CHAPTER – I

EFFECT OF *OCIMUM SANCTUM* LEAVES ON TESTIS, ACCESSORY ORGANS, MORPHOMETRIC ANALYSIS OF TESTICULAR CELLS AND FERTILITY TEST IN ALBINO RATS
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INTRODUCTION

Testis is the primary male sex organ with two well-established functions namely spermatogenesis and steroidogenesis. The anterior pituitary participates in the control of both these functions through secretion of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Steinberger and Steinberger, 1975; Sharpe, 1987). Based on the early work of Greep et al., (1936), FSH is held to be responsible for control of spermatogenesis and LH for testosterone production. Testis is basically composed of avascular seminiferous tubules consisting of non-proliferating germ cells lining the basal lamina of the seminiferous tubules (Sertoli, 1965). Sertoli cell is the primary target for FSH action in the testis (Means et al., 1978). Leydig cell synthesizes androgens, chiefly testosterone in response to LH (Dafau et al., 1984). Seminiferous epithelium in mammals consists of different generations of germ cells organized in a series of well-defined stages or cellular associations. Spermatocytes and spermatids are at various steps of their development and they succeed one another in time and space.

Spermatogenesis is a complex process, in which stem spermatogonia, through a series of events involving mitosis, meiosis and cellular differentiation, become mature spermatozoa (Russell et al., 1990; Sharpe, 1994). It is of interest that various generations of germ cells form associations with fixed composition or stages that constitute the cycle of seminiferous epithelium (Leblond and Clermont, 1952 b; Russell et al., 1990; Sharpe, 1994). In rat, there are 14 stages and at each stage the germ cells are in intimate association with Sertoli cells in a predictable fashion; the more primitive cells (i.e., spermatogonia) are located near the basal lamina and the most advanced cells (i.e., spermatids) are found near the tubular lumen (Leblond and Clermont, 1952 b; Russell et al., 1990; Sharpe, 1994).
The process of spermatogenesis is very complex but is highly organized. It involves a series of intricate cell-cell interactions, which are often cyclical between the Sertoli cells, germ cells, Leydig cells, Peritubular cells and vasculature (Sharpe, 1986). An alternative way of dissecting these interactions and studying their role in normal spermatogenesis is invivo, using approaches such as cell-specific destruction/inactivation and experimental alteration of the hormones, which regulate spermatogenesis. Analysis of the consequences of such treatment is completely dependent upon a close collaboration between physiologists, cell biologists and morphologists – the Latter to identify and quantify the changes in spermatogenesis (e.g., changes in germ cell numbers or in organization of the seminiferous epithelium/germ cell degeneration/failure of sperm release) and in the intertubular tissue (Changes in Leydig cell number & size, alterations in the vasculature, inflammatory responses, etc.); and the former to correlate this to changes in cell function/secretion.

Male reproduction is controlled by a system of hormones, which stimulate the spermatogenic, and androgenic functions of the testis. The male sex accessory tract comprises of a series of organs concerned with the transport of spermatozoa, maintenance of their metabolic requirements and participation of some initial steps of the fertilization. All of these organs share a sensitivity to sex steroids and depend on androgens for their structural and functional properties. Androgens consisting primarily of testosterone and dihydrotestosterone stimulate organ growth and secretory activity of testis and various accessory reproductive organs (Williams-Ashman and Reddy, 1972; Turner and Bagnora, 1976).

Androgens are capable of maintaining the progressive development of the germ cells in immature rats which have either been hypophysectomized (Lostroch, 1969) or
treated with compounds which interfere with the release and/or secretion of gonadotropins (Kalra and Prasad, 1967; and Steinberger and Steinberger, 1969). Similar results have also been obtained in adult male rats (Clermont and Harvey, 1967; Lacy et al., 1969). Studies on steroid metabolism *in vitro* by isolated seminiferous tubules of rats denuded of their germ cells by heat treatment has led to the view that not only Leydig cells but also Sertoli cells may be a major source of androgens (Collins et al., 1968; Lacy et al., 1969). Similar conclusions have also been reached from the studies on tubules in which the ratio of Sertoli cells to germ cells was altered artificially by irradiation. Since there appears to be two main sources of androgens in the testis, spermatogenesis may be dependent upon testosterone produced by the Leydig cells, Sertoli cells or by both (Ellis and Van Kampen, 1971).

Although it is widely accepted that testosterone is important for the maintenance of normal spermatogenesis, the mechanism by which testosterone exerts this drive remains unknown. A variety of approaches have been applied to this problem, with the aim of reducing the amount of testosterone available to the seminiferous tubules. The hypophysectomized rat model introduced by Smith (1927) has been used widely and was important in revealing the acute sensitivity of stage VII of the spermatogenic cycle (Leblond and Clermont, 1952b) to the withdrawal of testosterone (Russell and Clermont, 1977; Ghosh et al., 1991).

Although hypophysectomy is not an actually specific treatment, similar stage-dependent disruption of spermatogenesis has been confirmed in experiments that either selectively impaired LH production or action, or more specifically blocked the actions of testosterone upon the seminiferous tubules (Dym and Madhawa Raj, 1977; Russell et al., 1981). Acute and total withdrawal of testosterone has been achieved via selective destruction of the Leydig cells using ethane dimethane sulphonate (Sharp et al., 1990).
Taken together all of these studies have produced broadly comparable results that suggest that testosterone acts specifically at stage VII of the spermatogenic cycle.

Measurements of the extent of germ cell degeneration and the cytology of the Sertoli cells are then used as sensitive and quantifiable biological end point of the effects of testosterone withdrawal and replacement (Russell and Clermont, 1977; Russell et al., 1981, 1987; Sharpe et al., 1990; Ghosh et al., 1991).

Since Ocimum sanctum is known to be antiandrogenic and antispermaticogenic, the present experiment is designed to study the effect of Ocimum sanctum leaves (Benzene extract) on the testis and accessory organs. An attempt has also been made to delineate the histological changes within the seminiferous epithelium and fertility of male rats.
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 a). 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 10 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 (called B.E. after hereafter) (Ocimum sanctum leaves)/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 leaves (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 leaves (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.
Five animals from each group were used for fertility test.

The animals were sacrificed by giving mild ether anaesthesia, the testis, epididymis, vas deferens, seminal vesicle, ventral prostate, Cowper's, Coagulatory and ampullary glands were dissected out, blotted free of mucus and weighed to the nearest milligram.

The studies pertaining to graded dose effects have been reported by Kashinathan et al., 1971; Seth et al., 1981; and Mukhtar Ahmed, 1999). The dose of 250 mg/Kg used in the present study was based on standardization after preliminary studies. The treatment period of 48 day is fixed based upon the duration of three spermatogenic cycles in the albino rat for all the experiments.

1. **Histology and Histometry**

i) The testis were 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) After vascular perfusion, the testis were removed and fixed in 3% glutaraldehyde, which were prepared for transmission electron microscope, post fixed in 1% Osmium tetraoxide, embedded in araldite and semithin sections 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).
For histometrical studies, the calibrated ocular micrometer (Erma, Japan) was used. From each testis, 20 sections randomly were used in each group to record the following histometrical data:

a) Number of seminiferous tubules per microscopic field.
b) Diameter of seminiferous tubules.
c) Number of spermatogonia, spermatocytes, spermatids, Sertoli and Leydig cells.
d) Diameter of spermatogonia, spermatocytes and spermatids.
e) Nuclear diameter of spermatogonia, spermatocytes, spermatids and Leydig cells.

2. Fertility test

To assess the fertility rate with reference to the number of implantations, the female rats of proven fertility exhibiting regular estrous cycles and those in early proestrous and estrous stage were separately housed with the males of groups I, II, III and IV and left over night. The appearance of spermatozoa in the vaginal smear, next morning confirmed the mating and was considered as day 1 of pregnancy. After 8 days, the female rats were laparotomized under light ether anaesthesia and the number of implantations was recorded. After parturition number of pups and their weights on day 1 and after one week were recorded (WHO-protocol, MB-50, 1983 b).

3. Statistical Analysis

The data is 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 are considered as significant and highly significant, respectively.
OBSERVATIONS

I. Body and Organ weights (Table 1; Histogram 1)

The body weight of the rats did not differ significantly due to the treatment with Ocimum sanctum leaves (Benzene extract). However, there was slight but insignificant increase in the body weight two weeks after withdrawal of the treatment. Whereas, the weights of testis, epididymis and seminal vesicle decreased significantly as compared to the control, the weights of other accessory structures namely vas deferens, ventral prostate, Cowper’s gland, coagulatory gland and ampullary gland remained unchanged. One week after withdrawal of the treatment, the weight of testis, epididymis and seminal vesicle regained to the normal value. After two week’s of cessation of the treatment, the values are comparable to controls.

II. Histological changes (Tables 2-4; Histogram 2-4)

The testis of control rats consists of seminiferous tubules and intratubular elements. The seminiferous tubules displayed various stages of spermatogenesis with all cell types and well-developed interstitial cells. The tunica propria was normal and in the interstitium numerous Leydig cells with rounded nuclei and fibroblast like elements were present. Each seminiferous tubule has the tubular wall with the outermost basement membrane. The spermatogonia and Sertoli cells rest on the basement membrane. Towards the lumen, the primary spermatocytes, secondary spermatocytes, early spermatids and late spermatids were associated with Sertoli cells. Sperms were seen with their heads embedded in the cytoplasm of the Sertoli cells while their tails extended into the lumen of the seminiferous tubule. The sperm heads in relation to the Sertoli cells were discernible upto the level of the
zone relating to the primary spermatocytes. Spermatogenesis was advanced to Golgi phase spermatids and some showed cap phase spermatids. Towards the lumen, the arrangement of mature spermatozoa and formation of residual bodies could be observed under higher magnification (Figs. 1, 5 and 9).

The rats treated with *Ocimum sanctum* leaves showed atrophic tubules and spermatogenesis was very much suppressed arrested in majority of the tubules. The tunica propria was disintegrated. Basement membrane was thin and disrupted. In about 70 percent of the tubules, spermatogenesis was arrested either at the primary spermatocytes or the spermatogonial stage. In some tubules, complete cytolysis of the entire spermatogenic elements was seen and in some, the spermatogenesis progressed only upto the formation of primary spermatocytes and was arrested thereafter. The Sertoli cells showed vacuolization and cell debris due to cytolysis. The spermatogenesis did not advance beyond pachytene spermatocytes and few of these cells exhibited signs of degeneration and aggregate to form giant cells (arrows) (Figs. 6 and 10).

The intercellular spacing became wider, Leydig cells decreased in number. The interstitium contains mostly fibroblasts only occasional Leydig cells were discernible. There are preponderances of fibroblasts like elements over the Leydig cell with shrunken nuclei (Figs. 2, 6 and 10).

The increase in the seminiferous tubules per microscopic field was highly significant (Table 2, \( P < 0.001 \)). The diameter of seminiferous tubules decreased significantly (Table 3, \( P < 0.001 \)). There was highly significant decrease in the total count of spermatogonia, spermatocytes, spermatids, Leydig cells and Sertoli cells (Table 2, \( P < 0.001 \)). There was highly significant decrease in the cell (Table 3 & 4, \( P < 0.001 \)) and
nuclear diameter of spermatogonia, spermatocytes & spermatids. The nuclear diameter of Leydig cells also decreased significantly (Figs. 2, 6 and 10).

In the one-week recovery group, a partial recovery was observed in the process of spermatogenesis. In some tubules the spermatogenic process was resumed with all the cellular stages and the lumen contained sperms. But in others the spermatogenic process was advanced only upt to primary or secondary spermatocytes stage. Each seminiferous tubule revealed a typical adult organization of the spermatogenic cells and Sertoli cells with the tubular wall and the outermost basement membrane. In between the spermatogonial cells, the Sertoli cells were found (Figs. 3, 7 and 11).

The number of seminiferous tubules per microscopic field and diameter of the tubules were unchanged. The total count of the spermatogonia, spermatocytes, and Leydig cells were less, whereas the Sertoli cells were normal when compared to control animals. The cell and nuclear diameter of the spermatogonia, spermatocytes, spermatids and Leydig cells were also reduced when compared to control rats (Figs. 3, 7 and 11).

A complete recovery was observed in the process of spermatogenesis in two-week recovery group. Almost all the tubules regained the normal spermatogenesis and the lumen was full of sperms. Histological examination of the testis, revealed no particular effect on spermatogenesis or tubules. Leydig cells with rounded nuclei were observed in the interstitium. The spermatogenesis was advanced to pachytene spermatocytes, Golgi phase spermatids, cap phase spermatids and towards the lumen, elongated spermatids were normal as in controls. The spermatogenesis appeared qualitatively normal. Tubules contained many round spermatids with polarized nuclei, the majority of which were
correctly aligned, and few elongated spermatids and residual bodies were also apparent. Sertoli cells were normal. Leydig cells appeared normal with rounded nuclei (Figs. 4, 8 & 12).

III. Fertility test (Table 5; Histogram 5)

The female rats mated with control male rats, showed the following observations. The number of implantations are $10.20 \pm 1.07$ as revealed by laparotomy on day 8th of the pregnancy. After parturition the number of pups are $9.60 \pm 1.08$ and the body weight of one-day old pups were $5.61 \pm 0.05$ gm and those of one week old were $8.60 \pm 0.07$.

No implantations were observed in the female rats mated with male rats treated with *Ocimum sanctum* leaves.

In the female rats mated with one week recovery group male rats, the number of implantations were $8.20 \pm 0.73$, number of pups $7.60 \pm 0.68$ and body weight of one-day old pups were $5.81 \pm 0.13$ gm and one-week old $8.73 \pm 0.08$ gm, respectively. And female rats mated with two week recovery group male rats showed the number of implantations, $8.80 \pm 1.16$, number of pups were $8.80 \pm 1.16$ and body weight of pups on day one and one week old were $5.75 \pm 0.11$ gm and $8.78 \pm 0.08$ gm, respectively, indicating that a complete recovery of fertility towards the normal was attained, after cessation of two weeks of the treatment.
Table 1: Effect of treatment of *Ocimum sanctum* leaves on the body weight (g), testis and accessory organs weight (mg/100g body weight) and their recovery after withdrawal of treatment in albino rats (values are expressed in SEM of five animals).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight</th>
<th>Testis</th>
<th>Epididymis</th>
<th>Vasdefers</th>
<th>Seminal vesicle</th>
<th>Ventral prostate</th>
<th>Cowper’s gland</th>
<th>Coagulatory gland</th>
<th>Ampullary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>195.20 ± 0.80</td>
<td>571.20 ± 2.25</td>
<td>261.60 ± 1.06</td>
<td>53.90 ± 2.36</td>
<td>163.20 ± 0.75</td>
<td>94.70 ± 3.24</td>
<td>27.60 ± 1.56</td>
<td>21.30 ± 2.69</td>
<td>17.10 ± 0.99</td>
</tr>
<tr>
<td>II O.Sanctum</td>
<td>197.60 ± 1.12</td>
<td>553.80 ± 2.55***</td>
<td>249.50 ± 1.62 ***</td>
<td>52.40 ± 3.81</td>
<td>158.30 ± 1.10**</td>
<td>90.90 ± 6.74</td>
<td>26.30 ± 5.30</td>
<td>19.40 ± 3.38</td>
<td>17.60 ± 3.08</td>
</tr>
<tr>
<td>III One week recovery</td>
<td>197.20 ± 0.97</td>
<td>566.30 ± 1.16</td>
<td>259.20 ± 1.51</td>
<td>52.93 ± 3.18</td>
<td>162.57 ± 1.10</td>
<td>93.92 ± 1.17</td>
<td>28.96 ± 3.38</td>
<td>18.47 ± 2.77</td>
<td>17.47 ± 3.92</td>
</tr>
<tr>
<td>IV Two week recovery</td>
<td>198.80 ± 0.73*</td>
<td>569.43 ± 1.55</td>
<td>259.85 ± 2.51</td>
<td>54.59 ± 2.82</td>
<td>163.96 ± 1.05</td>
<td>94.65 ± 3.82</td>
<td>28.74 ± 3.76</td>
<td>20.16 ± 2.60</td>
<td>18.11 ± 1.49</td>
</tr>
</tbody>
</table>

* *P < 0.05; **P < 0.01; ***P < 0.001*
Table 2: Effect of treatment of *Ocimum sanctum* leaves on total count of seminiferous tubules, germ cells, Leydig cells and Sertoli cells and their recovery after withdrawal of treatment in the testis of albino rats (values are expressed in SEM of five animals).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seminiferous tubules in microscopic field (10 x)</td>
<td>Spermatogonia</td>
<td>Spermatocytes</td>
<td>Spermatids</td>
<td>Leydig cells</td>
<td>Sertoli cells</td>
</tr>
<tr>
<td>I Control</td>
<td>17.70 ± 0.26</td>
<td>116.90 ± 2.49</td>
<td>557.65 ± 38.41</td>
<td>967.45 ± 8.39</td>
<td>37.50 ± 0.79</td>
<td>22.70 ± 0.68</td>
</tr>
<tr>
<td>II <em>O. sanctum</em></td>
<td>22.25 ± 0.56***</td>
<td>91.05 ± 1.64***</td>
<td>310.35 ± 2.51***</td>
<td>759.95 ± 3.51***</td>
<td>25.80 ± 1.01***</td>
<td>15.35 ± 0.70***</td>
</tr>
<tr>
<td>III One week recovery</td>
<td>21.10 ± 0.65</td>
<td>101.70 ± 1.56***</td>
<td>436.35 ± 0.82*</td>
<td>852.35 ± 2.75***</td>
<td>30.30 ± 0.62***</td>
<td>22.85 ± 0.53</td>
</tr>
<tr>
<td>IV Two week recovery</td>
<td>19.20 ± 0.47</td>
<td>116.70 ± 2.96</td>
<td>554.60 ± 5.57</td>
<td>977.35 ± 5.15</td>
<td>36.45 ± 0.80</td>
<td>22.35 ± 1.06</td>
</tr>
</tbody>
</table>

* *P < 0.05; ***P < 0.001*
Table 3: Effect of treatment of *Ocimum sanctum* leaves on diameter of seminiferous tubules, germ cells (μm) and their recovery after withdrawal of the treatment in the testis of albino rats (values are expressed in SEM of five animals)

<table>
<thead>
<tr>
<th>Group</th>
<th>10 x</th>
<th>100 x</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seminiferous tubules (μm)</td>
<td>Spermatogonia (μm)</td>
</tr>
<tr>
<td>I Control</td>
<td>262.79 ± 3.79</td>
<td>11.20 ± 0.21</td>
</tr>
<tr>
<td>II O. sanctum</td>
<td>237.50 ± 1.18***</td>
<td>5.95 ± 0.18***</td>
</tr>
<tr>
<td>III One week recovery</td>
<td>254.20 ± 7.37</td>
<td>7.25 ± 0.80**</td>
</tr>
<tr>
<td>IV Two week recovery</td>
<td>261.32 ± 3.89</td>
<td>11.05 ± 0.20</td>
</tr>
</tbody>
</table>

**P < 0.01; ***P < 0.001**
Table 4: Effect of treatment of *Ocimum sanctum* leaves on nuclear diameter (μm) of the germ cells and their recovery after withdrawal of the treatment in the testis of albino rats (values are expressed in SEM of five animals).

<table>
<thead>
<tr>
<th>Group</th>
<th>Spermatogonia (100 x (μm))</th>
<th>Spermatocytes (100 x (μm))</th>
<th>Spermatids (100 x (μm))</th>
<th>Leydig cells (100 x (μm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>10.40 ± 0.21</td>
<td>7.75 ± 0.20</td>
<td>7.05 ± 0.09</td>
<td>7.95 ± 0.20</td>
</tr>
<tr>
<td>II <em>O. sanctum</em></td>
<td>5.75 ± 0.20***</td>
<td>3.95 ± 0.15***</td>
<td>4.00 ± 0.19***</td>
<td>3.80 ± 0.17***</td>
</tr>
<tr>
<td>III One week recovery</td>
<td>8.00 ± 0.45**</td>
<td>5.90 ± 0.37**</td>
<td>5.25 ± 0.16***</td>
<td>5.27 ± 0.53**</td>
</tr>
<tr>
<td>IV Two week recovery</td>
<td>10.55 ± 0.23</td>
<td>8.35 ± 0.24</td>
<td>6.95 ± 0.22</td>
<td>8.05 ± 0.18</td>
</tr>
</tbody>
</table>

** P < 0.01; *** P < 0.001
Table 5: Effect of treatment of *Ocimum sanctum* leaves on the implantations, number of pups and their body weight of female rats mated with treated and recovered male rats (values are expressed in SEM of five animals).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Implantations</th>
<th>No. of pups</th>
<th>Body weight of pups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>One day (g)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10.20 ± 1.07</td>
<td>9.60 ± 1.08</td>
</tr>
<tr>
<td>II</td>
<td><em>O. sanctum</em></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>III</td>
<td>One week recovery</td>
<td>8.20 ± 0.73</td>
<td>7.60 ± 0.68</td>
</tr>
<tr>
<td>IV</td>
<td>Two week recovery</td>
<td>8.80 ± 1.16</td>
<td>8.80 ± 1.16</td>
</tr>
</tbody>
</table>
Histogram 1: Body weight, testis and accessory organs weight
Histogram 2: Number of semiferous tubules, germ cells, Sertoli cells and Leydig cells

- Seminiferous tubules (10 X)
- Spermatogonia (100 X)
- Spermatocytes (100 X)
- Spermatids (100 X)
- Leydig cells (100 X)
- Sertoli cells (100 X)

- Control
- O. Sanctum
- One week recovery
- Two week recovery
Histogram 3: Diameter (μm) of seminiferous tubules and germ cells

- Seminiferous tubules (10X)
- Spermatogonia (100X)
- Spermatocytes (100X)
- Spermatids (100X)

Control □ O.Sanctum □ One week recovery □ Two week recovery
Histogram 4: Nuclear diameter (μm) of germ cells and Leydig cells

- Spermatogonia (100 X)
- Spermatocytes (100 X)
- Spermatids (100 X)
- Leydig cells (100 X)

Legend:
- □ Control
- ■ O.Sanctum
- □ One week recovery
- □ Two week recovery
The reproductive system in male is governed by a system of hormones, which stimulates the spermatogenic, androgenic functions of the testis and maintains the structural and functional properties of sex accessory glands (Williams-Ashman and Reddy, 1972; Turner and Bagnara, 1976).

The plant product affecting aspects of male reproduction, brings about the effects through either of two mechanisms namely, Estrogenic effect or Antiandrogenic effect (Kashinathan et al., 1972). Estrogenic hormones inhibit the type ‘A’ spermatogonia leading to suppression of spermatogenesis in the testis (Kalra and Prasad, 1969) and inhibit the secretion of male accessory gland and the effects are reversible after withdrawal of the treatment (Kalra and Prasad, 1969; Mann and Lutwak-Mann, 1981). Another mechanism of action of the estrogenic substance is inhibiting the testicular steroidogenesis (Samuel et al., 1967). The target organs for the estrogens of any origin in the males are the testis, epididymis, vas deferens, seminal vesicle and prostate (Van Beurdan-Lamers et al., 1974).

The leaves, flowers, fruits and seeds of several plants are known to possess estrogen or antiandrogen like substances, which act on the reproductive system of male and female inhibiting fertility and are reversible (Anjali Joshi and Nazeer Ahamed, 1996; Madhusudana Reddy et al., 1997; Chinoy et al., 1997 c; Shivayogi et al., 1997, 1999; and Ramesh et al., 2000). The leaves of Azadirachta indica fed to male albino rats, suppresses spermatogenesis resulting in the formation of cell debris in the lumen of seminiferous tubules and regress the Leydig cell and a complete recovery was observed after withdrawal of the treatment for 24 days (Anjali Joshi and Nazeer Ahamed, 1996). The leaves of Andrographis paniculata fed to male albino rats, suppresses the spermatogenesis and
regress the Leydig cells (Akbarsha et al., 1990). The leaves of Plumaria alba treated to male rats reduce the tubules diameter of the testis and suppresses spermatogenesis (Vyas and Jacob, 1986). The roots of Aristolochia indica in male mice cause decrease in diameter of seminiferous tubules, spermatogenesis was interrupted at various stages; and degenerative changes in the seminiferous germinal cell components with prominent nuclear degeneration (Pakrashi and Pakrashi, 1977).

The flower extract of Hibiscus rosasinesis reduces the weight of testis, suppresses the process of spermatogenesis, causes atrophy of the Leydig cells. A complete recovery was observed thirty days after withdrawal of the treatment in rats (Kholkute, 1977). In the bat Rhinopoma kinneari, it has the similar effect on the process of spermatogenesis and a partial recovery was observed seven days after withdrawal of the treatment (Singwi and Lall, 1980).

Chronic administration of Malvaviscus conzatti flowers on rats and dogs causes testicular lesions resulting in a mass atrophy of spermatogenic elements and reduction in nuclear diameter of Leydig cell (Dixit et al., 1978; Verma et al., 1980). Chakraborthy and Pakrashi (1991) repeated the study and found that step seven spermatids of stage VII of the spermatogenic cycle was reduced. Ethanolic extract of Gloriosa superba treated to male gerbils causes induction of testicular lesions and inhibition of spermatogenesis at the primary spermatocyte stage (Dixit et al., 1983). Chronic administration of Allium sativam (Garlic) on rats revealed degenerative changes in seminiferous tubules and the Leydig cell nuclei were shrunken. Spermatogenesis was arrested at the primary spermatocyte stage. Sertoli cells exhibit degenerative changes (Dixit and Joshi, 1982). Combined extract of Phyllanthus amarus and Balanites roxburghii in rat caused regressive changes in the seminiferous tubules and Leydig cells and spermatogenic arrest was also evident (Shah et
Various extracts of *Saussera lappa* decrease testis weight and arrest of spermatogenesis was also noticed (Reddy and Subbalakshmi, 1995). *Spirulina plantensis* in rats caused, the testis lesions accompanied by mass atrophy of the spermatogenic elements; spermatogonia and spermatocytes decreased in relative percentage (Sharma *et al.*, 1994). Leaf powder of *Tamarix diocia* treatment in rats results in arrest of spermatogenesis, deshaped seminiferous tubules, exfoliation of the germinal elements and hypoplasia of Leydig cells (Singh, 1992). Alkaloidal extract of *Tylophora asthamatica* in male rats cause an increased rhythm in spermatogenesis, sloughing of germinal elements and their accumulation in the lumen of the seminiferous tubules and origin of giant cells (Dikshith *et al.*, 1990).

Administration of total alkaloid of *Vinca rosea* in adult male rats and mice has been shown to bring about arrest of spermatogenesis and regression of Leydig cells (Chinoy and Geetha Ranga, 1983; Murugavel *et al.*, 1989; Murugavel and Akbarsha, 1991). *Vincristine*, an alkaloid isolated from the *Vinca rosea* causes total arrest of spermatogenesis and azoospermia in rat but also induced giant spermatogenic cell formation and regression of Leydig cell (Stanley and Akbarsha, 1992a, 1994). *Gossypol*, a constituent of cotton seed oil suppresses spermatogenesis and damages the germ cell of the hamsters (Waller *et al.*, 1981), shows necrotic changes in seminiferous tubules (Kaur *et al.*, 1988). *Plumbagin*, isolated from the roots of *Plumbago zeylanica* causes selective testicular lesions in dogs (Bhargava, 1984). *Mangiferin*, isolated from the stem bark of *Mangifera indica* caused inhibitory effect on the testicular activity, particularly spermatids at certain stages of development and mature spermatozoa were affected (Sharma *et al.*, 1994).

Oil extract of *Celastrus paniculata* from seeds caused germ cell depletion and arrest of spermatogenesis (Bidwai *et al.*, 1990). Ethanolic extract of *Citrullus colocynthis* fruit in
rats caused marked reduction in secondary spermatocytes, round and elongated spermatids and decrease in the nuclear area of Sertoli cell (Chaturvedi et al., 1994). Ethanolic extract of Euphorbia nerrifolia root caused decrease in weight of testis. Secondary spermatocytes, round spermatids and elongated spermatids decreased in relative percentage (Mali and Chaturvedi, 1994). Root extract of Echinops echinatas, inhibits spermatogenesis in many animals and its antifertility effect may be due to the effect on the developing spermatids as well as on the spermatozoal motility (Chaturvedi et al., 1995). Hexane extract of Curcuma comosa on rat reveals a decrease in testicular weight corresponded with a marked regression of spermatogonia and spermatids in the seminiferous tubules of rats (Piyachaturawat et al., 1999). Aqueous extract of Pfoffia glomerate administration revealed that there is degeneration and deciduation of spermiogenic cells in hamsters (Kohguchi et al., 1999). Petroleum ether, benzene and alcohol extracts of the seeds of Momordica charantia on rats showed the antispermatic activity as the number of spermatocytes, spermatids and spermatozoa decreased (Naseem et al., 1999). Chloroform extract of Carica papaya seeds in male rat results in complete regression of spermatogenesis coinciding with the reduced fertility (Lohiya and Goyal, 1992) and crude extract of this plant seeds in male rats caused degeneration of germinal epithelium and germ cells, reduction in the number of Leydig cells and the presence of vacuoles in the tubules (Udoh and Kehinde, 1999). Curcuma longa (50% EtOH) extract, altered testicular cell population dynamics and showed its antiandrogenic activity via affecting Leydig cell function (Purohit and Daradka, 1999 a). Effects of bitter kola (Garcinia kola) extract in rabbits causes significant but varying degrees of damage to the testis, ranging from mild to severe degeneration and necrosis of the spermatogenic cells (Akinloye et al., 2000).
The action of *Azadirachta indica* leaves on the testis may be due to its estrogenic property, as estrogen causes atrophy of testis, affect the seminiferous epithelium and androgen biosynthesis in testis (Samuel *et al.*, 1967; Kalra and Prasad, 1969). Paranjape and Paranjape (1992) presented evidence for spermatostatic action of neem oil suppositories and it has been suggested that intercellular spacing become wider, Leydig cells were reduced in number and basement membrane was thickened, disrupted and spermiogenesis was not seen. Spermatogenesis was adversely affected except for spermatogonial cells. Cellular debris were accumulated in the lumina of the collapsed seminiferous tubules (Manoranjitham *et al.*, 1993). Neem leaves (50% EtOH) results in reduced seminiferous tubule diameter and Leydig cell nuclear diameter. The antifertility effect is evident via affecting Leydig cell function (Purohit, 1999).

Spermatogenesis is a dynamic process which involves the transformation of the undifferentiated germ cells (type A spermatogonium) into a highly differentiated immature spermatozoa (Clermont, 1972; Sharpe, 1983). Spermatogenesis involves interplay of sex steroids and pituitary gonadotropins (Sharpe, 1987). Reduced testicular weight and maturational arrest of the primary spermatocyte manifest androgen deficiency (Samuel *et al.*, 1967). As androgen is essential for most of the stages of spermatogenesis, meiosis in particular, sperm production cannot proceed optimally to completion without a continuous androgen supply. Interference of testosterone production leads to the atrophy of the organ and impairment of spermatogenesis (Mann and Lutwak-Mann, 1981).

The present study on the effect of *Ocimum sanctum* leaves on the histoarchitecture of the testis reveals two principal impacts on the male reproductive system of albino rats namely, the antispermatic effect and antiandrogenic effect. The antispermatic effect is reflected in the arrest of spermatogenesis as seen by the adverse effect on the
spermatocytes, spermatids and cytolytic lesions in the germinal layers and cell debris in the lumen of the seminiferous tubules. The antiandrogenic action of this plant leaves is reflected in the regression of the Leydig cells and the maturational arrest of the spermatocytes stages.

It has been known for decades that in all mammals, testosterone secreted by the Leydig cells is essential for normal spermatogenesis and fertility. Without testosterone, spermatogenesis fails completely. Despite its central importance, it is virtually unknown how testosterone drives spermatogenesis except that this action is paracrine in nature and is mediated primarily by actions of the Sertoli cell and on the peritubular cells (Sharpe, 1983, 1986). In the rat, testosterone appears to act specifically at stage VII of the spermatogenic cycle. This has been deduced from observations in hypophysectomized rats (Russell and Clermont, 1977) and in rats treated with antisera to LH (Dym et al., 1977) or with a variety of agents known to severely reduce gonadotrophin secretion or to block the actions of testosterone on its target cells (Russell et al., 1981).

Stage VII or VIII of the spermatogenic cycle is known to be particularly sensitive to hormone deprivation/withdrawal (Russell and Clermont, 1977; Russell et al., 1981; Kerr et al., 1993; O'Donnell, 1996), consistent with this view is the observation of some degenerating pachytene spermatocytes and round spermatids in the present study.

The development of vacuoles occurred chiefly in stage VII or VIII tubules at or above the position of the Sertoli cell nuclei and in association with the appearance of cavities around the primary spermatocytes and towards the lumen and are similar to the vacuoles described in hypophysectomized and withdrawal of testosterone rats (Russell and Clermont, 1977; Ghosh et al., 1991 and Kerr et al., 1993). The histopathological changes within the testis were mainly confined to Sertoli cells and the histotoxicological changes...
include discontinuation in basal lamina, vacuolation at multiple sites and disruption of spermatogenic cycle treated with mercuric chloride in rats (Prakash et al., 2000). Crude seed extract of *Carica papaya* in rats showed degeneration of germinal epithelium, germ cells and presence of vacuoles in the tubules (Udoh and Kehinde, 1999) and triptolide, a diterpene triepoxide isolated from a Chinese medicinal plant, affecting the spermatogenesis in rats. The degree of damage ranged from apparently normal looking seminiferous tubules to flattened seminiferous epithelium lined by a single layer of cells consisting of Sertoli cells and few spermatogonia. Affected tubules exhibited intraepithelial vacuoles of varying sized multinucleated giant cells, and germ cell exfoliation (Huynh et al., 2000). Similar observations are made in the present work. It is not known if vacuole formation occurs as a direct consequence of germ cell necrosis or is a non-specific response of the Sertoli cells to androgen deprivation (Kerr et al., 1993). In the present study the concurrent appearance of numerous vacuoles of varying size represent a morphological indicator of Sertoli cell damage. Support for this idea has been provided by recent studies (Ghosh et al., 1991; Kerr et al., 1993).

The process of spermatogenesis is androgen dependent (Chowdhury and Steinberger, 1975). In the present study, significantly reduced number of Leydig cells, nuclear diameter and degeneration of these cells reflect the depletion of androgen level. Decreased number of germinal cells i.e., spermatocytes, spermatids and their nuclear diameters supports the observations/hypothesis/views, since these stages are completely androgen dependent (Dym et al., 1979). Thus in the present study, the histoarchitecture and morphometric analysis revealed the adverse effect of *Ocimum sanctum* leaves on the testis, including tubular atrophy, along with the abnormal histological appearance of the seminiferous epithelium and that of Leydig cells. This may be due to curtailing of androgen
supply within the testis or it may be a direct effect of this plant extract on the tissue. And it appears that *Ocimum sanctum* leaves effects on testis are possibly due to the gonadotrophic hormone deficiency.

It has been shown that the treatment of *Allium sativum* in rats reduces the weight of testis, epididymis and seminal vesicle significantly (Dixit and Joshi, 1982). *Andrographis paniculata* causes significant reduction in the weight and degenerative changes of accessory reproductive organs in male albino rats (Akbarsha et al., 1990). *Vinca rosea*, treated to male albino rats affects the histoarchitecture of epididymis, vas deferens, seminal vesicle and ventral prostate and exhibit potent antiandrogenic and antifertility effect (Chinoy and Geetha Ranga, 1983; Geetha Ranga et al., 1988). Recent studies have shown similar results in male rats treated with *Curcuma comosa* and *Piperine*, a major piper alkaloid (Piyachaturawat et al., 1998; Malini et al., 1999).

Hypophysectomy and castration reduce 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; Chinoy et al., 1997). Accessory system of male ducts and glands are morphologically and physiologically dependent upon the production of androgens (Williams-Ashman and Reddy, 1972). Therefore, in the present study, a significant reduction in the weight of the testis and accessory reproductive organs due to treatment of *Ocimum sanctum* leaves suggests the dwindling of androgen status.

The fertility studies reveal that the male rats treated with *Ocimum sanctum* leaves are unable to fertilize the females probably because the male gametes are affected, thereby, establishing the antifertility property of the plant studied.
However, a gradual recovery observed, after withdrawal of the treatment suggest that, the effect of the treatment is transient and reversible. So in the present investigations it is concluded that, the leaves of *Ocimum sanctum* possess reversible antiandrogenic and a possible antigonadotropic effect on the histology of the testis and fertility rate in adult male albino rats.