Chapter 1

INTRODUCTION

1.1 Introduction

Uterine leiomyomas, commonly known as **fibroids**, are well-circumscribed, non-cancerous tumors arising from the myometrium (smooth muscle layer) of the uterus. In addition to smooth muscle, leiomyomas are also composed of extracellular matrix (i.e., collagen, proteoglycan, fibronectin). Other names for these tumors include fibromyomas, fibromas, myofibromas, and myomas [1]. Leiomyomas are the most common solid pelvic tumor in women, causing symptoms in approximately 25% of reproductive age women. However, with careful pathologic inspection of the uterus, the overall prevalence of leiomyomas increases to over 70%, because leiomyomas can be present but not symptomatic in many women [1].

Leiomyomas are classified by their location in the uterus. **Subserosal** leiomyomas are located just under the uterine serosa and may be Pedunculated (attached to the corpus by a narrow stalk) or sessile (broad-based). **Intramural** leiomyomas are found predominantly within the thick myometrium but may distort the uterine cavity or cause an irregular external uterine contour. **Submucous** leiomyomas are located just under the uterine mucosa (endometrium) and, like subserosal leiomyomas, may be either pedunculated or sessile.

The two most common symptoms of fibroids are abnormal uterine bleeding and pelvic pressure. The most common bleeding abnormality is menorrhagia (prolonged and/or profuse uterine bleeding, also called hypermenorrhea). Normal menstrual periods typically last four to five days, whereas women with fibroids often have periods lasting longer than seven days. Although abnormal bleeding can occur with any of the three classes of fibroids, women with submucous fibroids seem particularly prone to this complication. Pelvic pressure results from an increase in size of the uterus or from a particular fibroid. Most women with leiomyomas have an enlarged uterus. Pressure on these structures can result in difficulty with bowel movements and constipation or urinary frequency and incontinence. Rarely, fibroids can also press on the ureters, which can lead to kidney dysfunction. Leiomyomas are also associated with a range of reproductive

dysfunction including recurrent miscarriage, premature labor, fetal malpresentations and infertility. [2]

Endometriosis, characterized histologically by the presence and growth of endometrial glands and stroma outside the uterine cavity, is a chronic recurring disease commonly encountered in women during their reproductive age [3]. Ectopic endometrial tissue can be found in different parts of peritoneal cavity, thus leading to three different conditions: ovarian, peritoneal, and deep infiltrative endometriosis. The occurrence of the disease is difficult to determine precisely, however, it is thought to affect up to an estimated 10% of women, with a higher prevalence in women who present with infertility (15–35%) and pain. Once symptomatic it can be an extremely debilitating condition causing chronic pelvic pain, infertility, dysmenorrhoea, deep dyspareunia, erratic pelvic pain, dyschezia (pain with defecation), and haematuria [3]. The symptoms are predominantly cyclical in nature increasing prior to and during menstruation but may be more vague and erratic.

Although the **initiating factors** that lead to the development of uterine leiomyomas and endometriosis are not known, there is a great deal of evidence showing that the ovarian steroids, estrogen and progesterone, are important factors for fibroid growth and endometriosis. Leiomyomas appear during a woman's reproductive years and usually regress following menopause. Biochemical and molecular studies have shown that leiomyomas have significantly increased levels of both estrogen and progesterone receptors when compared to normal myometrium, and are also capable of producing estradiol. Estradiol stimulates the proliferation of leiomyoma Smooth Muscles Cells, whereas progesterone appears to delay or inhibit programmed cell death (apoptosis) in uterine Smooth Muscles Cells [4]. Ovarian steroids, to which fibroids are responsive, influence the secretion of growth factor peptides and the expression of their receptors, with both estrogens and progestogens inducing expression of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF). All these growth factors promote the angiogenesis leading to proliferation, migration and differentiation of tumor cells. [5] Both bcl-2 protein and TNF-α expression is higher in fibroid cells than normal myometrium.

Because bcl-2 protein inhibits TNF-α-induced apoptosis it is believed that bcl-2 protein also has a role in fibroid growth by controlling TNF-α-induced apoptosis in fibroids. [6]

Current therapy for uterine fibroids and endometriosis is dependent on the degree of the patient's symptoms as well as the goals and objectives, keeping in mind the patient's age and the need to preserve her fertility. Treatment options include medical, surgical, and ablative therapies. [7]

Operative therapy**:** Operative therapies include Hysterectomy, and Myomectomy.

Hysterectomy: It is the removal of uterus by surgical means. However, for women who desire pregnancy or who wish to avoid surgery, hysterectomy is not a viable therapeutic option. Morbidity after hysterectomy can include bleeding, infection, injury to adjacent organs, and vaginal shortening. Hysterectomy with ovarian conservation is also associated with some loss of ovarian function and a decreased age of menopause. [7]

Myomectomy: For patients who desire future childbearing or uterine preservation, Myomectomy is an option. It can be performed via the vaginal route by laparotomy, laparoscopy, or hysteroscopy. However, myomectomy is associated with significant morbidity including hemorrhage, adhesion formation, leiomyoma recurrence, blood transfusion, bowel injury, and rarely hysterectomy. [7]

A variety of **thermal ablation techniques** have been applied to the treatment of uterine fibroids. This includes radiofrequency ablation, cryoablation, and laser ablation. The use of radiofrequency ablation (RFA) to achieve local control of a wide variety of tumors in many locations has become more accepted in recent years. However, the patient reviews revealed that outcomes were not as good as those seen after hysterectomy and there were no significant differences in quality of life before and after the radiofrequency ablation. [7]

Medical therapy includes the Non-Hormonal Therapy and Hormonal Therapy:

Although non-hormonal medical treatment does not deal with the disease process itself, it is an important part of the management of fibroids and endometriosis. Non-steroidal anti-inflammatory drugs are a main part of non-hormonal therapy for leiomyomas. NSAIDs are given for the symptomatic relief from the pain and inflammation that these disorder causes. [8]

Hormonal Therapy includes the use of several categories of the medications:

a) Combined estrogen and progestogens*:* A low-dose monophasic oral contraceptive when will render the endometrium hypoproliferative with hypotrophic glandular epithelium and atrophy of endometriotic tissue. Side-effects which may occur include: weight gain, headache and breast tenderness. However they lack the required efficacy for the management of the fibroids and endometriosis and the recurrence rate is high. [9]

b) Progestogens*:* Progestogens create a hypo-estrogenic, hyper-progestogenic state causing decidualization and atrophy of the endometrium. Norethisterone and Medroxyprogesterone acetate are well known agents of this class of medication. When prescribed for the treatment of endometriosis, progestogens are usually given for six to nine months. The side-effects of progestogens are excessive bleeding, increased appetite, weight gain, fluid retention/oedema, bloating, acne, breast tenderness, mood changes and reduced libido. [10]

c) Gonadotropin releasing hormone analogues: Gonadotropin-releasing hormone (GnRH) is the neuropeptide that regulates pituitary function in women. GnRH acts on the pituitary to increase the release of FSH and LH (two hormones that lead to ovulation and estrogen production by the ovary). GnRH-analogs overemphasize the action of GnRH. They suppress the production of LH and FSH by the pituitary gland. This results in decreased levels of estrogen. Also it has been reported that GnRH receptors besides being present in anterior pituitary gland, are also found to be expressed in ectopic endometrial cells and its proliferation can be directly inhibited by GnRH analogues like Leuprolide [11]. Treatment with these compounds causes a significant shrinkage in the size of

fibroids within three to six months. Analogues include Leuprolide, Goserelin, Buserelin, Nafarelin and Triptorelin. Leuprolide, Goserelin, Buserelin and Triptorelin are given subcutaneously or intramuscularly. Nafarelin is given by nasal route. The frequency of administration is weekly or monthly. However, these compounds cannot be used as a long-term therapy because they cause a significant decrease in bone mineral density when used for more than six months. Moreover, the therapy is too costly as these drugs are peptide based. Intramuscular or subcutaneous administration makes it a painful therapy lacking patient compliance. Add-back therapy equivalent to menopausal hormone replacement therapy (HRT) can be given either cyclically or continuously to prevent the decrease in bone mineral density. Of the all-available GnRH analogues Leuprolide acetate can be chosen over others due to its advantages like higher efficacy over other analogues, cost effectiveness and safety [12]. Although the effectiveness of both Goserelin and Leuprolide have been statistically proven similar, yet Leuprolide can be preferred primarily on the consideration of cost and its diverse clinical applications being well established drug for the treatment of uterine fibroids, endometriosis, and central precocious puberty [13]. Marketed formulation of Leuprolide includes Lupron depot® (microspheres), administered in a dose of 3.75 mg i.m. (weekly), or 11.25 mg i.m. (monthly) and Eligard® which is lyophilized powder for injection administered at doses, 7.5 mg s.c. (monthly) and 22.5 mg s.c. (three monthly). However, because of large volume of distribution (27 L), Leuprolide is not able to reach uterus in sufficient amount to act directly on GnRH receptors. This leads to various side effects like significant decrease in bone mineral density when used for more than six months, insomnia, decreased libido, headaches, mood swings, vaginal dryness, acne, muscle pains, dizziness, depression make it non patient acceptable. [14]

d) Selective estrogen receptor modulators (SERMs)

SERMs are another class of compounds that have been investigated as potential treatment option for uterine leiomyomas and endometriosis. SERMs are molecules that bind to the estrogen receptor but exhibit tissue-specific agonist or antagonist activity. Ideally, a SERM would provide the positive, estrogenic effects on bone, brain and the cardiovascular system but would act as an antagonist in the breast and uterus. [15]

Raloxifene is a benzothiophene derivative that does not show any agonist activity in the uterus. Raloxifene was specifically developed to maintain beneficial estrogenic activity on bone and lipids and antiestrogenic activity on endometrial and breast tissue. It is a USFDA approved drug for post-menopausal osteoporosis. Many researchers are now working upon Raloxifene to establish its potential use for treating uterine fibroids. It binds to the estrogen receptors over expressed on the leiomyoma cells, with high affinity and causes apoptosis of the cells. Oral administration has limited bioavailability of 2 %. Currently it is given by oral route as tablets (Evista ®) with the dose of 60 mg/day. Because of poor bioavailability it is not able to reach uterus in sufficient amount to elicit the direct action on estrogen receptors. The non-specific distribution throughout the body causes various systemic side effects like hot flashes, sweating, headache, dizziness, spinning sensation, leg cramps, joint pain, nausea, vomiting, and stomach pain [15,16] Raloxifene unlike Tamoxifene doesn't cause endometrial cancer. [17]

Hence, the surgical and ablative therapies are not preferred by women who wish to have pregnancy in future due to severe morbidities and mortalities. [18] Currently GnRH analogue Leuprolide acetate is administered by parenteral route making the delivery very painful and the systemic side effects make it non-patient acceptable. Similarly in the case of SERM, Raloxifene, the drug has very less oral bioavailability (2%) and so very lesser amounts may reach its site of action i.e. uterus. Hence, for both the type of agents vaginal delivery can prove to be a promising approach targeting the drug to the site of action.

The vagina, as a site for drug delivery, offers certain unique features that can be exploited in order to achieve desirable therapeutic effects. The currently available Vaginal Dosage Forms have limitations, such as leakage, messiness and low residence time, which contribute to poor subject or patient compliance. Attempts are being made to develop novel vaginal drug delivery systems that can meet the clinical as well as the user's requirements. [19, 20]

The **advantages** of the vaginal route of administration are:

1) The avoidance of hepatic first-pass metabolism.

2) A reduction in the incidence and severity of gastrointestinal side effects.

3) It overcomes the inconvenience caused by pain, tissue damage and probable infection by other parenteral routes.
4) The self-insertion and removal of the dosage form is possible.
5) By the advantage of First Uterine Pass Effect, drug can be directly targeted to Uterus via Vaginal Route. Hence, it can be a promising approach for treating uterine disorders.

Studies have reported that vaginal administration of progesterone produced uterine effects that could not be reasonably accounted for on the basis of circulating progesterone levels. [21] Similar findings have been reported for terbutaline (a uterine relaxant) and also subsequently for danazol (used to treat endometriosis). [22] The results suggested a mechanism whereby drugs administered vaginally were preferentially transported to the uterus through a so-called **first uterine pass effect**. It is an original concept that proposes that vaginally administered drugs are preferentially delivered to the uterus through some form of direct transport mechanism. According to this principle, vaginally administered substances are targeted to the uterus where their tissue concentration is amplified and systemic absorption is minimized which limits the circulating level and side effects. It can prove to be very advantageous for drugs whose actions are required in uterus. [23] Several theoretical mechanisms have been proposed to account for the phenomenon: (i) direct (passive) diffusion through the tissues; (ii) passage from the vagina to the uterus through the cervical lumen; (iii) transport through the venous or lymphatic circulatory systems; and (iv) countercurrent vascular exchange involving diffusion between adjacent uterovaginal veins and arteries. [24]

It has recently been demonstrated that the extent of the first uterine pass effect might be dependent on the exact location of the administered formulation within the vagina. A study performed showed preferential vaginal to uterine distribution of estradiol when a single estradiol tablet was placed in the upper third of the vagina, whereas no effect was observed for placement in the lower third. [25]

The **conventional vaginal dosage forms** includes Creams, gels, tablets, capsules, pessaries, foams, ointments, films, tampons and douches. Despite the variety of formulations for intravaginal therapy, their efficacy is often limited by a poor retention at the site of action due to the self-cleansing action of the vaginal tract. Also these medications often present problems like messiness and leakage being uncomfortable to use. Moreover they may not provide an exact dose because of non-uniform distribution. To overcome these limitations, novel delivery systems (microspheres, liposomes and niosomes) have attracted considerable attention for their opportunity to prolong the contact of drug with a mucosal surface, without inducing adverse local effects on the epithelium. [26, 27, 28] Certain formulation like liposomal carrier system is able to provide controlled and sustained release of appropriate drugs for the local treatment of gynecological diseases.

Rationale for chosing Liposomes as the formulation:

Liposomes are colloidal carriers, usually 0.05-5.0 μm in diameter, which form spontaneously when certain lipids are hydrated in aqueous media. Liposomes are composed of relatively bio- compatible and biodegradable material, and they consist of an aqueous volume entrapped by one or more bilayers of natural and/or synthetic lipids. Drugs with widely varying lipophilicities can be encapsulated in liposomes, either in the phospholipid bilayer, in the entrapped aqueous volume or at the bilayer interface. [29, 30] Liposomes of different sizes and characteristics usually require different methods of preparation. The most simple and widely used method for preparation of MLV is the thin-film hydration procedure in which a thin film of lipids is hydrated with an aqueous buffer at a temperature above the transition temperature of lipids. The drug to be encapsulated is included either in the aqueous hydration buffer (for hydrophilic drugs) or in the lipid film (for lipophilic drugs). Thin-film hydration method produces a heterogeneous population of MLV (1-5μm diameter), which can be sonicated or extruded through polycarbonate filters to produce small (up to 0.025 μm) and more uniformly sized population of SUV. [31]

Although thin-film hydration is a simple technique, one of the major disadvantages of this method is its relatively poor encapsulation efficiency (5-15%) of hydrophilic drugs.

Moreover, reduction of liposome size further decreases the amount of encapsulated drug. MLV with high entrapment efficiency can be prepared by freeze-drying preformed SUV dispersion in an aqueous solution of the drug to be encapsulated (Dehyrated Rehydrated Vesicles). [32]

Liposomes have been chosen as the formulation as they are lipid based systems which can be in nanometre size range. They will avoid the tissue necrosis and toxicity being biocompatible and bio degradable. Liposomes allows dual drug entrapment as well with Lipophilic drug loaded into its lipidic bilayer while Hydrophilic in to its aqeuous core. Liposomes will be able to easily diffuse through the vaginal cervix or uptaken by counter current exchange and enter the uterus for the direct effect of drug at the site of action by so called First Uterine Pass Effect. Moreover, liposomal system imparts stability to the drugs encapsulated. It provides targeted drug delivery or site specific drug delivery. It provides a controlled drug delivery.

Several aesthetic and functional qualities must be incorporated into the intravaginal formulations, which need to be designed for a specific prolongation of residence time and also for desirable distribution and delivery of the active substance for an extended period at a predictable rate and release pattern. Generally a prolonged vaginal residence time can be achieved by the use of Intravaginal Rings (IVR).

Intra Vaginal Rod Insert: Intravaginal rings are doughnut- shaped polymeric devices designed to provide a controlled release of drugs to the vagina for extended periods of time. Currently there are five commercially available IVR for contraception (NuvaRing®, Progering®, and Fertiring®) and hormone replacement therapy (Femring® and Estring®). [33]

Conventional vaginal ring technologies, comprising hydrophobic elastomeric polymers such as silicone or ethylene–vinyl acetate copolymers (PEVA), generally contain the active agent(s) homogeneously dispersed throughout the polymeric carrier (matrix-type IVR) or located in a drug-loaded central core over moulded by rate-controlling metering sheath (reservoir-type IVR). Active agents are released from hydrophobic, non-

biodegradable, elastomeric polymers via a permeation-controlled mechanism that depends upon the solubility and diffusivity of the active within the rate-controlling polymer. Consequently, the format of current IVRs devices are particularly well- suited to the sustained administration of therapeutically potent, small molecular weight and lipophilic actives. Hydrophilic and/or high molecular weight actives are generally poorly released from conventional IVR devices owing to poor permeation characteristics. A new vaginal ring device, the 'insert vaginal ring' (InVR), comprising a ring body into which various drug- loaded inserts can be placed has been recently developed. The device is effective for the sustained release of hydrophilic moieties. The ring device comprises one or more drug-loaded rods, fabricated from either modified silicone elastomer, a compressed solid tablet, or a lyophilized gel, that are inserted into cavities contained within a non- medicated silicone elastomer ring carrier. [34]

1.2. Aim and Objectives

The aim of the present study is to target the drugs available for fibroid and endometriosis treatment i.e. GnRH analogue and SERM to uterus by vaginal route to achieve the following objectives:

a) Targeting the drugs directly to the site of action and hence achieving maximum therapeutic effect by lowest possible dose.

b) Reduction in side effects of drugs by avoiding the systemic absorption.

c) Increasing the patient compliance by avoiding painful injectable route and self-medication being made possible by intra vaginal rings.

1.3 Hypotheses

Targeted delivery of Liposomal formulations containing drugs (Leuprolide acetate and Raloxifene Hydrochloride) specifically to uterus via intravaginal route will facilitate retention of drugs in uterus to act directly on the leiomyoma cells causing its apoptosis and thus, fibroid shrinkage. Targeting will improve therapy, reduce systemic side effects and provide a safe affordable dosage form.

1.4 Plan of Work

i) Literature search, procurement of drugs and excipients.

ii) Preformulation studies to establish the purity of drugs and assessment of drug excipient compatibility

iii) Analytical Methods for quantification of both the drugs

iv) Preliminary optimization of formulation and process parameters

v) To prepare and optimize Raloxifene Hydrochloride loaded liposomes by applying 3^2 Full Factorial Design

vi) To prepare and optimize Leuprolide acetate loaded Dehydrated Rehydrated Vesicles by applying 3^2 Full Factorial Design

vii) To Prepare and optimize dual drug (Raloxifene Hydrochloride and Leuprolide acetate) loaded Dehydrated Rehydrated Vesicles by applying 3^2 Full Factorial Design

viii) To prepare liposomal formulations loaded Rod Insert-Intravaginal Rings

ix) To characterize the prepared formulations for Vesicle size, zeta potential, % entrapment efficiency, % Loading (w/w) and Morphology by TEM and SEM

x) To determine the *in vitro* release properties of drugs from developed formulations.

xi) To perform the stability studies of prepared formulations as per ICH guidelines.

xii) To establish the safety profile of formulations by Histopathology studies.

xiii) To perform the cytotoxicity studies and apoptosis study of the formulations to evaluate the effect of different concentration and incubation time of formulations on % viability of cells.

xiv) To determine the *in vivo* performance of formulations by biodistribution studies in rabbits through Gamma Scintigraphy technique.

xv) Pharmacokinetic and Pharmacodynamic study to assess the fibroid regression over a period of time in rabbits by Ultrasonography method.

1.5 References

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