CHAPTER-I

INTRODUCTION

Since time immemorial, mankind has used plants and their product to cure different types of ailments and thus relieve him from physical agony (Ahmed et al., 2007). The healing power of folk medicine have been recognized and documented since earlier time. The Ayurvedic system of medicine has a record of about more than about 8,000 herbal remedies. 92 medicinal plants are recorded in Rig-Veda (5000 BC), 82 medicinal plant species recorded in Yajurveda and 28 medicinal plants recorded in Atharba-Veda (4500-2500 BC) (Bhattacharjya, and Borah 2008). Properties and uses of 1100 and 1270 species have been described in Charak Samhita (700 BC) and Sushrut Samhita (200 BC) respectively, and these are still in used in the conventional Ayurvedic system formulations (Thomas, 1997). Indigenous or folk medicine (also known as traditional medicine) comprises knowledge or information systems that have been developed over generations among various societies before the advent of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2008). Mostly in some Asian and African countries (when less developed countries are taken into account), about 80% of the world’s population relies on folk medicine for their primary health care needs, most of which involve the application of plant extracts and their part (Sandhya et al., 2006; Kaushik and Goyal et al., 2008). Another term associated with tradition medicine is the alternative medicine which means medicine when adopted outside of its traditional culture (WHO, 2008). In our country, the traditional system of medicine plays a vital role in health care
of most of the rural people. Evidences on the use and study of herbs dates back to 5,000 years among the ancient Sumerians, who described well-established plant medicine. Since then plants have been used therapeutically and even today plant-based medicines continues to play a crucial role in world health care (Lewington, 1993; Yadav et al., 2006). India is placed among one of the world’s 12 biodiversity centers and has about 45,000 plant species of which around 15000-20,000 medicinal plants are claimed to possess medicinal properties. However traditional communities are using only 7,000-7,500 plants species for curing different diseases (Dev, 1997; Samy et al., 2000). This knowledge is generally passed on orally through a community, family or individuals. Within the same culture group, elements of indigenous knowledge on medicine may be roughly known by many, and are practiced by a few (Acharya and Anshu, 2008). The most commonly acknowledged traditional systems of medicines include Ayurveda, Unani, Siddha medicine, traditional Chinese medicine, ancient Islamic medicine, traditional Korean medicine, Iranian medicine, Irani, Muti, Ifá, and traditional African medicine.

In India, prevalence of the traditional system of medicine to treat different types of ailments is still continuing in majority of the people in rural areas. These patients are basically treated according to three principle ancient systems of medicines; the Ayurveda, the Unani or Graeco-Arabic medicine and the Siddha system of medicine from South India. Based on these three systems of medicine, many of the traditional healers are rendering their health services to a large section of the people throughout the country. The use, mode of preparation and prevalence of traditional medicine varies from place to place based on cultural norms of the respective area (Bakx, 2008). The dawn of Yunani or Unani medicine, a term used for Perso-Arabic traditional medicine, practiced among Muslim culture in South Asia and present day Central Asia. This system is based on the wisdom of the Greek physicians Hippocrates (460-377 BC) and Galen
(Rahman, 2001). The fundamental principle of this system is based on Hippocrates’ Humoral Theory which suppose the presence of four humours in the body like blood (Dam), Phlegm (Balgham), Yellow bile (Safra), Black bile (Sauda). These humors remain in equilibrium. The health of a person depends on the balance of these humors in the body. If the normal equilibrium of humors of body (akhlat) is lost, it may lead to quantitatively or qualitatively deterioration leading to pathological change or disease condition. This affects different tissues thereby exhibiting clinical manifestations. By diagnosis of the clinical features, like symptoms, signs, laboratory features and temperament (mizaj), treatments are rendered. Siddha system of medicine is another oldest system of medicine which has gradually evolved along with the Dravidians’ culture and so this system is known as Dravidian system of medicine. It is practiced mostly in South India (especially in Tamil Nadu). The exponents of Siddha system of medicine (Siddhars) are considered as super human beings having incredible powers which they have acquired by way of Yoga practice, Meditation and rejuvenation. Through their supernatural powers (Siddhic powers) and findings of their experiments the Siddhars could explain and explore the reality of nature and its relationship with man. This system primarily is based on ‘Andapinda Thathuvam’ i.e., the relationship between human body and the universe. These two are interlinked through the five basic principles which are known as ‘Panchaboothas’. Purposeful assistance of these two is essential for the maintenance of health. Treatments in siddha system of medicine employs a variety of herbs and minerals and are classified into 64 types i.e. 32 external treatments which include fomentation, kizhi, steam etc. and 32 types of internal medications. Preventive measurements like taking proper diet in proper quantity, time, etc. are also clearly mentioned. Ayurveda is a system of medicine which has its historical roots in the Indian subcontinent. Plant-based treatments in Ayurveda may be derived from leaves, fruits,
bark, roots, or seeds and animal products include milk, bones etc. Therapies are naturally based on herbs while under the influence of rasa śāstra mineral and metal substances were also included. Ancient Ayurveda also taught surgical techniques, including rhinoplasty, the withdrawal of foreign objects and the suturing of wounds (Dominik, 2003; Mukhopadhyaya, 1913). However, little attention is being made towards exploration, documentation and development of methodology used by these people. In spite of the fact that this traditional healers in this region are divulging various challenges particularly in the management of urinary and liver disorder and even grave diseases like cancer (Mao, 2002). Therefore, the need of the hour is to systematically document these practices as is done in other countries (Pretorius, 1998; Letendre et al., 1998).

In addition to these traditional system of medicines there are many reports suggested by different workers who validate a variety of alternative therapies that can cure different types of ailments like acupuncture, homeopathy, nutrition and hypnotherapy. These complementary therapies although are not inevitably a cure for the diseases, they can definitely help promote or regulate the different organs of the body by bringing the body back into equilibrium. Helping the body to deal with the demands of modern life such as by employing acupuncture or sound dietary habit can do wonders and helps in stimulating and controlling the different organs of the body into action. Homeopathic remedies which are made from minerals, animal and plants are diluted down thousands of times so that each medicine contains only a hint of the original essence. The medicines, usually in tincture/powder or tablet form, produces symptoms similar to a specific disease and they 'kick-start' the body's defenses against the disease or problem. Homeopathic practitioners claim that they can help women with a variety of fertility problems like from blocked tubes, endometriosis, polycystic ovarian syndrome (PCOS) and are of the opinion that
they can aid fertility by giving women with the same hormone that is causing them the problem. There are also reports suggesting the formulation of different homeopathic products that are used to regulate ovulation, implantation and anti-spermatogenic activity in animals (Sundaram, 1999). Acupuncture is a process where several needles are inserted in the body into specific points on energy channels or meridians. It works on the theory that some disease/illnesses are resultant from an imbalance in the body's natural energies or 'chi'. The theory says that exhausted blood gets stored in a woman suffering from irregular periods and other reproductive infertility. This condition can hinder ovulation. So by encouraging or blocking fresh blood to specific points which influences our reproductive organs such as fallopian tubes, ovaries and uterus will help to bring the body back into balance and stimulate or sidetrack ovulation and enhance/reduce fertility in women.

These alternative therapies are found to have an influence on various types of disease including reproductive disorders. Reproduction is a biological process by which organisms produce genetically similar or identical copies of their own (asexual reproduction) or sexual interaction of two specialized morphologically distinct types of reproductive cells called gametes (male & female) that fuse together to form a zygote (sexual reproduction). The reproductive systems consist of primary and secondary organs and accessory glands (which add substances to the duct). In males these organs work together to produce sperm, the male gamete, and the other components of semen. These organs conjointly work together to deliver semen out of the body and into the vagina where it fertilizes egg cells to produce offspring. The female reproductive system consists of a pair of ovaries, which produce the egg cells or ova or oocytes; oviduct; the uterus that hosts the developing foetus, produces vaginal and uterine secretions, and can pass spermatozoon to the fallopian tubes; the vagina and the external genitalia. All these structures
have the primary function of producing egg cells, provides suitable ambience for fertilization of the ovum by the sperm and to protect and nourish the offspring until birth.

Reproduction and development are influenced and regulated by factors like hormones and the environment. Hormones produced by the endocrine system of the body are extremely dynamic and undergo frequent physiological fluctuation owing to diurnal variation and cyclic hormonal feedback system. These hormones are chemical substances which are either protein or steroids that affect the activity of another part of the body (target site). In real term, hormones serve as messenger for controlling and coordinating metabolism, growth, development and functioning of sex organs, maintenance of sexual activities, embryonic development, parturition etc. Hormones that are released into the bloodstream from their source will diffuse into the interstitial fluid neighboring the target cells and into the blood stream to reach the target sites. The target cell has receptors specific to a given hormone and gets activated by either a water-soluble (binds cell-surface receptor) or lipid-soluble hormone (permeable to plasma membrane). The reproductive cycle in both female and males is regulated by different hormones. Some of these hormones such as testosterone produced by the testes in males are responsible for stimulating the development of male secondary sex characteristics and spermatogenesis and estrogen produced by ovaries and stimulates the development of female secondary sex characteristics (like mammary glands) as well as initiating the thickening of the uterus lining and preparation for a possible pregnancy after the egg is released by the ovary. Progesterone is produced by yellow tissue called corpus luteum in the empty ovarian follicle of the ovaries in female. This hormone is responsible for maintaining the thickness of the uterus lining in case fertilization occurs and helps in development of the foetus. To carry out a proper healthy reproductive life the endocrine components (testis and the ovary) and the nervous system work together in an intricate mode of
co-ordination. The endocrine system undergoes recurrent physiological variation owing to environmental and diurnal deviation and cyclic hormonal feedback system. Any physiological, structural and secretory alteration in these organs leads to reproductive abnormalities, one of which may result in infertility in both male and female.

Apart from hormones there are several other factors which have influences on fertility in animals. Among them the endocrine disruptor is noteworthy. There is growing attention in the possible health hazard posed by endocrine-disrupting chemicals (EDCs), which are chemical substances present in our environment, food, and consumer products that has the capability to interfere with hormone biosynthesis, action or metabolism resulting in a deviation from normal homeostatic control or reproduction. An endocrine-disrupting compound was defined by the U.S. Environmental Protection Agency (EPA) as “an exogenous agent that interferes with synthesis, secretion, metabolism, binding action, transport or elimination of natural blood-borne hormones that exist in the body which are responsible for homeostasis, reproduction and developmental process”. The group of molecules recognized as endocrine disruptors is highly diverged and includes synthetic chemicals that are used as industrial lubricants or solvents and their byproducts [e.g. polychlorinated biphenyls (PCBs), dioxins, polybrominated biphenyls (PBBs)], plastics, bisphenol A (BPA), pesticides [e.g. methoxychlor, fungicides (vinclozolin), chlorpyrifos, dichlorodiphenyltrichloroethane (DDT)] and pharmaceutical agents [diethylstilbestrol (DES)]. Endocrine-disrupting chemicals (EDCs) were initially thought to exert their actions primarily through nuclear hormone receptors, including androgen receptors (ARs), estrogen receptors (ERs), thyroid receptors (TRs), and progesterone receptors among others. But today, basic scientific research has shown that the mechanisms are much broader than originally predicted. Thus, endocrine disruptors act through nuclear receptors, nonsteroid receptors (e.g.,
neurotransmitter receptors such as the dopamine receptor, norepinephrine receptor, serotonin receptor) nonnuclear steroid hormone receptors (e.g., membrane ERs), orphan receptors [e.g., aryl hydrocarbon receptor (AhR)—an orphan receptor], enzymatic pathways concerned in steroid biosynthesis and/or metabolism, and numerous other mechanisms that congregate upon endocrine and reproductive systems. Thus, from a physiological viewpoint, an endocrine-disrupting substance is a compound that is either natural or synthetic, which, through environmental or unfortunate developmental exposures, alters the hormonal homeostatic systems that permit the organism to converse with the environment and respond to it (Diamanti-Kandarakis et al., 2009). Natural products found in animal and human food (e.g., phytoestrogens, including coumestrol and genistein) can also act as endocrine disruptors. However, these substances are thought to have relatively low binding affinity to ERs, and are widely consumed by humans including the infant (Kuiper et al., 1988; Dickerson and Gore, 2007). A recent study have reported that urinary concentrations of the phytoestrogens daidzein and genistein were about 500-fold elevated in infants that are fed by soy formula compared to those fed with cow’s milk formula (Cao et al., 2009). Therefore, the possible for endocrine disruption by phytoestrogens needs to be well thought-out. Although EDCs can influence every possible cellular hormonal pathway, most of the information is available about interference of EDCs with the hormone receptors of the nuclear family. The NRs represent a group of structurally related transcription factors (Gronemeyer et al., 2004)). In mammals, 48 NRs have been acknowledged which are involved virtually in all crucial functions, e.g., reproduction, metabolism, fetal development, homeostasis, and response to xenobiotic substances (Swedenborg et al., 2009). Some EDCs has the capability of binding directly to these receptors either as agonists or antagonists, thus inhibiting or enhancing the effect of a hormone, respectively. However, other
mechanisms of EDC action particular the AhR which belongs to the family of the bHLH-PAS proteins, and is a key regulator of the cellular response to xenobiotic exposure is a ligand activated transcription factor. It is activated by organic compounds such as polychlorinated dibenzodioxins (dioxin), biphenyls (PCB) as well as polycyclic aromatic hydrocarbons (PAHs) like benzo[a]pyrene (BaP), 3-methylcholanthrene (3-MC), and benzoflavone (Poland & Knutson 1982). Transcriptional activation of AhR and NRs are very similar (Ruegg et al., 2008).

Most of the medical treatments of different types of reproductive diseases that are prevalent in our society are not free from side effects. Moreover, the cost involved in it is also a matter of concern. Treatments with alternative medicine such as the use of herbs are always preferred by the people due to its availability, low cost and lesser side effect. The problems related to fertility/anti-fertility are a major aspect of reproduction in our society.

So, the searching of indigenous plants with great potentiality influencing fertility is not only very essential, but also a great challenging tusk to the scientific community. Folk medicine have been claimed to have a very promising results, as far as reproductive disease is concerned. Considering the prevalence of traditional medicinal practices in our society and the efficacy of the plant extract in the treatment of different kinds of diseases, a study has been taken-up for further scientific investigation. Initially, a survey about the traditional medicinal practices was conducted on the basis of information collected from the traditional medicinal practitioners who prescribed the plant products among the Mising community for treatment of reproductive health related disorders in different villages of upper Assam district. From the survey, the plant *Plumeria acuminata* Ait. was selected, as this plant was found to be used by many of the traditional healers which is also supported by the available literature about the use of this plant for treatment of various types of diseases. So the present study was undertaken to validate
scientically about the effect of methanolic stem extract of the plant *Plumeria acuminata* Ait. on fertility in albino mice with the following objectives:-

1.1. OBJECTIVES OF THE PRESENT STUDY:

The present investigation was aimed to study the effect of *Plumeria acuminata* Ait. on female albino mice, which are traditionally used by the *Mising* tribes of upper Assam.

The objectives of the present study are:-

1: To study the effects of methanolic stem extract on the uterus in ovariectomized mice.

2: To study the effects of the extract on estrous cycle, uterus and ovary in albino mice.

3: To study the effects on implantation and litter size.