CHAPTER – III

EFFECT OF *OCIMUM SANCTUM* LEAVES ON CAUDA EPIDIDYMIS OF ALBINO RATS
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>58-61</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>62-63</td>
</tr>
<tr>
<td>OBSERVATIONS</td>
<td>64-65</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>66-70</td>
</tr>
</tbody>
</table>
INTRODUCTION

The epididymis forms one of the major male accessory reproductive organs. Along its length the ductus epididymidis is lined by tall columnar epithelium consisting of several cell types, namely principal, narrow, clear, basal and halo (intra-epithelial lymphocytes) cells. Not only does the height of the epithelium decrease along the length of the duct, but the various epithelial cellular constituents also change in quality as well as quantity in view of the inherent regional difference along the length, forming the anatomical segments, viz., initial segment, caput, corpus and cauda (Hamilton, 1975; Robaire and Hermo, 1988). The principal cell, which constitutes the major portion of the epithelium is concerned with the secretion of proteins, glycoproteins, inositol, immobilin, clusterin and inhibin, which are believed to interact with the spermatozoa, rendering them functionally mature (Hamilton, 1975; Robaire and Hermo, 1988; Cooper, 1992, 1993; Robaire and Viger, 1995). It is also involved with fluid-phase endocytosis as well as transcytosis of particulate and dissolved substances (Cooper et al., 1988; Robaire and Hermo, 1988; Yeung et al., 1991).

The narrow cell, confined to the initial segment, is the precursor of the clear cell of the distal parts of the ductus epididymidis (Robaire and Hermo, 1988). The clear cell, particularly of the cauda is believed to be concerned with the removal of the disintegrated products of the cytoplasmic droplets released from the spermatozoa, to be further acted upon by the lysosomal enzymes for ultimate removal (Hermo et al., 1988).

The role of the basal cell, the second largest constituent of the epithelium, is not yet clear though a protective role has been suggested (Robaire and Hermo, 1988; Veri et al., 1993). The halo cell is supposed to form an immunological barrier in the epithelium (Dym and Romrell, 1975; Wang and Holstein, 1983; Robaire and Hermo, 1988). Cooper (1990,
1993) has argued in defence of a function for the human epididymis, and according to him the fertilizing capacity of human sperm develops fully in the distal parts of the epididymal duct.

The epididymal duct contributes to the physiological maturation of the spermatozoa by way of secretion of several proteins, glycoproteins and small molecular weight substances. Modification of the luminal fluid through absorption and secretion of organic, inorganic ions, phagocytosis of dead and defective sperm and debris from the lumen (Robaire and Hermo, 1988; Hermo et al., 1988) assists in the maturation process. Moreover, through these activities the epididymal duct contributes to a changing luminal microenvironment, which is considered essential for the physiological maturation of the spermatozoa (Hinton and Palladino, 1995). However, the role of the various epithelial cell types in the processing of the spermatozoa and the fluid is not fully understood.

It is well established that the mammalian epididymis is adopted in an unknown way for prolonged storage of spermatozoa. It is also known that sperm survival, motility and fertilizing capacity are dependent on androgen (Elliot, 1965) either directly or via the epididymis. Androgens have been reported to prolong their survival time in the epididymis of castrated animals (Turner, 1966). Studies have demonstrated that the epididymis is capable of synthesizing and metabolizing androgens (Hamilton et al., 1969; Aafjes and Vreeburg, 1972). During recent years, the presence of compounds related to sperm motility and the effects of various chemical and physical agents on sperm motility both invivo and invitro systems have been studied extensively to reveal control mechanisms or causative factors of sperm motility more precisely (Alabi et al., 1986; Goeden and Zenick, 1985). Divergent views have been expressed in this regard. But from the results of various studies, it has become increasingly clear that sperm motility can be beneficially or detrimentally
influenced by a wide variety of physical and chemical factors. Several methods are now available to assess sperm motility under various experimental conditions (Amelar and Dubin, 1979; Nelson et al., 1980 b). Levin et al., (1980) has described a quantitative method for determining the effects of drugs on spermatozoal motility.

The ability of sperm to fertilize ova is required as they pass through a specific part of the epididymis. In rat, spermatozoa are fertile when they reach the proximal cauda region. The possession of motility alone does not infer that the spermatozoa also possess fertilizing ability.

The role that the epididymis plays in the maturation process has been controversial. It was proposed by Young (1931) that epididymal sperm maturation is an inherent process, begun in the testis and independent of the epididymis. This was based on his observation that the impregnation rate in guinea pig increased from 33% to 40% when ligation prevented spermatozoa from continuing their passage through the epididymis. If spermatozoa are retained in the epididymis just proximal to the region in which fertility is normally attained in the rabbit, these spermatozoa are able to develop fertilizing capacity (Bedford, 1967; Orgebein-Crist, 1967). Spermatozoa isolated in a more proximal region of the rabbit epididymis and in the hamster, caput and corpus epididymis fail to develop full maturity (Bedford, 1967; Orgebein-Crist, 1967; Igboeli and Foote, 1969). These data suggested the participation of particular region of the epididymis in sperm maturation.

The epididymis is an androgen sensitive gland which responds to even minor fluctuations in circulating androgens (Prasad et al., 1973). Androgens regulate the growth, structural and functional properties of the epididymis (Price and Ortiz, 1965). In rats epididymis serves two major functions, i) in the proximal region, spermatozoa undergo
maturation process where, they attain the fertilizing capacity, and ii) in the distal region, spermatozoa are stored before ejaculation (Cooper and Waites, 1974; Harris and Bartke, 1974, 1975). To effect these functions, it receives androgen by two routes from the bloodstream and from the testicular fluid which accompanies the spermatozoa (Vreeburg, 1975; Pujol et al., 1976). The importance of epididymis in sperm maturation and hence in male fertility has attracted much attention since long time (Mann and Lutwak-Mann, 1981) and is an ideal extragonadal site for the control of fertility in male by selective alternation of its functions (Prasad et al., 1970).

The present study is an attempt to evaluate the effect of Ocimum sanctum leaves (Benzene Extract) on the cauda epididymis and the approximate time required for its recovery after the withdrawal of the treatment in the laboratory rat.
MATERIALS AND METHODS

Fresh Ocimum sanctum leaves were collected from Buddanal forest nursery, which is 30 Km away from Dharwad and dried in shade. The dried leaves were coarsely powdered and subjected to soxheltation process to get the benzene extract. Benzene was separated and the extract thus obtained was allowed to dry and stored in a dessicator at 4 °C (WHO – protocol, LG-06, 1983). The benzene extract is then mixed with propylene glycol as required and administered orally (gavage) to the experimental animals.

Three months old adult male albino rats (Wistar strain) weighing 170-200 g, were obtained from the rat colony maintained in the Department. They were housed under well-ventilated light dark schedule with free access to food and water.

The animals were divided into four groups, each consisting of 5 animals.

**Group I** : The animals were given 1 ml propylene glycol/rat/day for a period of 48 days and served as control.

**Group II** : The animals were given 250 mg/kg body weight of Benzene extract (Ocimum sanctum)/rat/day for 48 days (The period of 48 days is selected/chosen keeping in mind 3 spermatogenic cycles of rat) and were autopsied 24 hours later. The group served as the test group.

**Group III** : The animals were given 250 mg/kg body weight of Ocimum sanctum (B.E.)/rat/day for 48 days and the treatment was withdrawn for 8 days and were autopsied on day 9th, after withdrawal of the treatment. The group served as the one week recovery group.

**Group IV** : The animals were given 250 mg/kg body weight of Ocimum sanctum (B.E.)/rat/day for 48 days and the treatment was withdrawn for 16 days and were autopsied on day 17th, after withdrawal of the treatment. The group served as the two week recovery group.
Twenty-four hrs. after the last dose, the control and treated animals were sacrificed by cervical dislocation. The epididymis was dissected out, blotted free from mucus and weighed to the nearest milligram.

1. **Histology and Histometry**

i) The cauda epididymis was fixed in aqueous Bouin’s fluid for 24 hours, washed thoroughly in 70% alcohol, dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. Sections of 5 μm thickness were obtained and stained in haematoxylin (Delafield’s) and eosin.

ii) For histometrical studies, the calibrated ocular micrometer (Erma, Japan) was used. From each cauda epididymis, 20 sections randomly were used in each group to record the following histometrical data:

a) Epithelial height; and

b) Nuclear diameter of the cells.

iii) After vascular perfusion, the cauda epididymis was removed and fixed in 3% glutaraldehyde, which were prepared for transmission electron microscope, post fixed in 1% Osmium tetraoxide, embedded in araldite and semi thin sections were obtained in Lieca LKB broma ultramicrotome. Such sections were immersed in 4% iron alum for half an hour to remove araldite (because, with araldite, the sections do not take stain with haematoxylin).

iv) **Statistical Analysis**

The data were presented as means ± SEM. The comparison of data for statistically significant differences was done using Student’s ‘t’ test and a probability level of P < 0.01 and P < 0.001 were considered as significant and highly significant, respectively.
OBSERVATIONS

Histological Changes (Table 1; Histogram 1)

The cauda epididymis of the control rats consisted of the tubules arranged compactly with a very little intertubular connective tissue. The epithelium was low cuboidal and ciliated along the luminal border. The cells contained prominent, spherical to oval nuclei very close to the basement membrane. The interstitium was normal with numerous interstitial cells with rounded nuclei and fibroblast like elements. The vascularity of the organ was normal. The tubular epithelium was pseudostratified, consisting of very tall columnar principal cells with long, nonmotile stereocilia and small basal cells. The stereocilia were visible. The lumen was wide and packed with evenly dispersed sperm (Figs. 1, 5 & 9).

In the treated rats there was a general reduction in the epithelial height and the nuclear diameter of the epithelial cells. The nuclei were pycnotic and the height of stereocilia was reduced. The lumen was devoid of sperm and filled with lymphocytes and debris of degenerated sperm. Intertubular fibrosis was evident. The basement membrane was thin and disrupted. The cells showed vacuolization and cell debris due to cytolysis. Few of these cells exhibited signs of degeneration. (Figs. 2, 6, 10, 11 and 12).

In one week recovery group, a partial recovery was observed in the tubules, with intertubular connective tissues. In epithelium the epithelial height and the nuclear diameter recovered partially. The lumen was wide and packed with sperm. The interstitial cells were normal as that of control. The intertubular fibrosis was reduced and the sperm appeared in the lumen. (Figs. 3, 7 and 13).
In two week recovery group, a complete recovery was observed in almost all the tubules. The epithelial height and nuclear diameter was fully recovered. The sperm were compactly arranged in the tubules. The interstitium was normal with numerous interstitial cells with rounded nuclei and fibroblast like elements. The intertubular fibrosis was completely absent and the lumen was full of sperm. The stereocilia were also seen. (Figs. 4, 8 and 12).
Table 1: Effect of *Ocimum sanctum* leaves (Benzene extract) on the body weight (g) and Epididymis weight (mg/100g body weight) and the epithelial height (µm) and the nuclear diameter (µm) of cauda epididymis of albino rats and its subsequent recovery (values are expressed as SEM of five animals).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight</th>
<th>Epididymis weight</th>
<th>100 x (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epithelial height</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(cauda)</td>
</tr>
<tr>
<td>I Control</td>
<td>195.20 ± 0.80</td>
<td>261.60 ± 1.06</td>
<td>23.35 ± 0.39</td>
</tr>
<tr>
<td>II O. sanctum</td>
<td>197.60 ± 1.12</td>
<td>249.50 ± 1.62**</td>
<td>16.95 ± 0.41***</td>
</tr>
<tr>
<td>III One week recovery</td>
<td>197.20 ± 0.97</td>
<td>259.20 ± 1.51</td>
<td>19.95 ± 0.60**</td>
</tr>
<tr>
<td>IV Two week recovery</td>
<td>198.80 ± 0.73*</td>
<td>259.85 ± 2.51</td>
<td>22.05 ± 0.52</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.001
Histogram 1: Body weight, epididymis weight, epithelial height and the nuclear diameter

- Body weight (gm)
- Epididymis weight (100 mg/Kg B.wt.)
- Epithelial height (cauda) (100X μm)
- Nuclear Diameter (cauda) (100X μm)

Legend:
- □ Control
- □ O.Sanctum
- □ One week recovery
- □ Two week recovery
DISCUSSION

The importance of epididymis in sperm maturation and hence in male fertility has attracted much attention since long time (Mann, Lutwak-mann, 1981) and is an ideal extragonadal site for the control of fertility in male by selective alteration of its functions (Prasad et al., 1970). Epididymis provides a favourable environment for motility, fertilizing ability, storage and survival of spermatozoa (Jehan et al., 1973). The epididymis provides a suitable environment for morphological and biochemical changes in spermatozoa (Orgebein-Crist, 1969). It performs both secretory and absorptive functions. Sperm maturation in the epididymis takes place because of the proteins synthesized and secreted by epididymal tissue (Klinefelter and Hamilton, 1985). Androgen deficiency causes a marked reduction in the tubular diameter, a general regression of epididymal epithelium, a rapid decline in the number of spermatozoa within the cauda epididymis and changes in the composition of epididymal plasma (Brooks, 1981).

The role that the epididymis plays in the maturation process has been controversial. It was proposed by Young (1931) that epididymal sperm maturation is an inherent process, begun in the testis and independent of the epididymis. This was based on his observation that the impregnation rate in guinea pig increased from 33% to 40% when ligation prevented spermatozoa from continuing their passage through the epididymis. If spermatozoa are retained in the epididymis just proximal to the region in which fertility is normally attained in the rabbit, these spermatozoa are able to develop fertilizing capacity (Bedford, 1967; Orgebein-Crist, 1967). Spermatozoa isolated in a more proximal region of the rabbit epididymis and in the hamster, caput and corpus epididymis fail to develop full maturity (Bedford, 1967; Orgebein-Crist, 1967; Igboeli and Foote, 1969). These data suggested the participation of particular region of the epididymis in sperm maturation.
It has been well documented that the epididymis is androgen dependent (Maneely, 1959). It has been demonstrated that sperm maturation is also androgen dependent (Dyson and Orgebein-Crist, 1973). Androgen is available either from the peripheral blood or from the luminal fluid in the epididymis (Setchell et al., 1969). Androgen may affect the sperm directly or through modification of the epididymal milieu. Several facts suggest a direct effect on the organ; spermatozoa of rats injected with tritiated testosterone are only lightly labelled compared to the epididymal tissue (Blaquier, 1971) and sperm retained in the caput epididymis remain immature even after prolonged storage in a medium rich in androgens (Bedford, 1967; Orgebein-Crist, 1967).

In present study, the treatment of Ocimum sanctum leaves on the histology of the epididymis reveals, a general reduction in the epithelial height and the nuclear diameter of the epithelial cell. The nuclei were pycnotic and the height of stereocilia was reduced. The lumen was devoid of sperm and filled with lymphocytes and debris of degenerated sperm. Intertubular fibrosis was evident. The basement membrane was thin and disrupted. The cells showed vacuolization and few of them exhibited signs of degeneration.

A partial recovery was observed one week after withdrawal of the treatment and a complete recovery was observed two week after withdrawal of the treatment. The histology of the cauda epididymis and the epithelial height reached almost normal conditions. The nuclear diameter and intertubular fibrosis being absent, the lumen was full of sperm.

The accessory reproductive organs are under the control of androgens (Mann, 1964; Akbarsha and Balasubramanian, 1982) and any change in the weight of these organs may reflect the androgenic status of the animal concerned (Aruldhas et al., 1990). In rats the weight of epididymis is androgen dependent (Brooks, 1976 a and b). Therefore, decrease in
the weight of epididymis in the present study suggests a probable dwindling of androgenic status in rats treated with the *Ocimum sanctum* leaves.

The epididymis is an androgen-sensitive tissue which responds to even minor fluctuations in circulating androgen (Prasad *et al*., 1973). Considerable evidence suggests that the tubular epithelium of epididymis is androgen dependent (Risley, 1963; Price and Ortiz, 1965; Brooks, 1976a and b) and androgen receptors with a high affinity for dihydrotestosterone are demonstrated in the epididymis of rat (Blaquier, 1971). Testosterone is necessary for the cellular and secretory integrity of the epididymis (Bishop, 1961; Mann, 1964) and for the maturation and viability of sperm (Dyson and Orgebein-Crist, 1973). Testosterone suppresses the oxidative metabolism of sperm in the male reproductive tract and thus preserves the viability of sperm (Murdoch *et al*., 1970). Castration and hypophysectomy affect the structure and metabolism of epididymis thereby, indicating androgen regulation of its functions (Bedford, 1975; Prasad and Rajalakshmi, 1977).

Androgen deficiency decreases the sperm count, the composition of epididymal plasma (Brooks, 1981) and causes involution of the lining of duct in rabbit epididymis (Jones *et al*., 1979). In hamster epididymis androgen insufficiency causes flattening of the principal cells and disappearance of the holocrine cells which were interspersed in between the principal cells (Lubicz-Nawrocki and Glover, 1973). The androgen deficiency results in the rapid exhaustion of spermatozoa from the epididymal lumen which can be ascribed to the enhanced rate of epididymal peristalsis and phagocytosis of spermatozoa in the epididymal lumen in albino rats (Sharma and Kanwar, 1981). The antiandrogen cryproterone acetate reduces the absorptive and secretory functions of the principal and the clear cells which would result in alternations in the composition of epididymal fluid which
inturn affect the sperm maturation in rats (Prakash et al., 1979). The leaves of Andrographis paniculata (Akbarsha et al., 1990), the flower extract of Malvaviscus conzatti (Dixit, 1977), the fruit extract of Balanity roxburghii (Dixit et al., 1981) and the shoot extract of Bambusa arundinacea (Manonayagi et al., 1989) reduce the weight and the epithelial height of epididymis and exhibit severe effect on the epididymal spermatozoa in rats. The leaf extract of Vinca rosea causes severe damage to the histoarchitecture of cauda epididymis in rats (Chinoy and Geetha Ranga, 1983). The seeds of Celastrus paniculatus reduce the weight and the epithelial height of epididymis in rats (Bidwai and Wangood, 1987).

*Azadirachta indica* leaves, affect the height of the epithelium and the diameter of the nuclei. In addition, sperms were packed at the centre of the lumen. Intertubular fibrosis was evident. The stereocilia were also reduced. A gradual and complete recovery was observed after withdrawal of the treatment in the male albino rats (Kasturi et al., 1995, Joshi et al., 1995). Similar observations have been made in our present study.

Various extracts of Hibiscus rosasinensis affect the internal milieu of the epididymis causing the change in the spermatozoa in treated albino mice (Madhusudana Reddy, 1997). Aqueous extract of Carica papaya seed has effect on microenvironment and sperm metabolism of cauda epididymis of rat and were reversible after withdrawal of the treatment for 60 days (Chinoy et al., 1997). Nicotine a tobacco extract, delays puberty in male rats affects the biochemical changes, reduces the diameter and epithelial cell height of cauda epididymis (Ramesh et al., 2000).

The chemical drugs like cis and trans clomiphenes decrease the weight of epididymis, reduce the epithelial height, cause pycnosis of the nuclei, degeneration of the sperms and increase intertubular fibrosis in caput and cauda epididymis of rats. Seven and
twenty one days after withdrawal of the treatment, a complete recovery was observed in the epididymis of rats treated with \textit{cis} and \textit{trans} clomiphenes, respectively (Rajalakshmi \textit{et al.}, 1970). \textit{\alpha}-chlorohydrin reduces the weight of epididymis and has effect on the epididymal spermatozoa in rats. A complete recovery was observed forty days after withdrawal of the treatment (Dixit and Lohiya, 1975). AY-22, 352 (4-chloromethyl-2 methyl-2-pentyl-1, 3-dioxolane) inhibits the fertilizing capacity of cauda epididymal spermatozoa in hamsters and recovery was observed five days after cessation of the treatment (Lubicz-Nawrocki and Chang, 1975).

Hypophysectomy and castration reduced the weight and secretory activity of epididymis and accessory reproductive organs and testosterone propionate treatment restores the normal conditions (Turner and Bagnara, 1976; Akbarsha and Balasubramanian, 1982). Accessory system of male ducts and glands are morphologically and physiologically dependent upon the production of androgens (Williams-Ashman and Reddy, 1972). The epididymis provides a suitable environment for the morphological and biochemical changes in spermatozoa (Orgebein-Crist, 1969). Physiological and biochemical integrity of epididymis are dependent upon androgen (Setty \textit{et al.}, 1977; Brooks, 1979). Therefore, in the present study, a marked reduction in tubular diameter, a general regression of epididymal epithelium, a rapid decline in the number of spermatozoa within the cauda epididymis and changes in the composition of epididymal plasma due to the treatment of \textit{Ocimum sanctum} leaves suggests the dwindling of androgen status or provides an indirect evidence for the antiandrogenic action of the plant extract. However, a complete recovery after sixteen days of cessation of the treatment indicates that the effects of the treatment are transient and reversible.