GENERAL INTRODUCTION
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Fertility control has come to the forefront as a topic of global concern with important medical, social and political considerations due to population increase. A number of contraceptive methods are now available to combat the unchecked rapid growth of the population. They include oral contraceptives, paper pills, steroid combinations, copper and medicated IUDs, vaginal rings with or without steroids, vaginal barrier contraceptives, cervical, vaginal, subdermal implants, immunological methods, biodegradable implants, antifertility drugs and plant extracts.

Apart from research for finding harmless chemical drugs as effective oral contraceptives in the western countries, the crude plant drugs used by tribal people are being closely looked into for their possible efficiency to find out safe and effective oral drugs for controlling human fertility. The relevant reviews of Henshaw (1953), De Laszlo and Henshaw (1954), Casey (1960) and Saha et al., (1961, a,b) are the outcome of the extensive research in this field, suggesting the usefulness of their systematic exploration to prepare the contraceptives which interfere with spermatogenesis in males and ovulation or implantation in females.

Research to develop safe, effective, affordable and acceptable methods of fertility regulation for men is being conducted by several international agencies, national research councils and pharmaceutical companies. The WHO task force on methods for the regulation of male fertility was established in 1972 and since then clinical and biomedical investigations have grown out of the basic physiological studies performed during the two preceding decades. Recently, through increased public awareness, statements supporting research on male methods and greater involvement of men in reproductive
been forthcoming from several quarters, including international women's organizations. The clinical and scientific basis for the research have been reviewed in recent years (Waites. 1994).

**Attention towards male contraceptives**

Male reproductive physiology is fairly simple, involving two basic mechanisms namely, spermatogenesis and androgenesis. Interference, particularly in the spermatogenic process would be theoretically simpler than the female systems. Hence, there is pertinent need to emphasize male fertility regulation in the context of birth control (Rajalakshmi, 1993).

There have been four major approaches towards male fertility control: they are (i) vasectomy, (ii) use of estrogens or antiandrogens, so as to disturb the hormonal mechanism of male reproduction, (iii) immunological approach were in monoclonal antibodies for sperm-specific antigens are used as antifertility vaccines and (iv) phytochemical approach. Partial success has been made in each of these approaches, but it is believed that the future of male contraceptive technology lies in the phytochemical approach (Akbarsha *et al.*, 1995 b).

**Use of plants in male antifertility**

From time immemorial, tribals are known to use plant materials and decoctions, both orally and locally, in attempt to prevent conception and hence they could be regarded as pioneers in the field of contraceptives. In the modern Indian literature, based on ancient Sanskrit and vernacular treatises on indigenous systems of medicine, many plants are listed to possess emmenagogue properties.
Recently, the use of plants in contraceptive development has been emphasized. A collaboration between the WHO task force on methods for regulation of male fertility and the Chinese national programme has been established to isolate, identify and screen pure compounds extracted from plants for their antifertility action. Numerous plant products and natural medicines are reported to inhibit male fertility and thereby have potential as chemical male contraceptives (Waites, 1994; Handelsman, 1994).

India is gifted with an abundance of natural remedies in the form of herbs, shrubs and mineral elements. About hundred plants have been reported to contain substances that affect human conception. These have been classified into three categories:

i) Crude plant substances called oral contraceptives to cause temporary sterility.
ii) Those plant drugs which interfere with implantation or gestation, and
iii) Those acting as emmenagogues affecting menstruation (Delaszlo and Henshaw, 1954).

Though many plant decoctions are used as folk medicines to prevent conception, some of them may not possess any such properties at all. But this does not lead to any positive conclusion that no plant possesses any antifertility property. As several valuable drugs have recently been isolated from plants, it should not be surprising if a few plants possess antifertility property. Hence, it is absolutely necessary that the plant would be searched extensively for the purpose.

During recent years a number of articles have been published, reporting a few contraceptives that would interfere with fertility, at any one of the stages of reproduction like, spermatogenesis, ovulation, fertilization or implantation of ovum in the uterus (Chinoy et al., 1995; Chinoy and Priya Padman, 1996; Chinoy et al., 1997 a; Chino et al.,
1997 b; Chinoy et al., 1997 c; Shivayogi et al., 1999; Ramesh et al., 2000; Aladakatti et al., 1999 & 2001; Poonam et al., 2001). Many of the plants, which are common in India, are reported to possess antifertility activity as spermicidal, abortifacient or antiandrogenic (Choudhary, 1990; Aladakatti and Nazeer Ahamed, 1999; Shivayogi et al., 1999; Aladakatti et al., 2001).

The leaves, flowers, fruits and seeds of several plants are known to possess estrogenic or antispermatic or antiandrogenic like substances, which act on the reproductive system of male or female and thus inhibit the fertility (Chinoy et al., 1997 c; Shivayogi P. et al., 1997; Madhusudana Reddy et al., 1997; Ramesh et al., 2000; Aladakatti et al., 2001; Poonam Raghuvanshi et al., 2001).

In past two decades extensive work has been done on the herbal extract and its products. Some of the important plants have been demonstrated on further lines.

The leaves of *plumeria alba* in male rats reduce the tubular diameter of the testis, suppress the spermatogenesis, cause reduction in tubule diameter of epididymis, lumen of cauda epididymis, weight of prostate and seminal vesicle and reduce the height of the secretory epithelium of prostate and seminal vesicle, while in female, it affects the implantation sites (Vyas and Jacob, 1986).

*Vinca rosea* leaf extract leads to suppression of spermatogenesis, Leydig cell regression and regression of male accessory reproductive organs (Murugavel and Akbarsha, 1991; Stanley and Akbarsha, 1992 a, 1994; Stanley et al., 1993), and affects the motility of sperm in rats and thus act as antispermatogenic (Chinoy and Geetha Ranga, 1983; Murugavel et al., 1989)
The leaves of *andrographis paniculata* fed to male albino rats induce a cessation of spermatogenesis, degenerative stages in the seminiferous tubules, regression of Leydig cells and degenerative changes in the epididymis, seminal vesicle, ventral prostate and coagulatory gland (Akbarsha et al., 1990).

The *Piper* betal leaf petiole causes decrease in sperm counts and motility of the sperm (Adhikary et al., 1989). The flowers of *Butea frondosa* induce significant changes in the weight of the testis, germinal epithelium, seminiferous tubules and Leydig cells in house sparrow (Singh et al., 1984).

Chaturvedi et al., (1994) studied the male antifertility effect of 15% ethanolic extract of *citrullus colocynthis* fruit in rats through oral feeding. Marked reduction in secondary spermatocytes, round and elongated spermatids, decrease in the nuclear area of Sertoli cell and sperm motility were noticed.

Crude ethanolic extract reduced fertility, suppressed cauda epididymal sperm count, motility and increased the abnormal sperms (Lohiya et al., 1992). Lohiya and Goyal (1992) reported complete suppression of spermatogenesis coinciding with the reduced fertility of epididymal spermatozoa with the chloroform extract.

The crude aqueous extract of *Carica papaya* seeds in male albino mice has shown no change in the weight of the testis, caput and cauda epididymis, vas deferens, seminal vesicle and prostate gland. The serum concentrations of Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), protein and cholesterol were also not much affected (Chinoy et al., 1994).
The aqueous extracts of four different varieties of *Carica papaya* seeds in male and female mice affect the sperm motility, fertility rate and both male and female mice and implantation sites in female mice (Chinoy *et al.*, 1996).

Aqueous extract of papaya seed on rat affect the sperm motility Adenosine triphosphatase (ATPase), Succinate dehydrogenase (SDH), Acid phosphatase, protein concentration, fertility test, after treatment and are reversible after withdrawal of the treatment. But there is no change in the histochemistry of testis and epididymis. Although the extract treatment brought about significant reduction in inorganic phosphate level, sperm pellet and reduction in spermatozoa (Chinoy *et al.*, 1997 c).

Oil extract of *celastrus paniculatus* seeds causes germ cell depletion and arrest of spermatogenesis (Bidwai *et al.*, 1990). Treatment with seeds of *Portulaca oleracea* in male rats produces a mass atrophy of spermatogenic elements and decrease in the weight of accessory reproductive glands (Verma *et al.*, 1982). The crude alcoholic and aqueous extracts of *Solanum xanthocarpum* seeds severely affect the spermatogenesis. Epididymal sperm count and motility decreased. Sperm morphology was affected and the circulating testosterone were decreased (Rao, 1988; Singh and Singh, 1994). Acetone extract of powdered seeds of *Dolichos biflours* and *Amaranthus spinosus* had combined decreasing effect on fertility, motility and counts of cauda epididymal sperm (Murugan *et al.*, 1993).

The root extract of *Echinops echinatus* exhibits antiandrogenic property (Sharma *et al.*, 1988). *Euphorbia nerifolia* ethanolic extract of root, causes decrease in testis and accessory organ weights. Germinal epithelium and epithelium of epididymis decreased in height, secondary spermatocytes, round spermatids and elongated spermatids decreased,
sperm motility and density decreased; Leydig cell population decreased. Fertility was 100% negative (Mali and Chaturvedi, 1994).

The shoot extract of *Bambusa arundinacea* in rats reduces the weight of the reproductive organs and libido (Vanitha Kumari *et al.*, 1989), decrease sperm motility, sperm count, epididymal epithelial height and weight of epididymis (Manonayagi *et al.*, 1989). *Mangiferin*, isolated from the stem bark of *Mangifera indica* affect, particularly spermatids of certain stages of development, and mature spermatozoa. Cauda epididymal sperm motility was totally arrested (Sharma *et al.*, 1994).

The leaves of *Azadirachta indica* possess emmenagogue, antiimplantational, spermicidal, anti-fertility and anti-spermatic activities (Sinha *et al.*, 1984 a, b; Choudhary *et al.*, 1990; Baradan *et al.*, 1991; Shaikh *et al.*, 1993). *A.indica* also causes the alteration in testicular cell population, histoarchitecture, decrease in the weight of accessory glands, such as the seminal vesicle and ventral prostate and these effects appeared reversible (Purohit and Dixit, 1991; Anjali *et al.*, 1996; Kasturi *et al.*, 1997). Changes in the morphology of head of rat spermatozoa and contain sperm parameters namely total sperm count, sperm motility, forward velocity, abnormal sperms and fructose content in the cauda epididymal fluid and alters the histoarchitecture of epididymal segment (Sampathraj, 1993; Aladakatti and Nazeer Ahamed, 1999; Aladakatti *et al.*, 2001).

In the recent year a combination of three herbal ingredients viz. *Azadirachta indica* leaves, *sapindus mukerossi* fruit and *mentha citrata* oil commonly called as praneem polyherbal pessary (PPP) has potent spermicidal action on human sperm *invitro* and *invivo*. When applied in the vagina before mating, it prevented rabbits from becoming pregnant (Poonam Raghuvanshi *et al.*, 2001).
These plants are known to possess emmenagogue, abortifacient estrogenic or antiandrogenic properties. Their crude or refined extracts have been believed to prevent the union of gametes and hence inhibit fertilization. In an extended sense, this list may include those plants whose product interferes with the growth of the fertilized ovum or implantation or capacity to produce offspring in female and act as antispermatogenic, spermicidal or antiandrogenic in the male.

Medicinal values, uses and chemical constitutions of *Ocimum sanctum*  
(The wealth of India. A dictionary of Indian Raw materials and industrial products. Raw materials, 1996, Vol. VIII: N-Pe; Pp. 87-89. CSIR publication, New Delhi)

*Ocimum sanctum* is commonly cultivated in gardens. The plant is held sacred by Hindus all over India and frequently grown in courtyards and temples. At least two types of *O. sanctum* are met with in cultivation; the green type (Sri Tulsi) is the most common; the second type (Krishna Tulsi) bears purple leaves. The plant is propagated by seeds (Gupta et al., 1942; Pakshit, Sci and Cult, 1940, Deshmukh and Muhmud, 1951).

Essential oil – (Ber-Schimmelu, Co; LP2, 1911, 1912, April, 87, April 95). Leaves yield 0.7% essential oil containing 71.3% eugenol, 3.2% carvacrol, 20.4% methyl eugenol and 1.7% carryophyllene used as an excellent antiseptic and insect repellent (pro-Indian Academy Science; Chem. Abstract 1940).

Decoction of eugenol (53.5), methyl eugenol (1.14), carryophyllene (19.26), unidentified sesquiterpenes (18.46) and terpenes (3.14%) in essential oil (PAFAI J. 1982; Chemical Abstract, 1983).

The leaves on steam distillation yield a bright yellow volatile oil possessing a pleasant odour characteristic of the plant with an appreciable note of cloves. The yield of oil varies with type, season and the place of origin. The yields and characteristics of the

8
distilled from leaves and flowering tops of plants grown in Ghazipur – type – Krishna Tulsi (Yield of oil 0.1-0.23%); Sp. gravity, 0.9421-1.0280; acid val, 1.0–1.6; phenols, 45-76% and aldehydes 15-25%. Jammu (yield of oil, 0.9%); Sp. gravity, 0.967, n^20, 1.5197; sap val; 86, sol. in all proportions 90% alcohol. A sample oil from Allahabad gave on analysis, eugenol 71%, eugenolmethyl ether 20%, and Carvacol 3%. The oil distilled from plants growing in Philippines is reported to possess a sweet anise-like odour; it contains methyl chavicol, cineole and linalool (Handa et al., 1955).

The oil is reported to possess antibacterial and insecticidal properties. It inhibits the \textit{in vitro} growth of mycobacterium tubercular activity, it has one tenth the potency of streptomycin and one fourth in that of isoniazid. It has marked insecticidal activity against mosquitoes, though it is not comparable to that of pyrethrum. The mosquito repellent action lasts for 2 hours (Gupta and Viswanathan, 1955; Gupta et al., 1942; Chopra et al., 1941; Joshi and Magar, 1952; George et al., 1947).

The plant is used as a pot-herb; leaves are used as condiment in salads and other foods. It is also reputed to have medicinal properties. Besides the volatile oil, the plant is reported to contain alkaloids, glycosides, saponins and tannins. The leaves contain ascorbic acid (83 mg/100 g) and carotene (2.5 mg/100 g) (Chem. Abstr, 1934, Basu et al., 1947).

The juice of leaves possesses diaphoretic, antiperiodic, stimulating and expectorant properties; it is used in catarrh and bronchitis, applied to the skin in ringworm and other cutaneous diseases and dropped in to the ear to relieve earache. An infusion of the leaves is used as a stomachic in gastric disorders of children. A decoction of the root is given as a diaphoretic in malarial fevers. The seeds are mucilaginous and demulcent, and are given in
disorders of genito-urinary system. They contain antistaphylocoagulase, which can be extracted with water and alcohol (Kirti and Basu, 1967; Bhat and Broker, 1954).

The plant *Ocimum sanctum* has been found to possess adaptogenic (anti-stress) properties when tested against a battery of tests in mice and rats. The drug increased the physical endurance (increased survival time) of swimming mice, prevented stress-induced ulcers in rats, induces a state of non-specific increased resistance against a variety of stress induced biological changes in animals. (Bhargava and Singh, 1981).

Restraint stress (RS) induced elevations in blood glucose and urea levels, were unaffected by *Ocimum sanctum* treatment. However, it lowered RS-induced cholesterol levels. *Ocimum sanctum* effectively lowered the RS-induced elevations in lactate dehydrogenase (LDH) and alkaline phosphatase. RS also induced (a) increased membrane protein clusterization, (b) increased membrane fluidity and (c) reduced membrane thickness in RBC membrane, whereas, the effects on the synaptosomal membrane were less marked. The RS induced changes in RBC membrane dynamics were attenuated/reversed by *Ocimum sanctum* in a differential manner (Sen et al., 1992).

**Botanical description of the plant**


<table>
<thead>
<tr>
<th>Plant</th>
<th><em>Ocimum sanctum</em> Linn., (Verna – Tulsi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>Labiatae</td>
</tr>
<tr>
<td>Habit</td>
<td>A perennial herb with typical aromatic smell.</td>
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<tr>
<td>Distribution</td>
<td>The plant is found throughout the world. Is being distributed both in temperate and tropical regions. The chief centre of distribution is the Mediterranean region. It is cultivated throughout India.</td>
</tr>
<tr>
<td>Stem</td>
<td>Erect, branched, quadrangular, some what woody, solid branches covered with soft hairs.</td>
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</tbody>
</table>
Leaf : Simple, opposite, short petioled, extipulate, ovate, serrate, acute, gland dotted, unicostate reticulate venation.

Inflorescence : Verticillaster, 6-10 flowered whorls present, emitting fine smell.

Flower : Pedicellate, bracteate, bracts small and caducous, purple, hermaphrodite, zygomorphic complete, hypogynous.

Clayx : 5 sepals, gamasepalous, bilabiate, petaloid (purple coloured), posterior lip broad and boat shaped, anterior lip with 4 small lobes possessing mucronate teeth, gland dotted, imbricate aestivation, inferior.

Corolla : 5 petals, gamopetalous, bilabiate, corolla tubeshort, upper lip four lobed, lower lip large, imbricate aestivation, inferior.

Androecium : 4 stamens, polyandrous, didynamous, epipetalous fifth posterior stamen completely suppressed, anthers bicelled, introse, dorsi fixed.

Gynoecium : 2 carpels (bicarpellary), Syncarpous, ovary bilocular in early stage but becomes tetralocular in later stages, ovary superior, four chambered, axile placentation. Single ovule in each loculus, gynobasic style (i.e. it arises from the base of the ovary). Stigma bifid.

Fruit : Schizocarpic, Carcerulus, 4 nutlets developed

Seeds : Exalbuminous or with scanty endosperm. The testa is thin; the embryo possesses flat cotyledons.

Pollination : entomophilous

Floral formula : % K (1+4), c (4+1), A 2+2, G(2)

Vernacular Names
(Kirtikar and Basu, 1933, Nadkarni, A.K. 1954)

> Sanskrit : Manjari, Krishna Tulsi;
> Bengali, Hindi, Gujarati and Konkani : Tulsi
> English : Holy Basil, Monks Basil, Rough Basil
> French : Basilic Saint
> Kannada : Vishnu Tulsi
> Malayalam : Trittavu
Additionally, the leaves of *Ocimum sanctum* significantly altered the weight of testis, while it did not have any significant effect on epididymis, seminal vesicle, prostate gland and vas deference. It was effective in reducing the sperm count and motility (Seth *et al.*, 1981). It causes a decrease in the pH, hypertonic environment and differences in concentration of chemical substances of biological importance i.e., mucoprotein, alkaline phosphatase and acid phosphatase (Kashinathan *et al.*, 1971) and reduce the mating behaviour of both male and female albino rats (Khanna *et al.*, 1986; Kantak and Gogate, 1992; Sardessai *et al.*, 1999).

As there is a little information concerning the influence of *Ocimum sanctum* leaves (Benzene extract) on the ultrastructure of the testis and cauda epididymis, the present investigations are designed to evaluate its effects on the testis and cauda epididymis histology, ultrastructure and sperm parameters.

The present work is divided into five chapters.

i) **Effect of *Ocimum sanctum* leaves on testis, accessory organs, morphometric analysis of testicular cells and fertility test in albino rats.**

ii) **Electron microscopic evaluation of albino rat testis treated with *Ocimum sanctum* leaves.**

iii) **Effect of *Ocimum sanctum* leaves on cauda epididymis of albino rats.**

iv) **Electron microscopic studies on epithelial cells and spermatozoa of cauda epididymis in albino rats treated with *Ocimum sanctum* leaves.**

v) **Effect of *Ocimum sanctum* leaves on sperm parameters and fructose content in albino rats.**

12