Chapter - 1

Introduction
1. INTRODUCTION:
1.1. Infertility:

Infertility is a major public health concern which imposes a strain on any marriage. It cannot be treated like other illness due to social stigma and emotional involvements. It often represents the very essence of masculinity and femininity of the couple. Infertility is defined as the inability of a couple to conceive after 12 months of unprotected, regular sexual intercourse. It is estimated to affect 10% to 15% of all couples. In almost half of such cases, a male factor is involved, but 15% to 24% have unexplained etiology (Sikka et al., 2001).

The pathogenesis of male infertility depends on two main factors:
- Inability to produce good quality and quantity of sperm - Sterility
- Inability to deposit sperm in the vagina and cervix - Impotency

Most of the infertile men are reported to have low sperm concentration and decreased motility of the sperm as the cause. Alterations in spermatogenesis even result in release of immature or abnormal spermatozoa in the ejaculate. Although the total sperm count may still be within the normal fertile range, these individuals may turn infertile due to large fraction of unfit spermatozoa (Mahdi et al., 1999).

Stress is one such potent factor to induce infertility in normozoospermic individuals. Defined by Mc Grady, more than half a century ago, the term “Stress” has been used to include a variety of responses elicited by noxious or potentially noxious stimuli which may sensitize stress response (Grady et al., 1984). The Hypothalmus-Pituitary-Adrenal (HPA) axis has been known to be involved in the stress response. Since HPA controls spermatogenesis, stress can be one possible contributor to the etiology of infertility (Shukla et al., 2009).

The problem of infertility is closely related to psychological stress due to several other reasons as well. As a couple fails to achieve the expected goal of reproduction, the feelings of frustration and disappointment raise the stress (Hardy et al., 2005). These feelings are compounded in couples experiencing infertility related disorders, requiring prolonged efforts to conceive.

Several other factors, such as environmental pollutants, infections, occupational exposure to various chemicals, life style changes and increased workload due to enormous competition at work place and economic recession accelerate psychological stress (Nagro Villar et al., 1993). Cortisol has been designated as the stress hormone. There are reports that gonadal and sexual dysfunction are associated with elevated cortisol levels (Cowen et al., 2002). Cortisol levels in circulation rises sharply in response to stress, resulting in testicular involution followed by significant drop in testosterone secretion (Shukla et al., 2008).
There are reports that elevated psychological stress which is associated with increased oxidant production and long term exposure to stress factors may enhance the generation of reactive oxygen species (ROS). As spermatozoa contain considerable amount of lipids, they are more susceptible to oxidative stress which may initiate lipid peroxidation because of paucity of cytoplasmic enzymes responsible for scavenging ROS (Bano et al., 1999). Stress and strain of modern living may make an individual human sexually neurasthenic and functionally impotent. This may lead to a strain of psychological complexes leading to sexual inferiority and its allied syndromes. Male animals have often been reported to yield semen of poor quality inspite of optimal management conditions including the best feeding schedules (Shivraj et al., 1971).

Males are exposed to the effect of various environmental factors which decreases their reproductive capabilities by affecting semen quality, change in culture, food habits, adulteration, exposure to various radiations, chemical hazards, pollution, smoking, alcohol and various diseases like hypertension, diabetes mellitus, obesity and resultant grave side effects of medication for these conditions, impacts the human being with dangerous and unexpected complications in life. Even recent studies reveal the fact that, the usage of electronic devices such as mobile phones and its electromagnetic wave radiations increases the free radicals that leads to lipid peroxidation resulting in change of the antioxidant activities of the sperm cells (Himanshu et al., 2012).

Cigarette smoking is known to be associated with sub fertility in males and may result in decreased sperm concentration and low motility (Ramlau Hansen et al., 2007). Chemical agents inhaled by smokers are known to affect male reproduction directly or by inhibiting testicular function and spermatogenesis. Disturbances of the Hypothalamus-pituitary-gonadal system or mild hypoxia caused by the disruption of the testicular microcirculation are possible explanations, but a direct toxic effect of many chemical components in the cigarette smoke on the germinal epithelium is a more likely explanation. Chemical stress due to introduction of foreign chemicals in the body adversely affects metabolism. Over production of ROS consequent to smoking, may be implicated as the main cause (Soares et al., 2008).

Epidemiological studies have shown correlation between heavy metals concentration in the body and human health. The body absorbs these toxic substances which are distributed into body systems and lead to different diseases. Testicular oxidative stress induced by different pollutants leads to male infertility.

Management of infertility requires the keenest insight, the tactfulness and utmost compassion (Arora et al., 1973). Around 15% of couples have difficulty in conceiving and the male factor is implicated as the main cause up to 50% of
cases (Kumar et al., 1994). The subfertile male has posed challenging problems to the medical profession. In case of sub fertility and improper spermatogenesis, the use of hormonal preparation has been well appreciated but only with warnings of grave side effects on prolonged treatment (Arora et al., 1973). The treatments of certain sexual disorders with gonadal and or pituitary hormones have not yielded favorable results (Hotchkiss et al., 1944; Grollman et al., 1964). Treatment with synthetic hormones alters the hormonal balance of the body and is detrimental to health (Swanson et al., 1950). In man, testosterone causes disappearance of the Leydig cells, atrophy of the tubules, arrest of spermatogenesis and pronounced hyalinization of the basement membrane (Heller et al., 1950). The therapeutic use of plants and their extracts may be promising approach for the treatment of different diseases.

Some of the common causes that induce sterility among males are senility, alcohol intake, stress and strains and diabetes. They are discussed in detail as follows:

1.2. **Senility induced sterility:**

Ageing is associated with the diminished function of various tissues in the body. This decline in the organism’s capacity for optimal functioning may be attributed to changes arising out of involution, wear and tear of the tissues. With age, there are also changes occurring in the cell membrane and chemicals, particularly in the cellular enzymes. The gonadal function declines with age. In males, there is a progressive atrophy of the sperm producing elements of the testes, resulting in diminished spermatogenesis. The trend for parenthood at an older age has also been seen in men. Since 1980, the infertility rate for men in their 30s has increased by 21% and for men aged 40 years, the rate has increased nearly 30%. In contrast, the fertility rate in men below the age of 30 years has decreased by 15%. Because motility is acquired during the sperm transit through the prostate and the epididymis, the decrease in motility is suspected to be due to age related decline in the function of these post testicular glands (Sartorius et al., 2010). Age related changes also cause alterations in sperm mitochondrial functioning (Aitken et al., 2007). Seminal volume evidence suggests there is a mild decrease in the volume with increasing age, although the clinical significance of this finding is marginal. The decrease in volume may be related to seminal vesicle insufficiency, because seminal vesicle fluid composes most of the ejaculate volume. Prostatic changes, including smooth muscle atrophy, may also affect semen volume and sperm motility (Kidd et al., 2001).

1.3. **Alcohol induced sterility:**

Infertility affects more than 80 million people around the globe. It is a ubiquitous phenomenon that transcends race and nationality (Anate et al., 1991).
Each male and female infertility factor accounts for about 40% cases of infertility. The remaining 20% is a combination of male and female infertility (Randolph et al., 2004).

Alcohol abuse is well known to impair reproductive performance in experimental animals and humans (Maneesh et al., 2007). Alcoholics are often found having fertility abnormalities with low sperm count and impaired sperm motility (Sigman et al., 1991). Chronic alcohol intake in men causes testicular atrophy and impaired testosterone production and shrinkage of the testes i.e., testicular atrophy (Adler et al., 1991). Ingestion of ethanol significantly augmented lipid peroxidation in the testis and inhibits the conversion of both dehydro-epi-androsterone and andro-stenedione to testosterone by decreasing the activities of 3 β-hydroxy steroid dehydrogenase and 17 β-hydroxy steroid dehydrogenase (Rosenblum et al., 1987; Rommerts et al., 1990). Mitochondrial enriched extracts from the testes of alcohol treated rats showed significant increase in the malondialdehyde formation; decrease in glutathione peroxidase and superoxide dismutase levels in the testes (Kumar et al., 1990; Fernandez et al., 1991; Mari et al., 2001). Ethanol induced oxidative stress produces testicular free radicals, which needs an antioxidant for stimulating testicular androgenesis.

Excessive use of alcoholic beverages results in a variety of medical and psycho-sociological disturbances that identify alcoholism as one of modern society’s major problems (Cebral et al., 1997). Most studies of ethanol induced fertility alterations have been conducted with male gender of both human and laboratory animals. Some studies have however reported that ethanol delays certain aspects of sexual maturation. Many agents are shown to have deleterious effects on the spermatozoa or the cyto-architectural pattern of the testes. Some of these agents include caffeine, nicotine, steroids, alcohol, anesthetic agents and insecticides. Researchers have expressed their concern over the rising cases of male spermatozoa abnormalities.

Numerous studies have indicated that alcohol abuse in men can cause impaired testosterone production and testicular atrophy. Those changes can result in impotence, infertility and reduced male secondary sexual characteristics. Testicular atrophy results primarily from the loss of sperm cells and decreased diameter of seminiferous tubules. Spermatogenic cells occupy 95% of testicular volume. Therefore, failure of spermatogenesis may be characterized by testicular atrophy associated with azoospermia. Increased ethanol consumption in teenager has led to concerns of significant hormonal changes during puberty. In rats, acute ethanol administration prior to puberty profoundly decreases serum LH levels, which decreases testosterone secretion and testicular weights. Ethanol promotes oxidative stress both by increased formation of reactive oxygen species and depletion of antioxidant status. These reactive oxygen
species cause destructive and irreversible damage to the cellular components, such as lipids, proteins and DNA. As there is no effective treatment for alcohol induced infertlity and testicular damage, the administration of antioxidants in patients with male factor infertility has begun to attract considerable interest.

1.4. Stress induced sterility:

Stress is a major factor in rising health care costs. Excessive amount of stress hormones in brain tissue cause the nerve cells or neurons in parts of the hippocampus to wither away and eventually die. Stress affects sexual desire in both men and women and can cause impotence in men. It appears to worsen the symptoms of premenstrual syndrome in women, cause amenorrhea or cessation of menstrual periods and affects fertility. Stress during pregnancy is associated with 50 % higher risk of miscarriage (Chidrawar et al., 2011).

Stress is one of the major causes that affect the health; excessive amounts of stress hormones in brain tissue cause the neurons to die. Stress affects sexual desire in men and cause impotence in men. In men stress interferes with the reproductive capacity adversely. Activation of the Hypothalamic-pituitary-adrenal axis by stressors is a presumable mechanism for the inhibition of male reproductive functions through a depression in the Hypothalamic-pituitary-testicular axis (Retana et al., 1996; Rai et al., 2004). Acute and chronic immobilization induced stress can decrease testosterone secretion in males, and as testosterone plays an important role in the sexual performance and sexual act, stress seems to be a predominant factor in the male infertility (Retana et al., 1996). Stress declines the semen quality, sperm concentration, morphology and percentage of motility resulting in correlation between the sperm concentration and fertility potential of males (Bonde et al., 1998). Exposure to stressful conditions leads to various patho-physiological conditions affecting endocrine system which activates Hypothalamic-hypophyseal-gonadal system as well as Hypothalamo-hypophyseal-adrenocortical system along with neuro-endocrine axis, which has a profound disruptive effect on male reproductive function and sexual act by the suppression of testosterone secretion, spermatogenesis and libido (Pandey et al., 2003). Stress decreases masculine sexual behaviour and sperm count in rats (Sato et al., 1996). It is well known that exposure to chronic stress leads to lower circulating levels of testosterone, prolactin, corticotrophin releasing hormones and also modulates Adreno-corticotrophin (ACTH), beta-endorphin and gluco-corticoids in Hypothalamic-pituitary-gonadal axis function and impairs Luteinizing-hormone-releasing hormone (LH-RH) co-ordination (Bharshankar et al., 2000).

In stressed rats, reduction in the number of spermatids and spermatozoa present in the tissue accounts for reduced testicular weight. Decreased weights
of accessory sexual organs like prostate gland, seminal vesicles, vas deferens and epididymis indicate the atrophy of glandular tissue, decreased secretory ability and low level of testosterone, since these organs are androgen dependent. It has been well established that the estrogen is formed from testosterone by the sertoli cells when they are stimulated by Follicle stimulating hormone (FSH). The combined activity of testosterone and estrogen is probably essential for spermatogenesis and improving the male sexual function, as estrogen has been widely used to abrogate male climacteric symptoms.

1.5. Diabetes induced sterility:

Diabetes Mellitus is a degenerative disease due to alteration in carbohydrate homeostasis that harmfully affects the male reproductive function (sexton et al., 1997; Amaral et al., 2006). Insulin-dependent diabetes results in hyperglycaemia and production of free radicals which leads to oxidative stress in different tissues (Vincent et al., 2002). High levels of free radicals could lead to damage of cellular organelles and increases the lipid peroxidation in the cellular membranes (Panchnadikar et al., 2003). As normal spermatogenic process requires extrinsic hormonal control like Follicle stimulating hormone and Luteinizing hormone as well as locally produced factors. The disturbances of the activity of the reproductive system in diabetes might be due to the influence on Hypothalamo-pituitary-testicular axis (Baccetti et al., 2002; Ballester et al., 2004). One of the diabetic complications is the production of advanced glycation end products (AGEs) that generates reactive oxygen intermediates, which might be deleterious to tissues (Ballester et al., 2004). Although the insulin treatment resulted in reversal of symptoms and bio-chemical abnormalities of diabetes, yet it had been shown to be unable to completely inhibit protein glycation, which increased the oxidative stress in diabetic.

Diabetes mellitus is a chronic disease affecting many tissues and systems of the body. Some of these manifestations were spermatogenic and steroidogenic alterations. Hyperglycemia induced production of Advanced Glycation End products (AGEs) which triggered the synthesis and release of cytokines that might cause more destruction of Beta cells (Amaral et al., 2006). AGEs are the important cause for diabetic complications as vascular dysfunction and testicular alteration (sexton et al., 1997).

1.6. Herbal medicines:

Medicinal herbs have been popular from ancient times among people and in recent years, a multilateral approach has emerged on using medicines with natural and especially herbal origin (Mohajera et al., 2008). Medicinal herbs like industrial drugs may cause some irretrievable tissue damage through its unwanted side effects. Evaluating toxic effects of medicament herbs by performing experimental tests on animal models will have an effective advantage
on identification and recognition of medicine’s harmful effects on humans (Mirhadi et al., 2011). On the other hand, recognizing the damages on different tissues and organs of body, following the use of medicament herbs will provide an appropriate strategy in order to specify the consumption of these drugs. Determining the dosage of medicines is very important too, in overdose of herbal medicines does not cure the illness and also cause irreparable damages (Smet et al., 1997).

Herbal medicines as preparations derived from naturally occurring plants with medicinal or preventive properties are a major component in all indigenous medicine. The World Health Organization (WHO) estimates that 4 billion people, about 80% of the world’s population, use herbal medicines for some aspect of primary health care (Heber 2007). It is estimated that 70% of indigenous populations in developing countries rely on phytotherapy (Wambebe 1998) and 25% of drugs in typical western pharmacies are plant derived (Gilani et al., 2005). The realities on ground, pose challenges to scientists and governments of developing countries with respect to the development and production of standardized herbal medicines. WHO has advocated for the proper identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies in medicare (Wambibe 1998). Pharmacological and toxicological evaluations of medicinal plants are essential for drug development (Ibarrola et al., 2000; Ahmed et al., 2006; Perera et al., 2010).

*Cycas circinalis* L. and *Iondium suffruticosum* Ging. with its taxonomy, geographical distribution, habitat and botanical description of the plant is collected from various literatures and summarized as below,

1.7. **Cycas Circinalis L.** (Fig - 1):

1.7.1. **Taxonomy of Cycas Circinalis L:**

- **Kingdom:** Plantae
- **Subkingdom:** Viridaeplantae
- **Phylum:** Tracheophyta
- **Subphylum:** Euphyllophytina
- **Infrahphylum:** Radiatopse
- **Class:** Spermatopsida
- **Subclass:** Cycadidae
- **Order:** Cycadales
- **Family:** Cycadaceae
- **Genus:** Cycas
- **Specific:** circinalis - L.
- **Botanical name:** - *Cycas circinalis* L.
1.7.2. Vernacular names:
Cycas Circinalis L. plant is known in Hindi as Jangli madan must ka phul; in Sanskrit Hintalah; in Tamil commonly known as Madana Kama Raja, Salaparai, Eentha panai (Murugesa Mudaliyar et al., 1996).

1.7.3. Parts of Cycas Circinalis, their uses and their actions:
The flowers, stems and seeds of the plant are used for treating skin diseases. The different parts of the plant acts as a narcotic, stimulant and aphrodisiac (drug that stimulates the sexual passion). A paste of Cycas seeds and coconut oil is used for the treatment of skin complaints, wounds, ulcers, sores, and boils.

1.7.4. Geographical distribution and habitat of Cycas Circinalis:
Cycas Circinalis L, an Indian endemic plant of Western Ghats, grows in the States of Kerala, Karnataka, Tamil Nadu, and the south of Maharashtra. It grows typically in dry, scrubby, woodlands in hilly areas. Cycas Circinalis is a deciduous plant in extremely dry seasons and dioecious with separate male and female plants. The female plant produces the seeds, and the male plant produces cones with pollens in them. The Sago palm is a cycad. The feathery leaves of these trees are arranged in a rosette pattern. The leaves are bright green, semi glossy. Male sago plants develop a cone of 30 cm tall coming from the center of the tree (Fig. 1). The cones are white or yellow in colour, rounded and produce abundant pollens. The seeds are quite large, brown or yellow in colour and are displayed on the feather-like seed-bearing leaves. Queen Sago plants are pollinated by a species of weevil, which finds protection in the plant's cone and seed-bearing leaves (Anita Vargese et al., 2008).

1.7.5. Botanical description of Cycas Circinalis:
Cycas Circinalis L, is always evergreen tree, dioecious, and mostly palm like. The botanical description is very much essential for identification of the particular species of Cycas. Leaves are borne at apex of trunk, arranged spirally in pinnate compound manner with new leaves erect. The leaflets are circinnate and have petiole with spines, base swollen and hairy. Pollen cones are borne at the apex of trunk, which are either in cylindrical or fusiform shape. Microsporophylls are numerous, scale like, spirally arranged along the axis of cone, with numerous micro sporangia in groups ab-axially; and each pollen tube produces 2 motile sperm cells. Mega sporophylls are numerous, arranged in alternate pattern with flushes of leaves. The ovules are usually 1 or 2-5 in number seen on each side of stalk. The Seeds are drupe like, compressed; with a seed coat of 3 layered, which consists of coloured sarcotesta, woody sclerotesta and cotyledons 2 found to be united at the base.
1.8. *Ionidium Suffruticosum* Ging. (Fig - 2):  
(Syn: *Hybanthus enneaspermus*)

1.8.1. **Taxonomy of *Ionidium suffruticosum* Ging:**
- **Kingdom:** Plantae
- **Subkingdom:** Viridaeplantae
- **Phylum:** Tracheophyta
- **Subphylum:** Euphyllophytina
- **Infraphylum:** Radiatopses
- **Class:** Magnoliopsida
- **Subclass:** Rosidae
- **Superorder:** Violanae
- **Order:** Malpighiales
- **Family:** Violaceae
- **Genus:** Ionidium
- **Specific epithet:** suffruticosum Ging.
- **Botanical name:** Ionidium suffruticosum Ging  
- **Syn:** Hybanthus enneaspermus

1.8.2. **Vernacular names:**

*Ionidium suffruticosum* Gin., are commonly known as Ratna Purush, in Hindi - Ratan Purus, in Sanskrit - Sooriyagandhi, Tamil - Orai Idhazh Thamarai (Murugesu Mudaliyar et al., 1996).

1.8.3. **Parts of *Ionidium suffruticosum* used and its action:**

The whole plant was used as an aphrodisiac, nutritive and diuretic.

1.8.4. **Geographical distribution of *Ionidium suffruticosum* and its habitat:**

*Ionidium suffruticosum* are the seasonal, sporadic, perennial herb, widely distributed in Africa, Madagascar, Srilanka, China, New Guinea, Australia and India. In India the plant are found to be commonly grown in south India.

1.8.5. **Botanical description of *Ionidium suffruticosum*:**

The botanical description is very much essential for identification of the particular species of Ionidium. The leaves of the plant are simple, usually arranged in alternate pattern, sometimes opposite, and with small leaf like stipules, petioles has margins which are entirely serrated. The flowers are bisexual or unisexual, actinomorphic or zygomorphic, spicate, paniculate with racemose inflorescences, having 2 bracteoles. Sepals are 5 in number which are imbricate and persistent. The petals are also 5 in number which are imbricate or convolute, unequal, and gibbous or spurred at the base. The stamens usually 5 in number with anthers erect and filamentous, but anterior 2 stamens have spur like nectary. The
ovaries are superior, loculed, with 3-5 carpels, syncarpous, 3-5 parietal placentae each with anatropous ovules. The fruits has loculicidal capsule, seeds are often carunculate with endosperm copious, fleshy and erect embryo (Murugesan Mudaliyar et al., 1996).

1.9. **Scope of the study:**

Perpetuation of one’s race is the dogma of all living organisms. All living organisms strive to achieve this through the process of reproduction, which is the vital process that enables a species to represent itself in the following generations in the form of its offsprings. Different contraceptive methods are in practice for family planning (population control) but at the same time there are couples who are facing the problems of infertility. Statistics reveals that more than 2 million married couples are now experiencing problems with infertility in India. About 30% to 50% of problems in infertile couples are due to male infertility.

Fertility in male depends on the viability and quantity of spermatozoa produced by the testes. Oligospermia, azoospermia, and necrospermia are the various pathological conditions which result in sterility of male. The scope of management of male infertility (sterility) is also very less and the treatment with hormonal preparations has been recommended, but they may have grave side effects on prolonged treatments. Inspite of great advances in modern medicine in recent decades, herbal plants still make an important contribution to healthcare. Numerous plants have been used traditionally to increase male fertility and modern scientific research has confirmed the fertility effects in some of the herbs, but still needs documentation of the research. This urged us to do a study on male infertility with 2 herbs, *Cycas circinalis* L. and *Ionidium suffruticosum* Ging, which was used traditionally in Indian System of Medicine, for arousing sexual desire and to increase the quality and quantity of sperm.

There is no scientific report documenting the fertility efficacy of *Cycas circinalis* L. and *Ionidium suffruticosum* Ging. till date. The present research work was taken to prove the fertility effect of these 2 herbs on male Wistar rats.
Fig. 1: *Cycas circinalis. L*

Fig. 2: *Ionidium suffruticosum. Ging*