CHAPTER I

INTRODUCTION

Pueraria mirifica Airy Shaw and Suvatabandhu is a herb that belongs to the Leguminosae family and has been used extensively in traditional Thai medicine for many years. Native Thai people used the roots as a rejuvenile drug, promoting breast enlargement, and improving health and skin. It is well known in the name of White Kwao Krua.

The estrogenic activity of *P. mirifica* was firstly recorded in 1958 by Jones and Pop (Jones and Pop, 1960). They have announced the isolation of the estrogen miroestrol from the tuberous roots found that this substance behaves as endogenous estrogens in growth changes of the reproductive tract in intact rats and mice. In the ovariectomized and ovariectomized-adrenalectomized rat, miroestrol produces a cornification of the vaginal epithelium that confirmed it own estrogenic effect, not pass through the stimulation of endogenous estrogen secretion by the ovaries or adrenal glands of the experimental animal (Jones and Pop, 1960). Forty years later many of estrogenic compounds from the root of *P. mirifica* were identified such as miroestrol, daidzein, daidzin, genistein, genistin, coumestrol and kwakhurin (Chansakaow, *et al.*, 2000).

Toxicities associated with *P. mirifica* have been reported. Acute toxicity studies showed that in mice fed with *P. mirifica* root powder, LD_{50} value was grater than 16 g/kg

BW (Chivapat, *et al*., 2000). Another study confirmed that the LD_{50} value was out of the range of 2 g/kg in mice (Cherdshewasart, 2003). Subchronic toxicity study in rats treated orally with *P. mirifica* suspension at the doses of 10, 100 and 1000 mg/kg/day for 90 consecutive days revealed that *P. mirifica* at doses of 10 and 100 mg/kg/day given orally in rats neither cause any abnormalities of hematological or biochemical parameters nor any dose-related histopathological changes in the visceral organs (Chivapat, *et al*., 2000).

The mechanisms and potencies of *P. mirifica* are not completely clarified and considered as a potential endocrine disrupter. It therefore should be caution when taking them. However, some of the endocrine disrupters and genetic toxicity potential of *P. mirifica* has recently been reported. The consumption of high doses of *P. mirifica* led to the infertility and reproductive disorders in rats (Langkalichan and Smitasiri, 1984), Japanese quails (Muangdet and Anuntalabhochai, 1986), and female dogs (Panthong, 1987). However, the problem whether *P.mirifica* can affect the fertility of female and male animals, particularly with non-toxic doses, has not been clarified. The aim of the present study was to investigate this clue in both sexes of mice. Chronic administration of *P. mirifica* in adult females and males may effect on the normal pattern of sex hormone synthesis and secretion. Thus, this study also investigated the long-term treatment of *P. mirifica* on sex hormone levels.

In Thai folklore story, both women and men used this herb as a rejuvenile drug (Anusansunthorn, 1931). Therefore, the animal model used in this study is the both sexes of mice. Mice were chosen to be a model for this study because of their small size, easily to control, short period of reproductive cycle gestation.

The ordinary dosage of *P. mirifica* for women and men is one of a pepper seed (Anusansunthorn, 1931) or 250 mg/50kgBW/day or 5 mg/kg BW/day (Thrupcharoen, 1998). The process of metabolism in rats is similar to human, but the metabolism rate is higher because the phenomenon of microsomal enzyme induction is highly induced in liver of rats. In addition, microflora, which can metabolize an orally administered compound, highly distributes in the intestine of rats when compare to human (Berkowitz and Katzung, 2001). The usage dose of drug for treatment in rats in the present study is therefore 5-10 times higher than that of human usage dose. Furthermore, Chivapat, *et al.* (2000) suggested that *P. mirifica* treated in rat at the dosages of 10 and 100 mg/kg BW/day is safety to use. From these reasons, the dosages of *P. mirifica* used in this experiment were 10 and 100 mg/kg BW/day.

To clarify whether this herb has the effects similar to estrogen hormone, the diethylstilbestrol (DES) was used as a positive control. DES is a synthetic estrogen, which has a higher binding affinity to estrogen receptor (ER), both α and β subtypes, and shows a potency of estrogenic biological activity than estradiol (Korach, *et al.*, 1978; Gutendorf and Westendrof, 2001). DES at the dosage of 200 μg/kg/day in corn oil was chosen for this study. There were reports that this dosage of DES can reduce number and motility of sperm in epididymis in male rat. The absolute weight of all reproductive organs, including the testis, head and body of the epididymis, tail of the epididymis, and seminal vesicle, and plasma testosterone (T) level were reduced. None of the females that cohabited with treated males (1:1) had a sperm plug or produced a pup (Goyal, *et al.*, 20001).

Aims of this study are as followed:

1. To study the effects of *P. mirifica* on fertility in adult female and male mice.
2. To study the effects of *P. mirifica* on sex hormone levels and reproductive organs in adult female and male mice.
3. To study the effects of *P. mirifica* on malformation of the offspring born from treated parents.