CHAPTER FOUR
EPIDIDYMIS

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Epididymis

4.1. Review of literature :

Various chemical compounds and plant preparations have been tried to explore their effects on structure and functions of epididymis.

4.1.1. Chemicals :

Many synthetic chemical compounds have been shown to alter the histological structure of epididymis in various animals. Following is the brief review of such compounds from available literature. These chemicals are estrogens and estrogenic compounds such as clomiphene (Nelson and Patanelli, 1962; Roy et al., 1964; Kalra and Prasad, 1967; Schally et al., 1970; Roy et al., 1976; Roy and Datta, 1976), Clomiphene citrate (Kalra and Prasad, 1967; Rajlakshmi et al., 1970), estrogens (Lacy, 1967; Steinbeck et al., 1971; Meistrich et al. 1977; Wang et al., 1980).

WIN - 18446 (Drobeck and Coulston, 1962; Beyler et al., 1965; Nag et al., 1976), Methoxamine (El-badwai and Schenk, 1962; Norberg et al., 1967; Hib, 1976; Ratnasooriya et al.,

1986), 17 - alpha - hydroxy - progesterone (Setty and Kar, 1966; Das et al., 1977), testosterone (Bose et al., 1977; Dinakar et al., 1977), alpha - chlorohydrin (Crabo, 1965; Peyre and Laporte, 1966; Gunn et al., 1969, 1970; Ericsson and Baker, 1970; Hoffer et al., 1973; Edwards et al., 1973; Vickery et al., 1974; Nag et al., 1976; Dixit 1976; 1977 a; 1979; Ford et al., 1977; Gurayya and Gill, 1977), Methallibure (Kar et al.,1968 a; Dixit, 1969), Cyproterone and Cyproterone acetate (Weichert and Neumann, 1965, 1966, 1977; Neumann, 1969, 1970; Meitkowaski and Lukaszyk, 1969; Prasad et al., 1970; Prasad, 1973; Morse et al., 1973; Rastogi et al., 1973; Rastogi et al., 1973, 1979; Karkun et al., 1974; Bose et al., 1975; Nag et al., 1976 a; Dinakar et al., 1977), Quinacrine hydrochloride (Zipper and Medal, 1970; Setty et al., 1972; Chandra et al., 1974; Malviya et al., 1974; Shivkumar and Sarkar, 1979), Chlorocyclizin (Wong et al., 1972), S.K. and F. 7690 (Lubicz - Nawrocki and Glover, 1973), $CdCl_2$ (Dixit, 1976; Chinoy and Sheth, 1977), Centchroman (Das et al., 1977), Progestin and androgen (Flickinger, 1977), cyclohexanol (Tyagi et al., 1979), dexamithazone metapiron, niridazol and damazol (Dixit, 1979), Chlormadinone acetate (Kaur and Mangat, 1979), testosterone propionate (Manjula and Kadam, 1980), oxyphenonium (Ratnasooriya, 1982), MGP (Rao et al., 1986), parachlorophenylalanine (Vanithakumari, 1986), PMHI (Dechamma and Sarkar, 1987), Formaldehyde (Shah

et al., 1987), Flutamide (Dhar and Setty, 1987), beta - sitosterol (Malini and Vanithakumari, 1988), Cyproterone acetate (Bhiwgade et al., 1990)

4.1.2. Plant Preparations :

There are very few plant, preparations with antispermatogenic potencies have been explored for their effects on the epididymis.

Aristolochia indica extract administration induced reduction in the diameter of epididymal tubules and density of spermatozoa (Pakrashi and Pakrashi, 1977). Malvaviscus conzanttii extract administration to house rat and gerbil caused sloughing off of epididymal epithelium, lumina devoid of spermatozoa and presence of oedomatous interstitial - tissues (Dixit, 1977, b). Garg (1979) administered Calotropis procera extract to gerbils and found reduction in the size of epididymal lumina which were devoid of sperms. Administration of Malvaviscus conzanttii extract to mice resulted into atrophy of lumina of cauda epididymis along with the presence of debris and reduction in the height of epithelial lining (Verma et al., 1980). Allium sativum powder when administered to rat caused insignificant reduction in wet weight of epididymis (Dixit and

Joshi, 1982). Bhargava (1984) with plumbagin (an active principle from Plumbago zeylenica) treatment reported regression of epididymal epithelium and absence of sperms in lumina in dogs. Toro (1984) administered Vinca rosea alkaloid to albino rats found reduction in the number of sperma in epididymis but no change in the height of the epithelial cells. Sohani (1985) with Vitex negundo and Shah (1985) with Daucus carota seed extract reported decrease in wet weight of epididymis and in significant decrease in height of epithelium. Butea monosperma leaves extract reduced the number of spermatozoa in epididymis of albino rats (Awati, 1985). Oral administration of Oscimum sanctum powder to albino rats caused significant decrease in wet weight of epididymis, sperm count and sperm motility (Khanna et al., 1986). Hiremath (1988) administered Piper betle petiole extract to alnino rats and reported to affect the luminal contents. Administration of plumbagin to albino rats caused reduction in wet weight of epididymis, decrease in tubular diameters and lumina devoid of sperms (Jadhav, 1988). Oleanolic acid (a compound extracted from Eugenia jambolana flowers) when administered to rats caused insignificant decrease in wet weights of epididymis (Rajsekaran et al., 1988). Malvadin chloride (active principle

from Malvaviscus conzanttii flowers) caused decrease in the wet weight of epididymis of langur monkeys (Bhargava, 1988). Rao (1988) reported many regressive changes in epididymis after the administration of alcoholic extract of Solanum xanthocarpum seeds to rats. Administration of Gossypol to rats found to decrease wet weight of cauda epididymis (Bhiwgade and Nair, 1989 a) Akbarsha et al., (1990) administered dry powder of Andrographis paniculata orally and found many degenerative changes in epididymis with reduction in its weight. Tylophora asthamatica alkaloid when administered to rats caused significant increase in the wet weight of epididymis (Dikshith et al., 1990) Patne (1990) administered Picrorhiza kurroa extract to albino rats and found decrease in weights of epididymis. He further reported slight decrease in cellular height and certain degenerative changes in luminal contents, basal lamina and interstitium. Syzygium cumini seed extract decrease weight of epididymis and induced alterations in epididymal histoarchitecture (Ambaldage, 1990).

4.2. OBSERVATIONS :

4.2.1. Alterations in wet weights of the epididymis :

A) Caput epididymis :

The alterations occurring in the wet weights of caput epididymis of control and experimental rats are recorded in Table No. 4 and illustrated in Graph No. 4.

Table No. 4 : Caput epididymis : Agnus castus extract induced alterations in wet weights.

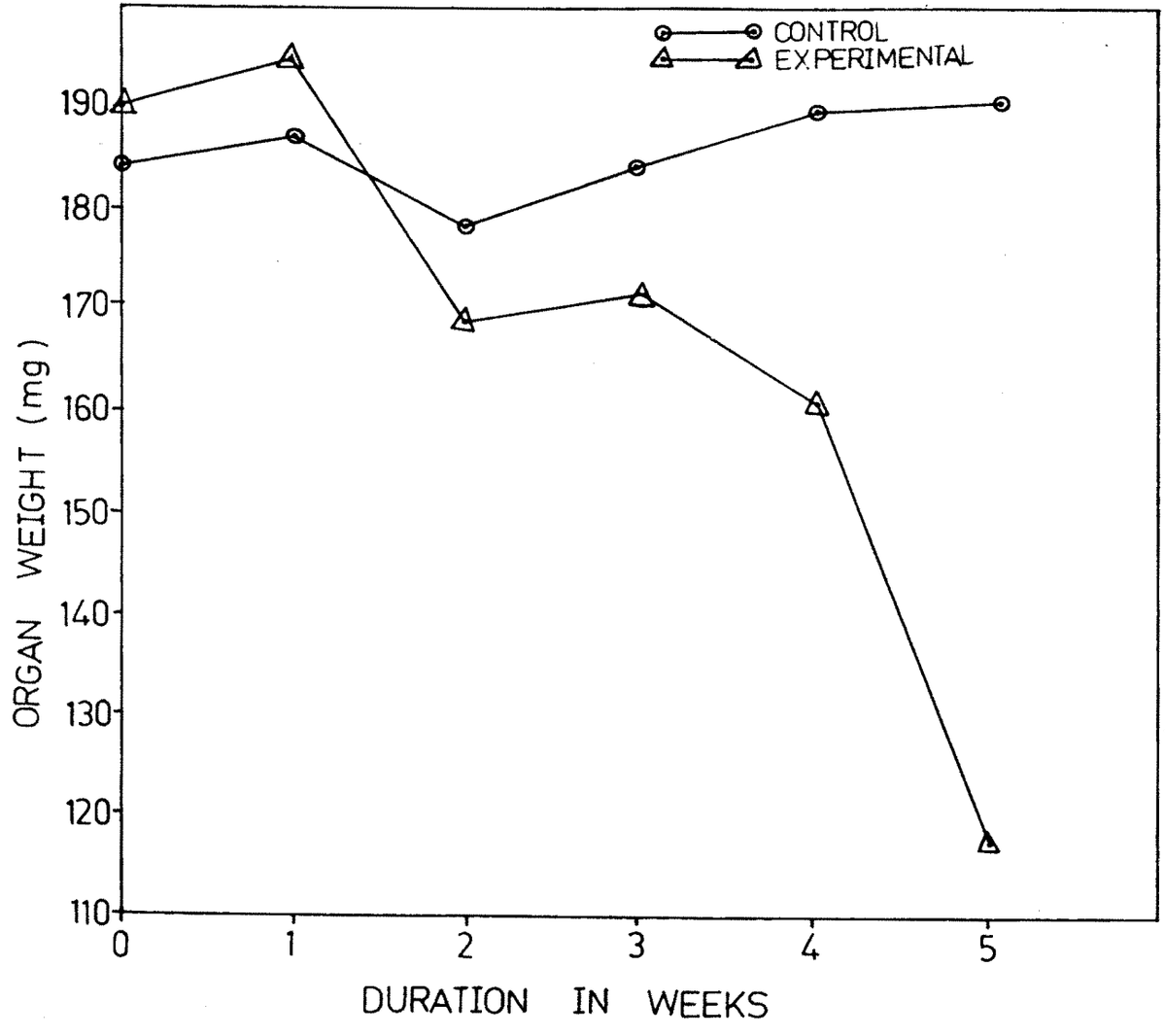
Duration in weeks	Control wt. of Caput (mg)	Experimental wt. of Caput (mg)
0	184 ± 5.2	190 ± 8.7
1	187 ± 5.7	195 ± 8.4
2	179 ± 7.5	168 ± 7.7
3	184 ± 5.2	171 ± 8.2
4	190 ± 8.1	160 ± 7.4
5	186 ± 7.4	117 ± 6.8

(Values are mean ± S.D. of three animals and are expressed in mg/100 g. of body weight).

As seen from the tabular and graphical illustrations, the weights of caput epididymis remained more or less constant in control rats which received only vehicle. But in the rats

CAPUT EPIDIDYMIS

ALTERATIONS IN WET WEIGHT (mg)



Graph No. 4.

receiving Agnus castus extract, the wet weights of caput decreased as a function of duration of the treatment.

Initially the wet weight of caput of control rat was 184 ± 5.2 mg/100 g. cf body weight. It showed insignificant variations to 187 ± 5.7 mg, 179 ± 7.5 mg, 184 ± 5.2 mg, 190 ± 8.1 mg. and 186 ± 7.4 mg. after 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} week respectively.

In experimental rats, initially, the weight of caput was 190 ± 8.7 mg/100 g. of body weight. It rose to 195 ± 8.4 mg. after 1^{st} week of the treatment. It decreased to 168 ± 7.7 mg. after 2^{nd} week of the treatment. It showed elevation to 171 ± 8.2 mg after 3^{rd} week of the treatment. Towards the end of the treatment there observed depletion in the weights. The values were 160 ± 7.4 mg and 117 ± 6.8 mg/100 g. of body weight after 4^{th} and 5^{th} week of the treatment respectively.

B) Cauda epididymis :

The alterations occurring in the wet weights of cauda epididymis and control and experimental rats treatment are recorded in Table No. 5 and illustrated in Graph No. 5.

Table No. 5 : Cauda epididymis : Agnus castus extract induced alterations in wet weights.

Duration in weeks	Control wt. of Cauda (mg)	Experimental wt. of Cauda (mg)
0	142 ± 3.6	149 ± 3.6
1	148 ± 4.4	156 ± 4.7
2	145 ± 4.8	135 ± 3.2
3	146 ± 3.5	143 ± 3.8
4	152 ± 4.3	118 ± 3.5
5	150 ± 3.8	103 ± 3.1

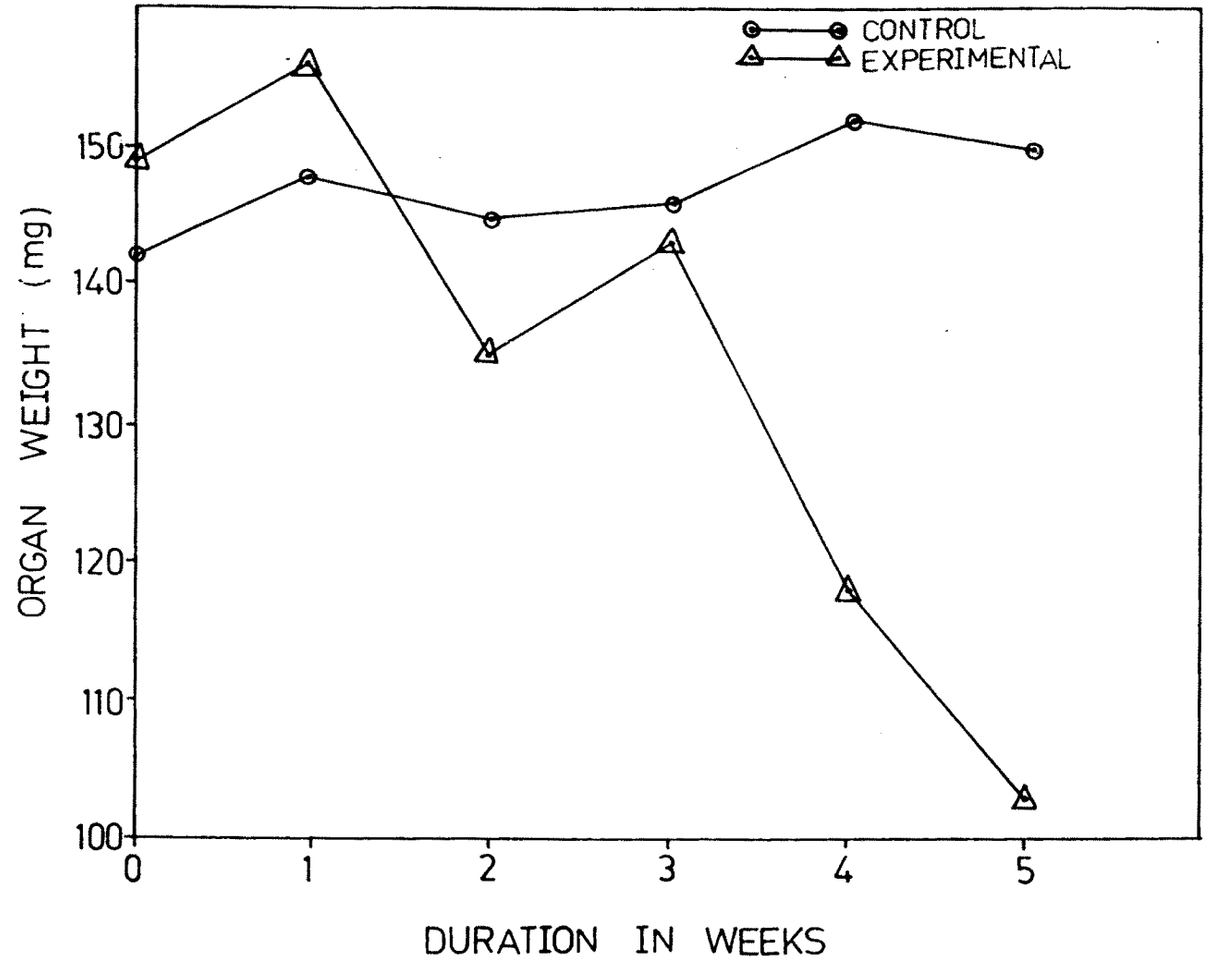
(Values are mean ± S.D. of three animals and are expressed in mg/100 g. of body weight).

As seen from the tabular and graphical illustrations, the wet weights of cauda epididymis remained fairly constant in control rats which received only vehicle. But in the rats receiving Agnus castus extract the wet weight of cauda decreased as a function of duration of the treatment.

Initially the weight of cauda epididymis of the control rat was 142 ± 3.6 mg/100 g. of body weight. It showed minor

CAUDA EPIDIDYMIS

ALTERATIONS IN WET WEIGHT (mg)



Graph No. 5

variations to 148 ± 4.4 mg, 145 ± 4.8 mg, 146 ± 3.5 mg, 152 ± 4.3 mg and 150 ± 3.8 mg/100 g. of body weight after 1st, 2nd, 3rd, 4th and 5th week respectively.

The weight of cauda epididymis of the experimental rats was 149 ± 3.6 mg/100 g. of body weight at the commencement of the treatment. It elevated to 156 ± 4.7 mg after the 1st week of the treatment. Then after 2nd week of the treatment the weight of cauda decreased to 135 ± 3.2 mg. It rose to 143 ± 3.8 mg after 3rd week of the treatment. The values, towards the end of the treatment, depleted to 118 ± 3.5 mg and 103 ± 3.1 mg/100 g. of body weight after 4th and 5th week of the treatment.

4.2.2. Alterations in Histology :

Mammalian epididymis is divisible into different regions, on the basis of structure and function. There are various opinions about the zonation of epididymis (Reid and Cleland, 1957; Maneely, 1959; Glover and Nicander, 1971; Flickinger, 1976). In the present investigation the terminology of Glover and Nicander (1971) is followed since it is simple and more logical. According to their opinion the mammalian epididymis is divisible into two broad regions. The first is caput which

is concerned with absorption and sperm maturation and the second is called as cauda which is concerned with sperm storage.

A) Caput epididymis :

Control :

The caput epididymis consisted of epithelium with principle and clear cells, luminal contents, basal lamina and interstitium (Plate No. 3, Fig. 2).

i) Epithelium :

Two types of cells line the caput epididymal tubules principal and clear cells.

a) Principal cells :

These were tall columnar cells with apical microvilli (stereocilia). Large number of apical vacuoles were seen. Stereocilia were longer than those of the caudal principal cells. Few lipid droplets were present in the basal regions of the cells. Few principal cells were binucleated.

b) Clear cells :

These cells were present in between principal cells.

These cells were common in initial segment of caput, while few were present in the later part of the segment of caput epididymis. Nuclei were elongated or pear shaped. These nuclei formed a fairly complete row in the basal part. Lipid droplets were seen in these clear cells, but these were absent in clear cells of cauda epididymis.

ii) Luminal contents :

Lumina of caput epididymis contained spermatozoa and immature spermatids. Spermatozoa were present in large number. The density of sperms was moderate in the lumina as compared with that in cauda epididymis. Occasionally few round or elongated spermatids were seen in the lumina, but their number was insignificant.

iii) Basal lamina and interstitium :

The basal lamina sorrounding the epididymal tubules was thin and eosinophilic interstitium exhibited few irregular shaped cells.

Experimental :

Agnus treatment induced few alterations in the histological structure of caput epididymis in treated rats at all

intervals. There observed no consistant progression of changes, the entire period of extract treatment is divided into two phases. The first phase consists of alterations taking place from commencement of treatment to third week of treatment and the second includes the alterations in 4th and 5th week of treatment.

I) First phase of treatment :

i) Epithelium :

a) Principal cells :

Histological alterations were few. The cells showed changes in height and structure. Their cytoplasm shows vacuoles, but number of which was insignificant. Near nuclear membrane found condensed chromatin which formed a rim around the central chromatin. Few large oval spaces seen at the end of this phase of treatment in the epithelium. Stereocilia did not show any change, they were normal in length.

b) Clear cells :

These cells showed poor and insignificant histological changes. Cytoplasm showed darkly stained granules. Condensed

chromatin matter was associated with nuclear membrane only, while the central area of nuclei was without any chromatin matter. Few intracellular vacuoles were also seen.

ii) Luminal contents :

Reduction in the number of sperms seen in the epididymal lumina. In some lumina few spermatogenic cells, such as spermatocytes and spermatids received from testis, were seen. Agglutinated masses of sperms found in many epididymal lumina towards the end of this first phase of treatment. Epididymal lumina decreased in diameter. (Plate No. 3, Fig. 3)

iii) Basal lamina and interstitium :

The basal lamina was wavy, thickened and showed high eosinophilia. The interstitial space appeared widened. Few cells were seen in it.

II) Second phase of treatment :

i) Epithelium :

a) Principal cells :

There observed appreciable decrease in height of these cells. The stereocilia decreased in length. While in some

tubules, towards the end of the treatment they became scanty and even lost. Cytoplasmic vacuoles increased number of tubules and principal cells showed vacuolation.

b) Clear cells :

Cytoplasmic vacuoles and darkly stained granules were present. Feulgen reactivity was decreased in some tubules.

ii) Luminal contents :

The alterations seen in the first phase of treatment became more conspicuous and spread in many more tubules in the second phase of the treatment. The sperms decreased in number in majority of tubules, while in some tubules these were disappeared. Desquamated cells appeared more in number in the lumina of tubules which towards the end of treatment reduced in number. Remnants of cellular debris was evident in some tubules while few tubules were more or less empty (Plate No. 3, Fig. 4).

iii) Basal lamina and interstitium :

The basal lamina showed thickening and high eosinophilia. Interstitial space appeared widned and showed few irregular cells.

B) Cauda epididymis :

Control :

The cauda epididymis consisted of epithelium with principal and clear cells, luminal contents and basal lamina and interstitium (Plate No. 3, Fig. 5).

i) Epithelium :

There were two types of epithelial cells principal and clear.

a) Principal cells :

These were cuboidal pseudostratified cells with short microvilli (Stereocilia). They had spherical or flattened nuclei with coarsely granular chromatin. Binucleation was more prominent than in caput epididymal principal cells. Perinuclear or infranuclear vacuoles were evident in the proximal regions.

b) Clear cells :

These were present inbetween principal cells. These were called as clear or light cells since they have less dense cytoplasm than that of the adjacent principal cells.

Apical region of cells showed many vacuoles. Clear cells were more in number in cauda than in caput epididymis.

ii) Luminal contents :

Cauda epididymal lumina contained large number of sperms. The sperm density was maximum in cauda. The sperms were arranged in bundles and unidirectional in orientation. The spermatids were not found caudal tubules.

iii) Basal lamina and interstitium :

The basal lamina was thin as compared to that of cauda. It showed eosinophilia. Interstitium was less compact and contained many irregular shaped cells.

Experimental :

As stated earlier, Agnus treatment induced few changes and there was no consistant progression in it, the entire period of the treatment is divided into two phases. The first phase consists of alterations taking place from commencement of treatment to third week of treatment and the second includes the alterations in fourth and fifth week of the treatment.

I) First phase of the treatment :

i) Epithelium :

a) Principal cells :

There was a slight reduction in cell height. There was seen cellular cytoplasmic vacuolation. No other change was evident.

b) Clear cells :

Poor and insignificant changes in the histological structure were seen in the clear cells. Chromatin matter was localized in nuclear membrane only.

ii) Luminal contents :

Number of spermatozoa was decreased. Density of sperm also decreased some tubules showed cellular debris containing degenerating spermatids and occasionally spermatocytes.

iii) Basal lamina and interstitium :

The basal lamina appeared thick and more eosinophilic as compared to that of control. It was seen detached from the tubular epithelium at certain places. Interstitium was less compact and with irregular cells.

II) Second phase of the treatment :

The alterations occurred in the histological structure of cauda epididymis, due to treatment are illustrated photomicrophically in Plate No. 3, Fig. 6.

i) Epithelium :

a) Principal cells :

Height of these cells reduced. Formation vacuoles in the cytoplasm was continued during this phase of treatment also. The vacuoles were infranuclear in position and appeared as a complete space in the epithelium.

b) Clear cells :

There observed no progression, in the alterations in these cells, even at the conclusion of the treatment.

ii) Luminal contents :

Lowering of sperm densities in many tubules was evident. In number of tubules, the sperms decreased and in few they disappeared totally. Cellular debris was seen in tubular lumina, but identification of the cells was not possible. The number of tubules with less or no sperms increased towards the treatment.

iii) Basal lamina :

The Basal lamina appeared wavy, thick and intensely eosinophilic. It was seen detached from epithelium at certain places in number of tubules. Interstitium was less compact with irregular cells scattered in it.

4.3. DISCUSSION :

In the present discussion Agnus extract induced alterations in the wet weight and histoarchitecture of epididymis of albino rats are discussed. We have more or less complete information about the extract induced alteration in epididymis. These alterations are proposed to be discussed at a comparative level with the available literature on the epididymal changes induced by chemicals and various plant extracts and to arrive at definite conclusions.

In the present investigation it is observed that there is decrease in the wet weights of caput and cauda epididymis due to the administration of Agnus castus extract. This finding finds a good parallel in the observations made by various workers who have worked estrogenic compounds (Kalra and Prasad, 1969), (Chang, 1942; Bacon and Kirman, 1955; Kar et al., 1965; Meistrich et al., 1977; Saxena et al., 1978) and

other chemical compounds alpha - chlorohydrin (Peyre and Laporte, 1966; Gunn et al., 1969; 1970; Ericsson and Baker, 1970; Hoffer et al., 1973; Ford et al., 1977; Dixit, 1977), endosulphan (Ansari and Gupta, 1981), Formaldehyde (Shah et al., 1969), Cyproterone acetate (Gupta et al., 1989; Bhiwgade, 1990).

Many plant preparations are also reported to reduce epididymal weights. These plants are Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Dixit, 1977 b; Verma et al., 1980; Bhargava, 1988), Calotropis procera (Garg, 1979), Papay (Das, 1980), Allium sativum (Dixit and Joshi, 1982), Daucus carota (Shah, 1985), Vitex negundo (Sohani, 1985), Butea monosperma (Awati, 1985), Oscimum sanctum (Khanna, et al., 1986), Piper betle (Hiremath, 1988; Adhikary et al., 1989), Plumbagin (Jadhav, 1988), Solanum xauthocarpum (Rao, 1986 a; 1986 b; Chinoy et al., 1986), Gossypol (Nair and Bhiwgade, 1989; Bhiwgade and Nair, 1989), Picrorhiza kurroa (Patne, 1990), Andrographis paniculata (Akbarsha et al., 1990), Syzygium cumini (Ambaldage, 1990).

The reduction in wet weight of epididymis may be due to less number of normal spermatozoa entering into epididymal lumina as a effect of Agnus treatment. The decrease in the

wet weights of epididymis may also be an indication of antiandrogenic action of the extract administration.

Agnus extract decreases height of the epithelial cells. This observation finds a good parallel in the work reported after administration of estradiol (Kumar et al., 1976), Methallibure niradazole (Dixit, 1979), Chlormadinone acetate (Kaur and Mangat, 1979), alpha chlorohydrin (Dixit and Lohiya, 1979), cyproterone acetate (Prakash et al., 1979), formaldehyde (Shah et al., 1988), methyl mercury chloride (Vachhrajani et al., 1988), beta-sitosterol (Malini and Vanithakumari, 1988). Reduction in height of epididymal cells is also observed after administration of plant preparations - Malvaviscus conzanttii (Dixit, 1977 b, Dixit and Bhargava, 1978; Verma et al., 1980); Aristolochia indica (Pakrashi and Pakrashi, 1977), Calotropis procera (Garg, 1979), Papaya (Das, 1980), Allium sativum (Dixit and Joshi, 1982), Plumbago zeylenica (Bhargava, 1984), Daucus carota (Shah, 1985), Vitex negundo (Sohani, 1985), Plumbagin (Jadhav, 1988), Andrographis paniculata (Akbarsha et al., 1990), Picrorhiza kurroa (Patne, 1990).

In the present studies it is observed that the Agnus extract treatment causes decrease in number of spermatozoa leading to oligospermia in the epididymis. This finding

resembles those reported with many chemical compounds such as estrogens (Lacy, 1967), Clomiphene citrate (Kalra and Prasad, 1967; Rajlakshmi et al., 1970), alpha - chlorohydrin (Carbo, 1965; Gunn et al., 1969; 1970; Ericsson 1969; 1970; Nag et al., 1970; Dixit, 1979), Methallibare (Kar et al., 1968 a), S.K. and F. 7690 (Lubicz - Nawrocki and Glover, 1973), WIN - 18446 (Coulston et al., 1960; Nag et al., 1976), $CdCl_2$ (Dixit, 1976; Chinoy and Sheth, 1977), Cyproterone acetate (Neumann et al., 1970; Prasad 1973; Bose et al., 1975; Flickinger and Loving, 1976; Rastogi et al., 1979), Prostaglandin (Tso and Lacy, 1975; 1978), Progestin and audrogen (Flickinger, 1977), Flutamide (Dhar and Shetty, 1987).

Oligospermia is also reported to be indused by various plant preparations. These are Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Dixit, 1977 b; Dixit and Bhargava, 1978; Verma et al., 1980), Calotropis procera (Garg, 1979), Papaya (Das, 1980), Allium sativum (Dixit and Joshi, 1982), Vinca rosea (Toro, 1984), Plumbago zeylenica (Bhargava, 1984), Daucus carota (Shah, 1985), Vitex negundo (Sohani, 1985), Butea monosperma (Awati, 1985), Oscimum sanctum (Khanna et al., 1986), Gossypol and Tripterygium wilfordii (Xu et al., 1987), Solanum xanthocarpum

(Rao, 1988 b), Piper betle (Hiremath, 1988), Plumbagin (Jadhav, 1988), Syzygium cumini (Ambaldage, 1990), Picrorhiza xurroa (Patne, 1990).

At the begining of the second phase of the treatment, cellular debris is observed in the lumina of many epididymal tubules. This observation finds a good parallel in the work of Flickinger and Loving (1976) with cyproterone acetate, Tyagi et al., (1979) with cyclohexanol, Ratnasoorlya et al., (1980) with Methoxamine. Some plant preparations also induced similar changes. These plants are Malvaviscus conzanttii (Verma et al., 1980), Vinca rosea (Toro, 1984), Butea monosperma (Awati, 1985), Daucus carota (Shah, 1985), Vitex negundo (Sohoni, 1985), Piper betle (Hiremath, 1988), Plumbagin (Jadhav, 1988), Picrorhiza kurroa (Patne, 1990), Syzygium cumini (Ambaldage, 1990).

Thickening of basal lamina and widening of interstitium are observed in the present work. Similar changes are reported after administration of Vinca rosea (Toro, 1984), Daucus carota (Shah, 1985), Vitex negundo (Sohani, 1985) Butea monosperma (Awati, 1985), Plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988), Picrorhiza kurroa (Patne, 1990),

Syzygium cumini (Ambaldage, 1990).

Thus Agnus extract induced changes in epididymis indicate that decrease in sperms seems most likely to be due to the decreased production of sperms by testes under the action of Agnus castus extract. The lumina of caput as well as of cauda contained cellular debris, which formed of various germ cells like spermatocytes, spermatids, multinucleated cells, degenerating spermatozoa, cytoplasmic masses. From the observations of the cellular debris, it appears that it is mainly derived from the damaged testes. With the progression of treatment the luminal cells alongwith spermatozoa decreased; and lumina became empty. The ultimate fate of the degenerating spermatozoa and cellular debris is not known, probably they are expelled through the remainder of the duct system.