CHAPTER – V

EFFECT OF OCIMUM SANCTUM LEAVES ON SPERM PARAMETERS AND FRUCTOSE CONTENT IN ALBINO RATS
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>103-107</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>108-112</td>
</tr>
<tr>
<td>OBSERVATIONS</td>
<td>113-114</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>115-119</td>
</tr>
</tbody>
</table>
INTRODUCTION

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). In recent years, the 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. After hypophysectomy, sperm motility was unchanged and few ova were fertilized. This suggests that as in the rabbit, sperm motility and fertilizing capacity develops independently (Bedford, 1967; Orgebein-Crist, 1967; Orgebein-Crist et al., 1972).
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, begins in the testis and independent of the epididymis. This was based on 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; Orgebin-Crist, 1967). Spermatozoa isolated from a more proximal region of the rabbit epididymis and in hamster, caput and corpus epididymis fail to develop full maturity (Bedford, 1967; Orgebin-Crist, 1967; Igboeli and Foote, 1969). These data suggested the participation of particular region of the epididymis in sperm maturation.

The similarity of the response to hypophysectomy and castration in rat points out to pituitary control of the epididymis mediated through the testis and testosterone support of sperm maturation strengthens this view. Castration has a more rapid effect than hypophysectomy on both sperm fertilizing ability and motility. The ability of rat testicular tissue to synthesize testosterone from progesterone 3.5 days after hypophysectomy (Steinberger and Ficher, 1969) and the absence of marked changes in the fine structure of the interstitial cells at 5 (Christensen, 1959) and 7 (Schwarz and Marker, 1965) days after hypophysectomy, leave open the possibility that limited androgen synthesis by the testis may take place after hypophysectomy. Such testicular androgen synthesis, if sufficient to maintain epididymal function, could explain the delay in onset of infertility after hypophysectomy compared with castration. After castration and hypophysectomy, sperm-fertilizing ability is affected before changes in sperm motility can be detected. The maintenance of sperm motility may require an even lower level of circulating androgen.
than the maintenance of sperm fertilizing ability and this may explain the lack of effect of hypophysectomy on sperm motility during the time period studied (Dyson and Orgebein-Crist, 1973).

It has been well documented that the epididymis is an 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). Hydroxysteroid dehydrogenases have been demonstrated histochemically in both epididymal sperm and the epididymal epithelium (Baillie et al., 1966). In vitro metabolism of steroid hormones by rat and rabbit epididymis (Inano et al., 1969; Frankel and Eik-Nes, 1970b) and synthesis of cholesterol dehydroepiandrosterone and testosterone from acetate by the epididymis of several species invites speculation that the epididymis may itself be able to contribute to the androgen pool (Hamilton, 1971). The decreased incorporation of acetate into cholesterol following castration and partial recovery with testosterone replacement raises the question that epididymal steroid synthesis may itself be dependent upon hormonal output from another organ. Failure of the ligation of the ductuli efferentes to influence sperm maturation and the complete recovery after castration with testosterone replacement indicated that circulating androgen is necessary for sperm maturation (Dyson and Orgebein-Crist, 1973).

Androgen may affect the sperm directly or through modification of the epididymal milieu. Several facts suggest a direct effect on the organ; spermatozoa of rat 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).
Furthermore, the uptake of testosterone by the epididymal tissue (Blaquier, 1971), the conversion of testosterone into dihydrotestosterone (Inano et al., 1969), the presence of dihydrotestosterone binding proteins in epididymal cytosol (Ritzen et al., 1971; Blaquier, 1971). The efficacy of cyproterone acetate in blocking the effect of exogenous testosterone gives additional evidence that testosterone acts on sperm maturation via the epididymal tissue (Dyson and Orgebein-Crist, 1973). The inhibition of acrosome development in the guinea pig epididymis by castration and recovery after testosterone replacement further support the importance of androgens in controlling the maturation process (Blaquier et al., 1972).

The mammalian spermatozoa show motility, which is brought about by their tail. Sperm motility forms one of the most important parameter in assessing the fertility potential of a semen specimen (Amelar and Dubin, 1979). Under physiological conditions, motility and fertilizing ability of spermatozoa are usually closely related to each other, but there are situations when these two functions of spermatozoa get dissociated, suggesting that sperm movement alone is not a sufficient criterion for fertilizing capacity (Mann, 1975). Mature mammalian spermatozoa present within the distal regions of the male reproductive tract show little or no movement in situ, but can become highly motile following their release into suitable salt solutions (Mann, 1964; Amelar and Dubin, 1979; Nelson et al., 1980 b; Mann and Lutwak-Mann, 1981).

Various plants, viz., Dolichos biflorus and Amaranthus spinosus (Murugan et al., 1993); Mangiferin, isolated from the stem bark of Mangifera indica (Sharma et al., 1994); Phyllanthus amarus and Balanites roxburghii (Shah et al., 1995); Solanum xanthocorpum (Rao, 1988; Singh & Singh, 1994); Vinca rosea (Chinoy & Geetha Ranga, 1983; Murugavel et al., 1989); Carica papaya (Chinoy et al., 1997c), Neem seed oil and Neem 106
leaves (Purohit and Daradka, 1999b; Aladakatti et al., 2001). *Curcuma comosa* (Piyachaturawat et al., 1998 & 1999); *Vaccinium macrocarpon* and *Rhododendron hymenathus* (Akbarsha et al., 1998) have been reported to possess anti-fertility activity by reducing the epididymal sperm count, motility, fertility, viability and increase in abnormal sperms in male rats (Huynh et al., 2000).

The purpose of the present study is to investigate the effect of benzene extract of *Ocimum sanctum* leaves on certain parameters namely, total sperm count, sperm motility, forward velocity, abnormal sperms, and the fructose content in the cauda epididymal fluid and seminal vesicle in male albino rats.
MATERIALS AND METHODS

Fresh *Ocimum sanctum* leaves were dried in shade and subjected to soxheltation process to get the benzene extract. Extract thus obtained was allowed to dry and stored in dessicator at 4 °C (WHO-Protocol, LG 06, 1983).

Adult male albino rats of Wistar strain, 3 to 4 month old and 190 to 200 gm body weight, were acclimatized to laboratory conditions and received a standard rat pellet diet (Gold Mohar, Hindustan Liver Ltd., Hyderabad) and water *ad libitum*.

The rats were divided into four groups comprising five animals each.

**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 *Ocimum sanctum* leaves (Benzene extract)/ rat/ day for 48 days and were autopsied 24 hrs later. The group served as the test group.

**Group III**: The animals were given 250 mg/kg body weight of *Ocimum sanctum* leaves (Benzene extract)/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 (Benzene extract)/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.
Sperm Analysis

For the standard sperm analysis, the cauda epididymis from each animal was chopped into Phosphate buffered glucose saline (PBGS: Composition: NaCl/50 m M/L. Na$_2$HPO$_4$ 200 m M/L, glucose 200 m M/L, KH$_2$PO$_4$ 26 m M/L). The debris was removed and a clear suspension viz.; the epididymal suspension was used for sperm analysis. The following observations were made.

1. Total number of sperm per ml.
2. Total number of motile sperm per ml.
3. Normal and abnormal sperm count (relative percentage).
4. Forward velocity of the sperm i.e., μm/sec.

The total sperm count was calculated by the method of Besley et al., (1980), using Neubauer haemocytometer. To increase the accuracy of sperm count, the epididymal plasma was diluted with a spermicidal solution, prepared by dissolving 5 g of NaHCO$_3$ and 1 ml of 40% formaldehyde in 100 ml of normal saline. A twenty times dilution was made using W.B.C. Pipette, which was thoroughly mixed and one drop was added to both sides of Neubauer haemocytometer. The sperms were allowed to settle down in the haemocytometer by keeping them in a humid chamber for one hour. The sperm count was done in R.B.C. counting 5 major squares designated E$_1$, E$_2$, E$_3$, E$_4$, and a central E$_5$. Each square is 1 mm long, 1 mm wide and 0.1 mm of height. The total volume represented by each major square E is thus 0.1 mm$^3$ or 10$^{-4}$ mm. Total number of sperm were counted in all the major squares and calculated as follows.

\[
\text{Total number of sperm/ml plasma} = \frac{\text{Total number of sperm/square (x)}}{\text{Total volume/square (10$^{-4}$)}} \times \text{dilution factor (20)}
\]
Similarly the total number of motile sperm were calculated, using PBGS instead of spermicidal solution.

The relative proportion of the normal and abnormal sperms were calculated by smear preparation according to the method of Bauer et al., (1974).

Equal volume of cauda epididymal plasma and 5% sodium bicarbonate were taken in a centrifuge tube, mixed well and centrifuged for 5 minutes at 4000 g. The supernatant was discarded and to the precipitate 5 ml of normal saline was added, mixed well and centrifuged again. The procedure was repeated 2 to 3 times and a clear precipitate was obtained. To the final precipitate few drops of normal saline were added, mixed thoroughly and a smear was prepared on a clean slide. The smear was dried at room temperature, fixed by heating it over the flame for two to three seconds. Then the smear was flushed with 95% alcohol, drained and dried. It was stained in Ziehl Neelson’s Carbol Fuchsin diluted with equal volume of 95% alcohol for 3 minutes and counter stained with 1:3 (v/v) aqueous solution of Loeffer’s methylene blue for 2 minutes (Gurr, 1956). After staining, the smear was rinsed in water and dried in air.

The abnormal sperms included categories like double tailed, detached head, detached tail, mid piece bending and irregular head. The relative proportions of the normal and abnormal sperms were expressed in terms of percentage.

To assess the forward velocity of sperms, the method of Ratnasooriya (1984) was adopted.

The epididymal plasma was suspended in PBGS, cleared the tissue debris and a clear solution was used for the assessment of average forward velocity of sperm. The
assessment was made under light microscope, fitted with a movable mechanical stage and a calibrated ocular micrometer, at 400 X magnification. A drop of sperm suspension was transferred to a clean glass slide and the initial place and time of each sperm was recorded. The time taken for forward movement of sperm from the initial place within microscopic field was recorded using a stop watch. The procedure was repeated for 10 spermatozoa in each sample and the average forward velocity of sperm was calculated and expressed as μm/sec.

Chemical Examination

To determine the fructose content in the cauda epididymal fluid, the method of Bauer et al., (1974) was adopted.

Reagents

1. Barium hydroxide and Zinc sulfate for the preparation of a Somogyi protein filtrate.

A. Zinc sulfate solution 5.0% 0.175 M : Dissolved 50 gm reagent grade zinc sulfate (ZnSO₄ 7H₂O) in distilled water and diluted to 1 litre. Zinc sulfate crystals tend to lose water on standing if the container is not tightly stoppered. Crystals covered with a white powder were not used which indicated a loss of water as the solution would then be too concentrated.

B. Barium hydroxide 0.3 N: Dissolved 95 gm barium hydroxide [Ba (OH)₂ 8H₂O] in distilled water and diluted to 2 litres. It is preferable to use distilled water that has been recently boiled and cooled to free it from carbon dioxide and used fresh crystals of barium hydroxide that have been exposed to air as little as possible. Avoid contact of the solution with air as much as possible.
2. **Resorcinol 0.1%**: Dissolved 100 mg resorcinol in 100 ml 95% alcohol stored in the refrigerator and is stable for 2 months.

3. **HCL, 10 N**: To 1 volume water, added 5 volumes of concentrated HCL.

4. **Fructose standard**

   A. Stock standard, 500 mg/100 ml Dissolved 50 mg pure fructose in 0.2% benzoic acid to make 100 ml. This solution is quite stable at room temperature.

   B. Working standard, 5 mg/100 ml. This is conveniently prepared as needed by diluting 1 ml stock standard to 100 ml with water. Since the seminal fluid is diluted 1:40 in the preparation of the filtrate, when the standard is treated similarly to an aliquot of the filtrate, it would be equivalent to 200 mg/100 ml fructose in the seminal fluid.

**Procedure**

Diluted 0.1 ml epididymal fluid and seminal plasma with 2.9 ml distilled water. Added 0.5 ml barium hydroxide, mixed and added 0.5 ml Zinc sulfate. Then mixed well, allowed to stand for a few minutes and centrifuged strongly. To separate tubes, add 2 ml supernatent, 2 ml working standard, and 2 ml of distilled water (blank). To each tube, added 2 ml resorcinol solution and 6 ml HCL, mixed well, heated tubes at 90 °C in water bath for 10 minutes and cooled in running water. Read standard and samples against blank at 490 nm. The average fructose content of fluid was calculated and expressed as mg fructose/100 ml.

Calculation: \[
\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200 = \text{mg fructose/100 ml.}
\]
OBSERVATIONS

Sperm analyses and Fructose content (Table 1; Histogram 1)

Analysis of sperm parameters, such as total sperm count, total number of motile sperm, forward velocity of the sperm, percentage of abnormal sperms and fructose level of the cauda epididymal fluid and seminal vesicle were carried out in the control as well as the treated groups.

The control rats showed $56.40 \times 10^4$ total number of sperm/ml epididymal fluid, $52.40 \times 10^4$ number of motile sperm/ml epididymal fluid with a speed of $127.63 \, \mu\text{m/sec.}$, $11.40\%$ of abnormal sperm were recorded. Fructose content of cauda epididymal fluid and seminal plasma were $81.48 \, \text{mg/100 ml}$ and $107.15 \, \text{mg/100 ml}$, respectively.

The Ocimum sanctum leaves (Benzene extract) treated animals showed a highly significant ($P < 0.001$) decrease in the total sperm count ($32.00 \times 10^4$/ml), total number of motile sperm ($24.00 \times 10^4$/ml), and the forward velocity of the sperm ($63.16 \, \mu\text{m/sec}$). There was a highly significant increase ($P < 0.001$) in the percentage of abnormal sperms (62.03$\%$) and highly significant decrease ($P < 0.001$) in the fructose content of both cauda epididymal fluid and seminal plasma ($26.67 \, \text{mg/100 ml}$ and $47.04 \, \text{mg/100 ml}$), respectively.

In one week recovery group the animals showed an increase ($P < 0.01$) in total sperm count ($46.40 \times 10^4$/ml), total number of motile sperms ($34.80 \times 10^4$/ml), forward velocity of sperm ($108.66 \, \mu\text{m/sec}$) and decrease ($P < 0.001$) in the percentage of abnormal sperms (28.32$\%$) and an increase in the fructose content of both cauda epididymal fluid and seminal plasma ($63.33 \, \text{mg/100 ml}$ and $85.07 \, \text{mg/100 ml}$), respectively.
In two week recovery group, the animals showed no change in the total sperm count (55.20 X 10^4/ml), total number of motile sperms (54.00 X 10^4/ml), forward velocity of sperm (127.86 μm/sec), percentage of abnormal sperms (11.53%) and fructose content in the cauda epididymal fluid & seminal plasma (78.52 mg/100 ml and 103.70 mg/100 ml), respectively when compared to control animals.
Table 1: Effect of treatment of *Ocimum sanctum* leaves on various sperm parameters in cauda epididymal fluid and fructose content in seminal vesicle and cauda epididymal fluid of albino rats and its subsequent recovery (values are expressed in SEM of five animals).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>Motile</td>
</tr>
<tr>
<td>I  Control</td>
<td>56.40 ± 1.39</td>
<td>52.40 ± 2.15</td>
</tr>
<tr>
<td>II <em>O. sanctum</em></td>
<td>32.00 ± 1.22***</td>
<td>24.00 ± 1.70***</td>
</tr>
<tr>
<td>III One week recovery</td>
<td>46.40 ± 1.73**</td>
<td>34.80 ± 1.44**</td>
</tr>
<tr>
<td>IV Two week recovery</td>
<td>55.20 ± 0.51</td>
<td>54.00 ± 1.14</td>
</tr>
</tbody>
</table>

** P < 0.01; *** P < 0.001.
Histogram 1: Sperm parameters and fructose content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>O. Sanctum</th>
<th>One week recovery</th>
<th>Two week recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motile sperms (No. X 10^4/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward velocity (μm/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal sperms (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose seminal vesicle (plasma) (mg/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose cauda epididymal fluid (mg/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

□ Control  ■ O.Sanctum  □ One week recovery  □ Two week recovery
DISCUSSION

In mammals highly synchronized and regular process of spermatogenesis gives rise to millions of spermatozoa. The spermatozoa attain their maturity and fertilizing capacity during their passage in the efferent ducts and highly convoluted tubular epididymis. The long epididymis provides a suitable environment for the spermatozoa to attain the fertilizing capacity (Hamilton, 1975). The epididymis provides a favourable milieu for the acquisition of fertilizing ability, motility, storage and survival of spermatozoa (Jehan et al., 1973; Brooks, 1979). Since spermatozoa are stored in the epididymis, this tissue could be a potential site of action for drugs affecting spermatozoa. Physiological and biochemical integrity of epididymis is dependent upon androgens (Setty et al., 1977; Brooks, 1979).

The epididymis is important because it 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).

It has been demonstrated that sperm maturation is also androgen dependent. Androgen is available either from the peripheral blood or from the luminal fluid in the epididymis. The efficacy of cryproterone acetate in blocking the effect of exogenous testosterone gives additional evidence that testosterone acts on sperm maturation via epididymal tissue (Dyson and Orgebein-Crist, 1973). Androgen may affect the sperm
directly or through modification of epididymal milieu. Several facts suggest a direct effect on the organ (Blaquier, 1971). It has been shown that androgens are essential for survival and motility of spermatozoa in the rat epididymis and cauda region appears to be the most favourable site of their survival (Jehan et al., 1973). Recent studies have shown that androgens can also maintain the fertilizing capacity of epididymal spermatozoa (Setty and Kar, 1970; Lubiz-Newrocki and Glover, 1970).

It may be useful to collect semen by the split ejaculate method to obtain good quality spermatozoa for therapeutic insemination from individuals having highly viscous semen as first portion of the split ejaculate is less viscous and has better sperm count and motility. Sputolysin or bromelin (dithiotheital in phosphate buffer saline) have been used to reduce viscosity (Upadhyaya et al., 1981; WHO, 1987). Hypo osmolality alters sperm morphology mainly leading to tail defects (Gopal Krishanan et al., 1989 a). Spermatozoa finally acquire their characteristic type of mature motility during their transit through the epididymis (Hamilton, 1977) and therefore, any reduced motility observed in the ejaculate could be due to epididymal dysfunction rather than due to intrinsic sperm defects (Chulavatonatal and Haesungcharevn, 1977).

In the present study, the effect of Ocimum sanctum leaves (Benzene extract) on the rats, indicate an adverse effect on the sperm parameters. This inference is based on the reduction in the sperm count, motility, forward velocity of sperm and an increase in relative percentage of abnormal sperms. Similar observations have been made by various authors for different parts of plants like Dolichos biflorus and Amaranthus spinosus (Murugan et al., 1993); Mangiferrin, isolated from the stem bark of Mangifera indica (Sharma et al., 1994); Phyllanthus amarus and Balanites roseberghi (Shah et al., 1995); Solanum xanthocorpus (Rao, 1988; Singh and Singh, 1994); Vinca rosea (Chinoy and Geetha...
Ranga, 1983; Murugavel et al., 1989); Carica papaya (Chinoy et al., 1997), Neem seed oil and Neem leaves (Azadirachta indica) (Purohit and Daradka, 1999 b; Aladakatti et al., 2001); Curcuma comosa (Piyachaturawat et al., 1999); Ursolic acid, a triterpine isolated from the leaves of Aractostaphylius uvaursi, Vaccinium macrocarpon and Rhododendron hymenathus (Akbarsha et al., 1998), and triptolide, a diterpene triepoxide isolated from a Chinese medicinal plant have been reported to possess antifertility activity by reducing the epididymal sperm count, motility, fertility, viability. The abnormal sperms increased in male rats (Huynh et al., 2000). It has been suggested that the extract causes androgen depletion at the target level, particularly in the cauda epididymis thereby, affecting physiological maturation of the sperm (Chinoy et al., 1995; Akbarsha et al., 2001). Thus the present study supports the view that androgen is essential for the survival, motility and fertility of spermatozoa in the epididymis.

It is known that sperm reproduction does not proceed optimally to completion without a continuous androgen supply (Brooks, 1981). Studies involving hypophysectomy, castration and androgen replacement therapy reveals that androgen is essential for physiological maturation and survival of the spermatozoa in the epididymis (Dyson and Orgebein-Crist, 1973; Setty et al., 1977; Davies and Danzo, 1981). Sperm possess two principle attributes, viz., motility and the fertilizing ability, which are prerequisites for fertilization. Any negative impact on motility would seriously affect the fertilizing ability (Murugavel et al., 1989). Semen sample per ejaculate containing more than 20 % of abnormal sperm is considered poorly fertile (Bauer et al, 1974).

The occurrence of morphologically abnormal spermatozoa is a diagnostic aid for infertility besides using other characteristics such as motility, density and viability. A high incidence of abnormality is associated with infertility (Macleod, 1970; Smith et al., 1977;
Amann, 1981). The relative distribution of the different morphological types of spermatozoa present in a sample provides the most significant clue to discriminate between fertile and infertile samples (Sherins et al., 1971; Gopal Krishnan et al., 1992). It has been reported that the sperm movement is morphologically different between normal and abnormal spermatozoa (Katz et al., 1982). The assessment of morphology is a good indicator of fertilizing ability of spermatozoa and the increased incidence of headless spermatozoa in the infertile group confirms that this is associated with infertility (Rogers et al., 1983; Gopal Krishnan et al., 1989b).

Sperm analysis is only one step in the investigation of male infertility (Joel, 1971). Seminal biochemistry is an indicator of the functional status of the accessory reproductive glands. Fructose levels are indicative of seminal vesicle function. Fructose levels are determined by the resorcinol method (Eliasson, 1971) or the more reliable enzymatic technique (Anderson et al., 1979). Glyceryl Phosphoryl Choline (GPC), carnitine, neutral and glucosidase have all been associated with epididymal function (Parez et al., 1985; Cooper et al., 1990). Concentrations of these substances in semen are variable due to a number of reasons and therefore, very little purpose is served by determining the concentrations of the chemicals in the semen.

In most mammals, fructose is the only sugar present in the semen (Turner and Bugnara, 1976). The sugar is an important source of energy for the spermatozoa and the rate of fructolysis correlates with the number of motile sperms present in semen and diminished level of fructose has been shown to parallel androgen deficiency (Mann, 1964). It has been suggested that diminished fructose content in cauda epididymal and seminal plasma may indicate semen pathology. The observations revealed that low fructose level respond to testosterone therapy, proves that a direct relation exists between the fructose
level and the testosterone level (Bauer et al., 1974; Chinoy et al., 1995; Aladakatti et al., 2001).

In the present study, the increased percentage of abnormal sperm, decreased sperm count, motility, speed and diminished fructose level may have resulted from the alteration in the epididymal milieu and diminished fructose level in the seminal vesicle probably due to androgen deficiency consequent to the antiandrogenic property of Ocimum sanctum leaves. A gradual recovery is observed after withdrawal of treatment. Thus the extract possesses reversible antifertility effects.