

## Treatment with *Pueraria mirifica* Extract Prevented Muscle Atrophy and Restored Muscle Strength in Ovariectomized Rats

(Rawatan dengan Ekstrak *Pueraria mirifica* bagi Menghalang Atrofi dan Memulihkan Kekuatan Otot Tikus Diovariectomi)

KOCHAKORN SUKJAN INTHANUCHIT, WANDEE UDOMUKSORN, EKKASIT KUMARNSIT, SURAPONG VONGVATCHARANON & URAPORN VONGVATCHARANON\*

### ABSTRACT

*Pueraria mirifica* (PM) is a phytoestrogen-rich plant that was tested to establish if its phytoosteroids could prevent estrogen dependent sarcopenia. The effect of PM on the estrogen levels, estrous cycle, toxicity, muscle mass, strength and endurance of extensor digitorum longus (EDL) and gastrocnemius muscles of ovariectomized rats was investigated. Adult female Wistar rats were divided into six groups: Sham-operated (SHAM); ovariectomized (OVX) fed with distilled water (PM0); OVX injected with 40 µg/kg estradiol benzoate (E40); (4-6) OVX fed with ethanolic extract of PM at doses of 50 (PM50), 500 (PM500) and 1000 (PM1000) mg/kg for 90 days. After treatment with all three doses of PM, no toxicity was detected to the hematopoietic system and liver function whereas the E40 group did show toxic effects. Treatment with 50 and 500 mg/kg of PM showed no effect on uterine hypertrophy and caused no arrest of the estrous cycle whereas treatment with estrogen and 1000 mg/kg of PM treatment did. The estrogen level, the cross sectional area of the EDL and the gastrocnemius muscle fiber strength and endurance were all significantly reduced in the PM0 group compared to that of the SHAM group ( $p < 0.05$ ) but were significantly increased in the E40, PM50, PM500 and PM1000 compared to that of the PM0 group ( $p < 0.05$ ). This indicated that the estrogenic activity of PM alleviated muscle atrophy and built up muscle strength and endurance. Thus, the 50 and 500 mg/kg of PM were suitable for treating estrogen dependent sarcopenia in ovariectomized rats.

**Keywords:** Estrogen; ovariectomy; *Pueraria mirifica*; sarcopenia

### ABSTRAK

*Pueraria mirifica* (PM) adalah tumbuhan kaya fitoestrogen yang diuji untuk menentukan jika fitosteroidnya boleh menghalang sarkopenia tergantung estrogen. Kesan PM pada tahap estrogen, kitaran estrous, ketoksikan, jisim otot, kekuatan dan ketahanan ekstensor digitorum longus (EDL) dan otot gastrocnemius tikus diovariectomi telah dikaji. Tikus Wistar betina dewasa dibahagikan kepada enam kumpulan: sham-dikawal (SHAM), ovariectomi (OVX) diberi air suling (PM0), OVX disuntik dengan 40 µg/kg estradiol benzoat (E40), OVX diberi ekstrak etanol PM pada dos 50 (PM50), 500 (PM500) dan 1000 (PM1000) mg/kg selama 90 hari. Selepas rawatan dengan ketiga-tiga dos PM, tiada ketoksikan dikesan pada sistem hematopoietik dan fungsi hati manakala kumpulan E40 menunjukkan kesan toksik. Rawatan dengan 50 dan 500 mg/kg PM tidak memberi kesan pada hipertrofi rahim dan tidak menyebabkan gangguan pada kitaran estrous berbanding rawatan dengan estrogen dan 1000 mg/kg rawatan PM yang menunjukkan kesan. Tahap estrogen, luas keratan rentas EDL dan kekuatan serta ketahanan gentian otot gastrocnemius berkurang secara signifikan dalam kumpulan PM0 berbanding dengan kumpulan SHAM ( $p < 0.05$ ) yang meningkat dengan ketara dalam E40, PM50, PM500 dan PM1000 berbanding dengan kumpulan PM0 ( $p < 0.05$ ). Ini menunjukkan bahawa aktiviti estrogenik PM mengurangkan atrofi otot dan membina kekuatan dan ketahanan otot. Oleh itu, 50 dan 500 mg/kg PM adalah sesuai untuk merawat sarcopenia bergantung estrogen kepada tikus diovariectomi.

**Kata kunci:** Estrogen; ovariectomi; *Pueraria mirifica*; sarkopenia

### INTRODUCTION

A decrease of estrogen levels, especially in menopause, results in a decrease of muscle mass, strength and endurance, a condition known as estrogen dependent sarcopenia (Messier et al. 2011). The loss of muscle mass and strength can lead to an increased risk of falls and subsequent injuries, causing a decrease in the quality of life (Norshafarina et al. 2013). Estrogen

replacement has been shown to improve skeletal muscle performance in ovariectomized rats (Bunratsami et al. 2015) and postmenopausal women (Sipila et al. 2001). A previous study showed that estrogen replacement increased the muscle mass, strength and endurance in the extensor digitorum longus (EDL) and gastrocnemius in ovariectomized rats (Bunratsami et al. 2015). However, only a high dose of estrogen (40 mg/kg) improved both EDL

and gastrocnemius functions, but this had toxic effects on the uterus (Bunratsami et al. 2015). In addition, estrogen treatment can increase the risk of cancer especially breast cancer (Amin et al. 2015; Marsden 2002). Therefore, phytoestrogen supplementation has been considered to be an alternative treatment. *Pueraria mirifica* Airy Shaw & Suvatabandhu (Leguminosae) known in Thai as “Kwao Krua Kao”, is a Thai medical phytoestrogen-rich plant. It is a traditional medicine use by menopausal women for rejuvenation and estrogen replacement (Suntara 1931). The plant tubers contain chemicals classified as phytoestrogens including isoflavones (Cherdshewasart et al. 2007a) and others such as miroestrol (Taylor et al. 1960) and deoxymiroestrol (Chansakaow et al. 2000). However, the isoflavonoids (daidzin, daidzein, genistin, genistein and puerarin) constitute the majority of the active chemical ingredients of the tubers (Cherdshewasart et al. 2007b). An estrogenic-like activity of *Pueraria mirifica* (PM) has been demonstrated on the reproductive organs of mammals and woman. An alcoholic extract of PM stimulated the proliferation of the epithelium of the vagina and uterus in female rats and woman (Pope et al. 1958; Malaivijitnond et al. 2004). Furthermore, PM has been demonstrated to provide other health benefits such as prevention of osteoporosis (Urasopon et al. 2008) and breast cancer (Cherdshewasart et al. 2007a). In the sarcopenia, atrophy of the predominantly type II fast twitch fibers was reported and led to a loss of muscle strength and power (Lang et al. 2010). The EDL and gastrocnemius are type II fast twitch muscle fibers. In order to avoid the loss of skeletal muscle mass and improve muscle strength, high replacement doses of estrogen are required. This may result in an increased risk of cancer. Therefore, this study aimed to investigate the effect of PM on estrogen levels, reproductive organs, chemical toxicity, mass and strength of the EDL and gastrocnemius in an ovariectomized rat. The results may help to assess the possibility for using PM to replace estrogen therapy to prevent sarcopenia during menopause.

## MATERIALS AND METHODS

### ANIMALS

Adult female Wistar rats, (12 weeks old, 250-300 g weight) were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. The experimental protocols described in this study were approved by the Animal Ethics Committee of the Prince of Songkla University for the care and use of experimental animals (MOE 0521, 11/175). The rats were randomly divided into six groups ( $n=10$  per group): sham-operated (SHAM), ovariectomized (OVX) fed with distilled water (PM0), OVX injected with 40  $\mu\text{g}/\text{kg}$  estradiol benzoate (E40), OVX fed with ethanolic extract of *Pueraria mirifica* (PM) at doses of 50 (PM50), 500 (PM500) and 1000 (PM1000) mg/kg for 90 days. The doses of treatment were modified from the work of Cherdshewasart (2003).

*Pueraria mirifica* was a product from St. herb Cosmetics International Co./Ltd, Pathumthani, Thailand (Lot no: A55050501). The extract contained puerarin 31.75 mg, daidzin 5.81 mg, gonistin 7.22 mg, daidzein 15.75 mg and genistein 8.35 mg/100 g as determined by HPLC analysis.

### EVALUATION OF THE ESTROUS CYCLE

A vaginal smear was performed daily for 5 days before the end of the experiment. The collected epithelial cells were stained with 0.5% methylene blue. The state of evaluation of the estrous cycle was determined according to the work of Wirakiat et al. (2012). The rats were in the stage of diestrus before ovariectomy, except for the SHAM group.

### MEASUREMENT OF MUSCLE PERFORMANCE

At the end of the treatment, strength and endurance of the extensor digitorum longus (EDL) and the gastrocnemius were evaluated. The procedure for measurement was modified from the work of Bunratsami et al. (2015). Briefly, rats were anesthetized by an intraperitoneal injection of 70 mg/kg thiopental. The EDL and gastrocnemius muscles were dissected and stimulated directly on the muscles using a bipolar electrode. The contractile force was converted to an analogue signal via a force transducer (Model 1030: AD Instruments, Australia). The signal was amplified using a Bridge Amplifier (Model 110, AD Instruments, Australia) and conveyed to a Chart program of the PowerLab system (Model 4/20; AD Instruments, Bella Vista, NSW, Australia) for converting into a digital signal. The data were stored for off line analysis.

### SAMPLE PREPARATION

Blood samples were obtained from the orbital plexus to evaluate serum estradiol levels using an electrochemiluminescence immunoassay (ECIA) kit (LKE2 1026, DPC, Gwyned, UK), modified from the work of Bunratsami et al. (2015) and a blood chemical analysis for a toxicity test. The EDL, gastrocnemius and uterus were removed and weighed.

### MEASUREMENT OF MUSCLE FIBER SIZE

The protocol for measuring the cross-sectional area was modified from the work of Bunratsami et al. (2015). Briefly, serial 20  $\mu\text{m}$ -thick frozen cross-sections of EDL and gastrocnemius were cut and stained with hematoxylin and eosin. The images of sections were captured with a digital camera (DP50) Olympus, Tokyo, Japan. The area of the fiber of the EDL and gastrocnemius were measured using Image-pro Plus 6.0 analysis software (Media Cybernetics, Belnesda, MD, USA).

### STATISTICAL ANALYSIS

Data are expressed as mean values  $\pm$  standard error of the mean. The statistical evaluation of the data was performed using one-way ANOVA and a least significant difference

test (LSD) to determine any significant differences between the mean values. A *P* value of <0.05 was considered to be significant.

#### DISCUSSION

Our data have demonstrated that a reduction of the estrogen level (in the PM0 group) induced body weight gain and treatment with 17 $\beta$ -estradiol or 50 or 500 or 1000 mg/kg of *Pueraria mirifica* (PM50, 500, 1000) prevented the excess weight gain (Table 1). This correlated well with previous reports by Urasopon et al. (2008). The result indicated that the PM exhibited an estrogen-like effect on the rat body weight. The previous report found that Genistein, the most abundant isoflavone in soybeans, reduced food intake, body weight and fat pad weight in ovariectomized female mice (Park et al. 2009). This indicated that the Genistein present in the PM may have the same effect in decreasing the body weight as found for feeding soy beans. Our data also demonstrated that the reduction of estrogen levels (in the PM0 group) increased the atrophy of the uterus, whereas estrogen (40  $\mu$ g/kg) and a 50, 500, or 1000 mg/kg of *Pueraria mirifica* replacement prevented atrophy of the uterus (Table 1). According to the work of Urasopon et al. (2008), treatment of 100 or 1000 mg/kg B.W./day of PM for 90 days increased the uterine weight in the ovariectomized rats. Our data correlated well with that study. The data on the estrous cycle (Table 2) and

estrogen levels (Figure 1) showed that in rats with a decreased estrogen level, only diestrus was found. A high level of serum estradiol was identified in the estrogen replacement group and only the proestrous and estrous were observed. In the high dose of PM replacement (PM1000) group, an increase of the estrogen level was observed similar to the SHAM group, the proestrous and estrous was identified as being similar to that of the estrogen replacement group. In contrast a low or medium dose of PM replacement (50, 500 mg/kg) increased the estrogen level less than that of the SHAM and the 3 stages of the estrogen cycle (proestrous, estrous and metestrous) were observed. These data have clearly demonstrated the estrogenic effects of *Pueraria mirifica*. Our data correlated well with the work of Malaivijitnond et al. (2004), when treatment with PM100 and 1000 led to an increase of uterine weight and vagina cornification, to indicate that PM can influence the female reproductive functions. A previous report by Pope et al. (1958) demonstrated that PM contained phytoestrogens that acted like estrogen in influencing vaginal cornification in ovariectomized rats. Puerarin is a major isoflavonoid component of the PM. It was found that treatment with 7.0 mg/kg B.W./day of puerarin for 140 days increased vaginal cornification (Malaivijitnond et al. 2010). It was also of interest, that treatment with PM increased the estrogen level. This finding indicated that the PM extract might stimulate hormone production from other sources. The hematological results (Table 3)

TABLE 1. The rate of weight gain, uterine weight, EDL and gastrocnemius weights in all treatment groups

Parameters	SHAM	PM0	E40	PM50	PM500	PM1000
Weight gain (g/month)	6.70 $\pm$ 0.41	13.46 $\pm$ 0.96*	4.53 $\pm$ 0.64**	5.50 $\pm$ 0.64 <sup>+</sup>	3.75 $\pm$ 0.67**	2.88 $\pm$ 0.48**
Uterine weight (g)	1.04 $\pm$ 0.08	0.31 $\pm$ 0.05*	1.12 $\pm$ 0.05 <sup>+</sup>	0.79 $\pm$ 0.07**	0.86 $\pm$ 0.09**	0.88 $\pm$ 0.05 <sup>+</sup>
Uterine weight/body weight ( $\times$ 100)	0.39 $\pm$ 0.03	0.10 $\pm$ 0.02*	0.44 $\pm$ 0.02 <sup>+</sup>	0.30 $\pm$ 0.02**	0.35 $\pm$ 0.04 <sup>+</sup>	0.36 $\pm$ 0.03 <sup>+</sup>
EDL weight (g)	0.100 $\pm$ 0.004	0.094 $\pm$ 0.005	0.106 $\pm$ 0.005	0.108 $\pm$ 0.008	0.111 $\pm$ 0.005 <sup>+</sup>	0.120 $\pm$ 0.006**
EDL weight/ body weight ( $\times$ 100)	0.038 $\pm$ 0.002	0.030 $\pm$ 0.001*	0.042 $\pm$ 0.003 <sup>+</sup>	0.041 $\pm$ 0.003 <sup>+</sup>	0.045 $\pm$ 0.002 <sup>+</sup>	0.049 $\pm$ 0.003**
Gastrocnemius weight (g)	2.976 $\pm$ 0.023	1.432 $\pm$ 0.023*	2.699 $\pm$ 0.050**	2.847 $\pm$ 0.104 <sup>+</sup>	2.574 $\pm$ 0.048**	2.611 $\pm$ 0.047**
Gastrocnemius weight/ body weight ( $\times$ 100)	1.092 $\pm$ 0.025	0.902 $\pm$ 0.025*	1.042 $\pm$ 0.026 <sup>+</sup>	1.056 $\pm$ 0.042 <sup>+</sup>	1.045 $\pm$ 0.029 <sup>+</sup>	1.037 $\pm$ 0.015 <sup>+</sup>

\*Significantly different from SHAM, *P*<0.05, \*\*Significantly different from the PM0, *p*<0.05, *n*=10

TABLE 2. Identification of the stage of estrous cycle in all treatment groups

Groups	Before treatment the stage of estrous cycle				After treatment the stage of estrous cycle			
	Proestrus	Estrus	Metestrus	Diestrus	Proestrus	Estrus	Metestrus	Diestrus
SHAM	+	+	+	+	+	+	+	+
PM0	-	-	-	+	-	-	-	+
E40	-	-	-	+	+	+	-	-
PM50	-	-	-	+	+	+	+	-
PM500	-	-	-	+	+	+	+	-
PM1000	-	-	-	+	+	+	-	-

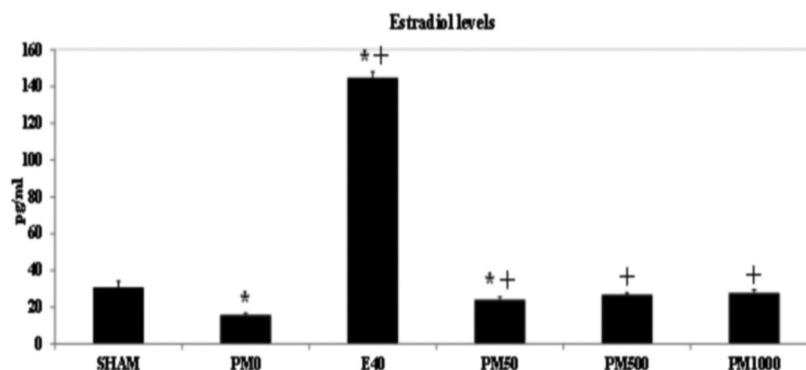


FIGURE 1. The serum estradiol levels for all treatments: SHAM, PM0, E40, PM50, PM500 and PM1000 groups

\*Significantly different from the SHAM group ( $P<0.05$ ), +Significantly different from the PM0 group, ( $P<0.05$ ), ( $n=9$ )

TABLE 3. Hematological tests in all treatment groups

Parameters	SHAM	PM0	E40	PM50	PM500	PM1000
RBC count ( $\times 10^6$ )/ $\mu$ l	7.63 $\pm$ 0.15	7.57 $\pm$ 0.18	6.76 $\pm$ 0.09*	7.26 $\pm$ 0.19	7.32 $\pm$ 0.18	7.18 $\pm$ 0.14
Hb (g/dl)	13.86 $\pm$ 0.10	13.54 $\pm$ 0.15	12.01 $\pm$ 0.23	12.44 $\pm$ 0.29	12.49 $\pm$ 0.24	12.28 $\pm$ 0.16
Hct (%)	41.52 $\pm$ 0.59	41.10 $\pm$ 0.99	37.78 $\pm$ 0.74*	39.74 $\pm$ 1.06	41.08 $\pm$ 0.74	39.28 $\pm$ 0.79
Platelet count ( $\times 10^3$ )/ $\mu$ l	693.80 $\pm$ 34.25	744.30 $\pm$ 76.05	789.50 $\pm$ 31.31	741.20 $\pm$ 46.50	788.90 $\pm$ 37.64	791.50 $\pm$ 47.48
SGOT (U/l)	174.80 $\pm$ 11.07	164.80 $\pm$ 6.69	190.40 $\pm$ 9.15	185.20 $\pm$ 16.35	160.7 $\pm$ 7.23	159.9 $\pm$ 13.13
SGPT (U/l)	94.80 $\pm$ 6.11	96.50 $\pm$ 3.36	147.90 $\pm$ 9.41*	108.10 $\pm$ 7.10	111.80 $\pm$ 7.70	99.00 $\pm$ 7.15

\* Significantly different from the SHAM,  $P<0.05$ ,  $n=10$

demonstrated that treatment with 40  $\mu$ g/kg of estrogen for 90 days caused a decrease of the erythrocytes (RBC) count, hemoglobin (Hb), % hematocrit (Hct) and increased SGPT levels. This indicated that a high dose of estrogen replacement may cause abnormalities of the hematopoietic system and liver function. In contrast, the treatment with 50, 500 and 1000 mg/kg of *Pueraria mirifica* (PM) showed no significant alterations of the RBC count, Hb, Hct, platelet, SGOT and SGPT. This indicated that a *Pueraria mirifica* replacement had no toxicity to the hematopoietic system and liver functions. According to the work of Manosroi et al. (2004), treatment of 100 mg PM for 6 months appeared to be safe in rats. The treatment of 100, 1000 mg/kg B.W./day for 90 days has also been shown to prevent bone loss in ovariectomized rats (Urasopon et al. 2008). Furthermore, Cherdshewasart (2003) has demonstrated that mice treated with the doses at 250, 500, 1000 or 2000 mg/kg B.W. for 14 day caused no acute toxicity. Therefore, any doses lower than 2000 mg/kg B.W. is relatively non-toxic (Cherdshewasart 2003). This was consistent with a previous *in vitro* study that confirmed the non-mutagenic but reasonably antimutagenic activities this plant extracts (Cherdshewasart et al. 2009). As for the skeletal mass and strength, a decrease of estrogen levels resulted in a decrease of the EDL weight/body weight, gastrocnemius weight and gastrocnemius weight/body weight (Table 1). In contrast, estrogen and 50, 500, 1000 mg/kg B.W./day of PM replacements preserved the EDL weight/body weight,

gastrocnemius weight and gastrocnemius weight/body weight (Table 1). This correlated with the size of the muscle fibers as observed using the light microscope (Figures 2 and 3). A decrease of the cross-sectional areas of the EDL and gastrocnemius were observed in the PM0 group (Figures 2-4). This indicated that the reduction of estrogen level may induce muscle fiber atrophy. It was of interest, that replacements with estrogen and 50, 500 or 1000 mg/kg B.W./day of *Pueraria mirifica* increased the cross-sectional area of both the EDL and gastrocnemius (Figures 2-4). This again indicated that PM exhibited an estrogen-like effect on rat skeletal muscle mass. The effects of estrogen are mediated through two estrogen receptors: ER $\alpha$  and ER $\beta$  (Hall 2001). Both receptors were identified in the nucleus of the EDL and gastrocnemius muscle fibers (Bunratsami et al. 2015). The nucleus ER $\alpha$  and ER $\beta$  act as transcription factors involving protein synthesis in the muscle fiber. Thus, a decrease of estrogen level may result in a reduction of protein synthesis causing a decrease of muscle fiber size (atrophy). Previous work from Boonchird et al. (2010) reported that *Pueraria mirifica* (PM) bound to both estrogen receptors (ER $\alpha$  and ER $\beta$ ) but more strongly to ER $\beta$  than to ER $\alpha$ . Thus, PM may bind with both of the estrogen receptors resulting in the induction of protein synthesis similar to that caused by estrogen. This may explain the effect of PM on preventing muscle atrophy of the EDL and gastrocnemius in the ovariectomized rats. The data of the muscle strength (the peak twitch tension, peak

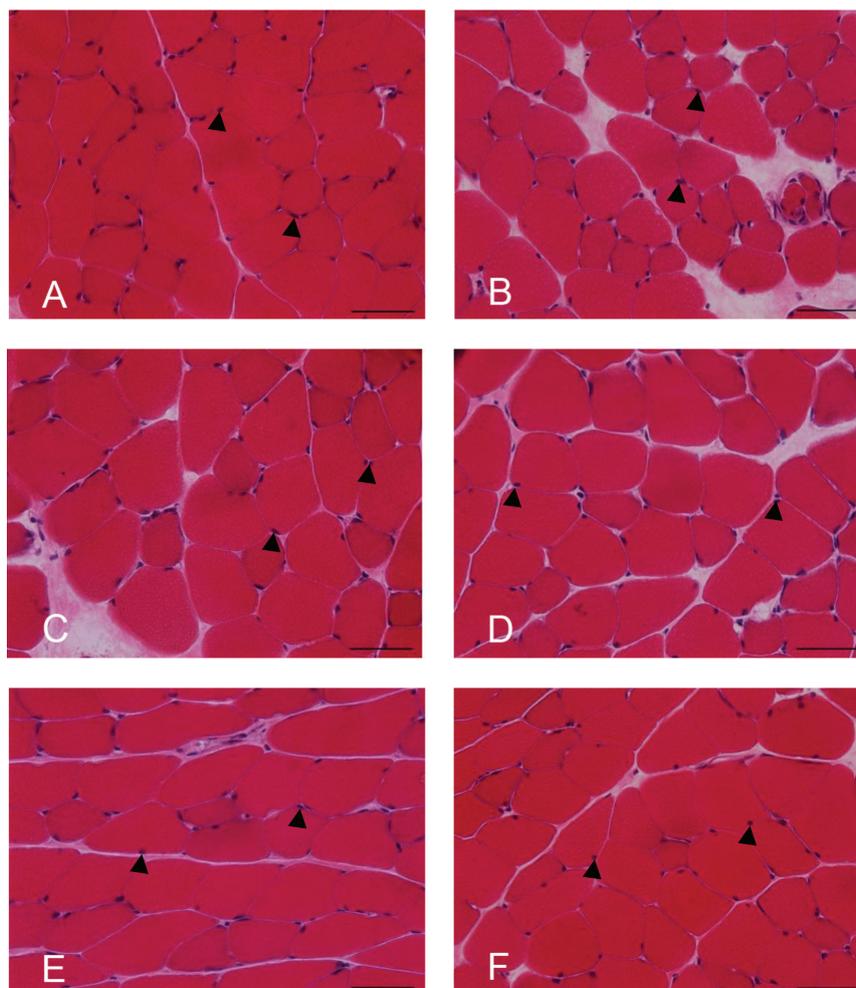


FIGURE 2. Cross-sectional micrographs of EDL in the SHAM (A), PM0 (B), E40(C), PM 50 (D), PM 100 (E) and PM 1000 (F) groups

H&E staining, arrow; nucleus, Bars = 50  $\mu$ m

tetanic tension), one-half relaxation time and endurance (time to fatigue) (Figures 5 and 6) demonstrated that a decrease of estrogen levels caused a decrease of the peak twitch tension, peak tetanic tension and time to fatigue and indicated the loss of muscle strength and endurance. In addition, a prolonged relaxation of EDL and gastrocnemius was found. This correlated well with the work of Bunratsami et al. (2015). The loss of muscle strength was due to alterations in the myosin function, especially, the fraction of the strong-binding myosin during contraction and was not due to a decline in the contraction proteins such as actin and myosin (Moran et al. 2006). According to the work of Bunratsami et al. (2015) the prolonged relaxation was due to a decrease of the parvalbumin (PV) protein. PV acts as a relaxing factor in the fast-twitch skeletal muscle. The reduction of PV caused a prolonged relaxation resulting in prolonging the contraction relaxation cycle. In contrast the estrogen and 50, 500, 1000 mg of *Pueraria mirifica* (PM) increased the peak twitch tension, peak tetanic tension and time to fatigue (Figures 5 and 6). Furthermore, estrogen and PM also improved this prolonged

relaxation. The estrogen replacement has been shown to improve a fraction of the myosin heads in the strong binding states during contraction (Moran et al. 2007) and increased the PV levels of the EDL and gastrocnemius (Bunratsami et al. 2015). This was one explanation for the mechanism of estrogen on enhancing the muscle strength and endurance. It was also of interest that, PM replacement improved the EDL and gastrocnemius strength and endurance. This may be due to its estrogenic effect. Thus it would be of interest to study the effect of PM on myosin function or PV levels to help to explain the mechanisms of PM that improve skeletal muscle strength and endurance. The data from this study have demonstrated that PM replacement is a potential approach to replace estrogen replacement for improving skeletal muscle atrophy and loss of strength and endurance especially in the estrogen dependent sarcopenia. Our data indicated that treatment with 40  $\mu$ g/kg estrogen protected against skeletal muscle atrophy, encouraged a build-up of muscle strength and endurance, however, high level of serum estrogen was found. This may cause toxicity to the hematopoietic system

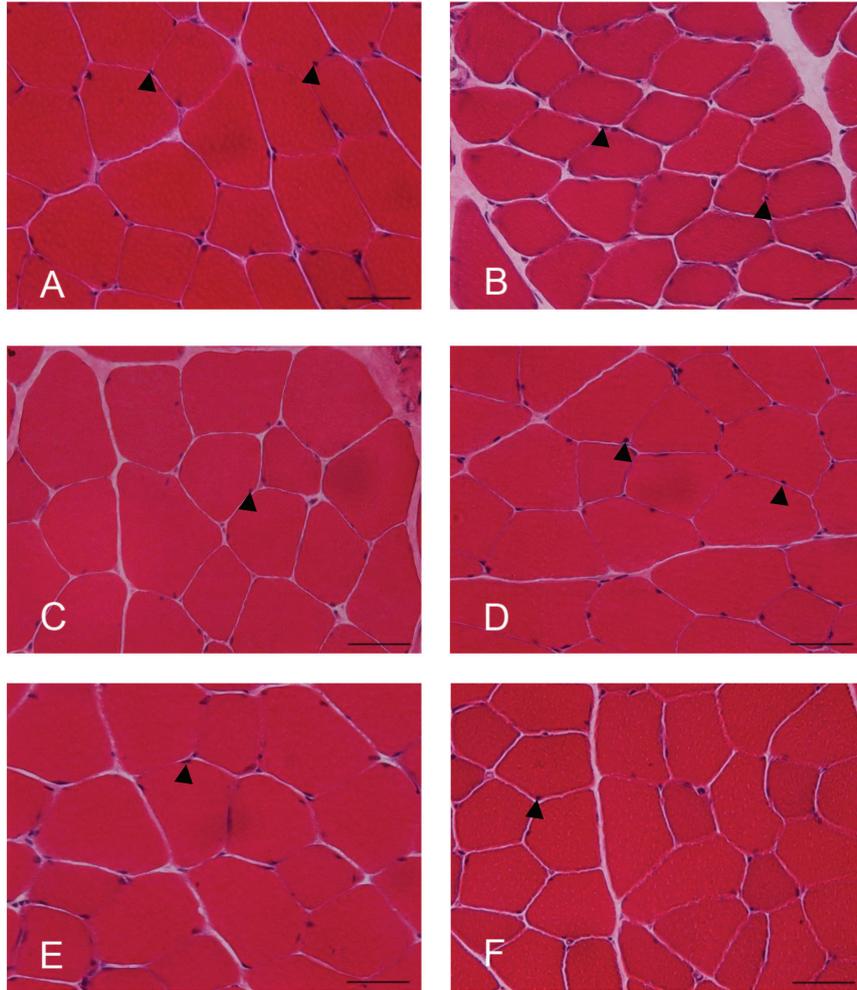


FIGURE 3. Cross-sectional micrographs of gastrocnemius in the SHAM (A), PM0 (B), E40(C), PM 50 (D), PM 100 (E) and PM 1000 (F) groups  
H&E staining, arrow; nucleus, Bars = 50  $\mu$ m

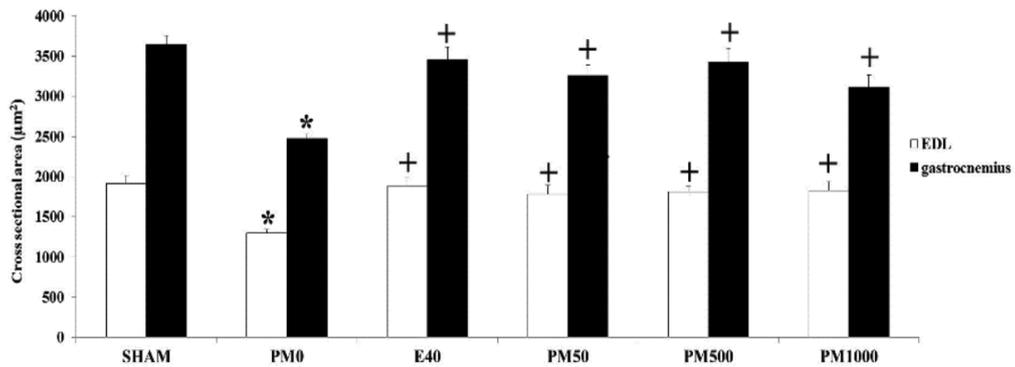


FIGURE 4. The cross sectional area in all treatment groups: SHAM, PM0, E40, PM50, PM500 and PM1000 groups  
\*Significantly different from the SHAM,  $P < 0.05$ ,  $n = 10$ , + Significantly different from the PM0,  $P < 0.05$ ,  $n = 10$

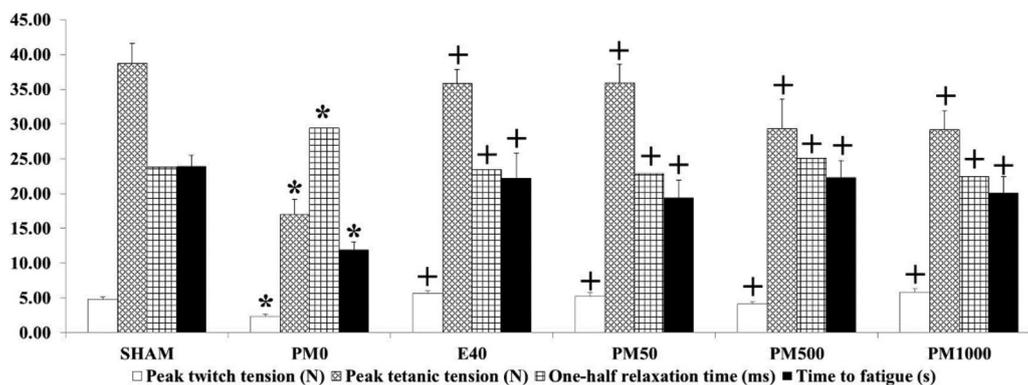


FIGURE 5. The muscle performance (the peak twitch tension, peak tetanic tension, the one-half relaxation time and time to fatigue) of the EDL all treatments: SHAM, PM0, E40, PM50, PM500 and PM1000 groups

\*Significantly different from the SHAM,  $P < 0.05$ ,  $n = 10$ , + Significantly different from the PM0,  $P < 0.05$ ,  $n = 10$

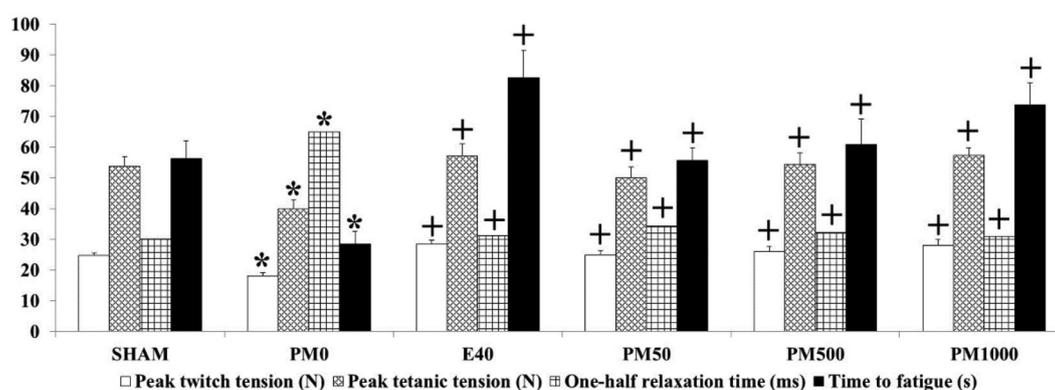


FIGURE 6. The muscle performance (the peak twitch tension, peak tetanic tension, the one-half relaxation time and time to fatigue) of the gastrocnemius from all treatments: SHAM, PM0, E40, PM50, PM500 and PM1000 groups

\*Significantly different from the SHAM,  $P < 0.05$ ,  $n = 10$ , + Significantly different from the PM0,  $P < 0.05$ ,  $n = 10$

and liver function. Furthermore, it arrested the estrous cycle and may cause hypertrophy of the uterus. In contrast the treatment with 50 or 500 or 1000 mg/kg of *Pueraria mirifica* also prevented muscle atrophy, restored muscle strength and endurance. It showed no toxicity to the hematopoietic system and liver function. In addition, the serum estrogen was not too high thus the estrous cycle was almost identical to the natural system after treatment with PM. However, treatment with 1000 mg/kg of *Pueraria mirifica*, induced only two stages of the estrous cycle. It also prevented uterine atrophy but did not cause hypertrophy of the uterus. Therefore, the 50 or 500 mg/kg of *Pueraria mirifica* treatment may be a possible dose for treatment of estrogen dependent sarcopenia in ovariectomized rats.

#### CONCLUSION

Treatment with 50, 500 or 1000 mg/kg of *Pueraria mirifica* for 90 days showed no toxicity, maintained the female rat reproductive system in ovariectomized rats in a natural

manner, prevented muscle atrophy and restored muscle strength and endurance.

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Kochakorn Sukjan Inthanuchit  
Faculty of Traditional Thai Medicine  
Prince of Songkla University  
Songkhla, 90110  
Thailand

Wandee Udomuksorn  
Department of Pharmacology, Faculty of Science  
Prince of Songkla University  
Songkhla, 90110  
Thailand

Ekkasit Kumarnsit  
Department of Physiology, Faculty of Science  
Prince of Songkla University  
Songkhla, 90110  
Thailand

Surapong Vongvatcharanon  
Department of Oral Surgery (Anesthesiology section)  
Faculty of Dentistry  
Prince of Songkla University  
Songkhla, 90110  
Thailand

Uraporn Vongvacharanon\*  
Department of Anatomy, Faculty of Science  
Prince of Songkla University  
Songkhla, 90110  
Thailand

\*Corresponding author; email: uraporn.v@psu.ac.th

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