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HISTOLOGICAL EFFECTS OF AQUEOUS LEAF EXTRACT OF CHAYA (CNIDOSCOLUS ACONITIFOLIUS) ON THE TESTES AND EPIDIDYMIS OF ADULT WISTAR RATS

SAKPA CHRISTOPHER LUCKY* UCHE-NWACHI O. EDWARD*

Abstract

The histological effects of aqueous leaf extract of Chaya (Cnidoscolus aconitifolius) on the testes and epididymis of adult wistar rats were studied. The rats were acclamatized for two weeks, weighed and assigned to four groups (A – D) of six rats in each group. The leaf extract was administered by gavage to each animal in treatment groups B, C and D at a dose of 1.5g/Kgbwt for two, four and eight weeks respectively. Group A served as control and had 1ml of sterile water by gavage daily. At the end of each study period, animals were sacrificed and the testes and epididymis were dissected out for histological studies. The tissues were weighed and then fixed in Boin's fluid, processed and stained with haematoxylin and eosin. Results showed significant decrease ($P<0.05$) in mean testicular and left epididymal weights for groups C and D animals with mild to severe disruption in the architecture of the testes and epididymis in all the treatment groups. There was also progressive disruption in the spermatogenic cell series with early release of immature sperm cells into the seminiferous tubules and epididymis.

Introduction

The paired testes are the male gonads which produce spermatozoa and secrete the sex hormones (testosterone and estrogen). However, these two products come from different regions of the testes.[1] The region of the testes that produces spermatozoa are the seminiferous tubules. Each testes has 250 to 1,000 seminiferous tubules. When unravelled, the seminiferous tubules are seen to begin blindly or by anastomotic loops. The tubules in rats are described as closed loops, opening at both ends into the tubuli recti.[2-4]

The seminiferous tubules consist of a tunic of fibrous connective tissue, a well-defined basal lamina, and a complex germinal or seminiferous epithelium. The seminiferous epithelium consists of two types of cells. The sertoli or supportive cells and cells that constitute the spermatogenic lineage. These later cells are stacked in 4 – 8 layers and are responsible for producing spermatozoa.[3]

Interstitial cells of Leydig secrete the hormones, testosterone and oestrogen. In the adult testes, Leydig cells occur as isolated or clustered cells in the spaces between seminiferous tubules in association with connective tissue, nerves, blood and lymphatic vessels.[5]

In most species, there are two generations of leydig cells; a foetal generation and an adult generation. [6,7] The foetal generation is stimulated by chorionic gonadotropin to secrete testosterone during gestation.[6] The epididymis temporarily stores spermatozoa produced in the testes before it is conducted to the female reproductive tract.

KEYWORDS: Cnidoscolus aconitifolius, Wistar rat, Testes, Epididymis.

Correspondence to:
Dr Sakpa Christopher Lucky,
Department of Anatomy, University of Benin,
Benin City, Nigeria. E-mail: sakpachristopher@yahoo.com

Cnidoscolus aconitifolius is commonly called Chaya or tree Spinach. It is a perennial shrub of the family Euphobiaceae commonly found in the tropics. It is a nutritious leafy vegetable crop domesticated in precolonial times and continues to be used today as food, medicine, a living fence post and ornamental plant by at least ten Maya groups as well as many Mexican and Meso-american Peoples.[9] It is one of the most productive green vegetables eaten in south western Nigeria where it is called Iyana Ipaja.[10] It is also eaten by the inhabitants of south eastern Nigeria where it is called "Hospital too far".[11]

Studies have shown that fresh Cnidoscolus Aconitifolius leaves contain per 100gram components including moisture (72.1 to 83.0), protein (4.17 – 6.82), fat (1.72 – 2.87), crude fibers (2.47 – 3.84), total carbohydrates (8 – 13), ash (2.5 – 2.8). Others are calcium (141 – 497mg), phosphorus (69 – 98mg), Iron (2.4 – 4.7mg), β- Carotene (10 – 18mg), ascorbic acid (287 – 318mg), hydrogen cyanide (27 – 42mg), [12] thianin (0.2mg), riboflavin (0.4mg), niacin (1.6mg).[13]

More recent studies have shown the presence of alkaloids, saponins, tannins and cyanogenic glycosides. This same study confirmed the presence of a cytotoxic component in the hot alkaloid fraction (AFH) of the leaf of Cnidoscolus aconitifolius.[14]

A wide variety of claims have been made as to the medicinal efficacy of Chaya as a treatment for numerous ailments, ranging from the ability to strengthen fingernails and darken grey hair. .[15] It has also been associated with anti-contraceptive properties.[16]

Inspite of these medicinal claims, more recent studies have shown that alcohol extract of Cnidoscolus aconitifolius is toxic and caused bone marrow destruction in a dose dependent pattern.[17] The ethanol leaf extract has also been shown to exhibit cytotoxic activity.[14] The present research was aimed at evaluation of the effect of the plant on the testes and epididymis and its potential on male fertility.

Materials and Methods

Fresh leaves of Cnidoscolus aconitifolius were collected from the premises of the Department of Biochemistry, University of Benin, Benin City and were identified and authenticated at the Forestry Research Institute, Ibadan, Nigeria and registered as FHI 109528 in the Herbarium. The aqeous leaf extract was prepared according to the method of Salahdeen, 2006.[18] The dose used for this study was 1,500mg/kg/body weight (mg/Kg.bwt) which was less than the calculated LD_{50} value for Cnidoscolus aconitifolius.[18]

Twenty four adult male wistar rats weighing 250- 300g bred in the animal house at the Department of Anatomy, University of Benin were used for this study. Animals were acclimatized for two weeks, weighed and assigned into four groups (A – D). The rats were fed with standard rat chow (Bendel Livestock Feed, Edo State, Nigeria.) and had free access to water throughout the period of the experiment.

Group A served as control. In addition to the normal feed and water, they had 1ml of sterile water daily by gavage to serve as control for stress of administration while groups B, C, and D represented two, four, and eight weeks treatment groups respectively. The study rats received 1.5grams/kg.bwt of the aqueous leaf extract daily by gavage.

At the end of each study period, the rats were anaesthetized with chloroform. The testes and epididymis were exposed through a ventral midline abdominal incision and dissected out. The organs were

fixed in Boin's fluid, processed and stained with Haematoxylin and Eosin. The slides were examined under a trinocular microscope with camera device (Procam D588E, Ningbo Guangbo Digital Technology Co. Ltd., China.) and photographs were taken at X400 magnifications and prepared as photomicrographs for study of histological changes.

Results

Weight of rats

Figure I showed the initial and final weight of rats in both the control and treatment groups. There was progressive increase in weight in all the groups throughout the period of experiment.

Testicular and Epididymal weight

Table I showed comparison of the right and left testicular weights of rats between the treatment and control groups while table II showed comparison of right and left epididymal weights of rats in the different treatment groups and control. There were statistically significant differences ($P<0.001$) in the mean right and left testicular weights, and left epididymal weights between the control and treatment rats. However, there was no statistical difference ($p=0.535$) between the right epididymal weight of all groups studied.

Seminiferous tubules

Figure IIa showed a section of seminiferous tubule of control rats with normal architecture and the spermatogenic cell series arranged sequentially. The lumina are filled with tufts of tails of spermatozoa.
Figure IIIa showed mild disruption of the epithelium and sequential arrangement of the cells of the spermatogenic series. The section also showed tufty tails of spermatozoa in the lumen of the seminiferous tubule.
Figures IVa and Va showed severe disruption of the epithelia and sequential arrangement of cells of the spermatogenic series in the seminiferous epithelium.
Figure IVa showed mainly immature spermatogenic cells in the lumen of the section while figure Va showed the lumen to be empty.

Caudal epididymis

Figure IIb showed sections of the caudal epididymis of control rats. The epididymal tubule demonstrated normal architecture and was lined by pseudostratified columnar epithelium of the ciliated variety and their lumina are filled with spermatozoa.
Figure IIIb showed section of the caudal epididymis for two weeks treatment group. The section showed normal architecture similar to that of the control but with widened spaces between the contained spermatozoa in the lumen and the epithelial lining.

Figure IVb showed sections of caudal epididymis containing clump of immature cells of the spermatogenic series. The epithelium appears atrophied with a widened space between it and the luminal content while figure Vb showed metaplasia of the cells of the epithelium with an empty lumen.

Discussion

This study showed that the oral administration of the aqueous extract of Cnidoscoulus aconitifolius for two, four and eight weeks at a dose of 1.5g/kgbwt resulted in progressive weight increases in the tested rats. The increase may be due to possible nutritional value of the plant.[20,21] It may also be as a result of natural growth phenomenon.

Observations from the testicular and epididymal histological studies showed progressive disruption in the architecture of the seminiferous tubules and in the sequential arrangement of the spermatogenic cell series in the epithelium of the treatment rats in groups B, C and D. By the fourth and eight weeks of the experiment, the disruptions had become quite severe with clumps of immature spermatogenic cells in the lumen of both the seminiferous tubules and epididymis at four weeks and empty lumina at eight weeks.

Appendix

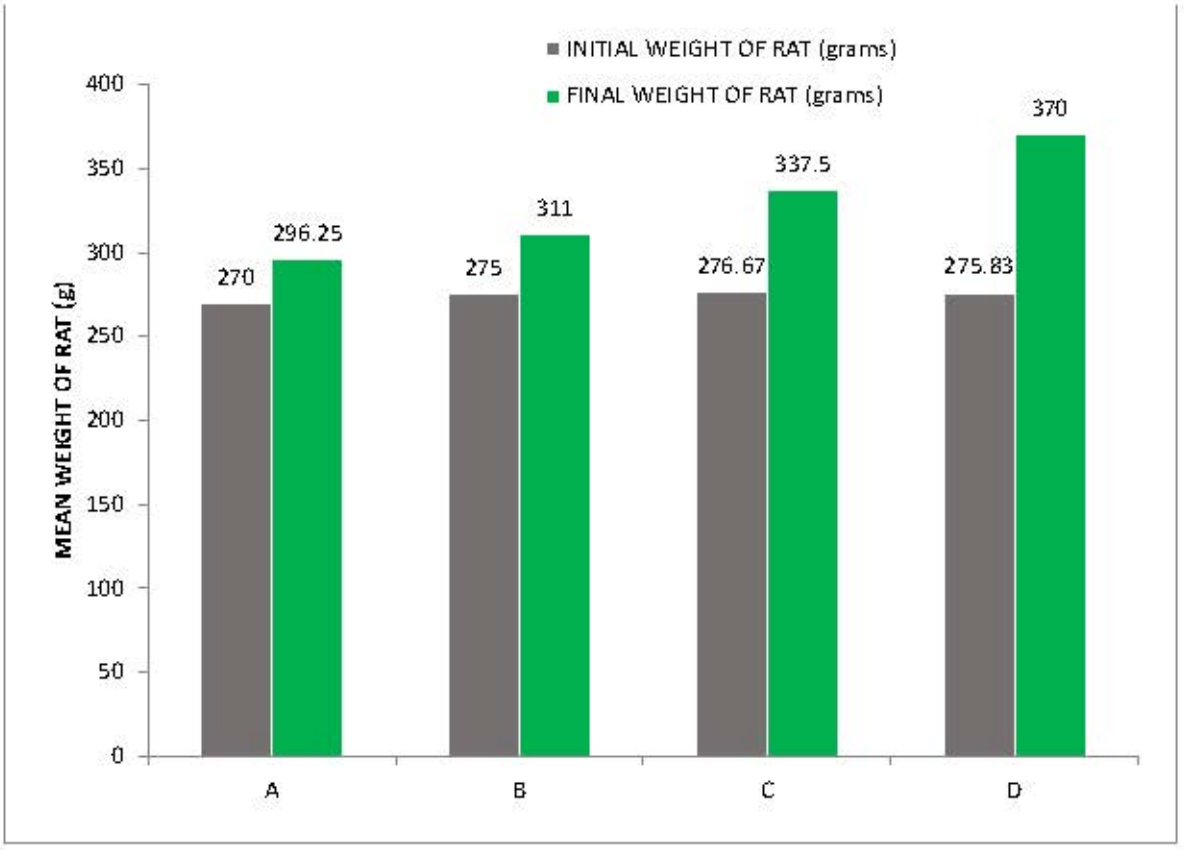

Figure I: MEAN INITIAL AND FINAL WEIGHT OF RATS {group A (control), group B, C and D (treatment groups at two, four and eight weeks respectively}

Table I: Comparison of the right and left testicular weights of the rats between the treatment groups and control

		Group A	Group B	Group C	Group D	p-value
Right Testicular weight	**Mean ± S.D**	**1.53 ± 0.08**	**1.62 ± 0.07**	**1.18 ± 0.07**	**0.90 ± 0.03**	**<0.001**
Left Testicular weight	**Mean ± S.D**	**1.53 ± 0.13**	**1.59 ± 0.06**	**1.21 ± 0.04**	**0.80 ± 0.02**	**<0.001**

Table II: Comparison of Right and Left Epididymal weights of rats in different treatment groups and control

		Group A	Group B	Group C	Group C	p-value
Right Epididymal weight	**Mean ± S.D**	**0.20 ± 0.22**	**0.18 ± 0.02**	**0.15 ± 0.01**	**0.11 ± 0.01**	**0.535**
Left Epididymal weight	**Mean ± S.D**	**0.20 ± 0.01**	**0.18 ± 0.01**	**0.14 ± 0.01**	**0.10 ± 0.01**	**<0.001**

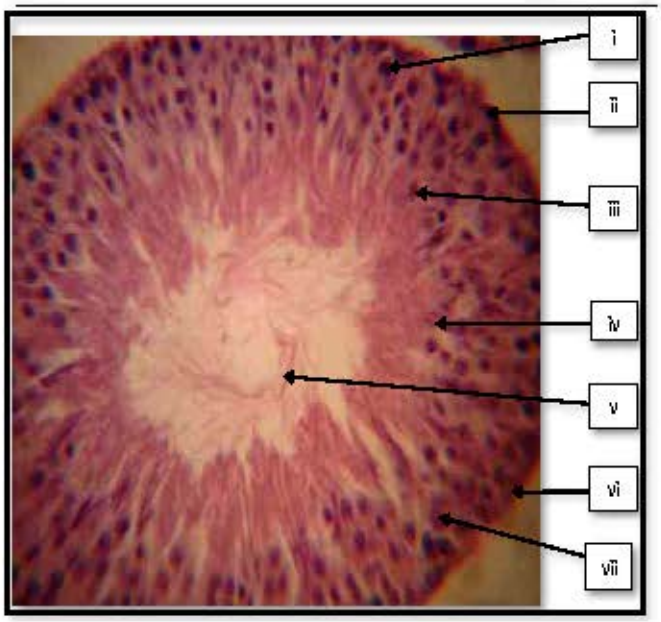

Figure IIa: Photomicrogragh of seminiferous tubule of Control rats (H&E X400): i. Primary spermatocyte ii. Spermatogonium iii: Spermatid iv: Spermatozoa v: tufty tails of spermatozoa vi: Peritubular (Myoid) cell vii: Sertoli cell

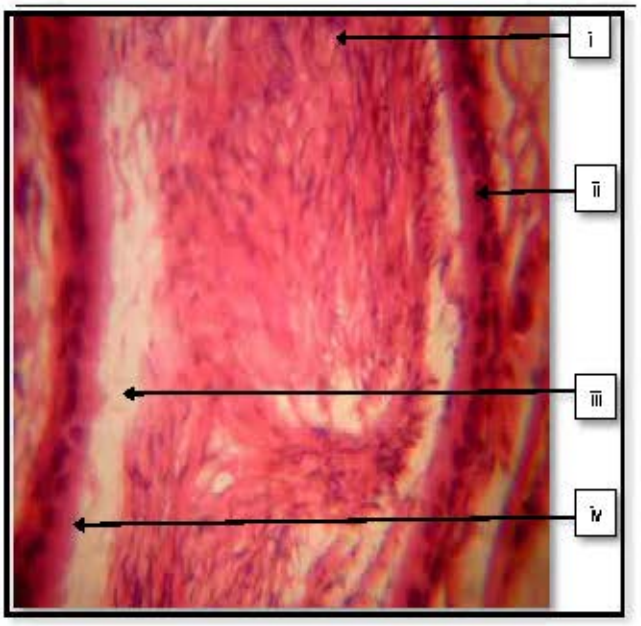

Figure IIb: Photomicrogragh of caudal epididymis of control rats (H&E X400) i: Spermatozoa ii: Nucleus of the epithelial lining of the epididymis iii: Space between the epithelium and the luminal content iv: stecocillia of the wall of the epithelium

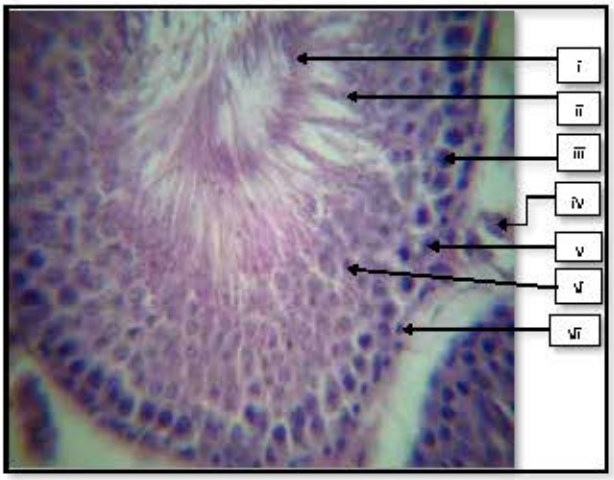

Figure IIIa: Photomicrograph of seminiferous tubules after 2weeks treatment with Cnidoscolus aconitifolius (H&E X400) showing mild disruption in the sequential arrangement of the cells of the spermatogenic series: i: Tufty tails of Spermatozoa ii: Spermatozoa iii: Primary Spermatocyte iv: Interstitial Cells of Leydig v: Sertoli Cell vi: Spermatid vii: Spermatogonia.

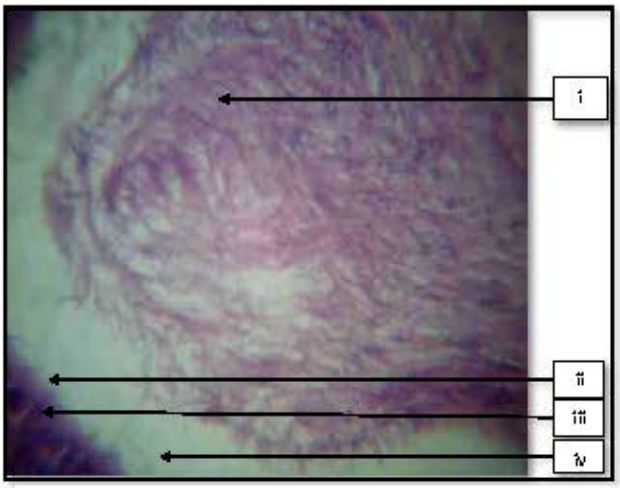

Figure IIIb: Photomicrograph of epididymis after 2weeks treatment with Cnidoscolus aconitifolius (H&E X400): i. Spermatozoa in the lumen of epididymis ii: Stereocilia iii: Nucleus of the epithelium of the epididymis iv: Space between the epithelium and the luminal content

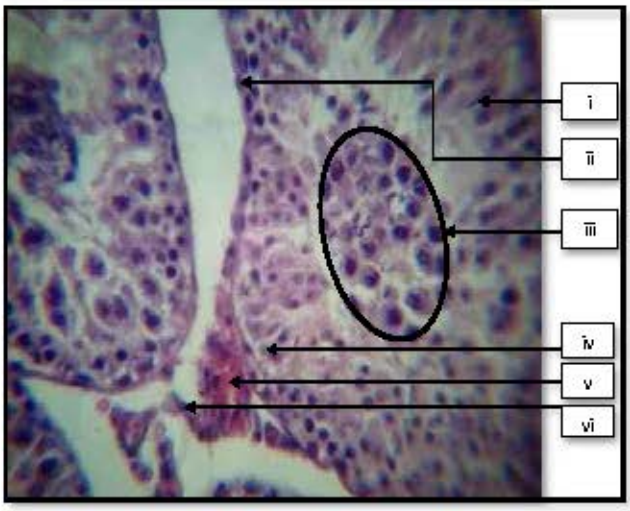

Figure IVa: Photomicrograph of seminiferous tubules after 4weeks treatment with Cnidoscolus aconitifolius (H&E X400) showing i: Spermatozoa ii: Spermatogonium iii: Clump of early spermagenic cells in the lumen of the seminiferous tubule iv: Sertoli cell v. Blood vessel vi. Interstitial (Leydig) Cells

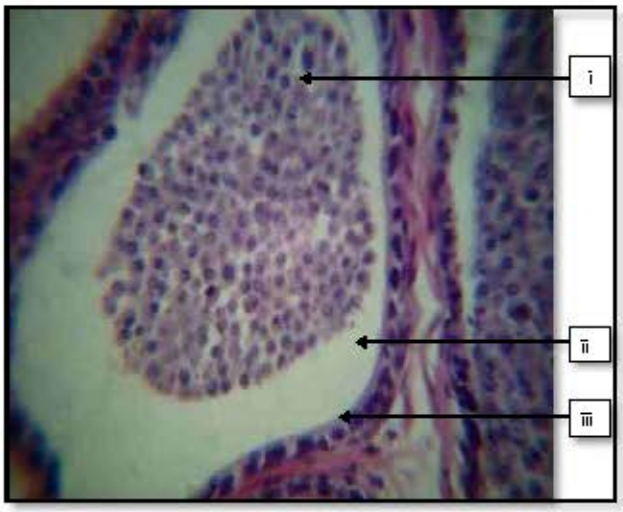

Figure IVb: Photomicrograph of caudal epididymis after 4weeks treatment with Cnidoscolus aconitifolius (H&E X400) showing i: Clump of immature spermatogenic cells ii. Epididymal lumen iii. Epithelial lining of the epididymis.

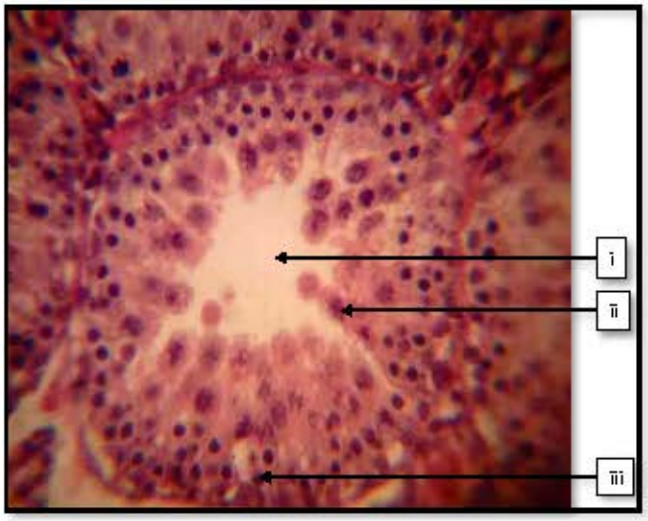

Figure Va: Photomicrograph of seminiferous tubules after 8weeks treatment with Cnidoscolus aconitifolius (H&E X400) i: lumen of epididymis ii: immature spermatogenic cell iii. primary spermatocyte

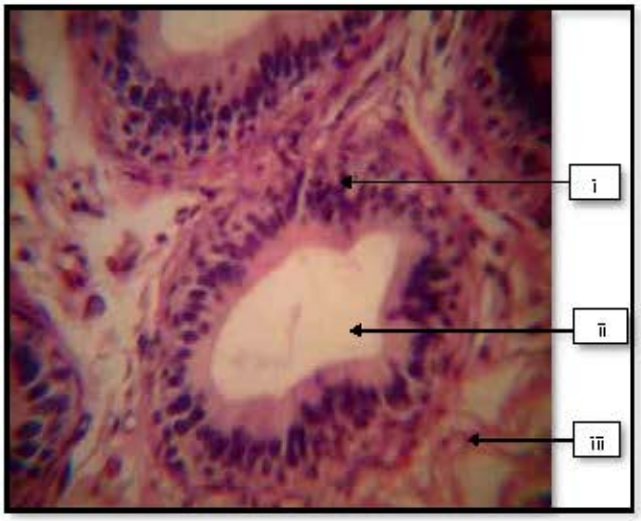

Figure Vb: Photomicrograph of caudal epididymis after 8weeks treatment with Cnidoscolus aconitifolius (H&EX400) showing i. Nucleus of the epididymal epithelium ii. Epididymal lumen iii: connective tissue in the interstitium of epididymis

There was progressive decrease in testicular and epididymal weights for all the treatment rats when compared with the control rats. The decrease for groups C and D rats were significant. A similar study has reported that extracts from different parts of Carica Papaya decreased the testicular weight of wistar rats when administered orally for eight weeks.[23]

The present study has shown that Cnidoscolus aconitifolius is not only associated with progressive decrease in testicular weight but also in epididymal weight which were both duration of treatment dependent. The histological photomicrographs of group C rats showed early release of rounded and elongated spermatids into the lumen of the seminiferous tubules and the presence of these cells in the epididymis (Fig IVa and IVb) Figure Va and Vb representing seminiferous epithelium and epididymis of eight weeks treatment showed arrest of spermatogenesis with failure of spermiogenesis. Consequently the lumina of the seminiferous tubules and epididymis were empty and devoid of spermatozoa, round or elongated spermatids. Previous studies have shown that luteinizing hormone (LH), through specific receptors found on the surface of Leydig cells controls the production and secretion of testosterone.[23,24] Several phases of germ cell development are known to rely on androgen action. The progression of germ cell development from spermatogonia through to the meiotic spermatocytes can occur in the absence of androgens. However, the survival of the pachytene spermatocytes (meiosis II) during the long meiotic prophase and entry into the final meiotic division require androgen action.[25-28]

Therefore, in the absence of androgen signaling via sertoli cells, spermatocytes cannot complete meiotic divisions and no haploid round spermatids are produced. The progression of haploid spermatids through spermatogenesis also relies on androgens and in the absence of androgens, spermatogenesis arrests at steps 7 – 8 due to defects in round spermatids survival and in the ability of the newly elongated spermatids to adhere to sertoli cells.[25-27] The final release of spermatids during the

process of spermiation is also sensitive to androgen and gonadotropin inhibition[32] since germ cells do not contain androgen receptors in the somatic cells. Many functions of the sertoli cells are known to be androgen dependent such as the maintenance of tight junctions at the blood testes barrier [33 – 35] and the production of androgen responsive mRNA[36] are likely to be necessary for supporting germ cell development. These assertions corroborate the histological findings of the present study, in which round and elongated spermatids were prematurely released into the seminiferous tubules and epididymis and these cell lines became absent at later stages (figures Va and Vb).

Conclusion

The findings of this research show that Cnidoscolus aconitifolius caused disruption in the architecture of the testes and epididymis with reduction in testicular and epididymal weights as well as caused progressive disruption in the spermatogenic cell series with early release of immature sperm cells. These effects may subsequently lead to azoospermia, since the seminiferous tubules and epididymis of treated rats at eight weeks were devoid of spermatogenic cells in their lumina.

References

1. Caceci T. Varterinary Histology, the male reproductive system. 2008. [online]. Available from;www.vetmed.vt.edu /education/curriculum/vm8054/Labs/lab27.htm
2. Clermont Y, Huckins C. Microscopic anatomy of the sex cords and seminiferous tubules in growing and adult male albino rats. Am J Anat 1961;108: 79-97.
3. Junqueira L C and Carneiro J. Basic Histology. Text and Atlas. 10th edition. Lange publishers.2003. ISBN: 0-07-137829-4.
4. Roosen-Runge EC. The Process of Spermatogenesis in Mammals. Boll Rev 1961;37: 343-77.
5. de Kretser D, Kerr J. The cytology of the testis. In: The Physiology of Reproduction, eds. Knobil E, Neill JD, Raven Press, New York. 1994: 1177-1290.
6. Pelliniemi LJ, Niemi M. Fine structure of the human foetal testes. The interstitial tissues. Z Zellforsch 1969; 99: 507-22.
7. Lording DW, de Dretser DM. Comparative ultrastructural and histochemical studies of the interstitial cells of the rat testis during foetal and postnatal development. J Reprod Fert 1972; 29:261-69.
8. Huhtaniami I. Studies on steroidogenesis and its regulation in human foetal adrenal testis. J Steroid Biochem 1977; 8:491-97.
9. Ross-Ibarra I, Molina-Cruz A. The Ethnobotany of Chaya (Cnidoscolus aconitifolius spp. Aconitifolius Breckon): A Nutritive Maya Vegetable. Economic Botany 2002;56(4):350-365.
10. Oyaghemi AA, Odetola AA and Azeez OI. Ameliorative effects of Cnidoscolus aconitifolius on anemia and osmotic fragility induced by protein-energy malnutrition. African Journal of Biotechnology 2008; 7(11): 1721-1726.
11. Iwalewe EO, Adewunmi CO, Omisore NO, Adebanji OA. Pro- antioxidant effect of vegetables in South West Nigeria. J Med food.2005; 8:531- 534.
12. Molina-Cruz A, Curley LM, Bressani R. Redesscubriendo el valor nutrirtvo de las hojas de chaya (Cnidoscolus aconitiifolius; Euphobiaceae). Ciencia en Accion, Universidad del valle de Guatemala 1997; 3:1-4.
13. INCAP-ICNND. Tabla de Composici6n de Alimentos para uso en America Latina. Instituto de Nutricion de Centro America Panama, Guate-mala, Guatemala. 1961.
14. Senjobi CT, Moody JO, Ettu AO. Antimicrobial and Cytotoxic Effects of Cnidoscolus aconitifolius. Journal of Agriculture and Biological Sciences 2011; 2(2):21-25.
15. Diaz BJ, Leon DGL. The Chaya plant marvellous food and medicine: Chronicethnobotany.Maya Ethnobotany. Marida, Mexico.1974.
16. Espinosa SAJ, (1985). Plantas Medicinales de la Huasteca Hidalguense. Tesis de licenciatura, Facultdad de Ciencias, UNAM, Mexico, DF.

17. Odokuma EI. Effects of Alcoholic Extract of Cnidoscolus aconitifolius on Bone Marrow Biopsy in Adult Male Wistar Rats: Basic Sciences of Medicine 2012; 1(1):6-8.

18. Salahdeen HM and Yemitan OK. Neuropharmacological effects of aqueous leaf extract of Bryophyllum pinnatum in mice. African Journal of Biomedical Research 2006; 9:101-107.

19. Adebiyi OA, Adebiyi OO, Ilasanmi OR, Raji Y. Sedative effect of hydroalcholic leaf extracts of Cnidoscolous acontifolius. International Journal of Applied Research in Natural Products 2012; 5(1):1-6.

20. Martin FW, Ruberte R. Cnodoscolus chayamansa includes composition and nutritional value, culture in Puerto Rico; vegetables of hot humid tropics. USDA, ARS. New Orleans, LA. 1978.

21. Kuti JO, Konuru HB, (2004). Antioxidant capacity and phenolic content in leaf extracts of tree spinach (Cnidoscolus spp.). J Agric Food Chem. 52(1):117-121.

22. Uche-Nwachi EO, Ezeokoli DC, Adogwa AO, Offiah VN. Effect of water extract of Carica papaya seed on the germinal epithelium of the seminiferous tubules of Sprague Dawley rats. Kaibogaku Zasshi 2001; 76(6):517-21.

23. De Kretser DM, Catt KJ, Paulsen CA. Studies on the in vitro testicular binding of iodinated luteinizing hormone in rats. Endocrinology1971; 88:332-7.

24. Loosfelt H, Misrahi M, Atger M, Salesse R, Vu Hai-Luu, Thi MT et al. Cloning and sequencing of porcine LH-hCG receptor cDNA: variants lacking transmembrane domain. Science 1989; 245: 525-528

25. Abel MH, Baker PJ, Charlton HM, Monteiro A, Verhoeven G, De Gendt K et al. Spermatogenesis and Sertoli cell activity in mice lacking Sertoli cell receptors for follicle-stimulating hormone and androgen. Endocrinology 2008; 149: 3279-3285.

26. Lim P, Robson M, Spaliviero J, McTavish KJ, Jimenez M, Zajac JD et al. Sertoli cell androgen receptor DNA binding domain is essential for the completion of spermatogenesis. Endocrinology2009; 150: 4755-4765.

27. De Gendt K, Swinnen JV, Saunders PT, Schoonjans L, Dewerchin M, Devos A, et al. A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc Natl Acad Sci USA 2004; 101: 1327-1332.

28. Chang C, Chen YT, Yeh SD, Xu Q, Wang RS, Guillou F, et al. Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. Proc Natl Acad Sci USA 2004; 101: 6876-6881.

29. O'Donnell L, McLachlan RI, Wreford NG, de Kretser DM, Robertson DM. Testosterone withdrawal promotes stage-specific detachment of round spermatids from the rat seminiferous epithelium. Biology of Reproduction1996; 55:895–901.

30. O'Donnell L, Pratis K, Stanton PG, Robertson DM, McLachlan RI. Testosterone-dependent restoration of spermatogenesis in adult rats is impaired by a 5-alpha-reductase inhibitor. J Androl 1999; 20: 109-117.

31. Holdcraft RW, Braun RE. Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development 2004; 131: 459-67.

32. O'Donnell L, Nicholls PK, O'Bryan MK, McLachlan RI, Stanton PG. Spermiation: The process of sperm release. Spermatogenesis 2011; 1: 14-35.

33. Meng J, Holdcraft RW, Shima JE, Griswold MD, Braun RE. Androgens regulate the permeability of the blood-testis barrier. Proc Natl Acad Sci USA 2005; 102:16696-16700.

34. McCabe MJ, Allan CM, Foo CF, Nicholls PK, McTavish KJ, Stanton PG, (2012). Androgen Initiates Sertoli Cell Tight Junction Formation in the Hypogonadal (hpg) Mouse. Biol Reprod 2012; 87: 38.

35. Willems A, Batlouni SR, Esnal A, Swinnen JV, Saunders PT, Sharpe RM et al, (2010). Selective ablation of the androgen receptor in mouse Sertoli cells affects Sertoli cell maturation, barrier formation and cytoskeletal development. PLoS One 2010; 5: e14168.

36. Nicholls PK, Harrison CA, Walton KL, McLachlan RI, O'Donnell L, Stanton PG, (2011). Hormonal regulation of sertoli cell micro-RNAs at spermiation. Endocrinology 2011; 152: 1870-1883.