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EPIDIDYMAL EFFECT ON WISTAR RATS TREATED WITH ETHANOLIC EXTRACT OF *Sida acuta* LEAVE

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Abstract: Ethanolic leaf extract of *Sida acuta* on the epididymis of adult wistar rats was evaluated in this study. Twenty five rats weighing between 180 – 220 g were assigned to five groups (control, olive oil control, low dose, medium dose and high dose) with five animals each. Normal control fed only on rat chow, olive oil control group received 0.5ml of olive oil. The experimental groups, low dose, medium dose and high dose received 500mg/kgBW, 1000mg/kgBW 1500mg/kgBW of the extract orally for 60days respectively. The animals were sacrificed at end of sixty days using chloroform anesthesia. The epididymis were dissected, sectioned for hematoxylin and eosin (H&E). Histological observations of the epididymis, showed dose dependent distortion of the normal cytoarchitecture of the organs, as the high dose revealed prominent distortion. This study thus suggests that consumption of *Sida acuta* at high dose may cause adverse effect on reproductive parameters and organs.
Keywords*: Sida acuta, epididymis, wistar rat, Histology*

INTRODUCTION

In diversity, plants are thought to be between 250,000 to 400,000 species spread across all continents from the Antartic to the Artic (Adesuyi*et al.,* 2011) and Africa has arguably one of the richest phytodiversities in the world. Africa's forests geographically span approximately 216, 634,000ha (Ijeh and Ejike, 2011). About 13,000 plant species are medicinally important worldwide. 3500 plant species in India are useful as a source of crude drug. About 2500 plants are ethnomedicinally important. Out of it, genus *Sida*is one of such important group of plants (Wake, 2012).

Traditional knowledge to solve health problems of mankind and animals exists in all countries of the world, with history dating back to as long as 3000 BC years ago (Sofowora, 1982; Rukangira, 2001). In most of the traditional medicine, the medicinal plant include the fresh or dried part, whole, chopped, powdered or an advanced form of the herb usually

made via extraction by a solvent such as water, ethanol or an organic solvent which play a major role and constitute the backbone of traditional medicine (Mukherjee, 2002). This system has undergone numerous transformations according to the prevailing cultural, traditional and social indices in the community but what has remained as a recurrent decimal across regions is the continuous interest by the scientific community into the proper identification of the relevant plant/herbs that are useful.

The male reproductive system is divided into factors that affect male function. These include: brain centers, which control the release of hormones from the pituitary and sexual behaviour; gonadal structures, which produce sperm and hormones; a system of ducts, which store and transport sperm; and accessory glands, which support viability of the sperm (Jayasurya, 1984). The main function of the male reproductive system is spermatogenesis, which results in the formation of the spermatozoa. Local regulation of spermatogenesis is carried out by extra-testicular stimuli provided by the hypothalamus and pituitary gland(Holstein *et al.*, 2003) which together with the testis form the hypothalamic-pituitary-gonadal-axis. Spermatogenesis is regulated by the pulsatile release of Gonadotropin-releasing Hormone (GnRH) from the arcuate nucleus of the hypothalamus. This then stimulates the release of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the anterior pituitary. The LH stimulates the Leydig cells to produce testosterone, which acts locally on the interstitium and seminiferous tubules, resulting in sperm production and maturation. FSH acts directly on the Sertoli cells to promote spermatogenesis (Manetti and Honig 2010).

Sida acuta is a small erect, much branched, perennial shrub or herb, ranging from 30 to 100cm in height with a strong tap root; stem and branches flattened at the extremities, fibrous, almost woody at times, leaves alternate, slender, lanceolate, acute, margins toothed, 1.2 to 9cm or longer, 0.5 to 4cm wide, lower surface smooth or with sparse, short branched starlike (Stellate) hairs with fairly prominent veins; petiole 3 to 6mm long, hairy with a pair of stipules at least one lanceolate- linear 1 to 2mm broad, three to six nerved, often curved, finally hairy, the other stipule narrower, one to four nerved.

The medicinal values of *Sida acuta*plant lies in some chemical substances that produce definite physiological actions on the human body. Some of

these bioactive constituents of plants are classified as alkaloids, tannins, flavonoids, saponins, phenolic compounds; and other compounds reported to possess diverse range of bioactivity (Edeoga*et al.*, 2005; Iwalewa*et al.*, 2007).

The epididymis is a tube that connects a testicle to a vas deferens in the male reproductive system. It is present in all male reptiles, birds, and mammals. It is a single, narrow, tightly-coiled tube bearing the length of 6 to 7 meters in adult humans, (Kim and Goldstein, 2010) connecting the efferent ducts from the rear of each testicle to its vas deferens. The epididymis can be divided into three main regions: The head which receives spermatozoa via the efferent ducts of the mediastinium of the testis. It is characterized histologically by a thin myoepithelium.

MATERIALS AND METHOD

Extract preparation

Fresh *Sida acuta* leaves was harvested from farms in Ugep, Yakuur Local Government Area of Cross River State and taken to the herbarium unit of the University of Calabar, Calabar for proper identification and authentication. The leaves were washed with water to remove debris and sand, air driedand grounded into powder using a table grinder and kept in air tight containers under dry conditions.

Modified method of Abdulrahman*et al.,* (2004) was used for extraction. Two hundred grams of grounded *Sida acuta* leave was macerated in 1000ml absolute ethanol for 72 hours properly covered and labeled. The extract was then be filtered with sterile filter paper (Watman No. 1). The filtrate was evaporated to dryness at 40°C in a vacuum using a rotator evaporator and stored at 50°C in a refrigerator until required for use. Approximate concentration of the extract was dissolved in 10ml of olive oil. The olive oil was used as a vehicle.

Experimental animals

The study constituted a total of twenty five male Wistar rats (8-10 weeks old, weighing180 -220 g) which was obtained from the Department of Pharmacology, University of Calabar, Calabar. The rats were housed in wire-wooden cages under controlled light schedule (12-hours light and 12-hours dark cycle) and were fed standard rat chow and water *ad libitum*. The animals were allowed to acclimatize for 2 weeks before the startof the administration.

Experimental design

The rats were randomly divided into 5 groups each containing 5 rats.Control group was fed with standard rat chow and water*ad libitum* without any administration of the extract. Olive oil control group received 0.5 ml of olive oil throughout the duration of the experiment. The low dose group received 500mg/kgBw, medium dose group received 1000mg/kgBw while the high dose group received 1500mg/kgBw of the ethanolicextract of *Sida acuta* orally by means of orogastric tube daily, for 60 days respectively

Termination of experiment

At the end of treatments, the animals were sacrificed under chloroform anesthesia. Epididymis were dissected out and fixed in Bouins fluid for 24hours for routine histological study.

Histological studies

The epididymis of the rats were removed under chloroform anaesthesia, preserved in 10% formal saline for 48hours and then dehydrated through ascending grades of alcohol, 2 changes of 70%, 90%, 95 %and absolute alcohol for one hour in each change. After dehydration the tissue were cleared in xylene, 3 changes, 1 hour in each. The tissues were placed in two 2 changes of paraffin wax for 20 minutes each for paraffin wax impregnation in an oven at 57 degree Celsius. They were embedded in molten paraffin wax inside the L shaped Leuckhartmould. The blocks were trimmed and mounted on wooden blocks. Serial sections were cut using a rotatory microtome at 5 thickness. Sections were floated in a water bath to spread out and later picked on albuminised slides and dried on a hot plate at 52 degree celcius. To stain, slides were put in staining racks and placed in staining wells containing xylene to dewax, then they were rehydrated in descending grades of alcohol, absolute alcohol (2 changes), 70% alcohol and then water for 5 minutes after which they were stained with haematoxylin for 5 minute. Excess haematoxylin was washed off with water and differentiated with 1% acid alcohol. Sections were counter stained with 1% eosin and washed off with water. They were dehydrated with 70%, 90% and absolute alcohol and finally cleared in xylene to remove water. A drop of mountant was placed on the surface of the slides and covered with a 22 by 22mm cover slip and observed under a light microscope.

RESULT

Histological study of the epididymis using haematoxylin and eosin staining method showed in the control group, normal histological features. The surface epithelium with stereocilia and sperm cells were found in the lumen spanning most of the surface area. The epithelial cells were supported on a basal lamina surrounded by smooth muscle cells, when contracted help to move the sperm along the duct and by loose connective tissues. (Plate 1)

Histological section of epididymis in the olive oil control group showed surface epithelium with stereocillia and lumen filled with sperm cells. The epithelial cells were supported on a basal lamina surrounded by smooth muscle cells and by loose connective tissues. There was no pathology seen (Plate 2). The animals in the low dose group which received 500mg/kgBw of *Sida acuta*ethanolic leaf extract, the epididymis showed surface epithelium with stereocilia surrounded by connective tissue and smooth muscle. The lumen was filled with matured sperm cells. No pathology was observed when compared with the control groups (Plate 3).

The epididymis in the medium dose group that received 1000mg/kgBw of the extract showed surface epithelium with stereocilia surrounded by connective tissue and smooth muscle. The storage centers showed early stages of granulomatous response, (Plate 4). Animals in the high dose group that received 1500mg/kgBw of ethanolic leaf extract of *Sida acuta*the epididymis showed surface epithelium with overlying stereocilia surrounded by connective tissue and smooth muscle. Part of the lumen showed onset of granulomatous response that destroys stored sperm cells. There was observable pathology when compared to the control and other treatment groups (Plate 5).

DISCUSSION

Histological features of epididymis in the normal, olive control groups, low and medium groups animals that received rat chow, 0.5ml of olive oil, 500mg/kg Bw and 1000mg/kgBw of ethanolic leaf extract of *Sida acuta* showed normal cytoarchitecture of the epididymis. The high dose group treated with 1500mg/kgBw of the extract showed cellular degradation evidenced in the lumen showing onset of granulomatous reaction that leads to destruction of stored sperm cells. This indicates the cytotoxic effect of *Sida acuta*on the epididymis which causes the degeneration of epididymal epithelium, necrosis and exfoliation of principal cells. This

effect is capable of reducing testosterone biosynthesis by the leydig cells (Wilma and Gary, 2014).

CONCLUSION

It is suggestive from the result of the study that consumption of *Sida acuta* at high dose may cause adverse effect on reproductive parameters and organs.

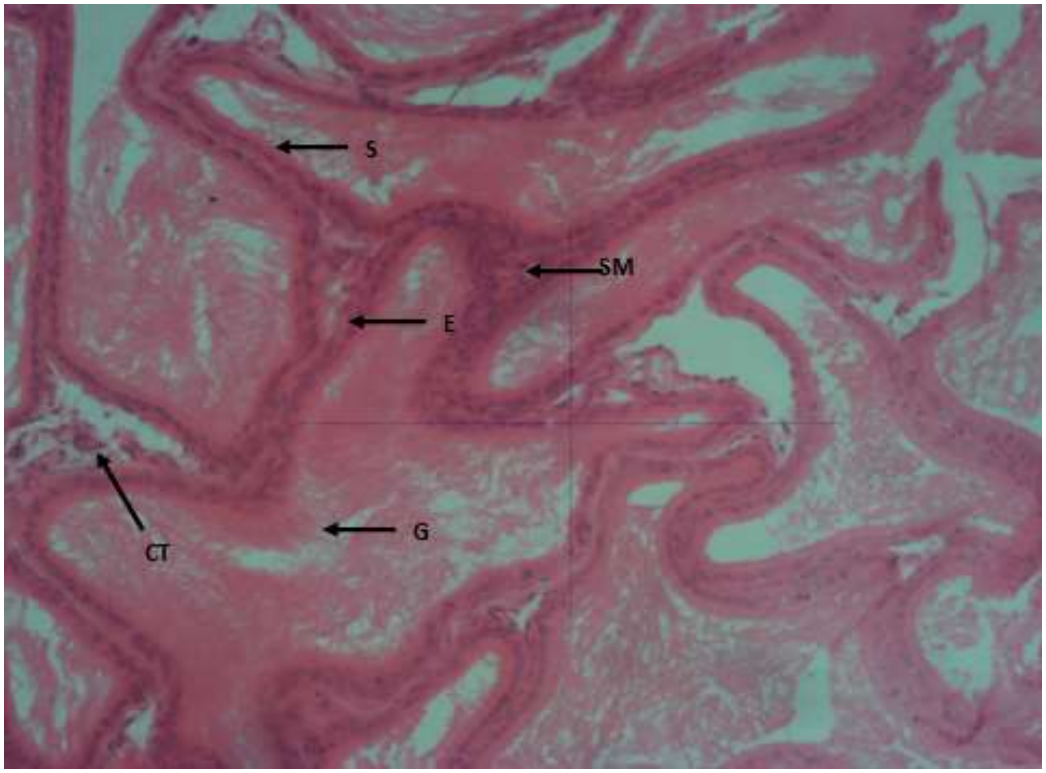

PLATE 1: Photomicrograph of epididymis of control group showing the surface epithelium (E) with stereocilia (S). The sperm cells (S) are seen in the lumen (G) spanning most of the surface area. The epithelial cells are supported on a basal lamina surrounded by smooth muscle cells (SM) and by loose connective tissues (CT). No pathology seen. X400.H & E

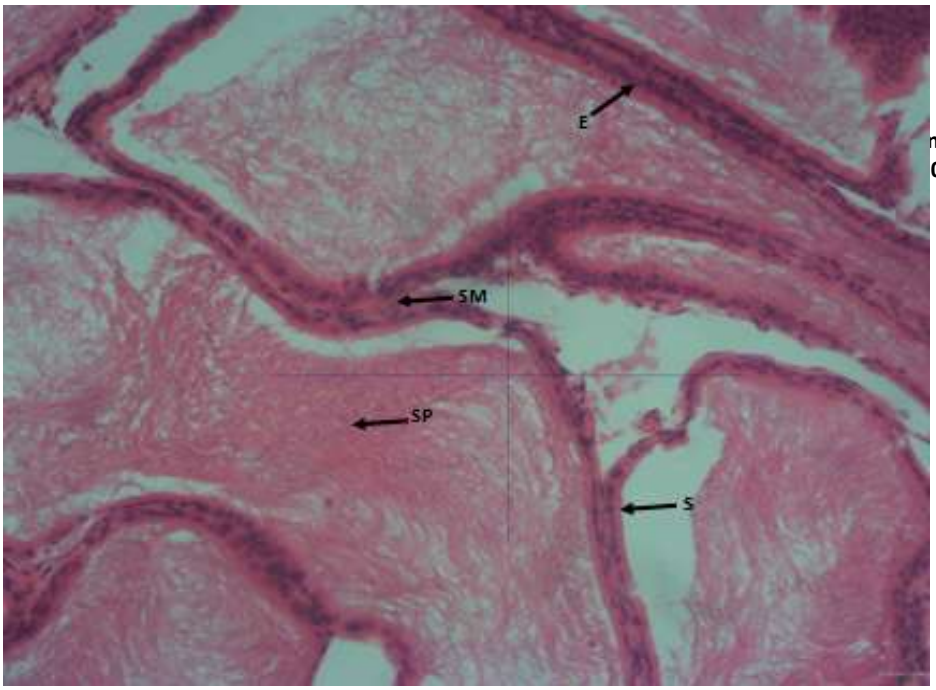

PLATE 2: Photomicrograph of epididymis of olive oil control group showing the surface epithelium (E) with stereocilia (S). The lumen filled with sperm cells (SP). The smooth muscle cells (SM) are intact. No pathology seen. X400. H & E

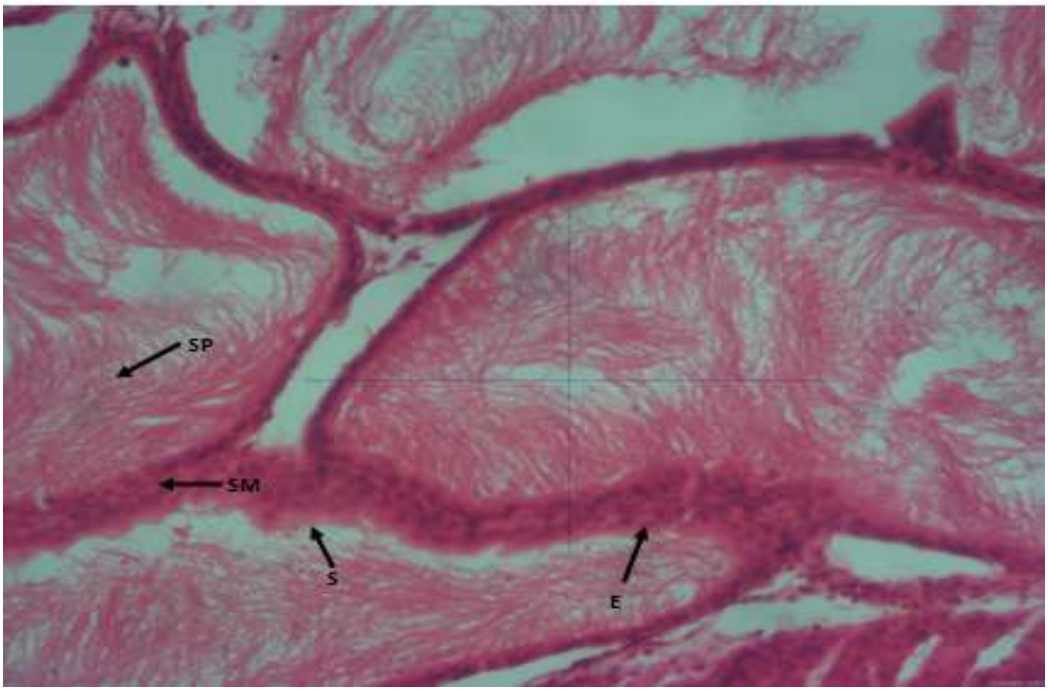

PLATE 3: Photomicrograph of epididymis of low dose group that received 500mg/kgBw of *Sida acuta*ethanolic leaf extract showing the surface epithelium (E) with stereocilia(S) and lumen filled with sperm cells (SP). The smooth muscle cells (SM) are also seen. No pathology seen. X400. H & E

PLATE 4: Photomicrograph of epididymis of medium dose groupthat received 1000mg/kgBw of the extract showing the surface area comprising the epithelium (E) and the stereocilia (E). Storage centers show early stages of granulomatous response (G). smooth muscle area (SM) appear normal. No pathology seen. MAG: X400. H & E

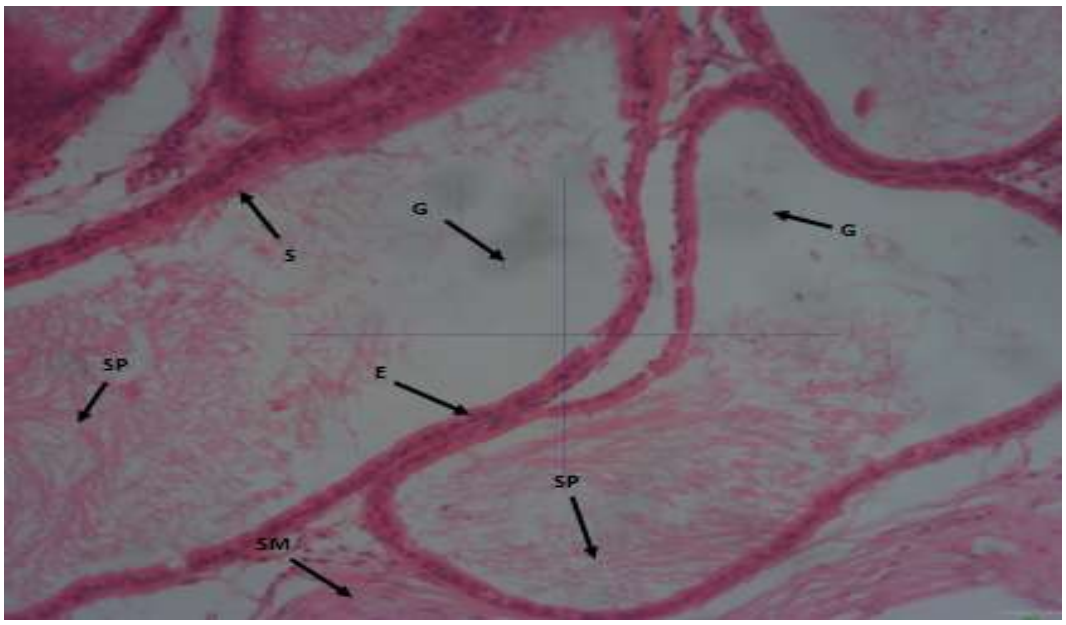

PLATE 5: Photomicrograph of epididymis of high dose group that received 1500mg/kgBw of ethanolic leaf extract of *Sida acuta* showing the surface epithelium (S) with the overlying stereocilia (S). Part of the lumen shows onset of granulomatous response (G) that destroys stored sperm cells (SP). The smooth muscle area (SM) appears normal. X400. H & E

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