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Full Length Research Paper

Antispermatogenic effects of aqueous ethanolic extract of *Hymenocardia acida* stem bark in Wistar rats

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Oral administration of aqueous ethanolic extract of *Hymenocardia acida* stem bark for eight weeks caused a significant reduction ($P < 0.05$) in the weights of testes, epididymides, ventral prostrate, seminal vesicles and vasa differentia compared to the control. The treatment related decreases in sperm motility, count, viability and serum testosterone returned to near normal levels on withdrawal of the extract. The groups that received doses of 100, 200 and 400 mg/kg body weight of the extract showed 40, 0 and 0% fertility, respectively. *H. acida* stem bark extract adversely affected spermatogenesis in Wistar rats.

Key words: Albino rat, antispermatogenic, *Hymenocardia acida*, male reproduction.

INTRODUCTION

In many African countries, herbal remedies play an important role in the health of millions of people, particularly the poor living in rural and peri-urban areas where medicinal plants are readily available, easily affordable and already integrated into the people's cultures. Generally, people begin treating themselves using medicinal plants before going to an herbalist or modern doctor. It is a common practice in Nigeria that herbal products are administered over prolonged period and by persons that have little or no knowledge of science (Ogbonnia et al., 2009). The constituents of these recipes elicit varied physiological activities. There has been a concern over adverse effects on reproduction or systemic toxicity due to prolonged use of medicinal products (Miller, 1998).

Hymenocardia acida (Tul.) is a small browse tree or shrub with palatable foliage, widely distributed within the savanna region of Nigeria. The antimicrobial activities of *H. acida* leaves and roots have been reported (Mann et al., 2008). Literature survey also revealed anti-sickling (Mpiana et al., 2007), anti-ulcer (Ukwe, 2004), anti-diarrhoeal (Tona et al., 1999), anti-HIV and anti-inflammatory (Muanza et al., 1995) activities of H. *acida*. All parts of the plant are useful as remedies for many ailments. Several plants, including *H. acida* are yet to be validated scientifically for the acclaimed efficacy and especially their effects on reproductive organs and functions. Since plant preparations play an important role in fertility regulation, assessment of the effect of common medicinal plants on reproductive functions remains a potential area of investigation.

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MATERIALS AND METHODS

Plant material

The stem bark of *H. acida* was collected within the premises of University of Agriculture, Makurdi and authenticated by Mr. Patrick Ekwuno of College of Forestry, University of Agriculture, Makurdi, Nigeria. Voucher specimen was deposited at the College herbarium. The stem bark was washed, air dried at room temperature for one week, pulverized and stored in air-tight container until required. One hundred grams of powdered material was soaked in 500 ml of 70% ethanol and stirred intermittently for 48 h at room temperature. The material was filtered using sterile cotton wool and Whatman (No. 1) filter paper; the residue was resuspended in the same amount of solvent and then filtered three more times. The pooled filtrates obtained were dried at room temperature under the electric

fan. The extracts were stored in air-tight containers at 4°C until needed.

Animals

Twenty five white albino rats of weight 120 to 150 g were obtained from the College of Health Sciences, Benue State University, Makurdi, Nigeria. The animals were kept in polypropylene cages under room temperature, with 12-h light and 12-h dark cycle and were allowed to acclimatize for two weeks. The animals were provided commercial feed (Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria) and clean water *ad libitum*. Protocols for this experiment was in accordance with the guidelines on the care and well being of research animals (NIH, 1985) and was approved by the Departmental Ethics Committee.

Experimental design

Twenty-five sexually mature Wistar rats weighing 120 to 150 g were randomly divided into five groups of five animals each as follows: Group I received the vehicle (0.5 ml distilled water) daily for 56 days and served as the control; Groups II - IV were administered the extract orally at 100, 200 and 400 mg/kg body weight daily for 56 days (8 weeks) respectively. Group V were administered 400 mg/kg body weight as in group IV but was allowed a recovery period of eight weeks. The body weight of each animal was recorded at the commencement of the study and at the time of autopsy. At the end of the eighth and sixteenth weeks, all the rats in groups I – IV and V were autopsied 24 h after the last dose of treatment, respectively.

Blood sample and organ collection

At autopsy, the testes, epididymides, vas deferens, seminal vesicles and ventral prostates were unraveled, dissected out, blotted free of blood and cleared of connective tissue or fat. The organs were weighed immediately (to the nearest 0.01 mg) using a metler balance. Blood samples were collected by cardiac puncture into sterile plain tubes and allowed to clot at room temperature. Serum samples were separated by centrifugation at 3000 rpm for 10 min and stored at - 20°C until testosterone assay.

Fertility test

Ten days to the end of the experiment, the male rats were paired with normal cycling females in a ratio of 1:1. The mated females were allowed to deliver at full term and numbers of litters were recorded. Percentage fertility was calculated as number of pregnant female rats divided by the number of mated females multiplied by 100.

Sperm collection and analysis

The rats were anaesthetized with diethyl ether. A scrotal incision was made to exteriorize the testis and epididymides. The epididymides were carefully dissected out of the testes and blotted free of blood. To prepare sperm suspension, epididymal sperm were obtained by mincing cauda epididymis of each rat in prewarmed beaker containing 2 ml of physiological saline (maintained at 37°C). Sperm characteristics were determined as previously described (Raji et al., 2005). Sperm motility was also assessed immediately by counting both motile and immotile spermatozoa per unit area at the magnification of ×40. Sperm count was done using the improved Neubauer's haemocytometer under the light microscope at ×400 magnification. The count was expressed as million/ml of suspension. Sperm viability was assessed using eosin-nigrosin test. The percentages of unstained (live) and stained (dead) spermatozoa were calculated by counting 200 spermatozoa per sample. Morphological appearance of normal and abnormal spermatozoa was determined by examining stained smears under the oil immersion (×100) and their percentages were calculated.

Histopathology

Tissues were prepared for histopathology as previously described (Raji et al., 2005). Serial sections (5 µ thick) were prepared, stained with haematoxylin (H) and eosin (E) dye and examined under the microscope (Olympus, Japan). Photomicrographs were taken in bright field.

Statistical analysis

Statistical evaluation of data was done using one –way analysis of variance (ANOVA). Means found to be significantly different at $P < 0.05$ were separated using Duncan multiple range test. The results were expressed as mean ± S.E.M. using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software, San Diego, California).

RESULTS

In vivo effects of *H. acida* on body and organ weights

Oral administration of the extract for eight weeks showed that the body weights of control and recovery groups increased significantly ($P < 0.05$) from 148.00 ± 5.82 and 149.40 ± 3.04 to 193.25 ± 4.15 and 188.25 ± 3.15, respectively. Significant decreases ($P < 0.05$) in weights of testes, epididymides, ventral prostrate, seminal vesicles and vas deferens were observed in the treated groups compared with the control as shown in Table 1. Both the absolute and relative organ weights did not differ significantly ($P > 0.05$) between the recovery group and the untreated control.

Effects of *H. acida* on spermatozoa

Table 2 shows the effects of *H. acida* on sperm characteristics. The sperm motility, sperm count and viability of sperm cells from the cauda epididymis were significantly ($P < 0.05$) lower in the extract treated rats than in the control. The effect was dose-related. The groups given the extract also showed greater ($P < 0.05$) abnormal morphologies. All these parameters were returned to near normal levels when treatment was withdrawn and the animals were allowed to recover.

Effects of *H. acida* on fertility of male rats

The ability of the animals to successfully fertilize the

Table 1. Effect of *H. acida* extract on body and reproductive organ weights.

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg	Recovery (400 mg/kg)
Body weights (g)					
Initial weight	148.00 ± 5.82	144.20 ± 3.95	149.20 ± 3.26	149.60 ± 4.25	149.40 ± 3.04
Final weight	196.25 ± 4.15^{a}	166.00 ± 4.42^{ab}	162.40 ± 4.01^{ab}	147.80 ± 4.08^{b}	188.25 ± 3. 15^{a}
Testes (g)					
Absolute weight	2.92 ± 0.01^{a}	2.16 ± 0.02^{ab}	1.95 ± 0.06^{b}	1.80 ± 0.05^{b}	2.18 ± 0.19^{ab}
Relative weight	1516.18 ± 40.33^{a}	1348.80 ± 44.17^{ab}	1219.20 ± 47.81^{b}	1216.97 ± 45.11^{b}	1409.80 ±47.54^{ab}
Epididymides (g)					
Absolute weight	0.99 ± 0.04^{a}	0.48 ± 0.09^{ab}	0.31 ± 0.05 ^{b}	0.25 ± 0.02^{b}	0.93 ± 0.05^{a}
Relative weight	486.53 ± 31.97^{a}	322.32 ± 24.37^{ab}	202.84 ± 29.81 ^{b}	140.71 ± 25.58^{b}	476.58 ± 28.93^{a}
Ventral prostate (g)					
Absolute weight	0.24 ± 0.01^{a}	0.17 ± 0.05^{b}	0.14 ± 0.02^{ab}	0.13 ± 0.02^{ab}	0.21 ± 0.03^{a}
Relative weight	122.12 ± 9.95^{a}	97.02 ± 6.58^{b}	83.74 ± 6.26^{ab}	82.68 ± 8.07^{ab}	121.08 ± 7.13^{a}
Seminal vesicles (g)					
Absolute weight	0.89 ± 0.10^{a}	0.32 ± 0.04^{ab}	0.27 ± 0.05^{ab}	0.18 ± 0.02^{b}	0.77 ± 0.08^{a}
Relative weight	436.91 ± 11.23^{a}	146.01 ± 12.28^{ab}	126.71 ± 10. 42^{ab}	115.84 ± 8.95^{b}	328.63 ± 12.80^{a}
Vas deferens (g)					
Absolute weight	0.32±0.02^{a}	0.21±0.01^{ab}	0.16±0.01^{b}	0.15±0.02^{b}	0.24 ± 0.02^{ab}
Relative weight	144.34 ± 4.81^{a}	131.81± 3.07^{ab}	107.36±4.79^{b}	121.96 ± 4.29^{b}	129.24 ± 2.39^{ab}

Values are expressed as means ± S.E.M.; N=5. Means with different superscripts in a row are significantly different (P < 0.05). "N" represents number of animals used in each group.

Table 2. Effect of *H. acida* on sperm characteristics of Wistar rats.

Groups	Sperm motility (%)	Sperm count	Viability (%)	Morphology (%)		Fertility test	Litter size
				Normal	Abnormal		
Control (mg/kg)	78. 00 ± 3. 74^{a}	70. 50 ± 4. 78 ^{a}	80. 80 ± 3. 22 ^{a}	83. 85 ± 3. 94 ^{a}	16. 15 ± 3. 94 ^{a}	100	6. 40 ± 1. 34 ^{a}
100	28. 80 ± 3. 80^{c}	31. 80 ± 4. 65 ^{b}	22. 00 ± 3. 52 ^{bc}	46. 18 ± 3. 96 ^{b}	53. 82 ± 3. 96 ^{b}	0	0. 00 ± 0. 00 ^{b}
200	23. 60 ± 4. 41^{d}	26. 60 ± 3. 37 ^{b}	27. 80 ± 4. 62 ^{bc}	43. 80 ± 2. 77 ^{b}	56. 20 ± 2. 77 ^{b}	0	0. 00 ± 0. 00 ^{b}
400	23. 00 ± 5. 15^{d}	20. 00 ± 3. 65^{c}	16. 40 ± 3. 44 ^{c}	43. 90 ± 3. 82 ^{c}	56. 10 ± 3. 82 ^{c}	0	0. 00 ± 0. 00 ^{b}
Recovery	65. 20 ± 4. 33 ^{b}	63. 33 ± 4. 41^{ab}	60. 20 ± 3. 90 ^{b}	65. 35 ± 2. 87 ^{b}	34. 65 ± 2. 87 ^{b}	60	4. 20 ± 1.80^{ab}

Values are expressed as means + S.E.M.; N=5; Means with different superscript in a row are significantly different (P < 0.05). "N" represents number of animals used in each group.

female and produce viable offspring showed 100% fertility for the control and 40, 0 and 0% fertility for rats that received doses of 100, 200 and 400 mg/kg body weight of the extract, respectively. However, the animals that received the highest dose of extract (400 mg/kg body weight) but allowed to recover for the same period of treatment recorded 60% fertility.

Effects of *H. acida* on serum testosterone concentration

There were decreases (P < 0.05) in serum testosterone concentrations of treatment groups relative to the control. The decreases were dose-dependent with the lowest concentration observed in animals that were given 400

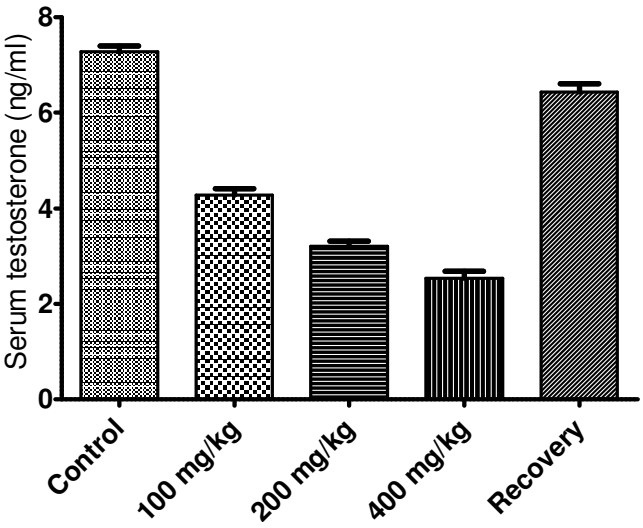

Figure 1. Effect of *H. acida* extract on serum testosterone.

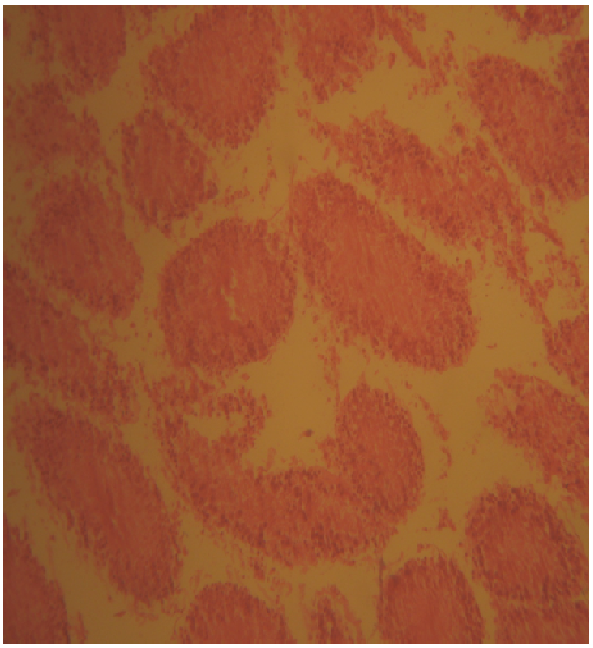

Figure 4. Photomicrograph of testis of rat treated with the extract showing necrosis of the seminiferous tubules, leucocytic infiltration into and oedema into the interstitium, pyknosis of the nuclei and general disorganization of the histoarchitecture of the testis (Transverse section, x200. H&E stain).

mg/kg body weight of the extract (Figure 1). The values were restored following withdrawal of the extract.

Histopathology

Necrosis of the seminiferous tubules, leucocyte infiltration into the interstitium, pyknosis of the nuclei and general disorganization of histoarchitecture as well as eosinophilic epididymitis and concretions in ductuli epididymis were observed in the extract treated rats (Figures 4 and 5). Photomicrographs of the untreated control showed normal histoarchitecture of these structures (Figures 2 and 3). There were no treatment related adverse effects on seminal vesicles, prostate gland and vas deferens.

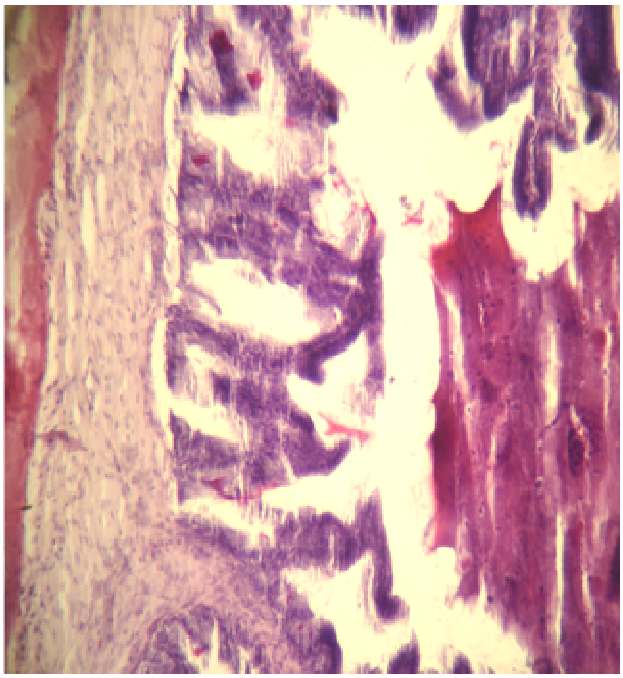

Figure 5. Photomicrograph of epididymis of rat treated with the extract showing epididymitis and concretions in ductuli epididymis (Transverse section, x400. H&E stain).

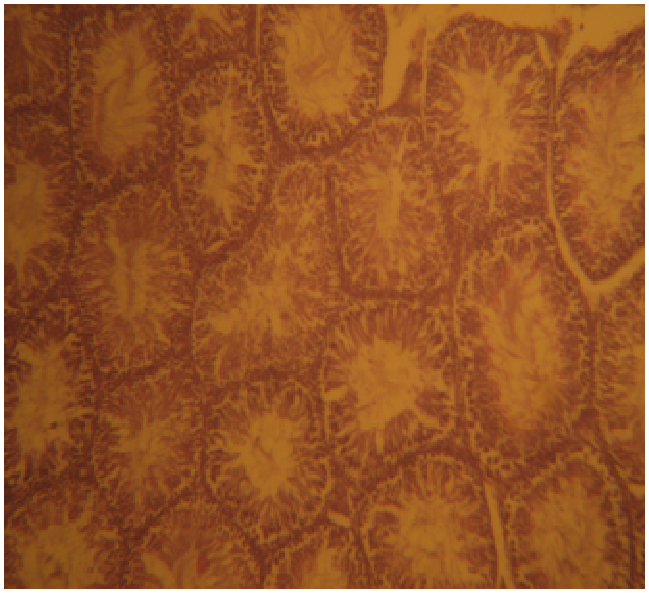

Figure 2. Photomicrograph of normal testis of rat showing well layered seminiferous tubules with different stages of spermatogenic cells (Transverse section, ×200. H&E stain).

DISCUSSION

The result of this study (Table 1) showed that oral administration of hydroethanolic extract of *H. acida* for a complete spermatogenic cycle caused a significant reduction ($P < 0.05$) in the weights of testes, epididymides, ventral prostrate, seminal vesicles and vasa differentia as well as a decline in biomarkers used for evaluation of spermatogenesis. The decreases in absolute or relative organ weights as observed in the present study are suggestive of biosensitivity to *H. acida* extract (Raji et al., 2005). The reduction in testicular

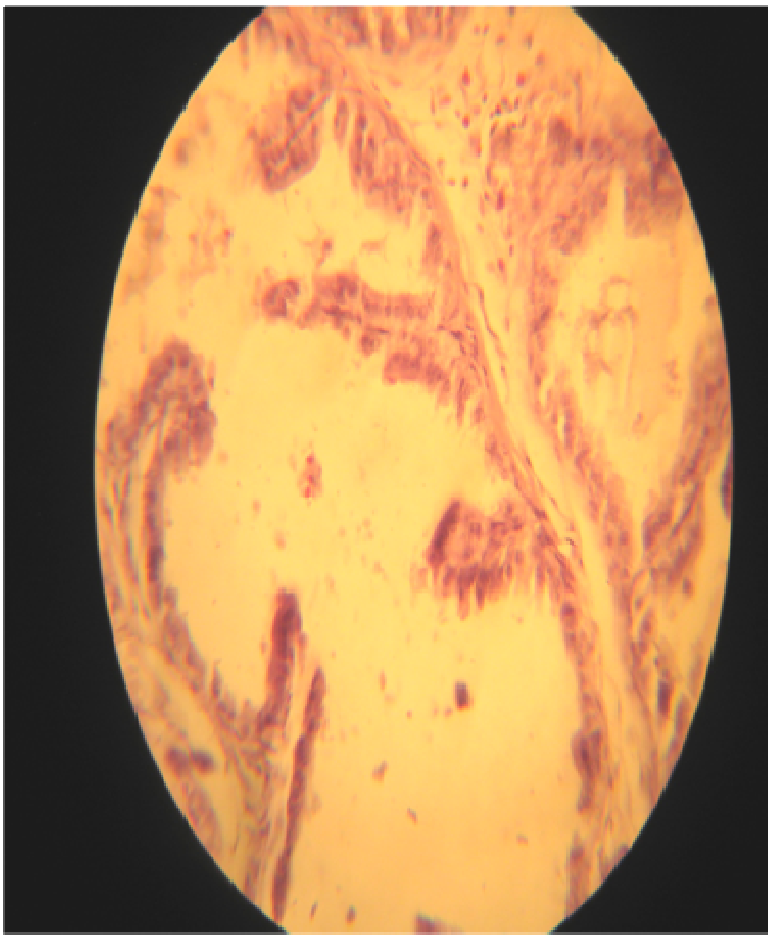

Figure 3. Photomicrograph of normal epididymis showing epithelial lining with cilia (Transverse section, x400. H&E stain).

weight might be attributable to a direct effect of the extract on the histoarchitecture of the testis (Figure 4) as reported by other investigators (Bone et al., 2000) leading to depletion of germinal or spermatogenic elements and ultimately to disruption of spermatogenesis. The seminiferous tubules make up about 90% of the wet weight of the testis (Mishra and Singh, 2008). The reduction could also be due to a reduction in serum testosterone concentration (Figure 1).

The extract caused a decrease in all the sperm parameters studied in a dose dependent manner (Table 2). Similar observations suggestive of impaired spermatogenesis were made following oral administration of benzene extract of *Carica papaya* seeds (Lohiya et al., 1994), ethanolic extract of *Canaga odorata* root bark (Pankajakshy and Madambath, 2009) and ethanolic extract of *Citrulus colocynthis* fruit (Chaturvedi et al., 2003).

It is well known that the blood - epididymis barrier selectively regulates the flow of substances into and out of the epididymal lumen (Hinton and Howards, 1981). Substances of larger molecular weight and many synthetic drugs do not pass freely through this barrier (Minsker et al., 1984; Uchendu, 2000). It is conceivable that *H. acida* stem bark in the present study was probably able to penetrate the blood-epididymis barrier, alter the microenvironment of the epididymis (Verma and Chinoy, 2001) and adversely affect the sperm parameters in the treatment groups.

Data from this study suggest impaired epididymal function due to compromised blood-testis, blood-epididymis barrier or inadequate supply of androgens to the epididymis (Ansari et al., 1998) as reported in the present study and supported by other investigators (Chauhan et al., 2007). The depletion of serum testosterone concentration is also responsible for the observed reduction in the weight of accessory sex organs as the development and functions of these organs are androgen dependent. Serum testosterone levels of extract treated rats were lower relative to the control (Figure 1). Similar observations on reduced concentrations of serum testosterone and sperm characteristics were made following treatment with extracts of *Quassia amara* (Faisal et al., 2006) and *Vernonia amydalina* (Oyeyemi et al., 2008). Udoh et al.

(2005) explained that the mechanism of action of anti-spermatogenic effect of *C. papaya* seed extract was due to interference with the anterior pituitary function as well as direct effect on the testis.

Energy for spermatozoal motility is derived from the oxidation of glucose and fructose. It is possible that hydroethanolic extract of *H. acida* stem bark inhibited the uncoupling reaction of oxidative phosphorylation (Kalla and Vasudeva, 1981) and hence rendered the spermatozoa immotile.

Results of this study also revealed significant reduction ($P < 0.05$) in sperm count and viability in the treatment groups indicating impaired spermatogenesis. The decline in viability of spermatozoa might be due to the spermicidal action of the extract. The decrease in spermatogenesis may be attributed to indirect effect of the extract on the hypothalamo-pituitary-gonadal axis and hence on gonadal function. Similar results on antifertility effect and reduced epididymal sperm count were reported with ethanolic extract of *Ricinus communis* (Sandhyakumary et al., 2003) and *Lagenaria breviflora* (Saba et al., 2009).

Increases in percentage of abnormal spermatozoa are a consistent finding in testicular damage. An animal is considered infertile if more than 10% abnormalities are observed in the semen sample (Zemjanis, 1977). The increase in sperm abnormalities (Table 2) suggests that the aqueous ethanolic extract of *H. acida* could also destroy the internal structure of the testis. Disorganization of the histoarchitecture of testis and degenerative changes observed in the present study were also reported with other medicinal plants found to have anti-spermatogenic property (Leigh and Fayemi, 2008; Raji et al., 2005). The correlation between the production of fertile spermatozoa and histological integrity of the testis is well documented (Hafez and Hafez, 2000). Histological findings also revealed eosinophilic epididymitis and presence of concretions in ductuli epididymis (Figure 5) It is well established that rapidly moving and progressive spermatozoa in sufficient number and free from abnormalities are necessary for fertilization (Aitken et al., 1984).

The extract of *H. acida* suppressed fertility in terms of quantal pregnancy and litter size (Table 2). The absence or reduced fertility observed in the treatment groups relative to the control might be attributable to low sperm concentration and motility as well as high sperm abnormalities.

Following the withdrawal of treatment, all the parameters measured had returned to near normal levels indicating that the effect of the extract was reversible. Hence, such plants could be exploited as male contraceptive agent of natural origin. *H. acida* stem bark extract could be a potential contraceptive agent as immotile spermatozoa may not be able to penetrate the zona pellucida or move from the site of deposition in the vagina to site of fertilization in the oviduct. The contraceptive potential of this plant is also inferred from its adverse effect on viability and morphology of the sperm cells.

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