CHAPTER THREE
TESTIS

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CHAPTER THREE

Testis

3.1. Review of literature :

3.1.1. Chemicals :

Many synthetic compounds have been reported to arrest spermatogenesis or induce functional sterility in laboratory male animals. These various compounds include triethylene melamine (Hendry et al., 1951; Jackson and Bock, 1955; Fox et al; 1963), WIN 13099, WIN 17416, WIN 18446 (Coulston et al., 1960), 6 - Medroxy progesterone acetate (Mc Leod, 1965), busulfan (Jackson, 1966; Ahlquist, 1966; Kar et al., 1968; Kanwar et al., 1970), Triethylinethiophosphoramide (Herschberger et al., 1969), S.K. and F. 7690 (Saunders et al., 1969), Monochlorohydrin (Gunn et al., 1970), Norgesterol (Singh et al., 1972), Trihydroxypregnenolene (Sud and Setty, 1973), 5 - thio - D - glucose (Whistier and Lake, 1972; Maughn, 1974; Zysk et al., 1975; Homm et al., 1977), alpha chlorohydrin (Cooper et al., 1974; Hundal and Mangat, 1978, Changamma and Reddanna, 1987).

Some estrogenic compounds also known to impaired spermatogenesis and caused sterility. These were Clomiphene (Holtkamp et al., 1960; Nelson and Patanelli, 1962; Roy et al., 1964; Nelson, 1965), Clomiphene citrate (Kaur and Mangat, 1979; Wang

et al., 1980), Centchroman (Das et al., 1977), Diethylstilbestrol (Kasinathan and Basu, 1974), estradiol 17 beta (Hunt et al., 1979).

Salts of various metals like cadmium, mercury, osmium, plutonium, zinc, silver, cobalt, lead etc. have also effects on reproductive organs of males (Gunn and Gold, 1970; Takeuchi (1972); Johnson, 1977; Roychowdhury and Arora (1982).

Rao et al., (1986) administered 2 - mercaptopropionyl glycine (M 6 P) in rat and found decrease in wet weight of testis, Leydig cell atrophy and distortion in normal organization of seminiferous epithelium.

Administration of testosterone oenanthate in mice decreased wet weight of testis and number of spermatogonia, pachytene spermatocytes and spermatids (Bansal and Davies, 1986). Follicle regulation protein (FRP) altered seminiferous epithelial function in albino rats (Iso et al., 1987; Nakumura et al., 1987). Shah et al., (1987) administered formalldehyde to albino rats and found no change in body weight but decrease in organ weights. Oral administration of Flutamide in male rats altered testicular histoarchitecture. Spermatogenesis was arrested at the spermatid stage; although few tubules showed normal spermatogenesis.

Leydig cells hypertrophied weight of testis was decreased (Dhar and Shetty, 1987).

Murcuric chloride treatment resulted in testicular degeneration, decrease in testicular wet weight, structural distortion of seminiferous tubules, atrophy of Leydig cells, distortion of spermatid and spermatozoa and pyknosis of spermatogenic cells in rats (Roy Chowdhury and Vachhrajani, 1987). Complete inhibition of spermatogenesis in field rats was reported by Dechamma and Sarkar (1987) with PMHI administration. Adrenalectomy resulted impairment of spermatogenesis in rats. The seminiferous tubules became shrunken and wavy in outline. Germinal epithelium was represented by only spermatogonia. The other cell types exhibited karyohexis and karyolysis. The interstitium was uncommonly large with degenerated Leydig cells. Lumina were full with cellular debris (Nair et al., 1987).

Administration of cyproterone acetate caused changes in spermatids, sertoli cells as well as in Leydig cells in albino rats (Bhiwgade et al., 1990). Ghosh et al. (1990) reported degeneration of germ cells and decrease in wet weight of testis in rats after the administration of Lithium.

Bhiwgade et al. (1991) administered Depot - medroxyprogesterone acetate (DMPA) and found degenerative changes in late

spermatids and atrophy of Leydig cells.

Gill and Sareen (1991) reported degeneration of testicular elements, after the administration of cimetidine in mice, with a significant decrease in seminiferous tubular diameter and germinal epithelial cell height.

3.1.2. Plant preparations :

Number of plants and plant products have been tried for their antifertility potency. Ethanolic extract of bark of Hippophae salicifolia showed antimitotic property in the testes of young rats (Joshi et al., 1965). Vinca rosea total alkaloids produced graded degenerative changes in the immature rats (Joshi and Ambay, 1968). Administration of Oscimum sanctum leaves when fed with diet resulted impairment of spermatogenesis in mice (Kasinathan et al., 1972). Oral administration of benzene extract of Hibiscus rosa - sinensis to albino rats caused arrest of spermatogenesis at the spermatid stage. Some of the tubules showed only spermatogonia and sertoli cells. Leydig cells showed atrophy. Endocrine function of testis was affected (Kholkute, 1977).

Administration of water soluble part of the chloroform fraction of Aristolochia indica in mice caused various

degenerative changes in the seminiferous germinal cells. Diameter of tubule was reduced. Lumina of some tubules filled with a debris of desquamated germinal elements. Sertoli cells and Leydig cells remain unaffected (Pakrashi and Pakrashi, 1977).

Dixit (1977, b) administered Malvaviscus couzanttii flower extract and found many degenarative changes in testes of house rat and gerbil. There was a loss of spermatogonia type A, spermatocytes, spermatids and spermatozoa. Seminiferous tubules were shrunken. Leydig cell cytoplasm was weakly eosinophilic and highly vacuolated with shrinkage in nuclear diameter.

Garg (1979) reported testicular necrosis after the oral administration of Calotropis procera flower extract.

Chauhan et al. (1979) fed ethanolic extract of Vinca rosea to mature rats and reported significant changes in testis.

Administration of Oscimum sauctum reduced sperm count and sperm motility in male rats (Seth et al., 1981).

Dixit and Joshi (1982) after administration of garlic powder reported that the body weights and testicular weights were reduced significantly. They also reported testicular lesions leading to spermatogenic arrest.

Hoffer (1983) administered gossypol, an active principle from cotton seed, and observed interesting effects. He noted presence of severely damaged as well as entirely normal seminiferous tubules adjacent to one another in the same section.

Bhargava (1984) reported arrest of spermatogenesis at spermatocyte in dogs after the administration of plumbagin, an active principle from Plumbago zeylenica.

Vinca rosea leaves alkaloid affect all spermatogenic cells with the exception of spermatogonia and mature spermatozoa in albino rats (Toro, 1984).

Vitex negundo leaves extract caused various changes in the testicular histology of albino rats (Sohoni, 1985).

Shah (1985) administered Daucus carota seed extract to albino rats and observed many alterations in the testis - and an arrest of spermatogenesis at primary spermatocyte level.

Effects of Butea monosperma leaves extract were studied by Awati (1985) who reported arrest of spermatogenesis at spermatid level.

Tulsi (Oscimum sanctum) leaves decreased sperm count, sperm motility and weight of male reproductive organs, when fed to albino rats (Khanna et al., 1986).

Solanum xanthocarpum after 60 days of treatment brought about depopulation of spermatogenic elements in seminiferous tubules in testis of rats (Rao, 1988).

Administration of Malvidin chloride, the colouring pigment of the flowers of Malvaviscus conzanttii -, caused decrease in weight of testis and impairment of spermatogenesis in langur monkeys (Bhargava, 1988).

Bhiwgade et al. (1988) reported inhibition of spermatogenesis, in albino rats after the administration of Gossypol.

Wangoo (1988) administered Celastrus paniculatus seed extract to albino rats and found arrest of spermatogenesis, shrinkage of seminiferous tubules, vacuolization in tubules, germ cell depletion and exfoliation.

Oleanolic acid extracted from Eugenia jambolane flowers, when administered to rats caused decrease in fertilizing capacity of the animals without any significant changes in body weight or reproductive organ weights. Spermatogenesis was arrested without causing any abnormality in the germinal cells, Sertoli cells and Leydig cells (Rajsekaran et al., 1988).

Chinoy et al. (1988) administered Vinca rosea leaves extract to male albino rats and showed that general testicular

histoarchitecture was affected.

Administration of plumbagin, an active principle from Plumbago zeylenica, to male albino rats resulted into asperma-togenesis. Except spermatogonia, all other germinal cells were affected (Jadhav, 1988).

Oral administration of dry powder of Andrographis panicu-lata leaves to albino rats resulted into cessation of spermato-genesis. The organ weights decreased slightly. The seminiferous tubular lumina contained phagocyte like cells. Interstitium appeared involuted (Akbarsha et al., 1988; 1990).

Piper betle petiole extract caused arrest of spermato-genesis at spermatid level in albino rats (Hiremath, 1988).

Administration of Piper betle petiole extract to albino rats decreased the wet weight of testes and changes in testi-cular elements. It also decreased number and motility of spermatozoa (Adhikary et al., 1989).

Syzygium cumuni seed extract when administered to albino rats caused decrease in wet weight of testis and alterations in histoarchitecture of testis (Ambaldage, 1990).

Picrorhiza kurroa extract administration resulted in decrease in wet weight of testis, reduction in diameter of

seminiferous tubules and many histological changes in albino rats (Patne, 1990).

Dikshith *et al*. (1990) administered *Tylophora asthmatica* alkaloid to male rats. They reported marked changes in the morphology of seminiferous tubules and spermatogenic activity. Sloughing of germinal cells and their accumulation in the tubular lumina evident. In some of the seminiferous tubules groups of giant cells were seen.

Administration of aquious extract of *Vinca rosea* leaves to mice resulted into complete arrest of spermatogenesis. Except a single layer of spermatogonia, all other germinal cell layers were detached and accumulated in lumina of seminiferous tubules. The detached cells were necrotic and appeared to undergo cytolysis and karyolysis. Regression and degeneration of Leydig cells were observed (Murugavel and Akbarsha, 1991).

3.2. OBSERVATIONS :

3.2.1. Alterations in body weight :

The alterations occurring in body weights of control and experimental rats are recorded in Table No. 1 and illustrated in Graph No. 1.

ALTARATIONS IN BODY WEIGHT (GMS)

Graph No. 1

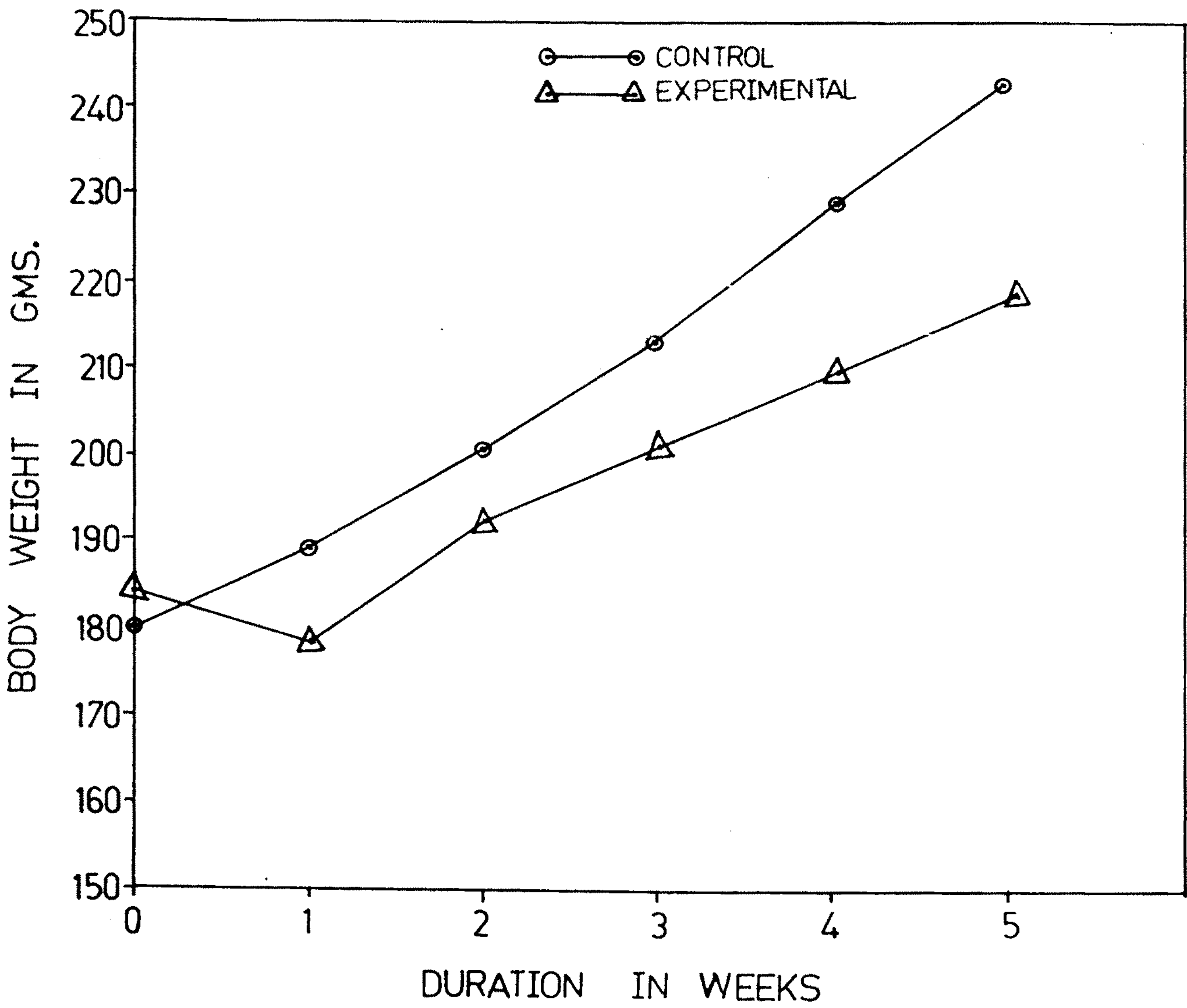


Table No. 1 : Body weights of control and Agnus castus treated rats.

Duration in weeks	Control body wt. (g)	Experimental body wt. (g)
0	180 ± 4.2	186 ± 3.7
1	189 ± 3.7	178 ± 4.0
2	201 ± 4.4	194 ± 3.5
3	216 ± 4.8	202 ± 4.7
4	229 ± 5.8	209 ± 4.3
5	245 ± 3.5	218 ± 4.9

(Values are mean ± S.D. of three animals and are expressed in g.).

As seen from the tabular and graphical illustrations, body weights showed a gradual increase in control rats but in experimental rats it was retarded.

Initially the weight of the control rat was 180 ± 4.2 g. It gradually increased to 189 ± 3.7 g., 201 ± 4.4 g., 216 ± 4.8 g., 229 ± 5.8 g. and 245 ± 3.5 g. on the 1st, 2nd, 3rd, 4th and 5th weeks respectivelly.

Initially the weight of the Agnus treat rat was 186 ± 3.7 g. After first week of the treatment it was decreased to

178 ± 4.0 g. The values then gradually increased to 194 ± 3.5 g. 202 ± 4.7 g., 209 ± 4.3 g. and 218 ± 4.9 g. on 2nd, 3rd, 4th and 5th weeks of treatment respectively.

3.2.2. Alterations in testis :

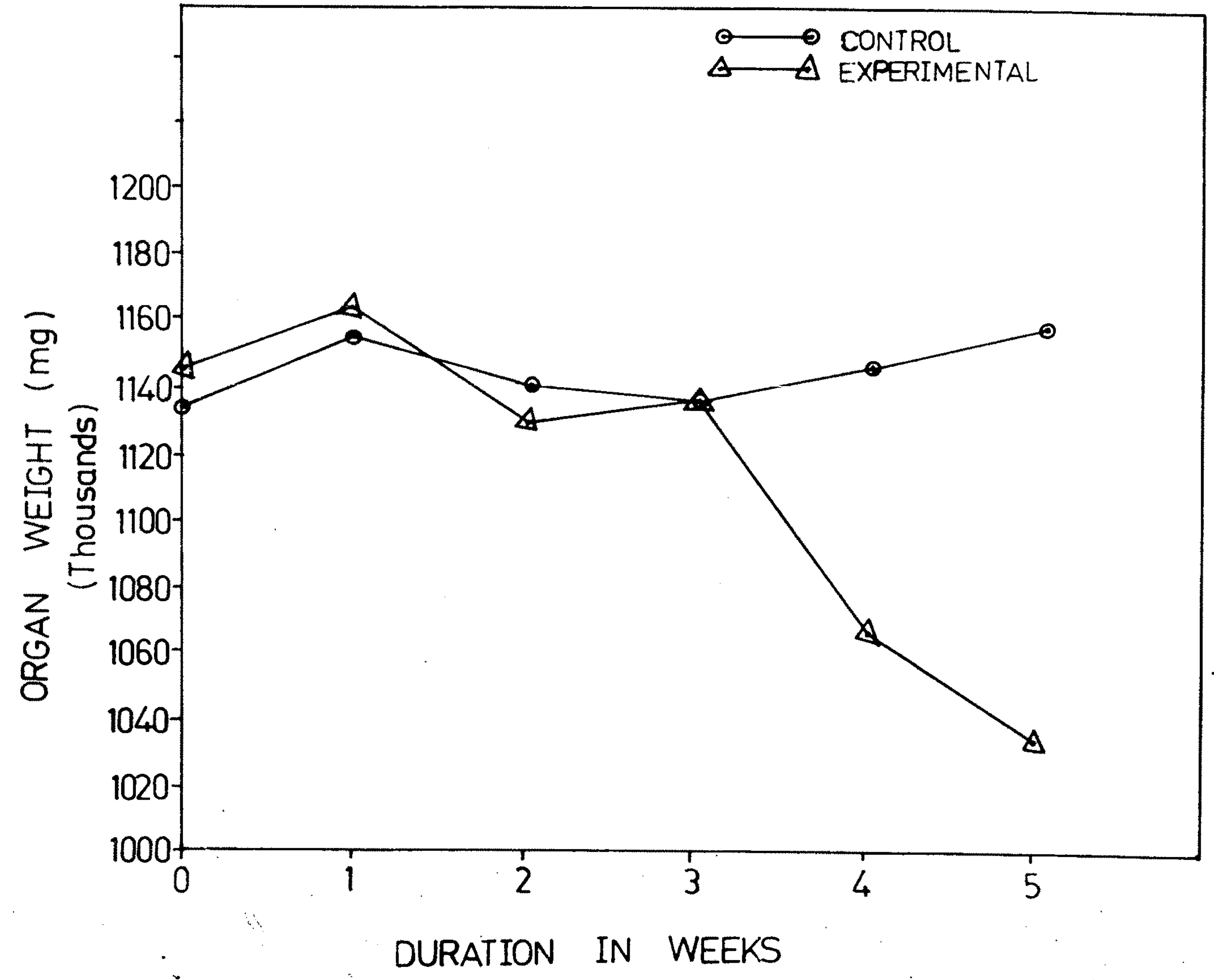
i) Alterations in wet weights :

The variations occurring in the wet weights of testis of control and experimental rats are recorded in Table No. 2 and illustrated in Graph No. 2.

Table No. 2 : Testis : *Agnus castus* extract induced changes in weights.

Duration in weeks	Control wt. of testis (mg.)	Experimental wt. of testis (mg.)
0	1137 ± 15.7	1145 ± 14.8
1	1155 ± 16.3	1161 ± 14.0
2	1140 ± 13.6	1130 ± 15.4
3	1136 ± 15.4	1138 ± 15.2
4	1147 ± 14.8	1067 ± 13.7
5	1159 ± 14.5	1037 ± 13.5

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


TESTIS : ALTERATION IN WET WEIGHT (mg)
CONTROL
EXPERIMENTAL
ORGAN WEIGHT (mg)
(Thousands)
1200
1180
1160
1140
1120
1100
1080
1060
1040
1020
1000
0
1
2
3
4
5
DURATION IN WEEKS
Graph No. 2

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

Initially the wet weight of testis of control rat was 1137 ± 15.7 mg per 100 g. of body weight. It showed insignificant variations to 1155 ± 16.3 mg., 1140 ± 13.6 mg., 1138 ± 15.4 mg., 1147 ± 14.8 mg. and 1159 ± 14.5 mg. after 1st 2nd, 3rd, 4th and 5th week respectively.

Initially the wet weight of testis of the experimental rat was 1145 ± 14.8 mg/100 g. of body weight. After 1st, 2nd and 3rd week of treatment values showed insignificant variations to 1161 ± 14.0 mg. respectively. After 4th week of the treatment weight of the testis depleted to 1067 ± 13.7 mg. At the end of the treatment there observed further decrease to 1037 ± 13.5 mg/100 g. of body weight.

ii) Alterations in diameter of seminiferous tabules :

The variations occurring in the seminiferous tubular diameter of testis of control and experimental rats are recorded in Table No. 3 and illustrated in Graph No. 3.

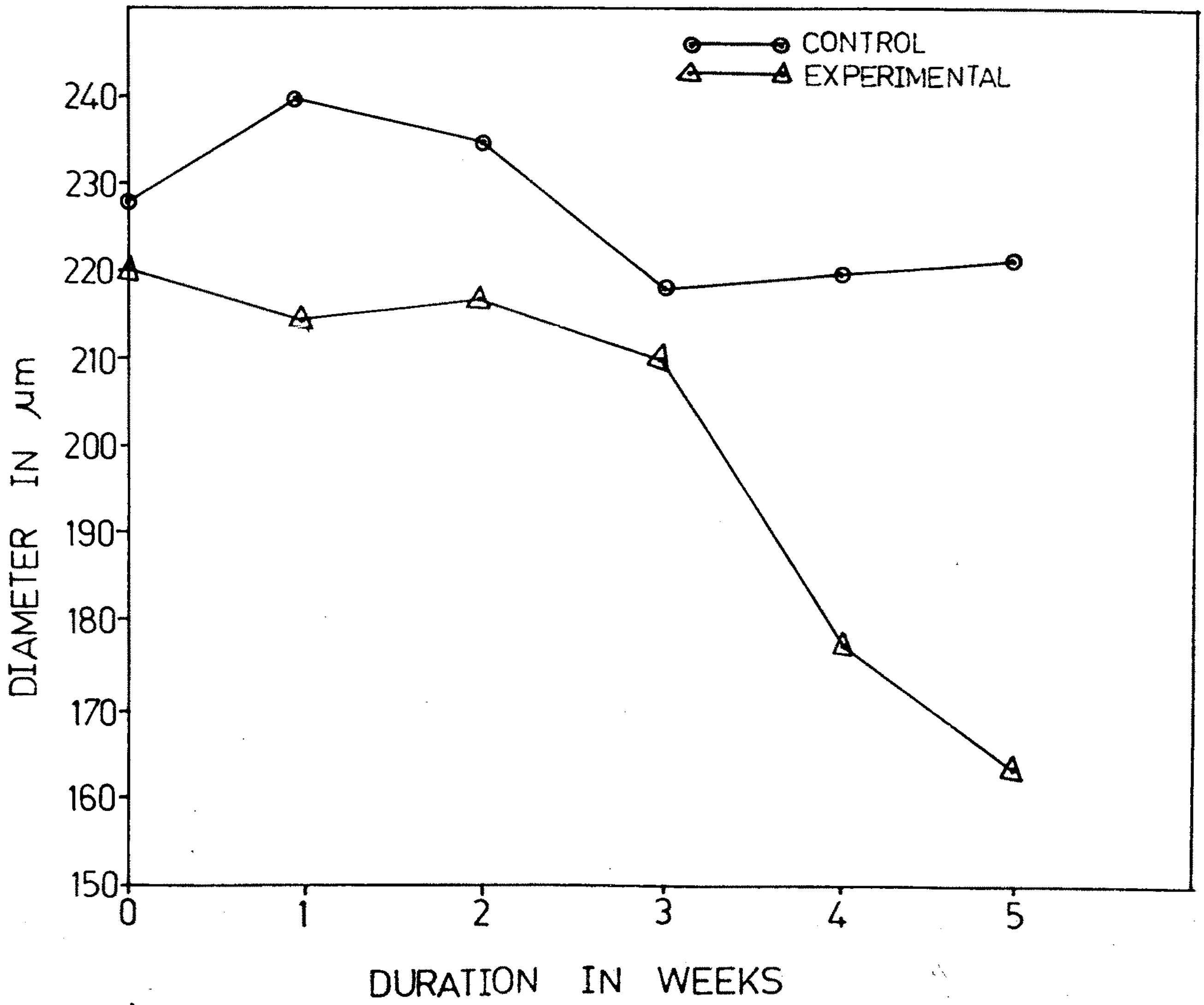
Table No. 3 : Agnus castus induced alteration in seminiferous tubular diameter.

Duration in weeks	Tubular diameter in μ m Control	Experimental
0	228 $\pm$ 16.2	220 $\pm$ 15.8
1	240 $\pm$ 18.5	215 $\pm$ 17.2
2	235 $\pm$ 15.4	217 $\pm$ 15.6
3	218 $\pm$ 16.8	210 $\pm$ 14.4
4	220 $\pm$ 16.2	178 $\pm$ 12.5
5	222 $\pm$ 14.1	164 $\pm$ 12.3

(Values are mean $\pm$ S.D. of 100 tubules. Tubular diameter is expressed in μ m.)

As seen from the tabular and graphical illustrations the diameter of seminiferous tubules (appearing round) of testes remained fairly constant - in control rats which received only vehicle. But in the rats receiving Agnus castus extract, the tubular diameter decreased as a function of duration of the treatment.

Initially the seminiferous tubular diameter of the testis of control rat was 228 + 16.2 μ m. It showed insignificant variations to 240 $\pm$ 18.5, 235 $\pm$ 15.4, 218 $\pm$ 16.8,


ALTERATIONS IN DIAMETER
OF SEMINIFEROUS TUBULES IN μm
CONTROL
EXPERIMENTAL
DIAMETER IN μm
240
230
220
210
200
190
180
170
160
150
0
1
2
3
4
5
DURATION IN WEEKS
Graph No. 3

220 ± 16.2 and 222 ± 14.1 μm. after 1st, 2nd, 3rd, 4th and 5th weeks, respectively.

At the commencement of the treatment, the seminiferous tubular diameter of testis was 220 ± 15.8 μm. It decreased to 215 ± 17.2 μm. after 1st week of the treatment. There observed an insignificant increase in tubular diameter to 217 ± 15.6 after 2nd week of the treatment. It gradually decreased to 210 ± 14.4, 178 ± 12.5 and 164 ± 12.3 μm after 3rd, 4th and 5th week of the treatment respectively.

3.2.3. Alterations in Histology :

Control :

Histological structure of the testis of control albino rats did not differ from the normal. Sertoli cells and germinal epithelial cells in different stages of development were seen in all seminiferous tubules. Each tubule has a basement membrane on which different germ cells rest. Tunica propria is an external covering of tubule. Interstitial elements including Leydig cells, blood vessels, lymphatic vessels and connective tissue cells were also normal (Plate No. 2, Fig.No.1).

Experimental :

The light microscopic histological changes in the testes of rats treated with Agnus castus extract were observed at all time intervals studied.

1) First week of treatment :

There was slight reduction in the seminiferous tubular diameter. The tunica propria and basement membrane showed no change. The spermatogonia, primary spermatocytes, round spermatids were normal. Pyknotic nuclei were not seen. Elongated spermatids, however, in some tubules, showed fusion. Spermatozoa were attached to the Sertoli cells and were normal. Sertoli cells also appeared normal.

Interstial space slightly increased. Leydig cells did not show any alterations. Their number, stainability, nuclear size, nature of cytoplasm resembled to that of the control.

Second week of treatment :

Diameter of seminiferous tubules showed insignificant variation after this stage of treatment. The tunica propria and basement membrane appeared slightly thickened. Many seminiferous tubules showed signs of moderate damage. The

spermatogonia, the spermatocytes and spermatids did not show any damage in majority of tubules. In few tubules giant cells and pyknotic nuclei were seen in spermatid layer. The giant cells are probably formed by fusion of certain spermatids. There were seen many vauoles or spaces in the spermatid layer probably formed by the phaygeytosis of giant cells by Sertoli cells, as well as because of sloughing off of germinal epithelial cells. The spermatocyte layer was seen to be separated from the spermatogonial layer, in few tubules. Number of spermatozoa was reduced in many tubules while in some tubules agglutinated masses of spermatozoa were seen in the lumina.

Interstitial spaces was increased Leydig cells were weakly affected (Plate No. 2, Fig. No. 2).

Third week of treatment :

Seminiferous tubules diameter showed fair reduction. The tunica propria and basement membrane appeared thickened, as observed in previous phase of treatment. In all the tubules the spermatogonia did not show any effect. In few tubules spermatocytes alongwith spermatids slonghed off from their positions and accumulated in lumina of seminiferous tubules and formed a cellular debris containing degenarating spermatozoa (Plate No. 2, Fig. 3). The spermatids when present, showed

fusion. In some tubules germinal epithelium did not whow any sign of damage. Sertoli cells remained unaffected.

Leydig cells were seen somewhat damaged.

Fourth week of treatment :

The degree of damage caused due to the extract was higher after this stage of treatment. The diameter of the tubules decreased significantly. The tunica propria and basement membrane thickened further. Most of the tubules showed moderate to severe damage. The spermatogenesis was arrested at primary spermatocytes. Overall depopulation of spermatogenic elements in seminiferous tubules with nuclear pyknosis was evident. There appeared several spaces, vacuoles in the gonial layers because of the exfoliation of all germinal cells except spermatogonia (Plate No. 2, Fig. 4). Spermatogonia remained unaffected. Lumina appeared to be widened H contained few degenerating germinal cells with spermatozoa. It also showed fragments of sperms. The number of spermatids decreased appreciably. No change was seen in Sertoli cells.

Interstitium showed presence edematous fluid. Leydig cells showed certain degenerating changes. Nuclear size appeared to be reduced.

Fifth week of treatment :

After this stage of treatment there observed a wide spread damage in testicular histoarchitecture. Diameter of seminiferous tubules decreased further. The tunica propria and basement membrane thickened. Though some tubules escaped from the destructive effects of the Agnus treatment, majority of the tubules showed marked degenerative changes (Plate No. 2, Figs. 5, 6, 7). In many tubules only spermatogonial layer remained intact. There observed asynchronization of different germ cell layers. These degenerating epithelial cells filled the lumina so that the lumina was abliterated completely. Sperms were not discrenible. In severaly damaged tubules, loss of tubular shape was noticed. All the gonial cells have been apperead to be detached en masse from the only intact layer of spermatogonial cells of the seminiferous tubules. Sertoli cells showed vacuolization.

Interstitium was increased and filled with edematous fluid. (Plate No. 3, Fig. 1). Leydig cells showed shrinkage. Their nuclei were reduced in diameter.

The salient features of the histological alterations induced by Agnus castus extract were as follows. Various alterations were seen at different intervals but there was no consistancy and sequential progression. There was significant

reduction in the diameter of seminiferous tubules. The tunica propria and basement membrane thickened. The spermatogenesis was arrested at primary spermatocytes. Except spermatogonia, rest of the tubular spermatogenic elements were affected by the treatment. Separation occurred between spermatogonial and spermatocytic layers. Round spermatids showed abnormalities. Formation of giant cells observed in some tubules. Cellular debris formed and disappeared in lumina of seminiferous tubules. Lumina of some tubules were obliterated due to accumulation of various degenerating germinal epithelial cells. Agglutination of spermatozoa was a common finding. Towards the end of the treatment severily of damage increased. Many seminiferous tubules lost their shapes. Sertoli cells under light microscopic observation revealed no appreaciable change. Interstitium widened with accumulation of edematous fluid. Leydig cells were slightly atrophied.

3.3. DISCUSSION :

The present dissertation was undertaken with a view of study alterations, induced by administration of *Agnus castus* berries extract in male reproductive organs of albino rats. Now we have full information on changes in body weight, organ weight and histomorphology. All the above information regarding

alterations is derived by employing well accepted techniques. In the present discussion the alterations are proposed to be discussed at a comparative level with the available literature and to arrive at definite conclusions.

Agnus castus extract reduced body weight of the animals. Such reduction in body weight is also reported by Sullivan and Smith (1957) and Chapman et al.,(1977) with estogen, Das and Smith (1977) with centchroman, Arora and Vijayraghavan (1989) with MIC. Some plant preparations are also reported to decrease the body weight. These include Allium sativum powder (Dixit and Joshi, 1982), Plumbago zeylenica (Bhargava, 1984), Vitex negundo (Sohani, 1985) Daucus carota (Shah, 1985), Plumbagin (Jadhav, 1988), Picrorhiza kurroa (Patne, 1990) and Syzygium cumini (Ambaldage, 1990).

The wet weight of testes is reduced appreciably after the administration of Agnus extract to the rats. This observation is well in accordance with that observed by many investigators who worked on antispermatogenic chemical and plant preparations. Chemicals such as estrogen estrodiol 17 beta (Chang, 1942; Bacon and Kirman, 1955; Saksena et al., 1978; Bansal and Mathur, 1984), WIN - 17416, WIN - 18446 (Coulston et al., 1960), buslphan (Ahlquist, 1966; Kar et al., 1968), Dorgesterol (Singh

et al., 1972), 5 - Thio-D-glucose (Zysk et al., 1975) centchroman (Das et al., 1977), alpha - chlorohydrin (Hundal and Mangat, 1978), endosulphan (Gupta and Ansari, 1981; Sing and Pandey, 1989), MGP (Rao et al., 1986), testosterone oenanthate (Bansal and Davis, 1986), Flutamide (Dhar and Shetty, 1987), formaldehyde (Shah et al., 1987), $HgCl_2$ (Roy Chowdhury and Vachhrajani, 1987), cyproterone acetate (Bhiwgade et al., 1990), Lithium (Ghosh et al., 1990) are shown to reduce the wet weights of testes. Similar observations, with various plant preparations are reported by Joshi et al., (1965) with Hippophae salicifolia; Joshi and Ambay (1968), Chauhan et al., (1984) with Vinca rosea; Kasinathan et al., (1972), Khanna et al. (1986) with Oscimum sanctum; Vyas and Singh (1976) with cannabis and opium; Kholkute (1977) with Hibiscus rosa sinensis; Dixit (1977) with Malvaviscus conzanttii, Pakrashi and Pakrashi (1977) with Aristolochia indica; Garg (1979) with Calotropis procera; Dixit and Joshi (1982) with Allium sativum powder; Bhargava (1984)with Plumbago zeylenica; Sohani (1985) with Vitex negundo; Shah (1985) with Daucus carota; Awati (1985) with Butea monosperma; Hiremath (1988) with Piper betle; Jadhav (1988) with Plumbagin; Adhikary et al. (1989) with Piper betle; Sinha and Mathur (1990) with Abrus precatorius; Akbarsha et al. (1990) with Andrographis paniculata; Patne (1990) with Picrorhiza kurroa; Ambaldage (1990) with Syzygium cumini; Murugavel and

Akbarsha (1991) with Vinca rosea. Thus it seems that aspermatogenesis, either caused by chemicals or plant preparations, affect the testicular weights significantly.

The normal testicular weight is mainly due to spermatids and supermatozoa. (Paul, 1953; Nelson and Patanelly, 1956). As Agnus extract unduces aspermatogenesis, these cells affected severely and reduced significantly in number; as a result fall in weight of testes occurred. Decrease in testicular weight may also be due to reduction in various metabolites induced by Agnus.

Agnus treatment caused decrease in the diameter of seminiferous tubules, in the present work. This alteration resembles those described for norgesterol (Singh et al., 1972) 5 - Thio-D-glucose (Zysk et al., 1975), formaldehyde (Shah et al., 1987) mercuric chloride (Roy Chowdhury and Vachhrajani, 1987) cimetidine (Gill and Sereen, 1991). Similar decrease in diameter of seminiferous tubules is reported with various plant preparation, which include cannabis and opium (Vyas and Singh, 1976), Aristolochia indica (Pakrashi and Pakrashi, 1977), Calotropis procera (Garg, 1979), Malvaviscus conzanttii (Verma et al., 1980), Vinca rosea (Toro, 1984), Daucus carota (Shah, 1985), Vitex

negundo (Sohani, 1985), *Butea monosperma* (Awati, 1985), *Piper betle* (Hiremath, 1988), Plumbagin (Jadhav, 1988), *Picrorhiza xurroa* (Patne, 1990), *Syzygium cumini* (Ambaldage, 1990), *Andrographis paniculata* (Akbarsha *et al*., 1990), *Vinca rosea* (Murugavel and Akbarsha, 1991). Such decrease in diameters of seminiferous tubules seems to be mainly due to the damage, observed in spermatogenic layers. The cellular elements spermatocytes, spermatids -, sloughed off in the lumina of tubules, due to *Agnus* extract, causing reduction in the diameter.

In the present investigation the thickening of tunica propria and basement membrane is seen. Similar observations were reported with heat treatment (Bawa *et al*., 1971; Kanwar *et al*., 1974), chlorpromazine (Bedwal and Mathur, 1980) and plant preparations *Hibiscus rosa sinensis* (Kholkute, 1977), *Butea monosperma* (Awati, 1985), *Vitex negundo* (Sohani, 1985), *Daucus carota* (Shah, 1985), Plumbagin (Jadhav, 1988), *Piper betle* (Hiremath, 1988), *Picrorhiza kurroa* (Patne, 1990), *Abrus precatorius* (Sinha and Mathur, 1990), *Sysygium cumini* (Ambaldage, 1990).

Most of the spermatogenic cells of the seminiferous tubules seems to be affected by *Agnus* extract administration.

A
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Spermatogonia :

Among the spermatogenic epithelium, they are relatively few in number. They seems to be remained unaltered by the extract treatment.

Spermatocytes :

These seems to be more suscepitible cells to the extract treatment. Spermatogenesis appeared to be arrested at primary spermatocytes. This observation finds good support in the earlier work with estrogen (Lacy, 1962), Clomiphene citrate (Kalra and Prasad, 1967; Kaur and Mangat, 1979), Cyproterone acetate (Meitokowiski and Lucaszyk, 1969; Flickinger and Loving, 1976), neregesterol (Singh et al., 1972), prostaglandins (Ericsson, 1972; TSO (1976), TSO and Lacy (1979), clomiphene (Flickinger, 1977). Similar observations are also made with plant preparations - Hibiscus rosa sinensis (Kholkute, 1977), Malvaviscus conzanttii (Dixit, 1979), Vinca rosea (Toro, 1984), Vitex negundo (Sohani, 1985), Butea monosperma (Awati, 1985), Daucus carota (Shah, 1985), Plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988), Picrorhiza kurroa (Patne, 1990), Syzygium cumini (Ambaldage, 1990).

The damage caused in the spermatocytes, after administration of Agnus extract, involve varying degree of damage.

Cellular nuclei became pyknotic and showed condensation of chromatin matter. The affected spermatocytes sloughed off and appeared in the lumina of seminiferous tubules. The extract seem to affect meiotic division of spermatocytes, resulting arrest of chromosomes which then merge together to form dense masses. Some of these cells get phagocytosed by Sertoli cells leaving clear spaces in their places. This is in accord with observations that sertoli cells phagocytose degenerating germ cells under other drug treatments such as estrogen (Lacy and Lofts, 1965; WIN 18446 (Reddy and Svobada, 1967), Cyproterone acetate (Flickinger and Loving, 1976).

Spermatids :

Round spermatids, as a result of treatment, unite together and form multinucleated giant cells. Such cells are seen at various time intervals. The cells nuclei show pyknosis (as indicated by dark staining with HE and feulgen techniques). Occasionally these cells appear in the lumina of tubules leaving spaces in the spermatid layer. These observations are in accordance with the observations made by Niemi and Kormano (1965) in cryptorchidism; Reddy and Svobada (1967) and De Martino et al. (1975) with WIN - 18446 treatment; Bawa et al.

(1971) with hypothermic shocks. Similar results are also shown after administration of plant extracts of Cannabis (Dixit et al., 1977), Malvaviscus conzanttii (Dixit and Bhargava, 1978), Calotropis procera (Garg, 1979), Allium sativum powder (Dixit and Joshi, 1982), Vinca rosea (Toro, 1984), Plumbago zeylenica (Bhargava, 1984), Daucus carota (Shah, 1985), Vitex negundo (Sohani, 1985), Butea monosperma (Awati, 1985), Plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988), Syzygium cumini (Ambaldage, 1990), Tylophora asthamatica (Dikshith et al., 1990), Picrorhiza kurroa (Patne, 1990).

Spermatozoa :

Fully matured spermatozoa remained unaffected. Spermatogenesis of damaged spermatids result into formation of damaged, abnormal spermatozoa. Sloughing off of premature spermatozoa into lumen is seen. More and more tubules showed lumina without sperms, as the duration of the treatment increased. Similar observations are recorded by administration of extracts of plants cannabis and Opium (Kholkate, 1977), Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Dixit, 1977), Oscimum sanctum (Sheth et al., 1981; Khanna et al., 1986), Vinca rosea (Toro, 1984), Butea monosperma (Awati, 1985), Daucus carota (Shah, 1985), Vitex negundo (Sohani, 1985),

Plumbagin (Jadhav, 1988), Celastus paniculatus (Wangoo, 1988), Solanum xanthocarpum (Rao, 1988), Piper betle (Hiremath, 1988; Adhikary et al., 1989), Syzygium cumini (Ambaldage, 1990), Andrographis paniculata (Akbarsha et al., 1990), Picrorhiza kurroa (Patne, 1990) and Abrus precatorius (Sinha and Mathur, 1990).

Desquamation of germinal cells in the lumina of seminiferous tubules leaving spaces or vacuoles in seminiferous epithelium is seen after administration of Agnus treatment. Such alterations are also witnessed with administration of ethionine (Livni and Yaffae (1974), Cyproterone acetate (Flickinger and Loving, 1976), prostaglandins (Tso and Lacy, 1979), Cyclohexanol (Tyagi et al., 1979), Orange - II (Singh and Khanna, 1979), thimet (Saxena and Sarin, 1979), Aspirin (Ratnasooriya and Lionet, 1984), Mercuric chloride (Roy Chowdhury and Vachhrajani, 1987), formaldehyde (Shah et al., 1987), DBP (Srivastava et al., 1990). Similar changes are also noted with various plant preparations by Joshi et al. (1965) with Hippophe salicifolia; Joshi and Ambay, (1968) and Toro (1984) with Vinca rosea, Kasinathan et al. (1972) with Oscimum sanctum, Dixit et al. (1977) with Cannabis, Dixit and Bhargava (1978) and Verma et al. (1980) with Malvaviscus conzanttii, Bhargava (1984) with

Plumbago zeylenica, Shah (1985) with _Daucus carota_, Awati (1985) with _Butea monosperma_, Hiremath (1988) with _Piper betle_, Wangoo (1988) with _Celastrus paniculatus_, Jadhav (1988) with plumbagin, Patne (1990) with _Picrorhiza kurroa_, Ambaldage (1990) with _Syzygium cumini_, Sinha and Mathur (1990) with _Abrus precatorius_, Akbarsha _et al_. (1990) with _Andrographis paniculata_.

Sertoli cells :

Light microscopic observations of _Sertoli_ cells showed no significant changes, except appearance of vacuoles in the cytoplasm. They remained in their original position.

Leydig cells :

Administration of _Agnus_ extract showed occasional atrophy of Leydig cells. Shrinkage of the cells and reduction of nuclei diameter is seen towards the end of the treatment. Such changes in Leydig cells are also observed after administration of various chemicals and plant preparations. The chemicals include are estrogen pelletes (Lacy, 1962), estradiol (Elkington and Blackshaw, 1971; Steinbeck _et al_., 1971), Chloromadinone acetate (Kaur and Mangat, 1979), Cyclohexanol (Tyagi _et al_., 1979), cyproterone acetate (Bhiwgade _et al_., 1990). Similar results are obtained by administration of plants _Malvaviscus_

conzanttii (Verma et al., 1979), Plumbago zeylenica (Bhargava 1984), Vitex negundo (Sohani, 1985), Plumbagin (Jadhav, 1988), Andrographis paniculata (Akbarsha et al., 1990), Syzygium cumini (Ambaldage, 1990), Abrus precatorius (Sinha and Mathur, 1990), Picrorhiza kurroa (Patne, 1990), Vinca rosea (Murugavel and Akbarsha, 1991).

This occasional Leydig cell atrophy may be resulting into some depletion in testosterone levels in the experimental animals. However effect of the extract on the functional state of the Leydig cells cannot be ascertained positively in the present investigation, since direct measurement of gonadotrophins and testosterone levels have not been carried out.

From the above discussion it can be concluded that :

a) Agnus castus berries extract affects testes and induce aspermatogenesis.

b) The basal lamina and lamina propria are thickened.

c) The spermatogonia remained unaffected and forms primary spermatocytes as that in control.

d) The primary and secondary spermatocytes are highly susceptible cells to the extract treatment.

e) Damaged spermatocytes may be getting converted into damaged spermatids.

f) Damaged spermatids, by spermatogenesis, may be leading to damaged spermatozoa. But the spermatozoa which are formed earlier do not show any effect of the extract administration.

g) Though Sertoli cells show signs of some changes, the nature of damage is not clear under our light microscopic studies.

h) Leydig cells are atrophied.

It seems that Agnus castus berries extract possibly affecting directly on the testes, especially gonial elements of the seminiferous tubules. Leydig cells also showed certain regressive changes which probably results in less secretion of androgen, which may also aid in the enchancement of aspermatogenesis. The changes observed in the present investigation, such as thickening of basal membrane, arrest of spermatogenesis at primary spermatocyte level, Leydig cell atrophy are observed after administration of different natural and synthetic estrogenic compounds, in various studies by number of workers - Bedwal and Mathur (1980) with chlorpromazine, Ericsson (1966) with nafoxidine hydrochloride; Kalra and Prasad (1967), Kaur and Mangat (1979), Wang et al., (1977) with clomiphene citrate; Bhargava (1984) with plumbagin - a estrogenic principle from Plumbago zelenica; Elkington and Blackshaw (1971), Steinbeck

_et al_. (1971) with estradiol; Lacy (1962), with estrogenic pelletes; Shah (1985) with _Daucus carota_ seed extract containing phytoestrogen - Coumarin Jadhav (1988) with plumbagin. _Agnus castus_ extract contain progesterene like substance which seems to be exerting effect of spermatogenesis by decreasing androgen level.