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Histomophological Effect of Chronic Oral Consumption of Ethanolic Extract of *Picralima Nitida* (Akuamma) Seed on the Caudal Epidydimis of Adult Wistar Rats

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ABSTRACT
Histomorphological effect of chronic consumption of ethanolic extract of *Picralima nitida* seed on the caudal Epididymis 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 Caudal Epidydidmis 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 showed normal cellular architecture of seminiferous tubules containing distinct Basal and Principal cell, distinct area of interstitium, the tubules were lined with Pseudo-stratified squamous epithelial cell with goblet cells enclosed in it are the spermatocytes stocked in the seminal fluid, there is no evidence of cellular abnormality seen. While in group 2 and 3 with Low and middle dose, there was no obvious cellular abnormality, though there is slight area of interstitial and tubular constriction as compared to control group and in group 4 (High dose), revealed cellular abnormality with evidence of cellular proliferation, abnormalities in the semniferous tubules, interstitium and epithelial lining as compared to control group.
In conclusion, *Picralima nitida* seeds does not pose cellular abnormality at low dose level when it is consumed with cautions, however prolong intake at high concentration has deleterious and adverse effect on cytoarchitecture of caudal epidydimis of male Adult wistar rats.
Keywords: *Picralima nitida*, Histopathology, wistar rat, and Caudal Epidydimis .

1.0 INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. The use of herbs in the treatment of ailments in Africa is an age long practice. The decreasing efficacy of synthetic drugs, cost, accessibility and increasing contradiction of their usage make the usage of natural drugs topical (Petrovska, 2012) .There is a growing awareness by scientific and medicinal communities of the importance of medicinal plants in the health care system of many developing countries (Ame and Salah, 1995) .Traditionally, it is believed that natural products are safe (Said *et al.*, 2005). This assumption to a large extent has influenced the indiscriminate use of these formulations by many particularly among the rural populace (Mbaka *et al.*, 2010). The safety of herbal medicines is of particular importance because the majority of these products is self-prescribed and is used to treat minor and often chronic conditions. Man's continuous reliance on herbs for therapeutic and nutritional benefits cannot be overemphasized. The ethno-medicinal and nutritional uses of wild plants are as old as men as they are sources of treatment, food security and income generation (Akubugwo *et al.*, 2007 ; Antia *et al.*, 2006; Ifon and Basssir, 1979). These wild plants serve not only as indispensable constituents of human diet but also as important medicinal tools for the treatment of various disease conditions (Aguwu *et al.*, 2010; Suresh *et al.*, 2008; Okorondu *et al.*, 2006).Traditional societies have over the years employed **medicinal plant**s in ethno-medicine for the treatment of various diseases without scientific knowledge of the physiologically active ingredients called phytochemicals which were responsible for the plants' medicinal and pharmacological potentials (Aja *et al.*, 2010; Akubugwo *et al.*, 2007; Adimoelja, 2000).

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 Epidydimis 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 Epidydidmis.

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.

Picralima nitida is an under storey tree in rainforest, also in mature secondary forest and semi-deciduous forest along river banks, up to 900 m altitude.

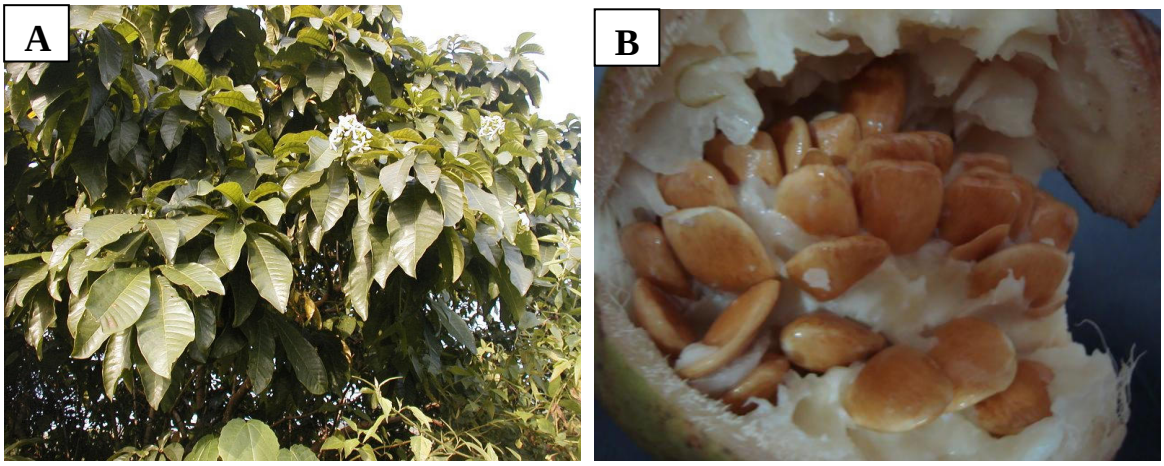

Figure 1a and 1b: *Picralima nitida* Plant and seeds within its fruit. Source: (www.gjournals.org)

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 function of the epididymis, including production of the epididymal specific microenvironment necessary for the maturation, storage, and survival of spermatozoa, is regulated by hormones and testicular growth factors (Swider- Al-Amawi *et al.,* 2010). More recently, it has been hypothesized that both testicular cancers and sub-fertility may be caused by the exposure of the developing male embryo to agents that disrupt normal hormonal balance (Sharpe & Skakkebaek, 1993; Sharpe, 2003; Izegbu *et al.,* 2005).

The epididymis is an important part of male reproductive system. It is a highly convoluted tubule that links the rete testes and the ductus deferens. The primary functions of transport, maturation and storage of spermatozoa released from the germinal epithelium of the seminiferous tubules are served by it (Flickinger *et al.,* 1978; Adebayo and Olurode, 2010). The acquisition of fertilizing capacity by the spermatozoa in the epididymis is an active process in a way that they have to be subjected to the micro environment of epididymis which is essentially controlled by the epididymal epithelium as it is responsible for the synthesis of proteins and sialic acid which are directly poured into the lumen (Chinoy *et al.,* 1995; Johnson *et al.,* 2000). Contraceptive efficacy of a number of plants have been studied in various animals (Lohiya *et al.,* 2002; Verma *et al.,* 2006; Jahan *et al.,* 2009; Mishra *et al.,* 2009; Abu *et al.,* 2012).

However, the information on the effect of ethanol extracts on the epididymal histology which play important role of conferring maturity to spermatozoa is 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 epidydimis.

This study revealed possible effects of ethanolic extract of *Picralima nitida* seed on the histomorphological architecture of the Epididydimis of adult male wistar rat. Histologists, clinicians and histopathologists will benefit from the findings and the outcomes could be widely used in drug design for male reproductive therapy, also to reveal the efficiency and mechanism of action of the extract on histomorphological architecture of caudal epididymal form of wistar male rat.

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^{o}C. The final extract weighing 100.2 grams, was collected in a semi-solid form and stored in a refrigerator at 4^{o}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.

Caudal Epidydimis 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 and histochemical 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.

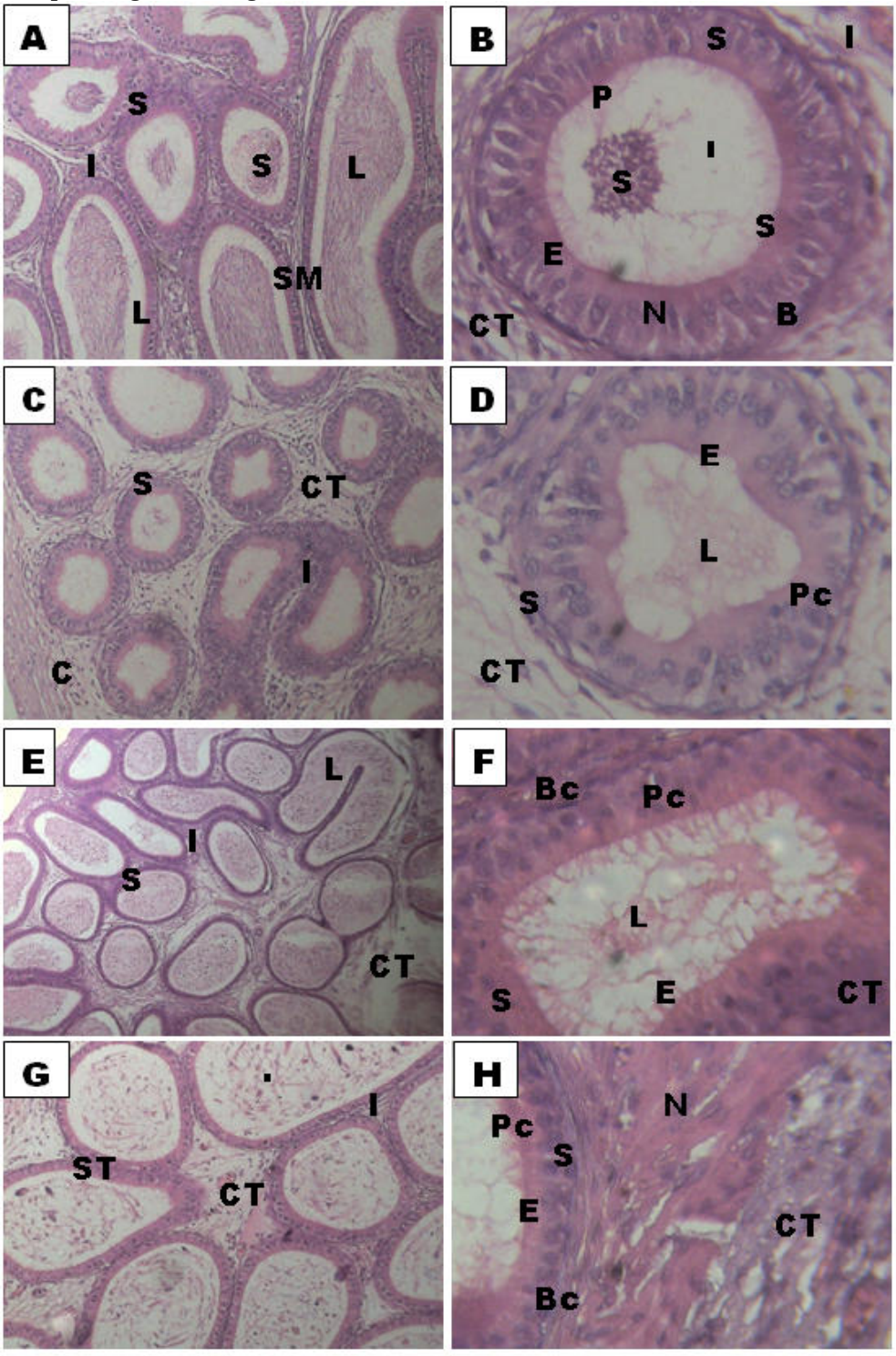

PLATE 1- A&B Photomicrographs of Epydidymis without administeration of the extract.of Picralima .Nitida**.**
PLATE 2- C&D Photomicrographs of Epididymis administered with 250mg/kg of the extract. of Picralima .Nitida
PLATE 3- E&F Photomicrographs of Epididymis administered with 350mg/kg of the extract. of Picralima .Nitida
PLATE 4- G&H Photomicrographs of Epididymis administered with 450mg/kg of the extract. of Picralima .Nitida.

Keys: ***ST*** *–Seminiferous Tubule,* ***L****- Lumen, S-Semen,* ***Bc*** *–Basal cell,* ***Pc*** *– Principal cells,* ***N*** *–Nucleus,* ***EL*** *–Epithelial lining,* ***CT*** *– Connective Tissues* ***SM****- Smooth muscle and* ***I****- Interstitium.*

Group 1 – Plate A(X100) and B(X400) of the **Control** Epididymis administered with normal rat feed and water revealed normal cellular architecture of seminiferous tubules containing distinct Basal and Principal cell, distinct area of interstitium, the tubules were lined with Pseudo-stratified squamous epithelial cell with goblet cells enclosed in it are the spermatocytes stocked in the seminal fluid, there is no evidence of cellular abnormality seen

GROUP 2 - Plate C(X100) and D(X400) of Epididymis treated with Low dose of 250mg/kg ethanolic extract of *Picralima nitida seed* revealed no cellular abnormality, though there is slight area of interstitial and tubular constriction as compared to control group.

GROUP 3 - Plate E(X100) and F(X400) Epididymis treated with Low dose of 350mg/kg ethanolic extract of *Picralima nitida seed* revealed no cellular abnormality, though there is slight area of interstitial and tubular constriction as compared to control group.

GROUP 4 - Plate G(X100) and H(X400) Epididymis treated with High dose of 450mg/kg ethanolic extract of *Picralima nitida* revealed cellular abnormality with evidence of cellular proliferation in the seminiferous tubules as compared to control group.

4.1 DISCUSSION

The Histopathological characteristics of the Epidydimis observed in cross sections of the cauda epididymis of both the control and experimental groups as illustrated in Plate 1 to 4 photomicrographs is an indication that the ethanol extract of *Picralima nitida* seed is not apparently harmful to the epithelium which is important in the synthesis of proteins and sialic acid of the epididymal fluid (Hinton and Palladino, 1995; Turner *et al.*, 1995), at low dose and middle dose but proved to be harmful at a very high concentration or dose levels (Plate 4 A and B). It is well known that the secretion of various proteins by the principal cells of the epididymis into the epididymal lumen influences sperm maturation (Verma and Chinoy, 2001; Almeida et al., 2006). Also, scanty collection of late spermatids in the lumen of the cauda epididymis of the same high dose experimental group treated with 450mg/kg of the extract results from the arrest of spermatogenesis at the germinal epithelium which is consistent with the findings of other investigators (Lohiya *et al.*, 1994; Udoh and Kehinde, 1999; Pathak *et al.*, 2000; Sharma *et al.*, 2001; Verma and Chinoy, 2001; Lohiya *et al.*, 2006).

4.2 CONCLUSION

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 caudal epidydimis which play a vital roles in sperm maturation and transportation, thereby jeopardizing its 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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