Chapter I

Introduction

White kwao krua is one of the indigenous Thai herbs that are classified into family Leguminoceae, subfamily Papilionoideae as soy and other legumes. It was firstly discovered and classified by Vatna in 1939 as red kwao krua (*Butea superba* ROXB) because of their superficial resemblance (Bounds and Pope, 1960). Later, in 1952, white kwao krua has been recognized as a new species and reclassified as *Pueraria mirifica* by Airy Shaw and Kasin Suvatabandhu. Other dialects of *P. mirifica* are tong-krua, tan-jom-tong, po-ta-goo, tan-krua, and jan-krua. This plant is widely found everywhere in Thailand, particularly in the deciduous forests of the northern Thailand especially in Chiang Mai province. It is a liana, which has tuberous roots. Flower color is bluish purple. Leaf shape is closely similar to that of red kwao krua, but thinner and smaller than it is. Its tuberous root with whitish starch granules has a round or ellipse-shape (Kashemsanta et al., 1957; Pisetpakasit, 1976).

The tuberous roots of *P. mirifica* have been analyzed by chromatography technique and found many chemical substances with estrogenic activities such as miroestrol, deoxymiroestrol, daidzein, genistein, coumestrol, puerarin, kwakhurin, and mirificin (Kashemsanta et al., 1957; Pope et al., 1958; Pisetpakasit, 1976; Ingham, Tahara, and Dziedzic, 1986, 1987, 1988, 1989; Chansakaow et al., 2000a, 2000b). These substances are included in phytoestrogens (Pope et al., 1958; Pisetpakasit, 1976; Barnes et al., 1998; Murkies, Wilcox, and Davis, 1998). Phytoestrogens are plant-derived substances with estrogen-like biological activity. Chansakaow (2000) noted that 100 gram of *P. mirifica* dry powder contains 46.1 mg of daidzein and 2-3 mg of miroestrol and deoxymiroestrol. Muangman and

Cherdshewasart (2001) also analyzed *P. mirifica* cultivar Wichai III, which is the same lot to our study, with the high performance liquid chromatography (HPLC) technique and found that this cultivar contains the significant amount of isoflavones (169.1 mg total isoflavones/100 gram of the dry powder) whereas small amounts of miroestrol, deoxymiroestrol, and other phytoestrogens present it.

Estrogens, sex steroid hormones, play the main function on reproductive system in women as well as female animals. There are three types of estrogens; estrone, estradiol, and estriol. Estradiol is the major estrogen that is secreted from the ovaries in women. Estrone and estriol are largely products of estradiol metabolism. During the reproductive year, the daily secretion of estrogen varies cyclically throughout the quasi-monthly menstrual cycle. Estrogen production is governed by two pituitary gonadotropins; follicle stimulating hormone (FSH) and luteinizing hormone (LH). Estrogen cooperated with FSH and LH regulates the growth and development of follicle and stimulates an ovulation. Estrogen and other ovarian hormones, including progesterone and inhibin, regulate FSH and LH secretion from the anterior pituitary gland by both the negative and positive feedback mechanisms (Rhoades and Pflanzer, 1996).

Several studies have been reported that phytoestrogens altered levels of gonadotropins and sex steroid hormones in women (Knight and Eden, 1996; Murkies et al., 1998; Setchell, 1998; Tham, Gardner, and Haskell, 1998). Functions and effects much differ with kind of phytoestrogens. Previous reports demonstrated that premenopausal women who consumed flax seed powder containing lignans, a kind of phytoestrogens, showed a longer luteal phase, but no changes in estradiol and progesterone levels (Phipps et al., 1993). Premenopausal women who consumed 45 mg of isoflavones extracted from soy for the duration of one menstrual cycle have an increase in follicular phase length and a decrease in peak levels of FSH, LH, and progesterone (Cassidy, Bingham, and Setchell, 1995). Although daily intake of

daidzein and genistein for 1 month did not increase significantly the menstrual cycle length, it decreased significantly the serum levels of estradiol and dehydroepi-androstendione sulfate in premenopausal women (Lu et al., 1996). The variation in the effects of phytoestrogens is the subject of research of importance. The phytoestrogens are considered an effective remedy for the various symptoms of estrogen deficiency.

Menopausal state is a state of failure in ovarian function and resulting in low rate of estrogen production. The reduction of estrogen production causes a loss of negative feedback mechanism on the secretion of gonadotropins at the pituitary levels; accordingly, the levels of gonadotropins progressively are increased during this time and kept elevated throughout the menopause (Smith et al., 1983; Gill et al., 2002). Moreover, the low level of endogenous estrogen is considered to be the prominent cause of bone loss. The osteoporosis mainly occurs during the first two decades after the natural menopause. Previous studies (Stock, Coderre, and Mallette, 1985; Khosla et al., 1997) noted that the menopausal state was associated with a state of negative calcium balance. Although the detailed mechanism has not been elucidated, the estrogen deficiency is the one of main factors. The intestinal calcium absorption is decreased, which makes the calcium balance negative, and then induced a secondary hyperparathyroidism. Hypersecretion of parathyroid hormone (PTH) stimulates high calcium release from bone and causes bone loss (Silverberg and Bilezikian, 1994). The administration of exogenous estrogen reduces, not only the gonadotropin secretion but also the rate of bone loss or bone fracture in menopausal women (Varma, Everard, and Hole, 1985; Lindsay et al., 1996; Pinkerton and Santen, 1999). However, the side effects of estrogen administration are concerned, for example nausea, breast tenderness, migraine headaches, hypertension, and carcinomas of endometrium and breast (Pinkerton

and Santen, 1999). That is why the phytoestrogens have attracted of researchers, who hope that they would have no such side effects.

Few studies have reported the estrogenic effect of phytoestrogens from soy in menopausal women. Daily consumption of soy decreased serum estrogen (Duncan et al., 1999) and FSH levels in menopausal women (Murkies et al., 1995). Isoflavones also decreased menopausal symptoms (Murkies et al., 1995; Vincent and Fitzpatrick, 2000). It reduced hot flushes and vaginal dryness and slightly increased the vaginal cell maturation (Wilcox et al., 1990; Baird et al., 1995). The epidemiological study in postmenopausal women found that Japanese women who highly consumed a soy diet have the estradiol and estrone levels lower than that of American women (Shimizu et al., 1990). In addition, some reports indicated that daily consumption of soy could reduce bone loss and bone resorption in oophorectomized rats (Draper et al., 1997; Arjmandi et al., 1998) and postmenopausal women (Yamori et al., 2002). These results suggested that consumption of phytoestrogen-rich soy affect reproductive system and bone in estrogen deficiency in menopausal women.

For more than twenty years, tuberous root of *P. mirifica* has been popularly used as a rejuvenating drug in aged persons in Thailand. They believed that *P. mirifica* contains some active substances alike female hormones (Kashemsanta et al., 1957; Pope et al., 1958; Pisetpakasit, 1976). Large quantities of its root were prepared by mixing with honey in a Thai traditional medicine. Native Thai people use it to remedy for various symptoms including cataract in the eyes, exhaustion, and emaciation due to starvation, flatulence, and cough with blood. They also use it to recover the black hair, promote an appetite, and increase the longevity (Wanadorn, 1933). In recent years, many products of *P. mirifica* in the forms of cream, tablet, and solution have been developed and widely used in normal cyclic women as an

age rejuvenation drug as well as cosmetics, for example, breast enlargement creams, skin moisturizers, and eye gels.

From these evidences, it is of interest to investigate the exact effect of *P. mirifica* on reproductive system in both adult cyclic women and aged menopausal women. However, studies on the effect of *P. mirifica* in humans may not yield valid results. Most of phytoestrogens can be found in a variety of daily human diet including soy and soy products; it is therefore very difficult to control the diet during experiment in humans. Moreover, the longitudinal determination of hormonal changes in humans is also difficult. Accordingly, the cynomolgus monkey (*Macaca fascicularis*) was chosen and used as an alternative model for the study of changes in reproductive hormones by *P. mirifica* treatment. Their physiological systems e.g. hormonal secretion patterns, menstrual cycle, reproduction, and bone metabolism are similar to those of humans (Chongthammakun and Terasawa, 1993; Krajewski et al., 2003). This study therefore examined the acute and long-term effects of *P. mirifica* on the menstrual cycle length in adult cyclic monkeys and on changes in serum levels of gonadotropins and sex steroid hormones. To find out whether *P. mirifica* had an estrogenic potency on bone in aged menopausal monkeys, serum calcium and PTH levels were also determined in these monkey groups.

However, the long-term study on changes in serum levels of hormone pose the limitations on frequency of sampling and on the volume of blood to be collected, because losses of high amount of blood and injury from frequent venipuncture may disturb the homeostasis of physiological system in subject monkeys. To avoid these problems, the assays of urinary hormone levels, the non-invasive method, should be the better choice. The changes in urinary levels of reproductive hormones in both adult cyclic and aged menopausal monkeys, which were treated by *P. mirifica* was also investigated in this study.

Objectives of the Studies

1. To determine the acute and long-term effects of *P. mirifica* on levels of FSH, LH, estradiol, and progesterone in serum and urine in adult female cynomolgus monkeys.
2. To determine the long-term effect of *P. mirifica* on levels of FSH, LH, and estradiol in serum and urine and levels of PTH and calcium in serum in aged menopausal cynomolgus monkeys.

Anticipated Benefits

1. To understand the mechanism of phytoestrogens on *P. mirifica* effect on reproductive system and bone
2. To transfer the knowledge of *P. mirifica* application obtained from monkey model to humans.