

*Full Length Research Paper*

# Estrogen bioassay of *Pueraria mirifica* Airy Shaw & Suvatabandhu

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*Pueraria mirifica* Airy Shaw & Suvatabandhu has been used by Thai women for centuries as a rejuvenating herb. The scientific claim for its phytoestrogenic property leads to the popular use of this plant in several herbal formulations. This research project was carried out to examine estrogenic activity of *P. mirifica* from Lampang Province, Thailand, one of the areas that it is harvested for using as raw material in herbal industries. Estrogen bioassay of aqueous extract from *P. mirifica* at concentrations of 0.1 and 0.2 g/ml was performed in immature ovariectomized mice in comparison to both the negative and positive control groups. Vaginal orifice of mice treated with the extract in all groups and with the positive control group which injected with estradiol benzoate (EB) opened earlier than the negative control mice and vaginal cornification was also found in these 3 groups only. Moreover, the extract significantly increased uterine weight and uterine epithelium height as compared to negative control group with the comparable degree of increment to those of EB treated group. The data of this present study indicated that dried powder of *P. mirifica* which is used as raw material for herbal products of Lampang Province, exhibited the potent estrogenic activity.

**Key words:** *Pueraria mirifica* Airy Shaw & Suvatabandhu, estrogen bioassay, mice.

## INTRODUCTION

*Pueraria mirifica* Airy Shaw & Suvatabandhu is a climbing plant known in Thailand as White Kwao Krua. The ancestral uses of its tuberous root in folklore medicine for promoting vigor and rejuvenating have been mentioned in Thai traditional text books. It has been established that the rejuvenating effect of *P. mirifica* was mediated by an estrogenic action of miroestrol (Pope et al., 1958; Cain, 1960) and several active isoflavones such as daidzin, daidzein, genistin, genistein and puerarin (Chansakaow et al., 2000). In recent years *P. mirifica* is gaining popularity in Thailand as researches continue to pour in supporting its potent estrogenic effect and non-toxicity (Cherdshewasart, 2003; Cherdshewasart et al., 2004; Malaivijitnond et al., 2004; Trisomboon et al., 2004). With respect to its beneficial properties based on its richly phytoestrogenic content, *P. mirifica* has been popularly used as a major ingredient in variety of cosmetic products, for instance, breast cream, eye gel, skin

moisturizer and hair tonic. Recently, it has been proposed as a new alternative to the use of postmenopausal hormone replacement therapy (Muangman and Cherdshewasart, 2001). Although a number of researches have been done in regards to estrogenic activity of *P. mirifica*, the variation of isoflavonoids in Kwao Kruas at different ages and different harvested locations has been reported (Manosroi and Manosroi, 2003). This study, thus, carried out to investigate the estrogenic activity of *P. mirifica* powder purchased from Lampang Province, one of the areas that this plant is harvested for using as raw material in herbal industries. The estrogenic activity test was conducted by bioassay in ovariectomized mice in comparison to genuine estrogen.

## MATERIALS AND METHODS

### Extraction of *P. mirifica*

The dried powder of *P. mirifica* tubers was purchased from Amphur Muang, Lampang Province, Thailand. 50 g of the powder were extracted with 250 or 500 ml of water using soxhlet extractor for

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**Table 1.** Vaginal opening (VO) and vaginal cytology of ovariectomized mice treated with extract from *P. mirifica* (PM) at concentrations of 0.1 and 0.2 g/ml for 7 days as compared to control and estradiol benzoate groups. Number of mice with VO in day 1-7 and stages of estrous cycle are presented (Es = estrous).

Group	Total number	Day (D) of experiment						
		D1	D2	D3	D4	D5	D6	D7
Control	5	0	0	0	0	0	0	0
PM extract 0.1 g/ml	5	0	0	0	1 (Es)	2 (Es)	1 (Es)	0
PM extract 0.2 g/ml	5	0	0	0	1 (Es)	2 (Es)	2 (Es)	0
Estradiol benzoate	5	0	0	0	1 (Es)	1 (Es)	2 (Es)	1 (Es)

**Table 2.** Uterine weights and luminal epithelial heights of ovariectomized mice treated with extract from *P. mirifica* at concentrations of 0.1 and 0.2 g/ml for 7 days as compared to negative control (distilled water) and positive control (estradiol benzoate) groups. (Mean  $\pm$  S.D.).

Treatments	Uterine weight (g%)	Luminal epithelial heights ( $\mu$ m)
Distilled water (1 ml/day)	0.1344 $\pm$ 0.0253 <sup>b</sup>	16.9 $\pm$ 0.0016 <sup>a</sup>
<i>P. mirifica</i> 0.1 g/ml/day	0.3112 $\pm$ 0.0246 <sup>c</sup>	32.2 $\pm$ 0.0012 <sup>b</sup>
<i>P. mirifica</i> 0.2 g/ml/day	0.2915 $\pm$ 0.0507 <sup>c</sup>	62.7 $\pm$ 0.0058 <sup>c</sup>
Estradiol benzoate (0.8 mg/kg BW)	0.2491 $\pm$ 0.0453 <sup>c</sup>	32.9 $\pm$ 0.0055 <sup>b</sup>

Superscripted letters indicate the significant difference from control at  $P < 0.05$ .

at least 10 h. The extracts were filtered and the 0.2 g/ml and 0.1 g/ml aqueous extract were then obtained.

### Animals

Immature female mice (*Mus musculus*), approximately 3 weeks of age and weighing between 23 - 25 g, were used in the present investigation. The animals were purchased from the National Laboratory Animal Center, Thailand. They were allowed to acclimatize in the departmental animal facility for one week prior to the day of the experiment. They had access to water and standard diet (C.P. 082). The study room was maintained of approximately 25  $\pm$  2°C in a 12 h light/dark cycle. All procedures involving animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Animal Care and Use Committee of Biology Department, Faculty of Sciences, Chiang Mai University, permission number Re 002/08.

### Experimental design

Mice were ovariectomized and acclimated for 7 days prior to initiation of treatment. The ovariectomized mice were then divided into 4 groups (5 each). Two groups were orally treated for 7 days with aqueous extract of *P. mirifica* at the concentrations 0.1 and 0.2 g/ml respectively. The positive control group was intramuscularly injected with 0.8 mg/kg BW estradiol benzoate (EB) while the negative control group received only distilled water (1 ml/day). The day of vaginal opening and vaginal cytology was recorded. At the end of experiment, all mice were sacrificed for measuring uterine weight. Six  $\mu$ m thick sections of uterine horn were prepared by the routine histological method (Brancroft and Cook, 1994) and epithelial height of uterus was measured.

### Statistical analyses

Mean values and standard deviations were determined for each treatment group. One-way ANOVA was used for uterine weights

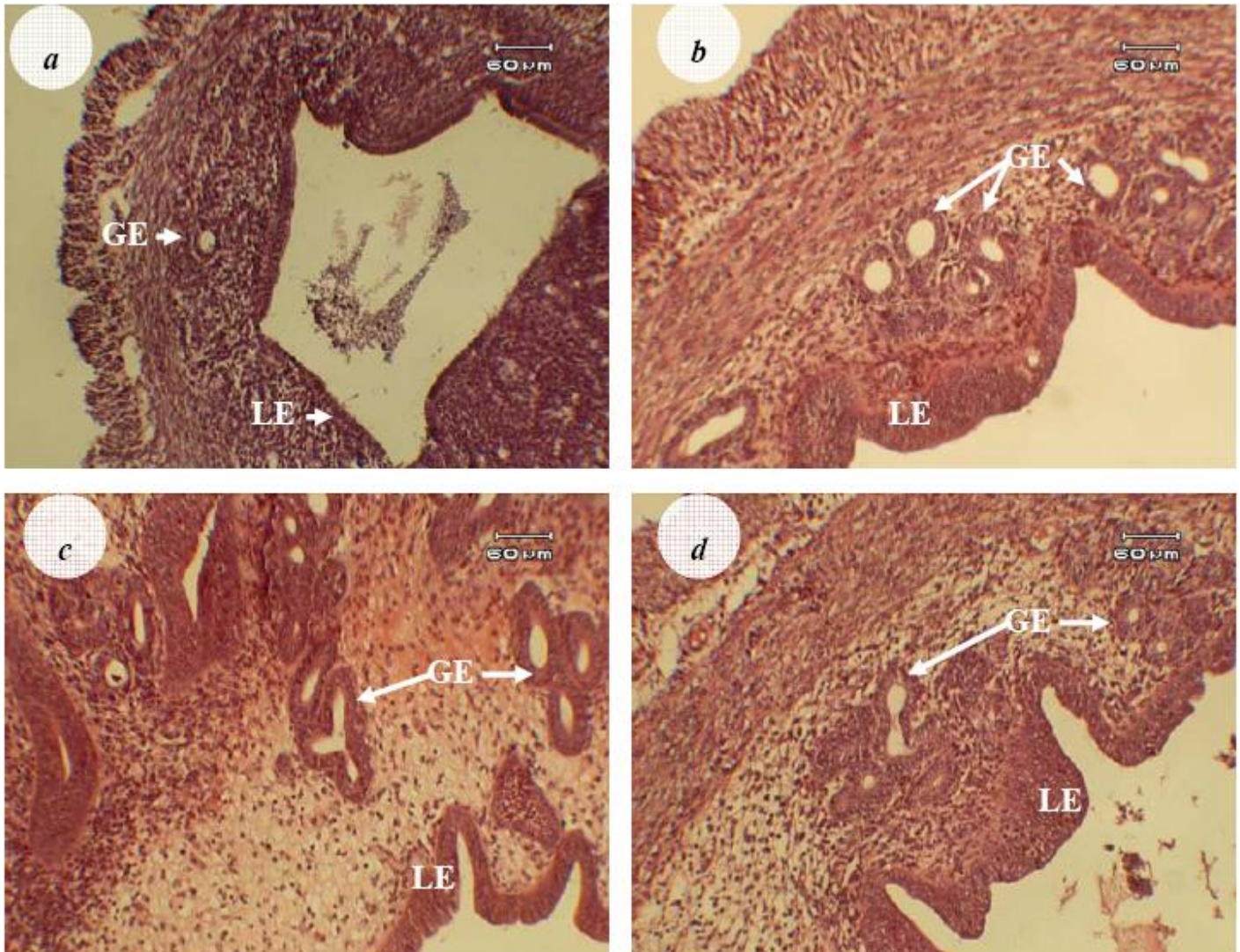
and epithelial heights. Least Significant Difference test was used for comparisons among treatment groups. Levels of significance are indicated by asterisks.

## RESULTS

The effects of aqueous extract from dried powder of *P. mirifica* on the timing of vaginal opening and vaginal cytology in ovariectomized mice are shown in Table 1. Vaginal opening of mice treated with the extract at both concentrations used and with EB occurred in day 4 of treatment, while control mice showed no signs of vaginal opening until day 7 of the experiment. Vaginal cytology revealed the stage of estrous in mice treated with the extracts and EB, showing abundant cornified cells. In contrast, vaginal cell cornification was not induced in control mice. Administration of aqueous extract from *P. mirifica* could induce a significant increase in uterine weight (Table 2). The uterotrophic effect of *P. mirifica* was also evident from the increase in the height of luminal epithelium and the proliferation of glandular epithelium (Table 2 and Figure 1). The group of 0.2 g/ml showed a tentative of higher uterotrophic activity than EB group.

## DISCUSSION

Due to its potent estrogenic activity, *P. mirifica* has been introduced commercially as promising products for breast enlargement. Later on, it has also been led to the cosmetic industries as products for skin nourishing and



**Figure 1.** Cross sections of ovariectomized mice's uterus treated with extract from *P. mirifica* at concentrations of 0.1 (b) and 0.2 g/ml (c) for 7 days as compared to negative control (a) and positive control (estradiol benzoate, d) groups. Notice the abundant of uterine gland with proliferating glandular epithelium (GE) and the increased size of luminal epithelium (LE) in groups b-c as compare to group a (H & E).

rejuvenating. The increased demand of *P. mirifica* in the word market has led to the heavy harvest of this plant in Thailand and Lampang Province is one of the popularly harvested areas. Dried powder from tuberous of this plant is generally sold to both local people and to the herbal industries. The estrogenic activity of bioactive compounds in phytoestrogenic plants is indicated by their ability to stimulate an estrogen receptor-dependent transcriptional response and to promote growth of estrogen-dependent MCF7 cells in culture (Cherdshewasart et al., 2004; Hiroko et al., 2006). Although estrogenic activity of *P. mirifica* powder from this area has been previously evaluated using vaginal cornification assay in rats and it was in the low rank. (Cherdshewasart et al., 2007) the supply of this plant from Lampang to herbal industries is still high. By using the combination of vaginal cornification

assay and the immature mice uterotrophic assay we found that *P. mirifica* from this area gave the appreciable estrogenic results. The aqueous extract, the form that normally used in herbal industry, from *P. mirifica* has shown highly significant estrogenic activity by showing all signs of estrogenic indices in ovariectomized mice. Time of vaginal opening and presence of cornified cells in vaginal smears are qualitative measurements of estrogen potency (Raju et al., 2007). Increase in uterine size and weight has been resulted by protein synthesis, water uptake and retention of fluid leading to ballooning of the uterus (Rifai et al., 2001). The uterotrophic changes such as increase in diameter of the uterus, thickness of endometrium, height of endometrial epithelium are resulted from estrogen action. An increase in height of uterine luminal epithelia found in this study is, therefore, a

remarkable index for estrogenic effect of this *P. mirifica* from our studied area. Our results also revealed that aqueous extract of *P. mirifica* was likely to have potencies comparable to estradiol benzoate. This plant species collected from Lampang Province, thus, could be a promising source of phytoestrogen-rich raw material for cosmetic and pharmaceutical products. Recently, *P. mirifica* has been introduced into the medical world by its effectiveness in the treatment of menopausal symptoms (Muangman and Cherdshewasart, 2001). Global movement towards the beneficial uses of phytoestrogen-rich plants as an alternative for synthetic estrogen rapidly increase and *P. mirifica* is among the most interesting candidate plants of these days.

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