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Chronic Oral Consumption of Ethanolic Extract of *Picralima Nitida* (Akuamma) Seed Induced Histopathological changes On the Testes of Adult Wistar Rats

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Abstract

Histomorphological effect of chronic consumption of ethanolic extract of *Picralima nitida* seed on the Testes of adult albino wistar male rats was investigated using 20 male albino wistar rats; they were distributed into 5 rats in each group. Group 1 was the control group while groups 2 to group 4 were the experimental groups. Group 1 was given distilled water and normal rat feed, Group 2 was given 250mg/kg serving as low dose, group 3 was given 350mg/kg as middle dose and group 4 was given 450mg/kg as High dose of *Picralima nitida seeds* extract orally for 21 days. At the end of administration, the rats were sacrificed and Testes from all the groups were carefully dissected out, fixed immediately in Bouin's fluid and sent to Laboratory for histopathological analysis. 2-3mm in thickness were section out, and re-fixed in neutral buffered formalin solution, processed to paraffin sections and cut at 5micron using Rotary microtome and evaluated under digital microscope. Result of histopathology of control group showed preserved cellular architecture of seminiferous tubules containing distinct interstitium harboring leydig cell, the tubules enclosed the germinal cell lining with primary and secondary spermatogonium, spermatocytes, spermatids, spermatocytes radiating towards the luminal semen, all within normal cellular profile, group 2 revealed no cellular abnormality, though there is slight area of interstitial and tubular constriction as compared to control group, there is moderate cellular abnormality, with degeneration of nuclear content of spermatogenic lining cell, slugging, interstitial and tubular constriction in group 3 as compared to control group while in group 4 there is severe cellular abnormality, degeneration of nuclear content of spermatogenic lining cell, leydig cells, myoid cell and sertolic cell, there is slugging, interstitial and tubular constriction as compared to control group. In conclusion, *Picralima nitida* seeds does not pose cellular abnormality at low dose when it is consumed with cautions, however prolong intake at high concentration has deleterious and adverse effect on Testicular morphology of male Adult wistar rats.

Keywords*: Picralima nitida, Histopathology, wistar rat, and Testes.*

1.0 Introduction

Picralima nitida (Akuamma) commonly called 'asewa' in Ibibio dialect is a rainforest tree rich in alkaloids occurring in African forest region. The plant has wide varied applications in Nigeria herbal medicine. *Picralima nitida* is a medicinal plant with diverse end-uses (Keay, 1989) extracts from its seeds, fruit rind and stem bark demonstrated anti-malarial activity (Iwu and Klayman, 1992), antimicrobial effect (Fakeye *et al*., 2002), anti-inflammatory and Ezeamuzieji *et al*., 1994; Iwu and Klayaman, 1992) among have reported the medicinal potentials of this plant.

Many anti-malarial agents have been shown to have anti-fertility effects. The evaluation of anti-malarial agents for possible toxicity and anti-fertility actions becomes imperative due to the global concern of malaria and infertility and thus relationships have to be established to guide the common man. In the absence of any information on the effect of *picralima nitida* seed on the Testes of albino male rats, this research is undertaken to determine effects on cyto-architectural components of the cells and tissues. To evaluate the effect of ethanolic extract of *Picralima nitida* seed on the histology of the Testes.

Picralima nitida is a species occurring in African forest region, spread through Ivory Coast to Uganda (NNMDA, 2008). *Picralima nitida* occurs south to Congo and Cabinda (Angola).

It is a tropical small bushy tree with white latex in all parts, hard bark, brittle, pale to dark greyish black or brown. *Picralima* is derived from the Greek word

'bitter'. Hutchinson and Dalziel (1963) revealed that it is a tree of about 15 meters high and with circumference of about 50 centimeters. It has large glossy leathery leaves, conspicuous white flowers and large orange-coloured fruits (Keay, 1989). At maturity, the leaves are pinnate with about 14 to 18 leaflets (Meyer *et al*., 2006). *Picralima nitida* bears white flowers (about 3 cm long) with ovoid fruits which at maturity are yellowish in colour. The leaves are broad (3-10 cm) and oblong (6-20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs. It has Berries which have an ellipsoid form, with large size and green in color. When they fall on the ground after maturity, they turn to yellow and the seeds germinate on the ground with many seedlings. These berries are also used in traditional medicines for treating typhoid and fight against muscular pain (Adjanohoun *et al*., 1996). Inside the berries are seeds. The seed is an object of commerce in the local market, and it is collected from the wild thus its availability has been severely threatened. *Picralima nitida* seeds, can be dried and stored for 0.5-2 years without loss of pharmacological activity.

Picralima nitida trees growing at the same location generally have the same height and are probably of the same age. The young plants have a high competition capacity. *Picralima nitida* can be found flowering and fruiting throughout the year. The flowers are visited by insects during sunny days.

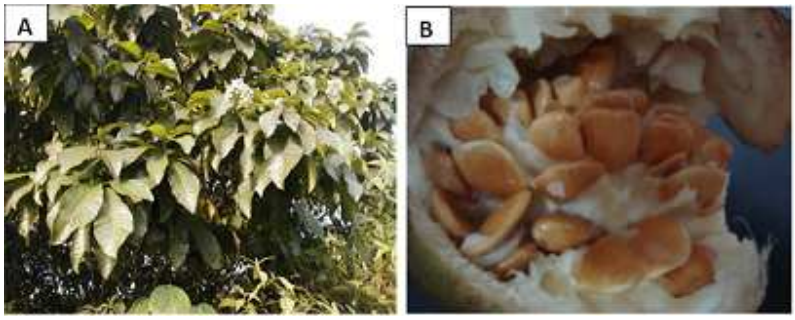

Fig. 1a and 1b: Picralima a nitida plant and seed within its fruit. Source (www.gjournals.org)

According to Meyer *et al*. (2006) assessed its medicinal composition. From laboratory analysis, they found that the active principle of *Picralima nitida* is formed by more than 10 alkaloids present in different tree parts (from bark, leaves, roots and fruits). Their names derive from the local name "Akuamma" (Okunji *et al*., 2006). These alkaloids play several biological roles (Meyer *et al*., 2006) such as: Anti-inflammatory (pseudo-akuammigine), Anti-fever Antimicrobial (against Gram-positive bacteria and fungi with use of root bark), Hypoglycemic control (with use of roots and fruits) and, Anti-malaria (with fruits) and Anti-leishmaniasis (with roots).

It is used in traditional medicines in the treatment of inflammation, otitis, pulmonary bronchitis and venereal diseases. The dried seeds from this plant are used in traditional medicine throughout West Africa, particularly in Ghana as well as in the Ivory Coast and Nigeria. The seeds are crushed or powdered and are mainly used for the treatment of malaria, (Kapadia *et al*., 1993) and diarrhoea, and as a painkiller. An enterprising Ghanaian hospital started manufacturing standardized 250 mg capsules of the powdered *P. nitida* seed, and sold them around the country where they became widely accepted as a safe and effective pain relief product. It is encapsulated and marketed in Ghana under the brand name 'Picap capsules'. In Cameroon the seeds, bark and fruits are commonly sold in local markets. Many herbalists have claimed to use the leaves, seed or stembark as treatment for various fevers, hypertension, jaundice, gastro-intestinal disorders and for malaria (Dalziel, 1961; Iwu, 1993). The seed stem and roots have been reported to be effective as a cough suppressant anodyne, as well as an aphrodisiac and hypoglycaemic agent in treatment of diabetes (Ayensu, 1978; Oliver, 1960).

The testes are the primary reproductive organ or gonads in the male. They are ovoid, reproductive and endocrine organs. They are suspended in the scrotum by scrotal tissues including the ductus muscle and spermatic cords. The testes are firm, mobile organs. Average testicular dimensions are 4-5cm in length, 2.5cm in breadth and 3cm in antero-posterior diameter, their weight varies from 10.5-14g. The left testis usually lies lower than the right testis (Moore, 2010). Each testis lies obliquely within the scrotum, the upper pole tilted antero-laterally and the lower postero-medially.

The testes have two functions which is to produce the male gametes or spermatozoa and to produce male sexual hormone (testosterone), which stimulates the

accessory male sexual organs and causes the development of the masculine extragenital sex characteristics. It derives its blood supply from the long testicular arteries which arises from the anterolateral aspect of the abdominal aorta just inferior to the renal arteries. They pass retroperitoneally crossing over the ureters and the inferior parts of the external iliac arteries to reach the deep rings and pass through the spermatic cord to supply the testis (Moore, 2010). The testis is drained by the pampiniform venous plexus: a network of 8-12 veins lying anterior to ductus deferens (Moore, 2010). The lymphatic drainage of the testis follows the testicular artery and vein to the right and left lumbar and preaortic lymph nodes (Moore, 2010). It is a compound tubulo-alveolar gland having both exocrine and endocrine portions. The gland is composed of stroma and parenchyma. The testis is invested by three coverings (tunics), Tunica Vaginalis (visceral layer): it is an outermost peritoneal covering lined with simple squamous epithelium. Tunica Albuginea: it is a tough, shinny, white connective capsule. Tunica Vasulosa: it is present internal to albuginea and consist of loose areolar connective tissue with rich capillary plexus, which collectively form the capsule of the testis. Within the septal are seminiferous tubules that are enclosed by a thick basal lamina and surrounded by 3-4 layers of smooth muscle cells (or myoid cells). The seminiferous tubules have a diameter of about 200 μm and contain the germinal epithelium (Singh, 2004). One or several highly convoluted seminiferous tubules form a lobule of the testis. The spaces between seminiferous tubules are filled by areolar connective tissue containing fenestrated blood capillaries, nerves, lymph vessels and Leydig cells that are rounded or polygonal with eccentric nuclei (containing 1-3nucleoli) and acidophilic cytoplasm, binucleated cells are common, the cells are rich in mitochondria, smooth endoplasmic recticulum, prominent golgi body and lipid droplets. These cells secrete testosterone. It consists of two general types of cells: spermatogenic cells and Sertoli cells. Within the seminiferous tubules are lumen, lined with epithelium that are of complex type of stratified epithelium, which is comprised of both cuboidal and columnar cells. It consists of many layers of two types of cells; (a) spermatogenic cells and (b) supporting or sertoli cells. The main components of the testes which play a role in sperm production are the seminiferous tubules, Sertoli and Leydig cells. There are also a series of ducts and tissues within the testis which play an accessory role in producing and/or transporting sperm from the testes which make them the principal organ of reproduction.

Considering the vital role of Testes in reproduction and fertility and *Picralima nitida* seed have demonstrated a lot of therapeutic usage while the effect on the testicular morphology has not been proved scientifically, information on the effect of ethanol extracts on the Testicular histology has been very scanty and this forms the stimulus for this study. Despite the widespread abundance and traditional use of *P. nitida* seeds, no systematic study has been done on the toxicological effects of this herb to the best of our knowledge. The present study was therefore designed to evaluate the safety/toxicity risk associated with the use of ethanolic seed extract of *P. nitida* based on functional indices and histology of rat Testes.

This study revealed possible effects of ethanolic extract of *Picralima nitida* seed on the histomorphological architecture of the Testes of adult male wistar rat. Animal scientists, Pharmacognosist, Pharmacist, Pharmacologists, Histologists, clinicians and histopathologists will benefit from the findings and the outcomes could be widely used in drug design for male reproductive therapy.

2.0 Materials and Methods

2.1 Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonade V, sodium bicarbornate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, distilled water, hutches, concentrate feed, syringe and hypodermic needles, EDTA treated bottles, latex hand glove, weighing scale, graduated vials, measuring tape, they were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

2.2 Procrument of *Picralima Nitida* (Akuamma) Seed

Picralima nitida (Akuamma) seeds were obtained from a local market in Nung Udoe Ibesikpo, Asutan Local Government Area in Akwa Ibom State, Nigeria. This plant was identified and authenticated at the Pharmacognosy Herbarium in Faculty of Pharmacy, University of Uyo. The seeds were removed from the fruit and washed to clear any adherent material from the pod and seeds were sun-dried. The thin covering of the seeds were carefully removed and pure seed content were stored in a glass container.

2.3 Preparation of Extract

Seeds were powdered by crushing (pounding). The powdered seed were weighed and the initial weight of seeds was 300.4 grams. *Picralima* seeds were put in a maceration tank in Pharmacognosy Laboratory,

Faculty of Pharmacy, University of Uyo, Nigeria. The *Picralima nitida* seed powder was macerated using 70% ethanol and kept for 72 hours. The solution was filtered and concentrated in a water bath at a temperature of 40°C. The final extract weighing 100.2 grams, was collected in a semi-solid form and stored in a refrigerator at 4°C. This extract had a brown color with a solubility characteristic in water.

2.4 Median Lethal Dose of *Picralima Nitida seed*

The LD_{50} (median lethal Dose) of *Picralima nitida* seed was determined when using Lorke's method. Lorke's method (LD_{50}) was calculated as geometrical mean of the maximum dose producing 0% mortality (a) and the minimum dose producing 100 % mortality (b). ($LD_{50} = \sqrt{ab}$ (Lorke, 1983).

The acute toxicity of *Picralima nitida* on wistar albino rats was determined by giving different doses of the plant extract based on body weight of the animals it was administered orally to the animals in five groups. Each group received 300mg/kg, 600mg/kg, 900mg/kg, 1,200mg/kg, and 1,000mg/kg body weight. The animal were monitored for the next three hours and examined after twenty-four hours for mortality.

2.5 Experimental Animals

Twenty sexually mature male wistar rats were used for the research work. Rats were gotten from University of Nsukka, Nigeria .The rats were left to acclimatize in the College of health sciences animal house in University of Uyo, Nigeria for seven (7) days. The rats were housed in a clean wooden cage and fed with rodent pelleted feed and clean drinking water *ad libitum*. Rats were identified by different color marking on their tails. All rats were handled according to guiding principles in the care and use of animal's standard care of laboratory animals.

2.6 Experimental Sites

The study was done at the College of Health Sciences Animal House, University of Uyo, Uyo, Akwa-Ibom State Nigeria.

2.7 Experimental Protocols

Rats were grouped into four (4) groups according to their weights with five rats housed per cage. Carefully grouped rats were labeled as follows.

Group 1: Control (Administered with distilled water only).

Group 2: Ethanolic extract of *Picralima nitida* seed at low dose (250mg/kg).

Group 3: Ethanolic extract of *Picralima nitida* seed at medium dose (350mg/kg).

Group 4: Ethanolic extract of *Picralima nitida* seed at high dose (450mg/kg).

2.8 Sample collection for Histopathological Analysis

At the end of the stipulated 21 days of administration of the extract, the rats were subjected to a 12 hours fast but had access to water and they were sacrificed using chloroform vapour. Testes were carefully harvested out from the rats, harvested organs were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were immediately fixed in Bouin's fluid transported to the Histopathology laboratory, After 72 hours, 2-3 mm in thickness were dissected out and post fixed in Neutral Buffered Saline and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly. The sections were designated "vertical sections". Serial sections of 5 µm in thickness were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

2.9 Photomicrography

Records of the Histological results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health Sciences, University of Uyo, Uyo, Akwa-Ibom, Nigeria as illustrated in Plate.1 to 4.

3.0 Results

3.1 Histopathological findings

Group-1 (Control)

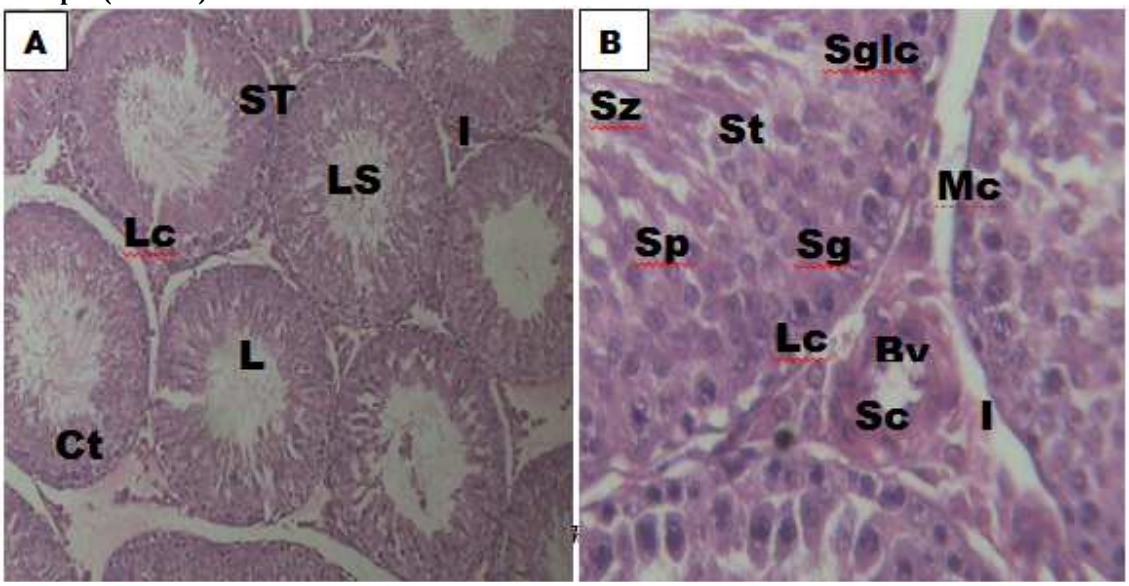

Plate 1 Photomicrograph of the control Testes without treatment at Magnification A (X100) and B (X400) stained with H and E technique

Keys: Sglc *–Spermatogenic lining cells,* ***I*** *– Interstitium,* ***Ls****- luminal semen,* ***Sz****- Spermatozytes,* ***Mc*** *– Myoid cells,* ***Sp*** *–spermatocytes,* ***ST*** *– Seminiferous tubules,* ***Sg*** *- spermatogonia,* ***L****- lumen,* ***St*** *– spermatids,* ***Sc*** *–sertolic cells,* ***Lc*** *– leydia cells,* ***Bv*** *– blood vessels,* ***Ct*** *– connective tissue.*

Group-2 (Low Dose)

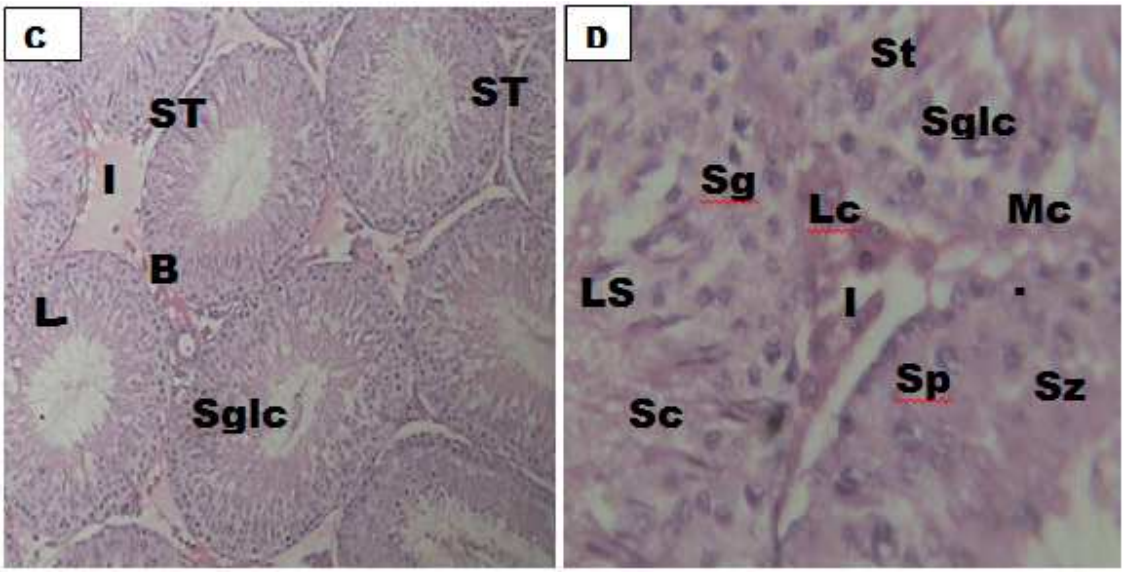

Plate 2 Photomicrograph of the Testes treated with 250mg/kg ethanolic extract of Picralima nitida at Magnification C (X100) and D(X400) stained with H and E technique

Keys: Sglc *–Spermatogenic lining cells,* ***I*** *– Interstitium,* ***Ls****- luminal semen,* ***Sz****- Spermatozytes,* ***Mc*** *– Myoid cells,* ***Sp*** *–spermatocytes,* ***ST*** *– Seminiferous tubules,****Sg*** *- spermatogonia,* ***L****- lumen,* ***St*** *– spermatids,* ***Sc*** *–sertolic cells,* ***Bv*** *– blood vessels.*

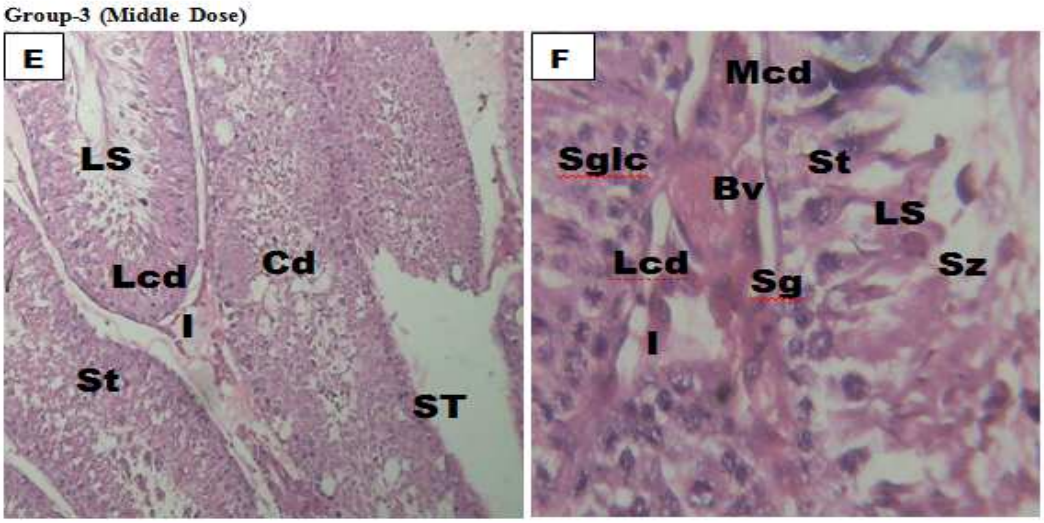

Plate 3 Photomicrograph of the Testes treated with 350mg/kg ethanolic extract of Picralima nitida at Magnification E (X100) and F (X400) stained with H and E technique
Keys: Sglc *–Spermatogenic lining cells,* ***I*** *– Interstitium,* ***Ls****- luminal semen,* ***Sz****- Spermatozytes,* ***Mc*** *– Myoid cells,* ***Sp*** *–spermatocytes,* ***ST*** *– Seminiferous tubules,****Sg*** *- spermatogonia,* ***L****- lumen,* ***St*** *– spermatids,* ***Sc*** *–sertolic cells,* ***Cd****– cellular degeneration,* ***Mcd*** *– myoid cell degeneration,* ***Lcd*** *– leydig cell degeneration.*

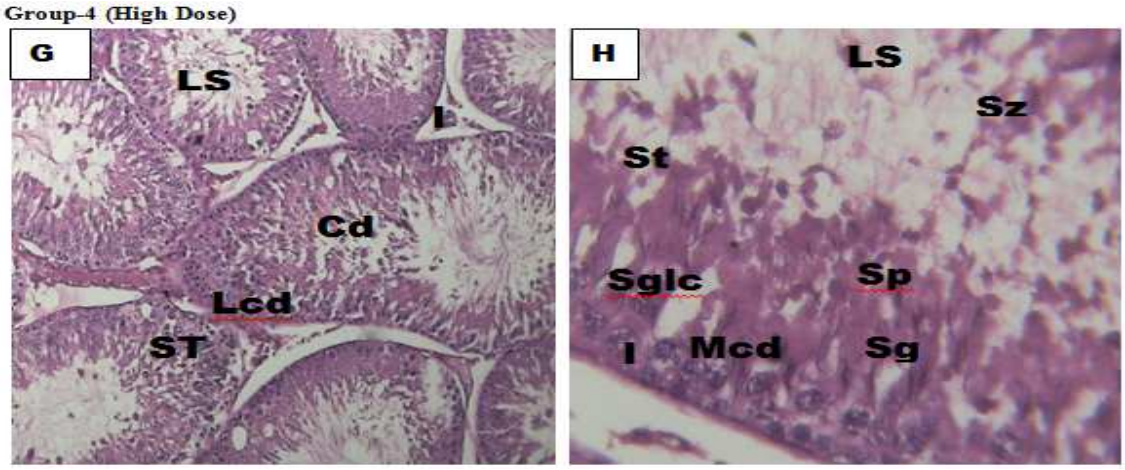

Plate 4 Photomicrograph of the Testes treated with 450mg/kg ethanolic extract of Picralima nitida at Magnification G(X100) and H (X400) stained with H and E technique
Keys: Sglc *–Spermatogenic lining cells,* ***I*** *– Interstitium,* ***Ls****- luminal semen,* ***Sz****- Spermatozytes,* ***Mcd*** *– Myoid cell degeneration,* ***Sp*** *–spermatocytes,* ***ST*** *– Seminiferous*

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4.0 Discussion

Medicinal plants can be used to treat almost any medical problem because the active compounds in drugs are extracted from plants but some of these plants also have the same potential to cause harmful side effects (Tapsell *et al*, 2006) In this study, effects of chronic oral administration of *Picralima nitida* on the histology of the testes of male albino wistar rats were evaluated. The results obtained from this research showed that *Picralima nitida* seed especially when taken at a high concentration causes severe abnormalities on the testes The histopathology of the testes from the control group (Plate A and B) showed preserved normal cellular architecture, group 2 (Plate C and D) which was treated with low dose revealed no cellular abnormality other than constrictions of the seminiferous tubules which could be easily reversed upon withdrawal of the extract as illustrated, In (Plate E and F), rats treated with middle dose of *Picralima nitida* revealed moderate cellular abnormality, with degeneration of nuclear content of spermatogenic lining cell, slugging, interstitial and tubular constriction while in (Plate G and H), rats treated with high dose of *Picralima nitida* extract revealed severe cellular abnormality, degeneration of nuclear content of spermatogenic lining cell, leydig cells, myoid cell and sertolic cell, there is slugging, interstitial and tubular constriction as compared to control group, considering the fact that predominant loss of epithelial lining and cellular distortion, diminished appearance of leydig cell and scanty sertolic cells revealed the toxic effect of the extract on the germinal cells and tubules in the group treated with high dose as these are basic hitopathological characteristic indication of the severity effect.

4.1 Conclusion

Despite the medicinal potentials of *Picralima nitida*, the extract still have some toxic effects on the testis of albino wistar rats. In conclusion, the result obtained from this study showed that *Picralima nitida* at low doses indicates no cellular abnormality and considered safe for consumption in animal model compared to the high dose which is toxic and could pose deleterious effect to the Testes which play a vital roles in sperm production, thereby jeopardizing its reproductive activities in male fertility.

Conflict Interests

The authors declared that they have no competing interests.

Authors' Contributions

All the Authors contributed equally.

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