CHAPTER I

INTRODUCTION

Osteoporosis, a major public health problem, is becoming increasingly prevalent with aging of the world population. Osteoporosis is a skeletal disorder characterized by low bone mineral density and deterioration of the microarchitecture of bone tissue, which predisposes the individual to increased risk of fractures of the hip, spine and other skeletal sites (Kanis et al., 1994). Bone fractures may lead to disability, decreased quality of life, and increased morbidity and mortality (Ray et al., 1997). It is estimated that worldwide cases of osteoporosis-related hip fractures will increase from 1.66 to 6.25 million persons each year by the year 2050 (Cooper et al., 1992). Asia has a large population. Possibly, it will have the highest absolute increment in the elderly population in the years ahead. Actually, 26% of all hip fractures occurred in Asia in 1990, and this rate could rise to 37% by the year 2025 and to 45% by the year 2050 (Gullberg et al., 1997). The high prevalence of osteoporosis has occurred recently in the world, especially in such countries where the human life-span has been prolonged. In women aged 40 – 44 years (a premenopausal age) (Marcus et al., 1994), the prevalence of osteoporosis is only 0.9% (Henry et al., 2000), however, it increases to over 30% at 70 years of age (Löfman et al., 2000) and to 87% for the age older than 79 years (Henry et al., 2000). In men, the prevalence is generally two to three times less than that in similarly aged women (Pande, 2001; Olszynski et al., 2004). Nevertheless, the incidence of osteoporosis also increases with the increase in male life-span. Therefore, bone loss in elderly men has began to receive much attention by researchers (Bilezikian et al., 1999; Melton, 2001).

In women, estrogen deficiency has been recognized as a key factor of osteoporosis development. It plays an important role in maintaining bone mass in adult women by exerting a tonic suppression of bone remodeling and maintaining the balance between bone formation and bone resorption. Thus, entering the menopause with the sudden loss of estrogen could result in decreases of bone mineral density (BMD) and bone mineral content (BMC) (Insogna et al., 1981; Riggs et al., 1982;

Orwol and Meier, 1986; Ohta et al., 2002). In men, androgen deficiency is considered to be one of the most important risk factors of osteoporosis (Baran et al., 1978; Orwoll and Klein, 1995; Behre et al., 1997) and testosterone therapy increases bone formation and bone mineralization (Baran et al., 1978). It is established that androgen withdrawal induced by orchidectomy (ORX) results in significant loss of bone mass in experimental animals (Vanderschueren et al., 1992; Prakasam et al., 1999; Erben et al., 2000) and androgen administration can prevent the decrease of BMD and BMC in the orchidectomized animals (Prakasam et al., 1999). However, during recent years, research results have provided evidence that estrogens play an important role in male bone homeostasis. The major source of estrogens in males is the extraglandular aromatization of gonadal and adrenal androgens. Men with a mutation in the estrogen-receptor gene (Smith et al., 1994) or those with a defective aromatase enzyme exhibit low bone mass (Morishima et al., 1995; Carani et al., 1997). In the latter, testosterone and other androgen levels are all greatly raised, while estradiol and estrone levels are undetectable; the subsequent treatment with estradiol increases bone mass at all bone sites (Carani et al., 1997; Rochira et al., 2000). During aging in males, not only serum testosterone levels are decreased, but bioavailable estradiol levels are also decreased (Janssens and Vanderschueren, 2000). Additionally, bone mass in elderly men is more significantly correlated with the serum estradiol level than the serum testosterone level (Slemenda et al., 1997; Szulc et al., 2001). Therefore, estrogen replacement therapy is proposed to prevent bone loss in men as well as in women (Turner et al., 1994; Rochira et al., 2000; Ockrim et al., 2003).

In women, the higher and the longer exposure to estrogen after menopause is considered to be a main risk factor for the development of breast cancer (Kenemans and Bosman, 2003; Fontanges et al., 2004) and endometrial cancer (Sulak, 1997; Canavan and Doshi, 1999). Use of testosterone or other androgens has been considered for prevention of bone loss, however, prostate cancer is highly dependent on stimulation by androgens (Janssens and Vanderschueren, 2000; Crawford, 2005; Gaylis et al., 2005). Breast cancer and prostate cancer are hormone-dependent neoplasms in women and men, respectively. The gonadal hormones induce viability or stimulate growth of tumor cells (Santen, 1992). Considering these problems, alternative drugs for bone loss therapy with few adverse effects should be sought.

Estrogens express their activities by binding two different estrogen receptors (ERs), ERα and ERβ. ERα dominates in some few specific tissues and is mainly involved in reproductive system, whereas ERβ is expressed in many tissues including bone (Gustafsson, 1999). Both types of ERs are found in rat and human osteoblasts (Hoyland et al., 1997; Onoe et al., 1997) in both cortical and trabecular compartments of bone (Onoe et al., 1997; Swindahl et al., 2000). There are no differences in distribution and expression levels of ERβ mRNA between female and male bones (Onoe et al., 1997). Although ERβ transactivates promoters containing estrogen responsive elements (ERE) in an estradiol-dependent manner, the molecular mechanism to regulate the transcriptional activity of ERβ appears to be distinct from that of ERα. Therefore, it is possible that estrogen exhibits its tissue-specific actions in an ERβ-dependent mechanism as well (Onoe et al., 1997). Intensive investigation is currently underway to identify selective estrogen receptor modulators (SERMs), which display desirable estrogenic effects but lacking or having less undesirable side effects. Thus, phytoestrogens are being tested for the SERMs activity.

Phytoestrogens exhibit estrogenic activity by binding to both ERs, with a higher binding affinity (Kuiper et al., 1998) and a higher induction of mRNA expression at ERβ (Onoe et al., 1997). Administration of soybean isoflavones, genistein or daidzein or their glycoside forms, significantly prevent bone loss in ovariectomized (OVX) rats and mice (Fanti et al., 1998; Ishida et al., 1998; Ishimi et al., 2000; Picherit et al., 2000), and in ORX mice (Ishimi et al., 2002). Phytoestrogen-rich plants, such as *Pueraria lobata*, *Epimedium brevicornum* and *Taxus yunnanensis*, prevent bone loss in OVX animals without a hypertrophic effect on uterus (Wang et al., 2003; Yin et al., 2006; Zhang et al., 2006). Based on these results, other herbs containing high amount of phytoestrogens are being sought as an alternative phytoestrogen source for bone loss therapy.

Pueraria mirifica, a Thai phytoestrogen-rich herb, has been thoroughly examined for its estrogenic effects on female and male reproductive organs (Malaivijitnond et al., 2004, 2006; Trisomboon et al., 2004, 2005, 2006a, 2006b). Feeding of *P. mirifica* induces a vaginal cornification and an increased uterine weight in OVX rats (Malaivijitnond et al., 2004, 2006). In adult female monkeys, long-term administration of *P. mirifica* prolongs the menstrual cycle length and suppresses

folliculogenesis and ovulation (Trisomboon et al., 2004, 2005). In aged menopausal monkeys, *P. mirifica* decreases serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels (Trisomboon et al., 2005, 2006a). Furthermore, *P. mirifica* could alleviate menopausal symptoms in women (Muangman and Cherdshewasart, 2001). In male, *P. mirifica* significantly decreases sperm numbers in epididymis and percentage of sperm motility in rats (Langkalichan and Smitasiri, 1985). It inhibits mating behavior and testicular development in male pigeons (Smitasiri and Sakdarat, 1995). From these numerous reports, it can conclude that *P. mirifica* has an estrogenic effect on reproductive systems in males and females. So far, no published reports of *P. mirifica* on bones have been found. Thus, effects of *P. mirifica* on bone loss in female and male rats was evaluated in the present study. Bone loss is induced by ovariectomy in female rats and by orchidectomy in male rats. The determination of bone loss therapy after *P. mirifica* treatment has been performed in both axial and the long bones, in trabecular and cortical compartments at metaphyseal and diaphyseal sites.

Most of *in vivo* studies evaluating estrogenic effects of phytoestrogens on bones are conducted in rodents, and most of rodent diets contain soybean products as a protein source. Additionally, soybeans contain high amount of isoflavone phytoestrogens. So far, no researchers have analyzed phytoestrogen contents in rat diets which are widely used in Thailand. In the present study, concentrations of major phytoestrogen substances as puerarin, daidzin, daidzein, genistin and genistein in standard and soybean-free rodent diets from S.W.T. Co., Ltd., Thailand, are analyzed. The phytoestrogen contents between two lots (collected on different days) of *P. mirifica* Cultiva Wichai-III are also compared.

The objectives of study

1. To study effects of *P. mirifica* on bone mineral density and bone mineral content in osteoporotic female and male rats.
2. To compare the efficacy of *P. mirifica* on bone loss prevention in female and male rats.
3. To investigate the phytoestrogen contents in rodent diets and *P. mirifica* powder.

Anticipated benefits

1. To understand the effect of sex hormonal deficiency on bone loss in female and male rats and the prevention by *P. mirifica* treatment.
2. To apply the knowledge gained from this study for future therapeutic use of *P. mirifica* in osteoporotic treatment in humans.