**CHAPTER FIVE**

5. **SEMINAL VESICLE**

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I. REVIEW OF THE LITERATURE:

5.1.1. Histological:

The seminal vesicles secrete a variety of substances into the semen ranging from small molecules to enzymes (Price and Williams-Ashman, 1961). In most species, a large contribution to the volume of semen is made by the seminal vesicles and hence they have become the subject matter of numerous histological, cytological, biochemical and histochemical investigations. Many synthetic chemicals, anti-fertility agents and plant extracts have been tried to explore the effects on seminal vesicles. Following is a brief review of the available literature.

The effects of castration on the rodent seminal vesicles have been reported by number of investigators. Cavazos and Melampy (1954) demonstrated statistically significant reduction in the height of the epithelial cells after castration in rats. In mouse seminal vesicles 5 days after castration there was reduction in cell height and loss of secretory granules (Szirmai and Van der Linde, 1962; Allison, 1964). Hypophysectomy resulted in considerable reduction in weights of seminal vesicles (Elkinton et al., 1972). Hypoxia caused loss of weight and corresponding changes in the secretory epithelium of seminal vesicles (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).

The effects of exogenous hormones on the epithelium of hamster seminal vesicle have been studied. In early treatment of four days with diethylstilbestrol and stilbestrol there was no change in the epithelium, which resembled that of untreated animals. The only change was increase in the number of autophagic bodies in the cells.
After six days of the hormone treatment these bodies were verified as lysosomes (Belt and Cavazos, 1967). Bovine anti-ICSH serum in rats decreased the weights of seminal vesicles, but antiserum of FSH had no effect on the weight (Gambal, 1967). Lamanowask et al. (1968) in their studies on seminal vesicles of rat following treatment with testosterone, reported that prolonged treatment reduced the secretion. SK and F 7690 treatment caused significant reduction in the weights of seminal vesicles with reduction in functional capacity resulting into decreased semen volume (Saunders et al., 1969). Estrogen administration caused reduction in the weights of seminal vesicles (Elkington and Blackshaw, 1971). Chlorocyclazine resulted in the formation of cytoplasmic vacuoles in the epithelial cells. The vacuoles became increasingly more and densely populated with time, accompanied by a reduction in the secretion in the lumen (Wong et al., 1972). Sing et al. (1972) studied the effects of norgesterol and reported reduction in the weights of seminal vesicles. With the administration of α-chlorohydrin there was marked regression of seminal vesicles within three weeks of treatment (Vickery et al., 1974). Cyproterone acetate caused reduction in height and width of cells (Dahl and Tveten, 1974). Agmo (1975) reported that cyproterone acetate reduced the activity of seminal vesicles with decrease in the volume of secretion. This view was supported by Bose et al. (1977), who reported reduction in secretion associated with reduction in weights, while Das et al. (1980) did not find any change in the weights of seminal vesicle of cyproterone acetate treated animals. Medroxyprogesterone treatment caused 15-25% reduction in weights of seminal vesicles at all intervals and the height of the epithelium was also found less than in control (Flickinger, 1977). Das et al. (1977) reported significant reduction
in the absolute weights of seminal vesicles of rats treated with centchroman for sixty days. Morphological examination with the treatment of CdCl₂ revealed smaller weight and size as well as histological change indicating a lower secretory activity of epithelial cells of seminal vesicles (Sakensen et al., 1977). Chlorohydrin induced marked regression in seminal vesicle (Hundal and Mangat, 1978). Dixit et al. (1979) demonstrated reduction in the weights of seminal vesicles of house rats and gerbils after administration of cyclonexanol. Kaur and Mangat (1979) observed regression in wet weights of seminal vesicle and inhibition of secretory activity after chloromadionine acetate treatment. Aspirin administration caused atrophy of seminal vesicles (Balsubrahmaniam et al., 1980).

Very few plants with antispermogogenic potencies have been explored for their effects on seminal vesicles. Oscimum sanctum extract administration caused change in the pH of seminal plasma of mice (Kasinathan et al., 1972). Oral administration of Malvaviscus conzantii extract caused decrease in the absolute weights of seminal vesicles in house rats as well as in gerbils (Dixit, 1977). Aristolochia indica, when fed orally to mice, caused a notable reduction in the size of seminal vesicle and reduction in its wet weight (Pakrashi and Pakrasi, 1977). They further reported that in treated mice there was no histological change but the lumen was devoid of secretion. Garg (1979) also noted reduction in weights of seminal vesicles of gerbils when fed with extract of Calotropis procera. Papaya seeds caused infertility without reduction in weight of seminal vesicle (Das, 1980). Malvaviscus conzantii extract administration for fifty days in mice caused decrease in relative weights of seminal vesicles and progressive reduction in the size. Histological picture
of drastically reduced seminal vesicles presented a significant inhibition in the arborization of the secretory epithelium. More marked was the reduction in cell height and the lumen was empty or with small amount of secretion (Verma et al., 1980). Allium sativum powder also reduced the weights of seminal vesicles in rats significantly (Dixit and Joshi, 1982).

In this laboratory Shah (1985) observed reduction in epithelial cell height as well as in the wet weight of the organ with Daucus carota seed extract in white rats. Toro (1984) and Sohani (1986) demonstrated no significant changes in epithelial cell height but reduction in wet weight of the organ after administrations of Vinca rosea alkaloid extract and Vitex negundo leaf extract to white rats respectively.

5.1.2. Enzymes:

a) Lysosomal enzymes:

Stimulation by androgen of the β-glucuronidase activity has been shown by number of workers in the seminal vesicles. Conchie et al. (1959) showed higher activity for β-glucuronidase in the mature animals than in the immature ones. Kind (1974) found reduction in the enzyme activity in both experimental and normal castration. Baile (1975) found highest level of β-glucuronidase activity during breeding period and lowest in the post-breeding period of bats and further showed enzyme rich granules in the epithelial cells of seminal vesicle of some bats.

Acid phosphatase activity has been shown in seminal vesicles by Wislocki (1949), Bern (1949), Rollison (1954), Brandes (1965), Maggi et al. (1970), Dounce (1950), Dixit and Niarai (1975), Goyal and Mathur (1976), Loving and Flickinger (1976) and by Hostorm (1977).
Stafford et al. (1949) got low acid phosphatase activity in the castrated rats and found that the testosterone treatment prevented the loss. Mann and Mann (1951) noted that after castration acid phosphatase underwent reduction in the activity, which could be restored by testosterone. Porter and Melampy (1952) observed that castration decreases acid phosphatase activity in the nuclei, cytoplasm and secretion granules, and further noted that there was remarkable increase after androgen administration. Similar observations have been made by Melampy and Cavazos (1963) and Cichonmikolajczyk (1969). Exogenous hormone stilbestrol treatment resulted in the increase in the number of autophagic bodies. These bodies were acid phosphatase positive and were verified as lysosomes (Cavazos, 1963). Kind-Hensel (1974) observed that strong activity of acid phosphatase present in the epithelium of seminal vesicle was reduced after experimental and hormonal castration in rats. Maggi et al. (1970) castrated and/or treated mice with estradiol. The enzyme activity temporarily increased as the gland regressed and then disappeared. Dixit and Niami (1975) studied acid phosphatase activity in the presence and absence of the pituitary. The growth and acid phosphatase activity were significantly increased in the nonhypophysectomized castrated rats, this was reduced in the absence of hypophysis. Verne and Herbert (1952) reported that hypophysectomy in rat caused a cessation of spermatogenesis and disappearance of esterase reaction with decrease in the height of epithelial cells of seminal vesicle after 12 days. Studies by Limanowski and Miskowiak (1970) indicated that the seminal vesicle esterases are dependent on testicular hormones. They observed increase in nonspecific esterases with testosterone treatment.
A few plant preparations have been tried for their effects on the histological structures of the seminal vesicles. But the available literature shows that there is no work on record on effects of plant extracts on biochemistry and histochemistry of lysosomal enzymes in seminal vesicles. In this laboratory Shah (1986), Toro (1984) and Sohani (1986) worked on Daucus carota, Vinca rosea and Vitex negundo respectively and found changes in β-glucuronidase, acid phosphatase and in nonspecific esterases. The details of the changes in these three lysosomal enzymes are given in discussion part of this chapter.

b) Alkaline Phosphatase:

Alkaline phosphatase activity is also found in the seminal vesicles. In rat after castration the enzyme activity decreased significantly as has been demonstrated by Stafford et al. (1949), Porter and Melampy (1952) and Cavazos and Melampy (1954). CdCl₂ administration resulted in significant decrease in the activity of alkaline phosphatase (Chinoy and Sheth, 1977). Treatment of chlorohydrin caused slight decrease in alkaline phosphatase activity (Hundel and Mangat, 1978).

Administration of Malvaviscus conzantill flower extract did not change the activity of alkaline phosphatase in the seminal vesicular epithelium of gerbil and rat (Dixit, 1977). This view was supported by Verma et al. (1980) in mice, where they reported that alkaline phosphatase activity of seminal vesicle remained unaltered. Thus the literature on the enzymatic alterations caused by the plant extracts is extremely poor. In this laboratory Sohani (1985) with Vitex extract and Toro (1984) with Vinca alkaloid extract showed an increase in alkaline phosphatase activity, whereas Shah (1985) with Daucus extract observed decrease in the activity of this enzyme in seminal vesicles of white rats.
5.1.3. Mucosubstances:

Though there are many reports on the biochemical and histochemical studies on seminal plasma, very few workers have focused their attention on the mucosubstances of the seminal vesicles in mammals. Hartree (1952), Odin (1955), Warren (1959) and Allison (1964) observed that sialic acid is secreted by the male accessory organs, which forms a normal constituent of seminal plasma in number of animals such as man, bull, boar and stallion. Leblond (1950) showed moderate to poor PAS reactivity in seminal vesicular epithelium of rat. Similar results were obtained by Melampy and Cavazos (1953) and Zegarese (1958) in rats. Warren (1959) demonstrated that major part of the sialic acid in semen is contributed by seminal vesicles. Feagans et al. (1961) showed moderate PAS reaction in hamsters. Rzeszowska (1960) identified acidic mucosubstances in seminal vesicular epithelium of rats. Grazyna (1969) showed acid mucopolysaccharides in the epithelia of seminal vesicles and gonads during postembryonic development in white rats. Fouquet (1972) studied the free sialic acids in the seminal vesicle secretion of the golden hamster. Karaginnidis (1972) also observed presence of sialic acid in human and bovine seminal plasma and observed that the concentration of sialic acid in seminal plasma was twice as high in bull as in man. Presence of glycogen, sialic acids and neutral polysaccharides in the epithelial cells and secretion of seminal vesicles of few bats was also shown by Pawar (1976) and Vibhute (1980).

Very few reports are available on the effects of antifertility agents on mucopolysaccharides of seminal vesicles. Aitken (1955) reported no change in the glycogen contents of seminal vesicles in ram even after one year or more following castration. Rzeszowska
(1966) found depletion in sialic acid levels of seminal vesicles in rats after castration, whereas testosterone administration reversed this effect. Similar results were obtained in rhesus monkeys by Bose and Kar (1968). Testosterone treatment increased the amount of mucosubstances (Limanowski and Miskowiak, 1970). Singh et al. (1968) found that a single injection of testosterone in rats caused increase in glycogen contents, but actinomycin pretreatment prevented the deposition of glycogen in the seminal vesicles. Depletion of glycogen, sialic acid and neutral mucopolysaccharides was shown during reproductive quiescent period in the breeding cycles of few bats (Pawar, 1976; Vibhute, 1980). Manjula and Kadam (1983) noted decrease in fructose in lorises after efferent ductule ligation.

Critical evaluation of the available literature on the effects of plant preparations on mucopolysaccharides of seminal vesicles clearly shows that no work on this aspect is on record. In this laboratory Shah (1985) found decrease in glycogen and sialic acid contents of seminal vesicles after administration of Daucus carota seed extract. Similarly Toro (1984) and Sohani (1985) also found reduction in these metabolites as an effect of administration of Vinca rosea alkaloid extract and Vitex negundo leaf extract to albino rats respectively.

5.1.4. Lipids:

Review of the earlier literature on lipids in mammalian seminal vesicles reveals that this metabolite has been studied by employing histochemical techniques by Mann et al. (1949) and Dubois (1964). Rajlakshmi et al. (1973) investigated lipids in hamster seminal vesicles. Gambal (1967) demonstrated that administration of ovine antk-ICSH serum to rats decreased the weight of testes and seminal
vesicles, and increased the concentration of total lipids, particularly all the phospholipids in the seminal vesicles, while FSH antiserum had no effect on the weight of the testes or seminal vesicles or the biosynthesis of phospholipids by the seminal vesicles, but it decreased the concentration of total lipid phosphorus and the incorporation of $^{32}$P in testicular lipids. Influence of testosterone in vivo on the metabolism of phospholipids in seminal vesicles of 30 days old rats was studied by Kargar et al. (1972). The relative content of PI was enhanced one hour after the hormone treatment and PC was markedly increased after 8 hours. Umapathy et al. (1979a) observed that cyproterone and cyproterone acetate brought about a marked decrease in total lipids by depleting neutral lipids markedly and phospholipids to a lesser extent. They also reported that cyproterone had its effect primarily on glycerides, cyproterone acetate brought about its action by inhibiting ester cholesterol formation as well. Medroxyprogesterone acetate treatment resulted in decrease in both phospho and neutral lipids bringing a fall in total lipids (Umapathy et al., 1979b).

Among the neutral lipids both cholesterol and glycerides were decreased. Testosterone administration resulted in an increase in the weight and synthesis of lipids in the seminal vesicles of rats, but weight and lipids were decreased due to oestrogen, progesterone and prolactin influence (Umapathy et al., 1979c). Castration caused reduction in total lipids due to reduction in MG and DG, PC and PE (Umapathy, 1980).

Very poor literature is on record on effects of plant preparations on lipids of seminal vesicles. In this department Sohani (1985), Shah (1985) and Toro (1984) worked on Vitex, Daucus and Vinca respectively and found changes in various components of lipids.
5.1.5. Proteins:

Seminal vesicles of mammals are known to secrete into semen a large variety of substances including proteins (Price and Williams-Ashman, 1961). Histochemically the sites of the formation of protein secretion were documented in the epithelium of seminal vesicles which have rough endoplasmic reticulum, Golgi apparatus and secretory vacuoles (Allison, 1964; 1969; Allison and Cearley, 1972; Belt et al., 1964; Cavazos and Belt, 1965; Dahnke, 1970; Dahnke and Mosebach, 1979; Deane, 1963; Deane and Porter, 1960; Szirmai, 1962; Dralandini, 1964; 1966; Tonner and Baillie, 1966; Riva, 1967; Wrobel, 1968; Flickinger, 1970; 1974; Maggi et al., 1970). Although there are many reports on the histochemical localization of the proteins in seminal vesicle, it seems that less attention has been paid to biochemical investigations on proteins. Following is a brief review of work on the effects of antispermatogenic agents on total proteins in seminal vesicles.

Nocenti (1968) reported that the level of proteins in the secretion of seminal vesicles was dependent on androgen production in animals. Chinoy and Sheth (1977) administered CdCl₂ in the rats and found that the level of proteins in seminal vesicle was increased. Further increase in the proteins was observed when the rats were administered with CdCl₂ and ascorbic acid in combination. Tyagi et al. (1979) administered cyclohexanol in gerbils and house rats, and observed decrease in the proteins of seminal vesicles.

Oral administration of Calotropis procera flower extract significantly reduced the level of proteins in seminal vesicles of gerbils (Garg, 1979). Alcoholic extract of Malvaviscus conzantii flowers when administered in mice caused significant reduction in proteins of seminal vesicles (Verma et al., 1980). Chronic administration of
Allium sativum in rats resulted in a significant reduction in proteins of seminal vesicles (Dixit and Joshi, 1982). Administration of Daucus carota seed petrolatum ether extract resulted in significant reduction in total proteins of seminal vesicle (Shah, 1985), while Vinca (Toro, 1984) and Vitex (Sohani, 1985) extracts did not exhibit a remarkable change in the proteins.
II. OBSERVATIONS:

Butea leaf extract induced changes in weights and histological alterations in seminal vesicle of rats were observed at all time intervals. Since no consistent progression of histological changes was detected in rats throughout the period of the extract administration, this period has been divided into two phases, the first being up to 45th day of treatment and second from 45th to 90th days of treatment. The salient features of different alterations are as follows:

5.2.1. **Histological**:

a) **Control**:

The structure of the epithelium of rat seminal vesicles has been described in detail by several workers (Price and Williams-Ashman, 1961; Brandes, 1966; 1974; Flickinger, 1974; Hamilton, 1975). The histological structure of seminal vesicles of control animals consists of mucosa, lamina propria, muscle coat and the secretion. The histology of the control did not differ from the normal seminal vesicle described by Copenhaver et al. (1971) and is shown in Plate No. 9, Fig. 1.

i) **Mucosa**: The epithelial cells of the mucosa are of two types, large columnar secretory cells and smaller basal cells. The mucosa is folded in a complicated manner called as arborization forming numerous irregular chambers or crypts. These folds project into the lumen. (Plate No.9, Fig. No.1). The epithelium is usually pseudostratified, the columnar cells of which reach the surface. The nucleus of each columnar cell is located in basal portion and is elongated in the direction of the long axis of cell, while the nucleus of basal cell is spherical in shape lying basally. Numerous short villi are present on the apical surface of columnar cells. The cytoplasm of columnar and basal cells contains secretion granules.
ii) **Lamina propria**: It is rich in elastic fibres and forms a continuous layer around the vesicle and also pierces the folds.

iii) **Muscular coat**: Outside the lamina propria lies muscle coat consisting of smooth muscle fibres. It is indefinitely divided into inner circular and outer longitudinal layers.

iv) **Luminal secretions**: The cavity of seminal vesicles is thrown into many lumina by muscular septa. The lumina are full of secretory material. The secretion is viscid in character and is also retained in the depths of the crypts and in patches adherent to the surface of the cells adjoining the main part of the lumen.

b) **Experimental**:

i) **Alterations in the wet weights**:

The seminal vesicle exhibited alterations in the wet weights during the period of ninety days of the extract administration. The variations occurring in the wet weights of the seminal vesicle and in body weights are recorded in Table No.21 and illustrated in Graph No.27.

As seen from the tabular and graphical illustrations the seminal vesicular weight did not exhibit any significant change in the control rats which received only vehicle, the average value being 186.3 ± 11.2 mg/100 g of body weight. In general in the rats receiving *Butea* extract the wet weight of the organ increased during the period of the extract treatment. The wet weight of the seminal vesicles showed an enhancement and the value was 191.6 ± 13.8 mg/100 g body weight of the animal on 15th day. On 30th day it decreased to 181.8 ± 15.7 mg/100 g body weight. The weight on 45th day of treatment was 207.4 ± 16.6 mg/100 g body weight. On 60th and 75th days the weights showed an increasing trend. The weights were 206.39 ± 14.2 and 211.22 ± 17.4 mg/100 g body weight on 60th and 75th days.
Table 21: Seminal Vesicle: *Butea monosperma* leaf extract induced alterations in wet weights.

<table>
<thead>
<tr>
<th>Duration (In Days)</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body wt. (g)</td>
<td>Weight of Seminal Vesicle (mg)</td>
</tr>
<tr>
<td>0</td>
<td>270 ± 5.30</td>
<td>186.3 ± 11.2</td>
</tr>
<tr>
<td>15</td>
<td>300 ± 5.90</td>
<td>186.66 ± 11.2</td>
</tr>
<tr>
<td>30</td>
<td>310 ± 6.18</td>
<td>184.83 ± 11.1</td>
</tr>
<tr>
<td>45</td>
<td>309 ± 6.16</td>
<td>185.35 ± 11.08</td>
</tr>
<tr>
<td>60</td>
<td>305 ± 6.08</td>
<td>182.6 ± 10.90</td>
</tr>
<tr>
<td>75</td>
<td>317 ± 5.32</td>
<td>181.13 ± 10.80</td>
</tr>
<tr>
<td>90</td>
<td>325 ± 6.48</td>
<td>180.92 ± 10.67</td>
</tr>
</tbody>
</table>

(Values are mean ± S.D. of five animals. Wet weights of seminal vesicles are expressed in mg per 100 grams of body weights. Values of body weights are in grams).
Seminal vesicle -
Alterations in wet weights
(mg/100 of body weight)
respectively. At the termination of treatment the weight of seminal vesicle was little less than in the previous phase of treatment but it was more than that in the control. The wet weight was $200.0 \pm 19.6$ mg/100 g body weight.

ii) Histological alterations:

Very few alterations were observed in the microscopic structure of seminal vesicles in the treated animals at all the intervals of the extract administration. As stated previously the entire period of the extract treatment is divided into two phases, the first phase being upto 45th day of the treatment and the second being from 45th to 90th days of the treatment.

I) Alterations upto 45th day of the treatment:

The changes observed in the mucosa, lamina propria, muscle coat and the luminal secretion are shown in the photomicrographs in Plate No.9 and Fig. No.2.

i) Mucosa: The epithelium which presented a folded appearance showed very little change. The folds which extended up to the centre of the lumen in control animals got shortened (Plate No.9, Fig. No.2). There was no change in the height and width of the columnar and basal cells. Nuclei of both the types of cells did not exhibit any change as indicated by Feulgan technique.

ii) Lamina propria: Lamina propria also did not exhibit any significant change. Slight thickening was seen in some places.

iii) Muscular coat: The inner circular and outer longitudinal muscle layers showed little thickening as indicated by bright eosinophilic staining.

iv) Luminal secretion: The lumen of vesicles contained secretory material. There was insignificant reduction in the amount
of secretion. But the secretion in few lumina showed cellular debris (Plate No.9, Fig. No.2). The secretory material was retained in the depths of crypts as seen in the control.

II) Alterations from 45th to 90th days of the treatment:

The changes seen in the histological structure of the seminal vesicles in this phase of the treatment are shown photomicrographically in Plate No.9, Fig. Nos. 3 and 4.

i) **Mucosa**: This phase of the treatment witnessed a gradual reduction in the height of the epithelial folds. The folds which were touching the centre of the lumen in control seminal vesicles were shortened. There appeared significant reduction in the arborization of the epithelium (Plate No.9, Fig. No.3). The height of the mucosal cells showed reduction.

ii) **Lamina propria**: The lamina propria was further thickened as indicated by high eosinophilic staining.

iii) **Muscular coat**: The inner circular smooth muscle layer immediately around the epithelium seemed to get detached from the outer layer and presented a wavy appearance (Plate No.9, Fig. No.3). The outer layer of smooth muscles showed a high degree of loose arrangement. In few vesicles this muscle layer was broken down at some places.

iv) **Luminal secretion**: As there was reduction in the height and the arborization of the epithelial folds in this phase, the lumen of seminal vesicle increased as a secondary effect. Lumina of few vesicles were full of secretory material with cellular debris, while majority of vesicular lumina were partially empty. The secretory material in few lumina showed less degree of density. In few lumina the secretion showed appearance of trabeculae or strands which were
seen stretched across the luminal periphery (Plate No.9, Fig. No.4). The secretion was highly eosinophilic.

5.2.2. **Enzymes**

5.2.2.1. **β-glucuronidase**

i) **Biochemical observations**

The alterations occurring in the β-glucuronidase activity in the seminal vesicles are given in Table No.22 and these alterations are graphically illustrated in Graph No.28.

In control animals the average enzyme activity was $4254 \pm 110$ F.U./g wet weight of tissue. After 15 days of the treatment the activity increased to $5458 \pm 99$ F.U./g wet weight. After 30 days of the treatment there was slight depletion in the enzyme activity to $5276 \pm 120$ F.U./g wet weight. There was slight increase on the 45th day of the treatment, when the enzyme activity was $5678 \pm 140$ F.U./g wet weight. On the 60th day of treatment the activity exhibited a significant depletion and it was below the control value. The activity was $3614 \pm 180$ F.U./g wet weight. The next thirty days witnessed a steady and significant increase in the enzyme activity. The activity was $7693 \pm 210$ and $8820 \pm 290$ F.U./g wet weight of tissue on 75th and 90th days of the treatment respectively.

ii) **Histochemical observations**

The results obtained with the histochemical techniques for β-glucuronidase enzymorphology in the control seminal vesicle and during various phases of the Butea leaf extract treatment are shown in Plate No.9, Fig. Nos. 5 and 6.

a) **Control**

i) **Mucosa**: In the seminal vesicles of control rats the major site of localization of β-glucuronidase in the mucosal cells was
<table>
<thead>
<tr>
<th>Duration (In Days)</th>
<th>Lysosomal Enzymes</th>
<th>Nonlysosomal Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$-glucuronidase</td>
<td>Acid phosphatase (µ moles)</td>
</tr>
<tr>
<td></td>
<td>(F.U.)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4254 ± 110</td>
<td>893 ± 60</td>
</tr>
<tr>
<td>15</td>
<td>5458 ± 99</td>
<td>878 ± 65</td>
</tr>
<tr>
<td>30</td>
<td>5276 ± 102</td>
<td>976 ± 105</td>
</tr>
<tr>
<td>45</td>
<td>5678 ± 140</td>
<td>1516 ± 110</td>
</tr>
<tr>
<td>60</td>
<td>3614 ± 180</td>
<td>1218 ± 100</td>
</tr>
<tr>
<td>75</td>
<td>7093 ± 210</td>
<td>1380 ± 114</td>
</tr>
<tr>
<td>90</td>
<td>8820 ± 200</td>
<td>1794 ± 160</td>
</tr>
</tbody>
</table>

(Values are mean ± S.D. of five animals and are expressed in Fishman Units for $\beta$-glucuronidase, in Esterase units for Non-specific esterase, in µ moles of p-nitrophenol for Acid and Alkaline phosphatases per gram wet weight of tissue).
Seminal vesicle -
Alteration in $\beta$-glucuronidase
(Fishman Units/g wet weight of tissue)
apical cytoplasm. The activity was in minute granular form and the staining intensity was moderate (Plate No.9, Fig. No.5). Basal cytoplasm showed poor staining, while nuclei remained unstained.

ii) Lamina propria: It exhibited moderate diffused staining.

iii) Muscular coat: It showed moderate diffused activity.

iv) Luminal secretion: It showed poor β-glucuronidase staining.

b) Experimental:

I) Alterations up to 45th day of the treatment:

i) Mucosa: β-glucuronidase activity was moderate in the mucosal cells during this phase of the treatment. Enzyme activity was observed in both apical and basal regions in the forms of minute granules. There was increase in the number of granules and also in their staining intensity.

ii) Lamina propria: In this layer slight increase in the enzyme activity was observed.

iii) Muscular coat: This layer exhibited intense reaction on 45th day of the treatment. The activity was diffused in nature.

iv) Luminal secretion: The lumen of the gland contained secretory material, which exhibited poor enzyme activity. The activity was diffused in form.

II) Alterations from 45th to 90th days of the treatment:

i) Mucosa: The staining intensity of the granules was more in the epithelial cells of mucosa as compared to the first phase of the treatment (Plate No.9, Fig. No.6). Darkly stained particles were found to have increased in majority of mucosal cells. Among the mucosal cells, those cells which were present towards the side of the lumina exhibited strong activity as compared to the mucosal cells which were present towards the lower side of crypt away from the lumina.
ii) Lamina propria: This layer exhibited increased activity than in the previous phase. Darkly stained particles were found to have increased in number.

iii) Muscular coat: The activity in muscular coat was moderate and diffused in form in this phase of the extract treatment.

iv) Luminal secretion: Secretion was more compared to the control and it exhibited poor diffused activity similar to the previous phase of the treatment.

5.2.2.2. Acid Phosphatase:

i) Biochemical observations:

The alterations occurring in acid phosphatase activity during various phases of the extract administration are given in Table No. 22 and these alterations are graphically illustrated in Graph No. 29.

In control animals the average enzyme activity was 393 ± 30 units/g wet weight of seminal vesicle. After 15 days of the treatment the enzyme activity decreased and it was little less than that in control rats. The activity was 878 ± 65 units. The 30th day witnessed an increase and the activity was more than values of the control. The activity was 976 ± 105. Then the activity increased significantly to 1518 ± 110 units on 45th day. The activity then depleted gradually, but the values were above those of the control. The value was 1218 ± 100 on 60th day. Towards the end of the experiment the enzyme activity showed a steady rise. The enzyme activities were 1380 ± 114 and 1794 ± 160 units on 75th and 90th days of the extract treatment respectively.

ii) Histochemical observations:

The results obtained with histochemical techniques for the distribution of the acid phosphatase in the control seminal vesicle
Seminal veicle-
Alterations in Acid phosphatase
(\(\mu\) moles \(p\) nitrophenol/g wet weight of tissue)
and during administration of Butea leaf extract are shown in Plate No.9, Fig. Nos. 7 and 8.

a) Control: The seminal vesicle of the control animals consisted of mucosa, lamina propria, muscular coat and luminal secretion.

   i) Mucosa: In the seminal vesicle of control rats the site of localization of acid phosphatase in the mucosal cells was apical cytoplasm. The activity was in minute granular form and the staining intensity was moderate to poor (Plate No.9, Fig. No.7).

   ii) Lamina propria: It exhibited poor staining.

   iii) Muscular coat: It showed moderate to poor diffused activity.

   iv) Luminal secretion: It showed poor acid phosphatase staining.

b) Experimental:

I) Alterations upto 45th day of the treatment:

   i) Mucosa: Acid phosphatase activity was more in the epithelial cells as compared to the control. Darkly stained particles were found to have increased in majority of mucosal cells (Plate No.9, Fig. No.8).

   ii) Lamina propria: In this layer slight increase in the enzyme activity was observed.

   iii) Muscular coat: This layer exhibited moderate to intense reaction. Darkly stained particles were found to be increased in number in muscular coat.

   iv) Luminal secretion: The lumen of the gland contained a secretory material, which exhibited poor enzyme activity in diffused form.

II) Alterations from 45th to 90th days of the treatment:

   i) Mucosa: Acid phosphatase activity was decreased in the mucosal cells during the second phase of the treatment. There was
decrease in the number of granules as well as in their staining intensity.

ii) Lamina propria : This layer also exhibited decrease in the enzyme activity.

iii) Muscular coat : Darkly stained particles were reduced in number. Their staining intensity was also poor.

iv) Luminal secretion : Secretion was more than in control and exhibited poor diffused acid phosphatase activity.

5.2.2.3. Esterase :

i) Biochemical observations :

The alterations occurring in the nonspecific esterase during the various phases of the extract treatment are given in Table No.22 and these changes are graphically illustrated in Graph No.30.

In control animals the nonspecific esterase activity was $14.2 \pm 2.3$ E.U./g wet weight of the seminal vesicle. After 15 days of the treatment the esterase activity was elevated above the level in controls. The activity was $20.4 \pm 4.2$ E.U./g. Then on the 30th day there was negligible decrease and the activity was $17.2 \pm 2.9$ E.U./g. Then it depleted suddenly below the control level in the next 15 days of the treatment, the activity being $10.7 \pm 1.5$ E.U./g on 45th day of the extract administration. The esterase activity again exhibited an enhancement over the control level. The activity was $16.8 \pm 3.1$ E.U./g. wet weight of tissue on 60th day. The activity then decreased gradually and it was $15.0 \pm 2.2$ and $13.5 \pm 1.9$ E.U./g wet weight of tissue on the 75th and 90th days of the extract administration respectively.

ii) Histochemical observations :

The results obtained with the histochemical techniques for nonspecific esterase enzymology in control seminal vesicle and during the induced aspermatogenesis are shown in Plate No.10, Fig. Nos. 1, 2).
Seminal vesicle -
Alterations in non-specific esterase
(Esterase Units/g wet weight of tissue)
a) Control:

1) **Mucosa**: In control rats, the nonspecific esterase activity was located throughout the cytoplasm of mucosal cells. The staining was from moderate to poor in granular as well as in diffused form (Plate No. 10, Fig. No. 1).

   i) **Lamina propria**: This layer exhibited moderate to poor diffused staining.

   ii) **Muscular coat**: In this layer, nonspecific esterase activity was moderate in the diffused form.

   iii) **Luminal secretion**: It exhibited no activity of the enzyme.

b) Experimental:

I) **Alterations up to 45th day of the treatment**:

1) **Mucosa**: Esterase activity was moderate to intense in the mucosal cells during this phase of the treatment (Plate No. 10, Fig. No. 2). Enzyme activity was observed in both apical and basal regions of the cytoplasm. There was increase in the staining intensity of granules.

   i) **Lamina propria**: There was no change in the intensity of staining, it was moderate to poor and diffused in form.

   ii) **Muscular coat**: There was increase in the activity of esterase. The activity was in minute granular as well as diffused in form.

   iii) **Luminal secretion**: The secretion exhibited no esterase activity.

II) **Alterations from 45th to 90th days of the treatment**:

1) **Mucosa**: In this phase of the extract treatment, the esterase activity in the mucosal cells got depleted. It was poor to moderate and diffused.
ii) Lamina propria: The staining was poor in this layer.

iii) Muscular coat: This layer exhibited poor staining.

iv) Luminal secretion: There was no staining for esterase in the secretion.

5.2.2.4. Alkaline Phosphatase:

i) Biochemical observations:

Alterations occurring in the alkaline phosphatase activity during induced aspermatogenesis caused by administration of *Butea* leaf extract are given in Table No.22 and these alterations are graphically illustrated in Graph No.31.

In control rats which were given only vehicle the enzyme activity was $5658 \pm 140 \mu$ moles/g wet weight of seminal vesicle. After 15 days of the treatment the alkaline phosphatase activity increased and it was $6865 \pm 175 \mu$ moles/g. On 30th day the enzyme activity decreased slightly to $6718 \pm 165 \mu$ moles/g. Then the 45th day of the treatment witnessed an enhancement in the activity to $8805 \pm 200 \mu$ moles/g. Again there was increase in the enzyme activity on 60th day when the activity was $10638 \pm 205 \mu$ moles/g. The enzyme activity then depleted gradually but the values were more than the control values. The values were $5790 \pm 146$ and $5910 \pm 176 \mu$ moles/g on 75th and 90th days of the extract treatment respectively.

ii) Histochemical observations:

The results obtained with the histochemical techniques for the alkaline phosphatase enzymorphology in control seminal vesicles and during the induced aspermatogenesis are shown photomicrographically in Plate No.10, Fig. Nos. 3 and 4.

a) Control:

i) Mucosa: The alkaline phosphatase reactivity in the seminal mucosal cells was located throughout the epithelium. It was moderate
Seminal vesicle -
Alterations in Alkaline phosphatase
(\(\mu\) moles p-nitrophenol/g wet weight of tissue)
granular and diffused in form (Plate No.10, Fig. No.3).

ii) Lamina propria: The staining was poor in this layer.

iii) Muscular coat: In this layer alkaline phosphatase staining was moderate in the form of granules.

iv) Luminal secretion: The secretion in the lumen exhibited moderate alkaline phosphatase activity.

b) Experimental:

I) Alterations upto 45th day of the treatment:

i) Mucosa: Alkaline phosphatase activity was moderate to intense in the mucosal cells during this phase of the treatment. Enzyme activity was observed in both apical and basal regions of the cytoplasm. There was increase in the number of granules (Plate No.10, Fig. No.4).

ii) Lamina propria: There was an increase in the staining intensity in this layer. The activity was granular. Associated with increase in staining intensity, increase in number of granules was also observed.

iii) Muscular coat: This layer also exhibited increase in staining intensity of alkaline phosphatase. The activity was intense and in the form of granules.

iv) Luminal secretion: The secretion in the lumen exhibited moderate enzyme activity in the granular form without any change.

II) Alterations from 45th to 90th days of the treatment:

i) Mucosa: The enzyme activity was moderate in the form of granules. It exhibited decrease in staining intensity during this phase of the treatment.

ii) Lamina propria: The intense activity seen in the first phase was decreased. The activity was moderate and granular.
iii) **Muscular coat**: This layer also exhibited depletion in the number of granules as well as in staining intensity.

iv) **Luminal secretion**: The secretion showed moderate to poor enzyme activity.

5.2.3. **Mucosubstances**:

a) **Control**:

The histochemical reactivities of various mucopolysaccharides observed in the seminal vesicles of control rats are recorded in Table No.23 according to the visually estimated intensity and shade, and shown photomicrographically in Plate No.10, Fig. Nos. 5 and 7.

1) **Mucosa**: The mucosal epithelial lining consists of columnar and basal secretory cells. These cells showed moderate PAS reactivity which was reduced by prior diastase digestion and which was also partly sensitive to prior phenylhydrazine treatment, thus indicating that these cells contained glycogen. These cells did not exhibit alcianophilia at pH 1.0, but at pH 2.5 they showed moderate alcianophilia. With C.I. the cells showed a positive reactivity. These observations indicated that carboxymucins were present, sulfate esters being absent in these cells. This was further supported by the following histochemical techniques. With AF-AB (pH 2.5) sequential staining they showed only moderate blue staining, thus, they reacted only with AB substantiating the presence of carboxymucins. With AB (pH 1.0)-PAS sequential staining, these glandular cells remained pink coloured only, while with AB (pH 2.5)-PAS and C.I.-PAS sequential staining they reacted with both the stains. These initial reactivities supported the presence of glycogen, diastase resistant PAS reactive neutral mucosubstances and carboxymucins in the mucosal epithelial glandular cells of the seminal vesicles. The confirmatory proof of the presence of carboxymucins was obtained by the loss of alcianophilia.
Table 23: Histochemical observations of mucous substances in the seminal vesicle of control rat.

<table>
<thead>
<tr>
<th>No.</th>
<th>Histochemical reactions</th>
<th>Lamina propria</th>
<th>Muscular coat</th>
<th>Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PAS</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>Phenyl + PAS</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Diastase + PAS</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>AB (pH 1.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td>C.I.</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>7.</td>
<td>AB (pH 1.0) + PAS</td>
<td>++P</td>
<td>+P</td>
<td>+P</td>
</tr>
<tr>
<td>8.</td>
<td>AB (pH 2.5) + PAS</td>
<td>++PB</td>
<td>+PB</td>
<td>++PB</td>
</tr>
<tr>
<td>9.</td>
<td>C.I. + PAS</td>
<td>++PB</td>
<td>+PB</td>
<td>++PB</td>
</tr>
<tr>
<td>10.</td>
<td>A.F.</td>
<td>-</td>
<td>-</td>
<td>+P</td>
</tr>
<tr>
<td>11.</td>
<td>A.F. + AB (pH 2.5)</td>
<td>++B</td>
<td>+B</td>
<td>+B</td>
</tr>
<tr>
<td>12.</td>
<td>C.E.C. (0.1 M Mg++)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>C.E.C. (0.2 M Mg++)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>T.B. pH(0.5 - 1.5)</td>
<td>+0</td>
<td>+0</td>
<td>-</td>
</tr>
<tr>
<td>15.</td>
<td>T.B. pH(2.5 - 4.5)</td>
<td>++M</td>
<td>+M</td>
<td>++M</td>
</tr>
<tr>
<td>16.</td>
<td>T.B. pH(4.5 - 5.0)</td>
<td>++M</td>
<td>+M</td>
<td>++M</td>
</tr>
<tr>
<td>17.</td>
<td>Mild methylation + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>18.</td>
<td>Mild methylation + Saponification + AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td>Active methylation + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20.</td>
<td>Active methylation + Saponification + AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>21.</td>
<td>Acid hydrolysis + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22.</td>
<td>Neuraminidase + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23.</td>
<td>Hyaluronidase + AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>24.</td>
<td>Pepsin + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Colours: P = Pink, B = Blue, PB = Purple blue, BP = Blue purple, O = Orthometachromacia, M = Metachromacia.
after mild and active methylations and its restoration after subsequent saponification. The alcianophilia was also abolished by the acid hydrolysis and neuraminidase digestion, which further confirmed the presence of sialic acid. These cells exhibited metachromacia with TB at pH 3.0 and above, but at lower pH they stained orthochromatically. Hyaluronidase treatment was without any effect on the alcianophilia, indicating absence of hyaluronic acid in the mucosubstances in these cells. Thus, the glandular epithelial cells of control rat seminal vesicles contained moderate amount of glycogen and diastase resistant PAS reactive neutral mucopolysaccharides and poor amount of sialomucins.

ii) Lamina propria: Lamina propria exhibited no reactivity with any of the histochemical tests employed in the present investigation. Thus this structure did not exhibit any staining reactivity with PAS (Plate No.10, Fig. No.5), AB (pH 1.0 and 2.5), C.I. and A.F. They did not exhibit any metachromacia with TB at any pH levels. Such negative results indicated apparent absence of any mucosubstances in the lamina propria. But from these negative reactivities absence of mucosubstances cannot be concluded, since there existed a possibility of presence of protein-masked mucosubstances. To verify this possibility, some of the sections were treated with pepsin for a prolonged period of 15 hours at 37.5° C, following which they were subjected to various aforementioned histochemical staining procedures. Even after pepsin digestion, the lamina propria failed to exhibit any PAS reactivity, alcianophilia at both the pH levels, reactivity towards C.I. and A.F. and in a similar manner it exhibited no metachromatic staining but stained orthochromatically with TB at all pH levels. It was, hence, concluded that the lamina propria of seminal vesicle of control rats did not contain any mucosubstances.
iii) **Muscular coat**: The muscular coat consists of smooth muscle fibres surrounding the lamina propria. The muscular coat of control rat exhibited moderate PAS reactivity (Plate No.10, Fig. No.5). The PAS reactivity could partly be blocked by prior phenylhydrazine treatment indicating presence of glycol groups. Furthermore, the PAS reactivity was partly sensitive to prior diastase digestion indicating presence of glycogen and diastase resistant PAS reactive neutral mucopolysaccharides in moderate amount in the muscular coat of the seminal vesicle.

Moreover, these sites exhibited weak alcianophilia at pH 2.5 and reacted positively with C.I., but remained unstained with AB (pH 1.0). These initial staining reactivities indicated the presence of carboxymucins although in poor quantity, but absence of sulfate esters. The muscular coat remained only PAS reactive in AB (pH 1.0)-PAS sequence and exhibited combined blue purple staining with AB (pH 2.5)-PAS and C.I.-PAS techniques. With AF alone it gave a faint purple staining and appeared only blue in AF-AB (pH 2.5) sequence, which also supported the above conclusion. The muscular coat exhibited weak blue orthochromatic staining with TB at low pH and exhibited moderate β-metachromasia at higher pH levels (pH 3.0 and above). Their alcianophilia at pH 5.6 could be completely abolished by the addition of 0.1 M Mg++. Both mild and active methylations blocked its alcianophilia, which could be completely restored after subsequent saponification, thus confirming the presence of carboxyl groups in poor quantity. Acid hydrolysis and neuraminidase digestion eliminated the alcianophilia in these cells, which confirmed the presence of sialic acid in the muscular coat. Hyaluronidase and pepsin digestions had no effect on their alcianophilia. All these histochemical
reactions indicated the presence of glycogen and diastase resistant PAS reactive neutral mucopolysaccharides in moderate quantity and sialic acid in poor quantity in muscular coat of control rat seminal vesicles.

iv) Secretion : The secretion in the lumen of vesicles exhibited intense to strong PAS reactivity which was partly labile to prior diastase digestion and also partly sensitive to prior phenylhydrazine treatment. The staining reactivity after diastase digestion and prior phenylhydrazine treatment was moderate to intense. These initial histochemical reactivities indicated presence of glycogen diastase resistant PAS reactive neutral mucopolysaccharide and also acidic mucosubstances in it. The secretion did not exhibit alcianophilia at AB (pH 1.0), but showed moderate alcianophilia at AB (pH 2.5) and also showed positive C.I. reactivity (Plate No.10, Fig. No.7). The secretion showed only purple staining with AB (pH 1.0)-PAS sequence, but blue-purple staining with AB (pH 2.5)-PAS and C.I.-PAS staining sequences. It showed only blue staining in AF-AB (pH 2.5) sequential staining. It exhibited blockade of alcianophilia in CEC technique by the addition of 0.1 M Mg++. Mild and active methylations abolished the alcianophilia, which could be restored by subsequent saponification. Acid hydrolysis and neuraminidase digestion abolished the alcianophilia indicating presence of sialic acid. Hyaluronidase treatment was without any effect. All these histochemical reactions indicated intense amount of saliva or diastase resistant PAS reactive neutral mucopolysaccharide, moderate quantity of glycogen and poor quantity of sialic acid in the secretion of the seminal vesicles of control rats.

b) Experimental :

As described earlier the alterations in the mucosubstances in
the seminal vesicles of rats administered with Butea monosperma leaf extract for ninety days, are described in two phases. The first phase deals with alterations up to forty-five days and the second with the remaining forty-five days.

I) Alterations up to 45th day of the treatment:

The results obtained with various histochemical staining techniques in the seminal vesicle during Butea leaf extract treatment in the first phase are recorded in Table No.24.

i) **Mucosa**: The epithelial cells of mucosa of seminal vesicle exhibited all the histochemical reactivities which were exhibited by the epithelial cells of the control rat seminal vesicles. Thus these cells in the treated rats also contained diastase resistant PAS reactive neutral mucosubstances, glycogen and sialic acid. But the intensities of staining were higher than in control, thus indicating an increase in the respective mucosubstances.

ii) **Lamina propria**: As in control the lamina propria did not exhibit any reactivity towards any of the histochemical techniques employed in the present study, thus indicating total absence of all mucosubstances in it.

iii) **Muscular coat**: The muscular coat in this phase of the extract treatment, exhibited moderate PAS reactivity, which was sensitive to prior treatment of phenylhydrazine and diastase digestion. Excepting slightly increased staining intensity, the results obtained with the remaining staining techniques were practically similar to the results of muscular coat of control seminal vesicles, which indicated increased amount of diastase resistant PAS positive neutral mucopolysaccharides and glycogen, and no change in the quantity of sialic acid in the treated rats.
Table 24: Histochemical observations of mucosubstances upto 45th day in the seminal vesicle of treated rat.

<table>
<thead>
<tr>
<th>No.</th>
<th>Histochemical reactions</th>
<th>Mucosa</th>
<th>Lamina propria</th>
<th>Muscular coat</th>
<th>Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PAS</td>
<td>++++</td>
<td>-</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>2.</td>
<td>Phenyl + PAS</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Diastase + PAS</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4.</td>
<td>AB (pH 1.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>6.</td>
<td>C.I.</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>7.</td>
<td>AB (pH 1.0) + PAS</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>AB (pH 2.5) + PAS</td>
<td>++PB</td>
<td>-</td>
<td>+PB</td>
<td>+++PB</td>
</tr>
<tr>
<td>9.</td>
<td>C.I. + PAS</td>
<td>++PB</td>
<td>-</td>
<td>+PB</td>
<td>+++PB</td>
</tr>
<tr>
<td>10.</td>
<td>A.F.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>A.F. + AB (pH 2.5)</td>
<td>++B</td>
<td>-</td>
<td>+B</td>
<td>+B</td>
</tr>
<tr>
<td>12.</td>
<td>C.E.C. (0.1 M Mg++)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>C.E.C. (0.2 M Mg++)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>T.B. pH(0.5 - 1.5)</td>
<td>+O</td>
<td>-</td>
<td>+O</td>
<td>-</td>
</tr>
<tr>
<td>15.</td>
<td>T.B. pH(2.5 - 3.5)</td>
<td>+++M</td>
<td>-</td>
<td>+M</td>
<td>+++M</td>
</tr>
<tr>
<td>16.</td>
<td>T.B. pH(4.5 - 5.0)</td>
<td>++++M</td>
<td>-</td>
<td>+M</td>
<td>++++M</td>
</tr>
<tr>
<td>17.</td>
<td>Mild methyilation + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18.</td>
<td>Mild methyilation + Saponification + AB (pH 2.5)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td>Active methylation + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20.</td>
<td>Active methylation + Saponification + AB (pH 2.5)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21.</td>
<td>Acid hydrolysis + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22.</td>
<td>Neuraminidase + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23.</td>
<td>Hyaluronidase + AB (pH 2.5)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24.</td>
<td>Pepsin + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Colours: P = Pink, B = Blue, PB = Purple blue, BP = Blue purple, 0 = Orthometachromasia, M = Metachromasia.
iv) **Secretion**: As in control rat seminal vesicles, in the treated rats also the secretion exhibited various histochemical reactivities indicating presence of diastase resistant PAS positive neutral mucosubstances, glycogen and sialic acid. The only change that was noted was in the intensity of staining, which was higher than in control, thus indicating increased amounts of these mucosubstances in the secretion at this phase of the *Butea* leaf extract treatment.

II) **Alterations from 45th to 90th days of the treatment**:

The results obtained with various histochemical staining techniques in the seminal vesicle during *Butea monosperma* leaf extract induced aspermatogenesis in the second phase are recorded in Table No. 25 and the histochemical distribution of mucopolysaccharides is illustrated photomicrographically in Plate No. 10, Fig. Nos. 6 and 8.

i) **Mucosa**: As in control rat, seminal vesicular epithelial cells in the treated rats during this phase showed presence of diastase resistant PAS positive mucosubstances, glycogen and sialic acid. One difference that could be noted was the increased intensities of staining towards various histochemical reactions of these mucosubstances in the treated rats. Thus in this phase of the treatment also the epithelial cells elaborated higher amounts of the typical mucosubstances, which were elaborated by these epithelial cells in control rats.

ii) **Lamina propria**: At this stage of the extract treatment also the lamina propria showed absence of all types of mucosubstances as in control rats.

iii) **Muscular coat**: The muscular coat showed increased concentrations of glycogen, and sialic acid as indicated by higher intensities
Table 25: Histochemical observations of mucosubstances up to 90th day in the seminal vesicle of treated rat.

<table>
<thead>
<tr>
<th>No.</th>
<th>Histochemical reactions</th>
<th>Mucosa</th>
<th>Lamina propria</th>
<th>Muscular coat</th>
<th>Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PAS</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>2.</td>
<td>Phenyl + PAS</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Diastase + PAS</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>AB (pH 1.0)</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>6.</td>
<td>C.I.</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>7.</td>
<td>AB (pH 1.0) + PAS</td>
<td>+P</td>
<td>-</td>
<td>-</td>
<td>+P</td>
</tr>
<tr>
<td>8.</td>
<td>AB (pH 2.5) + PAS</td>
<td>++PB</td>
<td>-</td>
<td>+PB</td>
<td>+++PB</td>
</tr>
<tr>
<td>9.</td>
<td>C.I. + PAS</td>
<td>++PB</td>
<td>-</td>
<td>+PB</td>
<td>+++PB</td>
</tr>
<tr>
<td>10.</td>
<td>A.F.</td>
<td>-</td>
<td>-</td>
<td>+P</td>
<td>+P</td>
</tr>
<tr>
<td>11.</td>
<td>A.F. + AB (pH 2.5)</td>
<td>++PB</td>
<td>-</td>
<td>+B</td>
<td>+B</td>
</tr>
<tr>
<td>12.</td>
<td>C.E.C. (0.1 M Mg++)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>C.E.C. (0.2 M Mg++)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>T.B. pH(0.5 - 1.5)</td>
<td>+M</td>
<td>-</td>
<td>+O</td>
<td>+M</td>
</tr>
<tr>
<td>15.</td>
<td>T.B. pH(2.5 - 3.5)</td>
<td>++M</td>
<td>-</td>
<td>+M</td>
<td>++M</td>
</tr>
<tr>
<td>16.</td>
<td>T.B. pH(4.5 - 5.0)</td>
<td>++M</td>
<td>-</td>
<td>+M</td>
<td>+++M</td>
</tr>
<tr>
<td>17.</td>
<td>Mild methylation +</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AB (pH 2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Mild methylation +</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Saponification + AB (pH 2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>Active methylation +</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AB (pH 2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>Active methylation +</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Saponification + AB (pH 2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>Acid hydrolysis + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22.</td>
<td>Neuraminidase + AB (pH 2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23.</td>
<td>Hyaluronidase + AB (pH 2.5)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>24.</td>
<td>Pepsin + AB (pH 2.5)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Colours: P = Pink, B = Blue, PB = Purple blue, BP = Blue purple, 0 = Orthometachromacia, M = Metachromacia.
of staining (Plate No. 10, Fig. No. 8), with the diagnostic histochemical reactions of these mucosubstances. The staining for diastase-resistant PAS reactive neutral mucosubstances did not show any change, thus indicating that this mucosubstance remained practically at the same concentration as in earlier phase of the treatment.

iv) Secretion: The luminal secretion of the seminal vesicles exhibited moderate to intense PAS reactivity (Plate No. 10, Fig. No. 6). All the remaining histochemical reactions exhibited similar results excepting increased staining intensity over that described for muscular coat in first phase of the treatment. The results of all these histochemical reactions clearly indicated increased amount of diastase resistant PAS reactive neutral mucosubstances, glycogen and sialic acid in secretion.

5.2.4. Lipids:

Biochemical observations:

Lipid analysis of seminal vesicle during various time durations of Butea leaf extract treatment implicated a duration dependent response.

a) Alterations in the TL values:

The quantitative alterations with statistical variations in TL of seminal vesicles during Butea leaf extract induced aspermato genesis are recorded in Table No. 26 and these alterations are graphically illustrated in Graph No. 32.

The value of TL of seminal vesicle of control rats was 75.984 ± 3.350 mg/g. After 15 and 30 days of the treatment the TL values decreased to 65.030 ± 1.860 and 58.821 ± 1.415 mg/g wet weight of the tissue. The next two time intervals witnessed enhancement but the values were somewhat less than the control TL values. The TL values
Table 26: Seminal vesicle: *Butea monosperma* leaf extract induced alterations in various lipid components.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Control</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
<th>60 days</th>
<th>75 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>75.98±3.50</td>
<td>65.03±1.86</td>
<td>58.82±1.415</td>
<td>72.2±2.38</td>
<td>72.89±3.106</td>
<td>78.12±3.79</td>
<td>80.69±3.98</td>
</tr>
<tr>
<td>PL</td>
<td>15.087±0.557</td>
<td>15.985±1.125</td>
<td>17.51±1.910</td>
<td>16.77±2.220</td>
<td>15.02±2.180</td>
<td>16.90±1.255</td>
<td>18.305±2.380</td>
</tr>
<tr>
<td>LPC</td>
<td>1.125±0.305</td>
<td>1.165±0.31</td>
<td>1.27±0.34</td>
<td>1.22±0.33</td>
<td>1.095±0.029</td>
<td>1.23±0.332</td>
<td>1.33±0.34</td>
</tr>
<tr>
<td>SPG</td>
<td>1.047±0.213</td>
<td>1.109±0.221</td>
<td>1.21±0.230</td>
<td>1.16±0.221</td>
<td>1.042±0.212</td>
<td>1.173±0.232</td>
<td>1.270±0.241</td>
</tr>
<tr>
<td>PC</td>
<td>5.215±1.110</td>
<td>5.55±1.110</td>
<td>6.04±1.345</td>
<td>5.7±1.126</td>
<td>5.345±0.835</td>
<td>5.869±2.26</td>
<td>6.321±2.49</td>
</tr>
<tr>
<td>PI</td>
<td>1.980±0.215</td>
<td>2.09±0.241</td>
<td>2.29±0.262</td>
<td>2.20±0.260</td>
<td>1.97±0.210</td>
<td>2.21±0.261</td>
<td>2.402±0.292</td>
</tr>
<tr>
<td>PS</td>
<td>1.675±0.385</td>
<td>1.76±0.396</td>
<td>1.937±0.410</td>
<td>1.849±0.362</td>
<td>1.663±0.388</td>
<td>1.871±0.370</td>
<td>2.021±0.428</td>
</tr>
<tr>
<td>PE</td>
<td>2.715±0.560</td>
<td>2.86±0.57</td>
<td>3.14±0.62</td>
<td>3.01±0.61</td>
<td>2.69±0.49</td>
<td>3.037±0.62</td>
<td>3.28±0.680</td>
</tr>
<tr>
<td>PA</td>
<td>1.330±0.254</td>
<td>1.40±0.261</td>
<td>1.54±0.282</td>
<td>1.47±0.267</td>
<td>1.32±0.251</td>
<td>1.49±0.281</td>
<td>1.61±0.28</td>
</tr>
<tr>
<td>NL</td>
<td>60.897±2.960</td>
<td>52.07±2.160</td>
<td>47.109±1.960</td>
<td>56.24±2.49</td>
<td>58.39±3.075</td>
<td>62.58±3.230</td>
<td>64.647±3.550</td>
</tr>
<tr>
<td>DG</td>
<td>17.615±1.005</td>
<td>15.05±0.920</td>
<td>13.62±0.821</td>
<td>16.26±0.980</td>
<td>16.88±0.992</td>
<td>18.09±1.12</td>
<td>18.69±1.220</td>
</tr>
<tr>
<td>CHO</td>
<td>3.810±0.430</td>
<td>3.256±0.670</td>
<td>2.947±0.412</td>
<td>3.518±0.355</td>
<td>3.653±0.385</td>
<td>3.925±0.545</td>
<td>4.44±0.605</td>
</tr>
<tr>
<td>CE</td>
<td>2.237±0.257</td>
<td>1.88±0.210</td>
<td>1.70±0.201</td>
<td>2.03±0.212</td>
<td>2.10±0.220</td>
<td>2.26±0.241</td>
<td>2.33±0.261</td>
</tr>
<tr>
<td>FFA</td>
<td>1.89±0.410</td>
<td>1.616±0.39</td>
<td>1.46±0.32</td>
<td>1.745±0.401</td>
<td>1.812±0.408</td>
<td>1.942±0.430</td>
<td>2.006±0.446</td>
</tr>
</tbody>
</table>

(Values are mean ± S.D. of five animals and are expressed in mg/g wet weight of the tissue).
Graph No-32

Seminal vesicle -
Alterations in TL, NL, PL.
(mg lipid/g wet weight of tissue)
were $70.2 \pm 2.38$ and $72.89 \pm 3.106$ mg/g wet weight of seminal vesicle on 45th and 60th days of the extract treatment. The 75th and 90th days showed an increasing trend in TL values but the increase was not significant. The TL values were $78.12 \pm 3.79$ and $80.69 \pm 3.98$ mg/g wet weight of tissue on 75th and 90th days respectively.

b) Alterations in PL values:

The quantitative changes with statistical variations in total PL in seminal vesicles at various time intervals are recorded in Table No.26 and these alterations are graphically illustrated in Graph No.32.

The average value of total PL of seminal vesicles of control animal was $15.087 \pm 0.557$ mg/g wet weight of tissue. First 30 days of the treatment resulted in an increase in PL values of seminal vesicles. The values were $15.985 \pm 1.125$ and $17.511 \pm 1.910$ mg/g wet weight of tissue on 15th and 30th days respectively. The values decreased and were $16.775 \pm 2.220$ and $15.024 \pm 2.180$ mg/g on 45th and 60th days of the extract treatment. Then there was increase in the values of PL, which were $16.900 \pm 1.255$ and $18.305 \pm 2.390$ mg/g on 75th and 90th days of the Butea leaf treatment respectively.

c) Alterations in individual components of PL:

The quantitative alterations with statistical variations in individual components of PL in seminal vesicles during the extract treatment are recorded in Table No.26 and illustrated graphically in Graph No.33. The separation of these components is shown in TLC Plate No.4.

The TLC separation of the PL components of seminal vesicles at various time intervals indicated the presence of LPC, SPG, PC, PI, PS, PE and PA. When the values of individual components are critically
Seminal vesicle -
Alterations in PL components
( mg lipid/g wet weight of tissue)

Graph No-33

Duration (in days)
studied from Table No. 26, it could be seen that at a comparative level quantitatively PC and PE were present in major concentrations, PI and PA were in moderate quantities, whereas LPC, SPG and PS occurred in poor concentrations.

The quantification studies on the individual constituents of PL indicated that their alterations during induced aspermatogenesis run parallel to the changes described for total PL. The values of PC in control rats was 5.215 ± 1.110 mg/g. First 30 days of the treatment witnessed an increase in the PC values which were 5.55 ± 1.110 and 6.04 ± 1.345 mg/g on 15th and 30th days of the treatment respectively. Then next 30 days exhibited gradual depletion. The values were 5.700 ± 3.115 and 5.345 ± 0.835 mg/g on 45th and 60th days of the treatment respectively. The PC values were increased on 75th and 90th days. The values were 5.869 ± 2.26 and 6.321 ± 2.49 mg/g wet weight of tissue on the mentioned days respectively. The alterations in PE, PS, PA and PI ran parallel to those described for total PL. The concentrations of LPC and SPG showed insignificant changes.

d) Alterations in NL values:

The biochemical changes in the quantity of the NL with statistical variations in the seminal vesicle during Butea leaf extract induced aspermatogenesis at different time durations are recorded in Table No. 26 and these alterations are illustrated graphically in Graph No. 32.

The alterations occurring in the NL paralleled those observed in the TL described earlier. The NL values of control seminal vesicles were 60.897 ± 2.960 mg/g wet weight of tissue. Like TL the NL values exhibited initial depletion in the first thirty days of the treatment, the values being 52.07 ± 2.160 and 47.109 ± 1.960 mg/g on 15th and 30th
days of the treatment respectively. The values increased in the next thirty days of the treatment and were 56.24 ± 2.49 and 58.398 ± 3.075 mg/g on 45th and 60th days of the treatment. Then there was gradual increase and the values were 62.588 ± 3.230 and 64.647 ± 3.580 on 75th and 90th days of the treatment respectively.

e) Alterations in individual components of NL:

The quantitative alterations with statistical variations in individual components of NL of seminal vesicles at various phases of the extract treatment are recorded in Table No.26 and these alterations are illustrated in Graph No.24. The alterations observed in individual components of NL at various time intervals in TLC separations are shown in TLC Plate No.4.

The TLC separations of the NL components from the seminal vesicles during the induced aspermatogenesis indicated the presence of MG, DG, TG, CHO, CE and FFA. When the values of these individual components are critically studied from Table No.26, it could be seen that at a comparative level TG was the most important component of NL. Next to TG came DG, MG and CHO, whereas CE and FFA were present in least concentrations. At a general level, it could be seen that the alterations occurring in the NL components run parallel to the alterations occurring in the total NL. TG and CHO exhibited an interesting behaviour during induced aspermatogenesis, so these are dealt with separately.

The response of TG of seminal vesicle to induced aspermatogenesis consisted of an initial decrease over the value of control. The value of TG in control rats was 24.535 ± 2.510 mg/g. On the 15th day of the treatment, the value decreased to 20.97 ± 1.910 mg/g wet weight of seminal vesicle. The 30th day of the treatment witnessed further decrease in TG value. The value was 18.982 ± 1.692 mg/g. On the 45th
Seminal vesicle
Alterations in NL components
(me lipid/g wet weight of tissue)

Graph No-3 4

Duration (in days)

mg lipid

CHO
CE
FFA

Duration (in days)
day there was an elevation in the value but it was below the control level and it was 22.66 ± 2.35 mg/g. The increase continued further for the next duration and the value was 23.53 ± 2.870 mg/g on the 60th day of the treatment. Then there was progressive increase and the values on 75th and 90th days of the treatment were 25.219 ± 2.520 mg/g and 26.048 ± 2.85 mg/g wet weight of tissue.

Though CHO was not the major component of NL when compared to the DG and MG, it also showed changes during induced aspermatogenesis. The value of CHO in the control seminal vesicle was 3.810 ± 0.430 mg/g wet weight of tissue. First thirty days of the treatment exhibited a gradual decrease and the values were 3.256 ± 0.670 and 2.947 ± 0.412 mg/g wet weight of the tissue on the 15th and 30th days of the treatment. Next thirty days of the treatment witnessed an increase, the values on 45th and 60th days of the treatment were 3.518 ± 0.355 and 3.653 ± 0.365 mg/g respectively. Last thirty days witnessed a progressive increase and the values were 3.925 ± 0.545 and 4.44 ± 0.605 mg/g wet weight of the tissue on 75th and 90th days of the treatment respectively.

The values of the remaining components of neutral lipids such as DG and MG exhibited alterations which were more or less similar to those in TG except for few minor variations. The concentrations of CE and FFA showed insignificant changes.

5.2.5. Proteins:

Biochemical changes in the proteins of seminal vesicles due to administration of Butea monosperma leaf extract are given in Table D and these alterations are graphically illustrated in Graph No.35.

The values of total proteins of seminal vesicles of control rats were 56.22 ± 2.83 mg/g wet weight of tissue. After fifteen days of the
Alterations in total proteins of Seminal Vesicle (mg/g wet weight of tissue)

Graph No-35

Duration (in days)

Total proteins (mg)
treatment the total proteins increased to $70.23 \pm 6.2$ mg/g wet weight of seminal vesicles. From 30th to 45th days of the treatment the values of proteins decreased, and were slightly below the level of control. On both days the value was $53.91 \pm 4.71$ mg/g of tissue. On 60th day of treatment the value increased and it was $62.23 \pm 5.61$ mg/g. The last two phases of treatment witnessed slight depletion, but the values were numerically more than the control values. The values were $57.75 \pm 4.99$ and $59.86 \pm 5.12$ mg/g wet weight of tissue on 75th and 90th days of extract treatment respectively.

Table D: *Butea monosperma* leaf extract induced changes in total proteins of seminal vesicles.

<table>
<thead>
<tr>
<th>Control</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
<th>60 days</th>
<th>75 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56.22</td>
<td>70.23</td>
<td>53.91</td>
<td>53.91</td>
<td>62.23</td>
<td>57.75</td>
<td>59.86</td>
</tr>
<tr>
<td>± 3.83</td>
<td>± 6.2</td>
<td>± 4.71</td>
<td>± 4.71</td>
<td>± 5.61</td>
<td>± 4.99</td>
<td>± 5.12</td>
</tr>
</tbody>
</table>

(Values are mean ± S.D. of five animals and are expressed in mg protein per gram wet weight of seminal vesicles).
III. DISCUSSION

In this and the next two ensuing chapters the organs discussed are of glandular nature and their main function is secretion. There is direct relation between the testes and these accessory sex glands. The secretion of these glands is concerned mainly with the viability of sperms and it is also useful as nutritious material and cleansing fluid. The secretion of these glands when mixed with spermatozoa form semen, in this milieu spermatozoa are motile. The epithelia of all these glands reflect alterations in the testosterone titre through cell atrophy, lysosomes formation and development of lipofuscin granules.

The characters of the secretions of these sex accessories differ greatly as the glands do in origin. In several species, including the rat, the seminal vesicle secretion contributes a substantial portion of the whole ejaculate compared with the prostatic fluid. The secretion of seminal vesicle is usually less acidic, sometimes distinctly alkaline, has a higher dry weight and contains more potassium bicarbonate, acid soluble phosphatase and proteins (Mann, 1964). In two species, man and stallion, a relationship has been claimed to exist between certain properties of the seminal plasma and male infertility (Rozin, 1961). In seminal plasma of stallion if nonprotein sulphhydryl concentrations are increased, it reduces the fertility (Werthessen, 1956; Marden and Werthessen, 1956; Hagg and Werthessen, 1956; Hagg, 1956).

*Butea monosperma* leaf extract induced histological, biochemical and histochemical alterations in seminal vesicle during aspermatogenic condition with all the parameters including wet weight changes are discussed below.
3.1. **Wet Weight and Histology**

In the present investigation it is observed that the wet weight of seminal vesicle is increased due to the administration of *Butea* leaf extract. Such increase mainly seems to be due to the accumulation of secretion in the lumina during the extract treatment. Except the work of Flickinger (1978) with testosterone administration, the present finding does not find any parallel. Most of the workers studying the effects of antifertility agents have reported decrease in wet weight of seminal vesicles. The antifertility agents which were tried are anti-ICSH serum (Gambal, 1967), SK and F 7690 (Saunders et al., 1969), estrogen (Elkington and Blackshaw, 1971), norgesterol (Sing et al., 1972), α-chlorohydrin (Vickery et al., 1974; Hundal and Mangat, 1978), cyproterone acetate (Prasad et al., 1977), medroxyprogesterone (Flickinger, 1977), centchroman (Das, 1977), CdCl₂ (Sakensen, 1977), cyclohexenol (Dixit et al., 1979) and chloromadinone acetate (Kaur and Mangat, 1979). Like these antifertility chemicals few plant extracts such as *Malaviscus conzamtii* (Dixit, 1977), *Aristolochia indica* (Pakrashi and Pakrasi, 1977), *Calotropis procera* (Garg, 1979) and *Allium sativum* (Dixit and Joshi, 1982) have also been reported to induce decrease in wet weight of this organ. Antiserum of FSH (Gambal, 1967) and *Papaya* seed extract (Das, 1980) do not induce any change in the wet weight of seminal vesicles.

*Butea* leaf extract seems to affect mucosal cells, muscular coat and luminal secretion with the possible exception of lamina propria of the seminal vesicles. The leaf extract induces many changes in all these structures. The epithelial cells lining the mucosa show slight reduction in height which is observed conspicuously in later phases of extract treatment. The mucosal folds which are highly arborized and reach up to the center of the lumen of seminal vesicles in the control
get reduced in height and in arborization. In some instances these folds appear as mere stubs. The muscular coat which consists of inner circular and outer longitudinal layers shows thickening in first 45 days and detachment of inner circular layer from outer layer in next 45 days. The muscles show high eosinophilic staining. Due to the reduction in the height of mucosal folds and decreased arborization the lumina of seminal vesicles appear to be increased. Many lumina contain secretion but few of them are devoid of secretion. In few lumina, the secretion is present only in the depths of the crypts. Opaqueness of the secretion appears reduced after the treatment.

The reduction seen in the height and the arborization of mucosal folds of seminal vesicles in the present work resembles that reported with *Malvaviscus conzantii* extract administration for 50 days in mice by Verma et al. (1980). They reported that the extract caused progressive reduction in the size of the seminal vesicles and significant inhibition in the arborization of the secretory epithelium. In present studies it is further observed that the height of the mucosal cells gets slightly reduced. This observation finds a good parallel in the work of Cavazos and Melampy (1954) on rats and Allison (1954) on mice. After castration they found statistically significant reduction in the height of the epithelium. Similar findings were also reported by Dahl and Tveter (1974) after administration of cyproterone acetate, Flickinger (1977) with mecroxyprogesterone treatment, after hypoxia by Riar and Malhotra (1977), by Verma et al. (1980) after administration of *Malvaviscus conzantii* extract to mice. In this laboratory similar results were observed by Shan (1988) after administration of Daucus carota seed extract, by Toro (1984) with *Vinca rosea* alkaloids and by Sohani (1985) with *Vitex negundo* leaf extract to rats.
In the present investigation it is also observed that the luminal secretion is reduced in few lumina due to extract treatment. This observation is in good accordance with the findings reported by various investigators such as administration of testosterone (Limanowask et al., 1968), SK and F 7690 (Saunders et al., 1969), chlorocyclazaine (Wong, 1972), cyproterone acetate (Agma, 1975; Prasad, 1977), CdCl₂ (Sakensen, 1977), a-chlorohydrin (Hundal and Mangat, 1978), chloromadinone acetate (Kaur and Mangat, 1979), Aristolochia indica extract (Pakrasni and Pakrasri, 1977), Malvaviscus conzantii extract administration (Verma et al., 1980). Similar observations are also made in this laboratory with Vinca rosea alkaloids (Toro, 1984), Vitex negundo leaf extract (Sohani, 1985) and Daucus carota seed extract (Shah, 1985).

An inhibitory influence on the functions of the rat seminal vesicles is shown with reserpine. It specifically acts on secretory epithelium leading to marked atrophic changes in these cells (Tuchman-Duplessis, 1956; Eranko et al., 1957). Such a significant atrophy in the mucosal cells of seminal vesicles is not observed in the present investigation. The vacuolization in the mucosal cells of seminal vesicles was reported by Wong (1972) and Balsubrahmaniam et al. (1980) after administration of chlorocyclazaine and aspirin respectively. After Butea monosperma leaf extract administration to rats such a type of vacuolization is not observed in the mucosal cells of seminal vesicles.

5.3.2. Enzymes:

a) Lysosomal enzymes:

Three lysosomal enzymes, β-glucuronidase, acid phosphatase and nonspecific esterases were selected for study in the present investi-
A critical comparative evaluation of the observations on the alterations in the structure of the seminal vesicle and in the lysosomal enzyme activities indicates that there is good relationship between the lysosomal enzyme activity and changes in the mucosal folds of seminal vesicles. The important features of these enzymatic alterations caused due to extract administration are (i) Initial enhancement in enzyme activities of β-glucuronidase, acid phosphatase and nonspecific esterase at the initiation of the extract administration up to 30th day of the treatment. (ii) The two lysosomal enzymes β-glucuronidase and acid phosphatase show continued enhancement up to 45th day, but they get depleted on 60th day of extract treatment. The nonspecific esterase activity decreases on 45th day but it is increased more than in control on 60th day. (iii) In the final phase of experiment gradual increase in β-glucuronidase and acid phosphatase, while gradual decrease in nonspecific esterase in last 30 days are witnessed. But the increase and decrease in nonspecific esterase is statistically very insignificant.

The available literature on alterations in these lysosomal enzymes after administration of various synthetic chemicals and anti-spermatogenic agents shows that these enzymes undergo certain interesting changes. Stafford et al. (1949) and Mann and Mann (1951) demonstrated low acid phosphatase activity after castration in rats. Porter and Melampy (1952) observed decrease in histochemical localization of acid phosphatase after castration. Stilbestrol treatment increased number of lysosomes in secretory cells of seminal vesicles, (Cavazos, 1963). Kind-Hunsel (1974) found reduction in acid phosphatase histochemically after castration in epithelium. Dixit and Niemi (1975) showed increased acid phosphatase activity in nonhypophysectomised
castrated rats. Histochemical reduction of esterase in epithelial cells after hypophysectomy was shown by Verne and Herbert (1952), while increase in nonspecific esterase after administration of testosterone was demonstrated by Limanowski and Miskowiak (1970), Scott and Persaud (1977) showed increase in acid phosphatase activity histochemically in the columnar epithelium of mucosa after administration of acetyl salisylic acid. The available literature shows that there is no work on record on effects of antifertility agents on seminal vesicular β-glucuronidase and effects of plant preparations on lysosomal enzymes.

At the termination of the experiment acid phosphatase activity is seen increased in the present investigation. Similar results were demonstrated by Cavazos (1963) after administration of stilbestrol, by Dixit and Niemi (1975) in nonhypophysectomized castrated rats, by Scott and Persaud (1977) after administration of acetyl salisylic acid, by Toro (1984) with *Vinca rosea* and by Sohani (1985) with *Vitex negundo* extract administration. In the present studies it is observed that the mucosa, muscular coat and secretion exhibit reduced histochemical activity of acid phosphatase. This observation finds good resemblance in the histochemical investigations on acid phosphatase after castration by Porter and Melampy (1952), Melampy and Cavazos (1963), Maggi et al. (1970) and Kind-Hensel (1974). In the present investigation it is also found that the nonspecific esterase gets reduced biochemically as well as histochemically, this is in accordance with the observations made by Verne and Herbert (1952) on hypophysectomised rat seminal vesicular nonspecific esterase. In this laboratory Shan (1985) with *Daucus* and Sohani (1985) with *Vitex* demonstrated increase, but Toro (1984) with *Vinca rosea* alkaloids found no changes in enzyme activity.
The work on castration by Stafford et al. (1949), Melampy and Cavazos (1963), Limanowski and Miskowiak (1970), Maggi et al. (1970), Kind-Hensel (1974) and Dixit and Niemi (1975) showed that the acid phosphatase and β-glucuronidase of seminal vesicles are under the control of testicular androgen. Further in their studies on β-glucuronidase Baile (1975) and Kanase (1978) also suggested its androgen dependency in seminal ampullae of seasonally breeding vertebrates especially bats. In the present investigation the earlier work on testes of Butea leaf extract treated rats has shown that the Leydig cells are not affected significantly. Exact information on the androgenic state of the Leydig cells cannot be discerned from such histological studies only. The observations on the acid hydrolases in seminal vesicle indicate that the androgenic levels in the treated rats might not be undergoing any significant change. Had there been any important effect on the Leydig cells and the androgenic production leading to depletion in the androgen levels, the seminal vesicular acid hydrolase levels would have fallen very significantly, and such depletion would have occurred progressively with time. But actually in the present observations it is found that the enzyme activities especially of β-glucuronidase and acid phosphatase increase very significantly in the last phase of the experiment i.e. on 75th and 90th days of the extract treatment.

Hence it has to be concluded that the alterations in the seminal vesicular acid hydrolases of the Butea leaf extract treated rats are not due to any androgenic imbalance, but mostly due to the changes occurring in the seminal vesicular structures. Part of the enzymatic alterations also appears to be due to alterations in the mucosal cells as seen from the histochemical observations. Such a behaviour of
enzymes in the present investigation is very much indicative of degenerative changes.

b) Alkaline Phosphatase:

A critical evaluation of the behaviour of the alkaline phosphatase activity during induced aspermatogenesis caused by Butea leaf extract shows (i) Initial enhancement in the enzyme activity in first 15 days of treatment. (ii) Then very insignificant decrease in next 15 days. (iii) Gradual increase in enzyme activity in next 30 days leading to two fold enhancement over control. (iv) Gradual decrease in the activity at the end of the experiment.

Histochemically the enzyme shows increase in first phase but significant decrease in the activity in the second phase showing overall decrease in staining in mucosa, lamina propria, muscular coat and luminal secretion.

A critical review of the behaviour of alkaline phosphatase in the induced interference in the male sterility shows many variations. Castration reduced the activity significantly in seminal vesicles (Stafford et al., 1949; Porter and Melampy, 1952; Cavazos and Melampy, 1954). Similar decrease was noticed after administration of CdCl₂ by Chinoy and Sheth (1977) and with treatment of α-chlorohydrin by Hundal and Mangat (1978). Dixit (1977) demonstrated no change in the enzyme in the epithelial cells of seminal vesicles of gerbils and rats with Malvaviscus conzantii extract. Similar observations were made by Verma et al. (1980) with same plant. Shah (1985) demonstrated decrease in the enzyme activity histochemically as well as biochemically after administration of Daucus carota seed extract, while Sohani (1985) with Vitex negundo leaf extract and Toro (1984) with Vinca rosea alkaloid showed insignificant changes in the enzyme activity.
Alkaline phosphatase activity in this investigation shows decreasing trend towards the end of the experiment biochemically. This observation slightly shares the findings of Stafford et al. (1949), Porter and Melampy (1952), Cavazos and Melampy (1954) who worked on effects of castration on alkaline phosphatase. In general it can be said that the alkaline phosphatase in treated rats does not show any significant changes.

5.3.3. Mucosubstances:

The present part of the discussion deals with the nature of mucopolysaccharides in the seminal vesicles of rats treated with Butea leaf extract. The significant paucity of the information on the mucosubstances in the seminal vesicles during impaired reproductive physiology has already been brought to notice in the introductory part of this chapter. The literature on the seminal vesicle mucosubstances in induced aspermatogenesis is also very poor in comparison with literature available on normal seminal vesicles.

Large amount of glycogen has been demonstrated in cells of seminal vesicles of ram (Aitkin, 1955), bull (Sajonski et al., 1972; Cons, 1959) and bats (Pawar, 1976; Vibhute, 1980). Seminal vesicular epithelium has a moderate PAS reaction in hamster (Feagans et al., 1961) and rat (Leblond, 1950; Melampy and Cavazos, 1953; Zagarese, 1958). Filotto (1962) observed two types of PAS substances, one was granular and intensely coloured with PAS and the second substance was diffusely distributed with less staining. Aughey (1969) reported that the secretory epithelium of the seminal vesicles secrete a complex carbohydrate in red deer. Rzeszowska (1966b) identified acid mucopolysaccharides in seminal epithelium of rats. In hedgehog seminal vesicles presence of mucoproteins, mucopolysaccharides and glycolipids
was shown by Bidwani and Bawa (1972). Fouquet (1971) detected sialic acid in seminal vesicle of Chinese hamster. Fournier (1973) showed that in man the principal source of sialic acid in semen is seminal vesicles. Histochemically Pawar (1976) and Vibhute (1980) showed the presence of sialic acid in ampullae of Henle and seminal vesicles of few bats. Biochemically the presence of sialic acid was also shown in number of animals, such as monkey (Bose and Kar, 1968), rat (Bose et al., 1966) and man (Warren, 1959). Presence of sulfomucins was not shown in seminal vesicles of animals except in the bats (Pawar, 1976; Vibhute, 1960). Karaginnidis (1972) observed sialic acid in human and bovine seminal plasma.

The literature is very scanty on effects of antifertility agents on mucosubstances of seminal vesicles. Aitken (1955) reported no changes in glycogen contents of seminal vesicles in ram even after one year following castration. Rzeszowska (1966) found depletion in sialic acid after castration, similar results were obtained in monkey by Bose and Kar (1968). Testosterone treatment increased the amount of mucosubstances (Limanowski and Miskowiak, 1970). Singh et al. (1963) found increased amount of glycogen after testosterone administration in rats. Depletion of glycogen, sialic acid and neutral mucopolysaccharides was shown during quiescent period in the breeding cycles of few bats (Pawar, 1976; Vibhute, 1980), while depletion in only glycogen and sialic acid was shown by Shah (1985) and Sohani (1985) after administration of Daucus carota seed extract and Vitex negundo leaf extract respectively.

In the present investigation it is found that the mucosal epithelial cells of the control rats contain more amount of diastase resistant PAS reactive neutral mucosubstance, glycogen and sialic acid. In the treated rats all these mucosubstances are present in increased
amounts in the mucosal cells. Similar observations have been made by only after testosterone treatment (Limanowski and Miskowiak, 1970; Singh et al., 1968).

The lamina propria of seminal vesicle of treated rats did not exhibit any type of mucopolysaccharides. The muscular coat of seminal vesicle after administration of *Butea* leaf extract shows increased amount of glycogen, sialic acid and no change in diastase resistant PAS positive neutral mucopolysaccharide. No work is on record about change in mucins of muscular coat of seminal vesicles except that done in this laboratory. Shah (1965) and Sohani (1965) made similar observations with the administration of *Baucus* seed and *Vitex* leaf extract respectively.

The luminal secretion of seminal vesicle also shows increased amount of diastase resistant PAS reactive neutral mucopolysaccharides, glycogen and sialic acid. This observation is in good accordance with the observations of Singh et al. (1968) and Limanowski and Miskowiak (1970) who observed increased amount of glycogen in testosterone treated animals. Increase in glycogen and sialic acid was also shown by Toro (1984) with *Vinca rosea* alkaloids in rats.

The results obtained following castration and hormone replacement indicate that the seminal vesicles also act as a sensitive parameter for androgen levels (Aitken, 1955; Singh et al., 1968; Bose and Kar, 1968; Rzeszowska, 1967; Limanowski and Miskowiak, 1970). The mucosubstances in the bat ampullae of Henle and seminal vesicles are also shown to be controlled by circulating androgen levels (Pawar, 1976; Vibhute, 1980).

The secretion of seminal vesicles shows an increase in the mucosubstances in the present work, thus, indicating that there is no
depletion in the androgen level in the bodies of the treated rats. It seems that the seminal vesicles of the *Butea* leaf extract treated rats elaborate more amounts of the mucosubstances and secrete them into the lumen.

5.3.4. **Lipids**

The present part of the discussion deals with the alterations taking place in the total lipids and in the components of neutral lipids and phospholipids of the seminal vesicles of rats administered with *Butea monosperma* leaf extract over the period of 90 days. Insipite of their important role in the testicular physiology, there is little information on the role of lipids and their components in the secretory process of male sex accessory glands. The literature on the lipids of seminal vesicle is poor and the review of the available literature dealing with changes in the seminal vesicular lipids during induced aspermatogenesis also shows that the literature on this aspect is also equally poor. Those workers who have studied the effects of plant preparations on reproductive tract have not given any attention to the changes in the seminal vesicular lipids. Man (1964) has also not mentioned the presence and role of lipids in the seminal vesicles in his studies on biochemical composition of seminal plasma of man, bull, boar, rat and guinea pig. Flickinger (1974) in his electron microscope radioautographic studies on secretion of seminal vesicle has not investigated lipids in the seminal vesicle. Mann et al. (1949) and Dubois (1964) have studied lipid histochemically.

When the lipid alterations of seminal vesicle during induced aspermatogenesis are viewed, it can be seen that first 30 days of the extract treatment witness a decrease in TL values. Then next 30 days show an enhancement in the TL values over the previous phase and last
30 days witness continued increase in lipid values. Maximum lipid values were seen at the end of the experiment. The alterations occurring in the NL parallel those observed in the TL and consist of initial depletion in the concentrations during first 30 days, then insignificant increase in the concentrations of NL on 45th and 60th days of the treatment and final increase in NL in last phase of 30 days. The values of NL are maximum at the end of the experiment.

When composition of NL is studied the significant fact which is immediately noticed is that during all the intervals of the treatment TG is the major NL components, DG, MG, CHO and CE being present in moderate concentrations. Out of all these six components TG and CHO show an interesting pattern of alterations. The TG shows initial decrease in first 30 days, next 30 days witness slight increase over previous phase and at the end of the experiment the values of TG are above the control values. The behaviour of CHO is also like that of NL and TG, and it also increases at the end of the experiment.

Total values of PL when viewed together it is seen that the PL also increases in the seminal vesicle of the treated rats. When compositions of PL is observed it is noticed that PC and PE are the major PL components, PA, PS and PI being present in moderate concentrations, while LPC and SPG exhibit poor concentration. The values of PC and PE are increased at the initiation of the extract administration during first 30 days. In the next 30 days the values of PC remain depleted and are more or less equivalent to control values, while PE values also remain stationary but above the control values. At the end of the experiment both PC and PE values show an enhancement.

The observations of all lipid components when viewed together it could be seen that the Butea leaf extract induces increase in the
values of TL, NL, PL, PC, PE, TG and CHO in seminal vesicle, while there is no change in the values of LPC, SPG, PI, PA and FFA.

Very few reports are available on effects of antifertility agents on lipids of seminal vesicles. Gambal (1967) demonstrated that bovine anti-ICSH serum treatment increased the concentration of TL and all components of PL, while FSH antiserum had no effect on PL. Karger et al. (1972) demonstrated increase in PC and PI after administration of testosterone. Umaphathy et al. (1979a) observed that cyproterone and cyproterone acetate brought about a marked decrease in TL by depleting NL markedly and PL to lesser extent, they also reported decrease in glycerides and CHO. Medroxyprogesterone acetate treatment resulted in decrease in TL, NL, PL, CHO and glycerides (Umaphathy et al., 1979b). Seminal vesicular lipids were increased after administration of testosterone, while they decreased due to estrogen, progesterone and prolactin influence (Umaphathy et al., 1979c). The castration of rats resulted in decrease in TL, MS, DG, PC and PE (Umaphathy, 1980).

In this laboratory Sohani (1982) with administration of Vitex leaf extract and Toro (1984) with Vinca alkaloids also observed changes in lipids mostly similar to those observed in the present investigation. Hence at a general level it can be said that aspermatogenesis caused either by hormonal treatment or plant extract treatment leads to some increase in seminal vesicular TL, PL, NL, TG, CHO, PC and PE. There appears to be an accumulation of these lipids in the seminal vesicles, after the induction of aspermatogenesis.

5.3.5. Proteins:

Although much work has been done cytochemically less attention seems to have been paid to biochemical investigations of seminal vesicular total proteins in the induced aspermatogenesis. The seminal
vesicles of mammals secrete protein into semen (Price and Williams-Ashman, 1961).

Biochemically it can be seen that the *Butea* leaf extract administration over 90 days induces increase in total proteins of seminal vesicle in first phase of the treatment, but a depletion in the next 30 days. The total protein values remain more or less similar to control in the last phase of the extract treatment. Thus, it seems that the *Butea* extract does not exert any significant influence over the total proteins of seminal vesicles.

The review of the available literature shows that there is poor information on the effects of antifertility agents on total proteins of seminal vesicles. Chinoy and Sheth (1977) found increased value of proteins after administration of CdCl$_2$ to rats, while Tyagi et al. (1979) demonstrated decrease in protein due to treatment of cyclohexanol. *Calotropis procera* extract (Garg, 1979), *Malvaviscus conzantii* flower extract (Verma et al., 1980), oral feeding of *Allium sativum* powder (Dixit and Joshi, 1982) and seed extract of *Daucus carota* (Shan, 1985) resulted in decrease in proteins, while *Vinca rosea* alkaloids (Toro, 1984) and *Vitex* leaf extract (Sohani, 1985) did not lead to any remarkable change in total protein.

In the present investigation it is observed that the total proteins do not exhibit any remarkable alteration after administration of *Butea* leaf extract. Similar observations were made by Toro (1984) and Sohani (1985).

When the alterations in various enzymes, lipids, mucosubstances and proteins in the seminal vesicles caused by administration of *Butea* leaf extract are viewed together, an interesting fact comes to light. As a result of *Butea* extract administration all the enzymes under
investigation and metabolites, such as lipids, mucins and proteins are increased, some of them vary significantly and other like proteins insignificantly. There are fluctuations in the values of enzymes and metabolites during 90 days of the treatment, but the final result is a definite enhancement. Such enhancement in enzyme and other metabolites is accompanied by some insignificant decrease in the height of the epithelial cells and distinct decrease in arborization. Thus the Butea leaf extract seems to induce a structural hypotrophy and a biochemical metabolic hypertrophy in the seminal vesicles. Practically similar results are also obtained in case of prostate glands and Cowper's glands as can be seen from the next two chapters. Finally it can be emphasized here that the conclusions drawn here are from circumstantial evidence at hand and also the available literature. To arrive at definite conclusion further work desirably at ultra-structural level is needed.