to be contained in the plant extract and the heavy metal, leading to selective immune modulatory effect and localized toxicity [48].

Groups	Basophil (%)	Eosino (%)	Neutro(%)	Lymph (%)	Mono (%)
A (Control)	0.40 ±0.24	3.80 ±0.92^a	19.60 ±1.68^a	69.60±1.47^a	6.60±0.40^a
B (100 mg/kg Extract)	0.40 ±0.24	3.60 ±0.24^a	22.60±0.24^b	71.80 ±1.49^a	1.60±0.24^b
C (200 mg/kg Extract)	0.40 ±0.20	4.00 ±0.55^a	22.40±0.90^b	71.20 ±1.46^a	1.60±0.75^b
D (400 mg/kg Extract)	0.40 ±0.24	4.40 ±0.24^a	23.40±0.24^b	68.00 ±1.25^a	2.80±0.68^b
E (2.5 mg/kg Cad)	0.20 ±0.20	6.20 ±0.37^b	29.40 ±0.60^c	60.40 ±0.60^b	3.80±0.87^c
F (5mg/kg Sildenafil)	0.40 ±0.24	4.20 ±0.20^a	21.60±0.60^a	70.00 ±0.45^a	3.80±0.37^c

Table 2: Mean values of the differential WBC count

[abc] = Different superscripts in a column indicate significant difference in the means of the groups *(P < 0.05)*.

3.3 Reproductive indices

3.3.1 Serum reproductive hormones

It has been considered that the structural and functional integrity of reproductive organs depends on the adequate bioavailability of testosterone [50]. The result of the mean values of some male reproductive hormones is presented in Figure 1. The result showed a non-significant increase *(P > 0.05)* in the mean serum testosterone level of the rats treated with 100 mg/kg BW of ethanolic stem bark extract of *Picralima nitida* when compared with the rats in the control group and the group treated with 5 mg/kg BW of Sildenafil citrate. Treatment with 200 mg/kg BW of the extract, 400 mg/kg BW of the extract, and 2.5 mg/kg BW of Cadmium chloride significantly *(P < 0.05)* lowered the mean serum testosterone level when compared with rats treated with 100 mg/kg BW of the extract, control group and the group treated with 5 mg/kg BW of Sildenafil citrate. 2.5 mg/kg BW of $CdCl_2$ created a further reduction in the serum testosterone level when compared with 200 mg/kg BW of the extract and 400 mg/kg BW of the extract. However, no statistical variation *(P > 0.05)* was observed in the mean serum FSH and LH across all the experimental groups. This implies that treatment with this extract at 100 mg/kg does not hinder testosterone synthesis. The interstitial cells of Leydig or Leydig cells are shouldered with the responsibility of the bulk of testosterone production in the male animals. Normal Leydig cell function and development are important for male sexual development, testicular steroidogenesis during puberty and adulthood, and hence normal fertility [51].The histopathology of the testis in Figure 3 also attests to this, owing to the fact that while cadmium chloride and higher doses of the extract elicited degeneration of the interstitium, 100 mg/kg, control and sildenafil treatment kept the interstitial tissues intact. The observed indifference in the mean serum FSH and LH across all the experimental groups could stem from the fact that time is needed for the reduction in testosterone level to signal the hypothalamic pituitary axis. The pituitary gland secretes FSH and LH in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus, which in turn is dependent on the feedback from the circulating testosterone [52].

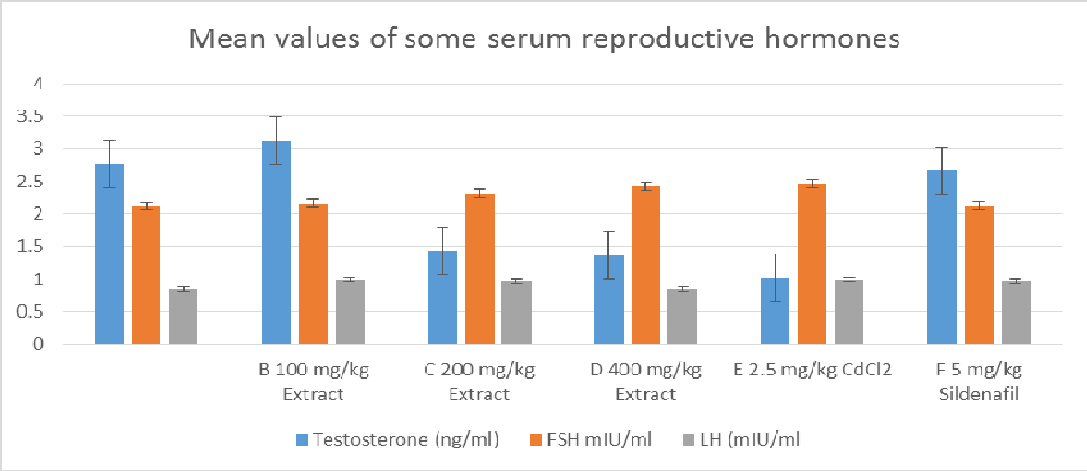


Figure 1: Mean values of some serum reproductive hormones

3.3.2 Testicular allometric weight (TAW)

The result of the testicular allometric weight is presented in Table 3. The result showed that there was no significant difference *(P > 0.05)* between the mean body weights of the control group when compared to

the treatment groups. However, treatment with 200 mg/kg body weight of *P. nitida* extract, 400 mg/kg body weight of *P. nitida* extract, and 2.5 mg/kg body weight of cadmium chloride significantly *(P < 0.05)* decreased the testicular weights and testicular allometric weights when compared to the control group, rats dosed 100 mg/kg body weight of *P. nitida* extract, and rats administered 5 mg/kg body weight of sildenafil citrate. Androgens have been shown to be necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands and studies have shown that the level of testosterone is positively correlated with the weights of testis and epididymis [50]. Therefore, the significant increase in testis and epididymis weight could be due to increased androgen biosynthesis as evidenced by a significant increase in serum testosterone levels in the extract-treated rats.

Table 3: Mean values of Testicular weights and Testicular allometric weights (TAW)

Groups	Body weight (g)	Testicular weight (g)	TAW(g)
A (Control)	122.40 ±3.63	1.16 ±0.03[a]	0.96 ±0.01[a]
B (100 mg/kg Extract)	130.80 ±5.23	1.18 ±0.05[a]	0.91 ±0.02[b]
C (200 mg/kg Extract)	135.60 ±12.64	1.09 ±0.02[b]	0.80 ±0.06[c]
D (400 mg/kg Extract)	129.60 ±3.54	1.06 ±0.03[b]	0.82 ±0.02[c]
E (2.5 mg/kg Cad)	138.80 ±16.14	1.06 ±0.09[b]	0.76 ±0.05[c]
F (5mg/kg Sildenafil)	125.20 ±6.01	1.16 ±0.01[a]	0.93 ±0.05[ab]

[abc] = Different superscripts in a column indicate significant difference in the means of the groups (p<0.05

3.3.3 Testicular sperm reserve (TSR), epididymal sperm reserve (ESR), % motility & Mass activity

Moreover, the number of stored spermatozoa determines the weight of epididymis. Sperm count was often used as a measure of sperm production, testicular function and/or male fertility. Low sperm count and high percentage of abnormal spermatozoa each have been associated with reduced fertility [50]. The result of the testicular and cauda epididymal sperm count, Percentage motility and Mass activity is presented in Table 4. The result showed that treatment with 100 mg/kg body weight of ethanolic stem bark extract of *P. nitida* significantly *(P < 0.05)* increased both the testicular sperm count and cauda epididymal sperm count when compared to the control group and the rest of the treatment groups. Treatment with 200 and 400 mg/kg body weight of the extract caused a significant reduction *(P < 0.05)* in the testicular and cauda epididymal sperm counts when compared to the control group and group treated with 5 mg/kg body weight of sildenafil citrate. The rats administered 2.5 mg/kg body weight of cadmium chloride had the lowest *(P < 0.05)* testicular and cauda epididymal sperm counts. 5 mg/kg body weight of sildenafil citrate produced the highest mean percentage motility of 90% followed by that of rats given 100 mg/kg body weight of extract with 80% motility, both being higher than the control with 70% mean percentage motility. Rats treated with 200 mg/kg and 400 mg/kg body weight both had mean percentage motility of 45% which were lower than that of the control group. Treatment with 2.5 mg/kg body weight of cadmium chloride produced no movement of the sperm cells i.e mean percentage motility of 0%. 5 mg/kg body weight of sildenafil citrate gave a very rapid mass activity (4+) followed by the control group which had 3+ mass activity. Treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg of extract all produced 2+ grade of mass activity. Treatment with 2.5 mg/kg body weight of cadmium chloride showed no activity/ movement (-). These findings is likely due to the toxic adverse effects posed by plant extracts especially at high doses due to heavy metals always associated with these plant materials. Gerhard *et al* [53] and Oliver *et al* [54] reported that the most frequent factors implicated in male fertility are occupational exposure to heavy metals, such as cadmium, lead, manganese, and mercury but according to Enuh *et al* [55], one of the major neglected sources of these substances is herbal remedies. The histopathology done in this research gave credence to these observations.

Groups	TSR ($x10^6$)	ESR ($x10^6$)	Motility (%)	Mass Activity
A (Control)	49.26 ±1.23[a]	92.11 ±0.98[a]	70	Active (3+)
B (100 mg/kg of Extract)	56.54 ±4.77[b]	97.92 ±1.03[b]	80	Medium (2+)
C (200 mg/kg of Extract)	45.81 ±1.86[c]	62.29 ±1.75[c]	45	Medium (2+)
D (400 mg/kg of Extract)	44.30 ±4.72[c]	51.67 ±1.17[d]	45	Medium (2+)
E (2.5 mg/kg BW Cadmium)	37.34 ±2.35[d]	37.84 ±1.03[e]	0	No movt (-)
F (5mg/kg Sildenafil citrate)	50.46 ±4.60[a]	95.14 ±2.40[a]	90	Rapid (4+)

Table 4: Mean values of Testicular and Epididymal sperm counts

[abc] =Different superscripts in a column indicate significant difference in the means of the groups *(P < 0.05)*.

3.3.4 Differential sperm cell count

In addition, the result of the differential sperm count presented in Figure 2 showed that treatment with 5 mg/kg body weight of sildenafil citrate and 100 mg/kg body weight of the extract significantly increased the mean normal sperm cells and mean percentage motility when compared with the control and the rest of the groups. Treatment with 200 and 400 mg/kg body weight and 2.5 mg/kg body weight of cadmium chloride caused a significant increase *(P < 0.05)* in the mean percentages of teratoid and immature sperm cells when compared with the control. Cadmium chloride-treated rats however created more disastrous impact on the mean differential sperm cell count. Also, treatment with 5 mg/kg body weight of sildenafil citrate increased the mass activity when compared to the control with the extract treated groups having less activity compared to the control. Treatment with 2.5 mg/kg body weight of cadmium chloride lead to no activity/no movement of the sperm cells. These effects shown by sildenafil treatment are due to its ability to affect sexual stimulation by enhancing the smooth muscle relaxant effects of nitric oxide (NO) in the corpus cavernosum [56]. NO then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine, producing inflow of blood to the male reproductive organs and thereby increasing the energy, motility and activity of spermatozoa. Similarly, the finding with 100 mg/kg body weight is in line with the work done by Adjanohoun *et al,* [12] which reported that the bark is used to improve male fertility although higher doses of the extract (200 and 400 mg/kg body weight) caused adverse effect on these parameters. These adverse effects could be said to be due to the toxic effects of the plants at high doses. The effects seen in the cadmium chloride treated rats were typical of effects of effect of heavy metals on male fertility [53, 54].

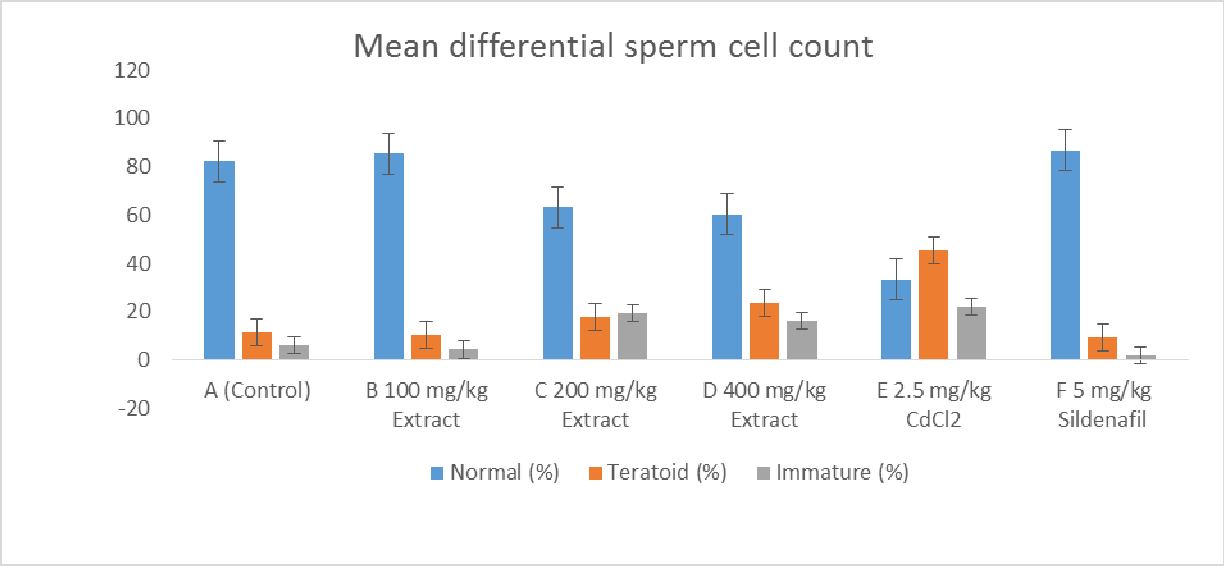
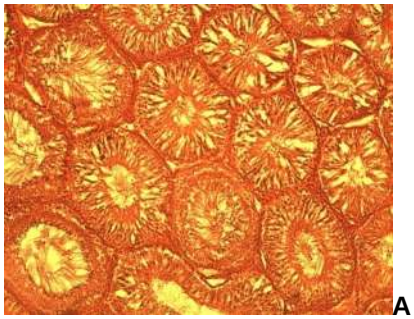


Figure 2: Mean differential sperm cell count.

3.3.5 Histopathology

Furthermore, histopathologic examination of the testis is the most sensitive means to detect effects on spermatogenesis [57]. The photomicrographs of sections of the testes presented in Figure 3 showed an increasing gradation in degeneration of the seminiferous tubules of rats administered 200 mg/kg body weight of *P. nitida* extract, 400 mg/kg body weight of *P. nitida* extract and 2.5 mg/kg bodyweight cadmium chloride with almost no evidence of spermatozoa in that of rats given cadmium chloride. However, those of rats in the control group, rats given sildenafil citrate and rats administered 100 mg/kg bodyweight of *P. nitida* were apparently normal with numerous spermatozoa seen in the lumen of the seminiferous tubule. It implies that the ethanolic stem bark extract of *P. nitida* is tolerable when administered at a dose of 100 mg/kg but have profound deleterious effects on the histo-architecture of the testes when administered at higher doses of 200 mg/kg body weight and 400 mg/kg body weight. Other researchers [48] have reported that the ethanolic extract of *P. nitida* seed when administered at low doses produced no cellular abnormality of the spermatozoa in the testes and considered safe for consumption in animal model compared to chronic administration of high doses which are toxic and could pose deleterious effects to the testes which play vital roles in sperm production, thereby jeopardizing its reproductive activities in male fertility. Similarly, cadmium has also been reported to cause testicular necrosis among other toxic potentials [58].


A

B

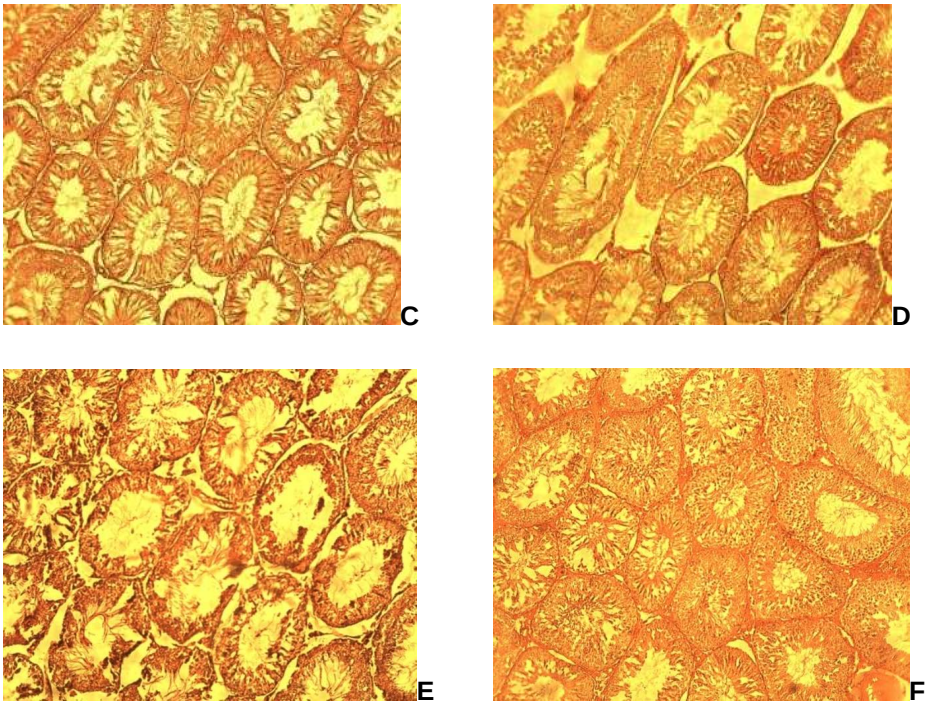


Figure3: Testicular Histomorphology: A (Control), B (100 mg/kg of extract) and F (5 mg/kg of sildenafil citrate) showing normal spermatogenic activity, normal germinal epithelia, high concentration of sperm cells in the lumen of the seminiferous tubules and intact interstitium. C (200 mg/kg of extract), D (400 mg/kg of extract) and E (2.5 mg/kg BW of cadmium chloride) showing slight, moderate and massive degeneration of the germinal epithelium and interstitial spaces and depletion of the lumen of the seminiferous tubules respectively

4. CONCLUSION

It is obvious from this study that *Picralima nitida* could only be used to alleviate male impotence and improve male fertility in male rats when administered at doses of 100mg/kg and probably below, but adversely affected these parameters at higher doses. The ethanolic stem bark extract of *P. nitida* at dose of 100 mg/kg bodyweight has the capacity to improve some of the haematological indices, serum testosterone level, testicular allometric weight, testicular/ epididymal spermatozoa concentrations and the integrity of the ultrastructure of the seminiferous tubules. Caution should be placed on the use of higher doses of this extract for any ethno medical intervention on the management of male sexual impotence as this can cause a deleterious effect on male reproductive physiology and blood picture.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Institutional ethics review committee of the Faculty of Veterinary Medicine, university of Nigeria, Nsukka.

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