



ANTIFERTILITY ACTIVITY OF SIMPLE ASCIDIAN, *MICROCOSMUS EXASPERATUS* - A HISTOPATHOLOGICAL APPROACH

Gomathy S.* and Meenakshi V.K.

Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin, Tamilnadu, India.

***Author for Correspondence: Dr. Gomathy S.**

Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin, Tamilnadu, India.

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ABSTRACT

One of the important concerns of today is problem of overpopulation which warrants immediate attention to control or check. The solution to this predicament is birth control. The present study was designed to find out the effect of ethanol extract of *Microcosmus exasperatus* on the fertility of male albino rats. The extracts were administered orally by using IGC at different doses of 50, 100 and 150 mg/kg for 14 days. Histopathological changes observed in the structure of seminiferous tubules, caput and cauda epididymis, vas deferens, seminal vesicle and prostate was recorded by photomicrograph. Histological analysis showed disrupted arrangement of seminiferous tubules, loosening of germinal epithelium and low counts of leydig cells, germ cells and sperm cells. Thickness of tunica albuginea, seminiferous tubule diameter and germinal epithelial height reduced significantly in treated compared to control. Histomorphology of the epididymus showed a decrease in tubule size, epithelial height and a reduction in sperm number in the tubular lumen. This study suggests that *Microcosmus exasperatus* at a higher dose (150 mg/kg) tends to suppress spermatogenesis and is hence liable to cause infertility in male rats.

KEYWORDS: Ascidian, *Microcosmus exasperatus*, Histopathology, Antifertility.

1. INTRODUCTION

Ascidians are marine sedentary, ciliary filter feeding animals. They occur as conspicuous and macroscopic members in many areas along the south east coast of India. *Microcosmus exasperatus* is a simple ascidian found widely distributed in all oceans. A review of literature shows that studies on the GC- MS analysis, toxicity, antidiabetic, vitamins by HPLC, phenolic compounds, flavonoids by HPTLC, antimicrobial, hepatoprotective, CNS depressant, antitumor, antifertility, pharmacognostical evaluation, protection against myocardial ischemia, antihyperlipidemic, anaesthetic, analgesic, antipyretic, anti-inflammatory, wound healing, nutritional value and biochemical components of *Microcosmus exasperatus* are available.^[1-18] But an insight into the histopathological changes occurring during antifertility activity of *Microcosmus exasperatus* has not been attempted at all which prompted the present study.

2. MATERIALS AND METHODS

2.1. Animal Material: Samples of *Microcosmus exasperatus* were collected from Thoothukudi coast, cleaned with sea water, shade dried and powdered. A voucher specimen AS 2240 has been deposited in the museum, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002. *Microcosmus*

exasperatus belongs to the Class: Ascidiacea, Order: Pleurogona, Suborder: Stolidobranchia and Family: Pyuridae. It has a hard leathery orange coloured tunic. Both siphons are clearly visible. The pharynx has 8-9 folds. There is one gonad in each side of the body, divided into three portions. It has been found to breed throughout the year.

2.2. Preparation of Extract: 100 gm powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature and evaporated in a rotary evaporator to get a residue. This residue was used for further studies.

2.3. Experimental Animal: Mature adult male Wistar albino rats weighing about 180 - 200 gm were selected for the study. They were maintained in a well ventilated animal house with constant 12 h of darkness and 12 h light schedule, room temperature (24±2 °C) and humidity (60-70%). Clean water and standard pellet diet "ad Libitum" (Hindustan Lever Ltd., India) were given to them. The animals were kept under fasting for 16 hours before the experiment.

2.4. Experimental Protocol and Histological analysis: Male albino rats were randomly divided into four groups consisting of 5 animals each. Group I served as control

and was given normal saline. Group II, III and IV were administered different doses of extracts orally by using IGC (50, 100 and 150 mg/kg body weight respectively) for 14 days. After the experimental duration of 14 days, testes were carefully dissected out following abdominal incision, fixed in 10% formal saline and processed routinely for paraffin embedding. 5 μ sections were obtained with rotary microtome and stained with Haematoxylin and Eosin (H / E). Sections were observed with light microscope and photomicrographs were taken for further interpretation.

3. RESULTS AND DISCUSSION

Histopathological changes observed in the structure of seminiferous tubules, caput, cauda of epididymis, seminal vesicle, vas deferens, prostate and sperm of male rats treated with the ethanolic extract of *Microcosmus exasperatus* is given in plate 1, 2, 3, 4, 5, 6 and 7.

The section of the control testis showed compactly arranged seminiferous tubules towards the margin. Round, oval or irregular leydig cells were present in large numbers in groups. The spherical or oval nuclei could be clearly observed in the cytoplasm of leydig cells. The sertoli cells were large in size. On treatment with the extract dose related degenerative effect was seen in the testis. In group II treated with 50 mg/kg bw of extract mild disorganized germinal epithelia and 3-5 giant cells in the lumen of seminal vesicle could be detected. Higher dose (group III) brought about deformation of the tubules and necrosis of primary spermatocyte. The number and size of leydig cells were small. The seminiferous tubules develop large lumen in which detached spermatogenic cells were visible. Moderate disorganized germinal epithelia and 10-15 giant cells in the lumen of tubules were noted. Group IV exhibited highly disorganized, atrophied germinal epithelia, with more number of giant cells in the lumen.

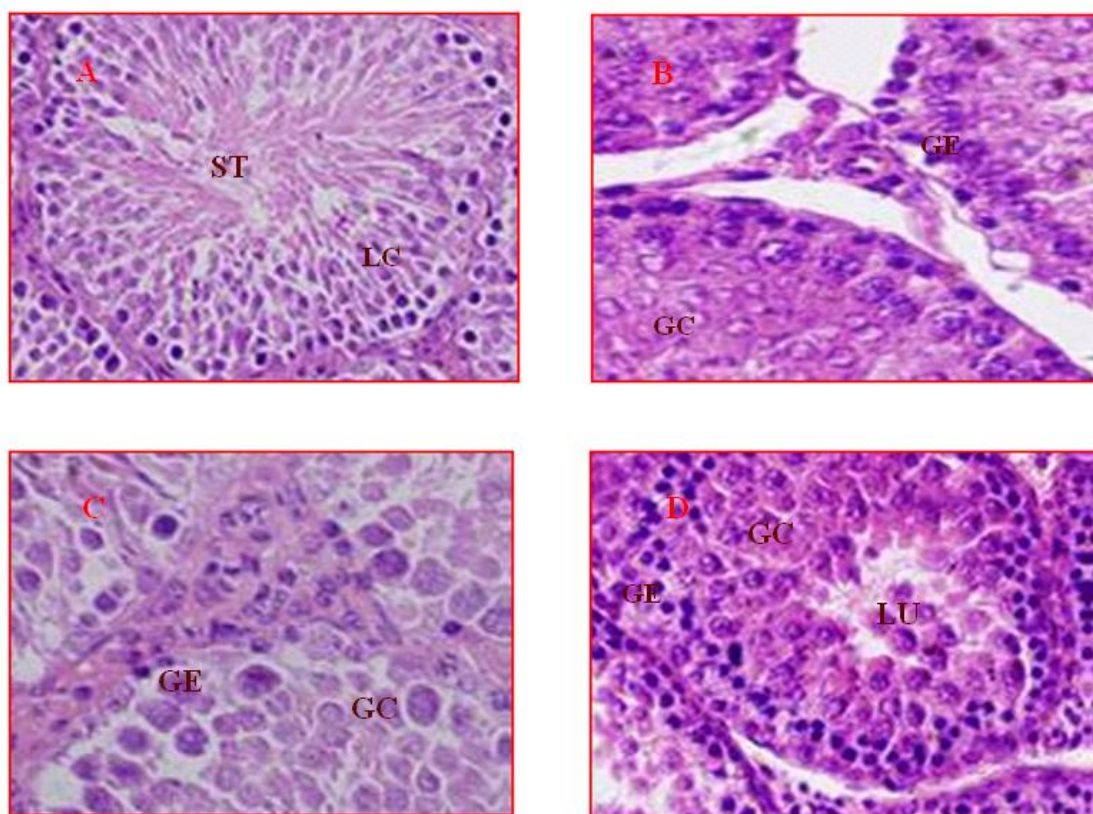


Plate 1: Histopathological changes in the Seminiferous tubules and Leydig cells

A - Group I: ST - Seminiferous tubules, LC - Leydig cells; **B** - Group II: GE - Germinal Epithelia, GC - Giant Cells; **C** - Group III; **D** - Group IV: LU - Lumen.

Normal histological structure with high sperm density was observed in caput epididymis of control rat. The groups administered with 50 mg/kg bw showed low sperm density and reduction of tubular diameter. Degeneration of germinal epithelial cells and very less sperm density in the lumen of caput epididymis was

observed in the groups treated with 100 mg/kg bw of extract. In the group treated with higher dose (150 mg/kg bw) of extract the caput epididymis showed drastic reduction of tubular diameter and absence of sperm in the lumen.

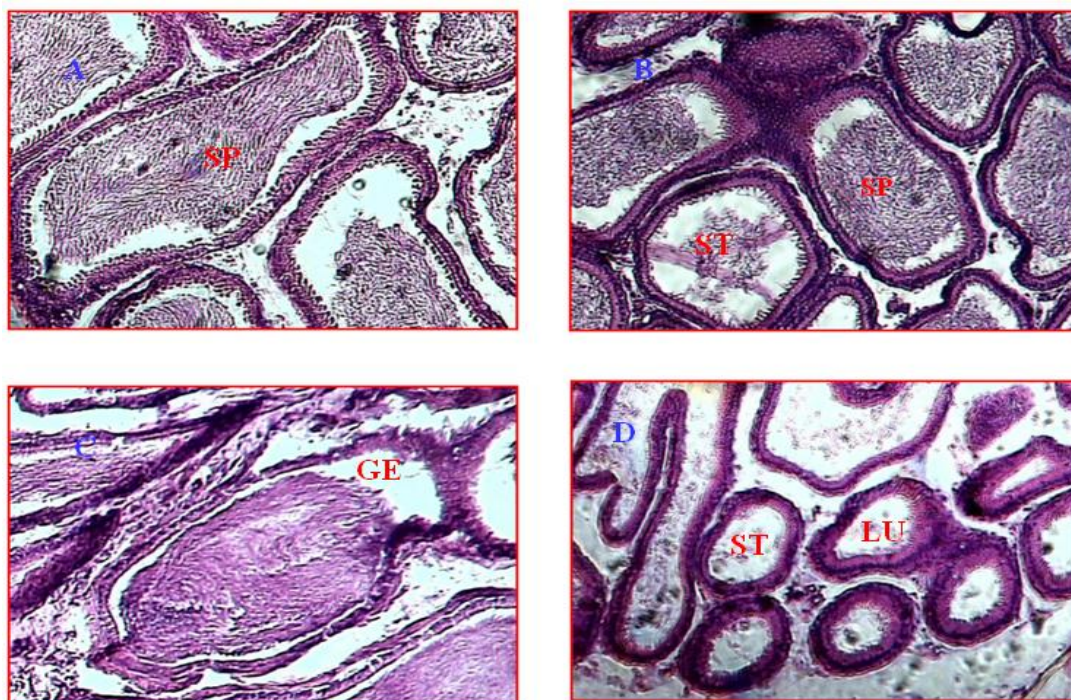


Plate 2: Histopathological changes in the Caput epididymis.

A - Group I: SP - Sperm; **B** - Group II: ST - Seminiferous tubules; **C** - Group III: GE - Germinal Epithelia; **D** - Group IV: LU - Lumen.

In control rat, the cauda epididymis exhibited normal histological structure with large tubules and normal sperm density. There was low sperm density, congested interstitial cells and vacuolation of few germinal epithelial cells in the group treated with 50 mg/kg bw of *Microcosmus exasperatus*. The group which received

100 mg/kg bw showed sloughing of some germinal epithelial cells, decreased tubular cell height with less connective tissue and very low sperm in the lumen. Necrotic change in the connective tissue and significantly low sperm count was noted in the group which was treated with 150 mg/kg bw.

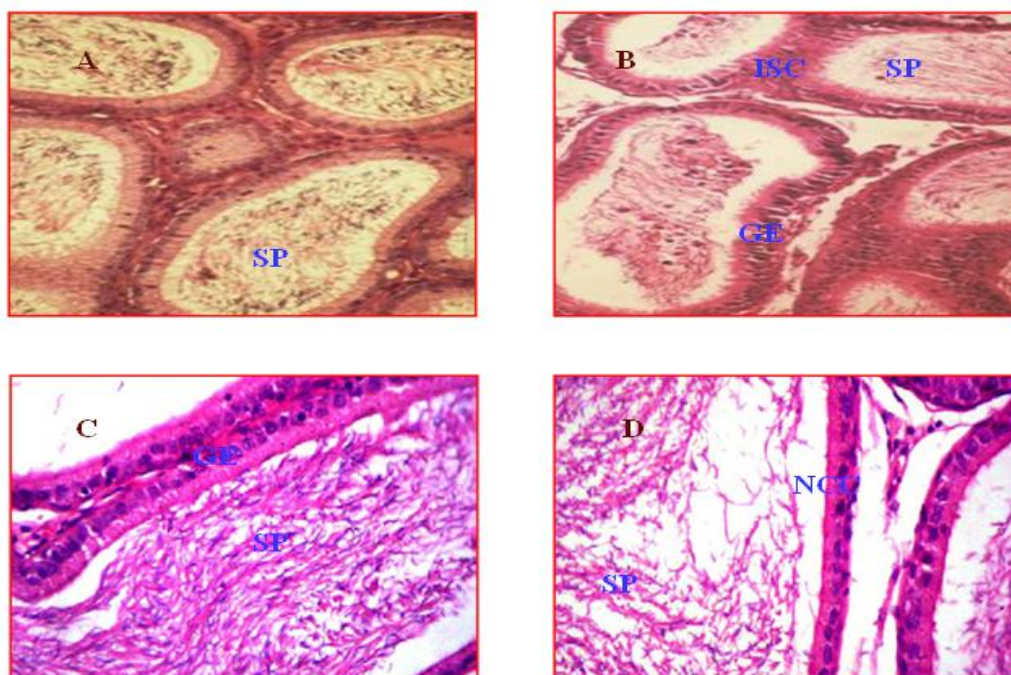


Plate 3: Histopathological changes in the Cauda epididymis.

A - Group I: SP - Sperm; **B** - Group II: ISC - Interstitial congestion, GE - Germinal Epithelia; **C** - Group III; **D** - Group IV: NCC - Necrotic changes in the connective tissue.

In group I, the seminal vesicle showed normal histological structure with tubular secretion. Reduction of secretory tubular diameter was noted in group II whereas seminal vesiculitis represented by leukocytic infiltrations, primary neutrophils, plasma cells and lymphocytes in the tunica serosa was identified in group III. In group IV, desquamation of some necrotic tubule and alveolar glandular epithelial cells was evident.

Normal histological structure of vas deferens with well dilated lumen was noted in group I. Treatment with the extract indicated significant morphological changes. Vas deferens showed moderate luminal reduction with cellular debris in group II. Invagination of germinal epithelial cells was observed in group III where as high necrotic changes in the connective tissue and absence of sperm in the lumen was recorded in group IV.

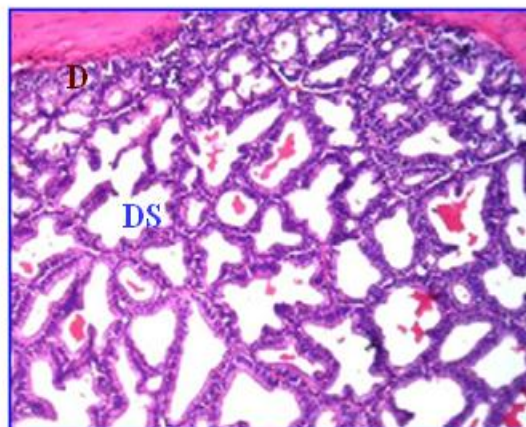
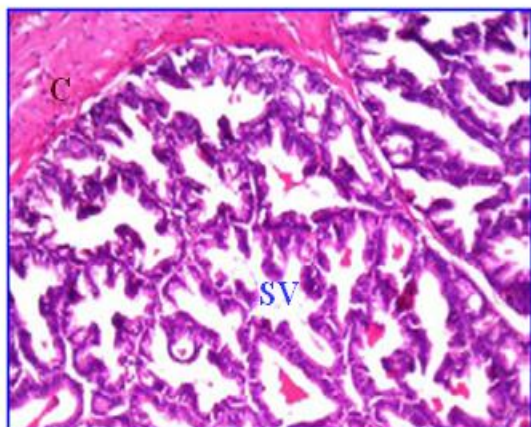
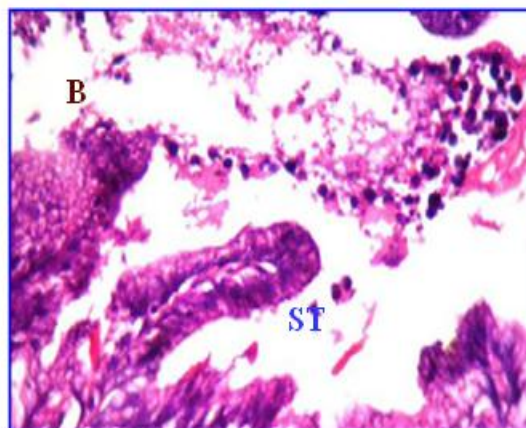
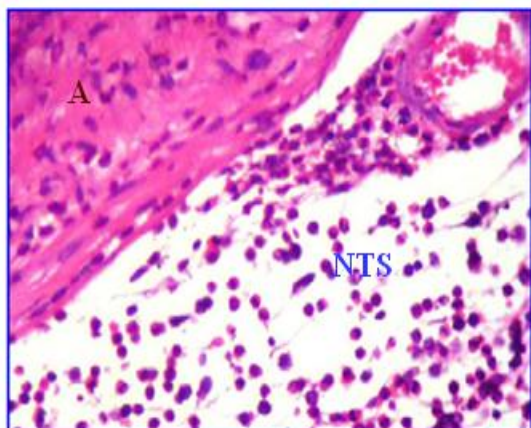
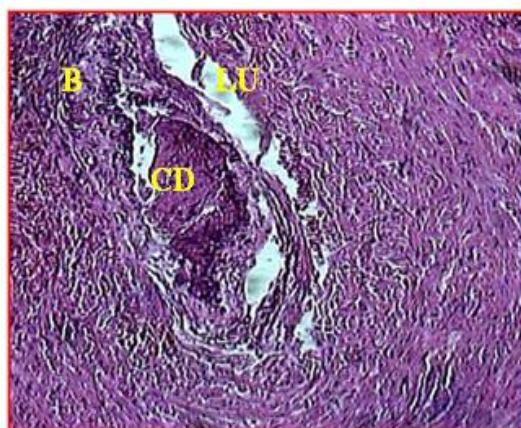
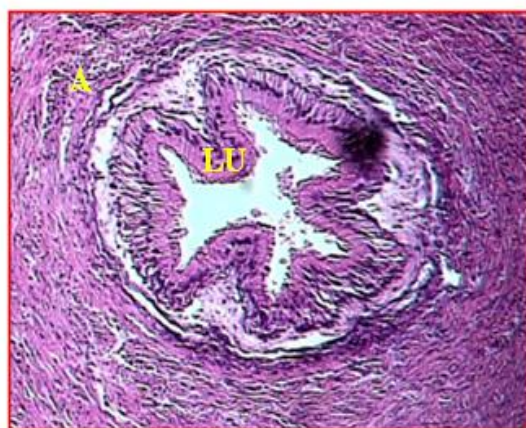


Plate 4: Histopathological changes in the Seminal Vesicle

A - Group I: NTS - Normal Tubular Secretion; **B** - Group II: ST - Secretory Tubule; **C** - Group III: SV - Seminal Vesiculitis; **D** - Group IV: DS - Desquamation.



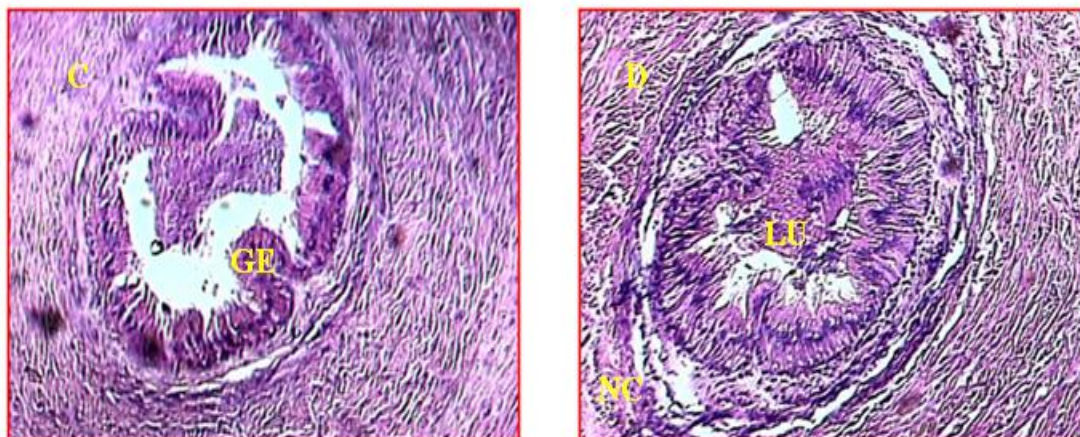


Plate 5: Histopathological changes in the Vas deferens

A - Group I: LU - Lumen; **B** - Group II: CD - Cellular Debris; **C** - Group III: GE - Germinal Epithelia; **D** - Group IV: NC - Necrotic Changes.

In the rats which received saline the prostate exhibited normal histological structure in the height of the epithelial cells of the parenchyma and typical invaginations. The prostate of the group administrated with 50 mg/kg bw showed interstitial congestion, edema with mild luminal secretions. Desquamation of the glandular epithelium of the prostate was noted in group

treated with 100 mg/kg bw. The group which received 150 mg/kg bw showed the presence of interstitial leucocytes in the wall and lumen of acini.

Normal sperm was noted in group I whereas abnormal sperm with malformed head and coiled tail were found in group II, III and IV.

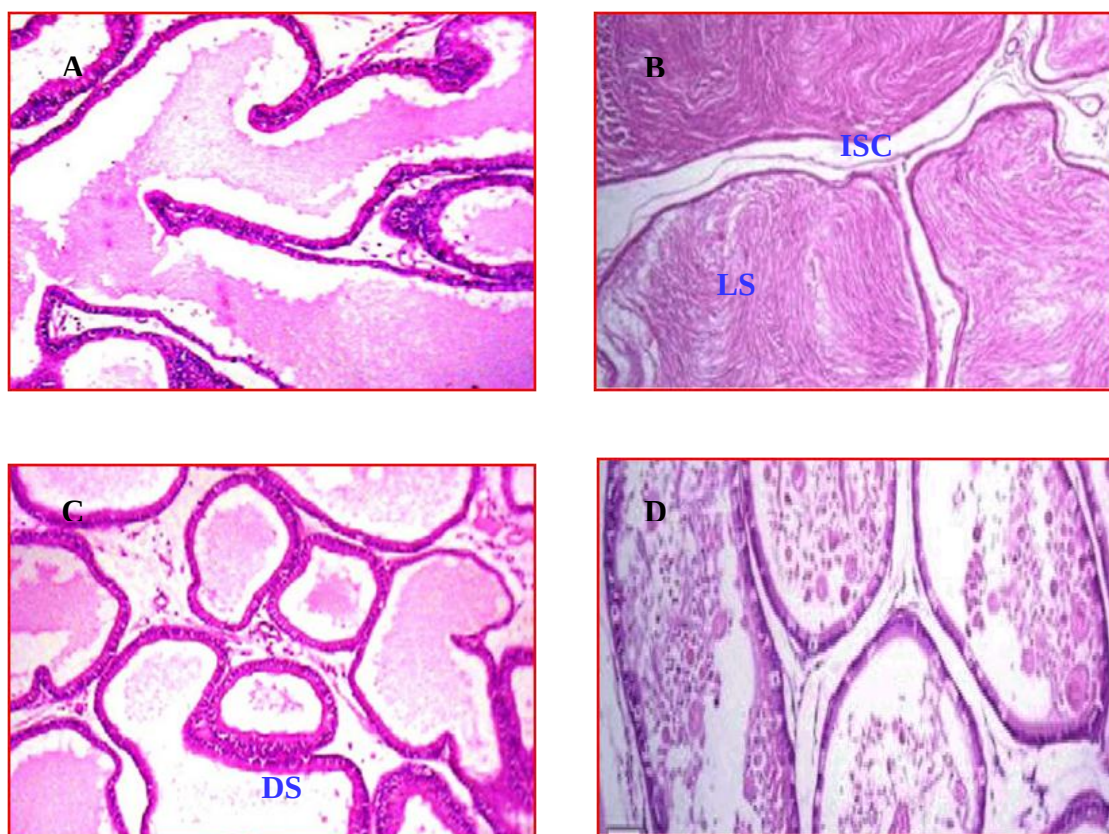


Plate.6: Histopathological changes in the Prostate

A - Group I; **B** - Group II: ISC - Interstitial Congestion; **C** - Group III: DS - Desquamation; **D** - Group IV.

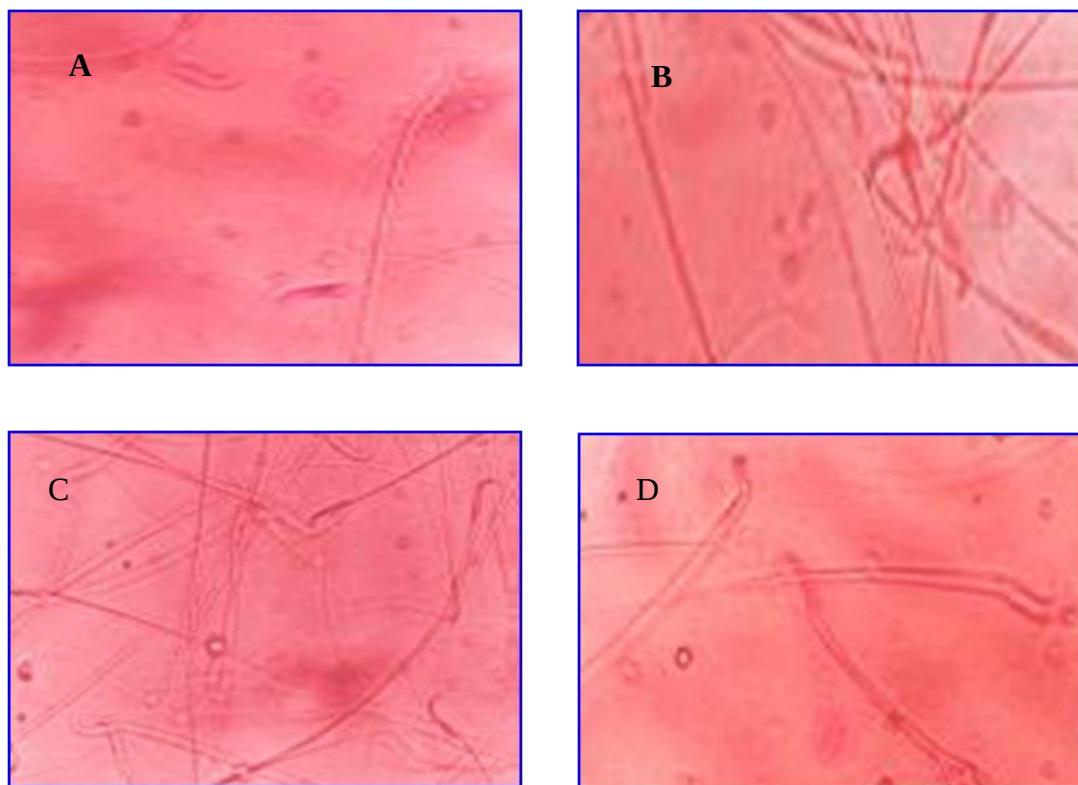


Plate 7: Photomicrograph showing sperm
A - Group I; B, C, D - Group II, III and IV.

Histological changes were more pronounced at high doses. Necrotic germ cells were found in the seminiferous tubules which may indicate that treatment caused severe impediment in the spermatogenic process as reported on treatment with *Ruta graveolens*.^[19] Caput and cauda epididymis showed low sperm density, reduction of tubular diameter and degeneration of germinal epithelial cells. This may be due to the deleterious effect on Leydig cell that might consequently have led to testicular and epididymal dysfunction. Seminal vesicle, vas deferens and prostate also exhibited abnormal morphology indicating failure of sperm production and maturation in the organs leading to loss of fertility in treated rats. Abnormal sperms were found in the lumen of the seminiferous tubule. The present histopathological studies show that one or more components present in the extract of *Microcosmus exasperatus* might have inhibited the normal process of spermiogenesis.

4. ACKNOWLEDGEMENT

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