CHAPTER - THREE
OBSERVATIONS

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III OBSERVATIONS

3.0 Introduction.

3.1 Review of literature.

3.1.1 Testes

3.2.2. Epididymes.

3.3.3. Seminal vesicles.

3.4.4. Prostate Gland.

3.5.5 Cowper's gland.

3.2. Alteration in body weights.

3.3. Alteration in Histoarchitecture of Organs.

3.3.1.Alterations in Testes

a) Wet weight

b) Tubular diameter.

c) Histology.

3.3.2. Alteration in caput epididymes

a) Wet weight.

b) Tubular diameter.

c) Histology.

3.3.3. Alteration in cauda epididymes

a) Wet weight.

b) Tubular diameter.

c) Histology.

I) Control:- Histologically the control caput epididymes under light microscope showed large number of compactly arranged epididymal tubules held together by a little interstitium.

The epididymal tubules were surrounded by thin, eosinophilic basal lamina made up of connective tissue containing smooth muscle fibres and elastic fibres.

The epithelial lining showed a single layer of tall, columnar cells made up of two types of cells such as principal cells and clear cells. Principal cells were tall, columnar cells with a spherical nucleus in each. They show microvilli towards the lumen sometimes called as "stereocilia" clear cells were low cuboidal cells placed in between the principal cells adjacent to basal lamina.

The lumen of the tubules was wide, but became folded due to coiling of tubules and completely full of immature spermatids and spermatozoa.

Interstitium was compact with a few irregular cells.

(Plate 2. Fig. 3, Fig. 4).

II) Experimental:- Light microscopic observations of *Ocimum* treated caput epididymes showed interesting alterations in different components at different dose levels as follows:-

a) 24 days treatment:- Basal lamina was thickened and more eosinophilic. It was affected, muscle fibres were separated from epithelial cells. (plate no. 2. Fig. 6.)

Epithelial cell height was not altered steriocilia were seen toward the ad luminal surface.Vacuolated cytoplasma and nuclei were seen in the epithelial cells. (plate 2. Fig. 5 and Fig. 6).

Lumen of most of the tubules showed abnormal sperms and decrease in the density of sperm population were also observed at this stage. (Plate no. 3. Fig. 1 & Fig. 2).

a) 48 days treatment:-

Basal lamina was densely thickened, undergone degeneration and highly eosinophilic.

Height of the epithelial cells was decreased considerably. Length of stereocilia was decreased to a greater extent most of the cells were degenerated.

Lumen of tubules showed no normal spermatozoa. All the spermatogenic elements coming from testis-undergone degeneration and fused together with degenerated sperms tails and cellular debris to form sperm coagulum in the lumen of all tubules.

Interstitium was very compact and showed very little CT with irregular cells. (Plate no. 3 Fig. 1, Fig. 2).

a) 72 days treatment:-

Basal lamina showed broken appearance and the broken pieces seen in Interstitium.

Epithelium at this stage was in general, regressed. The shape of cells was changed from tall columnar to more cuboidal one. The length of stereocilia was considerably decreased. At some place it was broken showing degenerative effects.

Lumen was spacious with no spermatids nor spermatozoa in it. Even though a few tubules showed degenerated vacuolated cellular fragments in the lumen, most of the tubules showed empty lumens (plate no. 3, Fig. 4).

Interstitium was loosened and widened due to degeneration of basal lamina of adjacent tubules. It contained broken fibres of connective tissue, irregularly dispersed, degenerated cells and pycknotic nuclei. (Plate no. 3, Fig. 3 & Fig. 4).

3.3.3 Alterations in Cauda epididymes.

A) Wet Weight

The wet weights of cauda epididymes in Oscimum treated experimental rats showed interesting variations from those of their control groups. They also changed along with the duration of time. Actual figures are recorded in Table no. 6.

Wet weights of cauda epididymes in control and Oscimum treated experimental rats.

Sr. No.	Duration in days.	Control cauda epididymes Weight in mg/100 gm. Of body weight.	Experimental Cauda epididymes Weight in Mg/100 gm of Body Weight.
1	0	105.15 ± 5.0	-------
2	24	113.96 ± 5.6	87.26 ± 8.4
3	48	118.89 ± 1.20	117.92 ± 4.8
4	72	120.89 ± 1.20	90.20 ± 6.9
5	120	119.00 ± 3.8	99.00 ± 4.6
	Termination of treatment (after 72 to 120 days)		

values are mean I. S. D. of weights for three animals. Wet weights of cauda epididymes are expressed in mg/100 gms of body weight.

In the beginning of experiment, the weight of cauda epididymes of control rat was 105.15 ± 5.0 mg. It gradually increased to 113.96 ± 5.6 mg, 118.89 ± 7.3 mg and 120.89 ± 1.2 mg after 24 days, 48 days and 72 days of treatment respectively. But after termination of treatment from 72 to 120 days the weight of canda epididymes decreased a little to 119.00 ± 3.8 mg.

In the experimental rats also, on 24th day treatment, the weight of canda epididymes was 87.26 ± 8.4 and it increased considerably on 48th day of treatment to 117.92 ± 4.8 mg. After 72 days of treatment the values reduced down to 90.20 ± 6.9 mg and after termination of treatment from 72 days onwards upto 120 days, the weight of caput epididymes again increased to 99.00 ± 4.6 mg.

Thus the weights of control cauda epididymes did not changed much but the Ocscimum administered rats showed reduction in the weight of cauda epididymes than thoes of respective control groups.

B. Tubular diameter

The changes observed in the diameter of cauda epididymal tubules during the experiment were recorded in Table no. 7. As shown below.

Table No. 7. Tubular diameter of cauda epididymes in control and Ocscimum treated experimental rats.

Tubular diameter in µm.

Sr. No.	Duration in days	Control	Experimental
1	0	257.9 ± 6.5	-----
2	24	273.69 ± 6.8	192.11± 8.6
3	48	273.69 ± 6.8	184.21 ± 6.7
4	72	250.01 ± 6.0	171.06 ± 6.9
5	120	252.64 ± 6.2	223.69 ± 7.3

After termination of treatment (From 72 to 120 Days).

Values are mean ± S. D. of 50 tubules. Tubular diameter of cauda epididymes are expressed in μm.

The diameter of tubules of cauda epididymes in control rats initially was 257.9 ± 6.5 μm. It increased to 273.69 to ± 6.8 μm on 24th day and remained same on 48th day of experiment on 72nd day of experiment, the control cauda epididynal tubules showed a reduction in diameter to 250.01 ± 6.0 μm and remained a little increased to after termination of treatment to 252.64 ± 6.2 from 72 to 120 days of experiment.

The diameter of tubules in experimental cauda epididymes was 192.11 ± 8.6 μm on 24th day of treatment. It showed gradual reduction in the tubular diameter on 48 days and 72 days of treatment to 184.21 ± 6.7 μm and 171.06 ± 7.3 μm respectively. But after termination of the treatment from 72 days up to 120 days, the tubular diameter showed an increasing trend towards those of control groups i. e. 223.69 ± 7.3 μm.

C. Histoloty

I) control:

Histologically the control cauda epididymes of all the dose levels under light microscope showed large number of closely packed epididymal tubules held together by a thin connective tissue containing interstitium.

The epididymal tubules were bounded by a thin, elastic, cosinophilic basal lamina made up of connective tissue, smooth muscle fiber and elastic fibers.

The epithelial lining showed a single layer of cells of two types namely, the principal cells and clear cells. The principal cells were cuboidal, pseudostratified and binucleated. They had very short stereocilia towards the adluminal surfaces.

The clear cells were interspersed between the principal cells having less dense cytoplasm. Their population is more in cauda than that of caput epididymes.

The lumen of the tubules in cauda contains large number of matured sperms, with their density more than that of caput epididymis.

The interstitium was compactly arranged having irregularly scattered cells. (Plate no. 3 fig. 5 and fig. 6)

II) Experimental

In experimental animals, treated with Ocscimum sanctum, the cauda epididymes showed varying degree of degenerative histological changes at different dose levels as follows:

1) 24 days treatment

Basal lamina was thin, eosinophilic and intact with connective tissue fibres. (Plate 4 fig 1 and fig. 2)

Epithelium lining the tubules was also normal like those of control tubules, but the coils of tubules were loosened, resulting into disappear-ance of folded nature of epithelium. The cells were cuboidal, pseudostratified in many places but become columnar at places. The length of their stereocilia was not affected.

Interstitium was compact with dense connective tissue and scattered cells. A few pycknotic nuclei were observed in the interstitium. (Plate 4 fig. 2)

Tubular lumen appeared to be widened due to uncoiling of the tubular epithelium. Large number of denatured, abnormal sperms coagulated together in the lumen of the tubule at this stage of treatment. Lumen was also obliterated with exfoliated nuclei (Plate 4. Fig. 2)

2) 48 days treatment

Basal lamina was affected more at this stage. It was degenerated in many of the tubules showing disturbed architecture of the organ. (Plate No. 4. Fig. 3 and fig. 4)

Epithelial lining of the tubules was also affected. It showed clearly pseudostratified nature at many places. (Plate No.4 fig. 4) the length of stercocilia was not affected but the cytoplasm as well as nucleus of the cells showed appearance of smaller and larger vacuoles due to degenerative effects. The nuclei became less dense due to dispersion of nuclear material with disappearance of nuclear membrane(Plate No.4,fig.4)

Interstitium showed a few pycknotic nuclei in intertubular connective tissue (Plate No. 4, fig. 4).

Lumen of the tubules was obliterated with degenerating nuclei and broken tails of denatured sperms. The number of sperms reduced. It also showed degenerating spermatids together forming a sperm coagulum in the lumen of the tubules. The interstitium was also affected. It also showed signs of degeneration of intertubular connective tissue with unidentified pycknotic nuclei in it.

All the ingredients of the tubules showed a severe damage.

72 days treatment

The basal lamina was highly thickened and eosinophilic in nature. It became folded, wavy and showed dettachment from tubular epithelium at certain places. (Plate No. 4 fig. 5)

The epithelium also showed degenerative changes at places. The cellular height of epithelial cells decreased considerably. Many vacuoles were seen in the cytoplasm of the epithelial cells. The nuclei showed densely stained rather heterochromatin material. Multinucleated appearance of tubular cells was observed. The height of stereocilia was a little affected.

Interstitium showed many irregular nuclei, network of connective tissue fibers and degenerated cell debris in it. (Plate No. 4 fig. 5)

Lumen of majority of tubules were empty. (Plate No. 4 fig. 6) eventhough sperm coagulum were still revealed in the lumen of some tubules.

Most of the observations found in the cauda epididymes were similar to those of caput epididymes.

3.3.4 Alteration in Seminal vesicle

a) Wet weight

The variations observed in the wet weights of seminal vesicles of control and Experimental rats were recorded in Table No 8

Table No 8

Wet weights of Seminal vesicles of control and Ocscimum sanctum treated experimental rats

Sr No.	Duration in days.	Control seminal vesicle weight in mg/100gm of body weight.	Experimental Seminal Vesicle Weight in mg/100gm of body weight.
1	0	269.76 ± 5.6	---
2	24	275.10 ± 5.9	263.53 ± 1.8
3	48	270.38 ± 6.8	214.55 ± 1.8
4	72	253.71 ± 12.3	203.87 ± 5.3
5	120 (termination of treatment after 72 to 120 days)	252.45 ± 2.6	251.55 ± 2.8

Values are Mean ± S.D for three animals. wet weights of seminal vesicles are expresed in mg/ 100gm of body weight.

The weight of seminal vesicles of control rats was 269.76 ± 5.6 mg in the beginng of the experiment. It increased to 275.10 I 5.9 on 24th day of treatment and the decreased slightly to 270.38 I 6.8 mg on 48th day of treatment and still decreased to 253.71 ± 12.3 on 72nd day of treatment. Lastly after termination of the treatment form 72 to 120 days, the control rats showed no significant change in the wet weight of seminal vesicles. They remained 252.45 ± 2.6 mg.

On the other hand in experimental rats, the weight of seminal vesicles was 263.53 I 5.4 mg on 24th day of treatment. It showed a gradual decrease in weight on 48th and 72nd day of treatment to 214.55 I 1.8 mg and 213.87 I 5.3mg respectively. But after termination the treatment from 72 to 120 days, the weight were not decreased but they showed a considerable increase in weight towards normal values to become 251.55 ± 2.8 mg.

A) Histology:-

I) Control :

Histologically, the seminal vesicles in all the control groups of the experimental animals showed more or less similar picture under light

microscope. The control seminal Vesicles in T.S showed muscular layer lamina propria, mucisa and lumen filled with secreation.

The adventitial coat formed an external, thin fibrous connective tissue covering Containing blood vessels, nerve fibress and connective tissue fibres.

Muscle layer was present inner to the coat surrounding the lamina propria. It was made up of inner circunlar and outer longitudinal layers of smooth muscle fibres.

Lamina propria is a spongy connective tissue layer bleow the muscle layer which extends deep in to the folds of mucosa.

Mucosa:- Is the innermost layer of glandular epithelial cells which is heavily folded. It showed two types of epithelical cells namely the large coulmnar secretory cells and small, triangular basal cells. The folds of mucous membrane apparently reduce the large lumen of the seminal vesicles.

The lumen was divided into many lumina (compartment) by speta of mucosa, which were full of secretion that even reaches the base of crypts of mucous folds. (Plate no 5 fig 1 fig.2)

ii) **Experimental :**

Histologically , seminal vesicles showed number of changes at different intervals of administration of dose as follows :--

a. **24 days treatment**

The advential coat was quite normal with a few breaks at intervals .The muscular layer showed normal cellular elements in a little disturbance in the thickness. (Plate no. 5 fig.3). The vascularity was also normal.

Lamino propria also showed a little decrease in the thickness of its conectivetissue with beginning of degeneration.

Mucosal epithelium showed that degenerative changes stated of take place in the secretory epithetial cells. A few vacuoles were observed in the cytoplasm as well as nucleus of epithelial cells. Height of the epithelial cells was also affected.

Lumen showed very less amount of secretion in it. The decrease in the secretion associated with degeneration mucosa seen in major parts of the section.

b. **48 days treatment**

Adventia - showed progressive damage at someplaces.

Muscular layer—showed thickening as well as degeneration side by side. At some places the ~~made~~ muscle. layers also undergone degenerative changes showing vacuolisation in between the muscle.

Lamina propria – also exhibitted some changes in its density. It showed grunnlar appearance (plate no.5,fig.4.)

Mucosa -- The secretory epithelial cells undergone progressive degeneration changes. Desquamation of the inner secretory columnar cell layer took place. Tall columnar cells were changed into low cuboidal cell resulting into shortening of mucous folds. Vacuolised cytoplasm and nuclei were more popular in secretory epithelial cells giving a differed look.

Lumen – The luminal space apparently increased with corresponding decrease in the amount of secretion resulted into empty lumena.

c. **72nd days treatment**

The advential coat disrupted at intervals with broken sheets of C.T. capsule extending in the inter tubular tissue.

The muscular layer – showed maximum vacuolisation due to increased degeneration of inner circular layers.

Lamina propria – completely disrupted and abolished at some places while patches of normal architecture showing functional lamina propria were also observed in adjacent areas as of same tubule.

Mucosa—The mucous membrane was most affected at this stage causing complete degeneration at some places and partial degeneration at other places. Height of the secretory epithelial cell decreased considerably . Vacuolisation of cytoplasm as well as appearance of pycknotic nuclei were also seen in damaged parts of epithelial cell. Depth of the mucous to wards periphery.

Lumen – showed secondary enlargement due to degeneration of mucous epithelium with disappearance of secretion in the lumen. This degeneration of secondary epithelium gave a diffused look without observation of secretion in the lumina.

3.3.5. **Alterationsin Prostate gland.**

a) Wet weight :--

The wet weights of prostate gland in experimental animals did not showed much variations as compared to those of control rats. The correct figers are recorded in Table No.9.

Wet weight of prostate gland of control and Oscimum sanctum treated experimental rats.

Sr. No.	Duration in days	Control prostate gland weight in mg/100gm body weight	Experimental prostate gland weight in mg/100gm body weight.
1	0	185.56±6.2	--
2	24	188.85±6.1	183.58±6.3
3	48	128.40±5.3	133.28±5.8
4	72	134.91±7.2	124.45±5.3
5	120	162.85±6.2	143.93±6.4
	Termination of treatment after 72nd to 120 days.		

Values are Mean ± S.D. of three animals. Wet weight of organs are expressed •inmg/100gm of body weight.

The wet weight of prostate gland in control animals in the beginning of the experiment was 185.56±6.2mg on 24th day of experiment it increased to 188.85±6.1mg and then decreased successively to 128.40±5.3mg and 134.91±7.2mg on 48th and 72nd days of experiment .After termination of treatment again the weight of prostate gland was increased to 162.85±6.2mg.

In experimental animals, the weight of prostate glands showed decrease in values progressively in 24th days treatment , 48 days and 72 days treatment from 183.58±6.3mg to 133.28±5.8mg and 124.45±5.3mg respectively. While , after termination of treatment after 72days up to 120 days , the weight increased considerably to 143.93±6.4mgs.

But all the experimental prostate weight were considerably less than those of their control groups.

b) **Histology**

1) Control:

The histological picture of control prostate glands at various dose levels were similar to those of normal and consisted of a connective tissue capsule covering the organ enclosing many branched prostatic

alveoli which are held together by inter alveolar connective tissue called stroma. The alveoli were lined by single layer of cuboidal epithelial cells with large number of secretary granules in the cytoplasm. The epithelial layer gets much folded projecting into the lumen alveoli.

The stroma is compact, containing numerous smooth muscle fibres and elstic fibres , fine branches of blood capillaries scattered in the connective tissue.

The lumen of each alveolus was full with eosinophilic secretory material which was condensed to give lamellated appearance (plate N0.6. Fig.1 and Fig.2)

3) Experimental :

Alteration in the histological structure of prostate gland after administration of Ocscimum sanctum were studied at different durations of dose. They showed drastic changes in the histological picture at later stages of treatment under light microscope as follows:

a) 24 days treatment—

The connective tissue capsule showed no changes Alveoli were normal, the epithelial lining of alveoli did not showed much variations in the granular cytoplasm and its folds were also normal.

The stroma was also compact similar to those of control animals.

The lumen of the alveoli were narrow containing eosinophilic secretion.

a) 48 days treatment –

The connective tissue capsule of the gland got thickened .

The alveoli with disturbed histoarchictecture. The cuboidal epithelial cells showed degeneration of cytoplasmic inclusions, reduction in the secretion granules and over all picture of the alveolar lining was distorted height of the cells also decreased. Many vacuoles and empty vesicles were observed inside and in between the alveolar epithelial cells.

The lumina of many alveoli were devoid of secretion but some alveoli contained decreased eosinophilic secretary substance in the lumen.

The stroma was also affected showing degeneration of inter alveolar connective tissue fibres and other cellular elements.(plateNo.6 Fig.5)

a) 72 days treatment ---

The connective tissue capsule covering the gland was considerably affected and showed many incisions.

The alveoli were complete-by empty and showed full degeneration changes at this stage. The thickness and height of the cells was reduced to a greater extent. The epithelial cells lost their normal morphography. (plate No.6. Fig. 6.) showing vacuolisation in degenerated cytoplasm.

The stroma appeared to be degenerated and mixed with degenerated cellular debri of epithelium forming a continuous thin sheet like appearance (plate No 6 Fig. 6).

The lumina of all the alveoli were devoid of secretion

Thus, the gland appears like empty network of degenerated sheets of epithelium and connective tissue.(PlateNo.6. Fig.6).

3.3.6. **Alteration in Cowper's gland**

a) Wet weight –

The wet weight of Cowper's glands of control and experimental rats treated with Ocscimum sanctum showed variations depending upon the duration of dose and they were recorded in Table No. 10

Table No 10.

Wet weight of Cowper's gland of conrtol and Ocscimum sanctum treated experimental rats.

Sr. No.	Duration in days	Control Cowper's gland weight in mMg/100gm body Weight.	Experimental Cowper's gland weight in mg/100gm body weight.
1	0	70.15±5.9	--
2	24	66.00±6.2	62.20±6.0
3	48	59.92±5.6	57.20±6.2
4	72	50.89±5.0	52.10±5.0
5	120 termination of treatment after 72 to120 days.	112.89±9.2	92.50±8.2

Values are Mean ± S.D. for three animals. Wet weight of Cowper's gland are expressed in mg/100mg of body weight.

In the begining of the experiment the weight of Cowper's gland of control rats was 70.15±5.9mg on 24th day of experiment, they reduced to 66.00±6.2mg and further reduction in the weights were observed to 59.92±5.6mg and 50.09±5.0mg on 48th and 72nd day respectively after termination of the treatment from 72nd day to 120 days, the weight again increased to 112.89±9.2mg .

In experimental rats, the weight of Cowper's gland on 24th day of treatment was 62.20±6.0mg. The weights reduced progressively with the increased duration of dose and became57.20±6.2mg and 52.10±5.9mg on 48th day and 72nd day of the treatment .But after termination of the treatment from 72 to 120 days, the weight of Cowper's gland again showed an increase to 92.50±8.2mg.

A) **Histology**

i) Control --

The histological structure of control Cowper's gland at various dose levels showed normal picture. It consisted of large number of alveoli held together by connective tissue. The alveoli were lined by a single layer of epithelium which was folded inside the lumen. The cells of epithelium were

tall columnar with a basalnuleus in each. The lumen of alveoli was filled with secretion in the form of glairy substance. (plate No.7 Fig.3.)

ii)Experimental

Under light microscope , the histological alterations observed Cowper's gland after administration of dose at different durations of time were as follows.

a) 24 days treatment –

The overall picture was similar to those of control ones.

Alveoli were lined by tall columnar cells which were quite normal. Lumina were filled with secretion that showed eosinophilia (plate No. 7. Flg.4)

a) 48 days treatment –

The histology of the gland was considerably affected at this stage.

Alveolar epithelium lost its coils. The cells showed degenerative changes eventually with degeneration of nearby connective tissue and decrease in the secretion.(plate No.7. Fig.5)

a) 72 days treatment

Alveolar epithelium decreased in its height of cells and majority of cells showed projected degeneration. Connective tissue in between the alveoli was increased in thickness showing more eosinophilia. Lumina of alveoli were devoid of secretion Plate No.7. Fig.6)

TULSHI

OSCIMUM SANCTUM

Plate no. 1 Captions To figures.

Fig. 1 T. S. (Control) Testis. Haematoxylene Eosin staining showing compactly arranged seminiferous tubules lined by germinal epithelium (G E). Lumen (L) containing maturing spermatids and spermatozoa X 32

Fig. 2 T. S. (Control) Testis Haematoxylene-Eosin staining showing all the germinal cell series such as spermatogonia (SPG), spermatocyles (SPC), Spermatids (SPD) and matured spermatoroa (SPZ) towards the lumen (L) of tubule. Interstifial cells (IC) were normal X (63 x 4)

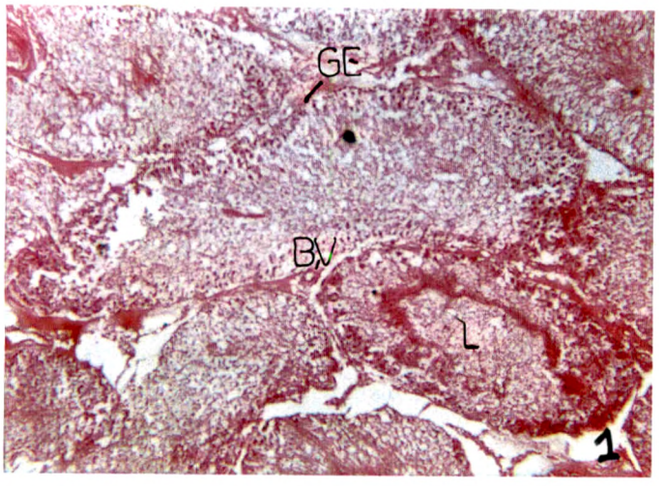
Fig. 3 T.S. Testis (24 days treatment) H and E x 32 Lumen (L) shows degenerated sperms and interstitium (IST) increased considerably.

Fig. 4 T. S. Testis (24 days treatment) H & E x 128.
Iltdefined space (ESP) seen in between the spermatogenic cells. Leydig cells (LC) were little affected.

Fig. 5 T. S. Testis (48 days treatment) H & E x 32
Tunica Propria (TP) separated from germinal epithelium. Illdefined spaces (ESP) were more prominent. Increased interstitium (IST).

Fig. 6 T. S. Testis (48 days treatment) H & E x 128
Cellular debris (CDB) and sperm coagulum (CLM) seen in the lumen (L) of the tubules Vacuoles (V) appeared in cytoplasm of spermatogenic cells. Giant multinucleated cells (GC) seen towards the lumen.

PLATE NO. 1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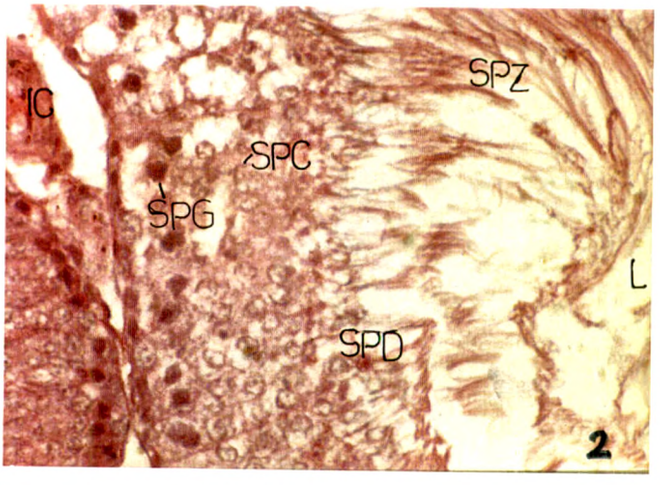
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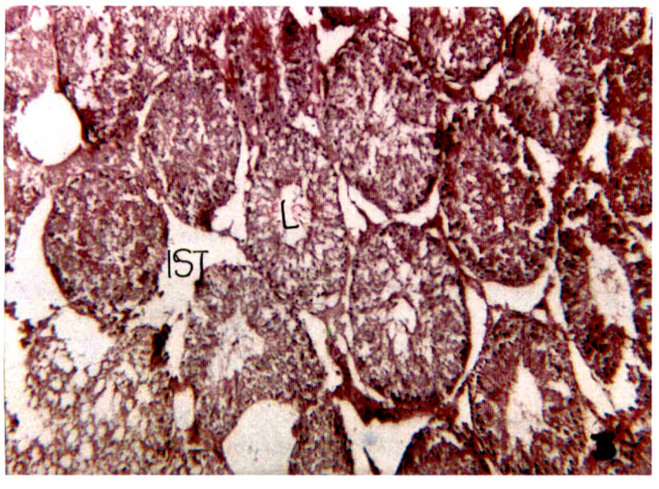
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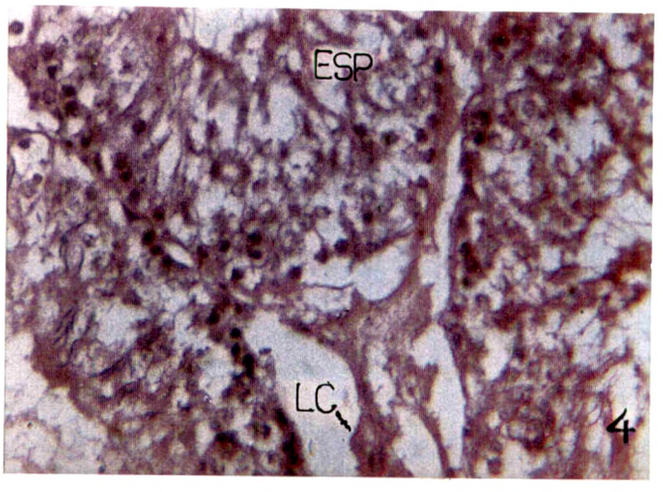
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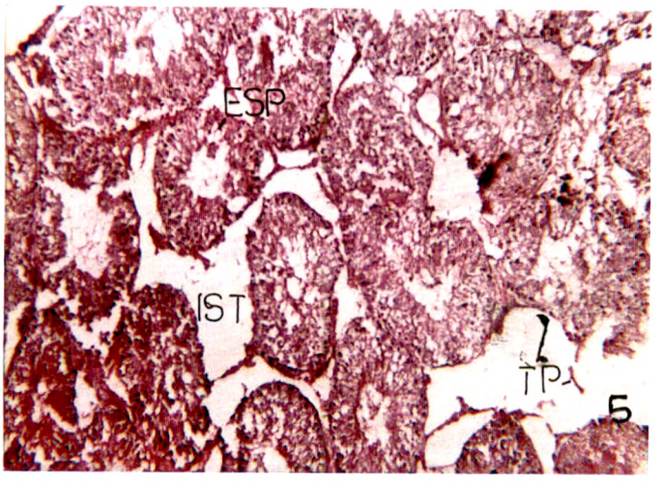
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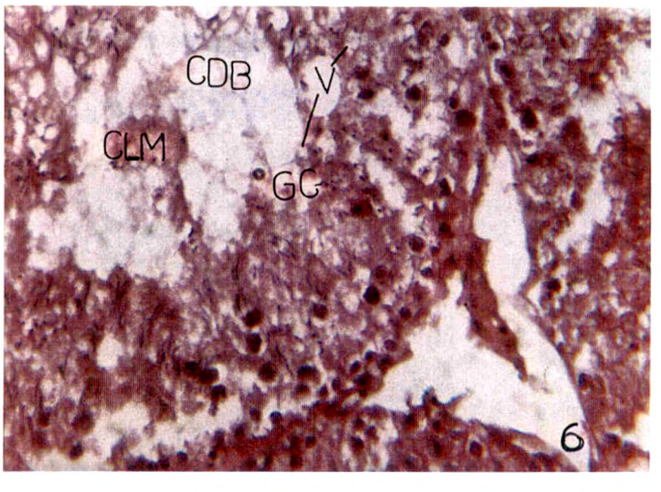

CDB
V
CLM
GC
6

Plate no. 2 Captions To figures

Fig. No. 1 T. S. Testis (72 days treatment) H & E x (6.3 x 4).
Most expanded interstitium (IST) due to shrinkage and decrease in tubular diameter. Pseudopodia like processes (PSD) given out from seminifererous tubules into the interstitium.

Fig. No. 2 T.S Testis (72 days treatment) H & E X (63 X 4) bare germinal epithelium, larger spaces within the tubule, vacuoles (V) inside the cytoplasing of cells, degenerated cell debris in the lumen of tubules and degenerated leyding Cell (LC)

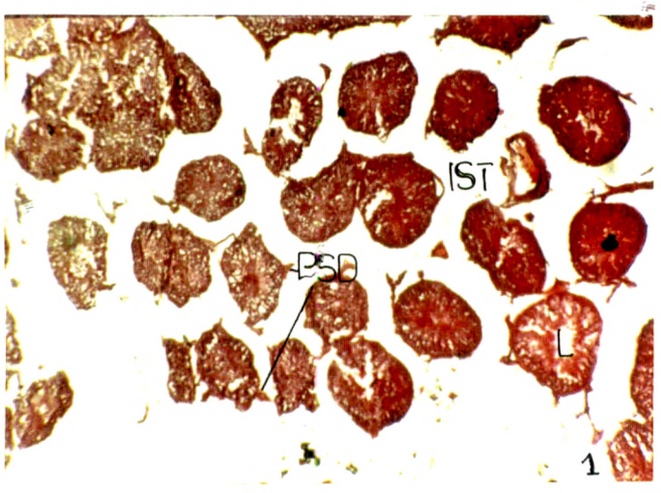
Fig No 3 T.S caput Epididymis control H&E X (6.3 X 4). Highly folded, compactly arranged epididymal tubules (ET) filled with maturing spermatids and matured spermatozoa (LSP) in the lumen. Normal intertubular connective tissue.

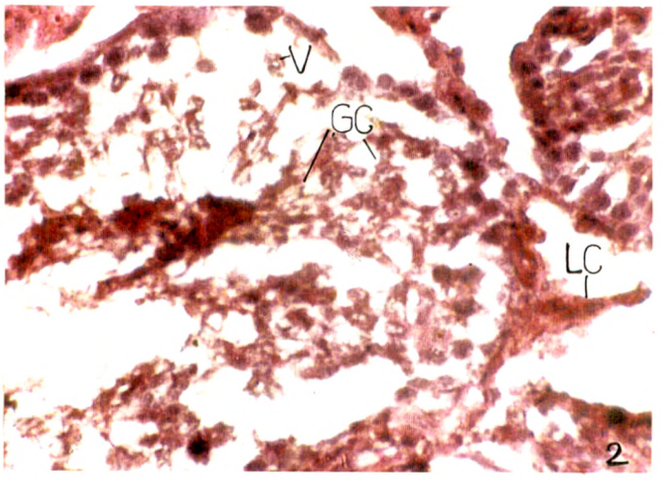
Fig No. 4 T.S caput epididymis control H&E X (63X4). Tall columnar epithlial cell (E P) lining the tubules with steriocilia (STC), towards adluminal surface. Nucleus (N) at the base of epithelial cells. Lumen of tubules filled with spermatozoa and maturing spermatids (LSP)

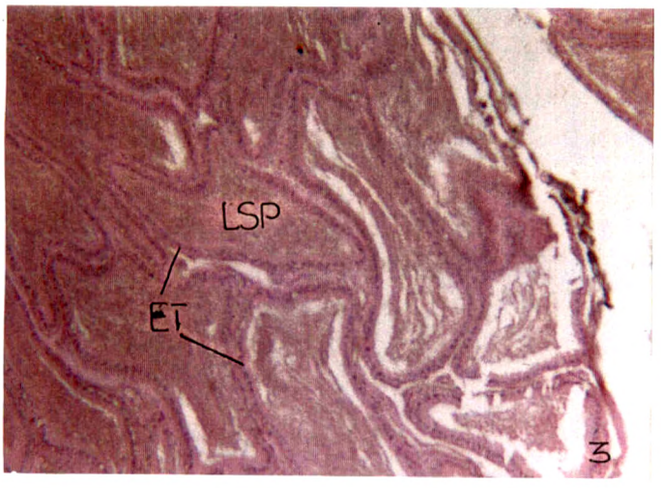
Fig No 5 T.S caput epididymis (24 days treatment) H&E X (63X4) Height of epithelium (EP) decreased, enlarged lumen with sperm coagulum (CLM) inside.

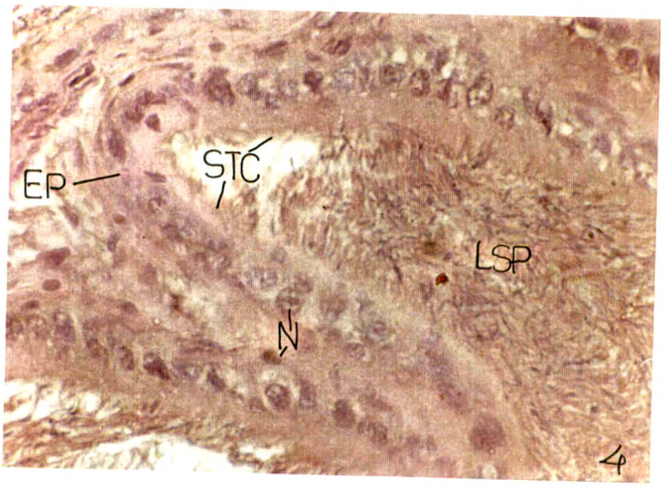
Fig No 6 T.S caput epididymis (24 days treatment) H&E X (63X4) decreased height of epithelium (EP) with vacuolated nucleus (N) and

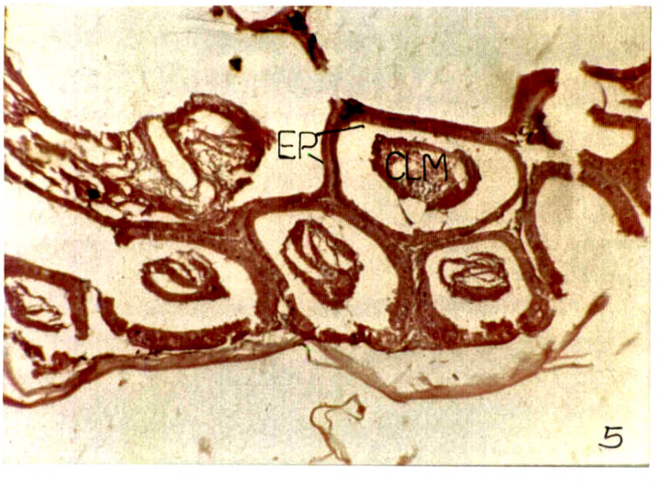
PLATE NO. 2











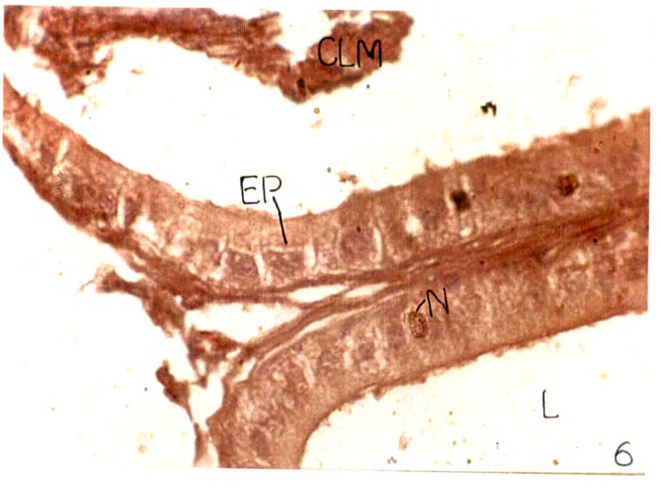


Plate No.3 : Captions To figures

Fig No.1 T.S caput epididymis (48 days treatment) H&E X (6.3 X 4) degenerating epithelium (EP) gets unfolded denigrated spermatozoa in the form of sperm coagulum (CLM) in the lumen.

Fig No 2 T.S caput epididymis (48 days treatment) H&E X (63 X 4) Epithelium with reduced height (EP) and very short stereocilia . Enlarged lumen (L) with a coagulum (CLM) made from degenerated spermatids, spermatozoa and spermtails.

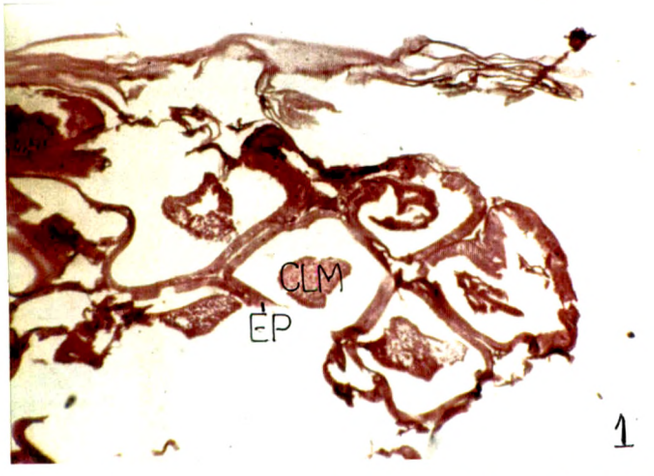
Fig No,3 T.S caput epididymis (72 days treatment) H&E X (6.3 X 4). Degenerating and broken epithelium (EP), empty lumen (L) with no sperms in many tubules very least inter tabular connective tissue.

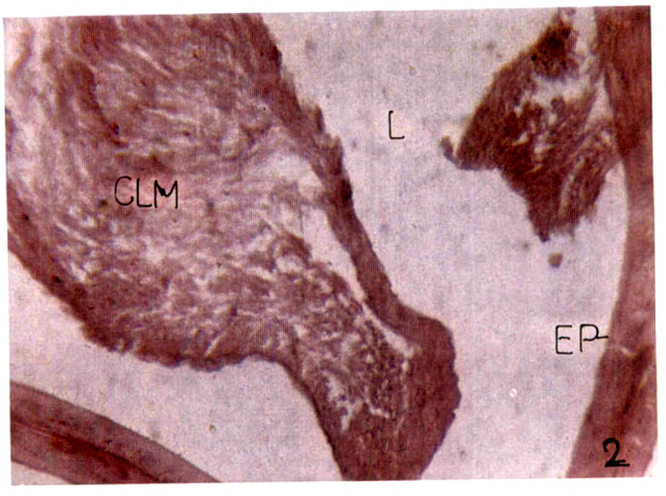
Fig No 4 T.S caput epididymis (72 days treatment) H&E X (6.3 X 4) very thin epithelium without stereocilia towards the lumen. Lumen (L) is empty without sperms. Intertabular connective tissue showing degenerated connective tissue cells and stroma become dense and eosinophilic.

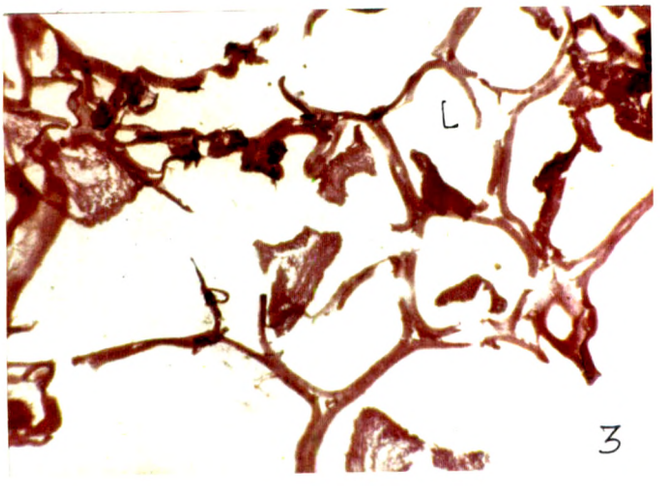
Fig No 5 T.S cauda epididymis (control) H & E X (6.3 X 4) . Highly Coiled ,compactly arranged epididymal tubules (ET) filled with matured spermatozoa in the lumen (LSP).

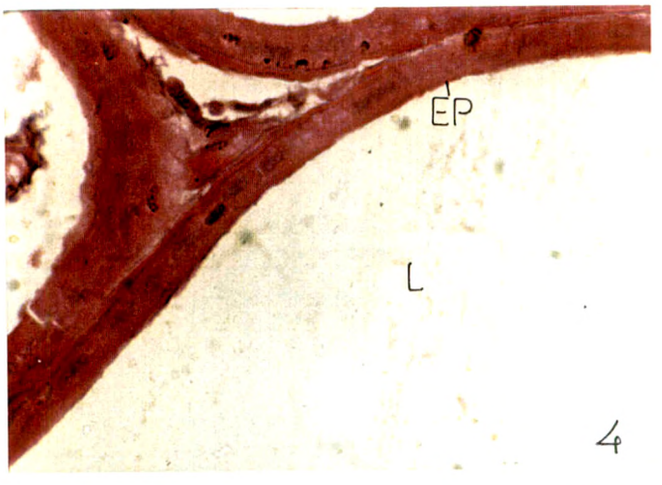
Fig No. 6 T.S cauda epididymis (control) H & E X (63 X 4) .Tall columnar epithelial lining (EP) of the tubules showed binucleated (N) cell. Lumen fillled with Sperms. (LSP) and normal interstial connective tissue with normal fibroblastd.

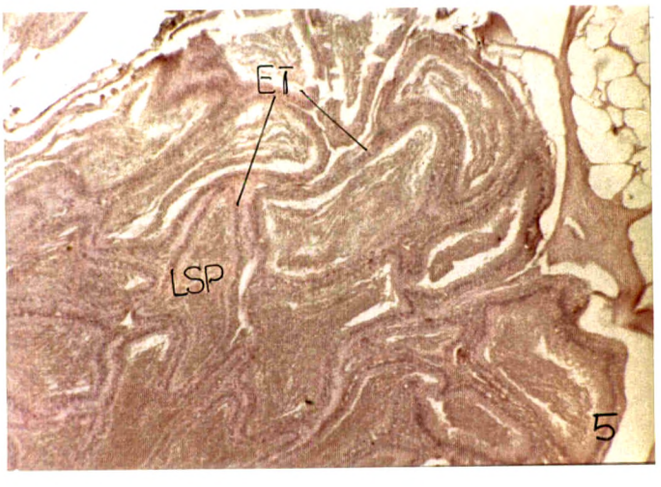
PLATE NO. 3











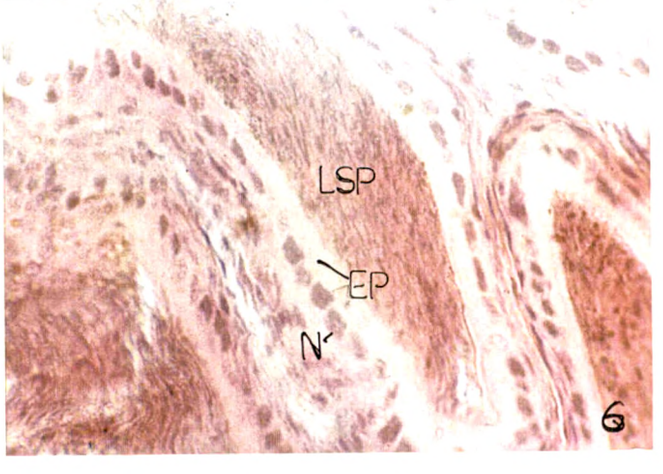


Plate No 4 : Caption To figures

Fig No 1 T.S cauda epididymis (24 days treatment) H & E X (6.3 X 4) .unfolded epididtmal tubules (ET) with Enlarged lumen containing sperm coagulum (CLM)

Fig No. 2 T.S cauda epididymis (24 days treatment) H & E X (63 X 4). Reduced height of epithlium (EP), enlarged lumen (L) and reduction in the height of stereocilia. Coagulum of degenerated sperms in the lumen (CLM), short stereocilia.

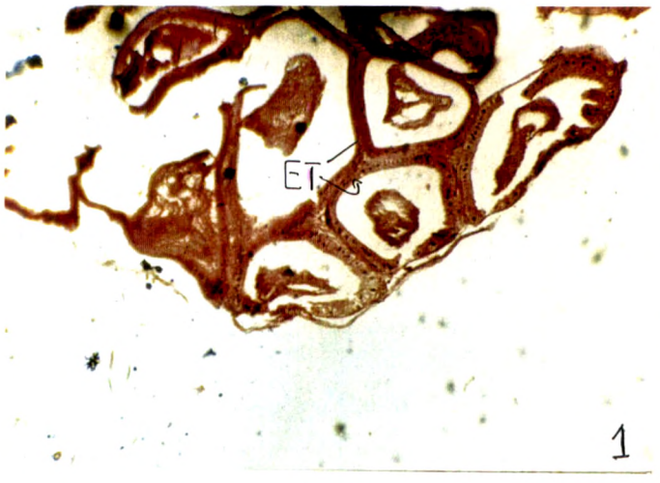
Fig No 3 T.S cauda epididymis (24 days treatment) H & E X (6.3 X 4) degenerated epithelinm of tubules, enlarged lumen (L) and decreased intersititium.

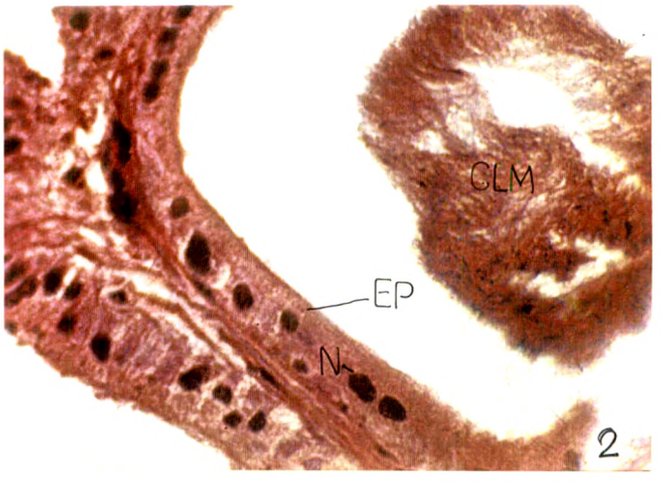
Fig No 4 T.S cauda epididymis (48 days treatment) H & E X (63 X 4) Degenerated epithelium showing vacuolated cytoplasm and nuclei (N) degenerating intertabular connective tissue with pycknotic nuclei in it lumen (L) enlarged with sperm coagulum (CLM) in it.

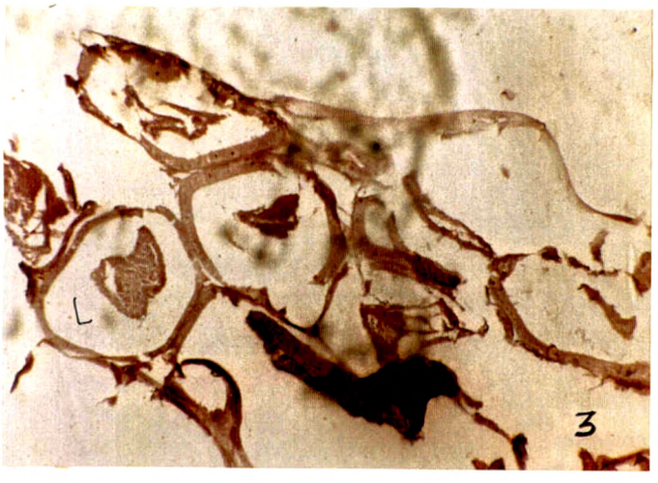
Fig No 5 T.S cauda epididymis (72 days treatment) H & E X (6.3 X 4) majority of tubules are empty. Epithclium is very thin (EP) and degenerated , empty lumen (L)

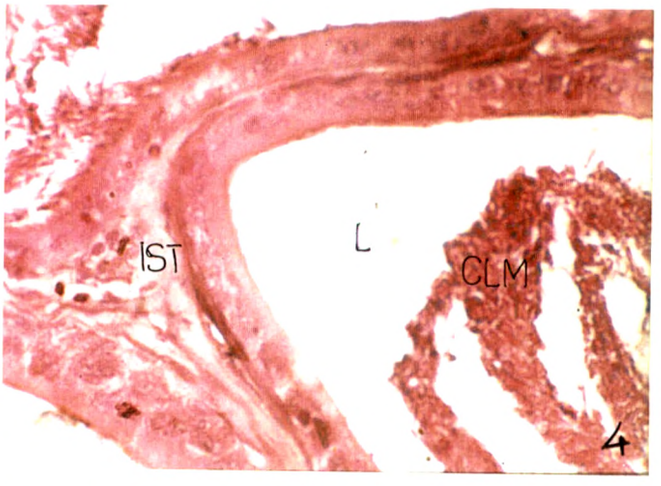
Fig No 6 T.S cauda epididymis (72 days treatment) H & E X (63 X 4) degenerated, eosinophilic epithlium (EM) with pycknotic unclear, empty lumen (L) stercolia absent.

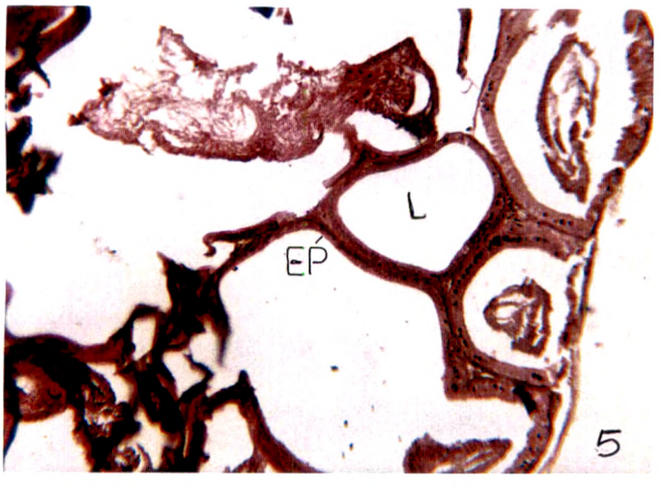
PLATE NO. 4











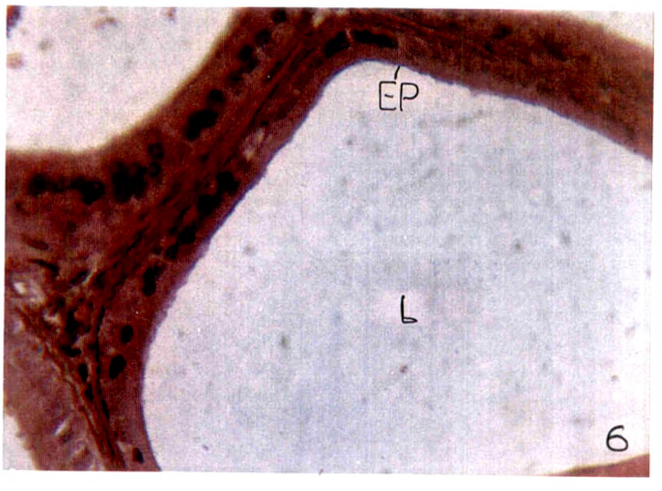


Plate No.5 Captions To Figures.

Fig No 1 T.S seminal vesicles (control) H & C X(6.3X 4). Thick muscular coat (ML), tunica propria. Surrounding the secretory epithelium. Lumen contains narrow spaces filled with secretion (S).

Fig No 2 T.S seminal vesicles (control) H & C X(63x4). Tall columnar cells in secretory epithlium (ep) which is highly folded. Nucleus (N) in each cell . Cell show granular cytoplasm.

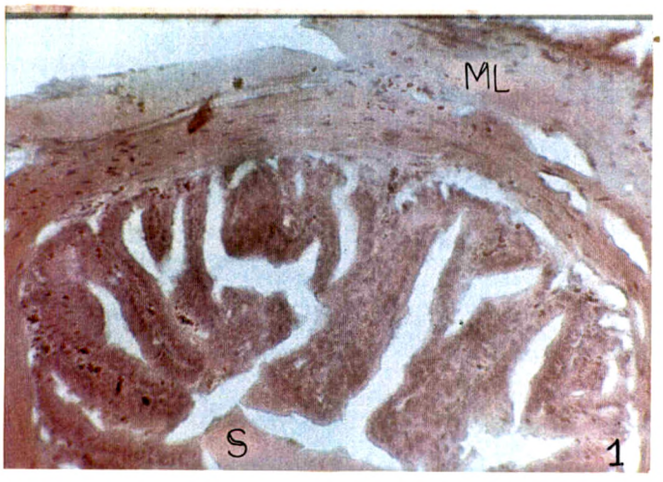
Fig No 3 T.S seminal vesicles (24 day treatment) H & C x (6.3X 4).Degenerated connective tissue capsule, and muscle layer. Mucus with folds, lumen with (L) decreased secretion empty spaces in the lumen.

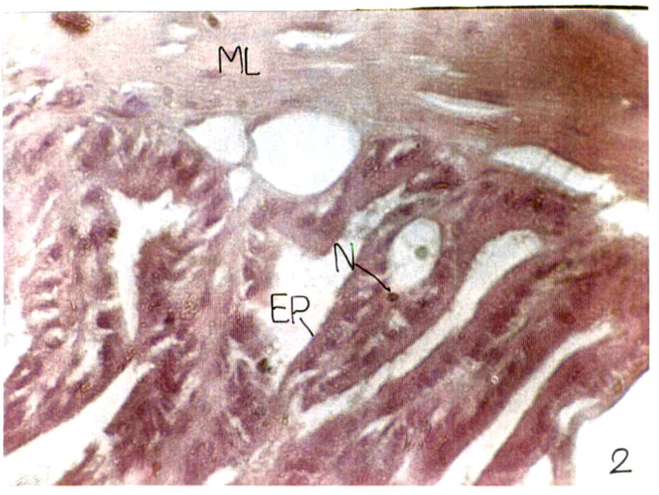
Fig No 4 T. S. Seminal vesicle (24 days treatment) H & E x (63x4) vacuolated muscle layer (ML), reduced connective tissue in the folds, degenerating epithelium (EP) with reduced secretion (S).

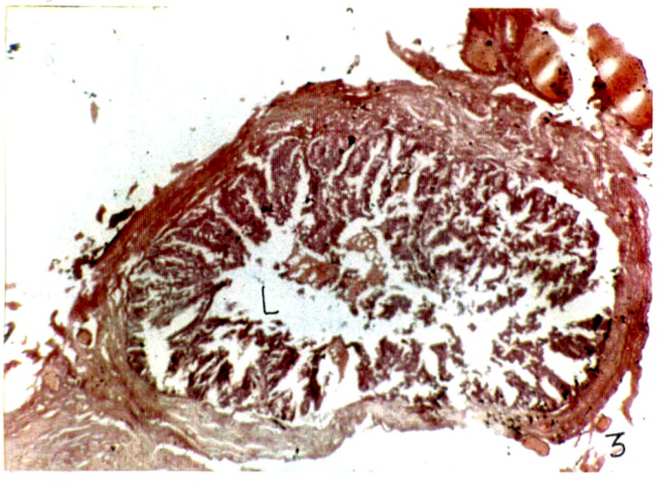
Fig. No 5 T.S. Seminal vesicle (48 days treatment) H&E x (6.3x4) Muscle layer showed (ML) patchy degeneration degenerating mucous folds, enlarged lumen (L) with reduced secretion (S)

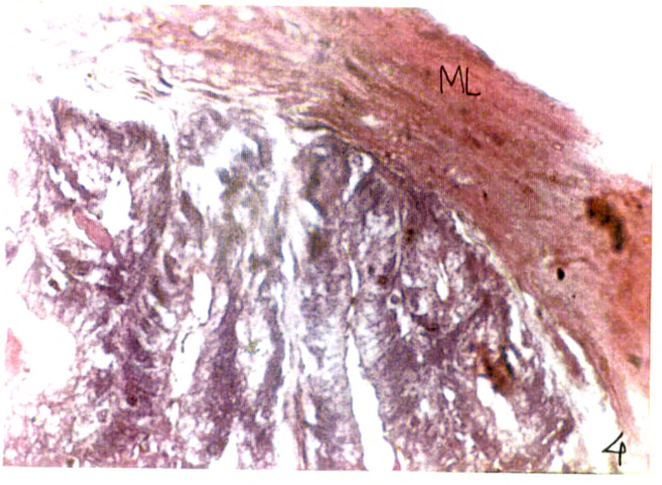
Fig No 6 T. S. Seminal vesicle (48 days treatment) H & E (63x4) Mostly vacuolated muscular (ML), vacuolated nuclei (N) in the secretory epithelium (EP), empty lumen (L) with no secretion.

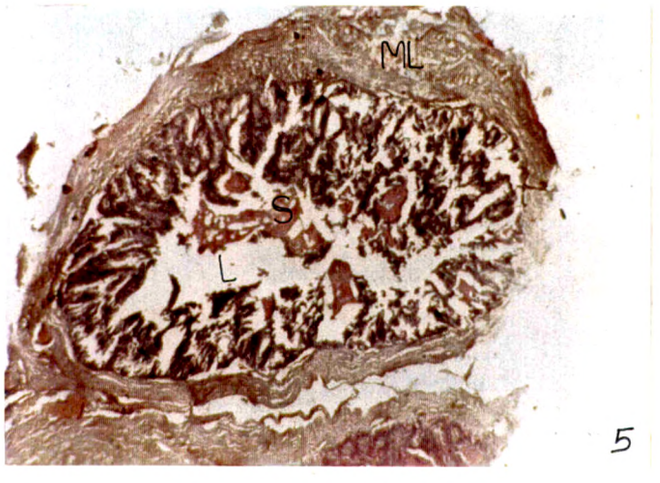
PLATE NO. 5











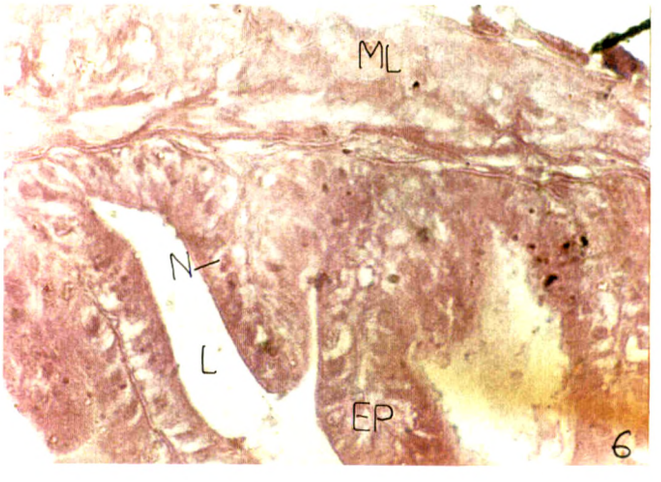


Plate No. 6 : Captions To Figures.

Fig No 1 T.S. seminal vesicle (72 days treatment) H&E x(6.3 x 4)
Degenerated patches of muscularis (ML) , degenerated folds of epithelium leaving spacious lumen (L) without secretion.

Fig No 2 T.S. seminal vesicle (72 days treatment) H & E x (6.3x4)
Breaks occur in degenerated epithelial folds (EP). The secretory cells were vacuolised. The muscualaris also showed empty spaces.

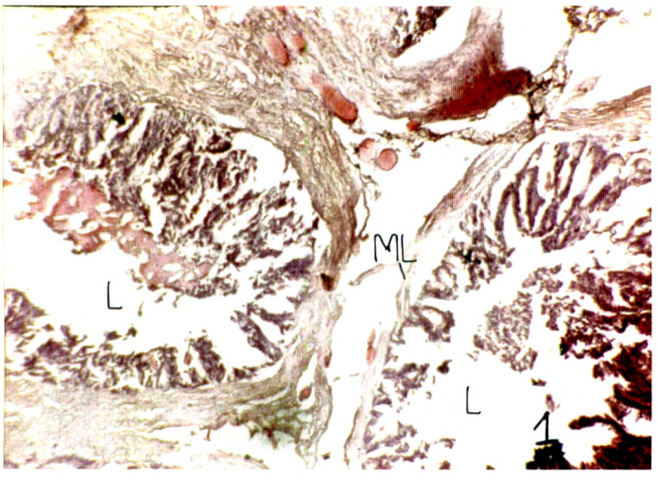
Fig No 3 T. S. Prostate gland (control) H & E x (6.3 x 4)
Prostatic alveoli (A) with interalveolar connective tissue septa. The cells granular, nucleated, lumen containing secretion.

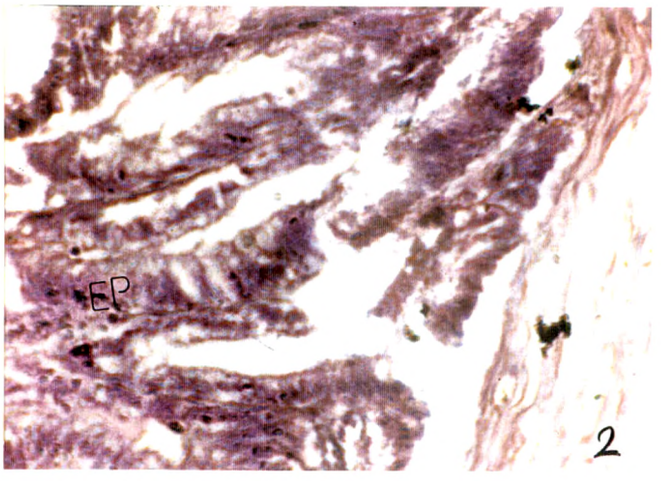
Fig. No 4 T.S. prostate gland (control) H & E x (63 x 4)
Prostatic alveoli (A) made of granular secretory cells, compact stroma and eosinophilic secretion (S) filled in the spaces.

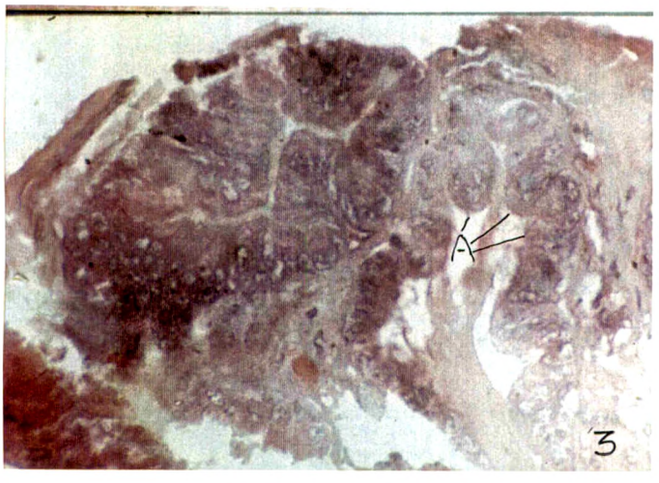
Fig. No 5 T. S. Prostate gland (48 days treatment) H & E x (63x 4)
Degenerating alveolar epithelium (A), enlarged lumen (L) with decreased secretion.

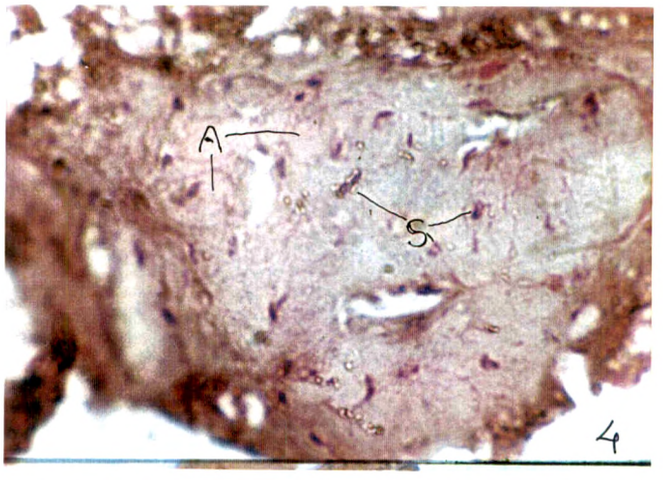
Fig. No 6 T.S. prostate gland (72 days treatment) H & Ex(63 x 4)
Very thin sheets of alveolar epithelium (A) undergone complete degeneration and empty lumen (L).

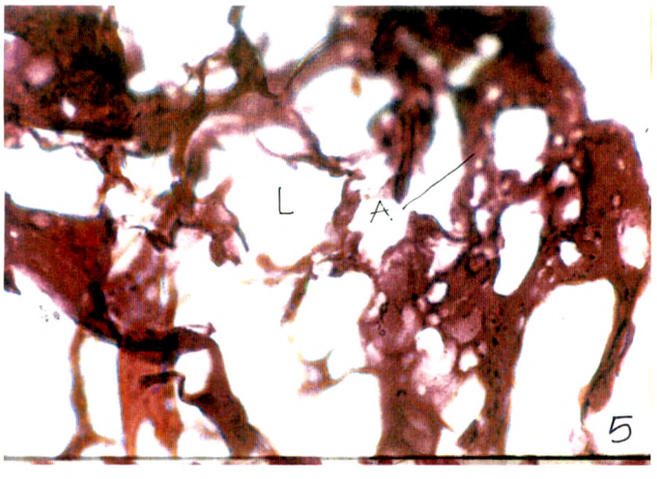
PLATE NO. 6











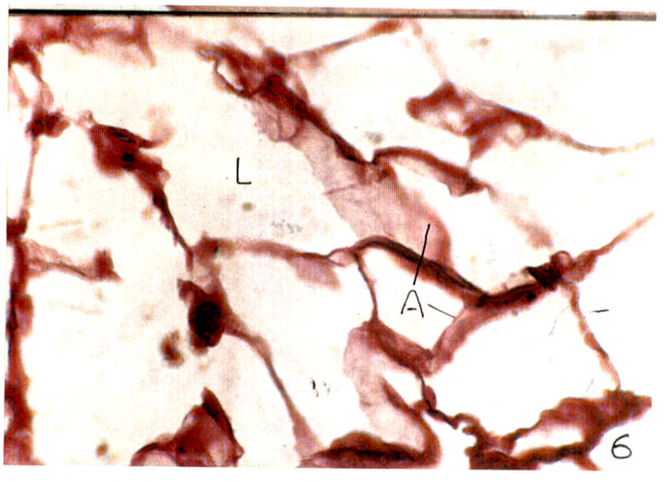


Plate No. 7 : captions To figures.

Fig. No 1 T. S. Testis (48 days treatment) H & E x64
Degenerated seminiferous tubules with pseudopodia like process (PSD) in the oedomatous interstitium (IST). Laydig cells (LC) were also degenerating.

Fig No 2 T. S. Testis (72 days treatment) H & E x (6.3 x 4).
Necrosed seminiferous tubule with tunicapropria (TP). Separated from germinal epithelium, illdefined spaces (SP) inside the tubule, vacnolated nuclei (N) in the degenerated spermatocytes and giant multinucleated cells (GC) in the lumen of tubule.

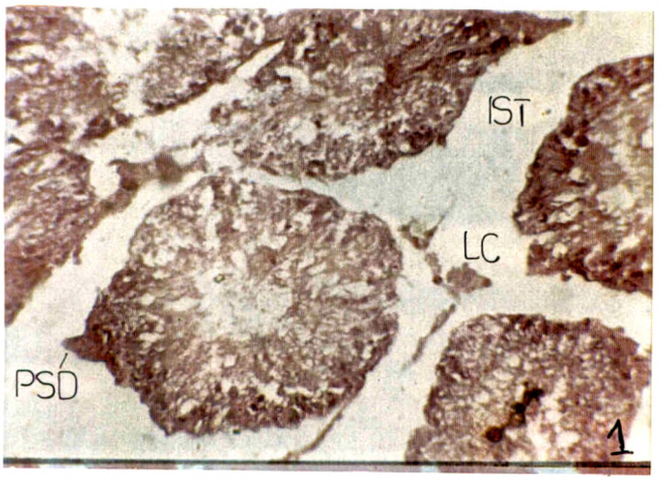
Fig No 3. T.S. Cowper's gland (control) H & E x (6.3 x 4)
The alveoli (A) were held together by connective tissue. Lumen filled with secretion.

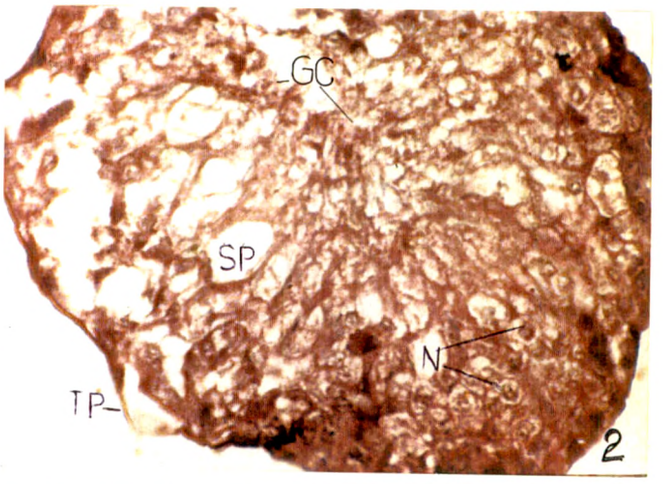
Fig No 4. T.S. Cowper's gland (24 days treatment)H & E x (6.3x4)
Columnar epithelium (EP) lining the alveoli, lumen (L) filled with secretion.

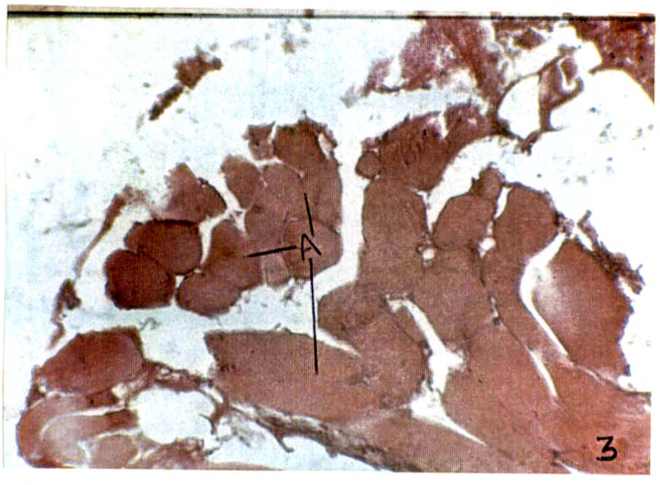
Fig. No 5. T.S. Cowper's gland (48 days treatment)H&Ex(6.3x 4)
Progressed degeneration of epithelial lining (EP) in the alveoli with lumen (L) containing least or no secretion.

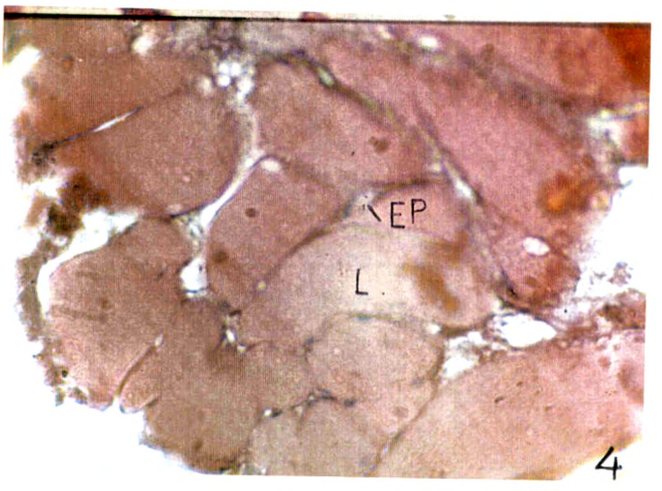
Fig. No 6. T. S. Cowper's gland (72 days treatment) H & E x (6.3x4)
Alveoli (A) were empty. With enlarged lumen (L). The gland shows maximum degeneration of epithelium (EP)

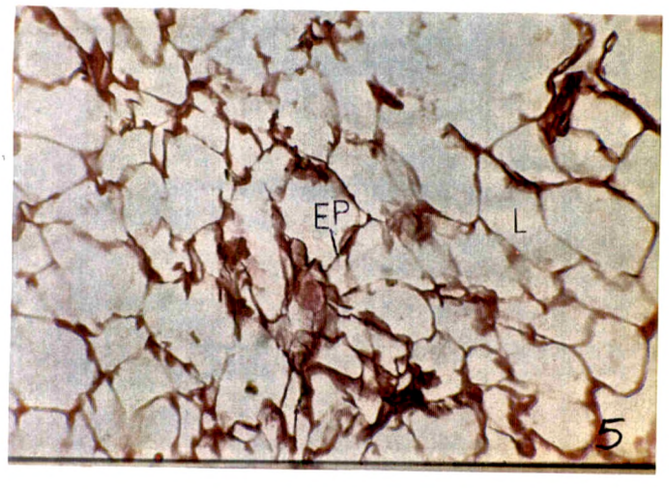
PLATE NO. 7











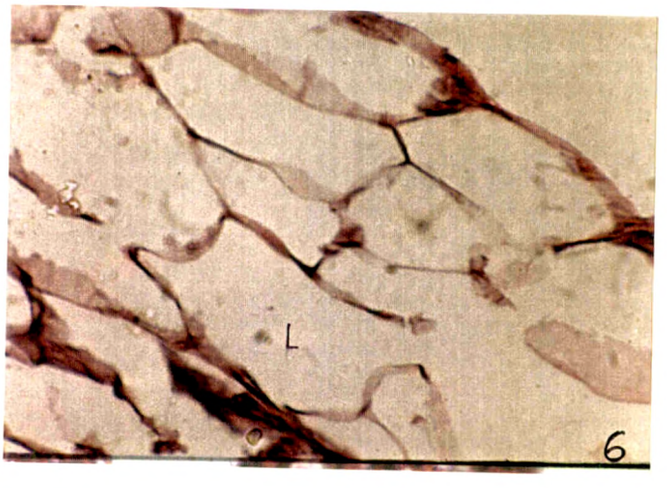


Plate No. 8 : Caption To Figure.

Recovery Tests : After 120 days.

Fig. No 1 T.S. Testis , H & E x 32 Regenerating semini ferous tubules with dense population of sperms in the lumen (LSP). Tunica propria regenerated at places (TP)

Fig. No 2 T.S. Testis , H & E x 128.
Regenerating tubules with developing tunica propria (TP), normal spermatogonia, spermatocyles and spermatids. Reduction in number of spaces.(SP). Regenerated Leydig cell (LC)

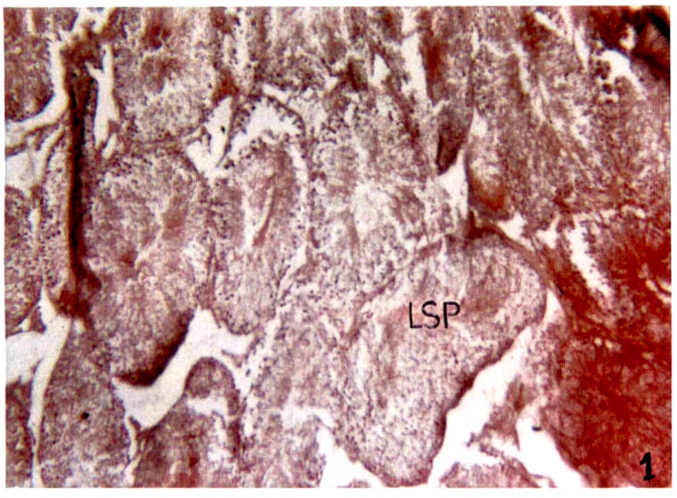
Fig. No 3. T. S. Caput epididymis, H & E x (63x4)
Compact epididymis tubules (ET) with tall, columnar epithelium (EP). Tubules showed large no of normal luminal sperms (LSP).

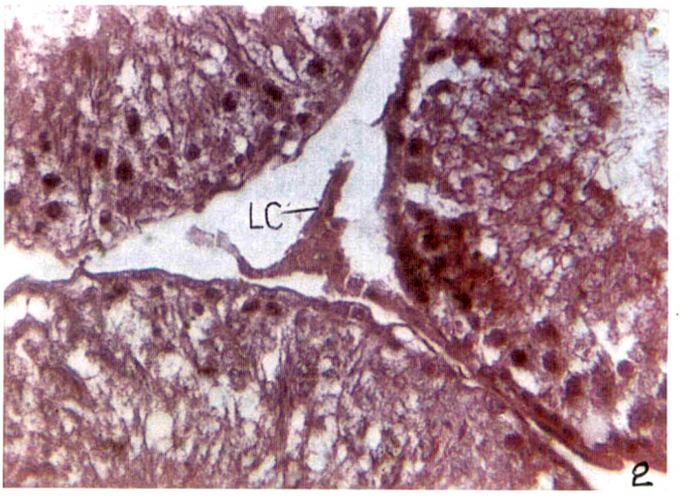
Fig No 4 T. S. Cauda epididymis, H & E x (63 x 4)
Compact epididymal tubules (ET) with dense, widened connective tissue in between. Normal binucleated epithelium (EP) with long slereocilia densely populated sperms (LSP) in the lumen.

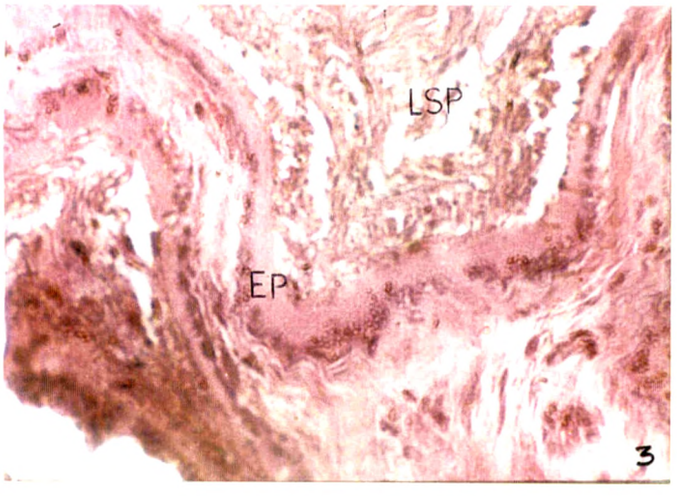
Fig. No 5 T. S. Seminal vesicle, H & E x (63 x 4)
Regenerating secretory epithelium, thikening muscularis (ML), lumen (L) with some secretion and full of secretion (S) in some parts.

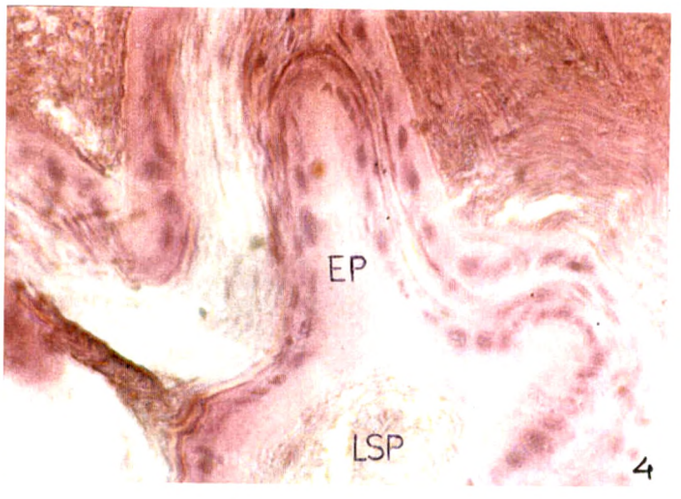
Fig. No 6 T.S. Prostate gland, H & E x (63x4)
The prostatic alveoli (A) were normal. Epithelium (EP) containing granular secretory cells with secretion (S) in between the spaces. Regenerated connective tissue capsule with septa. (CT)

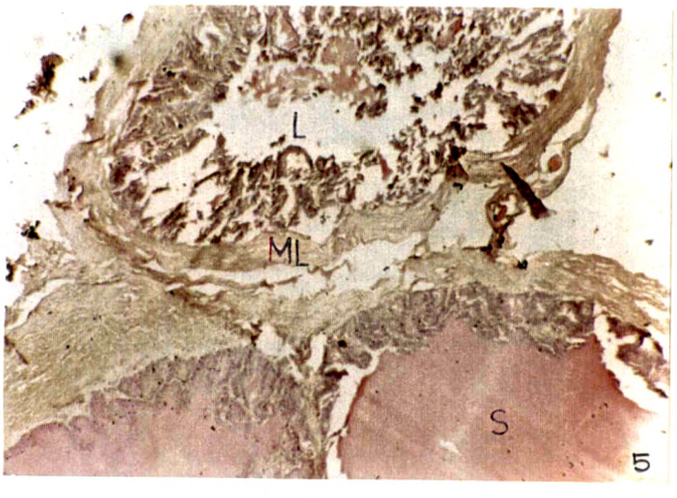
PLATE NO. 8

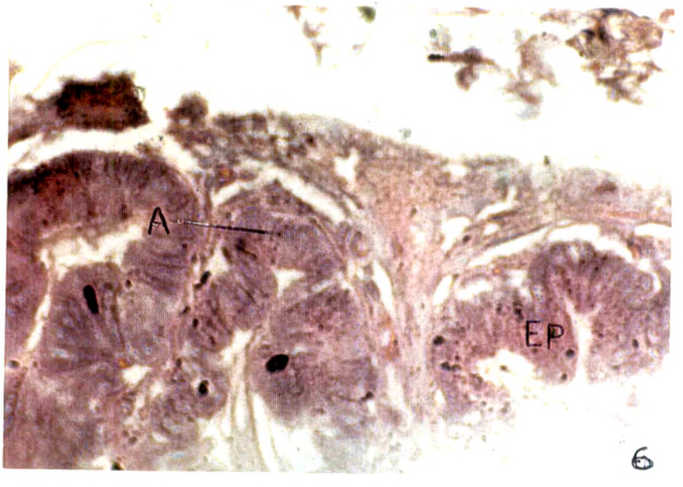












3.3.4. Alteration in seminal vesicle.

a) Wet weight.

b) Histiligy.

3.3.5. Alteration in prostate gland.

a) Wet weight.

b) Histology.

3.3.6. Alteration in cowper's gland.

a) Wet weight.

b) Histology.

3.4. Recovery Tests.

3.4.1. Testes

3.4.2. Caput Epididymes.

3.4.3. Cauda epididymes.

3.4.4. Seminal vesicle.

3.4.5. Prostate gland.

3.4.6. Cowper's gland.

3.0 Introduction:

Male reproductive systems of rat includes testes as the essential reproductive organs, epididymes, vas deferenses, seminal vesicles, prostate gland cowper's gland and penis.

Effects of large many number of synthetic compounds and natural drugs have been shown to cause arrest of spermatogenesis or complete sterility in males. Following if the brief review of literature available.

3.1. Review of literature : -

3.1.1. Testes

Number of synthetic compounds like triethylene melamine (Henry et al. 1960, Jackson and Bock 1955, Fox et al. 1963) , WIN 13099 , WIN 17416,WIN 18466 (Coulston et al. 1960) , 6 metroxyprogesterone acetate (Mc cleod 1965, Bhiwgade et al, 1991, Awari and Bhiwgade 1992) , busulfan (Jackson , 1966, kar et al., 1968 a ; Kanwar et al., 1974) , S. K. and F. 7690 (Sounder et al., 1969) , monochlorohydrin (Gunn et al., 1970) norgesterol (Singh et al., 1972) , trihydroxypregninolene (Sud and Setty , 1973) , 5-Thio-D-Glucose (Manghn (1974) and Homm et al., 1977), L-chorohydrin (Copper et al., 1974 ; Hundal and Managat –1978) endosulphan (Singh and Pandey 1989) , Vinca alkaloids namely Vinblastine and Vincristine have been studied for their antispermatogenic activity (Parvinan 1978; Toro and Varute 1989 ; T.murgavel and M.A. Akbarsha 1991). Effects of cemitidine and ranitidine on reproductive fundioning of mice were also studied (M.Gill et al ., 1991).

Some metalic salts have also been studied for their extensive antispermatogenic effect (Gun and Gould, 1970) , Orange II (Singh and Khanna 1979) , aspirin (Balsubramanian et al., 1980) , Cigarette smoking

(Viczin, 1968) etc have also been studied to effect damages to seminiferous tubules and cause antifertility effect. 2 mercapto Propionyl glycine (MPG) administration to albino rats resulted in alteration in the wet weights of testis.

Distortion of normal architecture of seminiferous tubules and atrophy of Leydig cells have also been reported by Rao et al., 1986). Administration of testesterone oenanthate in mice revealed decrease in weight of testes, decrease in number of spermatogonia, pachytene spermatocytes and spermatids (Bansal and Davies 1986). Shah et al., (1987) found decrease in organ weights without any significant changes in the body weight, reduction in the diameter of seminiferous tubules and disintegration of peritubular membrane in these rats. Treatment of flutamide (Dhar and Setty , 1987) in adult male rats caused alterations in histoarchitechire of testis. Arrest of spermatogenesis were identified at later stages of development of spermatids even though a few seminiferous tubules showed normal spermatids. They also revealed hypertrophy of Leyding cells. Alterations in the seminiferous tubular epithelium were also found in male rats after giving injections of Follicle regulating protein (Nakumura et al ., 1987).

Changes in testis after administration of PMHI to field rats (Dechamma and Sarkar 1987) , and to calotes versicolor (Anant laxmi and Sarkar 1994) , were also found to occur . Drastic reduction in the weight and size of testis with complete arrest of spermatogenesis and degeneration of germinal epithelium, along with formation of sperm coagulum in the lumen of the tubules of the testis were seen to occur. Effect of adrenalactomy in rats caused impairment of spermatogenesis shrunken seminiferous tubules, karyolysis and degeneration of Leydig cells with increased interstitium in testis. Tubular lumen get filled with debris . Effect

of injection of lithium chloride on testicular enzymes of B-hydroxy steroid dehydrogenase , acid phosphatase and gametogenesis were studied in Bufo. ((Ghosh et al., 1990) It caused significant decrease in activities of testicular enzymes and marked inhibition of spermatogenesis was observed in toads.

Intraperitoneal administration of P-aminodiphenyl amine induced decrease in the activities of testicular enzymes, hyaluronic acid and lactic acid indicating arrest of spermatogenesis (Singh et al., 1992). They also confirmed necrosis of the testis histopathologically. Effect of styrene on testicular enzymes (Shrivastav et al., 1992) also showed degenerative effects in growing rats. Effect of cyproterone acetate treated testes showed changes in sertoli cells, Leydig cells, germ cells and in speromatids . The levels of DNA, RNA cholesterol and glycogen in the testes were shown to decrease in these rats as compared to those of control groups. Effects of ascorbic acid (N. M Biswas et al., 1996) were also studied in rat testis showing degenerative changes.

Apart from attempts of finding effective chemicals and synthetic drugs, plant drugs of folklore medicines are also studied carefully and extensively for their possible efficacy in potential antifertility effect. Laszlo H. and Henshow P.S. (1954) after surveying the available literature, listed the names of 60 plants used by primittive people in controlling fertility. Later on number of plant drugs with antifertility properlies were experimentally studied by number of workers. East J. (1955) Verma et al., (1959) isolated meta-xylohydroquinone from Pisum sativum L., and reported to effect sterility in human females for the first time. Chopra et al., (1956) made a "Glossary of Indian Medicinal Plants)" and proved that the oils extracted from leaves of Oscimum have number of medicinal values. The water soluble portion of ethanotic extracts of barks of

Hippohea salicifolia (Joshi et al., 1965) has been shown to possess antimitotic properly in the testes of young rats. Joshi and Ambaye (1968) reported that intraperironeal administration of total alkaloids of vincarosea produced graded degenerative changes in the spermatogenic elements of the immature male rats. The leaves of O. sanctum when fed with normal diet (Kasinathan et al., 1972) caused slight impairment of spermatogenesis in experimental mice . Vyas and Singh (1976) studied the effect of cannabis and opium on columba livia. The extracts of both produced adverse effects like reduction in weight of testis and in diameter of spermatocytes and spermatozoa. It also showed proliferation of Leyding cells.

Benzene extracts of Hibiseus rosa sinensis (Kholkute , 1977) when administered orally to albino rats found to arrest spermatogenesis as well as endocrine functions of testis. Spermatogenesis was arrested at spermatid stage. Leyding cells were spares in number and atrophied after 30 days of treatment. Some of the seminiferous tubules showed only spermatogonia and sertoli cells, while rest of the germinal elements were totally absent.

Water soluble parts of chloroform extracts of Aristolochia indica (Pakrasi and Pakrasi, 1977) when fed orally caused degencrative changes of varying degrees in the seminiferous tubular components. Spermatogenesis was interrupted at various stages in different tubules. Necrobiosis and conspicuous nuclear degeneration of varying degrees was also seen. In some tubules, the tubular lumina were filled with cellular debris leaving small and large vacuoles in their places. Leydig cells were intact and morphologically unchanged.

Degenerative changes were also reported in testis of house rats and gerbils by administration of alcoholic extracts of Malvaviscus conzanttii flowers (Dixit, 1977). In gerbil, oral administration of Calotropis procera

flower extracts (Garg, 1979) caused widespread testicular necrosis. Ethanol extracts of Vinca rosea when given orally, caused significant changes in the testis of matured rats. (Chauhan et al., 1979). But both the groups have not reported histological damage to testis in rats. Das (1980) administered ripe papaya seed. Powder and reported no change in the histology of testis. But crude chloroform extracts of these seeds (Lohiya and Goyal, 1992) were shown to reduce fertility to zero percent after 40-60 days treatment). Dixit and Joshi (1982) reported the effects of garlic powder which caused significant reduction in the body weights and testicular weights. Hoffer (1983) administered gossypol, an active principle from Cotton seeds, and observed presence of severely damaged and entirely normal seminiferous tubules adjescent to one another in the same section. Effects of Plumbagin, an active principle from Plumbago zylencia (Bhargava, 1984) were reported in case of experimental dogs which arrested spermatogenesis at spermatocyte level. Toro (1984) administered alkaloids from Vinca rosea Linn. Leaves to adult male albino rats and reported that these alkaloids affected all the speronatogenic elements except spermatogonia and matured spermatozoa. He also reported that, sertoli cells and Leydig cells were rarely affected. Sohani (1985) administered leaf extracts of Vitex negundo to male albino rats and reported number of changes in testicular histology. Awati (1985) reported that the leaf extracts of Butea monosperma arrest spermatogenesis at spermatid stage, and Shah (1985) reported the arrests of spermatogenesis at spermatocyte stage by administering seed extracts of Daucus Carota.

Khanna et al., (1986) fed leaves of Oscimum sanctum to albino rats and showed the decrease in sperm count, sperm motility and the weights of male reproductive organs. Decrease in the population of spermatogenic elements in the seminiferous tubules were observed by Rao (1988) after

treatment with Solanum xanthocarpum for 60 days. Chinnoy et al., (1988) reported distorted histoarchitecture of testis by administration of leaf extracts of Vinca rosea. Bhiwgade et al., (1988) reported complete inhibition of spermatogenesis by oral administraction of Gossypol for 8 weeks. Toro and Hiremath (1988) and P. Adhikary et al., (1989) administered petiole extracts of piper betle to male albino rats and reported decrease in the wet weights of testis and degenerative changes in the seminiferous epithelium. Akbarsha et al., (1988 and 1990) after administration of dry leaf powders of Andrographis paniculata (Nees) reported suppressed spermatogenesis, decrease in organ weights and involuted interstitium with phagocytic cells in the lumen of the tubules. He also observed regression of Leydig cells. They reported both antispermatogenic and antiandrogenic effects of the plant. D. V. Choudhary et al., (1988) studied antifertility effects of leaf extracts of some plants in male rats. They reported that leaf extracts of Azadiracta indica (Neem) and Chordia dichotoma (bahuvarka) resulted into abolition of sexual desire in all experimental male rats.

Short term administration of Ocimum sanctum caused significant decrease in sexual behaviour score (Kantak and Gogate, 1992). The testicular sperm counts after administration of chloroform extracts of Carica papaya Linn. Seeds in male rats were seen to be considerably decreased. (Lohiya et al., 1992). Effects of Azadiracta indica leaves on testis and its recovery in albino rats were studied by Anjali Joshi et al., (1996). Singh et al., (1996) carried out chemical and pharmacological studies on fixed oil from Oscimum sanctum, and reported

Madhusudan Reddy (1997) studied the effects of Hibiscus rosa sinensis on the testis. He also reported decrease in diameter of seminiferous tubules in proportion to the weight of testis and have

undergone destrophy. All the spermatogenic elements decreased in treated animals as compared to those of control groups. They also revealed increased interstitial space due to Shrinkage of Seminiferous tubules.

3.1.2. Epididymes

The secretory and aobsorptive function of epididymes are believed to be related with the maturation and storage of sperms (Hamilton 1975). Hence it has become the object of number of histological and biochemical studied as seen from the reviews by Nicander (1957) , Reid and Cleland (1957) , Maneely (1959) , Risley (1963) , Holstein (1969) , Martan (1969) and Clermant and Flannery (1970) .

Many chemicals have been tried in different animals and proved to possess potent antifertility ability and explored their effects on epididymes in the process of aspermatogenesis. Following is the brief review of existing work. A few of them are clomiphene (Nelson and Patenelli , 1962; Ray et al., 1964, Kalra and Prasad , 1967., schalley et al., 1970., Roy and Dalla 1976; Roy et al., 1976) , Dexamethazone , Metopiron, ninidazol, danazol (Dixit, 1979) , Chloromadione acetate (Kaur and Mangat, 1979), Ethylene dimethane suplhonate (copper and Jackson,1970) , U 29409 (Ericesson, 1971) Cadmiumchloride (Dixit, 1976,Chinney and Seth, 1977), Progestin and Androgen (Flickinger, 1977) cyclohaxanol (Tyagi et. al. 1979), testeronapropionate (Manjula and Kadam 1980), and oxyphenonium (Rathasoorya, 1982). All these chemicals have been shown to alter the epididymal architechture by reducing the cell height. These chemicals have also reduced the number of spermatozoa in the lumen, although the degree of reduction of spermatozoa varies with chemicals employed. All these results were shown to be dose dependent.

Cyproterone and cyproterone acetate (Newmann et al., , 1969,70 ; Meitkowiski and Lukaszyk 1969 , Pruoad et al., 1970 ; Rajlaxmi et al., 1971 ; Steinbeck et al., 1971 ; Prasad 1973 ; Rajlaxmi and Prasad 1975 ; Bose et al., 1975 ; Nag et al., 1976 a, 1977 ; Dinkar et al., 1977 ; Rastogi et al., 1979) , α-chlorohydrin (craubo 1965 ; Peyre and Laporte, 1966 ; Ericson and Baker, 1970 ; Hoffer et al., 1973; Vickery et al., 1974 ; Edwards 1976 ; Ford et al., 1977 ; Guraya and Gill , 1977 ; Dixit , 1979 , Nag et al., 1976 , Kreider and Dutt , 1970 etc. have been shown to alter the epididymal historachitecture and reduce the number of spermatozoa especially in caput epididymes .

Long term treatment of MPG in adult male rats suggested decrease in the weight of epididymis. Changes were also observed in the structure and secretory activity of epididymal epithelium (Rao et al., 1986). Dhar and Setty (1987) studied the effect of fiutamide on male rats. They reported reduction in the tubular diameter no change in the epididymal epithelium., decrease in the number of sperms. There was also a reduction in the weight of cauda epididymis. PMHI when administered to field rats showed caput and corpus epididymal tubules completely devoid of sperms without affecting the histology of luminal epithelium, similar degenerative effects on sperms without affecting the luminal epithelium were also found by Dechamma and Sarkar, 1987, Shah et al., (1987) reported a decrease in the wet weight of epididymes after administration formaldehyde to male albino rats. They also found significant reduction in the tubular diameter of tubules and height of epithelial cells in cauda epididymes. After administration of β-Sitosterol to albino rats, Malini and Vanita kumari (1988) also reported a reduction in the wet weight of cauda epididymes . They have also found a marked atrophy in the tubular epithelium, a

decrease in the height of tubular cells as well as stereocilia and reduction in the number of luminal spermatozoa of both caput and cauda epididymes.

Ultrastructural and biochemical changes in the epididymis of gossypol treated rats Bhiwgade et al. (1989). They reported a significant reduction in the weight of cauda epididymis and effects in the sperm such as vacuolisation and complete degeneration of the mid piece mitochondria and plasma membrane . A specific effect of Vincristine sulphate on epididymis of albino rats were studied (Horne Averal, Stanley, Murugaian and Akbarsha , 1994 , 1996) it caused conspicuous pathological changes in the principal and apical cells of caput and the clear cells of cauda epididymis suggesting impairment of epididymal function , particularly concerning sperm maturation. Impact of prolactin on epididymal lipid profile were studied in castrated rats (Basvadatta Ray et al., (1994).

Very few plants with antispermatogenic potencies have been explored for their effects on epididymis.

Aristolochia indica extract administration induced reduction in the diameter of epididymal tubules , reduction in the density of spermatozoa and deposition of fat droplets in the lumen of tubules (Pakrasi and Pakrasi , 1977). Dixit (1977 b) observed sloughing off of the epididymal epithelium, despermatization of lumen and oedamatous interstitium in the epididymes of house rats after administration of extracts of Malvavisous conzantti. Verma et al., (1980) have also reported atrophy of lumen of cauda epididymes in mice with M. conzanttii. Garg (1979) found reduction in the size of epididymal tubules and number of spermatozoa by administration of extracts of Calotropis procera to gerbils. Administration of powder of Allium sativum resulted into significant reduction in the wet weight of epididymis of rats.(Dixit and Joshi, 1982). Bhargava (1984)

reported regressed epididymal epithelium and despermatized lumen after treatment with Plumbagin. Toro (1984) administered alkaloid fractions of leaf extracts of Vinca rosea and found reduction in the number of spermatozoa in epididymis but no change in the height of cells lining the tubules. Sohani (1985) administered Vitex negundo in the wet weights and height of epithelial cells of epididymes. Awati (1985) found reduction in the spermatozoan population by administering Butea monospermia extracts while, Shah (1985) reported many alterations in the histoarchitecture of caput and cauda epididymes after administration of seed extracts of Daucus Carota. Khanna et al., (1986) studied effect of long term feeding of Ocimum sanctum on reproductive performance of adult male rats and reported a significant decrease in the wet weights, sperm count and motility of sperm in epididymes. Toro and Hiremath (1988) reported accumulation of cellular debris in the tubules by administration of Piper betle petiole extracts to male albino rats. Ethanoloic extracts of Terminalia belirica fruits (Rao, 1988) were shown to cause degenerating effects on epididymis. Akbasha et al., (1988) studied the effect of administration of Andrographis paniculata leaves in albino rats and showed reduction in the cellular height of epithelium, Rao, (1988) studied the effects of alcoholic extracts of Solanum xanthocarpum seeds in adult male rats and found that the sperms from cauda epididymes had characteristically acquired sickle-shaped head and straight mid piece and tail. They also reported decapitation and swelling in sperms at mid piece region and found regressive changes in epididymes with loss in weight.

Crude chloroform extracts of carica papaya Linn. Seeds (Lohiya and Goyal , 1980) were investigated for contraceptive efficacy and related side effects in male albino rats. The extracts suppressed the motility of sperms in cauda epididymes after treatment and decreased sperm count in

epididymes without any alterations (Side effects) in clinical parameters and libido of the animals.

3.1.3 Seminal Vesicle

In most of the species, seminal vesicles contribute more to the volume of semen so, they have become the subject of numerous histological cytological , biochemical and histochemical investigations, Many synthetic chemicals tried to explore the effects on the seminal vesicles. According to the available literature, a brief review is given below.

Cavazos and Melampy (1954) demonstrated statistically a significant reduction in height of the epithehial cells of seminal vesicles after castration in rats. Five days after castration, these was reduction in cell height and loss of secretory grannules in mouse seminal vesicles (Szirami and Van der Linde , 1977 ; Allison, 1964).Hypoxia caused loss of weights and corresponding changes in the sceretory epithelium of seminal vesicles (Riar and Malhotra,1977) Saunders et al., (1969) , found significant reduction in the weights of seminal vesicles with reduction in semen volume by treating with and F. 7690 Elkington and Blackshaw (1971), reported reduction in the seminal vesicular weight after estrogen administration. Chlorocyclizin treatment resulted in the formation of cytoplasmic vacuoles in the epithelial cells, which became increasingly more and densely populated , accompanied by a reduction in the secretion with time. (Wong et al., 1972), Singh et al., (1972) studied the effect of norgesterol and reported reduction in the weight of seminal vesicles, α-chlorohydrin administration resulted in marked regression in seminal vesicles (Vickery et al., 1974). Cyproterone acetate caused reduction in height and width of epithelial cells (Dahl and Tveter, 1974). Agmo (1975)

reported reduced activity of seminal vesicle with decrease in the volume of secretion after cyproterone acetate administration. This view was supported by Bose et al., (1977) . Medroxyprogesterone treatment caused 15-25% reduction in weight of seminal vesicles and in the height of epithelial cells (Flickinger , !977) , Das et al., (1977 b), reported significant reduction in weights of seminal vesicles in the rats treated with centochroman. Morphological examination of seminal vesicles after administration of cadmium chloride revealed reduction in weight , size and secretory activity of epithelial cells. (Hundal and Mangat , 1978). Tyagi et al., (1979) demonstrated reduction in in the weights of seminal vesicles of house rats and gerbils after administration of cyclohexanol Kaur and Mangat (1979) observed regression in weight. Balsubramanian et al., (1980) reported atrophy of seminal vesicles by treating with aspirin. Shah et al., (1987) administrated formaldehyde to white rats and showed significant decrease in weight of seminal vesicle without affecting the body weights. Dhar and Setty, (1987) reported marginal reduction in the weight of seminal vesicle by giving high dose of flutamide to male albino rats.[M.V.Rao ,Shah and chinnoy studied the long term effects of 2-MPG on reproductive organs of adult rats and found decrease in the weight of treated seminal vesicles] K Venkata, Rami Redday and S Govindappa (1986) studied the effects of prolactin on male reproductive organs. They reported a significant decrease in the thickness of muscular layer of seminal vesicle in all the age groups of treated rats than those of control rats. Indicating increased biosynthesis in glandular elements of the organ. Mangayarkkarasi et al., (1988) studied the effects of intraperitoneal administration of ovine prolactin in hypoprolactinemic adult bonnet monkey and showed decrease in the weight of seminal vesicles. G Vanitakumari studied the effect of short-term treatment of eugenol on the

seminal vesicles adult albino rats and showed that, eugenol exerts an adverse effect on the histology of the seminal vesicle by causing degeneration of the secretory epithelium. However , an increase in the vesicle weight was also observed.

Very few plants having antispermatogenic activity have been explored for their effects on seminal vesicles .S. Kasinathan et al., (1972) reported change in the pH of seminal plasma of seminal vesicles by administration of Oscimum sanctum extract to mice oral administration of Malvaviscus conzanttii extract caused decrease in the weights of seminal vesicles in house rats as well as in gerbils (Dixit , 1977) Oral administration of Aristolochia indica (Pakrasi and Pakrasi 1977) caused significant reduction in the size and weight of seminal vesicles of mice. They further reported no histological changes but the lumen was devoid of secretion Garg (1979) also reported reduction in the weights of seminal vesicles of gergibls when fed with extracts of Calotropis Procera (Verma et al., 1980) observed decrease in the relative weight and size of seminal vesicles by administration of Malvaviscus Conzantti ; extracts. They also reported intibition in the arborization of secretory epithelium , reduction in cell height and in secretion Powder of Allium Sativum (Dixit and Joshi 1982) also showed reduction in weight of seminal vesicles in rats. Toro (1984) reported decrease in the height of epithelial cells after administration of vinca alkaloids. Shah (1985) with seeds of Daucus carota and Awati (1985) with leaf extracts of Butea monosperma reported reduction in the wet weights of seminal vesicles. Sohani (1985) got similar results with vitex negundo extract S. Khanna, Gupta and Grover (1986) observed effect of long term feeding of Oscimum sanctum Linn on reproductive performance of adult albino rats and reported significant reduction in the weight of seminal vesicles of Tulsi treated rats. Similar

result were reported by Seth et al., (1981). Hiremath (1988) observed decrease in the weight of seminal vesicles of rats treated with petiole extracts of Piper betle. Akbasha et al., (1988) studied the effect of Andrographis paniculata leaves in male albino rats and reported degenerative changes and reduction in the lumen of seminal vesicles. Chinnoy et al., (1988) reported that administration of Vinca rosea to albino rats significantly affected the histoarchitecture of seminal vesicles. Jadhav (1998) reported reduction in the weight of seminal vesicles and histological alterations such as reduced thickness in muscle layer and lamina propria, shorteining of epithelial folds and empty lumen of the tubules in seminal vesicles by treating with extracts of Plumbugo.

Reddy et al., (1997) found slight decrease in the weight of seminal vesicles in rats treated with Hibiscus than those of control groups.

3.1.4. Prostate Gland.

Prostate gland secretes a variety of substances which contribute to the volume of semen in mammals. (Price and Williams – Ashman, 1961 , Mann 1964). Many studies have been devoted to the prostate gland in man and other mammals because it is a large organ subjected to various pathological disorders. The light microscopy of prostate and its histochemistry in mouse have been extensively reviewed by Brandes (1966), Many synthetic chemicals antifertility agents and plant extracts have been tried to explore the effects on prostate gland. A brief review of available literature is as follows.:

Price et al., (1955) found that large doses of progestational agents had a stimulating effect on the accessory sex glands in castrated rats. Later studies showed that progestin causes decrease in weight and alteration in function of accessory sex glands (Patanelli and Nelson , 1959) Similar

results were obtained in the prostate of castrated or estrogen treated rats by Price et al., (1961) , Brandes et al. (1962),Helminen and Ericsson (1971) . Dahl & kjaerheim (1973) and in rats treated with cyproterone acetate (Dahl and Tveter ,1974) and Loving and Flickinger (1976) Kar et al., (1968) confirtred the effect of busulphan on the weight and functional capacity of prostate. Depression in weight of prostate gland in rats administered with norgesterol resulted due to androgenic defficiency of testis (Singh et al., 1972). Rajlaxmi (1972) administered cyproterone acetate in rats and found decrease in the activity of prostate gland complex. Cadmium chloride administration to rats decreased the weight of prostate (Chinnoy and Seth, 1977).

Removal of epididymal fat in albino rats resulted into significant decrease in weight of prostate gland (Shriniwasan et al., 1986) In ventral prostate the diameters of the alveoli, lumen and height of epithelium were increased after administration of prolactin suggesting the participation of this hormone on activation, growth and maturation events of prostate (K.Reddy and S Govindappa 1986). Flutamide administered to prepubertal and adult rats showed a significant reduction in the weight of ventral prostate in immature rats while, it failed to show any significant changes in case of adult rats Dhar and Setty (1987). Paraamino diphenyl amine induced no considerable changes in the ventral prostate (R.L.Singh et al., 1990) . A significant decrease in prostate and other accessory sex organ weights was confirmed in lithium treated rats.(Bhiwgade et al., 1989) The weights of ventral prostate decreased in 2-MPG treated rats than those of control. (Roa et al., 1986).

Chronic administration of flower extracts of Malvaviscus conzanttii showed reduction in the weights of prostate glands (Dixt , 1977 b). Administration of extracts of Aristolochia indica resulted in decrease in

weight and secretion of prostate gland in mice without any change in the histology (Pakarsi and Pakarsi , 1977) Chauhan et al , (1979) reported reduction the weight of prostate and Toro (1984) reported insignificant reduction in the height of epithelial cells after treatment with Vinca rosea extracts . Shah (1985) reported decrease in the wet weight and secretion without any changes in epithelial cells after administration of Daucus carota seed extract. Sohani (1985) observed reduction in the wet weight, epithelial cell height and secretion after administration of Vitex negundo to the rats. Khanna et al., (1986) administered Ocimum sanctum to male albino rats and found significant reduction in weight of ventral prostate. Chinnoy et al., (1988) found the effects of Vinca rosea leaf extracts which altered some androgen sensitive parameters of prostate in albino rats. Dry leaf powder of Andrographtis paniculata caused significant decrease in the weight of prostate. The lumen was diminished by infoldings of epithetium. Nuclear extrusion and phagocytic activity in the lumen of the prostatic tubules was insignificant (Akbarsha et al., 1988). Hiremath (1988) administered petiole extracts of Piper betle to albino rats and reported decrease in weight of prostate gland. Purnima Adhikary , Julie Bauerji et al., (1990) also reported decrease in the weight of androgen dependent accessory sex organs. Jadhav (1988) also confirmed simillar results after administration of Plumbagin to albino rats. Reddy et al., (1997) studied significant increase in the prostate of rats treated with Hibiscus.

3.1.4 Cowper's gland

Comparatively less attention has been paid to the detailed study of Cowper's gland. Various workers have studied the changes in wet weight of glands in different mammals.

Oritz (1953) showed atrophy of bulbouerethral glands in rats within ten days after castration. Latalski (1969) observed changes in the cells of bulbourethral glands of rats after prostatactomy. Jeffery (1967) observed involution of secretory epithelium of cowper's gland after estrogen administration. The changes in wet weight of cowper's glands of rat after administration with cis-or trans-clomiphene citrate were noted by Rajlakshmi et al., (1970), cyproterone acetate treatment caused very little changes in the weight of the glands (Rajlakshmi and Prasad, 1971). Sharma et al. (1976) observed decline in the weight of glands after chloromadinone acetate treatment. Latalski and Pruch (1976) found regression and degenerative changes in the cowper's glands of albino rats after administration of a synthetic estrogen releasing compound. The changes in weights were some what pronounced after treatment with ORF-1616 and cyproterone acetate in combination. (Rajlakshmi and Prasad , 1971). Decrease in the weight of cowper's glands after treatment with cadmium chloride were also reported (Chinnoy and Seth 1977). Reduction in the weights of the gland were also observed up to 20% in castrated Loris, (Majula and Kadam 1980) and by administration of WIN 18446 (Singh and Dominic 1980). Long term effects of 2- MPG were studied in albino rats (Rao M.V. , Shah V.C. and Chinnoy 1986) and the weight of coagulating glands were seen to be decreased in treated rats. R.L.Singh , Khanna and G.B.Singh (1992) studied Para-amino diphenylamine induced biochemical changes in the coagulating glands of albino rats. They observed no significant change in fructose content of coagulating glands and activities of some enzymes supporting androgenic status even though the drug induced partial necrosis in testis M.N. Ananthlaxmi et al., (1994) studied the effect of PMHI on the reproductive system of male

lizard, calotes versicolor. They found decrease in the weight and size of the acessory reproductive glands.

Absolutely very few plants have been studied regarding their effects on histomorphology and cytochemistry of Cowper's glands in rats. Awati (1985) showed increase in wet weight no change in epithelium and stroma and a slight increase in secretion after administration of Butea monosperma extract. Sohani (1985) found reduction in the height of epithelial cells and secretion after administration of Vitex negundo extract. Toro (1984) showed reduction in secretion decrease in wet weight of Cowper's glands of rats treated with Vinca rosea leaf extract. Shah (1985) also reported reduction in the secretion of Cowper's glands after administration of Daucus carota extract. Jadhav (1988) showed reduction in weight of Cowper's gland of plumbugin treated rats and Hiremath (1988) also reported decrease in the weight of cowper's glands treated with petiole extracts of Piper betle in male albino rats. M A Akbarshah et. al., (1990) studied antifertility effect of Andrographis paniculata (Nees) in male albino rats. They reported regressive and degenerative changes in the coagulating glands along with reduction in the weight and fluid content of the gland.

3.2. Alteration in body weights:-

The total body weights of albino rats treated with Ocscimum sanctum showed considerable changes as compared to those of control groups after different intervals of administration of dose. All the rats of both experimental and control groups showed normal health with no mortality. The body weight showed a gradual increase in the control and experimental rats.

Actual records of body weights are shown in Table no.1.

Table No.1

Body weight of control and Oscimum sanctum treated experimental rats.

Sr. No.	Duration in days.	Control body weight in gms.	Experimental body weights in gms.
1	o	210±1.80	212±1.98
2	24	248±2.03	255±2.96
3	48	273±1.36	302±1.33
4	72	320±3.44	370± 5.31
5	120	320±2.38	378±3.40
	Termination Of treatment (from 72nd to 120th day)		

(values are Arithmetic mean ± S.D. of three animals and are expressed in gms.)

In the begining of the experiment, the total body weights of control rats were 210 ±1.80 gms. They gradually increased to 248±2,03 gms. 273±1.36gms. and 320±3.44 gms. after 24 days, 48 days and 72 days of treatment respectively. And after treatment of the up to 120 days, the body weights of rats similarly increased to 352±2.38 gms.

On the same line, the body weights of experimental rats were 212 ± 1.98 gms. in the beginning of experiment. They increased to 255±2.96 gms., 302±1.33gms and 370±5.31 gms. after 24 days , 48 days and 72 days

of treatment up to 120 days , the weight were increased a little i.c. from 370±5.31 gms. (on 72nd days) to (378±3.40 gms.(on 120th day)

3.3. Alterations in Histoarchitecture of Organs

3.3.1 Alteration in Testes.

Testes exhibitted interesting alteration in the wet weight during the period of administration of the dose. The variations occurring in wet weights of testes recorded in table no 2.

Table No.2

Wet weight of testes of control and Oscimum sanctum treated Experimental rats.

Sr. No.	Duration in days.	Control testis weight in mg/100gm Of body weight	Experimental testes weights in mg/100gm of body weight.
1	o	943.25±5.38	--
2	24	928.54±6.67	798.17±3.49
3	48	804.50±4.364	685.20±5.2
4	72	815.73±4.2	603.00±5.42
5	120	880.53±6.8	701.87±5.2
	Termination of treatment (after 72nd to 120th day)		

Values are Mean ± S.D. of three animals. Wet weight of testes are expressed in mg/100gm. Of body weight.

In the beginning of the experiment , the weight of control testes was 92.3.25±5.38mg/100gm of body wet on 24th days of treatment , the weight of control testes became 928.54±6.67mg/100gm bodyweight. It decreased significantly to 804.50 ± 4.364 on 48th day while it increased a little on 72nd day to 815.73±4.2 mg. But the increase in control rats after termination of treatment up to 120 days i.c. 880.53±6.8mg of body weight.

On the 24th day of treatment , the wet weight of testes of experimental rat was 798.17±3.49mg. It showed a gradual decrease in weight to 685.20±5.2mg and 603.00±5.42mg on 48th day and 72nd day of treatment respectively. But it showed a sudden increase in weight up to 701.87_5.2 mg after termination of treatment on 120th day.

B. Tubular diameter

The diameter of seminiferous tubules of testes in the experimental animals changed much more than those of control rats The correct figures of variations are given below in Table No.3.

Table No.3

Tubular diameter of seminiferuos tubules of control and Ocscimum sanctum treated experimental rats.

Sr. No.	Duration in days.	Tubular diameter in µm. control	Experimental
1	0	268.5±11.0	---
2	24	270.62±10.4	232.00±.5
3	48	273.00± 5.4	198.37±5.3
4	72	267.88±10.4	165.79±10.5
5	120 Termination of treatment (after 72 to 120 days)	269.87± 7.9	209.12±4.4

Values are mean ± S.D. of 50 tubules , diameters are expressed in μm.

In the beginning of the experiment , the diameter of normal rat seminiferous tubules was 268.5 ± 11.0 μm.

On 24th day of experiment , the tubular diameter of control testes became 270.62± 10.4μm and showed no significant change in control rats after 48 days (273.0± 5.4μm) and 72 days (267.88±10.4μm) of treatment and after termination of treatment (269.87±7.9μm) on 120 day of experiment on the other hand , the diameter of seminiferous tubules in experimental rats treated with oscimum sanctum showed significant decrease with progress of the duration of dose. It was 232.00±8.5μm on 24th day. And it decreased to 198.37±5.3μm on 48 day and on 72nd day of treatment it showed marked decrease to 165.79±10.5μm

While , after termination of treatment up to 120 days , the tubular diameter again showed a progressive increase to 209.12±4.4 μm in oscimum treated rat testes.

C. Histology

1) Control: - The histoarchiteture of testes of control rats did not altered much from those of normal albino rats.

Each testis had a fibrous connective tissue capsule , tuhica albugenia. It penetrates with in the body of testis to from mediastinum which sends many septa to divide the testis into many compartments. Each compartment shows 1-4 seminiferous tubules held together by connective

tissue containing groups of interstitial cells often called as Leyding cells with neighbouring blood vessels and lymphatics.

Seminiferous tubules were well developed, entire with maximum tubular diameter. The wall of seminiferous tubules was lined by a single layer of cubiodal germinal epithelium lying on the basement membrane wrapped externally by tunica propria of fibroelastic connective tissue. In between the germinal epithelium, were a few sertoli cells. The tubules showed all the cells of spermatogenic series. Tubular profiles containing all the stages of spermatogenesis such as spermatogonia primary spermatocytes, secondary spermatocytes, round spermatids, elongating spermatids and soermatozoa could be recognised from outside within. Large bundles of concentrically arranged spermatozoa were seen in the lumen of the tubules.

Sertoli cells were normal, tall, pyramidal resting on basement membrane. The nuclei were oval. They provide nourishment to developing spermatozoa (Plate no 1 fig.1).

The germinal cells of the seminiferous tubules undergo spermatogenesis to give rise to large number of spermatozoa. During the process, spermatogonia are seen just inside the basement membrane in several layers. They are spherical or cuboidal cells with a chromatin rich rounded nucleus in each. They undergo mitotic divisions to give rise to new spermatogonia and later on get differentiated into primary spermatocytes by growth and increase in size.

The primary spermatocytes present just inside the spermatogonia are larger in size . They undergo maturation division to produce smaller secondary spermatocytes. Each secondary spermatocyte divides into two spermatids which lie inner to them. Each spermatid is a smaller cell with a

dense nucleus that gets stained with heamatoxylene and contains one or two dark nucleoli.

The spermatids undergo metamorphosis to from matured spermatozoa which lie in the lumen of the tubules. Each spermatozoa consists of a head , a middle piece and a tail or flagellum. They remain closely adhered to the sertoli cells with their heads while their tails projecting into the lumen of the tubule like a whorl (Plate no.1 Fig. 1 and Fig.2)

2) Experimental: -

The histological observations in T.S. of Testes treated with Ocscimum sanctum in experimental rats as observed under light microscope showed remarkable variations from those of control rats. They showed graded differeness with the duration of time as follows. The results are illustruted in plate No 1. And 2

a) 24 days treatment

Tunica propria and basement membrane get separated from each other in some tubules even though they remain simply thickenened in some tubules. Patchy degeneration of seminiferous tubules showed desquamation and sloughing off of the gametogenic epithelial cells. Illdefind spaces appeared in between the spermatogenic elements due to degeneration of cells.(plate no.1 fig.3 and fig.4). The interstitium was normal with vascularity with a little increase in it. But the Leyding cells gathered together showing some disorganisation (Plate no 1 fig.3 and fig.4) Sertoli cells were normal.

b) 48 days treatment :-

Overall population of spermatogenic elements was affected at this stage. Patchy degeneration of seminiferous tubules progressed and showed cellular debris in some tubules. (Plate no 1 fig.6). But some seminiferous tubules adjacent to the necroscd one were apparently normal in appearance (Plate no 1 fig.5).

Shrinkage of the seminiferous tubules progressed with more increase in the interstisium. The Leyding cells were also shrunken in appearance resulting into dark staining (Plate N0 1. Fig.6). The tubules were totally devoid of spermarids, structure of spermatozoa were also affected. Degenerating spermatids and tail remainents of degenerating sperms accumulated in the lumen of tubules in the form of cellular debri and sperm coagulum (Plate No1. Fig.6.)

D. **72 days treatment.**

At this stage of experiment , there was maximum damage to the testicular elements. Majority of seminiferous tubules were necrosed.

Tunica propria and basement membrane showed dense thickening and breakage at intervals. Population of spermatogenic cells was greatly affected. The seminiferous tubules showed some normal spermatogonia at the periphery and spermatocytes scattered in between the smaller and larger vacuole like ill defined spaces. Vacuoles appeared even in the cytoplasm and nuclei of spermatocytes. (plate N0. 2. Fig. 2.)

Shrinkage of the seminiferous tubules resulted in to enlargement of interstitium to its maximum. Interstitium was filled with an oedomatous fluid (plate No.2. Fig.1.) The seminiferous tubules showed many pseudopodia like processes.

3.3.2. **Alteration in caput epididycis.**

Wet weight:-

The caput epididymes of experimental rats treated with Ocscimum sanctum showed varying degree of reduction in wet weight with progressive duration of dose while those control rat epididymes on other hand did not showed any decrease in their weight. Actual figures recorded during the experiment are given in Table no 4

Table No. 4

Wet weight of caput epididymes of control and Ocscimum sanctum treated experimental rats.

Sr. No.	Duration in days.	Control caput epididymes Wet weight in mg/100gm body weight.	Experimental caput epididymes wet weight In mg/100gm body weight.
1	0	176.96±2.46	---
2	24	172.00±2.46	137.20±5.8
3	48	165.09±2.4	140.25±2.8
4	72	159.75±2.4	132.01±3.7
5	120 termination of treatment up to 120 days.	155.75±3.8	129.48±4.7

Values are mean ± S.D. of three animals wet weight of caput epididymes are expressed in mg/100gm of body weight.

Initially , the value of wet weight of caput epididymes in control rats was 176.96±2.46mg before the treatment , on 24th day of treatment , the wet weight became 172.00±2.46mg showing insignificant further reduced to 165.90±4.2 and 159.75±2.4 after 48 days and 72 days of treatment in control rats respectively. And at the end of experiment on termination of treatment also the wet weight of control epididymes was 155.75 ± 3.8 mg. These values indicate an insignificant decreases in the wet weights of control caput epididymes.

On the 0ther hand, in experiment group of rats the weight of caput epididymes on 24th day of treatment was 132.20 ± 5.8 mg. The value increased to 140.25 ± 2.8 mg on 48th day of treatment. There after the weight decreased to 132.01 ± 3.7 on 72nd of treatment and finally more or less remained the same after termination of treatment i.e. 129.48 ± 4.7 mg on 120th day of experiment.

All these weights of experimental caput epididymes showed earlier increase in weight followed by a gradual decrease in wet weights at later periods of treatment. All the values were considerably less than those of control groups.

B) Tubular diameter:

The diameter of epididymal tubules in the caput epididymes in Ocscimum sanctum treated experimental animals changed much more than those of control groups. Actual recorded figures at various dose levels are given in Table no.5. Tubular diameter of caput epididymes in control and Ocscimum treated experimental rats.

Table No. 5.

Tubular diameter in µm.

Sr No.	Duration in days.	Control	Experimental.
1	0	278.96 ± 9.3	------
2	24	294.75 ± 9.1	223.69 ± 7.3
3	48	293.75 ± 7.9	192.11 ± 8.6
4	72	276.32 ± 7.0	184.21 ± 6.7
5	120 (Termination of Treatment (after 72 to 120 days)	289.48 ± 8.9	250.01 ± 6.1

- (Values are mean ± S.D of 50 tubules. Tubules diameters are expressed in µm)

Initially, the tubular diameter of caput epididymes in control rats was 278.96 ± 9.3 . On 24th day of treatment it showed a little increase to 294.75 ± 9.1 μm. On 48th day of treatment the values of control rats remained unaltered while on 72nd day a little decrease to 276.32 ± 7.0 μm was seen. After termination of the treatment from 72nd to 120th day, finally the diameter of caput edpididymal tubules not changed much only a little increased to 289.48 ± 8.9 was seen.

On the other hand, in case of Oscimum Sanctum treated experimental rats, the tubular diameter in caput epididymes was 223.69 ± 7.3 μm on 24th day of treatment. It showed a gradual decrease significantly with increasing period of dose, and the values was 192.11 ± 8.6 μm and 184.21 ± 6.7 μm on 48th and 72nd day of Oscimum treatment respectively. All the experimental values were much less than those of their control groups.

While, after termination of the treatment after 72 days upto 120 days finally the diameter of tubules again increased to 250.01 ± 601 μm.

C) Histology.

3.4 Recovery Test

3.4.0 Introduction.

After 72 days of treatment of Oscimum sanctum dose to the albino Rats, majority of organ showed degenerative structural alteration which may result into functional dystrophy. So, it was decided to stop the treatment and to check the recovery of the effect if any in the testes and associated reproductive organs in albino rats.

The treatment of dose was terminated after 72days and rats were maintained on control diet and water at libido up to 120th day of experiment.

The observation of alterations in wet weights and histology of the organs were recorded.

3.4.1 Testes —

A) Wet weight :

The wet weight of control testes increased after termination of treatment from 815.73±4.2mg (on 72nd day) to 880.53±6.8mg (on 120th day).

In experimental rats also the wet weight of testes increased from 603.00±5.4mg (on 72nd day) to 701.87±5.2mg (on 120th day) (Table No 2)

B) Tubular diameter.

Tubular diameter of seminiferous tubules in the control rats during recovery test were 267.88±10.4μm. And they were 165.79±10.5 μm in

experimental. This showed an increase in the diameter of seminiferous tubules considerably. upto 209.12 ± 4.4 μm after termination of treatment upto 120 days in expermental rats. (Table No 3)

C) Histology :

Histological observations of control and experimental testes treated with O. sanctum after termination of treatment showed following observations.

Control testes were similar to those of normal testes and showed normal simininiferous tubules with all the elements of spermatogenic series. Interstitial cells were also quite normal Sertoli cells were also normal.
The experimental testes also showed regeneration of distorted tissues due to termination of treatment.

More than 80 percent seminiferous tubules showed spermatids and spermatozoa in the lumen of the tubules , even though a few tubules still showed degenerated tunica propria at places. (plate No 8 Fig.1)

The overall population of spermatogenic cells was considerably increased with decrease in the number of vacuoles in the cytoplaosm of cells. (plate No 8, Fig. 2)

The size of intercellular spaces also reduced. Leyding cells showed normal development with reduced interstituim due to reincrease in the size and diameter of seminiferous tubules. (plate No.8 , Fig. 1 and Fig.2)

3.4.2 Caput epididymes.

A) Wet weight –

The wet weight of caput epididymes in control as well as treated rats showed very little decrease in their values The weight of control caput epididymes changed from 159.75±3.4mg to 135.75±3.8mg on 128 days and that of treated caput changed from 132.01±3.7mg to 129.48±4.7mg on 128 days of experiment. (Table No.4)

B) Tubular diameter.

The tubular diameter of caput epididymes tubules in control groups after termination of treatment was 289.48±8.9µm and it was 250.01±6.1µm in treated cauda epididymes .(Table No, 5)

These values showed that the diameter of control tubules were more or less similar to those of other control groups. But the considerable significant increase in the weights from 171.06±6.3mg (on 72nd day) to 223.69±7.3mg (on 120th day) the end of experiment (Table No.5) was due to regeneration of all the tubular elements evidenced by histological studies.

C) Histology :

Histologically, the caput epidiydmes of control rats after termination of treatment (from 72 to 120 days) showed normal stucture.

But the experimental caput epididymes after termination of treatment (from 72 to 120 days) also became quite normal.

The epididymal tubules under went regenerative changes. Thickness and height of the epithelial cells increased to normal. Vacuoles in the cytoplasm and nucleus of the epithelial cells also decreased in number and size. The stereocilia were of normal size.(Plate No.8 Fig.3)

Inter tubular tissue also regenerated with increased stroma containing connective tissue fibres and fibroblast cells with normal vascularity (Plate No 8. ,Fig.3).

The lumen of the tubules showed large number of luminal sperms and maturing spermatids which were quite normal. The empty lumens found in the 72 days treated caput epididymes were not seen after termination of treatment. (Plate No.8. , Fig.3)

3.4.3 **Cauda epididymes.**

A) Wet weight :--

The wet weight of cauda epididymes in control and treated rats showed a little increase in values after termination of treatment from 72 to 120 days of experiment .

The wet weight of control cauda epididymes does not altered much from 120.89±5.3mg (on 72nd day) to 119.00±4.2mg (on 120th day)

The weight of experimental cauda epididymes increased from 90.20±6.9 mg (on 72nd day) to 99.00±4.6 mg (on 120th day).

Eventhough the weight of experimental epididymes incresed after termination of treatment , they were less than those of the control epididymes after termination of treatment.

B) Tubular diameter:--

The tubular diameter of epididymes tubules in control epididymes after termination of treatment was 252.64±6.2μm It was 223.69±7.3μm in treated cauda epididymes after termination of treatment from 72 to 120 days of experiment. (Table No.7) Values were increased in treated rats from .Those of 72 days treated rats to became normal due to the regeneration of tissue (Table. No.7)

A) Histology:--

Histologically the cauda epididymes of control rats after termination of treatment from 72 to 120 days also showed normal events and the experimental cauda epididymes of treated rats after termination of treatment from 72 to 120 days also revealed quite normal events under light microscope.

The epididymal tubules showed reverse effects of the dose. Regeneration of all the tissues was evident. Thickness and height of the epithelial cells was more or less nearer to those of normal. Again we can identify the normal binucleated, cuboidal epithelial cells transforming into columnar ones. Streiocilia could be identified with a little low height towards the adluminal surfaceof the epithelium (Plate No 8. Fig.4).

Inter tubular connective tissue was also widened with regenerated nature. The fibroblasts were normal with connective tissue fibres.(Plate No 8.Fig.4)

The lumen of cauda epididymal tubules again became full of matured spermatozoa with little spaces in between. The luminal sperm coagulum observed in 72days treated cauda epididymes were vanished and replaced by normal functional spermatozoa .(plate No. 8., Fig.4)

The appearance of pycknotic nuclei was not evident form observation in these sections after termination of treatment at the end of experiment.

3.4.4 Seminal Vesicles.

A) Wet weight:--

The wet weight of seminal vesicles of control rats after termination of the treatment was 252.45±2.6 mg and those of experimental rats were more or less same i.e. 251.55±2.8 mg.

In control rats the seminal vesicular weights were not more different from those of other control groups of 72 days treated rats which were 203.87±5.3 mg showing a considerable increase in weight more like those of control groups. (Table. No.8)

A) Histology

Internally , the seminal vesicles of control rats after termination of treatment from 72 to 120 days showed more or less normal picture under light microscope.

But the seminal vesicles of experimental rats showed a good deal of regeneration in tissues with more normal like picture.

The outer connective tissue capsule showed normal structure with broken segments in between. It was a little wavy.

Muscle layer surrounding the mucosa and lamina propria became thicker at intervals. But degenerated patches showed spaces in between the circular muscle fibres.(Plate No 8 , Fig.5)

Lamina propria was not regenerated efficiently.

Mucus membrane was regenerated considerably in many region of the vesicle with granular cytoplam. The depth of its folds was also increasedin the center. (Plate No.8, Fig.5)

3.4.5. **Prostate gland**.

A) Wet weight :--

The wet weight of prostate gland of control rats after termination of treatment at the end of experiment was 162.85±6.2mg. It increased considerably than those of 72 days treated rats from 134.91±7.2mg Similarly the weights of experimental prostate glands on 72 days treatment were 124.45±5.3mg and the weight of prostate gland increased significantly after termination of the treatment in these animals to 143.93±6.4mg (Table.9) This indicates the increase in prostate weights due to regeneration of cellular elements of the gland after stopping the administration of dose.(Table No.9.).

Histology :

Postate glands of control glands after termination of the treatment from 72 to 120 days showed normal histological structure like those of all other control groups.

But the prostates of experimental animals after termination of the treatment from 72 to 120 days showed interesting regenerative alterations in their histology (plate No. 8, Fig. 6)

The external connective tissue capsule was partially regenerated. The septa of connective tissue developed deep into the gland with widened connective tissue.

Prostatic alveoli again showed a good deal of regenerative changes. The epithelium of alveoli again showed normal cuboidal cells with a basal nucleus in each. Secretion granuales reappeared in the cells. Vaccuoles were comparatively less (Plate no 8, Fig. 6)

The lumina of many alveoli showed darkly stainable secretory substonce with reduction in space due to folded in growth of the alveolar epithelium (plate no 8, Fig. 6)

3.4.6. **Cowper'gland**

A) Wet weight

The wet weight of Cowper's gland of control rats after termination of the treatment increased from 50.09±9.2mg (in72 days treatment) to 112.89±9.2mg (after termination of treatment for 72 to 120days). Similarly the weight of experimental prostate glands in treatment terminated rats also doubled (92.50±8.2mg) than those of 72 days treated rats (52.10±5.9mg) (Table No 10).

Even though the weight are increased double, they were still less than those of respective control groups. Which indicates partial recovery after termination of the treatment.

A) Histology :--

The Cowper's gland of control rats after termination of the treatment showed same histological structure like those of normal like those of other control groups.

But in the experimental rats, the Cowper's gland was well developed with normally developed alveoli. The cells of alveolar epithelium did not show vacuolated degenerative effects. Secretion was observed again in the lumen of alveoli after termination of the treatment from 72 to120 days at end of experiment.