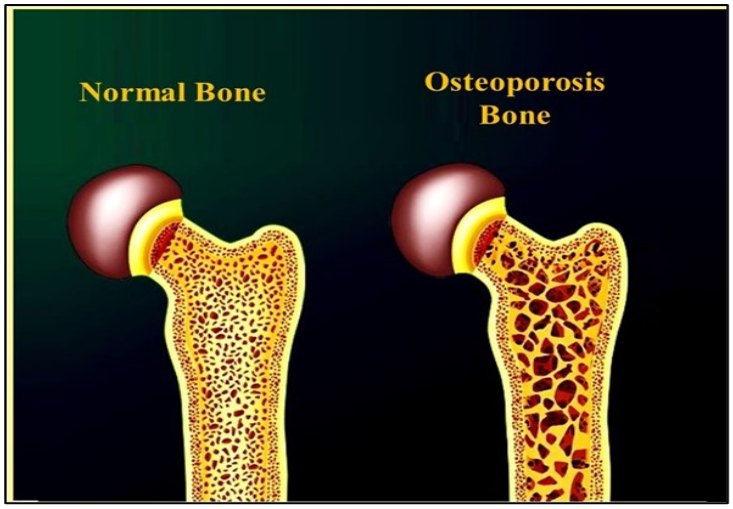

Chapter-1

Introduction

1.1. OSTEOPOROSIS:

1.1.1. Introduction:

Osteoporosis is a major public health problem and global healthcare burden characterized by reduction in bone mass and structural deterioration of bone tissue leading to impaired skeletal strength. It has clinical implications because of the morbidity, mortality and treatment cost related with osteoporotic fractures. The dramatical increase in fracture risk with age in both men and women, leads to increase in frailty of bones. About 16 % and 24 % of 50 years or older men and women respectively suffer each year globally. According to statistical survey of International Osteoporosis Foundation, 1 out of 3 women above 50 years age and 1 out of 5 men above 50 years age will suffer osteoporotic fracture in their life worldwide. It is one of the major public health issues which has been estimated to rise from 1.66 million in 1990 to 6.26 million by 2050 [1-4].

According to estimates, 158 million people were at high risk of fracture in 2010; by 2040, it was predicted that this number would double due to demographic changes [5, 6]. In India, there were around 26 million instances of osteoporosis in 2003, but by 2023, 80 million persons either had low bone density or were osteoporotic. Hip fractures occur more often in men and women over 50 in India, with yearly incidence rates of 163 and 121 per 100,000, respectively [7]. One in three women over 50 years and one in five men over 50 years may experience an osteoporotic fracture in their lifetime, according to the international osteoporosis foundation's statistics study [8]. This shows that osteoporosis is a serious global healthcare burden and a public health problem.

An imbalance between bone formation and bone resorption markers is an underlying mechanism in all cases of osteoporosis [9, 10]. The bone mineral density decreases, altering various transcription factors, growth factors, cytokines, etc., and bone microarchitecture deterioration occurs [9]. Three main mechanisms develop osteoporosis: excessive bone resorption, inadequate bone mass or strength during growth, and inadequate formation of new bone during remodelling [11]. Normally, bone matrix remodelling is constant. The remodelling of bone is based on coupled action of osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells). The bone remodelling cycle involves three consecutive phases: (a) in the resorption phase;

osteoclasts digest old bone; (b) reversal phase, when mononuclear cells appear on the bone surface; and (c) in the formation stage, when osteoblasts lay down new bone until the resorbed bone, is completely replaced [12].

Osteoblast and bone marrow stromal cells produce two cytokines that are essential for osteoclastogenesis. Receptor Activator of Nuclear factor-κB (RANK) is the receptor for Receptor Activator of Nuclear factor-Kappa B Ligand (RANKL) and is expressed on mononuclear osteoclast precursor. Osteoprotegerin (OPG), also produced by stromal cells and osteoblasts, is a natural decoy receptor for RANKL and thus antagonizes the osteoclastogenic action of RANKL. The other growth factors and transcription factors include VEGF (vascular endothelial growth factor), TNF (tumour necrosis factor), TGF (transforming growth factor), BMPs (bone morphogenic proteins), OTX (osterix) etc. Many other factors have been studied for their potential use in osteoporosis [11].

The defects in various hormonal levels lead to increase bone resorption by osteoclasts and decreased bone formation by osteoblasts. In menopause, lack of estrogen increases bone resorption and reduction in bone formation that takes place in the normal weight-bearing bone. Estrogen exhibits both skeletal and extra skeletal activities. In direct skeletal activity based upon estrogen receptors on osteoblasts and osteoclasts. At the same time, indirect activities of estrogen are mediated by estrogen receptors on various cells, including stromal cells and cells of immune systems that influence bone homeostasis. In addition, estrogen, calcium and vitamin D deficiency significantly impair bone deposition. Other than that, the secretion of parathyroid hormone by parathyroid glands reacts to a decrease in calcium level, which increases bone resorption. The hormones that regulate bone metabolism, which increases bone resorption, are glucocorticoids, thyroid hormones, and high doses of vit. D while decreasing bone resorptions are calcitonin and estrogen [3, 10].

1.1.2. Clinical risk factors for Osteoporosis [13]:

Many important clinical risk factors, several disorders and drugs have been identified through epidemiological studies. Clinical risk factors, BMD, and fractures were assessed using the WHO's Country Specific Fracture Risk Assessment Tool (FRAX). The tool calculates a 10-year probability of hip fracture or major osteoporotic fracture

(combined hip, spine, humerus, or wrist) utilizing BMD measurement and clinical risk factors, hence identifying people at high fracture risk.

When identifying whether individuals with osteopenia are at a higher risk of fracture, the FRAX tool is extremely useful. The FRAX method is helpful in determining a person's absolute fracture risk and, as a result, in personalized therapy, but it has certain drawbacks that keep it from being the mainstream standard. It is designed for postmenopausal women and men over the age of 40 who have not previously received bone protective treatment. Second, it does not take into consideration dosage responses for a variety of risk variables, including glucocorticoid use. Third, falls or an increased proclivity to fall are still not considered in the algorithm. Fourth, because only the BMD of the femoral neck is utilised to calculate fracture risk, absolute fracture risk in patients with the lowest BMD at the spine may not be correct.

Age, Sex, body weight, height.

Glucocorticoid therapy.

Femoral neck bone mineral density.

Smoking and alcohol consumption.

Parental history of hip fractures.

Rheumatoid arthritis.

Secondary Osteoporosis-

Premature menopause, malabsorption, chronic liver disease, hyperthyroidism, inflammatory bowel disease etc.

Medicines:

Heparin, immunosuppressants, chemotherapy, antiseizure medications etc.

1.1.3. Diagnosis of Osteoporosis [13]:

Bone mineral density (BMD) is the gold standard for osteoporosis diagnosis, measured by dual X-ray absorptiometry (DXA). In BMD, a comparison to sex-matched young health adults (T-score) or Sex matched and age-matched healthy population (z-score). The WHO defines a T-score of less or equal to -2.5 and osteopenia as a T-score between -1.0 to -2.5. Several studies in men and women have

shown that BMD is a specific predictor of fracture risk. Individuals with T-scores less than -2.5 are at a higher risk of fracture. Patients with T-scores more than -2.5, on the other hand, may still be at high risk of fracture. It is predicted that more than 50% of patients with hip fractures and 35% of patients with vertebral fractures had a T-score greater than -2.5. As a result, combining BMD and clinical risk factors for fracture predicts fracture risk better than BMD alone. Several laboratory tests are available to diagnose osteoporosis as follows:

- Initial laboratory tests:

  Renal profile

  Blood calcium, phosphorous and magnesium level

  25-hydroxyvitamin D

  Parathyroid hormone

  Thyroid-stimulating hormone

- Additional laboratory test:

  Bone turnover markers

  Sex hormones (includes testosterone, oestradiol etc.)

  Rheumatoid factors

  Serum tryptase and histamine levels

1.1.4. Treatment and management of Osteoporosis:

The major problem of osteoporosis management is that majority of those at high fracture risk are not diagnosed or treated, despite the availability of safe and effective diagnostic tools and therapeutics. This applies to both women and men, although more evident in men. Treatment aims to reduce future fracture risk and should be tailored to individual persons [14]. The management and treatment of osteoporosis include:

Non-pharmacological treatment:

The non-pharmacological treatment includes the change in general lifestyle factors, viz. balanced diet containing sufficient calcium and vit. D. The other factors include

avoiding excess alcohol consumption and smoking; regular weight-bearing and muscle-strengthening exercises should be encouraged. Walking was the easiest and simplest program to implement and most effective on the bone mineral density of the spine and hip. The role of calcium and Vit D supplementation in reducing falls and fractures are not clear. Bone loss in elders occurs either due to a reduction in calcium intake or absorption or due to deficiency of vit D. The daily intake of 1000 - 1200 mg of calcium, while 600 - 800 IU of Vit D, pronounced more anti-fractured effect or reduced risk of any fracture [14].

Pharmacological treatment:

Many drugs and combinations of drugs are now approved for the treatment and management of osteoporosis. The current therapies for osteoporosis are designed to increase bone formation and decrease bone resorption [8, 14][8,14]. The therapeutic agents for the treatment of osteoporosis are as follows:

Bisphosphonates:

Bisphosphonates are the most widely therapeutic agents for the treatment of osteoporosis. The bisphosphonates include alendronate, Risedronate, zoledronic acid, ibandronate, etidronate etc. These are synthetic analogues of pyrophosphate, which have a high affinity for bone minerals and reduced osteoclastic activity [8, 11, 14].

Strontium ranelate:

It is also commonly used to reduce non-vertebral and vertebral in postmenopausal osteoporotic women, but it weakens bone resorption and bone formation. It was approved by EMA [13].

Teriparatide:

It is a recombinant human parathyroid hormone (PTH-fragment of 1-34) used to treat osteoporosis with a high risk of fractures. It has a "black box" warning due to the occurrence of osteosarcomas in rats treated with a very high dose of teriparatide. The duration of treatment is a limited period of 24 months. The candidate is used only at very high risk of fractures or who have failed or are intolerant to other anti-osteoporotic agents [8, 11].

Selective estrogen receptor modulators:

This class of drugs belongs to the estrogen agonist and antagonist type. Raloxifene belongs to the SERM class and exhibits dual agonist and antagonist properties in estrogenic pathways. It acts as an estrogenic agonist on bone by increasing bone mass density and decreasing bone resorption [14].

Statins:

Statins are HMG-CoA reductase inhibitors that produce an anabolic effect by increasing in production of bone morphogenic proteins-2, which helps to increase bone formation. The statins exhibit both anabolic and anti-resorptive agents that may be important in osteoporosis management. The active mechanism of statins on bone includes proliferation, osteoblast protection, and osteoclastogenesis decrease [15, 16].

Other therapeutic agents:

Romosozumab is a human monoclonal antibody that inhibits sclerostin, secreted by osteoclasts to increase bone resorption. Odanacatib belongs to selective inhibitors of CatK, which is released by osteoclasts and promotes collagen degradation in bones. Tibolone is a systemic steroid used to manage and treat postmenopausal women [8, 11, 14].

1.2. Nanocarriers for drug delivery:

Nanocarriers gained have extreme attention due to their ability to diagnose and treat various chronic diseases. They have been used to overcome the problems associated with conventional drug delivery systems. Nanocarriers help to improve the therapeutic efficacy and bioavailability of drugs. Nanocarriers must owe to their high surface area to volume ratio and ability to alter the drug's basic properties and bioactivity. The aim of nanocarriers is to effectively treat disease with minimum side effects. Nanocarriers are expected to be easy and reasonably cheap to prepare, have a small particle size, possess high loading capacity, and ideally, increase bioavailable fraction [17, 18].

The various self-assembled or engineered construct nanocarrier systems with nanometres ranging up to 500 nm [17]. The nanocarriers include liposomes, glycerosomes, polymeric, lipid and metal nanoparticles, micelles, polyelectrolyte complex nanoparticles, niosomes, transferosomes etc. [19, 20]. The drug or therapeutic agents are

either covalently attached, adsorbed or encapsulated in nanocarriers [21, 22]. Numerous nanocarriers have been proposed in favour of the transport of drugs through the skin, enabling drug retention and, in several cases allowing a controlled release. Developing nanocarriers for poorly bioavailable drugs is a great challenge, and it still faces some unresolved issues [23]. The greater challenge with transdermal drug delivery is the permeation of drugs through the skin, which restricts the entry of most drugs. Skin penetration is important for drug delivery to the skin through skin care and protection [24-26]. Nowadays, nanocarriers have been tried and tested to overcome the barriers of SC to achieve higher permeability. They are designed to avoid immune system rejection and to reach the target site [23]. Nanocarriers can penetrate biological membranes to deliver drugs to the bloodstream [23]. In this present thesis, we have worked on drug-loaded glycerosomes and polyelectrolyte complex nanoparticles.

1.2.1. Glycerosomes:

Glycerosomes are novel vesicular systems for dermal and transdermal drug delivery. Glycerosomes are composed of different phospholipids, cholesterol, and high concentration of glycerol. Glycerosomes are flexible vesicular drug delivery systems that contain cholesterol which enhances their lipidic bilayer stability [17, 27, 28]. A new approach to enhance the permeability of vesicles is by adding a high concentration of glycerol and named the novel vesicular preparations are named as “glycerosomes”. Glycerol is a harmless and fully acceptable short-chain alcohol that can improve the fluidity and deformability of the vesicular bilayer, thus improving the ability of preparations to penetrate through the skin.

1.2.2. Polyelectrolyte complex nanoparticles (PECN):

PECN is formed by stoichiometric combinations of the polycation and polyanionic compounds, which are association complexes formed between oppositely charged polyelectrolytes (e.g., Polymer-polymer, polymer-drug, polymer-drug-polymer etc.) [29]. Generally, oppositely charged polyions undergo electrostatic interactions (polycations or polyanions), resulting in PECN formation [30, 31]. The advantage of PEC is that they can be formed without chemical cross-linking agents, making the synthesis process very simple and straightforward [30].

1.3. Drug candidate selection:

1.3.1. Risedronate Sodium (Bisphosphonate):

Risedronate sodium has low permeability (belongs to the BCS-III) and low oral bioavailability (under 1%), which are the most critical challenges of Risedronate sodium [32]. It also causes various side effects like upper GI irritation, ulceration of the oesophagus etc. [33]. Risedronate contains a phosphate–carbon bond resistant to most chemical agents and inert to enzymatic degradation [34].

1.3.2. Atorvastatin (statins):

Atorvastatin exerts beneficial effects on bone by acting as a rate-limiting step in cholesterol synthesis by blocking the conversion of HMG-CoA to mevalonate, increasing the expression of bone morphogenic protein-2 (BMP-2) in osteoblasts, inducing osteoblast differentiation and subsequently stimulating bone formation [35, 36]. The low bioavailability of Atorvastatin (12% orally) is due to its poor aqueous solubility, low dissolution rate and extensive first-pass metabolism (belong to BCS class II drugs) [35].

1.4. Aim and Objectives:

The present research aims to formulate, optimize and evaluate anti-osteoporotic drugs loaded nanocarriers for improved treatment of osteoporosis by enhancing permeability and bioavailability through transdermal route.

The objectives of the present work are:

- To enhance permeation of drugs through the skin, resulting in enhanced bioavailability.
- To formulate novel nanocarriers for delivery of anti-osteoporotic drugs (like glycerosomes and polyelectrolyte complex nanoparticles).
- To minimize side effects and enhance anti-osteoporotic drugs' efficacy by formulating novel nanocarriers.
- To optimize the formulation and process variables of the nanocarriers using the design of experiment.
- To characterize and evaluate the optimized formulations.
- To incorporate optimized drug-loaded nanocarriers into transdermal patch.

- To optimize, characterize and evaluate transdermal patch.
- To perform *in vitro, ex-vivo* and in vivo studies of drug-loaded nanocarriers incorporated transdermal patches.
- To assess the stability study of drug-loaded nanocarrier formulations and transdermal patches.

1.5. Hypothesis:

It is hypothesized that the transdermal patches containing drug-loaded nanocarriers (glycerosomes and polyelectrolyte complex nanoparticles) for the treatment of osteoporosis will enhance the permeability of the drug through the skin and deliver them to the bloodstream, which in turn will enhance their bioavailability and also avoid the gastrointestinal intolerance associated with the drugs.

1.6. Plan of work:

- Procurement of drugs, excipients and other chemicals and reagents.
- Preformulation study of drugs and screening study of material and process parameters.
- Development of analytical method for quantification of drugs.
- Development and optimization of nanocarriers (glycerosomes and polyelectrolyte nanoparticles)
- Physico-chemical characterization of prepared formulations.
- Particle size, PDI and zeta potential.
- Surface and shape analysis.
- % Drug Entrapment Efficiency (EE).
- % Drug loading.
- Deformation index determination.
- Stability study.
- *In vitro* drug release study of nanocarriers.
- *Ex vivo* drug permeation study of nanocarriers.
- *In vitro* cell-line study of the formulations.
- Incorporation of nanocarriers into transdermal patch.
- Characterization and evaluation of transdermal patch.
- Stability study of the transdermal patches.

- *Ex vivo* skin permeation studies.
- In vivo pharmacokinetic and Pharmacodynamic study.

Chapter 1 -Introduction

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