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# Preclinical studies and clinical evaluation of compounds from the genus *Epimedium* for osteoporosis and bone health

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ABSTRACT

The morbidity and mortality associated with fractures due to osteoporosis or "porous bone" contributes significantly to global healthcare costs and will increase exponentially with ageing populations. In menopausal women, the onset of menopause and rapid estrogen withdrawal leads to osteoporotic fractures. Healthy bone requires the coordinated remodeling function of osteoclasts, osteoblasts, and osteocytes in the basic bone multicellular unit, regulated by estrogen, RANKL/OPG, ROS, growth factors, and other kinase signaling pathways. Anti-osteoporotic drugs in current use such as hormone replacement therapy, selective estrogen receptor modulators, and bisphosphonates are designed to target these pathways, but all have their limitations. Extracts of the dried aerial parts of the traditional Chinese medicinal plant *Epimedium* (Berberidaceae) has long been used for bone health. Some nine *Epimedium* prenylflavonoid compounds have been reported to target estrogen signaling and other bone morphogenesis pathways in mesenchymal stem cell, osteoblast, and osteoclast cell lineages. *Epimedium* prenylflavonoids and enriched extracts can exert beneficial effects on bone health in estrogen-deficient and other osteoporosis animal models. The development of sensitive and rapid mass chromatographic techniques to quantify compounds extracted from *Epimedium*, including icariin and icaritin, has been used to standardize production and to study the pharmacokinetics and metabolism of *Epimedium* in animal models and humans. Recent clinical trials have reported positive effects on bone health, suggesting that compounds or extracts of *Epimedium* have the potential to be developed as agents, alone or in combination with other drugs, to prevent or delay the onset of osteoporosis and reduce the risk of hip fractures.



**Contents**



*Abbreviations:* BMP, bone morphogenetic protein; ER, estrogen receptor; EXD, Er Xian decoction; HRT, hormone replacement therapy; MAPK, mitogen-activated protein kinase; MCSF, macrophage colony stimulating factor; MSC, mesenchymal stem cell; NFκB, nuclear factor kappa B; NFATc1, nuclear factor of activated T-cells, cytoplasmic 1; OPG, osteoprotegerin; OVX, ovariectomized; RANK, receptor activator of NFκB; RANKL, receptor activator of NFκB ligand; ROS, reactive oxygen species; RUNX 2, Runt-related transcription factor 2; WHO, World Health Organization; XLGB, Xian Ling Gu Bao.

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## 1. Introduction

Osteoporosis or "porous bones" occur when an excessive amount of protein and minerals, particularly calcium, is lost from skeletal tissue. Bone loss gradually and asymptomatically occurs with aging. In women, osteoporosis accelerates after menopause due to the drastic drop in endogenous estrogen production. The morbidity and mortality associated with osteoporosis is secondary to the fractures that occur. More than 200 million people worldwide are estimated to suffer from osteoporosis, with annual costs exceeding approximately 10 billion dollars (Reginster & Burlet, 2006). Moreover, it is projected that about 50% of all osteoporotic hip fractures will occur in Asia by the year 2050 (Gullberg et al., 1997). In Singapore, the incidence of osteoporosis in those over the age of 65 is projected to increase from 6% to 19% from the period of 1990 to 2030 (Committee on Ageing Issues, 2006). Mortality rates after a hip fracture can be up to 20–24% in the first year (Cooper et al., 1993; Leibson et al., 2002). Among hip fracture survivors, there is significant loss of function and independence, with 40% being unable to walk independently and 60% requiring assistance a year later (Magaziner et al., 1990).

## 2. Osteoblasts, osteoclasts, and bone metabolism

Osteoporosis results when bone metabolism is unbalanced with bone resorption exceeding bone formation. Bone is a dynamic tissue that undergoes constant remodeling in response to mechanical load, and remodeling is the physiological mechanism whereby old damaged bone is replaced (Boyce et al., 2012). Bone remodeling occurs in the basic multicellular unit, which comprises the osteoblasts, osteoclasts, and osteocytes, covered by a canopy of bone lining cells (Fig. 1).

*Osteoblasts and osteocytes* are derived from mesenchymal stem cells (MSCs), which are multipotent cells originally found in the bone marrow that have the potential to differentiate to osteoblasts, adipocytes, myocyte, neurons, and chondrocytes (Fig. 2). Osteoblasts that are trapped within calcified bone collagenous matrix form osteocytes. These cells extend dendritic processes that facilitate communication with the bone lining cells and osteoclasts. Osteoblasts express receptor activator of NFκB ligand (RANKL), which binds to its receptor, receptor activator of NFκB, on the surface of osteoclasts and their precursors (Fig. 1). It has been shown that apoptosis of osteocytes increases osteoblast expression of RANKL, which binds to its cognate receptor in osteoclasts (Tatsumi et al., 2007).

*Osteoclasts* are the cells responsible for bone resorption (Fig. 3). These multinucleated cells originate from the hematopoietic lineage and are formed by the fusion of mononuclear progenitors such as monocytes and bone marrow macrophages (Boyle et al., 2003). The binding of RANKL to its receptor leads to differentiation and fusion of the mononuclear precursors into differentiated multinucleated osteoclasts. Osteoclast activation and survival both normally and in most pathologic conditions are associated with increased bone resorption. Osteoprotegerin (OPG), a decoy peptide, is secreted by osteoblasts and osteogenic stromal stem cells and prevents excessive bone resorption by mopping up RANKL to reduce binding to RANK. Thus, the RANKL/OPG ratio in the bone marrow is an important determinant of bone mass in normal and disease states (Boyce & Xing, 2007).

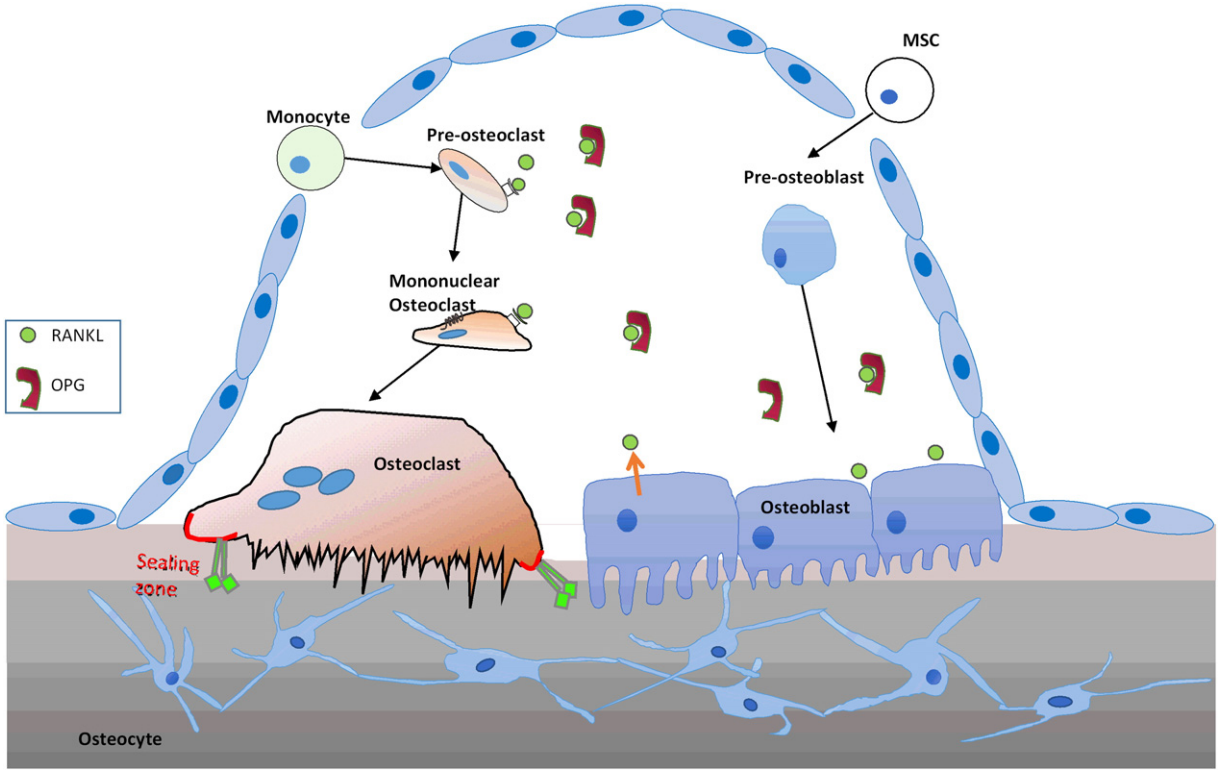


**Fig. 1.** Bone remodeling, basic multicellular unit. The basic multicellular unit is the site where bone remodeling occurs. During bone turnover, the bone lining cells shift away from the bone surface allowing the osteoblasts and osteoclasts to perform their respective functions. Monocytes enter the remodeling site and undergo differentiation in the presence of RANKL to become a mature osteoclast, which then initiates bone resorption. The osteoblast precursors, MSCs, also enter the remodeling site and undergo osteogenic differentiation into mature osteoblast. Functional osteoblasts would then begin to mineralize the areas it is adhered to. In addition, osteoblasts express RANKL, necessary for osteoclast differentiation, as well as OPG, which are decoy receptors that bind RANKL. The osteoblasts therefore modulates osteoclast differentiation via its relative expression of RANKL: OPG.