INTRODUCTION

Bone is a mineralized connective tissue that supports our body movements, gives protection and serves as a reservoir for minerals (Giudiceandrea et al., 1998; Lee et al., 2008; Soicher et al., 2010). Because of its role as a structure that is strong and provides framework for locomotion, bone is having an unfortunate reputation of being an inert and static material. However, bone is capable of adapting its mass and morphology according to functional demands; it regenerates without leaving a scar (Sabolinski et al., 1996) and it functions as the largest reservoir of the minerals and regulates their metabolism, making it rather the best “dynamic” tissues of all; making it the best example of “forms follow function” (Sullivan et al., 1947).

Bone is made up two components; cells that make the bone dynamic and matrix, which is a mineralized extra cellular component that gives rigidity to bone. Bone cells are of 3 types: osteoblasts, osteocytes and osteoclasts. Osteoblasts develop from preosteoblastic cells present in the lining of the osteoid, also called as periostium. Osteoblastic cells perform the function of matrix synthesis, and when the mineralization is completed, 90% of the osteoblasts undergo apoptosis, while remaining 10% osteoblasts differentiate into mature osteocytes. These osteocytes communicate with each other through number of extra cellular processes and play a key role in bone remodeling and regeneration. Osteoclasts are bone resorbing cells that resorb the bone and play a significant role in mineral metabolism. Bone formation during the normal process of skeletal remodeling is initiated by the migration of preosteoblasts to sites of osteoclastic bone resorption, which precedes the laying down of new bone (Baron, 1996). These preosteoblasts subsequently differentiate into mature osteoblasts and eventually deposit and mineralize osteoid protein. Hence, this entire cyclic activity of osteoblasts and osteoclasts maintains our bone integrity.

Bone matrix is a calcified collagen matrix, lightweight and formed of both organic and inorganic constituents. Collagen is the main protein, while hydroxyapatite is the chief mineral constituent of the inorganic part (Rho et al., 1998). Collagen gives strength to the matrix in the same way steel is used in reinforced concrete. Collagen is made up of fibrils that are made up of 3 polypeptide chains and each about 100 amino acid long. These chains are bundled together to form a rope like structure which gives tensile strength to the bone (Lowenstam and Weiner, 1985). Mineralization of hydroxyapatite on this fibrils, makes the bone hard and one of the strongest biological material
Introduction

on this planet (Rho *et al.*, 1998; Kerschnitzki *et al.*, 2010). At micro level bone is forming repetitive lamellar structures which are bathed by periosteal fluid (Weiner and Wagner, 1998). This lamellar arrangement makes bone more dynamic, having greater torsion strength, making it harder to break.

Three different types of cells can be found within the bone matrix: osteoblasts (which produce matrix and synthesize bone); osteoclasts (which resorb the bone); osteocytes (residential cells of the bone which account for 90% of adult bone skeleton). Osteocytes are considered as specialized and fully differentiated osteoblasts (Ducy *et al.*, 2000), osteoblasts are described as differentiated fibroblasts. It is the multipotent mesenchymal stem cells that give rise to fibroblasts, osteoblasts, adipocytes and osteocytes (Aubin 1998). Compared to these, osteoclasts are hematopoietic in origin and they develop from monocytic cells in the bone marrow (Fujikawa *et al.*, 1996).

Osteoblastic cells are the edifice squad of the bone. They not only form and synthesize the matrix, but they also regulate the mineralization and development of mature bone. They are cuboidal cells located at the bone periphery in the form of a tight layer. Bone homeostasis is an orchestral phenomenon dependent on variety of intrinsic and extrinsic factors such as important proteins integrins, connexins and cadherins responsible for cell to cell contact or cell-matrix contact. Other than these varieties of receptors and hormones, cytokines and growth factors are also equally important. This entire assembly controls the function of osteoblasts and regulates the mineralization; maintaining the bone homeostasis (Lecanda *et al.*, 1998; Ferrari *et al.*, 2000). Life span of an osteoblast varies depending upon the species and individual. Life span of a rabbit osteoblast is about 3 days (Jowsey, 1977), while that of the human may be as long as 8 weeks (Lecanda *et al.*, 1998). After a long process of this bone synthesis, 90% of the osteoblasts undergo apoptosis, while remaining gets trapped inside the calcified matrix and change their phenotype into osteocytes. Compared to normal osteoblasts they reduce their organelle load and stops bone synthesis. They remain connected with other osteocytes and the osteoblasts which are lining the periostium, creating an extensive network of cells for intercellular communication. This communication is the one that plays a key role in bone regeneration and healing.

Osteoclasts are multinucleated cells, characterized by their ability to resorb fully mineralized bone. Osteoclasts develop from hematopoietic stem cells and they are highly migratory,
multinucleated and polarized cells. They carry an armory of acids and enzymes which bring about the resorption of bone (Teitelbaum, 2000). They are unique compared to hematopoietic cells as they carry pleomorphic mitochondria, large number of lysosomes, filled with acids and hydrolytic enzymes and vacuoles (Walker, 1972). At the apical membrane they form tight junction with the calcified bone at the site of resorption; also called as Howship’s lacunae. At this site lytic enzymes are secreted, with lowering of pH by the action of proton pumps, which continuously pump H+ ions in the resorption pit. This leads to acidic pH between the range of 2 – 4 and activation of acidic hydrolytic enzymes such as Tartrate resistant acid phosphatase (Blair et al., 1989). Osteoclasts are so energetic in bone resorption that they resorb bone 200,000 more potently then osteoblastic bone synthesis (Sommerfeldt and Rubin, 2001). Hence, bone formed by 7 to 10 generations of osteoblasts is resorbed by one single osteoclast; in its limited life span of 15 – 20 days (Albright and skinner, 1987).

It was established in 1997 that formation and activity of osteoclasts depend on secretion of paracrine factors by osteoblastic cells. Secretion of osteoprotegrin (OPG) by osteoblastic cells is governed by many hormonal factors. This OPG binds to OPG ligand and then binds to RANK receptor on osteoclasts precursor cells. This initiates a cell signaling mechanism that leads to gene inactivation and inhibition of osteoclasts maturation. Opposite to this, another ligand, RANK Ligand, binds to RANK receptor and activates genes of osteoclastic development and maturation. Hence, OPG has negative effects, while RANK L has positive effects on osteoclastic development and maturation. RANK L and OPG competitively inhibit each other and ultimate development of osteoclasts is determined by the ratio of concentration of OPG and RANK L (Hofbaur et al., 2000). Secretion of OPG and RANK L is controlled by variety of hormones and growth factors, which are the structure troupe of bone metabolism. Hence, significant power of bone metabolism is controlled by the concentrations of RANK L and OPG. OPG has entered clinical trials, merely 3 years after its discovery (Hofbauer and Heufelder, 2000).

The primary job of hormones responsible for controlling bone remodeling is to regulate the mineral homeostasis rather than controlling skeleton morphology. Though hormones like PTH, calcitonin and vitamin D$_3$ are the major hormones responsible for this remodeling, they exert their effects by either stimulating osteoblastic bone formation or by inhibiting osteoclastic bone resorption. Osteoblastic stimulation is a direct process, while control on the osteoclasts is
achieved by OPG RANK L system. For example, PTH increases serum calcium by increasing the
expression of OPG L on osteoclastic cell membrane or by directly inhibiting OPG secretion (Lee
and Lorenzo, 1999; Väänänen et al., 2000; Wise et al., 2002; Irie et al., 2007). OPG and its ligand
are also believed to be involved in the action of Vit D3 on bone cells. In addition, Vit D3 is also
increasing the reabsorption of calcium from intestine and hence increases serum Calcium and
suppresses secretion of PTH from Parathyroid glands, leading to lowered osteoclastic activity.
Similarly estrogen also protects bone by increasing OPG levels in osteoblasts and inhibits the
osteoclasts development and activity (Hofbauer et al., 2000).

It was Fuller Albright who defined osteoporosis as a disease with his very famous statement “too
little bone in the bone, but what bone there is, is normal bone”. In last few decades there is an
increase in average life span and popularization of western life style in the Asian countries. Due
to this altered life style there is increase in the occurrences of chronic diseases like type II
diabetes, dementia and osteoporosis (Czerwiński et al., 2007). Osteoporosis is a bone condition
defined by low bone mass, increased fragility, decreased bone quality, and an increased fracture
risk (WHO, 1994). Although the disease historically has been reported mostly in white women, it
can affect individuals of either sex or all ethnic groups. Although osteoporosis traditionally has
not been listed as a complication of diabetes, patients with either type 1 or type 2 diabetes are
among those at increased risk for this disease (Christensen et al., 1999; Kemink et al., 2000).

Peak bone mass, the highest of bone density is attained in humans by the adulthood. This peak
bone mass is maintained for few years and then it starts dropping due to inequality in bone
formation and bone resorption rates. These conditions are much more worsened in case of females
who sustain increased bone loss due to menopause (Marcus et al., 1983; Melton and Riggs, 1989;
Kelly et al., 1989; Tremollieres et al., 1993; Bismar et al., 1995; Mei et al., 2001; Gallagher and
Christopher, 2007; Hussain, 2009). Though this accelerated bone turnover and loss of bone
integrity is more prominent in post menopausal osteoporosis, it is the decrease in bone synthesis
that leads to development of osteoporosis. Hence, due to this problem, though osteoporosis is
more prominent in post menopausal conditions, osteoporosis like conditions are observed in
elderly men as well (Eastell et al, 1998; Scane et al., 1999; Amory et al., 2004; Sadat-Ali et al.,
2006; El-Desouki et al., 2007; Francis, 2007).
Osteoblasts and osteoclasts, cells of these remodeling units are controlled by variety of systemic hormones, cytokines and other local paracrine factors such as PTH, calcitonin, estrogen, and Vit D (Lurkt and Raisz, 1990; Stavros and Jilka, 1995). During menopause estrogen deficiency leads to alteration in the levels of specific cytokines such as IL-1, tumor necrosis factor-a, granulocyte-macrophage colony stimulating factor, and IL-6 (Hurwitz, 1993). These factors promote osteoclasts formation and increase bone resorption (Cumming et al., 1995; Bauer et al., 1993; Prince et al., 1991).

There are many factors, apart from hormonal factors that affect progression and severity of osteoporosis. It has been an established fact that physical exercise promotes bone health and increases bone density, makes bones stronger and reduces bone loss. During menopause patient who have lesser bone mass and body weight, tend to have more bone loss (Kanis and Pitt, 1992). Several other factors like irregular menses, early menopause, other hormonal complications, caffeine ingestion, alcohol consumption and smoking badly affects bone health (Seeman et al., 1983; Jensen et al., 1985; Aloia et al., 1985; Spencer et al., 1986; Hazes et al., 1990; LaCroix et al., 1993; Baron et al., 2001; Albrand et al., 2003; ). Similarly genetic constituent of the person also affects the bone. For example men have higher bone mass compared to women and similarly, Blacks and Hispanics have more bone density compared to Whites and Asians (Bell et al., 1991; Gilsanz et al., 1991; Russell-Aulet et al., 1993; Ross et al., 1996; Maalouf et al., 2000; Shilbayeh et al., 2003; Barret et al., 2005; Louise, 2005; Sharma et al., 2005; Cheng et al., 2007).

Two specific types of osteoporosis has been described, where type I is associated with loss of sex gland functioning and associated osteoporosis, while type II is associated with normal aging of the person and associated bone loss (Whyte et al., 1982; Melton and Wahner, 1986; Rico et al., 1993; Buckley et al., 1996; Czerwinski et al., 2007; Lee, 2007).

Type I osteoporosis develops in the cases of decrease in the circulating levels of sex hormones, especially in women, where there is loss of ovarian function due to menopause. During menopause, there is decrease in the levels of estrogen which fails to check the serum levels of various cytokines and leads to development of increased procurement, proliferation and development of osteocalsts. These events lead to development of more prolific osteoclasts which resorb the bone and thin trabecules. Due to this thinner trabecules, these patients present with more risks of fractures. As the long bones and vertebral bodies are bearing more stress, their
probabilities are higher of breaking under as a stress (Stavros and Jilka, 1995; Yamato et al., 1998; Cavolina et al., 1997; Zhang et al., 2002; Leung et al., 2002; Heo et al., 2009; Arlot et al., 2005; Pivonka et al., 2012).

Contrary to this type II osteoporosis occurs with normal aging process and it is observed in men and women typically after the age of 70 (Melton and Riggs, 1989; Hurwitz, 1993; Feik et al., 1997; Miyakoshi et al., 1999; Zebaze et al., 2002 Pivonka et al., 2008). Normal aging process leads to lower number of osteoblasts as the reservoir of preosteoblastic cells gets depleted and hence the formation of new bone is less and the person suffers from osteoporosis (Parfitt, 1982). Thus, type II osteoporosis is associated with less bone formation and not over activity of osteoclastic cells, as compared to type I.

Osteoporosis can be studied using variety of experimental models and various biochemical markers as well histomorphometric evaluations. Biochemical markers of osteoporosis includes serum alkaline phosphatase (ALP), osteoblastic alkaline phosphatase (ALP), type I procollagen carboxy terminal peptide and osteocalcin (Taylor et al., 1994; Charles et al., 1992; Eriksen et al., 1993). Osteoclastic resorption rates can be estimated using products of osteoclastic degradation. For example type I procollagen carboxy terminal peptide is measured in serum and urine to learn about the activity of osteoclasts (Kushida et al., 1995). Majority of the osteoclastic degradation products are excreted in the urine and hence estimation of hydroxyproline, hydroxylysine and glycosylated hydroxylysine and 3 hydroxy-pyridinium crosslink compounds gives a better picture of bone resorption (Parfitt et al., 1987; Uebelhart et al., 1990; Beardsworth et al., 1990; Panigrahi et al., 1994). Similarly, elevated levels of serum alkaline phosphatase indicate the increased activity of osteoblastic cells (Panigrahi et al., 1994; Jenkins et al., 2001; Nawaw et al., 2001; Rangrez et al., 2011; Parikh et al., 2009; Parikh and Rangrez, 2012). This marker is considered to be the most significant marker of understanding osteoblastic activity, except for the patients with liver disease, as liver also contributes towards the serum alkaline phosphatase levels. Alternative biomarker for osteoblastic activity is osteocalcin, a 49 aminoacid chain protein which constitutes about 20% of the total non collagenous proteins of the bone. It is widely accepted as a marker of osteoblastic activity and one of the important factors that induce mineralization of the matrix. However, this marker is less useful compared to ALP levels, as its levels tend to vary with slight onset of other metabolic disorders (Eriksen et al., 1993; Charles et al., 1992; Taylor et al., 1994).
For osteoclastic bone resorption serum levels of type I procollagen carboxy terminal peptides had shown promising results. They are found to be in high accordance with trabecular bone resorption, histomorphometry and osteoclastic activity. Urinary markers of osteoclastic activity include hydroxyproline, pyridinium cross linked peptides and increased levels of tartrate resistant acid phosphatase (TRAcP) (Cheng et al., 1996; Garnero et al., 1998; Watts, 1999; Farley et al., 2002; Chailurkit et al., 2001; Vesper et al., 2002).

Diabetes is the world’s largest disease involving metabolic disorders of carbohydrate, protein and fats. According to WHO projections, the number of diabetes if patients is expected to be doubled to 300 million in the year 2025 (Boyle et al., 2001). The mechanism of bone loss in type 1 diabetes is still unknown, although several theories exist based on animal and cellular models. Insulin-like growth factors and other cytokines may influence diabetic bone metabolism (Bullion, 1991). Among many systemic and local factors involved in the regulation of bone metabolism, insulin and insulin-like growth factor-I (IGF-I) are known to play important anabolic roles (Canalis, 1993; Thomas et al., 1997). Patients with insulin deficiency as exemplified by type 1 diabetes are associated with osteoporosis (Krakauer et al., 1997; Piepkorn et al., 1997); those with Laron syndrome caused by IGF-I deficiency also exhibit this condition (Laron et al., 1999). A reduction in IGF-I is also implicated as an important factor in the etiology of involutional osteoporosis, especially of age-related bone loss (Nicolas et al., 1994; Rosen, 1994; Reed et al., 1995; Canalis, 1997). Insulin and IGF-1 signaling is the most important factors in diabetes, but is also important regulators in bone metabolism. Insulin and IGF-1 have anabolic effects on osteoblasts in vitro and an association of diabetes with abnormal bone metabolism has been reported.

IRSs (insulin receptor substrates) are substrates for both insulin and IGF-I receptors. Insulin and IGF-I are known to be bone anabolic factors. IRS-1 deficient mice exhibited severe low turnover osteopenia with decreased bone anabolic and catabolic action, while IRS-2 deficient mice showed osteopenia with decreased anabolic and increased catabolic action. IRS-1 signaling plays an important role for maintaining bone turnover while IRS-2 signaling maintains anabolic balance in osteoblasts (Akune, 2004). IRS, main target molecule of insulin/IGF-1 receptor signaling, have been shown to play important roles in maintaining normal bone turn-over by skeletal analysis of IRS-1 and -2 knock-out mice. Skeletal analysis of disruptive AKT, a downstream molecule of IRS, in mice also revealed that AKT was established as a crucial regulator of osteoblasts and
osteoclasts by promoting their differentiation and survival to maintain bone mass and turnover (Ogata and Kawaguchi, 2008). Insulin and insulin-like growth factor-I (IGF-I) are potent bone anabolic factors through the balance of distinct signals of the two adaptor molecules, insulin receptor substrate IRS-1 and IRS-2; IRS-1 for maintenance of bone turnover by up-regulating anabolic and catabolic functions of osteoblasts, while IRS-2 for retaining the predominance of the anabolic function over the catabolic function (Kawaguchi, 2009). Diabetic retinopathy, advanced cortical cataracts, and diabetic neuropathy have all been associated with increased fractures (Ivers et al., 2001 and Piepkorn, 1991). These are also risk factors for increased falls because of visual impairment and alterations in balance or gait.

Type 2 diabetes was previously believed to provide bone protection because of its associated normal to increased BMD. These reports were primarily based on the concept of BMD alone and were generally not from prospective controlled large trials. However, considering the associated risk factors of osteoporosis, patients with diabetes generally have an increased risk of accidents because of peripheral neuropathy, possible hypoglycemia, nocturia, and visual impairment. Because many type 2 diabetic patients are obese and sedentary, coordination and balance factors that are protective in falls may be absent. Thus, patients with generally larger body size and relatively high bone mass may have higher fracture rates. Conversely, patient groups with low BMD, such as Asians, may have lower fracture rates when one considers all factors in a risk assessment. Bone quality changes may also be affected by microvascular events common in diabetes (Vogt et al., 1997). Schwartz et al., (1997) in a large prospective study of older women obtained from the Study of Osteoporotic Fractures, confirmed that women with type 2 diabetes experience higher fracture rates in regions of the hip, humerus, and foot than do non-diabetic women. Bone loss has been observed to be greater in patients with poorly controlled diabetes than in those whose diabetes is in good control (Gregorio, et al., 1994).

Research has lead to the general agreement among physicians and researchers that the progression of bone loss can be halted in post menopausal women with Estrogen Replacement Therapy (ERT) and long been thought to be the treatment of choice for osteoporosis because it improved in BMD at the hip and spine by 5% and 2.5%, respectively. However, views on this issue have changed recently as recommended by the recent consensus statement from the North American Menopause Society (NAMS, 2000). Moreover, effectiveness of estrogen well documented in controlled
clinical trials, but due to new data on harms, risk generally outweighs benefit for the indication of osteoporosis prevention. (Gaby, 1994; Gaby and Wright, 1990; Andersson et al., 2002 and Weil, 2005). Raloxifene (RLX, Evista) is a selective estrogen receptor modulator (SERM) that has indications for both prevention and treatment of osteoporosis. 25 Trials involving SERMs have shown a 55% reduction in vertebral fractures. Both estrogens and SERMs have a small increased risk of various other side effects including thromboembolic and cancer (Cauley et al., 2001; Cummings et al., 2002; Bushnell and Goldstein 2004; Vogel, 2006; Premkumar et al., 2006).

Current treatments for osteoporosis include those pharmacological agents that check the osteoclastic bone resorption. Very fewer therapies target to increase the osteoblastic bone formation, like treatment with thyroid hormone. Antiresorptive drugs include calcium, bisphosphonates and vitamin supplementation, hormonal replacement therapy and administration of calcitonin through parenteral rout or nasal routs. Osteoblast stimulating agents such as sodium fluoride, androgens and parathyroid hormones are used more for the treatment of type II osteoporosis.

However, these conventional therapies possess various side effects including virulization and hepatotoxicity, more bone and joint pain, development of uterine and vaginal cancers. One of the most accepted supplementation therapies is calcium and its formulations. It decreases the bone resorption and increases bone mineral density. Lee (2007) in his review article has mentioned that calcium supplementation of the normal diet increases bone mass in adolescents and reduces bone loss associated with advancing age (Nagant et al., 1983; Prince et al., 1991; Buckley et al., 1996). Calcium supplements when given alone to women who have established osteoporosis have shown variable results in reducing fracture risk in controlled trials. Relative vitamin D deficiency, like calcium deficiency, is also a problem in postmenopausal women, and some studies have shown that vitamin D3 and calcium seems to reduce the risk of hip and other nonvertebral fractures by as much as 43% in elderly women (Chapuy et al., 1992). However, calcitriol therapy, though safe, requires careful monitoring of serum and urine calcium levels to avoid hypercalcemia and significant hypercalciuria.

Estrogen deficiency is a major cause of bone loss in postmenopausal women (Mosekilde et al., 2000). In young women who have amenorrhea or ovulatory disturbances, cyclic medroxyprogesterone has been demonstrated to increase bone density, further exhibiting the central role that female sex hormones play in osteoporosis. Estrogen replacement therapy is
associated with increased bone mass, decreased urinary biochemical resorption markers, and reduced fracture rate in many studies (Ronderos et al., 2000; Torgerson et al., 2001; Nelson et al., 2002).

Therapeutic regimens that include estrogen are more effective in protecting against osteoporosis than those that contain calcium alone. Estrogen replacement seems to be most effective at maintaining bone mass when started soon after menopause and continued. This is due to the accelerated period of bone loss during early postmenopause during which women may lose bone mass at the rate of up to 3% to 5% per year for approximately 5 to 7 years. Unfortunately, estrogen therapy has significant risks of either uterine cancer or breasts cancer (Lafferty and Fiske, 1994; Cauley et al., 1995; Stampfer and Colditz, 1990; Steinberg et al., 1991; Colditz et al., 1995; Kawas et al., 1997; Eitan et al., 2009; Rossouw et al., 2002; Glassset et al., 2007). Furthermore, recent data have demonstrated that use of hormone replacement therapy is associated with an increased risk for acute myocardial infarction and stroke (Baldereschi et al., 1998; Rau et al., 2003; Deborah et al., 2000; Heckbert et al., 1997). Thus the use of hormone replacement therapy has been relegated to only short-term use at low dose for patients who have significant perimenopausal symptoms.

Complementary therapy is an area of health care that is growing in popularity and many people find such therapies beneficial in offering relief from pain and improving their quality of life (Moyad et al., 2002). Complementary therapy is increasingly being used along with conventional medicine as part of a person’s pain management plan. Herbal medicine is one of the oldest forms of complementary therapy having been used in all cultures and civilisations for centuries. For centuries, various human cultures have used plants to treat several common health conditions, including bone fractures. Recently, active compounds extracted from the roots, leaves, flowers, and seeds from these plants have been shown to have anti-reabsorptive or anabolic properties for bone.

Plants benefit bones through different pathways: some have agents that decrease systemic levels of the pro-inflammatory cytokines associated with bone loss; others contain high levels of calcium, while some others act in the gastrointestinal tract to enhance calcium absorption. A common vegetable with bone protective properties that is consumed worldwide is onion (Allium cepa). The aqueous residue after aqueous ethanol fractionation from dried onion bulb has been found to decrease bone reabsorption and to increase bone mineral content (BMC), trabecular
thickness, and trabecular BMD in growing male rats. Ethanolic extracts from onion have also been shown to decrease bone loss in female osteoporosis models and to inhibit the reabsorption activity of osteoclasts. Another related vegetable that is commonly consumed worldwide is garlic. Its oil extracts have been shown to prevent ovariectomy-induced bone loss and to modulate bone turnover in young rats (Muhlbauer et al., 2003). Recently, safflower seed components have been scientifically shown to have bone-protective and bone-forming properties. Both crude and aqueous extracts of safflower seeds stimulate osteoblast differentiation in culture, (Jang et al., 2005) and defatted safflower seeds prevent ovariectomy-induced bone loss in rats (Kim et al., 2002). Methanolic extracts of safflower seeds have bone-forming properties that may be mediated by IGF-1 in young rats (Lee et al., 2009). Safflower seed oil protects trabecular bone at the proximal tibial metaphysis of adult rats. It also increases insulin growth factor-I (IGF-I), IGF-II, IGF binding protein-3, and bone alkaline phosphatase in treated ovariectomized (OVX) rats (Alam et al., 2006). In animal models for osteoporosis and in in vitro studies, green tea has been positively correlated with bone health both by increasing bone formation and decreasing bone reabsorption (Delaissé et al., 1986; Nakagawa et al., 2002; Tokuda et al., 2007; Yun et al., 2007). Of the many chemical agents from plants that have beneficial properties on bone, phytoestrogens stand out and have been studied extensively. These compounds have estrogen-like activity and have been incorporated into drug treatments for estrogen replacement in menopausal women; as a result, their positive effects on bone maintenance and low incidence of side effects are now documented. The most commonly recognized phytoestrogens are:

1) The isoflavones, which are naturally occurring organic compounds in plants, and
2) The isoflavonoids, which are related to isoflavones and are derived from the flavonoid biosynthesis pathway (Anderson et al., 1999).

Many studies have reported that consuming these compounds benefits bone. Specific components of the soy isoflavone group of compounds can have bone-protecting effects when used alone. In young OVX rats, for example, bone loss was prevented by genistein, a selective estrogen receptor modulator (SERM) analog.
Extracts from the stems and leaves of a plant from the grape family, Cissus quadrangularis (CQ) have been used in traditional Ayurvedic medicine to heal bone fractures. In rats, this plant is known to enhance fracture healing (Singh and Udupa, 1962) increase bone mineralization (Irving, 1973), promote accumulation of mucopolysaccharides at the site of fracture, and increase calcium
uptake (udupa and Prasad, 1964). In OVX rats, it also increased the biomechanical properties of bone (Shirwaikar et al., 2003) and bone mass while reducing osteoclastic activity (Potu et al., 2009).

In conclusion, one can say that many food plants and spices contain agents that show promising effects for bone maintenance in age-related and postmenopausal osteoporosis. However, with the exception of phytoestrogens, the mechanisms by which their protective effects are exerted are still not fully understood. Some of the commonly consumed plants are very promising for bone health, for example, onions, dried plums, curcumin, green tea, and phytoestrogens. There are sporadic reports of other plants that have shown bone-protective properties, and investigating these plants further may be worthwhile in order to understand their mechanisms as well as to explore their effects on humans. Among the options for plant-based complementary medicines, a few stand out: *Cissus quadrangularis*, FLL, and safflower seed extracts. *C. quadrangularis* has been used in Ayurvedic medicine for a long time to enhance fracture healing, (Singh and Udupa, 1962; Shirwaikar et al., 2003) and it protects against ovariectomy induced bone loss; it is also consumed as a vegetable in Africa and Southeast Asia. It is important to note that no reports were found of adverse side effects following very high-level consumption of these plants. The scientific literature includes very promising reports of plant nutrients and fatty acids that are both beneficial to bone and commonly consumed in many parts of the world. As life expectancy around the world continues to increase, the number of people at risk of developing osteoporosis is rising as well. There is consequently a growing need for therapies that:

1) Provide a balance between bone resorption and bone formation,
2) Increase the strength of bone, and
3) It should have few or no side-effects.

A closer look at and selection of plant-based agents, particularly those that may ameliorate age-related and postmenopausal bone loss, is therefore needed, and studies on the mechanisms of action (for the most promising agents) should be conducted.

The genus *Moringa Adans.* (Family Moringaceae) has more than 13 species (Verdcourt, 1985), of which two species viz. *M. oleifera* Lam. (syn. M. pterygosperma Gaertn.) and *M. concanensis* Nimmo occur in India. *Moringa oleifera* Lam., (MO) a medium sized tree species has gained importance due to its multipurpose usage and well adaptability to dry and hot climates of north-
western plains, central India and dry regions of peninsular India. For centuries, many cultures have looked to MO as a general remedy and healing agent. It has been referred to as the Miracle Plant. MO’s benefits are both broad and compelling. Legend has it that MO’s effectiveness is known for treating more than 300 conditions and has been heavily utilized in folk medicine to treat a variety of health conditions. Apart from being a good source of vitamins and amino acids, it has medical uses (Makkar and Becker 1999; Francis et al 2005). MO, otherwise regarded as a “miracle tree”, is reputed to have many medicinal properties, although many have not been scientifically substantiated. It has been used in the treatment of numerous disease conditions (Pal et al 1995; Makonnen et al 1997; Ghasi et al 2000 and Matthew et al 2001), including heart disease and obesity due to its hypocholesterolemic property (Ghasi et al 2000). Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics. MO provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, MO is very important for its medicinal value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor (Bharali et al., 2003), antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant (Leelalavinothan et al., 2007), antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

It is considered as one of the World’s most useful trees, as almost every part of the MO tree can be used for food, medication and industrial purposes (Khalafalla et al., 2010). People use its leaves, flowers and fresh pods as vegetables, while others use it as livestock feed (Anjorin et al., 2010). This tree has the potential to improve nutrition, boost food security and foster rural development (Hsu, 2006). Almost every part of MO can be used for food and as forage for livestock (Ram, 1994). The leaves can be eaten fresh cooked or stored as dried powder for several months the pods, when young can be cooked; eaten like beans (National Research Council, 2006). Its oil and micronutrients have been reported to contain antitumour, antiepileptic, antidiuretic, antiinflammatory and venomous bite characters (Hsu, 2006).
Introduction

Recently, a high degree of renewed interest was placed on the nutritional properties of MO in most countries where it was not native (Reyes et al., 2006; Oduro et al., 2008). This could be due to the claims that it increases animal productivity as it has nutritional, therapeutic and prophylactic properties (Fahey, 2005). Studies from other countries indicate that the leaves have immense nutritional value such as vitamins, minerals and amino acids (Anwar et al., 2007).

Recently it was reported that MO leaf possesses neotropics ctivity (Mohan et al., 2005) and hence can enhance memory. MO leaves contain significant amounts of vitamins A, B and C, calcium ions, iron, potassium, proteins, traces of carotenoids. Saponins, pylates (Ferreira et al., 2008) and phenolic constitituents (Siddhuraju and Becker, 2003). In addition, leaf extracts have been shown to regulate thyroid status (Tahiliani et al., 2000) and cholesterol levels in rats (Ghasi et al., 2000). Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids (Bennett et al., 2003; Aslam et al., 2005; Manguro and Lemmen, 2007; Amaglo et al., 2010; Gowrishankar et al., 2010).

Fruits of MO have also been scientifically examined for their use in hypercholesterolaemia (Ghasi et al., 2000; Mehta et al., 2003). The MO aqueous pod (fruit) extract contain high amount of tannin, phenolic compounds and flavonoids (Biswas et al., 2012). Several bioactive compounds were isolated from MO pod and seed such as niazirin, niazimicin, niazicin A, benzyl-isothiocyanate, benzyl-thiocarbamate, and glucomoringin (Francis et al., 2004; Brunelli et al., 2010; Cheenpracha et al., 2010). The isothiocyanate from glucomoringin, an active compound found in MO seeds, was found to reduce myeloma growth in nude mice (Brunelli et al., 2010).

Flowers of MO are reported to be used in treating cold and anemia and report to contain powerful antibiotic pterygospermin which has fungicidal properties (Sreenivasan and Jyotsna, 2000). The flowers show effective hepatoprotective effect due to the presence of quercetin. A decoction of the flowers is used as a cold remedy (Orwa et al., 2009). Flowers have also proved to possess the antimicrobial activity against E. coli, K. pneumoniae, Enterobacter species, Proteus mirabilis, P. aeruginosa, Salmonella typhi A, S. aureus, Streptococcus and Candida albicans (Nepolean et al., 2009).
Introduction

Recently fruit of this plant has been explored for its osteoprotective effect. Preliminary results indicate that it is having positive effect on bone (Burali et al., 2010; Rangrez et al., 2011). These reports are of in vivo studies and no reports are available to understand the probable mechanism behind its activity. Though, all the components of this plant are proven to be having many pharmacological effects, but one fruit is explored for their osteoprotective effect. Hence, there is an urge to explore this plant for its osteoprotective effect and identifying new pharmacological agents from it. Keeping this aim in mind we have designed a study to explore this plant and its components for their osteoprotective effect.

Aims of the present studies are:

1. To have an insight in to hematological, biochemical and histological alterations in hyperglycemic ovariectomized rats and understand the effect of hyperglycemia in postmenopausal bone loss (Chapter I).
2. To explore MO plant components for their ameliorating effect on hyperglycemic OVX bone loss (Chapter II)
3. To look into the effects of different parts of MO for their osteoprotective potency by taking into consideration various biochemical markers as well as histoarchitecture (Chapter III)
4. To explore various components of MO for their phytochemical constituents. (Chapter IV)
5. To explore different components MO for their osteoclast inhibiting potential. (Chapter V)
6. To explore different components of MO for their osteoblast stimulating potential. (Chapter VI)