

## *Asian Journal of Food and Agro-Industry*

ISSN 1906-3040

Available online at [www.ajofai.info](http://www.ajofai.info)

### **Organic *Pueraria mirifica* tuber : Its potent estrogenic potency and its effects on some organs and vaginal histology of aged female mice**

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#### **Abstract**

The test of estrogenic potency of dried *Pueraria mirifica* (PM) tuber powder from one organic source at the Organic Medicinal Plant Garden of Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Maerim district, Chiang Mai and from two nonorganic sources located at Wiang Kaen district, Chiang Rai and Dok Kham Tai district, Phayao were investigated in bilaterally ovariectomized immature mice revealed that dried organic PM source pronounced highest estrogenic potency when compared with dried PM tuber powder from two nonorganic sources. The test on dried organic PM effects on some organs weight and vaginal histology of aged female mice for 14 consecutive days indicated that dried organic PM tuber powder could significantly increase uterine, liver and spleen weight and could induce stratified squamous epithelium hyperplasia of the vagina whereas there was no effect on vaginal epithelium in the control group. It was concluded that dried organic PM tuber powder pronounced its estrogenic potency more potent than dried nonorganic PM tuber powder and it had some effects by increasing of some organs weight and could induce vaginal epithelium hyperplasia in the aged female mice.

**Keywords :**

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## Introduction

*Pueraria mirifica* Airy Shaw et Suvatabandhu is a plant in Family Leguminosae. PM tuber could pronounce estrogenic activity (Cain, 1960 ; Jones and Pope, 1960 ; Kashemsanta et al, 1963 ; Nilanidhi et al, 1963 ; Pope et al, 1958), mammogenic activity (Benson et al, 1961), antifertility activity (Smitasiri, 1993 ; Tanachai and Smitasiri, 1987), postcoital antifertility activity (Smitasiri et al, 1986), antioxidative activity (Phansawan et al, 2003), antitumor activity (Jeon et al, 2005). According to the high potent estrogenic activity of PM tubers, then very high consumption of PM tubers are needed for many purposes in the forms of various products such as herbal drug, food supplement, breast cream etc. It was reported that PM tubers collected from different natural sources pronounced different estrogenic activities (Tongprong and Smitasiri, 1987) or collected from various months pronounced different estrogenic activity (Smitasiri et al, 2003). In 2004, PM were firstly grown at the Organic Medicinal Plant Farm of Faculty of Agricultural Technology, Chiang Mai Rajabhat University. The estrogenic activity of PM tuber from this source was needed to investigate for its estrogenic activity. At the same time, PM tubers from a new nonorganic source at Wiang Kaen district of Chiang Rai province were also found whereas PM tubers from the nonorganic source at Dok Kham Tai district of Phayao province were also used. In this project, the effects of dried PM tuber powder on vaginal tightening of aged female mice was investigated because there was an evidence that miroestrol, a potent estrogenic substance, isolated from PM tuber could effectively induce vaginal cornification in the rat (Jones et al, 1961). Then the aim of this project was firstly to compare the estrogenic activity between dried PM tuber powder from one organic and two nonorganic sources in bilaterally ovariectomized immature mice and secondly to study the two-week effects of dried PM tuber powder from the source which pronounced the highest estrogenic activity on some organs weight and vaginal histology of the aged female mice.

## Methodology

### Experimental animals

Two lots of ICR mice i.e. 24 immature female mice (3-week old) and 12 aged female mice (32-week old) were sent from National Laboratory Animal Center, Mahidol University at Saraya, Nakhon Pathom to Chiang Mai International Airport by air. Then the mice were transferred to the controlled Laboratory Animal House (  $24 \pm 1^\circ\text{C}$  / 12-hr light and 12-hour dark light cycle) at Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Maerim District, Chiang Mai. All of the experimental mice were fed with mice pellets (CP No.082) and water *ad libitum*.

### *Pueraria* species tested

*Pueraria mirifica* Airy Shaw et Suvatabandhu tubers from various sources were digged and used in this project i.e. *Pueraria mirifica* tubers from nonorganic source at Wiang Kaen District of Chiang Rai Province (PM-WK), *Pueraria mirifica* tubers from organic source at Organic Medicinal Plant garden, Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Maerim District of Chiang Mai Province (PM-CMRU) and *Pueraria mirifica* tubers from nonorganic source at Dok Kham Tai District of Phayao Province (PM-DKT) then PM from each source was processed into dried tuber powder and kept in three dried and clean glass bottles.

### Experiment 1 : Experimental study in immature female mice

Two-day after arrival, all of the immature female mice were bilaterally ovariectomized by the method described by Zarrow et al (1964). Fourteen days post-ovariectomy, all of the ovariectomized immature mice were divided into four groups (6 mice/group). The first group

was control group receiving 0.5 ml. distilled water/mouse/ day once daily for two consecutive days, but the second, third and fourth groups did the same as control group but receiving dried PM-WK, PM-CMRU and PM-DKT respectively at the dosage of 20 mg / mouse/day. On Day 3, each mouse was anaesthetized with ether then body weight, uterine wet weight and uterine dry weight were measured using an electronic balance (Ohaus Adventurer<sup>TM</sup>). Uterine liquid content were recorded and statistically analysed using ANOVA and LSD.

### **Experiment 2 : Experimental study in aged female mice**

Aged female mice were divided into two groups (6 mice/group). The first group was control group receiving 1 ml distilled water/mouse once daily for 14 consecutive days, but the second group did the same as control but receiving dried PM tuber powder from the source which pronounced highest estrogenic activity chosen from Experiment 1 at the dosage of 25 mg/mouse. On Day 15, after weighing, each mouse was anaesthetized with Nembutal injection intraperitoneally then laparotomy was done and the mammary gland, ovaries, uterus, kidney, liver, spleen in each mouse were weighed, recorded and statistical analysed. The vagina was also removed and fixed in 10% buffered neutral formalin solution then sectioned and processed for histopathological section. All of the slides were examined and reported by expert histopathologist from Veterinary Diagnostic Laboratory, Faculty of Veterinary Medicine, Chiang Mai University.

Note : Both experiments in these laboratory mice of this project were done under the regulation guided by National Research Council of Thailand (1999) for regulating the ethically use of laboratory animals for experimentation .

### **Results**

The results from Table 1 revealed that the highest estrogenic potency was found from PM tuber collected from organic source (PM-CMRU) by consideration from the uterine wet weight, uterine dry weight and uterine liquid content of the ovariectomized immature mice whereas the estrogenic potencies of PM tubers from two nonorganic sources i.e. from Wiang Kaen source (PM-WK) and from Dok Kham Tai source (PM-DKT) pronounced lesser estrogenic activities than PM tubers collected from organic source (PM-CMRU). According to the results obtained then PM-CMRU tuber, which pronounced the highest estrogenic activity was chosen for the study in the second experiment in aged female mice. And the results from Table 2 revealed that treatment of PM-CMRU tuber at the dosage used for 14 consecutive days did not pronounce significant effects on body weight ; mammary gland, ovarian and kidney weights in aged female mice but could significantly increase the uterine, liver and spleen weights. Vaginal histopathological study from PM-CMRU-treated aged mice for 14 consecutive days revealed that in PM-CMRU-treated aged mice. There was stratified squamous epithelium hyperplasia and some haemorrhage found and there was some infiltration of neutrophils in this group. But in the control group, even though the infiltration of neutrophils was also found but with stratified squamous epithelium was normal.

### **Discussion and Conclusion**

The results clearly shown that PM tuber grown in organic source pronounced higher estrogenic potency than from the two nonorganic sources. However, the mechanism by which organic source can increase the estrogenic potency of this plant is still unknown but this may involve with the content of estrogenic substances (Chansakaow et al, 2000 ; Jones and Pope, 1960 ; Kashemsanta et al, 1963 ; Nilanidhi et al, 1963) and this need some further investigation. Then plantation of PM tuber in organic farm is recommended for its high estrogenic activity from this study. However, organic PM tuber seemed to pronounce its effects on some organs weight

i.e. uterine, liver and spleen weights. The effects of PM tuber on uterine weight might due to the stimulation of uterine endometrial growth as reflected from every parameters of uterine weight. These results support the work of Jones and Pope (1960). It was notable that organic PM tuber could significantly increase the liver weight, this might be due to hepatic cell

**Table 1** Comparison between the estrogenic potency of dried *Pueraria mirifica* tuber powder collected from various sources.

Groups (2-day treatment)	No.of mice	Body weight (g)	Uterine wet weight (mg%)	Uterine dry weight (mg%)	Uterine liquid content (mg%)
DW 1ml/mouse/D	6	26.67 ± 2.16 NS	35.31 ± 1.63 a	11.52 ± 1.28 a	23.78 ± 0.76 a
PM-WK 25 mg/mouse/D	6	26.00 ± 1.55 NS	161.31 ± 46.23 b	28.64 ± 6.92 b	132.67 ± 39.52 b
PM-CMRU 25 mg/mouse/D	6	25.50 ± 2.07 NS	219.19 ± 21.71 c	39.42 ± 4.73 c	179.76 ± 19.84 c
PM-DKT 25 mg/mouse/D	6	25.83 ± 2.40 NS	172.63 ± 36.49 b	28.60 ± 4.92 b	144.03 ± 31.76 b

NS = non-significant difference ; Mean ± S.D. followed by different letter is significantly different at P < 0.05

**Table 2** Effects of dried organic *Pueraria mirifica* tuber powder from Organic medicinal plant farm, Chiang Mai Rajabhat University on some reproductive and various organs weight of aged female mice on Day 15. Data were collected from six mice.

Paramaters studied	DW 1 ml/mouse/D 14 D	PM-CMRU 25 mg/mouse/D 14 D
Body wt. (g)	41.33 ± 5.47	40.33 ± 3.20 NS
Mammary gland wt.(mg%)	1,434.21 ± 221.66	1,212.10 ± 145.41 NS
Ovarian wt. (mg%)	41.08 ± 7.32	40.81 ± 13.94 NS
Uterine wt. (mg%)	430.34 ± 153.30	696.08 ± 85.65**
Kidney wt. (mg%)	1,398.51 ± 129.30	1,567.92 ± 215.12 NS
Liver wt. (mg%)	5,720.79 ± 605.88	7,928.59 ± 1,034.90 **
Spleen wt. (mg%)	359.80 ± 74.78	534.75 ± 103.07**

Mean ± S.D. ; NS = non-significant difference ; \*\* P < 0.01

injury etc. (Langkalichan,1984) and the increasing of spleen weight might be due to its direct or indirect effects on this organ. This requires further investigation.

Histopathological study on the vagina of aged mice revealed that organic PM tuber powder could induce vaginal tightening when considered from hyperplasia of stratified squamous epithelium. This finding support the earlier work of Jones et al (1961) who found the effects of PM on vaginal cornification in rats. Results from histopathological findings also revealed that organic PM tuber could induce the infiltration of neutrophil and found some haemorrhage, it was possibly that the evidene found might be like those from vagina of mice during metestrus or estrus stages (Zarrow et al, 1964).

It was concluded that dried organic PM tuber powder pronounced strong estrogenic activity and was more potent than dried PM tuber powder from two nonorganic sources. Dried organic PM tuber powder could induce vaginal stratified squamous epithelium hyperplasia in aged female mice, and also had pronounced effects on organ weight.

### **Acknowledgement**

This project was financially supported by a grant from Ms. Krongthong Chutima via Luang Anusarnsunthorn Kwao Keur Research grant No.KC-006/2008 and MITARI Research grant No.MR-002/2008.

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