CHAPTER I

INTRODUCTION

Rationale for the study

Menopause is the cessation of menstruation resulting from the dysfunctional ovarian activity. The ovaries produce most of the estrogen that activate all of the reproductive system such as regulation of the menstrual cycle, stimulation of breast and maintainance of bone mineral density. Therefore, the risk of breast, ovarian and endometrial cancers and osteoporosis has been exhibited by estrogen deficiency (La, et al., 1992; Schildkraut, et al., 2001; Francucci, et al., 2008). Menopause commonly occurs between the ages of 40 and 50, and typically perimenopausal period spans 8 to 10 years. In 2010, there have been about 740 million menopausal women in the world. It is predicted that in the next 10 years, there will be approximately 1.0 billion women aged 50 and older. This number is projected to increase to 1.2 billion by the year 2030 (Hill, 1996). Women aged 50 can be expected to live for more than 30 years. As people live longer, women are spending more of their lives in menopause, affected by a variety of menopause-related health effects such as osteoporosis, cardiovascular disease, breast cancer and other symptoms that decrease the overall quality of life.

A gradual reduction in the amount of estrogen can cause symptoms of menopause. In general, menopausal symptoms may include insomnia, hot flushes of the skin, vaginal atrophy and drying and for some women, menopause may bring on changing of the mood and may lose the elasticity of the skin. Also, the decrease of estrogen may cause loss of bone mass and lead to osteoporosis with increased risk of bone fracture (Elizabeth, 2008). Osteoporosis generally develops in the bone of the hips, distal forearms, and particularly in vertebral columns.

Many women receive hormone replacement therapy (HRT) to relieve symptoms of menopause and to protect against menopause-related health effects such as osteoporosis. HRT is mainly the supplement of estrogen or estrogen plus progestin. Estrogen and progestin are usually given together in the woman with intact uterus because the risk of endometrial cancer is significantly higher among women who used

estrogen alone (Estrogen replacement therapy or ERT) for many years (Rogerio, 1995).

The Women's Health Initiative (WHI) and several studies confirmed that prolonged use of estrogen plus progestin decreased the risk of fracture but HRT with estrogen alone or estrogen plus progestin can potentially increase the risk of cancer, particularly in the breast and endometrium, and thromboembolic disease (Ingemar, 1996; Grady, et al., 2002; Mara, et al., 2005). The recent study (Susan, 2010) found that there was no increase in the number of cases of cancer in women taking combination HRT in the beginning two years of therapy. However, over five to six years the number increased significantly. Hence, several women against using or stop taking HRT because of these adverse effects.

Selective estrogen receptor modulators (SERMs) are a group of drugs that may be good replacements for HRT which have been generally used to treat menopausal symptoms. Later, many studies found that SERMs-like Raloxifene did not stimulate the endometrium and had beneficial effects on cardiovascular system by reducing cholesterol. In contrast, it produced significantly trouble breathing, chest pain and vision change. SERMs-like Tamoxifen has been shown to increase a woman's risk of uterine bleeding, uterine cancer (Adjuvant Hormonal Therapy, 2010) and another potentially serious adverse effect is the risk of developing a thromboembolic event causing heart attack or stroke (Garber, 2010). A report in September 2009 from Health and Human Services Agency for Healthcare Research and Quality suggested that invasive breast cancer was reduced in women taking SERMs.

Nowaday many women are interested in the natural estrogen for an alternative treatment of menopausal symptoms. Phytoestrogens are currently being used as a natural estrogen. Phytoestrogens can bind to the estrogen receptor (ER) and induce estrogen like effects in animals and humans. The major phytoestrogen groups are isoflavones, coumestans, and lignans. Some studies (Anderson, Smith and Washnock, 1999; Xiao, 2008) indicate that isoflavones in soy bean and soy bean products have health benefits including potential reduction in the cancer of breast, prostate, the risk of cardiovascular disease, possibly protection against osteoporosis and menopausal symptoms. Furthermore, both flavonoid and lignan phytoestrogens have antioxidant activity (Cassidy, 2003). Many natural dietary products are contained

phytoestrogen, such as Dong quai, as being responsible for the menstrual cycle and to stop menstrual pain (Francesca and Edzard, 2010); black cohosh, was used for hot flushes, night sweats and vaginal dryness (Francesca, et al., 2008); sesame, shown to be estrogenic associated with the use in preventing osteoporosis (Boulbaroud, et al., 2008). Phytoestrogens have been effective in treating menopausal symptoms but some phytoestrogen plants can produce adverse effects White Kwao Khruea has been associated with proliferative lesions and tumors of the uterus (Jefferson, et al., 2002) even if white Kwao Khruea may be useful for the treatment of osteoporosis in menopausal women (Urasopon, et al., 2008).

Nowadays, several herbs have been proven to be useful for osteoporosis and the effects related to menopause. *Asparagus racemosus* (AR) is locally known in Thai as Samsib. It is well known for its phytoestrogenic properties to be useful for female rejuvenation. Its root has long been used in Thai traditional medicine to promote fertility and reducing menopausal symptoms. There are evidences that the root extract of AR has been used to increase milk secretion in pregnant rats (Pandey, et al., 2005), increase the weight of mammary glands and uterus (Sabnis, et al., 1968). Moreover, Rao (1981) found that AR exhibited a decrease in tumour incidence on 7,12 - dimethylbenz[α]anthracene (DMBA)-induce mammary carcinogenesis in rats. However, no scientific trial explains the beneficial effects of root extract of AR on bone loss and reproductive organs. Therefore, the aim of this study is to evaluate the protective effects of AR root extract on bone loss and effects on reproductive organs in ovariectomized (OVX) adult female rat.

Main objective of the study

The objective of this study is to evaluate the effects of AR root extract on bone and reproductive organs in ovariectomized rats.

Specific objectives of the study

1. To examine the effect of AR root extract on the length, thickness and weight of femur
2. To examine the effect of AR root extract on histology of femur

3. To examine the effect of AR root extract on the level of β-CTx, P_1NP, calcium, phosphorus, ALP and estradiol

4. To examine the effect of AR root extract on macrostructure and microstructural change in uterus and mammary gland of rat after ovariectomy

The scope of the study

Female Wistar rat model of bone loss induced by ovariectomy was used to evaluate the effect of AR on bone and reproductive organs. The dose of AR at 100 or 1000 mg/kg body weight (B.W.) was administered for 90 days before evaluating of the protective effect on bone loss and effect on reproductive organs. Biochemical analysis of serums was performed to evaluate the levels of calcium, inorganic phosphate, alkaline phosphatase (ALP), estradiol and the bone turnover markers for bone resorption, β-crosslaps (β-CTx) and bone formation, total procollagen type 1 amino-terminal propeptide (P_1NP). The effect on histological changes of bone and reproductive organs was examined using Hematoxylin & Eosin (H&E) staining of femur bone, uterus and mammary gland.

Hypothesis

Estrogen deficiency is an important risk factor in the pathogenesis of osteoporosis. The OVX rat has been well established as a model for studying menopausal bone loss. Ovariectomy may be resulted in a decrease in uterine weight and femoral parameters. OVX rats leading to estrogen decline may develop similar characteristics like increased bone turnover. β-CTx and P_1NP, the markers for bone formation and resorption used in this study may be increased in OVX group. Histologically decalcified bone sections may be revealed the thinning of trabeculae and widening of intertrabecular spaces.

To test the hypothesis that AR has a beneficial effect on bone of rats, the reduction of β-CTx and P_1NP levels in AR treated rats should be exhibited compared to the OVX group. AR treated rats should be revealed the thicker elongated trabeculae with narrow intertrabecular spaces compared to OVX group. Moreover, feeding with AR should not exhibit the uterotrophic effect and proliferative effect on reproductive organs.

Research place

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Research plans

Table 1 Research plans

Activities	1st-3rd month	4th-6th month	7th-11th month	12th-13th month	13-14th month	15th-16th month	17th-18th month
Review of related literature and research	↔						
Plant material and plant extraction		↔					
Animal preparation, ovariectomy and treatment with AR			↔				
Biochemical analysis				↔			
Histological analysis					↔		
Statistical analysis						↔	
Conclusion and discussion							↔