3. HISTOLOGY

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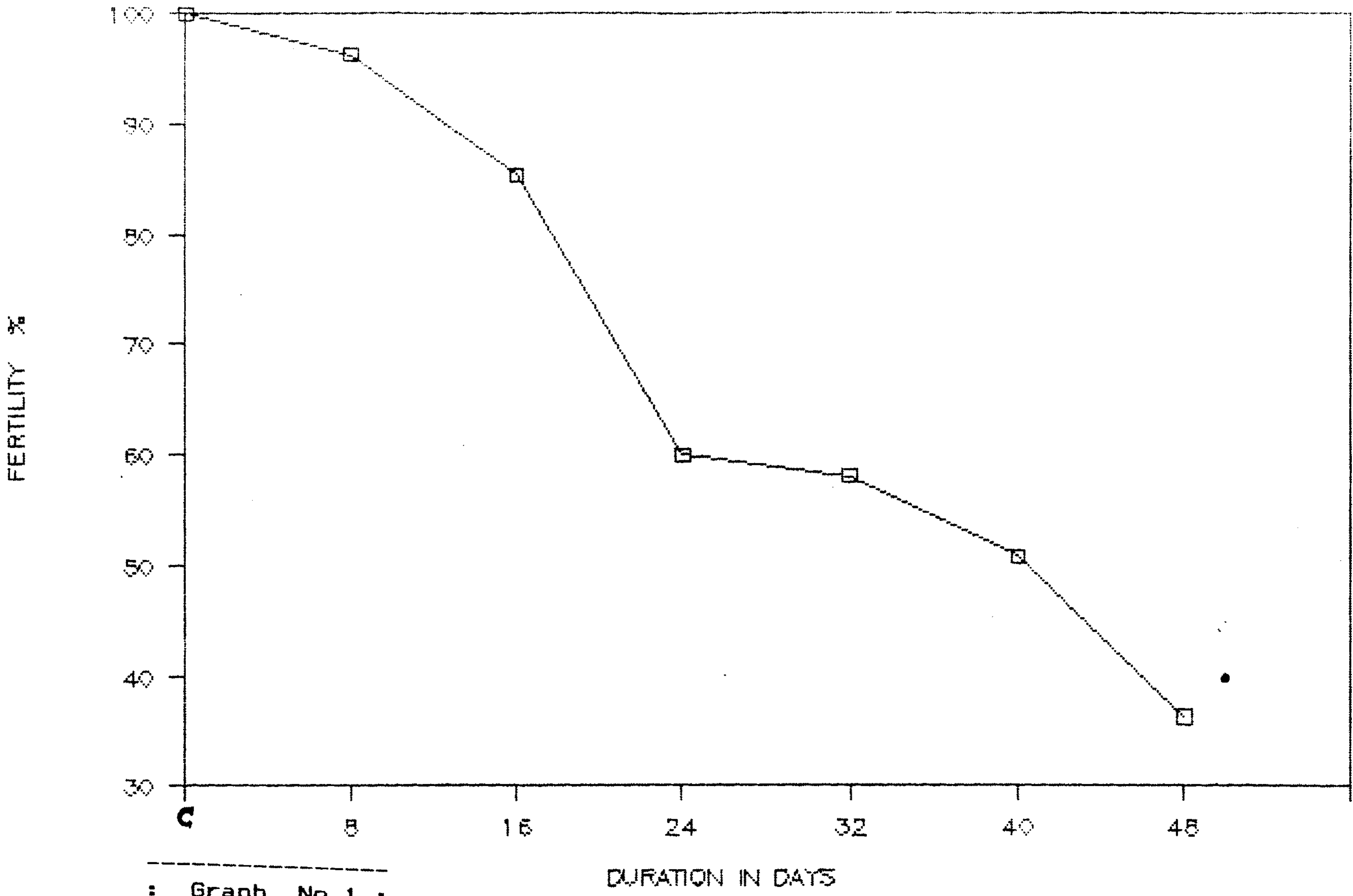
HISTOLOGY

3.1 FERTILITY TEST :

Fertility tests were carried out to find out the infertility induced in the male rats by the administration of Picrorhiza kurroa extract. The results of control & treated rats are recorded in Table No. 1, and illustrated in Graph No. 1.

The control and treated rats were mated with the estrous females of proven fertility one day before they were sacrified for histological and biochemical studies. On the following morning vaginal smears were examined for the presence of spermatozoa which indicated, copulation had occurred. After 21 days of gestation period the number of the delivered young ones was calculated.



: Graph No.1 :

TABLE NO. 1

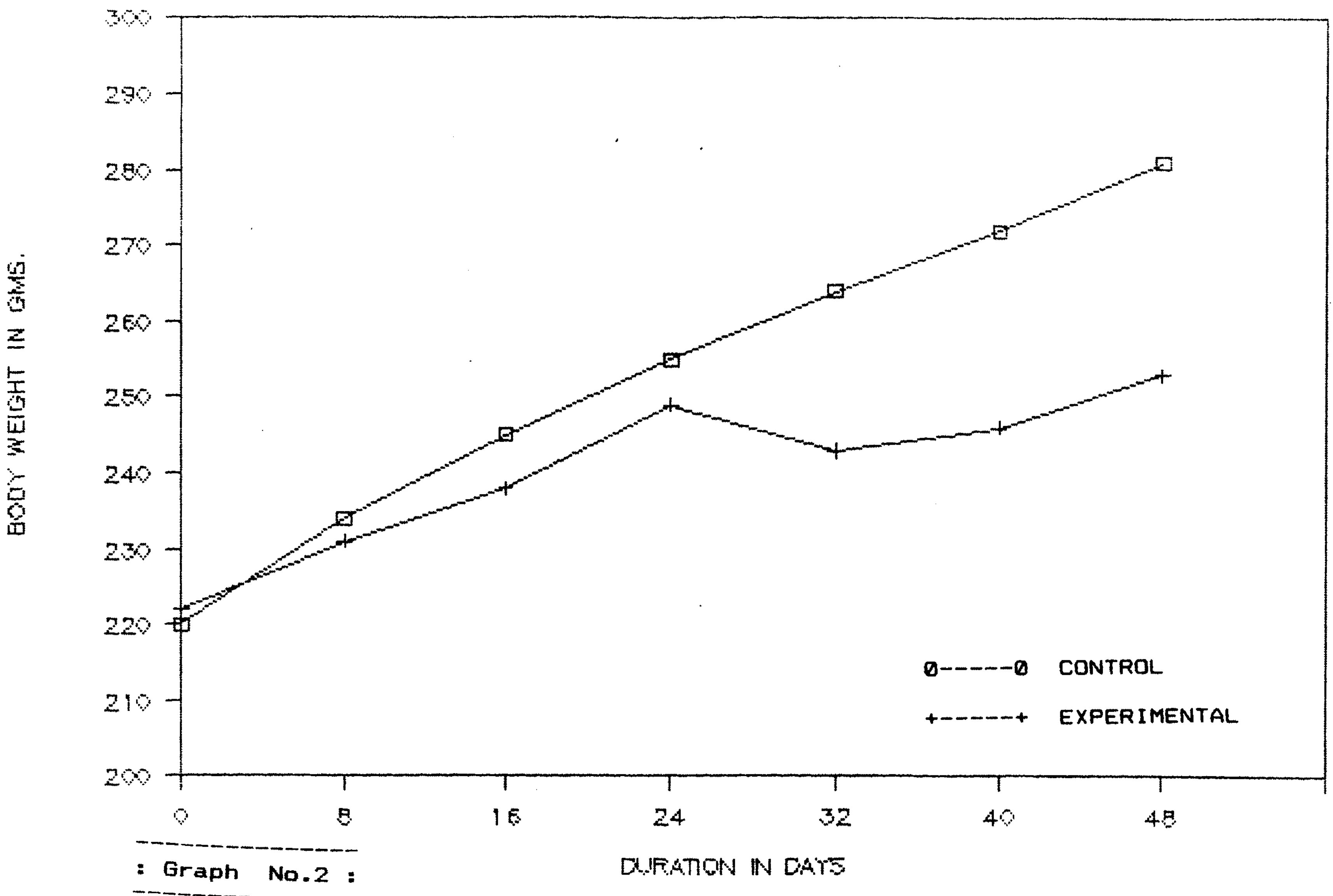
Fertility of Males (revealed by the number of young ones delivered by the mated females).

Duration in days	No.of young ones delivered by females					Total	Average	Fertility %
	F1	F2	F3	F4	F5			
C	11	12	10	11	11	55	11.0	100.0
8	9	10	12	11	11	53	10.6	96.3
16	12	0	11	12	12	47	9.4	85.4
24	11	10	0	0	12	33	6.6	60.0
32	10	0	11	11	0	32	6.4	58.1
40	0	10	9	0	9	28	5.6	50.9
48	0	0	5	8	7	20	4.0	36.3

It is very clear from the tabular and graphical illustration that the control rats which received only vehicle,the fertility is 100 %, but in the rats administered with the Picrorhiza kurroa extract, the fertility of the males decreased to 36.3 % only.

3.2 ALTERATIONS IN BODY WEIGHT :

The alteration occurring in the body weights of control and experimental rats are recorded in Table No. 2 and illustrared in Graph No. 2.



: Graph No.2 :

TABLE NO. 2

Body weights of control and Picrorhiza kurroa treated rats.

Duration in days	Control body Weight in gm	Experimental body weight in gm.
0	220 ± 4.8	222 ± 3.8
8	234 ± 4.2	231 ± 3.7
16	245 ± 3.6	238 ± 4.3
24	255 ± 3.5	249 ± 3.1
32	264 ± 4.8	243 ± 4.0
40	272 ± 5.8	246 ± 4.5
48	281 ± 5.5	253 ± 4.6

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

As seen from the tabular and graphical illustrations, weights of control rats increased gradually and the percent increase in body weight (age related) was 23.7 g; while in extract treated rats it was only 14.26 g.

3.3 TESTIS

3.3.1 REVIEW OF LITERATURE :

A) CHEMICALS :

Spermatoginic arrest or induction of functional sterility in male animals is caused with many synthetic

compounds. Such compounds include synthetic estrogess like diethylstilbestral (Kasinathan and Basu, 1974), Clomiphene (Holtkamp et al;, 1960, Nelson and Patanelli, 1962m Roy et al;, 1964, Nelson, 1965), Clomiphene citrate (Kaur and Mangat, 1979 Wang et al;, 1980), Centchroman (Das et al;, 1977), other synthetic compounds, tried are Triethylene melamine (Hendry et al;, 1951, Jackson and Bock, 1955, Fox, et al;, 1963), Win 130991, Win 17416, Win 18446 (Coulston et al;, 1960). 6 medroxy progesterone acetate - (Mc Leod, 1969), busulfan (Ablquist, 1966, Jackson, 1966, Kar et al;, 1968, Triethylinethiophosphoramide (Herschberger et al;, 1969), S.K. & F. 7690 (saunders et al;, 1969), Monochlorohydrin (Gunn et al;, 1970), Norgesteral (Singh et al;, 1972), Trihydroxy - pregnenolene (Sud and Setty, 1973), alpha-chlorohydrin (Cooper et al;, 1974), 5-thio-D-Glucose (Maughn, 1974; Zysk et al;, 1975, Homm et al;, 1977), Hundal and Mangat, 1978).

Salts of various metals such as Cadmium, mercury, Osmium, Plutonium, Zinc, silver, Cobalt, Leat etc. have also effects on reproductive system (Gunn And Gold, 1970).

Cigarette Smoke inhalation (viczin, 1968), Orange II (Singh and Khanna, 1979), aspirin (Balasubramanian et al;, 1980), have been reported for their antispermatoginic activity. Vanithakumari (1986) administered para-

chlorophenyl alanine in rats and found degenerative changes followed by immense necrosis of germ cells lead to complete breakdown by seminiferous tubules. But leydig cells remained unaffected. 2 mercaptopropionyl glycine (MGP) resulted in alteration of wet weight of testis of rat. It disturbed normal orgaization of Seminiferous epithelium. Leydig cells atrophied (Rao et al;, 1986).

Bansal and Davies (1986) reported decrease in wet weight of testis and also decrease in spermatogonia, pachytene spermatocytes and spermatids after administration of testosterone oenanthate in mice. Follicle regulating protein (FRP) altered seminiferous epithelial function in white rats (Iso et al;, 1987; Nakumura et al;, 1987). Oral administration of Flutamide (Dhar and Shetty, 1987) in adult male rat altered histology of testis. Spermatogenesis was arrested at the spermatid state; although few tubules showed normal spermatogenesis. Leydig cells hypertrophied; weight of testes decreased. (Shah et al;, 1987) administered formaldehyde to white rats. They reported no change in body weight but decrease in organ weights.

Diameter of seminiferous tubules was reduced. Lumina contained cellular debris. Nair et al, (1987), showed impairment of spermatogenesis in rats after adrenalectomy. The seminiferous tubules were shrunken and Wavy in outline. Germinal epithelium was evident with only spermatogonia. The

other cell types exhibited karyohexis and Karyolysis. The interstitium was uncommonly large with degenerated Leydig cells. Lumina were with cellular debris. Dechamma and Sarkar (1987) administered PMHI to field rats and showed complete inhibition of spermatogenesis. Bhiwgade *et al*;, (1990) reported changes in spermatids, sertoli cells as well as in Leydig cells with cyproterone acetate. Ghosh *et al*;, (1990), administered Lithium, to albino rats and found degeneration of testicular germ cells as well as decrease in wet weight of testis.

B> PLANT PREPARATIONS :

As compared to chemical compounds, very few plants have been tried for their antispermatogenic potency. The water soluble portion of ethanolic extract of bark of *Hippophae salicifolia* (Joshi *et al*;, 1965) have been shown to possess antimitotic property in the tests of young rats. Administrations of total alkaloids of *Vinca rosea* produced graded degenerative changes in the immature rats (Joshi and Ambay, 1968). Impairment of spermatogenesis in mice was resulted after feeding leaves of *Oscimum sanctum* with normal diet (Kasinathan *et al*;, 1972).

Kholkute (1977) administered orally, benzene extract of *Hibiscus rosa-sinensis* to albino rats and found to affect the spermatogenesis and endocrine function of testis.

Spermatogenesis was arrested at the spermatid stage. Some of the tubules showed only spermatogonia and sertoli cells. Leyding cells were atrophied.

Administration of Malvaviscus conzanttii flower extract produced many degenerative changes in tetes of house rat and gerbil (Dixit, 1977b). 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.

Pakrashi and Pakrashi (1977) when fed orally the water soluble part of the chloroform fraction of Aristolochia indica in mice reported degenerative changes of varying degree in the seminiferous germinal cells. Diameter of tubule was reduced. In some tubules the lumina were filled with a debris of desquamated germinal elements. Sertoli cells and Leyding cells show no change morphologically.

Oral administration of Calotropis procera flower extract caused testicular necrosis (Garg,1979). Diameters of seminiferous tubules and Leydig cell nuclei were reduced.

Mature rats when fed with ethanolic extract of Vinca rosea caused significant changes in testes (Chauhan et al;, 1979).

Oscimum sanctum reduced sperm count and sperm motility in male rats (Seth *et al*;, 1981).

Garlic powder reported to cause reduction in body weight and testicular weights (Dixit and Joshi, 1982). The powder caused testicular lesions leading to spermatogenic arrest of primary spermatocyte stage. Degenerative changes were also seen in sertoli cells.

Gossypol, an active principle from cotton seeds, showed interesting results. Gossypol severly damaged the seminiferous tubules but adjacent to it found normal tubules (Hoffer, 1983).

Plumbagin, an active principle from *Plumbago zeylenica* reported to arrest spermatogenesis at spermatocyte in dogs (Bhargava, 1984).

Toro (1984) administered alkaloid from leaves of *Vinca rosea* in albino rats and reported that except spermatogonia and mature spermatozoa all other spermatogenic cells were affected.

Awati (1985) reported arrest of spermatogenisis at spermatid stage in albino rats with *Butea monosperma*.

Sohani (1985) administered *Vitex negundo* leaf extract to male albino rats and found many changes in the testicular histology.

Shah (1985) reported arrest of spermatogensis at primary spermatocyte level after administration of Daucus carota seed extract in adult male albino rats.

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

Malvidin chloride, the colouring pigment of the flowers of Malvaviscus conzattii when fed to Langur monkeys, caused decrease in weight of testis and impairment of spermatogenesis (Bhargava, 1988).

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

Rajsekaran et al;, (1988) administered Oleanolic acid present in the flowers of Eugenia jambolana, in the male rat. They reported 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 spermatoginic cells, Leydig cells and sertoli cells.

Oral administration of Gossypol to albino rats resulted inhibition of spermatogenesis (Bhiwgade et al; 1988).

Celastrus paniculatus seed extract after administration in albino rats caused arrest in

spermatogenesis. The seminiferous tubules were shrunken and showed vacuolization, germ cell depletion and exfoliation (Wangoo, 1988).

Vinca rosea leaf extract resulted into many alterations in the histomorphology of testes in male albino rats (Chinoy et al, 1988).

Piper betle petiole extract administraton in albino rats caused decrease in wet weight of testes and many degenerative, changes in the seminiferous epithelium (Hiremath and Toro, 1988).

Jadhav (1988) administered plumbagin, an active principle from Plumbago zeylenica to male albino rats and found all spermatogenic cells affected except spermatogenic leading to aspermatogenesis.

Powder of Andrographis paniculata leaves when fed to albino rats, resulted into cessation of spermatogenesis (Akbarsha et al;, 1988, 1990).

Hiremath (1988) reported arrest of spermatogenesis mainly at spermatid level in the albino rats after administration of Piper betle petiole extract.

Adhikary et al; (1989) administered Piper betle petiole to albino rats. They reported in male animals decrease in the wet weight of testes and changes in testicular elements, decrease in number and motility of spermotozoa.

Ambaldhage (1990) with <u>Syzygium</u> <u>cumini</u> seed extract reported decrease in wet weight of testis and many alterations in testicular histoarchitecture.

3.3.2 OBSERVATIONS :

I) ALTERATIONS IN WET WEIGT OF TESTES :

The alterations occurring in the weights of testis of control and experimental rats are recorded in Table No. 3 and illustrated in Graph No. 3.

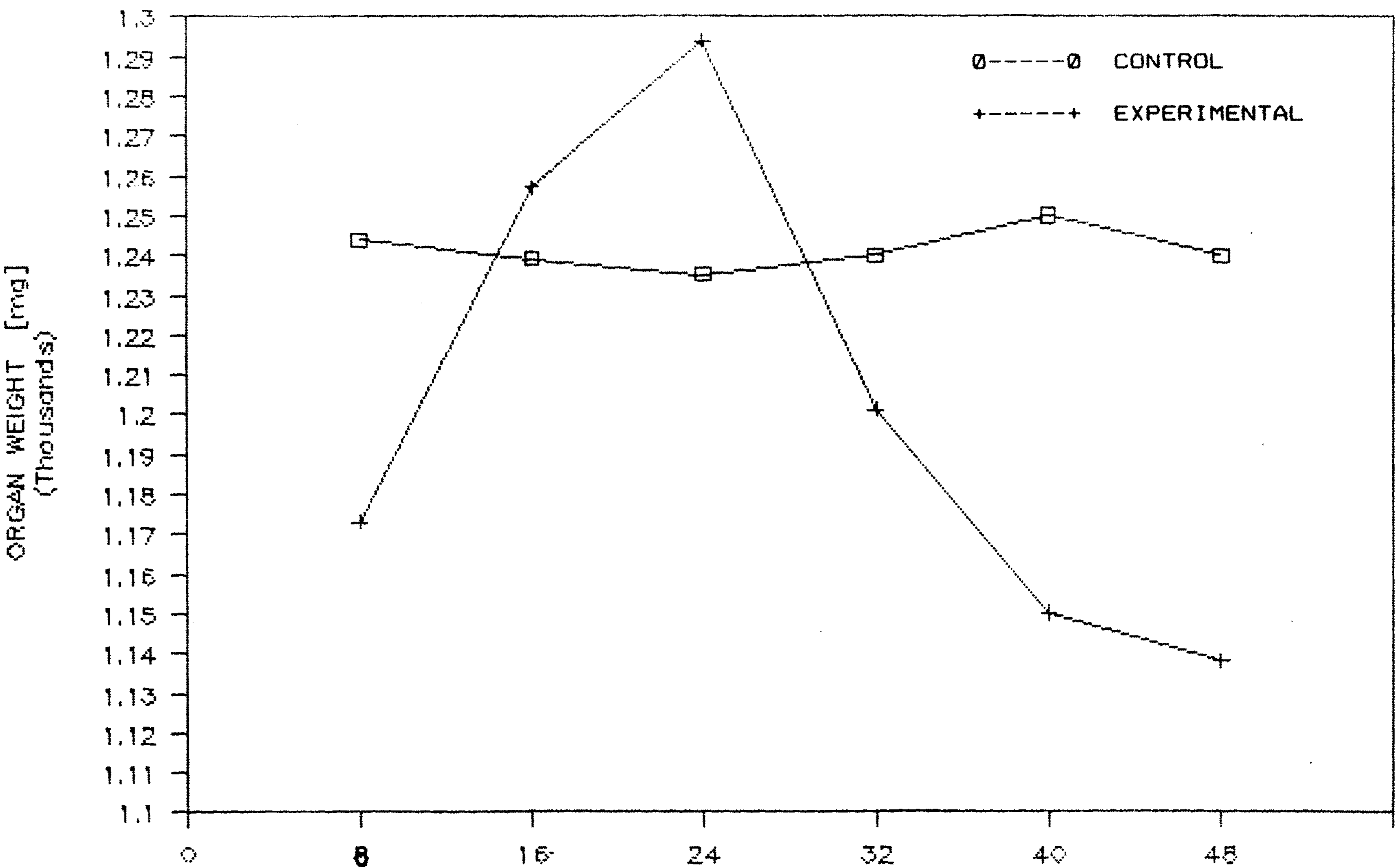
TABLE NO. 3

Testis : *Picrorhiza kurroa* extract induced changes in weight of Testis.

Duration in days	Control weight of testis (mg)	Experimental weight of testis (mg)
8	1244 ± 16.3	1173 ± 10.6
16	1239 ± 10.6	1257 ± 15.5
24	1235 ± 12.2	1294 ± 13.2
32	1240 ± 15.4	1201 ± 14.8
40	1250 ± 10.5	1150 ± 10.6
48	1240 ± 13.4	1138 ± 10.5

(values are mean ± S.D. of five animals. Weight of testis are expressed in mg per 100 gm. of body weight).

As seen from the tabular and graphical illustrations, the wet weight of testis remained fairly



: Graph No.3 :

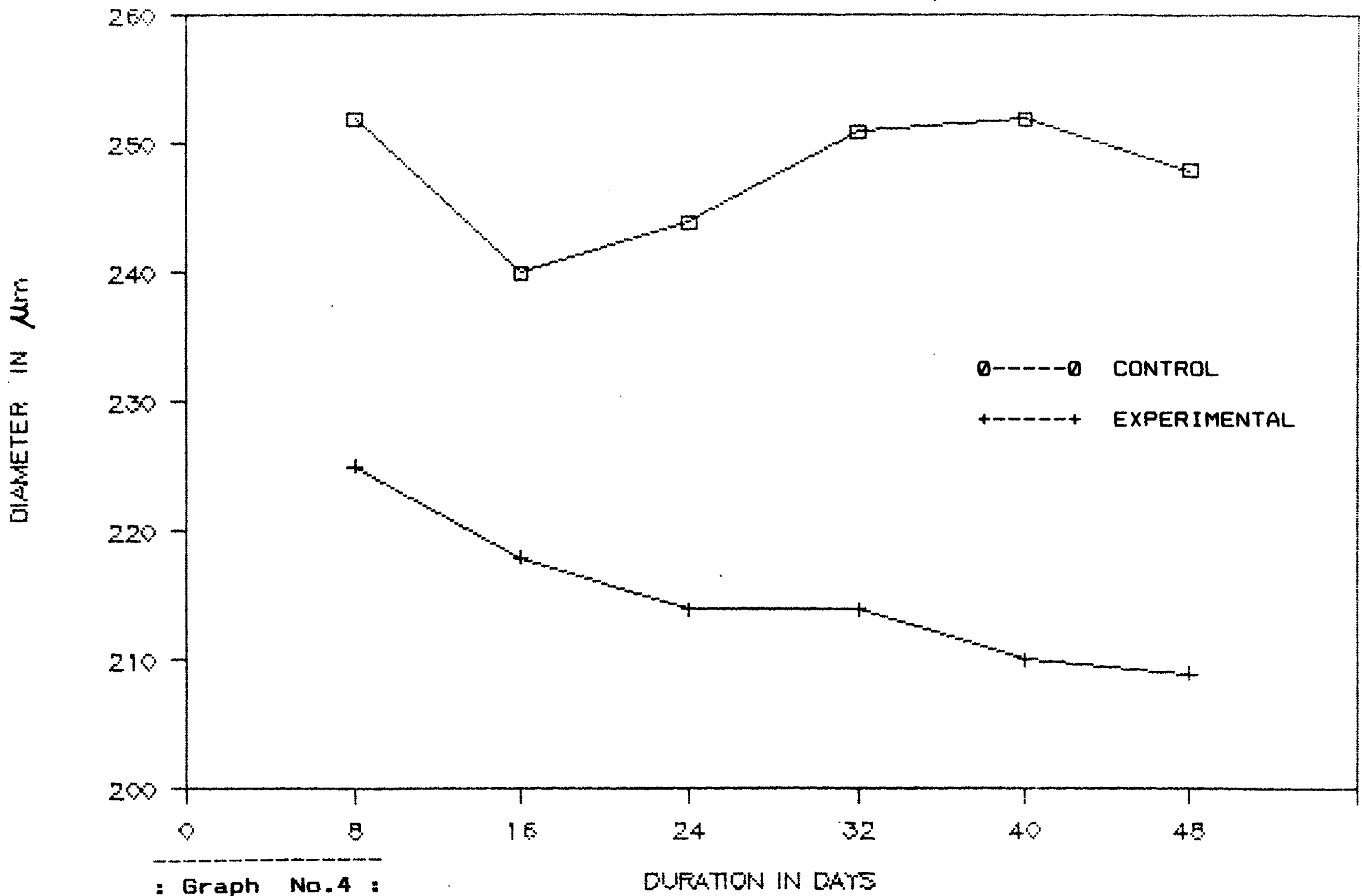
constant in control rats which received only vehicle. But in the rat receiving Picrorhiza kurroa extract,the wet weight of testis though not significant, decreased appreciably as a function of duration of the treatment.

The wet weight of testis of the control rat was 1244 ± 16.3 mg on 8th day. It showed insignificant variations to 1239 ± 10.6 mg, 1235 ± 12.2 mg, 1240 ± 15.4 mg, 1250 ± 10.5 mg and 1240 ± 13.4 mg on 16th, 24th, 32nd, 40th and 48th days.

The wet weight of testis of the experimental rat was decreased to 1173 ± 10.6 mg. It gradually increased to 1257 ±15.5 mg and 1294 ± 13.2 mg on 16th and 24th days of treatment. Then it steadily depeted to 1201 ± 14.8 mg, 1150 ± 10.6 mg and 1138 ± 10.5 mg on 32nd, 40th & 48th days of treatment. Thus the percent decrease in the testis of treated rats is 8.2 mg.

Alterations in diameter of seminiferous tubules :

The alterations occurring in the diameter of seminiferous tubules of testis of control and experimental rats are recorded in Table No. 4 and illustrated in Graph No. 4.



: Graph No.4 :

TABLE NO. 4

Diameter of Seminiferous tubules, Picrorhiza extract induced changes.

Duration in days	Tubular diameter (μm) Control	Experimental
8	252 ± 14.1	225 ± 12.3
16	240 ± 12.4	218 ± 14.6
24	244 ± 13.5	214 ± 10.5
32	251 ± 10.7	214 ± 8.4
40	252 ± 9.5	210 ± 11.7
48	248 ± 13.8	209 ± 9.3

(values are mean ± S.D. of 100 tubules and are expressed in μ.m).

As seen from the tabular and graphical illustrations, the diameter of seminiferous tabules of testis remained fairly constant in control rats which received only vehicle. But in the rats receiving Picrorhiza extract, the tubular diameter decreased as a function of duration of the treatment.

The seminiferous tubular diameter of the testis of control rat was 252 ± 14.1 μm, on 8th day. It showed minor variations to 240 ± 12.4 μm, 244 ± 13.5 μm, 251 ± 10.7 μm, 252 ± 9.5 μm and 248 ± 13.8 Um on 16th, 24th, 32nd, 40th and 48th days.

The seminiferous tubular diameter of testis of experimental rat was decreased 225 ± 12.3 μm on 8th day of the treatment. It gradually decreased throughout the treatment. Thus, it was decreased 218 ± 14.6 μm and 214 ± 10.5 μm on 16th and 24th day of the treatment. It remained unaltered on 32nd day of treatment. It then decreased to 210 ± 11.7 μm and 209 ± 9.3 μm on 40th and 48th day of the treatment. Thus the percent decrease in the diameter of seminiferous tubules of testis of extract treated rat was 15.7 μm.

II) HISTOLOGICAL ALTERATIONS IN TESTIS :

A) CONTROL :

The histological structure of the testis of control male albino rats did not differ from normal albino rats, (Plate No.2, Fig. No.1). Seminiferous tubules, which forms the bulk of each testis, were entire intact and well spread and arranged in compact manner.Tubular diameter was maximum. The tubules showed different germinal cell types, from periphery to lumen of the tubule were spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Non-germinal cells i.e. sertoli cells of the tubules were normal and tall, pyramidal, extend from basement membrane to the lumen. Each sertoli cell contain single avoid nucleus, finely dispersed chromatin material

PLATE NO.2 : CAPTIONS TO FIGURES

Fig.1 : Testis :(Control) :Haematoxylene-Eosin Staining. Single Seminiferous tubule (ST) showing normal Spermatogonia (SPG), Spermatocytes (PS), Spermatids (SPT), Luminal Spermatozoa (SPZ), Leydig cells (LC). X200.

Fig.2 : Testis:(16 days treatment):Haematoxylene-Eosin Staining. Note wavy counter(Arrows) of seminiferous tubule, Basement membrane(B.M.), Leydig cells (LC) normal X 240.

Fig.3 : Testis :(16 days treatment): Haematoxylene-Eosin staining. Note many spaces (S) in spermatogenic cellular layers. Lumen (L) contain desquamated cells (CD) and few spermatozoa (SPZ) X260.

Fig.4 : Testis:(24 days treatment):Haematoxylene-Eosin Staining. Note separation of spermatocyte layer from spermatogonia layer (Arrows). Fused spermatids (SPT) are seen. Clear spaces (S) evident in cellular layers. Leydig cells normal, X240.

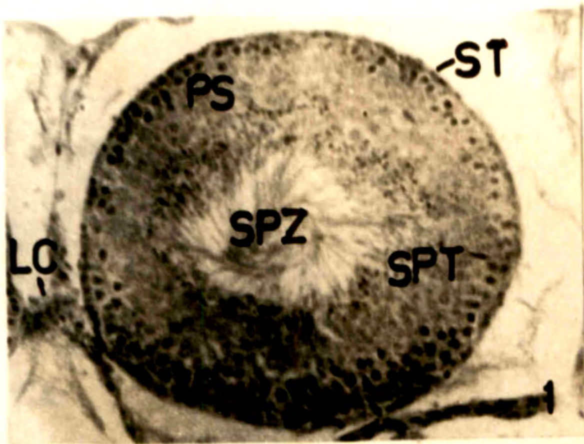
Fig.5 : Testis :(24 days treatment):Haematoxylene-Eosin staining. Note luminal mass (AM) containing degenerating cells and spermatozoa. Clear spaces (S) are seen in tubule, X200.

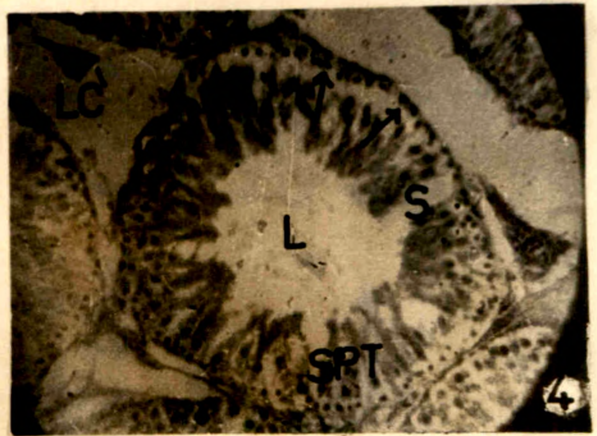
Fig.6 : Testis:(32 days treatment):Haematoxylene-Eosin Staining. Note empty lumina(EL) in many tubules. Desquamation of cells resulted many clear spaces (S) in tubules. Basal membrane (BM) thickened. Leydig cells(LC) reduced, X100.

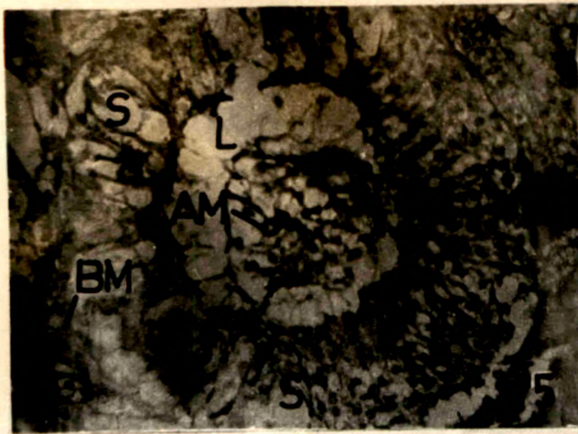
Fig.7 : Testis:(40 days treatment): Haematoxylene-Eosin staining. Note shrinkage of seminiferous tubules (ST) and pseudopodial projections (PP). Leydig cells (LC) atrophied, X240.

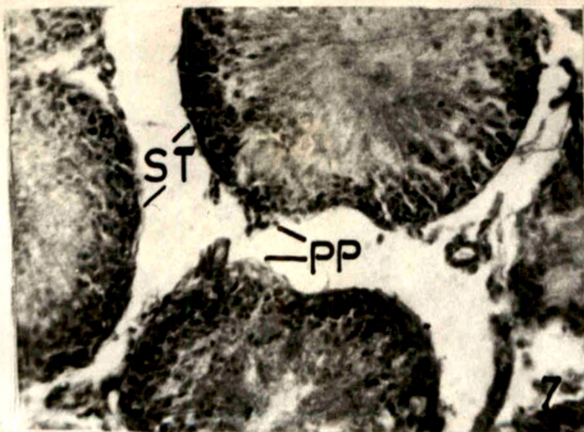
Fig.8 : Testis: (48 days treatment):Haematoxylene-Eosin Staining. Note severe damage in Seminiferous tubule showing many clear spaces (S). Lumen (L) contain negligible number of spermatozoa (SPZ). Basement membrane (BM) thickened. Leydig cells (LC) show reduction, X240.

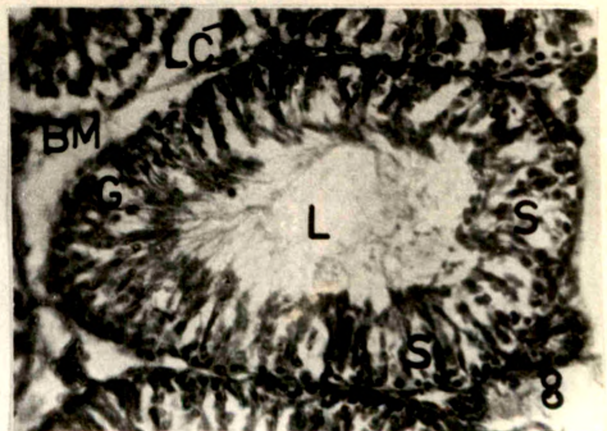
PLATE NO.2











with one or two nucleoli. Spermatozoa were attached by their heads to the sertoli cells. Each tubule has basement membrane on which different cells rest and tunica propria as an external covering.

Interstitium was normal, Leyding cells, blood vessels, lymphatic vessels and connective tissue were found in the interstitium. Leydig cells were avoid or polygonal, large and were arranged in clumps.

B) EXPERIMENTAL :

The light microscopic histological changes in the testes treated with Picrorhiza extract were observed at all time intervals. The changes which occured in different tubules at different time intervals had no any consistency in progression of damage. But towards the end of the treatment, the severity of damage was high.

1. 8TH DAY OF THE TREATMENT :

There was no detectable change at this phase of treatment. Majority of seminiferous tubules were Virtually normal; except that of tubular diameter decreased appreciably. Tubular elements spermatogonia, spermatocytes, round spermatids and elongated spermatids were normal and showed no change, when compared with those of the control. Sertoli cells were normal and bundles of spermatozoa were attached to them.

Interstitium with Leydigs cells was also seen to be unaffected.

Tunica propria and basement membrane also appeared normal.

2. 16TH DAY TREATMENT :

At this phase some significant changes started appearing. Many seminiferous tubules showed wavy contours, (Plate No. 2, Fig. No. 2).Tubular diameter further reduced. Spermatogonia & spermatocytes were normal. Round and elongated spermatids, both showed fusion. Many gonadal elements desquamated and found in the lumina. Most of them were spermatids and few were spermatocytes. Because of degeneration and desquamation of spermatogenic cells, many clear spaces appeared in many tubules (Plate No.2 Fig No.3). The number of spermatozoa was also reduced. Sertoli cells were normal.

Tunica propria and basement membrane were appeared normal.

Leydig cells with other interstitial elements remained unchanged, after 16th day of <u>Picrorhiza</u> treatment.

3. 24TH DAY OF TREATMENT :

Some tubules showed further damage. Spermatogonia were unchanged. Separation of spermatocyte layer from the spermatogonia was an unique feature of this treatment. Occasional damage to spermatocyte was seen (Plate No. 2,

Fig. No.4). Clear spaces were seen around majority of cells. At certain instances large vacuoles were also evident in the seminiferous layers due to sloughed off cells. Diameter of the tubules decreased. Seminiferous tubular lumina contained cellualar debris with residual sperms. Fragments of various degenerating cells observed in the luminal mass (Plate No.2, Fig. No.5). Sertoli cells appeared unaltered.

Tunica propria and basement membrane appeared thickened. Leydig cells were without any appreciable change.

4. 32ND DAY OF TREATMENT :

The diameter of seminiferous tubules decreased, similar to that observed in the previous phase of treatment. A process of spermatogenic inhibition continued. Arrest of spermatogenesis seen at spermatocyte level. The spermatogonia were not affected. Giant cells were seen. Conspicuous vacuolization observed in the cellular layers. Luminal space widened. In most of the tubules the lumina were empty (Plate No.2, Fig.No.6), while some tubules showed very few residual spermtozoa.

Tunica prapria and basal membrane thickned. Sertoli cells were normal.

Leydig cells were seen to be reduced in size.

5. **40TH DAY OF TREATMENT :**

Further decrease in diameter of seminiferous tubules observed. Many conspicuous histological changes exhibited by the testis of the treated rats at this phase. Most of the tubules showed shrinkage. The contours of seminiferous tubules showed pseudopodial projections which were made up of tunica propria, basal membrane and spermatogonia (Plate No.2, Fig,No.7). Spermatogonial cells were normal. Spermatocytes occured in few layers, but the layer were not continmous, since many clear spaces formed because of sloughing of spermatogenic cells. Occasionally Sertoli cells displayed Vacuolization in cytoplasm.

Tunica propria and basal membrane showed further thickening.

Shrinkage of the tubules led to widening of the interstitium. Leydig cells were fairly affected.

6. **48TH DAY OF TREATMENT :**

Maximum alterations were noticed during this phase of treatment in testis (Plate No.2, Fig.No.8). Most of the tubules showed severe damage. Diameter of the tubules decreased significantly (normal 248 $\pm$ 13.8 μm, control - 209 $\pm$ 9.3 μm). The germ cells were seen in the state of maturation only upto primary spermatocytes, hence only spermatogonia were normal. Spermatids were almost negligible

PLATE NO.3, CAPTIONS TO FIGURES

Fig.1 : Testis (48 days treatment): Haematoxylene-Eosin staining. Note majority of Seminiferous tubules with empty lumina (EL) Heavy sloughing off of gonial elements created spaces (S). Spermatogonia (SPG) normal. Basement membrane (BM) thickened. Leydig cells (LC) a trophied, X 400.

Fig.2 : Caput epididymis: (Control): Haematoxylene-Eosin staining. Note epithelium (EP), Stereocilia (ST) Mass of spermatozoa (SPZ) in lumen, Basal lamina (BL), Interstitium(I) normal, X 240.

Fig.3 : Caput epididymis : (32 days treatment): Haematoxylene-Eosin staining. Lumina (L) contain cellular debris (CD) with spermatozoa. Epithelium (EP) reduced slightly. Stereolilia (ST) normal. Interstitium (I) widened, X260

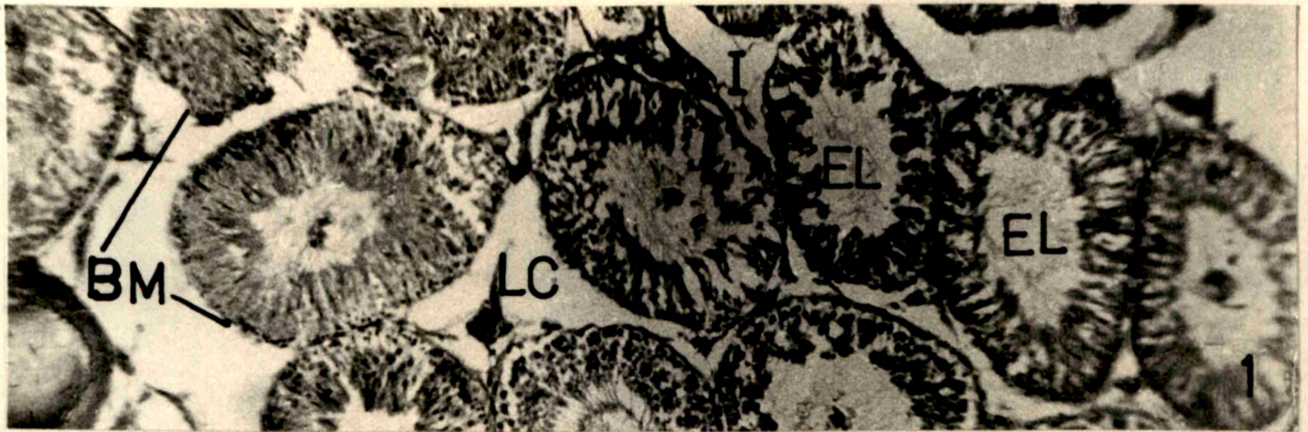
Fig.4 : Caput epididymis (40 days treatment): Haematoxylene-Eosin staining. Note spermatozoa alongwith cellular elements (DM) degenerating. Stereocilia (ST) scanty. Interstitium (I) increased. Basal lamina (BL) thickened, X240.

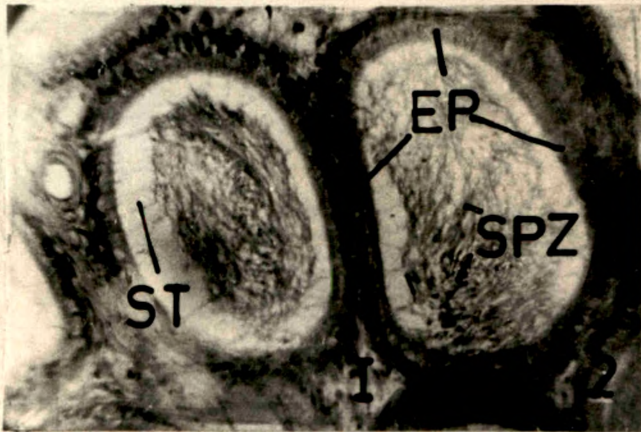
Fig.5 : Caput epididymis: (48 days treatment): Haematoxylene-Eosin staining. Majority of tubular lumina contain degenerating mass (DM) of cellular components and spermatozoa. Basal lamina (BL) thickened. Interstitium (I) widened, X150.

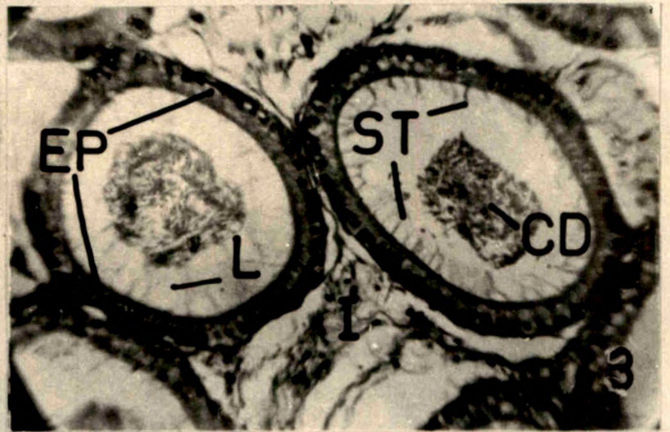
Fig.6 : Cauda epididymis : (Contool): Haematoxylene-Eosin staining. Note Epithelium (EP), Stereocilia (ST), Interstitium (I), density of spermatozoa (SPZ) normal, X100.

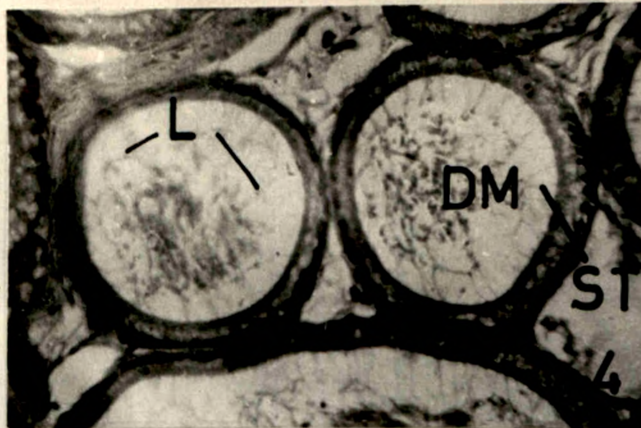
Fig.7 : Cauda epididymis (48 days treatment): Haematoxylene-Eosin staining. Note spermatozoa and other contents of lumina (DM) show degeneration. Interstitium (I) widened. X100.

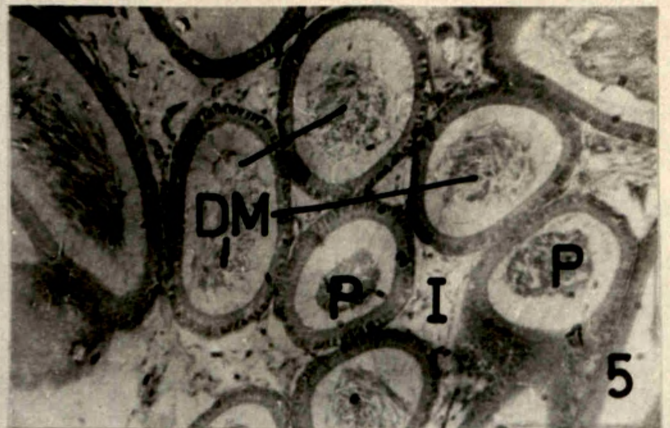
PLATE NO. 3

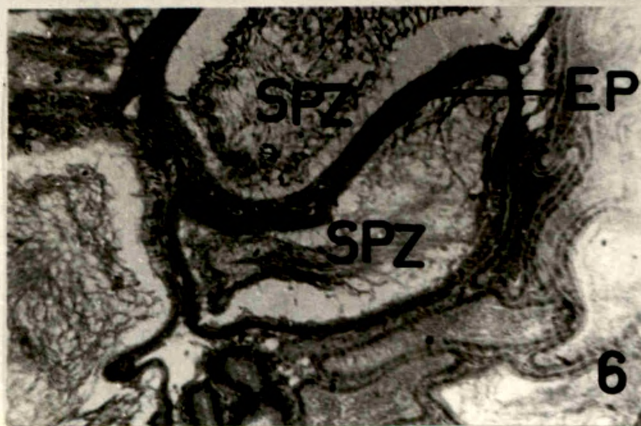


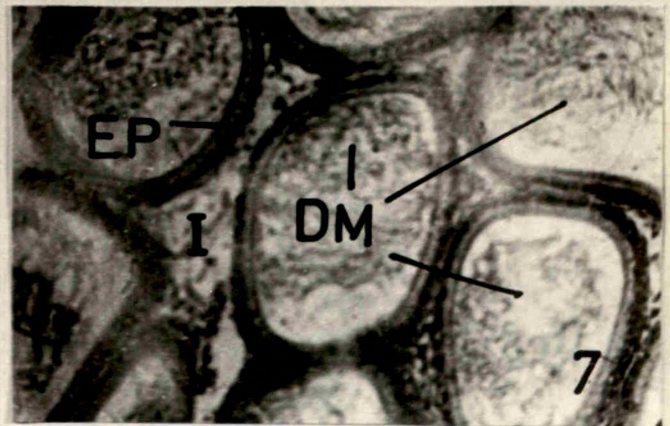












in number, in many tubules.

Giant cells were evident in many seminiferous tubules. Some tubules showed regions of cytolytic lessions - resulting into formation of clear spaces - due to phagocytosis or sloughing of degenerated cells. Majority of tubules showed empty lumina (Plate No.3, Fig. No.1). Sertoli cells showed varying degrees of vacuolization in cytoplasm.

Tunica propria and basal membrane thickened and intensely stained.

Interstitium was enlarged due to shrinkage of seminiferous tubules. Leydig cells showed pycnosis.

Thus Picrorhiza treatment altered histological features of testis of rats. In nutshell these were -

Seminiferous tubular diameter was Significantly reduced.Spermatogensis was arrested at primary spermatocytes level. Appearance and subsequent disappearance of giant cells, formation of clear space between spermatogonia and spermatocytes, vacuolizaton among spermatogenic cells. Formation and subsequent lysis of cellular debris in the lumina of seminiferous tubules, thickening of tunica propria and basal membrane, appreciable atrophy of Leydig cells. Shrinkage of seminiferous tubules and enlargement of interstitium. All these alterations were noticed in different tubules at different time intervals, but there was no consistent sequential progress in damage.

Severity of damage increased towards the end of the treatment.

3.3.3 DISCUSSION :

The investigation was undertaken with a view to study alterations induced by Picrorhiza kurroa extract treatment in albino rats, in fertility, body weight, organ weights and histoarchitectural changes occuring in the male reproductive system. Now we have before us a more or less complete information on the aforesaid change induced by the extract and all this information regarding the alterations is derived by employing both well accepted and recent 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.

Fertility :

It can be seen from the fertility test carried out that about 64 % infertility resulted in male albino rats due to administration of Picrorhiza extract. Similar reduction in fertilizing capacity observed with chemical like alpha-chlorohydrin (Ford et al;, 1977), Methaxamine (Rathasooriya et al;, 1980), aspirin (Ratnasooriya and Lionet, 1984), FRP (Isao et al;, 1987), mercury chloride (Rao, 1988a), lead acetate (Rao et al;, 1988) and plant preparations - Vinca rosea alkloids (Toro, 1984), Butea monosperma (Awati, 1985),

Vitex negundo (Sohani, 1985), Daucus carota (Shah, 1985), Gossypol and Tripterygium wil fordi (Xu et al;, 1987), Gossypol, (Nair et al;, 1988), Carica papaya (Sondarva and Chinoy, 1988), Piper betle (Hiremath, 1988;, Adhikary et al;, 1989), plumbegin (Jadhav, 1988), Solanum xanthocarpum (Rao, 1988b), Terminalia bellirica (Rao, 1988c), Kemoferol (Kumar et al;, 1989), Azadirachta indica, Emblica-officinalis and Piper nigrum (Reedy et al;, 1989) and Syzygium cumuni (Ambaldhage, 1990) administration.

The probable cause of the decrease in fertility could be attributed to androgen deprivation effect of Picrorhiza extracts, which contain a phytosteroid kutkisterol.

Picrorhiza extract caused reduction in body weight. This could be due to in aniation. Similar decrease in the body weight was observed by administration of estrogen sullivan and smith (1957), chapman et al;, (1977), centechroman (Das and Smith, 1977), MIC (Arora and Vijayaraghav, 1989). Plant preparaton Allium sativum powder (Dixit and Joshi, 1982), plumbagin, an active principle from Plumbago zeylenica (Bhargava, 1984) also reduced the body weight.

Administration of Picrorhiza extract reduced the wet weight of testes appreciably in this investigation. Such as decrease in the weight of the testes was observed in the work of many investigators who worked with various chemicals

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and plant preparations. Chemicals such as estradiol 17 Beta (Chang 1942, Bacon and Kirman, 1955, Sakeena *et al*;, 1978), WIN 17416, WIN 18446 (Coulston *et al*;, 1960), busulphan (Abliquist, 1966, Kar *et al*;, 1968), norgesterol (Singh *et al*;, `972), 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), estrogen (Bansal and Mathur, 1984), MGP (Rao *et al*;, 1986), Formaldehyde (Shah *et al*;, 1987), Flutamide (Dhar and Shetty, 1987), Methyl mercury (Rao, 1988), Lithium (Ghosh *et al*;, 1990),Cyproterone acetate (Bhiwgade *et al*;, 1990) are reported to reduce the wet weight of testes. Plant preparations such as *Hippophae salicifolia* (Joshi *et al*;, 1965), *Vinca rosea* (Joshi and Ambay, 1968, Chauhan *et al*;, 1979; Toro, 1984), *Oscimum sanetum* (Kasinathan *et al*;, 1972; Khanna *et al*;, 1986) Cannabis (Vyas and Singh, 1976), *Hibiscus rosa-sinensis* (Kholkute, 1977), *Malvaviscus conzanttii* (Dixit, 1977), *Aristolochia indica* (Pakrashi and Pakrashi, 1977), *Calotropis procera* (Garg, 1979), Garlic powder (Dixit and Joshi, 1982), *Plumbago zeylenica* (Bhargava, 1984), *Daucus carota* (Shah, 1985), *Vitex negundo* (Sohani, 1985), *Butea monosperma* (Awati, 1985), plumbagin (Jadhav, 1988),*Piper betle* (Hiremath, 1988, Adhikary *et al*;, 1989), *Andorgraphis paniculata* (Akbarsha *et al*;, 1990), *Abrus precatorius* (Sinha and Mathur, 1990) also showed

decrease in weight of testis, after their administration in various chemicals.

Various spermatogenic cells, spermatozoa, and Leydig cells together form the major part of testicular weight in control rats. Picrorhiza extract severly damage and reduce the number of some of these cells, especially post meiotic cells, i.e. the spermatids and spermatozoa considerablly, leading to depletion in the weight of testis, (Paul, 1953, Nelson and Patanelly, 1956). Decrease in weight may also be due to reduction in various metabolites induced by Picrorhiza extract. Therefore decrease in testicular weight is but a natural effect.

Reduction in the diameter of seminiferous tubules is also observed in the present investigation, due to Picrorhiza treatment. Similar results have been obtained with norgesterol (Singh et al;, 1972), 5-Thio-D-glucose (Zysk et al;, 1975), Formaldehyde (Shah et al;, 1987). Various plants are also reported to decrease the diameter of seminiferous tubules, these are cannabis (Vyas and Singh, 1976), Aristolochia indica (Pakrashi and Pakrashi, 1977), Calotropis procera (Garg, 1979), Malvaviscus conzanttii (Verma et al;, 1980), Andrographis paniculata (Akbarsha et al;, 1980). Picrorhiza extract affected the testicular cells significantly, therefore these were sloughed off in the

lumina of seminiferous tubules, so it appeared that because of absence of normal spermatogenic cell layers, the diameter of seminiferous tubules were reduced.

Picrorhiza extract altered the histoarchitecture of the testis, it affects all the cellular elements except spermatogonia. Damage done to the various cell types by the extract during 48 days of treatment is as follows ---

1. SPERMATOZOA :

Matured spermatooa, which are alreadly formed seems to be not affected. At certain instances sloughing of premature spermatozoa from sertoli cells into the lumen of seminiferous tubules is seen. The extract reduced the number of sperms in the lumina and most of the tubules with empty lumina. These observations are similar to the administration of many plants such as cannabis and Opium (Vyas and Singh, 1976), Hibiscus rosa-sinensis (Kholkute, 1977), Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Dixit, 1977), Oscimum sanctum (Seth et al;, 1981; Khanna et al;, 1986), Vinca rosea (Toro, 1984), Daucus carota (Shah, 1985), Butea monosperma (Awati, 1985), Vitex negundo (Sohani, 1985), Celastrus paniculatus (Wangoo, 1988), Piper betle (Hiremath, 1988, Adhikari et al;, 1989), Solanum xanthocarpum (Rao, 1988), Plumbagin (Jadhav, 1988), Andorgraphis paniculata (Akbarsha et al;, 1990), Abrus

precatorius (Seema Sinha and Mathus, 1990), Syzygium cumuni (Ambaldhage, 1990).

2. **SPERMATIDS :**

Some of the spermatocytes seem to be escape from the damage and become spermatids. Their number towards the end of the treament was negligiable. One interesting aspect of the Picrorhiza treatment is that it affect round Spermatids and form multinucleated giant cells. These cells seen at various time intervals. The giant cells occasionally found in lumina, leaving a clear space in their original position in the spermatid layer. The formation of giant cells after Picrorhiza administration is also observed earlier with WIN 18446 (Reddy and Svoboda, 1967, De Martino et al;, 1975), Cannabis (Dixit et al;, 1977), Malvaviscus conzanttii (Dixit and Bhargave, 1978), Calotropis procera (Gerg, 1979), Allium sativum powder (Dixit and Joshi, 1982), Vinca rosea alkaloids (Toro, 1984) Plumbago zeylenica (Bhargava, 1984), Butea monosperma (Awati, 1985), Vitex negundo (Sohoni, 1985), Daucus carota (Shah, 1985), Piper betle (Hiremath, 1988), Plumbagin (Jadhav, 1988), Tylophora asthamatica (Dikshith, et al;, 1990), such a giant cell formation may be due to damage of intercellular cytoplasmic bridge as shown by Dym and Fawcett (1971).

3. SPERMATOCYTES :

Picrorhiza extract inhibit the spermatogenesis at primary spermatocyte level. These observtions are similar to that observed by Jackson *et al*,(1961), with methylene suplhonate; Lacy (1962) with estrogen; Kalra and Prasada (1967), Kaur and Mangat (1979) with clomiphene citrate; Meitokowiski and Lukaszyk (1969) and Flickinger and Loving (1976) with Cyproterone acetate; Singh *et al*; (1972) with norgesterol; Ericsson (1972), TSO (1976), TSO and Lacy (1979) with prostaglandins. Similar results are also witnessed by administration of plant extracts such as Hibiscus rosa Sinensis (Kholkute, 1977), Malvaviscus conzanttii (Dixit, 1979), Vinca rosea alkaloids (Toro, 1984), Vitex negundo (Sohani, 1985), Daucus carota (Shah, 1985).

Spermatocytes sloughed off from their location leaving clear spaces and vacuoles and utimately they appeared in the lumina. These changes are seen more conspicvously in the last phase of the treatment.

Picrorhiza extract, arrested the spermatogenesis at primary spermatocyte level. Regarding mechanism of such arrest of spermatogenesis, it seems that it is concerned with the inference in the action of · hormones controloing spermatogenesis. The extract may be interfering normal androgen funciton. It is known that androgen is of vital importances for normal spermatogenesis, (Greep, 1939, Mann

and Mann, 1951; Steinberger, 1971, Brooks, 1981, Stevens and Steinberger, 1983). During spematogenesis, the meiotic division appear dependent on an adequate level of testosterone (steinberger, 1971, Hansson _et al_;, 1976, Ramaswamy, 1983). For transformation of primary spermatocyte (prophase) into metaphase and then into secondary spermatocyte as well as for Maturation of Spermatozoa, testosterone is needed. The observation in the present investigation show that _Picrorhiza_ extract induce atrophy of the Leydig cells (see later) and thus interfere in the production of testosterone. The extract may have another action of antiandrogenic nature. This result in the depletion of testosterone level in the treated rats. Under such depleted androgen condition, the transformation of prophase spermatocytes into metaphase and then into secondary spermatocytes may be getting, affected, which inturn may be responsible for arrest of spermatogenesis at metaphase of primary spermatocytes as seen in the present investigation.

Picrorhiza extract contain a phytosteroid-kutki-sterol which might be responsible for decreased level of testosterone and hence caused arrest of spermatogensis at primary spermatocytes level.

4) Spermatogonia :

Picrorhiza extract seems to be not affected the spermatogonia throughout the treatment for 48 days.

5) Sertoli cells :

Sertoli cells were found to be in their normal position. They exhibited occasional presence of vacuoles in their cytoplasm.

6) Leydig cells :

The extract affected the Leydig cells. It induced appreciable atrophy in the late of the treatment. Similar results are seen by De La Blaza (1962) with estrogen, Steinbeck _et al_;, (1971) Elkington and Blackshaw (1971) with estradiol, Tyagi _et al_;,(1979) with Cyclohexanol, Kaur and Mangat (1979) with chloromadinone acetate, Verma _et al_;, (1980) with _Malvaviscus conzanttii_ , Bhargava (1984) with _Plumbago zeylenica_, Shah (1985) with _Daucus carota_, Jadhav (1988) with plumbagin, Kumar _et al_;, (1989) with Kempferol, Bhiwgade _et al_;,(1990) with cyproterone acetate, Akbarsha _et al_;, (1990) with _Andrographis paniculata_ and Sinha and Mathur (1990) with _Abrus precatorius_. Leydig cell atrophy indicates lesser activity of androgen Mann (1964). Spermatogenesis is dependent on testosterone through its action on the sertoli cells (Stevenge and Steinberger, 1983). Therefore testosterone deficiency will be expected to interfere with the completion of meiosis by a

direct action on the germ cells. (Steinberger 1971). As stated previously phytosteroid-kutki-sterol present in the Picrorhiza extract resulted atrophy of Leydig cells which ultimately must have been reduced the testosterone level causing interference in the spermatogenesis.

Picrorhiza extract resulted into aggregated masses of desquamated germinal elements in the lumina of seminiferous epithelium layers. These observations are in accordance with observed previously with ethionine (Livni and raffae, 1974) , Cyproterone acetate (Flickinger and Loving, 1976), Cyclohexanol (Tyagi et al;,1979), Orange II (Singh and Khanna, 1979), thimet (Saxena and Sarin, 1979), prostaglandins (TSO and Lacy, 1979), aspirin (Ratnasooriya and Lionet 1984), Formaldehyde (Shah et al;,1987), di-n-butyl phthalate (Srivastava et al;, 1990). Similar results are shown by Joshi et al;, (1965) with Hippophae Salicifolia; Joshi and Ambay , (1968), Toro (1984) with Vinca rosea, Kasinathan (1972) with Oscimum Sanctum, Dixit et al;,(1977) with Cannabis; Garg (1979) with Calotropis procera; Dixit and Bhargava (1978),Verma et al;, (1980) with Malvaviscus conzanttii; Bhargava (1984) with Plumbago Zeylenica; Shah (1985) with Daucus carota; Sohani (1985) with Vitex negundo, Awati (1985) with Butea monosperma, Hiremath (1988) with Piper betle; Jadhav (1988) with a

plumbagin; Wangoo (1988) with Celastrus paniculatus; Akbarsha et al;, (1990) with Andrographis paniculata. Seema sinha and Mathur (1990) with Abrus precatorius. Tunica-propria and basement membrane, in this investigation, seen thickened. These findings are similar to those described for heat treatment (Bawa et al;, 1971, Kanwar et al;, 1974), chloropromazine (Bodwal and Mathur, 1980), di-n-butyl phthalate (Srivastava et al;, 1990). Similar results are also observed after administration of Hibiscus rosa sinensis (Kholkute, 1977), Daucus carota (Shah, 1985), Vitex-negundo (Sohani 1985), Butea monosperma (Awati, 1985), Plumbagin (Jadhav, 1988), Abrus precatorius (Seema sinha and Mathur, 1990).

The contours of many seminiferous tubules showed pseudopodia like projections in some instances in the present investigation. Such observations are also made by Sohani (1985) with Vitex negundo, Awati (1985) with Butea monosperma, Jadhav (1988) with plumbagin and Srivastava et al;, (1990) with di-n-butyl phthalate.

Picrorhiza extract treatment caused shrinkage of most of seminiferous tubules. This observation is well in accordance with that observed with administration of di-n-butyl phthalate (Srivastava , et al;, 1990) and plant extracts of Daucus carota (Shah, 1985) Piper betle (Adhikary et al;, 1989).

Thus the results indicate Picrorhiza kurroa extract in male albino rats cause-

1) The spermatogenic cells to develop upto primary spermatocyte stage which then degenerate and die.

ii) Degerating cells are phagocytosed by sertoli cells (Lacy and Lofts, 1965, Reddy and Svobada, 1967, Flickinger and Loving , 1976).

iii) Sertoli cells affected little and showing vacuolization in cytoplasm.

iv) Basal membrane and tunica propria become thickened.

v) Shrinkage of seminiferous tubules and,

vi) Atrophy in Leydig cells.

Picrorhiza contain phytosteroid-kutki-sterol which affect the germinal cells along with Leydig cells. Atrophy in Leydig cells indicate reduction in androgen level of testis (Mann, 1964). This antiandrogenic action of Picrorhiza is reflected in the regression of Leydig cells and germ cells.

3.4 EPIDIDYMIS :

3.4.1 REVIEW OF LITERATURE :

Various chemicals and plant-preparations are known to alter structure and function of epididymis. Following is brief review of the existing work.

A>CHEMICAL :

Many chemicals have been shown to affect the histoarchitecture of epididymis in various animals. Testosterone (Ortiz, 1953, Karkun et al;, 1974, Dinkar et al; 1977, Bose et al;, 1967), WIN-18446 (Coulston et al;, 1960, Drobeck and Coulston, 1962, Beyler et al;, 1965, Nag et al;, 1976) Methoxamine (E I Badwai and Schenk, 1962, Norberg et al;, 1967, Hib, 1976, Rathasooriya et al;, 1980), Clomiphene (Nelaon and Patauelli, 1962, Roy et al;, 1961, Kalra and Prasad, 1967, Schally et al;, 1970, Roy and datta, 1976, Roy et al;, 1976), 17 alpha-hydroxy progesterone (Macleod, 1965), Shetty and Kar, 1966, Terner and Mac Laughlin, 1973, Das et al;, 1977), alpha-chlorohydrin (Crabo, 1965, Peyre and Laporte, 1966, Gunn et al;, 1969, 1970, Ericsson and Baker, 1970, Kreider and Dutt, 1970, Hoffer et al;, 1973, Edwards et al;, 1973, Vickery et al;, 1974, Nag et al;, 1976), Dixit, 1976, 1977a, 1979, Ford et al;, 1977, Gurayya and Gill, 1977), Cyproterone and Cyproterone acetate (Wiechert and Neumann, 1965, 1966, 1971, Neumann, 1969, 1970, Mietkowaski and Lukaszyk, 1969, Markawitz et al;, 1969, Prasad et al;, 1970, Prasad, 1973, Morse et al; 1973, Rastogi et al;, 1973, 1979, Karkun et al;, 1974, Bose et al;, 1975, Nag et al;, 1976a, Dinakar et al;, 1977, Clomiphene citrate (Kalra and Prasad, 1967, Rajlakshmi et al;, 1970, estrogen (Lacy 1967, Steinbeck et

al;, 1971, Meistrich et al;, 1977, Wang et al;, 1980), bushulphan (Kar et al;, 1968a), Methallibure (Kar et al;, 1968a, Dixit, 1979) quinacrine hydrochloride (zipper and medel, 1970, Shetty et al;, 1972, Chandra et al;, 1974, Malaviya et al;, 1974, Shivkumar and Sarkar, 1979), U 29409 (Ericsson, 1971), S.K.F. 7690, (Lubicz-Nawrocki and Glover, 1973), 2-alpha-chloromethylene-6-chloropregne-4, (Nag et al;, 1976), $CdCl_2$ (Dixit, 1976, Chonoy & Sheth, 1977). Centchroman (Das et al;, 1977) progestin and androgen (Flickinger, 1977), Cyclohexauol (Tyagi et al;, 1979), dexamethazone metapiron, niridazol and damazol (Dixit, 1979), Chloornadinone, acetate (Kaur and Mangat, 1979), tesosterone propionate (Manjula and Kadam, 1980), Oxyphenonium (Ratnasooriya,. 1982), MGP (Rao et al;, 1986), para - chlorophenylalanine, Vanitha kumari (1986), Flutamide (Dhar and Shetty, 1987), PMHI (Dechamma and Sarkar, 1987), Formaldehyde (Shah et al;, 1987), Beta-sitosterol (Malni and Vanithakumar, 1988). All these chemicals have been reported to alter the histological structure of epididymis and reduce number of sperm present in its lumina.

B> PLANT PREPARATIONS :

Available literature reveals that there are very few plant preparations with antispermatogenic potencies have been explored for their effects on the epididymis.

Pakrashi and Pakrashi (1977) Administered *Aristolochia indica* extract to mice and found reduction in the diameter of epididymal tubules, reduction in the density of spermatozoa and deposition of fat droplets in the lumen. *Malvaviscus conzanttii* extract administration to house rat and gerbil showed sloughing off, of epididymal epithelium, lumen devoid of sperms and oedamatous interstitial tissues (Dixit, 1977b), *Calotropis procera* extract found to reduce the size of lumina of epididymis and was devoid of spermatozoa in gerbils (Garg, 1979). In mice Verma *et al*;, (1980) with *Malvavisucs conzsanttii* extract found atrophy of lumina of cauda epididymis along with the presence of debris and reduction in the height of epithelial lining. Papaya seeds did not alter histology of epididymis (Das, 1980). Dixit and Joshi (1982) reported insignificant reduction in wet weight of epididymis, with *Allium sativum* extract administration in rat. Administration of plumbagin (active principle from *Plumbago zeylenica*) induced regression of epididymal epithelium and the Lumen was without any sperms (Bhargava, 1984). *Vinca rosea* alkaloids reduced the number of spermatozoa in epididymis but no change in the height of the epithelial cells in white rats (Toro, 1984). Similar results were reported by Awati (1985) with *Butea monospema* extract in albino rats. Shah (1985) with *Daucus carota* seed extract and Sohani (1985) with

Vitex negundo leaf extract reported decrease in wet weights of epididymis and insignificant decrease in height epithelium. *Oscimum sanctum* when fed to albino rats, caused significant decrease in wet weight of epididymis, sperm count and sperm motility (Khanna *et al*;, 1986). Jadhav (1988) with plumbagin administration reported reduction in wet weight of epididymis, decrease in tubular diameters, and lumina devoid of spermatozoa, *Piper betle* petiole extract in white male rats reported to affect the luminal contents of epididymis (Hiremath, 1988). *Malvidin chloride* (active principle from *Malvaviscus conzanii* flower) treatment caused decrease in the wet weight of epididymis of Langur Monkeys (Bhargava, 1988). Oleanolic acid (a compound from *Eugenia jambolana* flowers) administration resulted insignificant decrease in wet weights of epididymis in rats (Rajsekaran, *et al*;, 1988).

Alcoholic extract of *Solanum zanthocarpum* seed induced many regressive changes in epididymics (Rao, 1988). Bhiwgade and Nair (1989a) administered Gossypol to male rats and found decrease in wet weight of cauda epididymis. *Andrographis paniculata* leaves dry powder fed male albino rats showed decrease in wet weight and many degenerative changes in epididymis (Akbarsha *et al*;, 1990). *Syzygium cumini* seed extract administration showed decrease in wet

weight and many degenerative changes in epididymis (Ambaldhage, 1990).

3.4.2 OBSERVATIONS :

1) ALTERATIONS IN THE WET WEIGHTS OF CAPUT AND CAUDA EPIDIDYMIS :

The alterations in the wet weights of caput and cauda epididymis during the period of the extract treatment are recorded in Table No. 5 and illustrated in Graph No. 5 & 6.

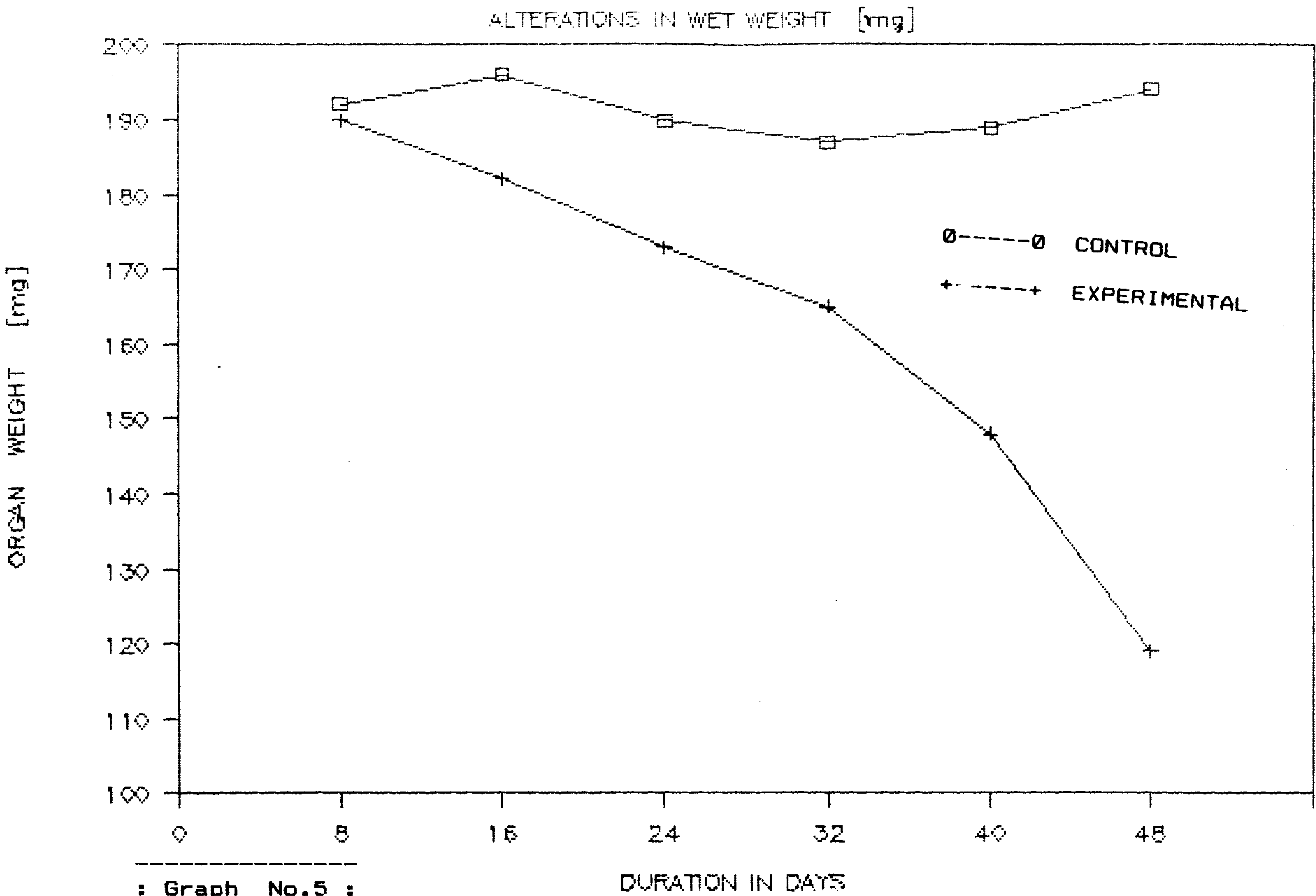
TABLE NO. 5

Epididymis : Picrorhiza kurroa extract induced changes in wet weight (mg).

Duration in days	Control		Experimental	
	Caput	Cauda	Caput	Cauda
8	192 ± 3.0	151 ± 4.4	190 ± 4.8	158 ± 4.6
16	196 ± 4.3	146 ± 4.1	182 ± 3.4	140 ± 5.3
24	190 ± 3.8	157 ± 4.2	173 ± 3.9	149 ± 4.7
32	187 ± 4.7	150 ± 3.9	165 ± 4.7	146 ± 4.2
40	189 ± 4.5	145 ± 4.1	148 ± 4.4	114 ± 5.3
48	194 ± 4.7	155 ± 4.3	119 ± 3.8	112 ± 4.8

(values are mean ± S.D. of five animals and are expressed in mg per 100 gm. of body weight).

As seen from the tabular and graphical illustrations, the wet weight of caput and cauda epididymis remained fairly constant in control rats which received only vehicle. But in



: Graph No.5 :

the rats receiving Picrorhiza kurroa extract, the wet weights of caput and cauda decreased as a function of duration of the treatment.

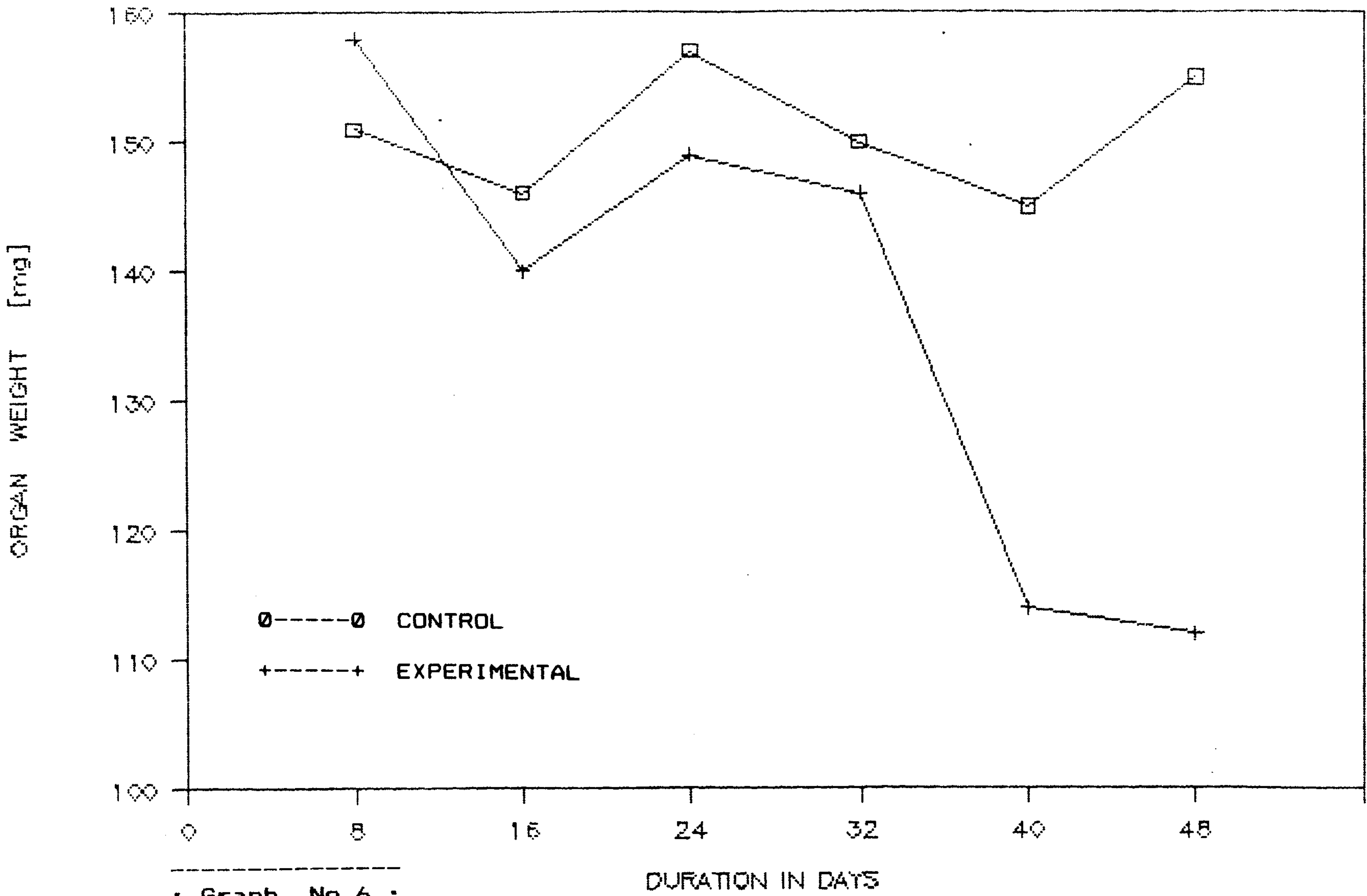
i) CAPUT EPIDIDYMIS :

The wet weight of caput epididymis of the control rat was 192 ± 3.0 mg on 8th day. It showed minor variations as 196 ± 4.3 mg, 190 ± 3.8 mg, 187 ± 4.7 mg, 189 ± 4.5 mg, 194 ± 4.2 mg on 24th, 32nd, 40th and 48th days respectively.

The wet weight of caput epididymis of the experimental rats showed decrease, the values were 190 ± 4.8 mg, 182 ± 3.4 mg and 173 ± 3.9 mg on 8th 16th and 24th days of treatment respectively. This depletion then continued throughout the remaining period of the treatment. Thus the values were decreased to 165 ± 4.7 mg, 148 ± 4.4 and 119 ± 3.8 mg after 32nd, 40th and 48th days of the treatment respectively.

ii) CAUDA EPIDIDYMIS

The wet weight of cauda epididymis of control rats was 151 ± 4.4 mg on 8th day. During the entire period of the experiment it showed minor variations. It decreased to 146 ± 4.1 mg on 16th day. It rose to 157 ± 4.2 mg on 24th day. It slightly decreased to 150 ± 3.9 and 145 ± 4.1 mg on 32nd and 40th days respectively. It again rose to 155 ± 4.3 mg on 48th day.



: Graph No.6 :

The wet weight of cauda epididymis was inreased initially to 158 ± 4.6 mg on 8th day of treatment. It decreased to 140 ± 5.3 mg on 16th day of treatment. It is elevated to 149 ± 4.7 mg on 24 th day of treatment. It depleted to 146 ± 4.2 mg, 114 ± 5.3 mg and 112 ± 4.8 mg on 32nd, 40th and 48th days of treatment.

II) HISTOLOGICAL ALTERATION IN EPIDIDYMIS :

1) CAPUT EPIDIDYMIS :

A> Control :

Caput epididymis consisted of epithelium (with two main cells principal cells and clear cells), basal lamina, luminar contents and interstitium (Plate No.3, Fig. No. 2).

i) EPITHELIUM : It consisted of two types of cells viz. principal and clear.

a)Principal cells - These were tall, columnar cells with apical microvilli called stereocilia. They showed apical vacuoles, spherical or ovoid nucleus in the basal region. Few cells were binucleated.

b) Clear Cells - These were low cuboidal cells present in between the principla cells. Nucleus was elongated and situated in basal region.

ii) LUMINAL CONTENT : Lumina of caput contained both spermatozoa and immature spermatids. The spermatozoa were more in number and sperm density was moderate.

iii) BASAL LAMINA AND INTERSTITIUM : Thin, eosinophilic

basal lamina surrounded the epididymal tubules. It contained connective tissue with smooth muscles and elastic fibres. interstitium was compact and few irregular cells were seen.

B> Experimental :

1. 8TH DAY OF TREATMENT :

Picrorhiza extract treatment at this phase did not show any signiicant alterations.

i) EPITHELIUM : Both principal and clear cells were normal in structure and height.

ii) LUMINAL CONTENT - Spermatozoa were observed normal in sperm density and its structure.

iii) BASAL LAMINA AND INTERSTITIUM - Basal lamina, like that of control, appeared thin eosinophilic, Interstitium was also with irregular cells.

2. 16TH DAY OF TREATMENT :

1) EPITHELUM : Insignificant decrease in height evident. Stereocillia length did not alter.

ii) LUMINAL CONTENTS : Lumina showed spermatozoa and few immature spermatids.

iii) BASAL LAMINA AND INTERSTITIUM : Slight thickening appeared in basal lamina and interstitium was widened.

3. 24TH DAY OF TREATMENT :

i) EPITHELIUM : It was normal except in few tubules, cytoplasm of some cells showed valuolization and slight

decrease in height.

ii) LUMINAL CONTENTS : Number of spermatozoa appeared to be decreased. Immature spermatids were more than observed in control.

iii) BASAL LAMINA AND INTERSTITIUM : Basal lamina become more thick and eosinophilic than that observed in the previous phase of treatment. Interstitium widened.

4. 32ND DAY OF TREATMENT :

i) EPITHELIUM : Reduction in the height of cells observed but stereocilia were normal in length. Cytoplasmic vacuoles were evident in few cells.

ii) LUMINAL CONTENT : Number of spermatozoa present in lumina was dwindled, increase in immatured spermatids seen. Giant cells also appeared in the lumina. All these formed a cellular debris in the lumina of some tubules (Plate No.3, Fig. No. 3).

iii) BASAL LAMINA AND INTERSTITIUM : Thickening of basal lamina and widening of interstitium seen.

5. 40TH DAY OF TREATMENT :

i) EPITHELIUM : Height of the cells seen to be decreased appreciably. Few cells from the epithelium have lost their cilia. Extrusion of nucleus was seen in few cells.

ii) LUMINAL CONTENT : The luminal spermatozoa were in the state of degeneration (Plate No.3,Fig. No.4). The cellular

debris seen contained few spermatocytes, spermatids and giant cells.

iii) BASAL LAMINA AND INTERSTITIUM : Basal lamina thickened and show intense eosinophilic. Interstitium widened and contained scattered, irregular cells.

6. 48TH DAY OF TREATMENT :

Various changes were seen after this phage of treatment (Plate No.3., Fig. No.5).

i) EPITHELIUM : The cells showed decrease in height in most of the tubules. Stereocilia appeared scanty. Nuclei of few cells appeared pycnotic. Nuclear extrusion from cells also observed occasionally. Cytoplasmic vacuolization also evident in the epithelium.

ii) LUMINAL CONTENTS : Agglutinated mass of spermatozoa and various germ cells desquamated from testis appeared in the lumina. This cellular debris reduced in volume. In many tubules spermatozoa were disappeared while no contents were found in some tubules.

iii) BASAL LAMINA AND INTERSTITIUM : Basal lamina seen thickened and highly eosinophilic and the interstitium was with irregular cells.

Thus Picrorhiza treatment induced many alterations in the caput epididymis.

2) **CAUDA EPIDIDYMIS :**

A> **CONTROL :**

Histological features of Cauda revealed epithelial cells of two types viz. principal cells and clear cells, luinal contents, basal lamina and interstitium (Plate No.3, Fig. No.6).

i) Epithelium : It consisted of two types of cells,

a) Principal cells - Thsese were cuboidal, pseudostratified cells. Nuclei were flattened or spherical, binucleation was more conspicuous that in caput cells. Stereocilia were short than those of caput.

b) Clear cells - In between principal cells, these clear cells were present. They were called as light or clear cells because they had less dense cytoplasm than that of the adjacent principal cells. Number of these cells were more than that in the caput.

ii) Luminal content - Number and density of sperms was maximum in the lumina of cauda. Sperms arranged in unidirection manner in the bundles in lumina. The spermatids were not found.

iii) Basal lamina and interstitium - Basal lamina was thinner than that observed in caput and was eosinophilic. Interstitium showed many irregular cells.

B> EXPERIMENTAL :

Cauda epididymis of Picrorhiza, extract treated rats showed changes as follows :

1. **8TH DAY OF TREATMENT :**

   i) Epithelium - The cells did not show any change.

   ii) Luminal contents - Sperms in the lumina were large in number and its density was maximum.

   iii) Basal lamina and interstitium - Basal lamina was thin and eosinophilic. Interstitium contained few irregular cells.

2. **16TH DAY OF TREATMENT :**

   i) Epithelium - Except appearance of vacuoles in cytoplasm the cells were normal.

   ii) Luminal contents - The number of sperms present in the lumina appeared to be decreased.

   iii) Basal lamina and interstitium - Basal lamina thickened and interstitium slowed no alterations.

3. **24TH DAY OF TREATMENT :**

   i) Epithelium - The cells showed same result as observed in the previous phase of treatment.

   ii) Luminal contents - Tubular lumina contained further reduction in the number of sperms. Occassionally immature germ cells appeared.

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iii) Basal lamina and interstitium - Basal lamina appeared thickened and the interstitium was normal.

4. **32ND DAY OF TREATMENT :**

i) Epithelium - The cells showed insignificant reduction in height. Vacuolization in cytoplasm of many cells seen Stereocilia were normal.

ii) Luminal contents - Progressive disappearance of sperms was seen in few tubules, while in many tubules germ cells, reveived from testis, found in the process of degeneration.

iii) Basal lamina and interstitium - Basal lamina thickened and showed eosibophilic. Interstitium was with irregular cells as well as certain vacuoled cells were also observed.

5. **40TH DAY OF TREATMENT :**

i) Epithelium - The cells exhibited alterations which were similar to that observed at 32nd day of treatment with further progression in it.

ii) Luminal content - Many tubules showed very meager amount of sperms. In some tubules lumina contained cellular debris.

iii) Basal lamina and interstitium - Basal lamina showed intense staining and was thickened.

Interstitium was not as compact as seen in the control.

6. **48TH DAY OF TREATMENT :**

Picrorhiza extract induced maximum alterations at this phase of treatment.

i) Epithelium - Height of cells appeared to be decreased appreciably. Some cells showed no stereocilia. Vacuoles were observed in few cells. Nuclear extrusion also occured occasionaly in few cells.

ii) Luminal content - Lumina of majority of tubules showed either low density of sperms or agglutinated mass of degenerating germ cells, indentification of which was not possible because of severe damage, with fragments of sperms (Plate No.3, Fig. No.7). Some tubules were virtually empty.

iii) Basal lamina and interstitium - At certain instances basal lamina appeared to be detached from the tubules. It was thickened. It was irregular and wavy in tubules with empty lumina. Interstitium was sparse with many irregular cells.

Thus Picrorhiza extract caused alterations in caput and cauda and they were decrease in height of cells,

vacuolization in cells, low density of sperms or emptying of lumina, thickening of basal lamina and sparse interstitium.

3.4.3 DISCUSSION :

In the present discussion Picrorhiza induced changes in the wet weights and histological structure of epididymis of albino rats are discussed. We have more or less complete information about the extract induced alterations in epididymis. These changes are proposed to be discussed at a comparative level with the available iiterature on the epididymal changes induced by the other plant extract and synthetic antispermatogenic agents and to arrive at definite conclusions.

The wet weight of both caput and cauda epididymis are decreased after administration of Picrorhiza extract. Such depletion is in accordance with the observatons made by the administration of estrogen compounds (Chang, 1942, Bacon and Kirman, 1955, Kar et al;, 1965; Kalra et al;, 1978), alpha-chlorohydrin (Peyre and Laporte,1966,Gunn et al;, 1969 and 1970, Ericsson and Baker, 1970; Hoffer et al;, 1973; Ford et al;, 1977, Dixit, 1977) endosulfan (Ansari and Gupta, 1981), Formaldehyde (Shah et al;, 1987), Flutamide, EDP, STS-557 (Gupta et al;,1969), cyproterone acetate (Gupta et al;, 1989, Bhiwgade, 1990). Similar results are also reported with the administration of plant preparations

Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Dixit, 1977, Verma et al;, 1980, Bhargava 1988), Colotropis procera (Garg, 1979), Papayseeds (Das, 1980). Allium sativum (Dixit and Joshi, 1982), Vinca-rosea (Toro, 1984), Daucus carota (Shah, 1985), Butea-mnonosperma (Awati, 1985), Vitex negundo (Sohani, 1985), Plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988; Adhikary et al;, 1989), Gossypol (Bhiwgade and Nair, 1989; Nair and Bhiwgade, 1989), Andrographis paniculata (Akbarsha et al;, 1990), Syzygium cumini (Ambaldhage, 1990).

Decrease in weight of epididymis is an indication of antiandrogenic action of the extract (Prasad et al;, 1973; Rajlakshmi and Prasad, 1975, Umapathy and Rai, 1980, Baijal and Mathur, 1981; Baijal et al;, 1981). Kutki-sterol is a phytosteroid present in the Picrorhiza extract, which seems to be antiandrogenic in nature causing reduction of the weight of epididymis.

Decrease in weight of epididymis may also be due to less number of normal spermatozoa entering the lumina of epididymis because of the interference of Picrirhiza extract in the spermatogenesis as described earlier under discussion on testis.

Picrorhiza extract caused many histological alteration after its administration in epididymal

histoarchitecture into male albino rats. These are as follows -

The histoalterations seen after Picrorhiza administration are more significant as compared those found found in the initial phases of the treatment. In caput as well as in cauda, the epithelial height appeared to the decreased appreciably. The epithelium shows cytoplasmic vacuolization. Occasionally nuclear extrusion from cells also observed. Stereocilia are scanty. Lumina in initial stages of treatment showed reduced number of spermatozoa with many immatured and desquamated spermatogenic cells. Towards the end of the treatment the lumina contained cellular debris of degenerating spermatogenic cells and spermatozoa, cellular contents of the debris could not be identified because these are derived mainly from damaged testes. Lumina of severely damaged tubules contain no sperms. In caput, basal lamina seen thickened and highly eosinophilic and interstitium was with irregular cells, while in cauda, basal lamina thickened and appeared to be detached from the tubules. It was irregular and wavy in tubules with empty lumina. Interstitium was sparse with irregular cells.

In present investigation it is observed that epithelial cells show though not significant appreciable decrease in height. This observation is similar to that

observed by Jehan _et al;_, (1973) with castrated animals, Dixit and Lohiya (1975), with alpha-chlorohydrin, Kumar _et al;_, (1976) with estradiol, Dixit (1979) after administrtion of Methallibure, niridazole and danzole, Kaur and Mangat (1979) with chloramadinone acetate treatment, Prakash _et al;_, (1979) with cyproterone acetate, Shah _et al;_, (1987) with Formoldehyde, Vachhrajani _et al;_, (1988) after administration of Methyl mercury chloride and Malini and Vanithakumari (1988) with beta-Sitosterol treatment. Many plant preparations are also shown to decrease height of epithelial cells, these are _Malvaviscus conzanttii_ (Dixit, 1977b, Dixit and Bhargava, 1978, Verma _et al;_, 1980), _Aristolochia indica_ (Pakrashi and Pakrashi, 1979), _Calotropis procera_ (Garg, 1979), Papaya seeds (Das, 1980), _Allium sativum_ powder (Dixit and Joshi, 1982), _Plumbago zeylenica_ (Bhargava, 1984), _Vitex negundo_ (Sohani, 1985), _Daucus carota_ (Shah, 1985), plumbagin (Jadhav, 1988), _Andrographis paniculata_ (Akbarsha _et al;_, 1990), i.e. derease in oligospermia, number of spermatozoa is seen in epididymal lumina in _Picrorhiza_ treated rat.This observation finds a good parallel in the work with administration of WIN-18446 (Coulson _et al;_, 1960"), alpha-chlorohydin (Crabo, 1965, Gunn _et al;_, 1969, 1970; Ericsson, 1969, 1970, Nag _et al;_, 1976, Dixit, 1979), Cyporterone acetate, (Morkewitz)

et al;, 1969, Prasad *et al*;,1970, Prasad, 1973, Bose *et al*;, 1975; Flickinger and Loving, 1976; Rastogi *et al*;, 1979;, Nag *et al*;, 1976), estrogens (Lacy, 1967), *Clomiphene citrate* (Kalra and Prasad, 1967; Rajlakshmi *et al*;, 1970), Methallibure (Kar *et al*;, 1968a), 5-dihydrotestosterone (Beaulieu *et al*;, 1968, Blaquier, 1973), S.K.F. 7690 (Lubicz-Nawrocki and Glover, 1973), $CdCl_2$ (Dixit, 1976; Chinoy and Sheth, 1977), prostaglandins (Tso and Lacy 1975, 1978), progestin and androgen (Flickinger, 1977) and Flutamide (Dhar and Shetty, 1987). Many plant preparations are also reported to induce such oligospermia in the epididymis. These are *Aristolochia indica* (Pakrashi and Pakrashi, 1979), *Malvaviscus conzanttii* (Dixit, 1977b, Dixit and Bhargave, 1978, Verma *et al*;, 1980), *Calotropis procera* (Garg, 1979), Papaya seeds (Das, 1980), Gossypol (Coulson *et al*;, 1980), *Allium-sativum* powder (Dixit and Joshi, 1982), *Vinca-rosea* (Toro, 1984), *Plumbago zeylenica* (Bhargava, 1984), *Butea monosperma* (Awati, 1985), *Vitex negundo* (Sohani, 1985), *Dacus carota* (Shah, 1985), *Oscimum sanctum* (Khanna *et al*;, 1986), Gossyupol and *Tripterygium wilfordii* (xu *et al*;, 1987) and *Solanum xanthocarpum* (Rao, 1988b).

Another important feature of *Picrorhiza* extract treatment is the presence of cellular debris in epididymal lumina of albina rats. This observation is similar to that

observed with synthetic chemicals by Flickinger and Loving (1976) with cyproterone acetate, Tyagi et al;, (1979) with cyclohexanol, Ratnasooriya et al;, (1980) with Methoxamine, similar observations are also made with the administration of various plant preparations, these are Malvaviscum conzattii (Verma, et al;, 1980), Vinca-rosea alkaloids (Toro, 1984), Daucus carota (Shah, 1985), Butea monosperma (Awati, 1985), Vitex-negndo (Sohani, 1985), Plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988).

In the present investigation, basal lamina is appeared to be thickened and especially in cauda it is seen to detached from tubules. Intersctitium was sparse with irregular cells. These observations were also reported by Toro (1984) with Vinca rosea alkaloids, Awati (1985) with Butea monosperma, Sohani (1985) with Vitex negundo and Shah (1985) with Daucus carota.

Thus in the present investigation, the results indicate that in rat Picrorhiza extract cause the germinal cells in testes to develop upto primary spermatocytes which than start exfoliating from their place of formation and make their appearance in lumina of seminiferous tubules from where they pass to lumina of epididymis. In the later phase of treatment lumina were with few or no spermatozoa and epithelial cells, basal lamina, interstitium all showed degenerative or regressive changes. Such regressive changes

in epididymis reflects the antiandrogenic nature of the plant extract. Epididymis being androgen dependent, regressive changes after Picrorhiza treatment provide indirect evidence for antiandrogenic nature of that extract, because such changes result due to androgen deprivation (Oscar *et al*;, 1980; Tse and Wong, 1980). Picrorhiza contain a phytosteroid Kutki-sterol which seems to have antiandrogenic properties which resulted in the alteration of normal strucure and function of epididymis.

3.5 SEMINAL VESICLE :

3.5.1 REVIEW OF LITERATURE :

Seminal Vesicle, secretion of which contribute large part of semen, have become the subject of many histological, cytological histochemical, and bichemical investigations. Various chemicals, antifertility agents and plant preparations have been tried to explore their effects, on seminal vesicles. Following is the brief review of the available literature.

A) CHEMICALS :

Reduction in height of the epithelial cells of Seminal Vesicles after castration is reported in various mammals (Cavazos and Melampy, 1954, Szirmai and Van der Linde, 1962, Allison, 1964). Hypophysectomy resulted in considerable reduction in weights of seminal vesicles

(Elkington et al;, 1972). Hypoxia caused loss of weight and changes in the Secretory epithelium of Seminal vesicle (Riar and Malhotra, 1977). Placement of epididymis of orchidectomized rats in the abdomen resulted in the reduction in the weights of seminal Vesicle after two weeks (Das and Roy, 1977).

Bovine anti-ICSH serum in rats decreased the weight of semnal vesicle but antiserum of FSH has no effect on the weight (Gambal, 1967). Administration of S.K. and F. 7680 (Sauuders et al;, 1969); estrogen (Elkington and Blackshaw, 1971), Chlorocyclizin (Wong et al;, 1972), norgesterol (Singh et al;, 1972). Alpha-chlorohydrin (Vickery et al;, 1974), Hundal and Mangat, 1978), Cyprogerone acetate (Dahi and Tveter, 1974), Agmo 1974, Bose et al;, 1977), Medroxy progesterone (Flickinger 1977), Centchroman (Das et al;, 1977), $Cdcl_2$ (Dixit, 1976), Cyclohexanol (Dixit et al;, 1979), chloro-madianone acetate (Kaur and Mangat, 1979), aspirin (Balasabramanian et al;, 1980), Formaldehyde (Shah et al;, 1987), Flutamide (Dhar and Setty 1987), Ovine prolactine (Mangayarakkarasi et al;, 1988), S T S - 557, estradiol dipropionate (Gupta et al;, 1989),Lithium chloride (Ghosh et al;, 1990), in various animals caused reduction of wet weights of seminal vesicles lowering of secreatary activity, reduction in height of epithelium and or marked regression in the seminal vesicle.

B> **PLANT PREPARATIONS :**

Very few plants with antispermatogenic potencies have been explored for their effects on seminal vesicle. Administration of Oscimum sanctum extract caused change in the PH of seminal plasma of mice (Kasinathan et al;, 1972).

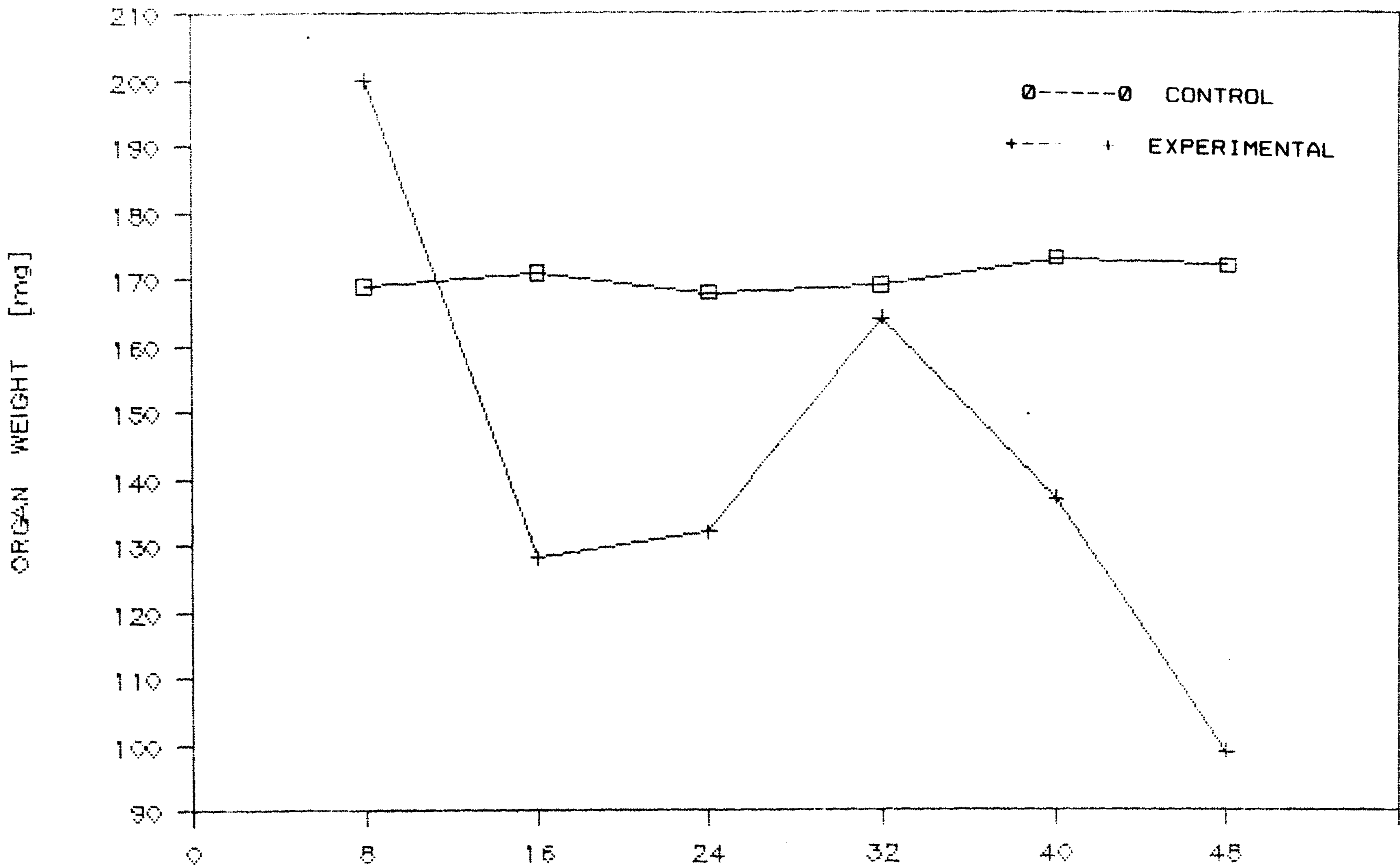
Oral administration of Malvaviscus conzanttii extract decreased the absolute weights of seminal vesides in house rats and gerbils (Dixit, 1977b). Mice fed with Aristolochia indica showed reduction in the size and weights of their seminal vesicles. Lumina were without secretion with no histological alterations (Pakrashi and Pakrashi, 1977). Garg (1979) reported reduction in weights of seminal vesicles of gerbiils fed with extract of Calotropis procera. Papaya seeds caused infertility without reduction in weight of seminal vesicle (Das, 1980). Malvaviscus canzanttii extract decreased relative weight and size and seminal vesicles and inhibition in the arborization of the secretary epithelium with reduction in cell height, lumen was empty (Verma et al;, 1980). Allium sativum powder decreased weight of seminal vesicles in rat Significantly (Dixit and Joshi, 1982). Toro (1984) observed reduction in epithelial height and arborization of mucosal folds of seminal vesicles but increase in the weight of seminal vesicle with Vinca rosea alkaloid in albino rats . Sohani (1985) with Vitex negundo

extract, Awati (1985) with Butea monosperma extract reprted no significant change in epithelium but reduction in weight of seminal vesicles in white rats. Administration of Daucus carota seed extract caused reduction in epithelial height as well as wet weight of seminal vesicle in male albino rats (Shah, 1985). Oral administration of Oscimum sanctum to white rats resulted into significant decrease in weight of seminal vesicle in rats after administration of oleanolic acid, a compound extracted from Eugenia jambolana. Hiremath (1988) administered extract fo Piper betle petiole to white rat and found decrease in weight of seminal vesicle. Vinca rosea affect general histological structure of seminal vesicle in albino rats (Chinoy et al;, 1988a, 1988b). Plumbagin administraton to albino rats reduced height and arborization of mucosal folds of seminal vesicles (Jadhav, 1988). Akbarsha et al;, 1990) have reprted decrease in weight of seminal vesicle with many degenerative changes in its histoarchitecture. Ambaldhage (1990) with Syzygium cumini extract reprted decrease in weight and histological alterations in seminal vesicles of rat.

3.5.2 OBSERVATIONS :

I)ALTERATION IN THE WET WEIGHTS OF SEMINAL VESICLE :

The alterations in the wet weights of seminal vesicle during the period of the extract treatment are recorded in Table No. 6 and illustrated in Graph No. 7.



: Graph No.7 :

TABLE NO. 6

Seminal Visicle : Picrorhiza kurroa extract induced changes in wet weights.

Duration in days	Control weight of seminal vesicle(mg)	Experimental weight of seminal vesicle(mg)
8	169 ± 3.6	200 ± 4.9
16	171 ± 4.2	128 ± 3.8
24	168 ± 3.7	132 ± 3.8
32	169 ± 3.5	164 ± 4.1
40	173 ± 4.0	137 ± 3.6
48	172 ± 3.8	99 ± 3.7

(values are mean ± S.D. of five animals and expressed in mg per 100 gm. of body weight).

As seen from the tabular and graphical illustratons, the wet weight of seminal vesicle remained fairly constant in control rot swhich received only Vehicle. But in the rats receiving Picrorhia kurroa extract, the wet a weights of seminal vesicle decreased as a function of duration of the treatment.

The wet weight of seminal vesicle of control rat was 169 ± 3.6 mg on 8th day. It varied to 171 ± 4.2 mg, 168 ± 3.7 mg, 169 ± 3.5 mg, 173 ± 4.0 mg and 172 ± 3.8 mg on 16th, 24th 32nd, 40th and 48th days of treatment respectively.

PLATE NO.4 : CAPTIONS TO FIGURES

Fig.1 : Seminal Vesicle (Control): Haematoxylene-Eosin staining. Mucosa (M) shows folds (Arrows). Lamina propira (L P), Muscular coat (MC) normal. Lumen with full secretion(S), X 60.

Fig.2 : Seminal vesicle (32 days treatment): Haematoxylene-Eosin staining. Note reduction in secretion (S) keeping part of lumen (L) empty. Lamina propria (LP) normal.X100.

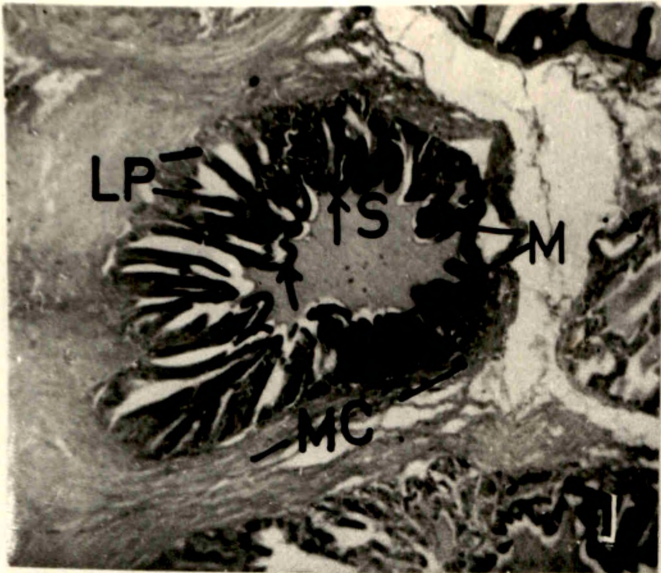
Fig.3 : Seminal Vesicle (48 days treatment): Haematoxylene-Eosin staining. Mucosal (M) folds reduced. Lumen (L) is without secretion(S). Arrows indicate detachment of epithelium from muscular coat, X100.

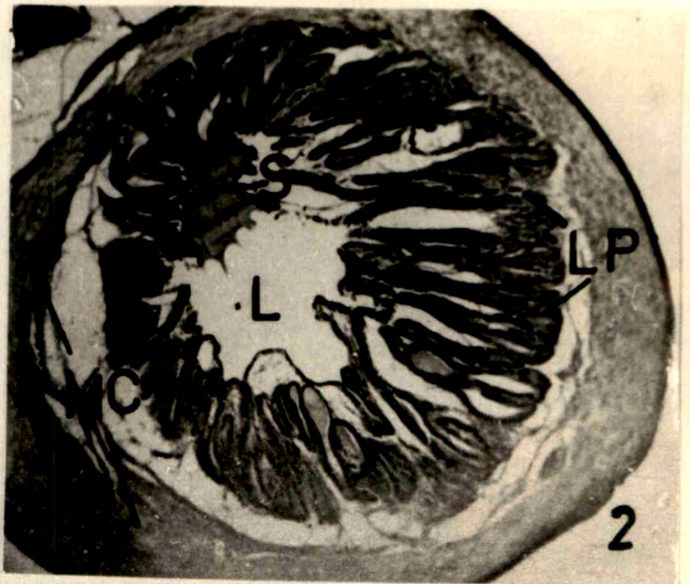
Fig.4 : Prostate gland: (Control):Haematoxylene-Eosin staining: Note acinar epithelium (AE), interacinar tissue (I) normal. Lumina with full secretion (S), X 60.

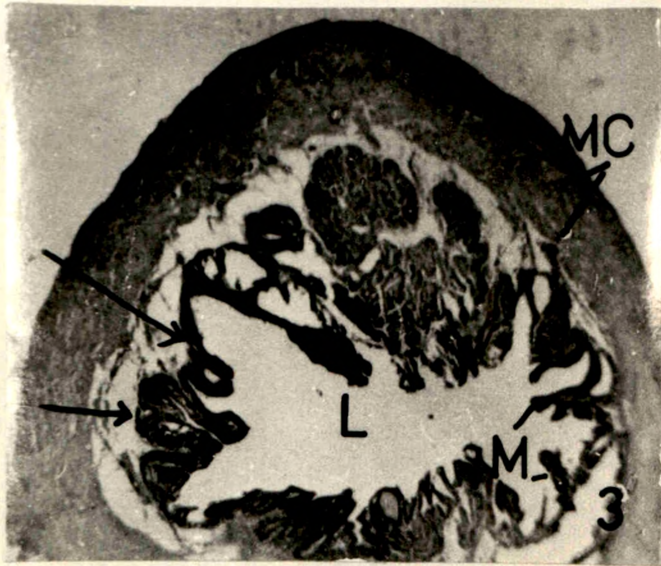
Fig.5 : Prostate gland :(40 days treatment): Haematoxylene-Eosin staining. Epithelium (EP) reduced. Lumina (L) contain reduced amount of secretion (S). Interacinar tissue (I) widened, X 150.

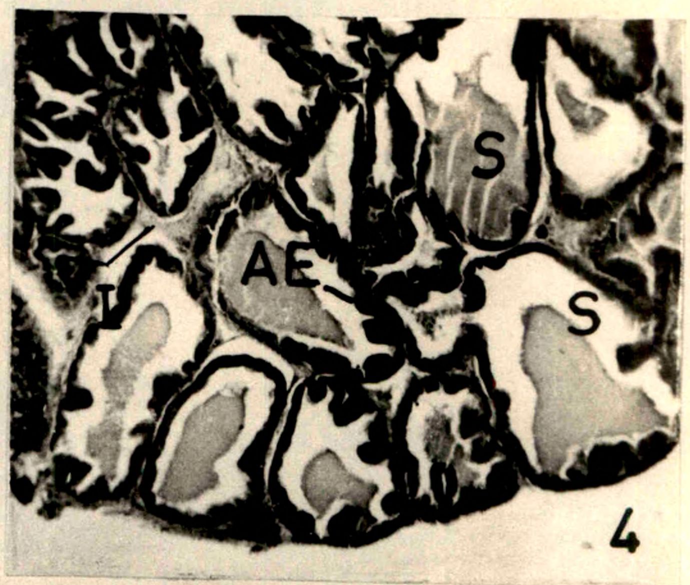
Fig.6 : Prostate gland (48 days treatment):Haematoxylene-Eosin Staining. Note majority of acini show empty lumina (EL). Very few lumina contain little amount of secretion (S). Interstitium (I) widened, X 150

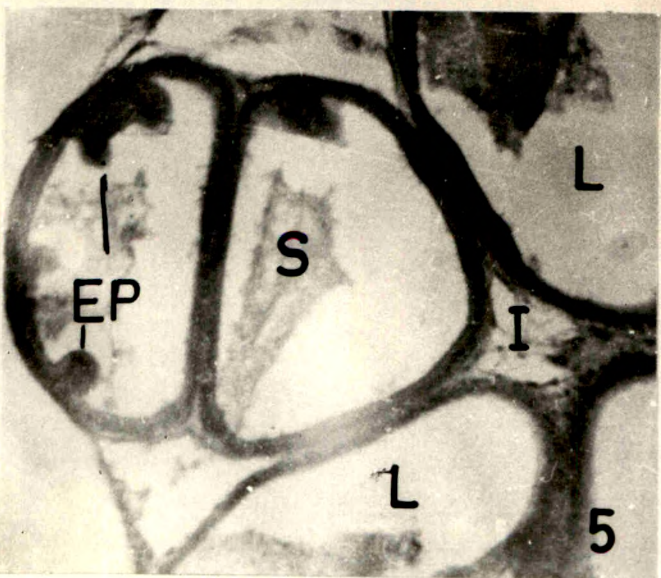
PLATE NO.4

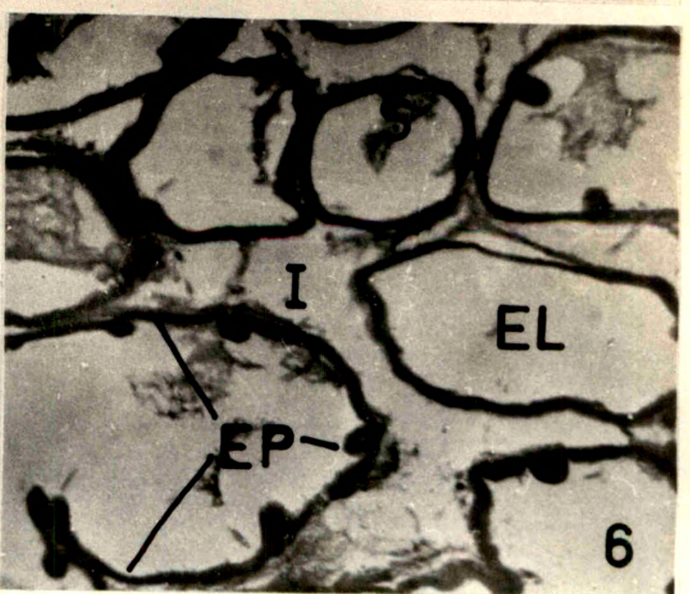












The wet weight of seminal vesicles of experimental rat rose to 200 ± 4.9 mg on 8th day of treatment. But after initial increase the values remained below the of controls. Thus it decreased to 128 ± 3.8 mg on 16th day of treatment. It increase to 132 ± 3.8 mg and 164 ± 4.1 mg on 24th & 32nd day of treatment respectively. It declined to 137 ± 3.6 mg on 40th day of treatment. At the termination of the treatment i.e. on 48th day of treatment it further depleted to 99 ± 3.7 mg.

II) HISTOLOGICAL ALTERATIONS IN SEMINAL VESICLE :

A) CONTROL :

The histological structure of seminal vesicle consisted of mucosa, lamina propria, muscle coat and secretion (Plate No.4, Fig. No. 1).

i) Mucosa : There were two types of epithelial cells viz large columnar secretory cells and smaller basal cells Entire mucosa was thrown into extensive folds into lumen, this was called arborization. Epithelium was pseudostratified usually. Columnar cells had elongated nuclei and basal cells had spherical nuclei. Both the cells have cytoplasm with secretion granules.

ii) Luminal secretion :

Its wall full of secretory material. The secretion of viscoid in nature and found to retained in the depths of crypts.

iii) Lamina propria :

It formed a continous layere around vesicle and also piecred the folds. It consisted of elastic fibres.

iv) Muscular coat :

Lamina propria was encircled by a muscular coat. It consisted of inner circular and outer longitudinal layers.

B> EXPERIMENTAL :

Very few alterations were observed in the microscopic structure of seminal vesicle in the treated animals at all the intervals of the Picrorhiza extracts treatment. As no consistent progression of changes was detected throughout the treatment, the changes in individual interval are not described and illustrated separately. The entire period of the extract treatment is divided into two phases, the first phase beign upto 24th day of treatment and the second beign from 32nd to 48th day treatment.

1> ALTERATION UPTO 24TH DAY OF TREATMENT :

i) Mucosa :

Insignificant change was seen in the folded appearance of epithelium. Apparent change in the height and width of the columnar and basal cells was evident.

ii) Luminal secretion :

There was secretory material in the lumina but it showed reduction in its amount as compared to control. Like

control, during this phase of treatment, the secretion was retained in depths of crypts (Plate No. 4, Fig. No. 2).

iii) Lamina propria :

Lamina propria did not show any significant change.

iv) Muscular coat :

The layers of muscles showed slight reduction.

2. **ALTERATONS FROM 32ND TO 48TH DAY TREATMENT :**

The changes in the histological structure of the seminal vesicles in this phase of treatment are shown photo micrographically in plate No. 4, Fig No. 3.

i) Mucosa :

The mucosal folds and height of cells reduced considerably. There appeared significant alteration in the arborizationn of epithelium. At certain phases, mucosa appeared to be degenerated.

ii) Luminal contents :

Because of the reduction in the height and arborization of the epithelial folds in this phase of treatment, the Lumen of the seminal vesicle increased as a secondary effect. Lumina of majority of the vesicles showed no or very little amount of secretion, although in few vesicular lamina fair amount of secretion was evident.

iii) Lamina propria :

It showed apparent reduction in majority of vesicles.

iv) Muscular coat :

In most of the vesicles the inner layer of circular muscles detached from the epithelium. In general muscular coat was reduced in thickness.

3.5.3 DISCUSSION :

In the present discussion Picrorhiza kurroa extract induced changes in the wet weights and histological structure of seminal vesicles of albino rats are discussed. Now we have more or less complete information about the extract induced alteratons in seminal vesicles. These chages are proposed to be discussed at a comparative level with the available literature on the seminal vesicular changes induced by the other plant extracts and synthetic antispermatogenic agents and to arrive at definite conclusions.

The wet weight of seminal vesicle is decreased after administration of Picrorhiza extact. Such depletion is in accordance with the observations made by the administration of S.K. and F 7690 (saunders et al;, 1969, estrogens (Elkington and Blackshaw, 1971), norgesteron (Singh et al;, 1972), alpha-chlorohydrin (Vickery et al;, 1974, Hundal and Mangat, 1978), Centroman (Das, 1977), Medroxyprogesterone

(Flickinger, 1977), Cyproterone acetate (Prasad et al;, 1977), $CdCl_2$ (Sakensen, 1977), clomiphene citrate (Kaur and Mangat, 1979), cyclohexanol (Tyagi et al; 1979, Dixit et al;, 1979), endosulfan (Ansari and Gupta, 1981), Formaldehyde (Shah et al;, 1987) Flutamide (Dhar and Shetty, 1987), acetyl mercury chloride (Rao, 1988), STS-557 Flutamide and cyproterone acetate (Gupta et al;, 1989), Lithium chloride (Ghosh et al;, 1990a, 1990b), similar decrease in weight of seminal vesicle is also reported with the administration of plant preparations Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Dixit, 1977b), Calotropis procera (Garg, 1979), Allium sativum (Dixit and Joshi, 1982), Vitex negundo (Sohani, 1985), Dausus varota (Shah, 1985), Butea monosperma (Awati, 1985), Oscimum sanctum (Khanna et al;, 1986), Gossypol and Tripterygium wilfordii (Xu et al;, 1987), plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988; Adhakary et al;, 1989), Andrographis paniculata (Akbarsha et al;, 1990), Syzygium cumini (Ambaldhage, 1990).

Picrorhiza kurroa extract induced certain changes in histoarchitecture of seminal vesicles of male albino rats. The mucosal epithelial cells showed reduction in height. The mucosal folds which are highly arborized and reach upto the centre of lumen of seminal vesicle in the control ,get reduced.At certain places these folds reduced drastically to

form a mere stub like structures. Lumen is appeared to be increased, but it is as secondary effect as there occur reducion of mucosal folds and its arborization. Lumina of majority of vesicles are devoid of secretion, though few vesicles contain less amoount of secretion. Muscular coat is reduced in theckness. Its inner layer of circular muscles is detached from the epithelium.

Picrorhiza extract reduced mucosal folds and its high arborization in the lumina of seminal vesicle. Such parallel observations are reported earlier with chlorocylizine (Wong et al;, 1972), aspirin (Balsabramanian et al;, 1980), methyl mercury chloride (Vaccharajani et al;, 1988). Some plant preparations are also showed similar observation. These are Malvaviscus conzanttii (Verma et al;, 1980), Vinca rosea alkaloids (Toro, 1984), Vitex negundo (sohani, 1985), Butea monosperma (Awati , 1985), Daucus carota (Shah, 1985), Andrographis paniculata (Akbarsh et al;, 1990) and Syzygium cumini (Ambaldhage, 1990).

In this present investigation it is found that mucosal epithelium showed occasionaly degeneration. This observation finds parallel in the observations made by Wong et al;, (1972) with chlorocyclizine; Balsubramanian et al;, (1980) with aspirin, Sohani (1985) with Vitex negundo leaves extract. But in addition to this, they also observed vacuolization in the epithelial cells, such vacuoles are not

found in the mucosal epithelium of seminal vesicles of Picrorhiza extract treated male albino rats.

Lumina of seminal vesicles are with very little secretion and majority of tubular lumina were without any secretion. These observatons are well in accordance with the treatment of S.K.F. 7690 (Saunders et al;, 1969), Cyproterone acetate (Agmo, 1975), $CdCl_2$ (Chinoy and Sheth, 1977), alpha - chlorohydrin (Hundal and Mangat, 1978), chloromadionine acetate (kaur and Mangat, 1979), and plant preparations such as Aristolochia indica (Pakrashi and Pakrashi, 1977), Malvaviscus conzanttii (Verma et al;, 1980), Vinca rosea (Toro, 1984), Vitex negundo (Sohani, 1985), Butea monosperma (Awati, 1985), Daucus carota (Shah, 1985), plumbagin (Jadhav, 1988), Piper betle (Hiremath, 1988) , Androgeraphis paniculata (Akbarsha et al;, 1990), Syzygium lumini (Ambaldhage, 1990).

Thus in the present investigation it is observed that weight of seminal vesicles decreased and many histological alterations are also noticed after administration of Picrorhiza extract into the male albino rats. These observed changes may be due to the depletion in androgen level. Weight of accessory reproductive organs is a good indicator of circulating androgen (Moore, et al;, 1930), and for the normal functioning of seminal vesicles,

like other accessory sex organs, androgen is necessary (Bedwal and Mathur, 1980). *Picrorhiza* contain a phytosteroid Kutki-sterol. Phytosteroids are reported to induce antiandrogenic effects ,by decreasing the level of androgens and affecting all the accessory organs (Garg, 1979; Bhargava, 1984; Adhikary *et al*;, 1989; Sinha and Mathur, 1990). It seems that the antiandrogenic substance in *Picrorhiza* decreases the testosterone level (as also indicated earlier by atrophied Leydigs cells) and causes decrease in weights and partial regressive change in the seminal vesicles.

Decrease in weight, partial regressive chages in seminal vesicles and absence of secretion in the lumina of most of the tubules, clearly indicate that the *Picrorhiza* extract induce a functional hypotrophy in the treated albino rats.

3.6 PROSTATE GLAND :

3.6.1 REVIEW OF LITERATURE :

Many synthetic chemicals and plant preparations have been tried to explore their effects on prostate gland. Following is a brief review of the available literature.

A> CHEMICALS :

Castration resulted cellular regression and loss of secretion in prostate of rats (Moore *et al*;, 1930). Prostate (Kar *et al*;, 1968), Busulphan affect weight of S.K. & F.

7690 Significantly reduced the weight and functional capacity of prostate in rat (saunders et al;, 1969). Various steriods found to decrease the weight of prostate gland in rats (Singh et al;, 1971). Administration of cyproterone acetate in rats reduced activity of prostate complex (Rajlakshmi, 1972). Wong et al;, 1972, administratered chlorocyclizine to rats and found decrease in weight of prostate, atrophy of the epithelium and reduction in the amount of secretion in the lumina. Centchroman treatment decreased the weight of prostate in rats (Das et al;, 1977,a,b). Similar results were found in rats with $Cdcl_2$ treatment (Chinoy & Sheth, 1977). Chloromadinone acetate treatment reduced weight of prostate in rats (Kaur and Mangat. 1979).

Weight of prostate gland decreased after removal of epididymal fat in albino rats (Shrinivasan et al;, 1986). Administraton of Flutamide to prepubertal and adult rat, found to decrease weight of ventral prosate in immature rats but not in adult rats (Dhar and Shety, 1987). Lithium chloride treatment significantly decrease weight of prostate in albino rats (Ghosh et al;, 1990).

B> PLANT PREPARATIONS :

Malvaviscus canzanttii flower extract resulted decrease in weight of prostate in rats and gerbils (Dixit,

1977b). Pakrashi and Pakrashi (1977) reported decrease in weight of prostate and its secretion in mice, with no histological change with Aristolochia indica. Chauhan et al;, (1979) adminstered Vinca rosea extract to rat and found reducton in weight of prostate.Toro (1984) reported increase in weight and reduction in cell height and secretion of prostate in albino rats treated with Vinca rosea alkaloids. Butea monosperma extract resulted increase in weight of prostate, and its secretion in albino rats (Awati, 1985). Sonani (1985) reported reduction in weight, epithelial cell height and secretion of prostate gland in albino rats treated with Vitex negundo extract.

Administration of Daucus carota seed exract resulted decrease in weight of prostate and its secretion without any change in epithelial cells (Shah 1985). Oscimum sanctum extract reduced weight of ventral prostate significantly in albino rats (Khanna et al;, 1986). Chinoy et al;, (1988) reported many changes in ventral prostate after administration of Vinca rosea leaf extract to albino rats. Piper betle petiole extract when administered to albino rats caused decrease in weight of prostate (Hiremath 1988). Plumbagin treatment resulted decrease in weight of prostate, its secretion and many changes in epithelium (Jadhav 1988). Akbarsh et al;, (1990) reprted decrease in weight and many

regressive chages in prostate with the administration of Andrographis paniculata in male albino rats, Ambaldhage (1990) reprted decrease in weight and secretion in prostate after administration of Syzygium cumini.

3.6.2. OBSERVATIONS :

I) ALTERATIONS IN THE WET WEIGHT OF PROSTATE GLAND :

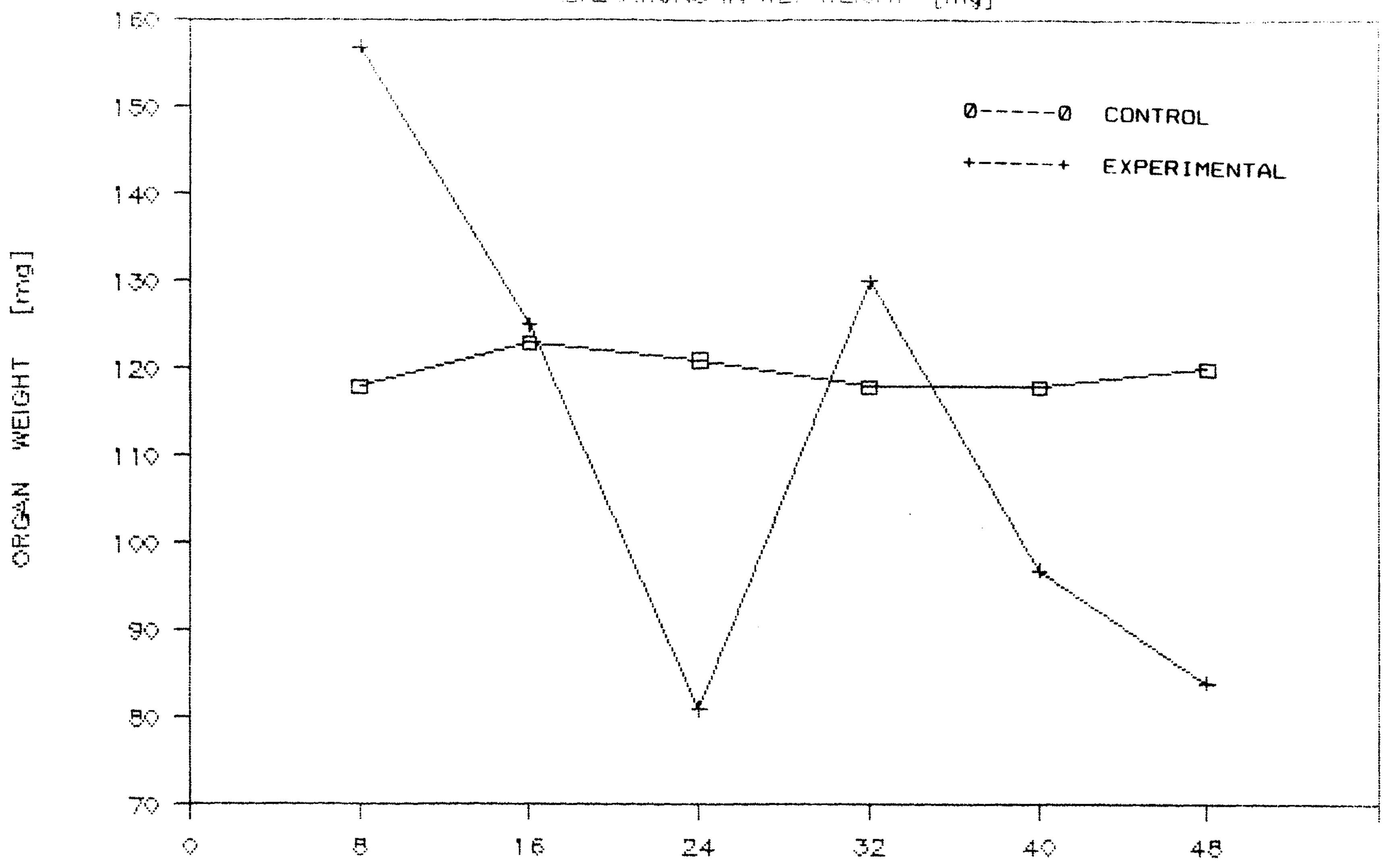
The alterations in the wet weight of prostate gland during the period of extract treatment are recorded in Table No. 7 and illustrated in Graph No. 8.

TABLE NO. 7

Prostate gland : Picrorhiza kurroa extract induced changes in wet weight.

Duration in days	Control weight of prostate(mg)	Experimental weight of prostate(mg)
8	118 ± 4.1	157 ± 5.1
16	123 ± 3.6	125 ± 4.7
24	121 ± 5.0	81 ± 2.9
32	118 ± 4.6	130 ± 4.8
40	118 ± 4.4	97 ± 3.6
48	120 ± 3.8	84 ± 3.1

(values are mean ± S.D. of five animals and expressed in mg per 100 gm. of body weight).



: Graph No.8 :

As seen from the tabular and graphical illustrations, the wet weight of prostate gland remained fairly constant, in control rats which received only vehicle. But in the rats receiving Picrorhiza kurroa extract, the wet weights prostate glands depleted as a function of duration of the treatment.

The wet weight of prostate gland of the control rat was 118 ± 4.1 mg on 8th day. It increased to 123 ± 3.6 mg and 121 ± 5.0 mg on 16th and 24th day respectively. It was 118 ± 4.6 mg on 32nd day. It remained constant on 40th day, while on 48th day it was 120 ± 3.8 mg.

The wet weight of prostate gland of experimental rat elevated initially to 157 ± 5.1 mg on the 8th day of the treatment. It gradually depleted to 125 ± 4.7 mg and 81 ± 2.9 mg on 16th day and 24 the day of treatment. On 32nd day of treatment it again increased to 130 ± 4.8 mg. It decreased to 97 ± 3.6 mg and 84 ± 3.1 mg on 40th and 48th days of treatment.

II) HISTOLOGICAL ALTERATIONS IN PROSTATE GLAND :

A> CONTROL :

The histological structure of prostate of control showed acini, secretion and interacinar tissue, which is photomicrographically represented in Plate No.4, Fig. No.4.

i) ACINI : There were number of acini which were lined by simple cuboidal or tall columnar type of epithelial cells.

The acini showed great variations. Certain cells showed bleb like protrusions. The epithelium and adjacent stroma form folds occasionally, which project into the lumina of the acini. The cytoplasm of epithelial cells contain secretory granules.

ii) SECRETION :

Lumina of acini were full of secretion. The secretion was viscid in nature.

iii) INTERACINER TISSUE :

The gland was surrounded by fibroelastic tissue containing many smooth muscle fibres from which broad septa penetrated into the interior forming abundant stroma. Acini were separated by this stroma.

B) EXPERIMENTAL :

The alterations were very few, in the prostate of <u>Picrorhiza</u> treated rats at all intervals of the treatment, since no consistent progression of changes was detected throughout the period of the treatment, the changes in the individual intervals are not described and illustrated separately. The entire period of the treatment, as stated previously is divided into two phases, the first phase being upto 24th day of the treatment and the second being from 32nd to 48th day of the treatment.

1. ALTERATIONS UPTO 24TH DAY OF TREATMENT :

i) ACINI :

There was apparent change in the epithelial cells with respect to height, width and secretory activity.

ii) SECRETION :

The density of secretion was decreased. Many acini still found to be full of secretion.

iii) INTERACINAR TISSUE :

It remained unaltered.

2. ALTERATIONS FROM 32ND TO 48TH DAY OF TREATMENT :

The changes in the histological structure of the prostate in this phase of the treatment are shown photomicrograhically in Plate No.4 , Fig. No. 5.

i) ACINI : The height and width of epithelial cells was appeared to be reduced. No other significant change was detected.

ii) SECRETION : Most of the acinar lamina were without any secretions. While in some tubules it was reduced (Plate No.4, Fig. No. 6). Few tubules were with regular amount of secretion but it appeared to be opague.

iii) INTERACINAR TISSUE : Interacinar tissue was appeared to be thickened and widened.

3.6.3 DISCUSSION :

In the present discussion *Picrorhiza kurroa* extract iduced changes in wet weights and histological structure of Prostate gland of albino rats are discussed. Now we have more or less complete information about the extract induced alterations in prostate glands. These changes are proposed to be discussed at a comparative level with the available literature on the prostatic chnges induced by the other plant preparatons and synthetic antispermatogenic agents and to arrive at definite conclusions.

The wet weight of the prostate decreased after administration of *Picrorhiza* extract. Such a depletion in the weight of prostate is reported to be obsered after administration of estrogen (Patanelli and Nelson, 1959), S.K. & F. 7690 (Saunder *et al*;, 1969), various steriods (Singh *et al*;, 1972), Chlorocyclazine (Wong *et al*;, 1972), Cyproterone acetate (Rajlakshmi, 1972; Dahl and Tveter, 1974), Centchroman (Das *et al*;, 1977), Provera (Flickinger, 1977), $CdCl_2$ (Chinoy and Sheth, 1977), Chloromadinone acetate(Kaur and Mangat, 1979),Coumarin (Gupta *et al*;, 1980), bromocryptine (Arunnakaran *et al*;, 1985) M G P (Rao, *et al*;, 1986), Flutamide (Dhar and Shetty, 1987), Methyl mercury chloride (Rao, 1988), antiandrogens Esradiol-dipropionate, Flutamide, Cyproterone acetate, STS-557 (Gupta

et al;, 1989), anethole (Farook *et al*;, 1989), Lithium chloride (Ghosh *et al*;, 1990).

Many plant preparations are also induced decrease in weight of prostate, these are *Malvaviscus conzanttii* (Dixit, 1977b), *Aristolochia indica* (Pakrashi and Pakrashi, 1977), *Vinca rosea* (chauhan,*et al*;.1979),*Allium sativum* powder (Dixit and Joshi,1982),*Vitex negundo* (sohani,1985),*Davcus carota* (Shah,1985),*Oscimum sanctum* (Khanna *et al*., 1988), Plumbagin (Jadhav, 1988), *Piper betle* (Hiremath, 1988, Adhikary, *et al*;, 1989), *Andrographis paniculata* (Akbarsha *et al*;, 1990), *Abrus precatorius* (Sinha and Mathur, 1990), *Syzygium cumini* (Ambaldhage, 1990).

Acinar epithelial cells are appeared to be reduced in height. This observation find a good support in the work of Wong *et al*;, (1972) with chlorocyclazine. Flickinger (1977) with Provera, Kaur and Mangat (1979) with chloromadinone acetate. Some plant preparations are also shown to decrease the height of epithelial cells. These are *Vinca rosea* alkaloids (Toro, 1984), *Vitex negundo* (Sohani, 1985), *Daucus carota* (Shah, 1985), *Piper betle* (Hiremath, 1988), Plumbagin (Jadhav, 1988), *Andrographis paniculata* (Akbarsha *et al*;, 1990).

Picrorhiza treatment reduced the acinar secretion. Some acini are with very little amount of secretion, while

most of the acini were nearly empty. Similar observations are reported with Castration (Moore et al;, 1930), S.K.F. 7690 (Saunders et al;, 1969), Chlorocyclazine (Wong et al;, 1972), Cyproterone acetate (Dahl and Tveter, 1974). Such depletion in secretory activity is also reported with plant preparations Aristolochia indica (Pakrashi and Pakrashi, 1979), Vinca rosea (Toro, 1984), Daucus carota (Shah, 1965), Vitex negundo (Sohani, 1985), Piper betle (Hiremath plumbagin (Jadhav, 1988), Andrographis paniculata (Akbarsha et al;, 1990), Syzygium cumini (Ambaldhage, 1990).

In this investigation it is observed that the weight of prostate gland is decreased and few histological changes are observed due to administration of Picrorhiza kurroa extract in male albino rats. It is known that for normal functioning of prostate, like other accessory sex organs, the presence of androgen is necessary (Bedwal and Mathur, 1980). Weight of accessory reproductive organs is a good indicator of circulating androgen (Moore et al;, 1930). Picrorhiza contain a phytosteroid kutki sterol. Phytosteroids are reported to induce antiandrogenic effects by decreasing the level of androgens and affecting all the accessory organs (Garg, 1979; Bhargava, 1988; Adhikary, et al;, 1989; Sinha and Mathur, 1990). It seems that androgenic substance in Picrorhiza decreases the testosterone level and causes depletion in weights and histological changes in

prostate gland.

Thus decrease in weight, partial histolterations with partical or total absence of secretion in acini, clearly indicate that Picrorhiza extract induce a functional hypotrophy in prostate of treated animals.