การศึกษาเภสัชจลนศาสตร์ของสารพูรารินในหนูแรท

นางสาวพิลาสลักษณ์ ภู่

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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PHARMACOKINETIC STUDY OF PUERARIN IN RATS

Miss Pilaslak Poo

A Thesis Submitted in Partial Fulfillment of the Requirements
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พิลาสลักษณ์ ภู่ : การศึกษาเภสัชจลนศาสตร์ของสารพูรารินในหนูแรท (PHARMACOKINETIC STUDY OF PUERARIN IN RATS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ภก. ดร.พิสิฐ เขมาวุฒฒ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.สุจินดา มาลัยวิจิตรนนท์, 50 หน้า.

กวาวเครือขาว (*Pueraria candollei* var. *mirifica*) เป็นพืชที่มีสรรพคุณทางยาที่ได้รับการสนับสนุนจากรัฐบาลไทยให้เป็นผลิตภัณฑ์สมุนไพรต้นแบบ โดยมีสารพูราริน (Puerarin) ซึ่งเป็นสารสำคัญหลักของพืชชนิดนี้ โดยมีการศึกษาฤทธิ์ทางเภสัชวิทยาที่หลากหลาย รวมถึงมีผลการศึกษาที่เกี่ยวข้องกับโรคในผู้สูงอายุ แต่ข้อมูลการศึกษาทางด้านเภสัชจลนศาสตร์สำหรับสนับสนุนผลการศึกษาฤทธิ์ทางเภสัชวิทยาดังกล่าวยังมีข้อมูลไม่เพียงพอ ดังนั้นการศึกษานี้มีวัตถุประสงค์เพื่อศึกษาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ รวมไปถึงการศึกษาขบวนการดูดซึม การกระจายไปยังเนื้อเยื่อบริเวณต่างๆของร่างกาย, กระบวนการเมแทบอลิซึม และการขับออกของสารพูรารินในหนูแรทเพศเมีย นอกจากนี้การศึกษานี้ยังเป็นการศึกษาแรกที่ศึกษาการกระจายของสารพูรารินไปยังสมองส่วนฮิปโปแคมปัส, กระดูกแข้ง, กระดูกน่อง และต่อมน้ำนม ซึ่งเกี่ยวข้องกับฤทธิ์ทางเภสัชวิทยาที่ใช้ในการป้องกันและการรักษาโรคเกี่ยวกับระบบประสาท, โรคกระดูกพรุน และโรคมะเร็งเต้านม ตามลำดับ โดยใช้หนูแรทเพศเมียโดยได้รับสารพูรารินทางหลอดเลือดดำขนาด 1 มิลลิกรัม/กิโลกรัม และในรูปแบบรับประทานขนาด 5 และ 10 มิลลิกรัม/กิโลกรัม จากนั้นเก็บตัวอย่างเลือด อวัยวะ ปัสสาวะ และอุจจาระภายหลังได้รับสาร และวัดระดับสารสำคัญโดยใช้เทคนิค Liquid chromatography-tandem mass spectrometry จากการศึกษาพบว่าสารพูรารินมีระดับความเข้มข้นของยาสูงสุดในพลาสม่าภายใน 1 ชั่วโมงหลังได้รับสารในรูปแบบรับประทาน และมีค่าชีวประสิทธิ์ผลประมาณ 7% การกระจายตัวของสารพูรารินในเนื้อเยื่อพบว่า สารพูรารินสามารถแพร่กระจายไปยังอวัยวะต่างๆได้ดี รวมถึงสมองส่วนฮิปโปแคมปัส, หัวใจ, ปอด, กระเพาะอาหาร, ตับ , ต่อมน้ำนม, ไต, ม้าม, กระดูกแข้ง และกระดูกน่อง โดยพบว่า 50% ของขนาดสารที่ได้รับทางหลอดเลือดดำจะถูกขับออกจากร่างกายผ่านทางปัสสาวะในรูปของ Puerarin glucuronide ซึ่งข้อมูลดังกล่าวเป็นข้อมูลพื้นฐานที่มีความจำเป็นสำหรับการวิจัยและพัฒนาสาพูรารินและสารสกัดกวาวเครือขาวให้เป็นผลิตภัณฑ์ยาสมุนไพร สำหรับใช้ในการป้องกันและรักษาโรคในผู้สูงอายุต่อไป

ภาควิชา	เภสัชวิทยาและสรีรวิทยา	ลายมือชื่อนิสิต .....
สาขาวิชา	เภสัชวิทยาและพิษวิทยา	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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PILASLAK POO: PHARMACOKINETIC STUDY OF PUERARIN IN RATS. ADVISOR: ASST. PROF. PHISIT KHEMAWOOT, Ph.D., CO-ADVISOR: PROF. SUCHINDA MALAIVIJITNOND, Ph.D., 50 pp.

Pueraria candollei var. *mirifica* is a medicinal plant which has been promoted as a “Champion Product” by the Government of Thailand. Puerarin, the major bioactive component of this plant, has been an interest of pharmacodynamic activity against aging diseases, but there is little pharmacokinetic information to support further study of this activity. Therefore, the aim of this study was to determine the pharmacokinetics of puerarin, including absorption, distribution, metabolism and elimination, in female rats. Moreover, this is the first study to examine the tissue distribution of puerarin in the hippocampus, femur and tibia, and mammary gland based on recently the pharmacodynamic effects reported for neurodegenerative disease, osteoporosis, and breast cancer, respectively. Adult female rats were administered puerarin at 1 mg/kg intravenously or 5 and 10 mg/kg orally. Blood, tissue, urine and feces were collected and analysed by liquid chromatography-tandem mass spectrometry. Puerarin reached a maximum concentration in blood within 1 h after oral dosing, and had an absolute oral bioavailability of approximately 7%. Puerarin was widely distributed in several tissues, including the hippocampus, heart, lung, stomach, liver, mammary gland, kidney, spleen, femur, and tibia. Approximately 50% of the intravenous dose was excreted as glucuronide metabolites via the urinary route. These results are useful for the development of puerarin and *Pueraria candollei* var. *mirifica* as phytopharmaceutical products for the prevention and treatment of aging diseases.

Department:	Pharmacology and Physiology	Student's Signature .....
		Advisor's Signature .....
Field of Study:	Pharmacology and Toxicology	Co-Advisor's Signature .....
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LIST OF ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion
ALT	alanine aminotransferase
ATP	adenosine triphosphate
AST	aspartate aminotranferase
cAMP	cyclic adenosine monophosphate
C_{max}	maximum plasma concentration
dL	deciliter
DMSO	dimethylsulfoxide
ESI	electrospray ionization
g	gram
h	hour
HDL	high density lipoprotein
HPLC	high-performance liquid chromatography
ICR	imprinting control region
IV	intravenous
kg	kilogram
LC-MS/MS	liquid chromatography-tamdem mass spectrometry
LDL	low density lipoprotein
LLOQ	lower limit of quantitation
mg	milligram
min	minute
mL	milliliter
ng	nanogram
PI3K/Akt	phosphoinositide 3-kinase/protein kinase B
PO	per oral
QC	quality control
ROS	reactive oxygen species
UV	ultraviolet

µg	microgram
XlogP	partition coefficient

CHAPTER I
INTRODUCTION

1.1 Rationale and significance

Puerarin (7,4′-dihydroxy-8-C-glucosylisoflavone) is one of the major active phytoestrogens in *Pueraria candollei* var. *mirifica*, an endemic Thai plant of the family Leguminosae. This plant has been reported to relieve postmenopausal symptoms [1,2], prevent and ameliorate bone loss [3-5], inhibit the growth of breast cancer [6] and alleviate cardiovascular diseases in preclinical and clinical studies [7-8]. There is also increasing evidence of a beneficial role of puerarin and *Pueraria candollei* var. *mirifica* in the prevention of and therapy for diseases of aging such as osteoporosis, neurodegeneration, diabetes and cardiovascular disease [3, 8-10]. However, there is little information on the oral bioavailability and tissue distribution of puerarin, with respect to its pharmacodynamic activities.

Puerarin has an aqueous solubility of 0.46 mg/mL [11] and a partition coefficient of 1.95. In pharmacokinetic studies using oral dosing in rat and dogs models, puerarin reaches a maximum plasma concentration (C_{max}) in 0.45-5.00 h and has an absorption half-life of 0.80-1.00 h after dosing [12-16]. Distribution of puerarin to the liver, spleen, kidney, lung, heart and brain occurs within about 1 h after single oral administration at 20 mg/kg. In the tested organs, C_{max} was reached at 2.5 h post-treatment [17]. Prasain et al. (2004) suggested that puerarin undergoes hepatic phase I metabolism via a cytochrome P450 and proposed that the metabolite was dihydroxylated puerarin [18]. However, Luo et al. (2010) reported that the major metabolic pathway of puerarin after intravenous administration is phase II glucuronidation to give puerarin-7-O-glucuronide and puerarin-4′-O-glucuronide, through glucuronidation at 7-OH and 4′-OH [19]. Excretion of puerarin has been

proposed to occur mainly via the urinary system, with negligible amounts of puerarin and its glucuronide metabolites excreted via the hepatobiliary route [20].

With the increasing significance of a potential beneficial role of puerarin in prevention and therapeutics of aging diseases [3, 8-10], there are still lack of sufficient reports for oral bioavailability and tissue distribution of puerarin in related to these pharmacodynamic activities**.** The aim of this study was to determine the pharmacokinetics and absorption, distribution, metabolism and elimination (ADME) properties of puerarin in female rat. The tissue distribution of puerarin in the hippocampus, femur and tibia, and mammary gland was investigated, based on the pharmacodynamic effects reported for neurodegenerative disease, osteoporosis, and breast cancer, respectively. The puerarin doses used in this study were based on estrogenic effects on reproductive organs [21] and bone [22] in female rats. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to determine the puerarin level in biological samples after administered puerarin at 1 mg/kg intravenously (IV) and 5 and 10 mg/kg orally (PO) in healthy female rats.

1.2 Objective

1.2.1 To determine the pharmacokinetics and absorption, distribution, metabolism and elimination properties of puerarin in female rats.

1.2.2 To investigate the tissue distribution pattern of puerarin and puerarin glucuronide in female rats.

1.3 Hypothesis

Puerarin has suitable pharmacokinetic profiles including;

- Absolute oral bioavailability more than 1%.

- Good tissue distribution into pharmcological related organ with tissue to plasma ratio more than 1.00.

- Multiple metabolic pathways and route of excretions.

1.4 Expected benefits from the study

1.4.1 This study provides the pharmacokinetic informations of puerarin in female rats. These data should be beneficial development of puerarin and *Pueraria candollei* var. *mirifica* as phytopharmaceutical products of Thailand.

1.4.2 The tissue distribution results should be useful for further pharmacological study of puerarin.

CHAPTER II

LITERATURE REVIEW

2.1 *Pueraria candollei* Wall. ex. Benth. var. *mirifica* (Airy Shaw & Suvat.) Niyomdham

Pueraria candollei var. *mirifica* or Kwao Krua Khao in Thai belongs to Family Leguminosae which can be found in mixed deciduous forest of the North, West and Northeast part of Thailand. It is a tropical climber that can grow up to 5 meters in height (Figure 1A). It has a round and long tuberous root with a brown peel and white texture (Figure 1B). This tuberous root has been widely used in Thai traditional herbal medicine.

1A  1B 

Figure 1 Morphological characters of Pueraria candollei var. mirifica.
A: Trunks and leaves B: Tuberous root.

Pueraria candollei var. *mirifica* has been reported to relieve postmenopausal symptoms [1,2], prevent and ameliorate bone loss [3-5], inhibit the growth of breast cancer [6] and alleviate cardiovascular diseases in preclinical and clinical studies [7-8].

Pueraria candollei var. *mirifica* contains five major isoflavonoids, including puerarin, daidzin, daidzein, genistin and genistein [23]. Puerarin is a major isoflavone in *Pueraria candollei* var. *mirifica*. The *Pueraria candollei* var. *mirifica* extract contains puerarin in the range of 53.20 - 870.50 μg/g, as analyzed by HPLC-UV [24].

2.2 Physical and chemical properties of puerarin

Puerarin (7,4'-dihydroxy-8-C-glucosylisoflavone) is one of major bioactive ingredients in *Pueraria candollei* var. *mirifica*, with the chemical structure shown in Figure 2. Puerarin has an aqueous solubility of 0.46 mg/mL [11] and a partition coefficient of 1.95. Puerarin is in class IV of the Biopharmaceutical Classification System, indicating low solubility and low permeability, and is a weak acid that is ionised at high pH. Thus, it has lower solubility in the acid condition in the stomach of rats, which may cause precipitation [25].

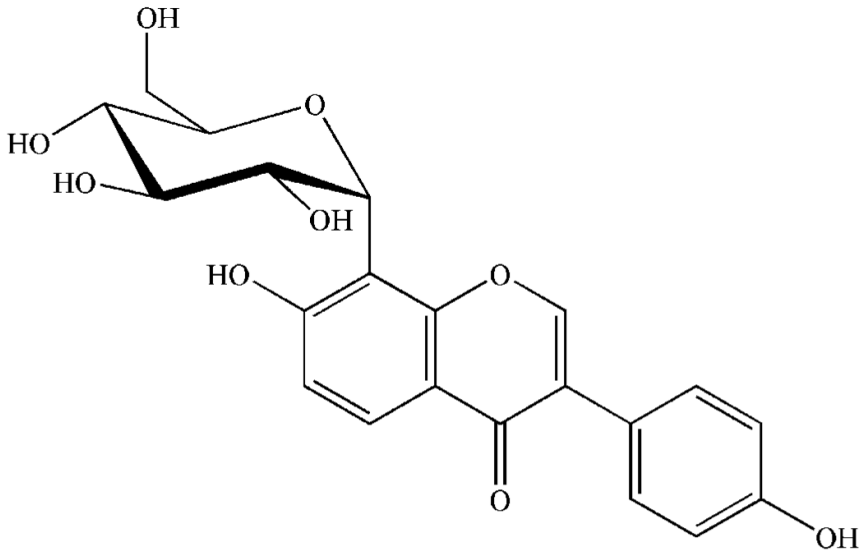

Figure 2 Chemical structure of puerarin.

2.3 Pharmacological actions of puerarin

Puerarin has a variety of therapeutic activities such as anti-hypertension, vasodilation, anti-ischemia, anti-apoptosis, anti-diabetes, anti-hypercholesterolemia and anti-inflammation [8, 26-34]. In recent years, several pharmacological effects and activities of puerarin on various organ systems were reported as follows:

2.3.1 Cardiovascular system

The anti-hypertensive effect of puerarin was investigated in spontaneous hypertensive rat models and puerarin can reduce heart rate, blood pressure, and affect on the plasma renin activity [8]. Moreover, puerarin has demonstrated its vasodilatory activity which may be related to endothelium-dependent pathway via nitric oxide system, and ATP-sensitives K^+-channel and endothelium-independent pathway via the cAMP pathway [8]. Puerarin has also shown cardioprotective effect against ischemia and reperfusion in rats. It can reduce infract area in heart, and induce angiogenesis in rat with myocardial infraction at a dose of 120 mg [8]. Puerarin has been widely used for treatment of acute ischemic stroke in Chinese population. The recommended doses of puerarin is 400-600 mg/day for 1-2 weeks [8].

2.3.2 Phytoestrogenic activity

The estrogenic activity of puerarin on the reproductive organs was investigated by subcutaneous injection of puerarin at 0.7 mg/kg for 14 days in immature overiectomized rats at 7 mg/kg for 140 days in mature female rats. The results showed that puerarin increased the number of uterine glands in immature overiectomized rats and increased the percentage of cornified cells in mature female rats [21]. Additionally, ovariectomized rats fed with pelleted food supplemented with

44.2 ± 1.20 mg of puerarin for 3 months improved urethral closure mechanism in ovariectomized rats [27].

2.3.3 Diabetes and inhibition of diabetic complications

In vitro study, puerarin significantly increased glucose uptake into the insulin sensitive cell [28]. Puerarin has increased the glucose exertion and decreased plasma glucose level in both streptozocin-induced diabetic rats and type 2 diabetes mellitus rats [29-30].

2.3.4 Anti-Alzheimer's disease activity

The preventive property of puerarin has been studied using mitochondrial transgenic neuronal cell cybrid models of sporadic Alzheimer's disease. This model had increased of reactive oxygen species (ROS) accumulation, cell apoptosis, and activation of caspase-3. The results demonstrated that pretreatment with puerarin could block cell viability loss and apoptosis via the oxidative stress-related signaling pathway [31]. Moreover, administration of puerarin showed the neuroprotective effect against ischemic brain injury in rats [32].

2.3.5 Anti-osteoporotic activity

Puerarin markedly enhanced the rate of bone formation by promoting osteoblast proliferation and differentiation via the PI3K/Akt pathway [32]. In animal model, streptozocin-induced diabetic rats which has lowered bone mineral density and elevated caspase-3 expression, showed a reversed of caspase-3 expression in osteoblasts after 100 mg/kg intra peritoneal injection of puerarin [34].

2.4 Toxicity, safety and tolerability of puerarin

The genotoxic effect of puerarin using *in vitro* Ames test with *Salmonella typhimurium* strains revealed that 50 µL of puerarin had no mutagenicity and significantly reduced the mutagenic effect induced by 4-nitroquinoline-1-oxide up to 41%. The result from bone marrow micronucleus test using ICR mice suggested that puerarin at a dose 500 mg/kg can induce the formation of abnormal erythrocytes *in vivo* [35].

Oral administration of puerarin at doses of 0, 50, 250, 500 and 2,000 mg/kg for 4 weeks to rats showed that puerarin at a dose of 2,000 mg/kg/day can reduce physical activity in both males and females but one female died during the experiment with no lesions observed in necropsy test. All given doses of puerarin did not affect body weight, food and water consumption. Hematological parameters were unchanged. However, at a dose of 250 mg/kg/day, male rats had significantly lower platelet counts. Puerarin at a dose higher than 500 mg/kg/day decreased HDL, LDL and total cholesterol levels in male rats and some abnormalities of liver histology such as swollen hepatocyte and occasional appearance of double nuclei were detected. The investigation of toxicity shows that consumption of puerarin is safe at dosages below 250 mg/kg/day [36].

Table 1 Previous pharmacokinetic studies of puerarin.

Research group	Li Y [12]		Ren F [13]		Zhiguo Y [14]	Liu X [15]		Cao L [16]		
Journal	Drug Development and Industry Pharmacy		Pharmaceutical and Biomedical analysis		Pharmaceutical and Biomedical analysis	Chromatography B		Fitoterapia		
Year	2006		2006		2007	2010		2013		
Subject species	5 Sprague-Dawley rats		6 dogs		6 male and female Wister rats	7 Male Sprague-Dawley rats		6 Sprague-Dawley rats	6 pregnant Sprague-Dawley rats (GD-10thday)	6 pregnant Sprague-Dawley rats (GD-20thday)
Weight range	200 ± 20 g		13.60 ± 2.10 kg		200-220 g	230-250 g		230-270 g		
Dose	400 mg/kg Puerarin	400 mg/kg Puerarin-Phospholipid complex	130 mg /dog in sustained release tablet	130 mg /dog in Yufengning-xin tablet	8 mg/kg	20 mg/kg Puerarin	20 mg/kg PEGylatedpuerarin conjugate	250 mg/kg		
Method	HPLC-UV		HPLC-UV		LC-MS/MS	HPLC-UV		HPLC-UV		
Route of administration	Oral administration		Oral administration		Intravenous administration	Intravenous administration		Oral administration		
T_{max}(h)	0.89 ± 0.52	0.43 ± 0.26	4.00 ± 0.98	1.50 ± 0.32	N/A	N/A	N/A	0.45 ± 0.11	0.70 ± 0.27	0.50 ± 0.12
C_{max}(μg/ml)	1.37±0.58	2.20 ± 1.28	674.90 ±91.00	917.00 ± 123.20	N/A	N/A	N/A	3.04 ± 1.34	2.64 ± 0.77	2.66 ± 1.22
K_e(1/h)	N/A	N/A	0.26 ± 0.08	0.48 ± 0.12	N/A	N/A	N/A	N/A	N/A	N/A
$T_{1/2}$(h)	N/A	N/A	2.83 ± 0.84	1.52 ± 0.37	N/A	26.9 min	139.3 min	N/A	N/A	N/A
$T_{1/2}\alpha$(h)	0.86 ± 0.68	1.23 ± 1.61	N/A	N/A	0.17±0.03	N/A	N/A	0.75 ± 0.39	1.08 ± 0.78	0.90 ± 0.31
$T_{1/2}\beta$(h)	3.70 ± 1.22	4.67±1.59	N/A	N/A	2.46±1.24	N/A	N/A	8.27±1.35	6.60 ± 2.10	8.41 ± 3.18
AUC $_{0-t}$ (μg h/ml)	N/A	N/A	N/A	N/A	23.29±4.13 (t=96 h)	N/A	N/A	14.34 ± 4.64 (t= 24 h)	14.82 ± 2.39 (t= 24 h)	11.08 ±3.86 (t= 24 h)
AUC $_{0-\infty}$ (μg h/ml)	5.80 ± 1.66	8.45 ± 0.44	4.85 ± 877.30	3.75 ± 565.80	N/A	N/A	N/A	N/A	N/A	N/A

2.5 Pharmacokinetics of puerarin

2.5.1 Absorption

The pharmacokinetic studies of single dose of puerarin at the range of 100 mg to 400 mg were conducted in various animal models. For oral administration, puerarin was reached C_{max} around 0.45-4.00 h after dosing in rats and dogs [12-16]. In addition, a single oral administration of puerarin at a dose of 400 mg to healthy human had a maximum plasma level at around 3 h after dosing [37]. Moreover, the pharmacokinetic profile of puerarin after administration at a dose of 500 mg of Kudzu (*Pueraria labota*) extract (equivalent to 23.75 mg of puerarin) to a healthy volunteer had C_{max} at 2 h and a half-life approximately at 4.3 h [38].

2.5.2 Tissue distribution

The distribution of puerarin in tissues was appeared almost at 1 h post single oral administration at a dose of 20 mg/kg to rats. Puerarin was found in liver, spleen, kidney, lung, heart and brain. Of the organs tested, the C_{max} appeared at 2.5 h post-treatment [17]. In addition, single oral administration of puerarin to rats at a dose of 50 mg/kg was widely distributed to various organs, especially being highest in lung [39].

2.5.3 Metabolism

The metabolism of puerarin was investigated in rats after a single oral administration of puerarin at a dose of 20 mg/kg. There were two monoglucuronidated metabolites of puerarin, puerarin-4′-O-glucuronide and puerarin-7-O-glucuronide. They were detected in plasma and urine as shown in Figure 3 [40].

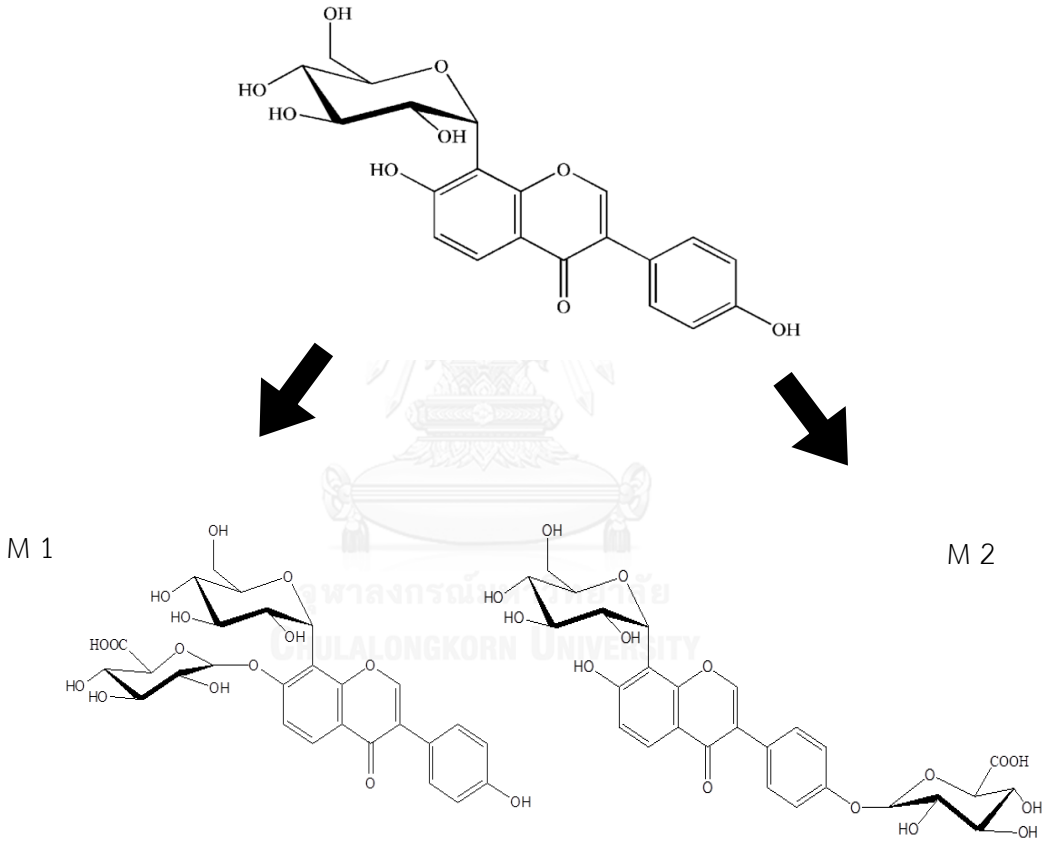

Figure 3 Chemical structure of puerarin metabolites [40]
M1: puerarin-4′-O-glucuronide, M2: puerarin-7-O-glucuronide.

Prasain et al. (2004) found that the concentration of unchanged puerarin was decreased, but the concentrations of daizein, dihydrodaizein and eqoul were increased after puerarin 50 mg/kg multiple oral administrations. Therefore, they

proposed that puerarin is hydrolyzed by microbial metabolism to daidzein, then it was reduced to dihydrodaidzein and eqoul [18]. Thus, puerarin might has multiple metabolic pathways, started from phase I hydrolysis in GI-tracts and phase II glucuronidation in both enterocytes and hepatocytes. *In vivo* study, puerarin was mainly presents as glucuronide in plasma and urine [17]. Recent study found that puerarin-4′-O-glucuronide and puerarin-7-O-glucuronide were the major metabolites in plasma and urine. Human UGT studies showed that UGT 1A1 catalyzed 7-glucuronidation of puerarin [41].

2.5.4 Urinary and fecal excretion

Negligible amount of unchanged puerarin was excreted via feces and urine at 0-72 h following oral administration [12]. The compound might be biotransformed to other products such as hydrolysis products and glucuronide metabolites before excretion into bile or urine.

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

3.1.1 Animals

Sixteen female Sprague-Dawley rats aged 12 weeks old were obtained from the National Laboratory Animal Centre, Mahidol University, Thailand. The animals were housed for 4 weeks at 25±2 ºC under a 12 h light/dark cycle with free access to food and water. They were moved to a metabolic cage one day before the experiment started and kept in this cage for another 72 h after the experiment ended. Rats weighing 300 to 400 g were used in pharmacokinetic studies. The sample size was four animals per experiment, based on OECD guidelines for testing chemicals (2008). Experiments were conducted under the protocol approved by the Ethical Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (approval number: 15-33-001, approval date: April 22, 2015).

3.1.2 Chemicals

- Puerarin power for pharmacokinetic experiments was purchased from Pure Chemistry Scientific, Inc., USA.

Analytical standards of chemicals use in LC-MS/MS analysis were purchased from following companies.

- Puerarin (Sigma-Aldrich, Corp., USA)
- Daidzin (Sigma-Aldrich, Corp., USA)
- Daidzein (Sigma-Aldrich, Corp., USA)
- Equol (Sigma-Aldrich, Corp., USA)

- Glycyrrhetinic acid (Wako Pure Chemical Industries, Ltd., Japan)
- Dimethyl sulfoxide (Sigma-Aldrich, Corp., USA)
- Isoflurane (Minrad, Inc., USA)
- Heparin (LEO Pharma A/S, Denmark)
- 0.9% Normal saline (General Hospital Products Public, Co., Thailand)
- Methanol (Honeywell Burdick & Jackson International, Inc., USA)
- Water HPLC grade (Honeywell Burdick & Jackson International, Inc., USA)
- β-Glucuronidase from *Escherichia coli* Type VII-A (Sigma-Aldrich, Corp., USA)

3.1.3 Equipment

Animal experiments

- Metabolic cage 3701M081 (Tecniplast, S.P.A., Italy)
- Insulin syringe, size 1 mL (Nipro, Corp., Thailand)
- Stopwatch (Canon, Co., Ltd., China)
- Gavage needle, 13G size 3 inches (BiolascoThai Co., Ltd., Thailand)

Sample preparation

- Microliter centrifuge, model MIKRO (Andreas Hettich, GmbH & Co. KG, Germany)
- Tabletop centrifuge, model EBA 20 (Andreas Hettich, GmbH & Co. KG, Germany)
- Micropipette (Labnet International, Inc., USA)
- Homogenizer, model Yellowline DI 18 Basic (IKA-Werke GmbH & Co. KG, Germany)
- Homogenizer, model WT-130 (Success Technic, Malaysia)
- Vortex mixer, model VX-200 (Labnet International, Inc., USA)
- Analytical balance, model AG135 (Mettler-Toledo International, Inc., Switzerland)

- Analytical balance, model UMT2 (Mettler-Toledo International, Inc., Switzerland)
- Chest freezer, -20℃ (Singer, SdnBhd, Malaysia)

LC-MS/MS analysis

- QTRAP 6500 LC-MS/MS system (AB Sciex, Pte. Ltd., USA)
- HPLC C18 column, model SynergiTM Fusion-RP (Phenomenex, Inc., USA)
- Guard C18 column, model SecurityGuardTM Fusion-RP (Phenomenex, Inc., USA)

3.2 Pharmacokinetic studies

Twelve rats were divided into three groups of four animals each for the administration of puerarin at the doses of 1 mg/kg IV, and 5 and 10 mg/kg PO. The puerarin solution for administration to animals was made in 20% DMSO in normal saline solution. Rats were anesthetised with isoflurane by the chamber induction method before IV administration and blood collection. Blood samples (300 µL) taken from the lateral tail vein at 0, 0.08, 0.25, 0.50, 1, 2, 4, 8, 12 and 24 h after administration were collected in heparinised tubes, centrifuged at 1,500×*g* for 10 min, and kept at -20℃ for further analysis. Urine and faeces were collected in three periods of 0-24, 24-48 and 48-72 h after IV and PO administration and stored at -20℃.

To examine the tissue distribution of puerarin, 16 rats received 1 mg/kg puerarin IV and were subsequently sacrificed four animals each by cervical dislocation at 0.08, 1, 2 and 4 h after administration, respectively. Major organs including brain (particularly hippocampus), liver, kidney, spleen, stomach, heart, lung, small intestine, mammary gland, femur and tibia were collected, rinsed with ice-cold saline, and stored at -20℃ until analysis.

3.3 Sample preparation

All biological samples were extracted by the protein precipitation method of Prasain et al. [18], with slight modification. In brief, 50 µL of plasma and urine samples were mixed directly with 200 µL of methanol containing 10 ng of glycyrrhetinic acid as the internal standard, whereas 50 mg of tissue and feces samples were chopped into small pieces, homogenised and mixed in methanol 200 µL containing 10 ng of glycyrrhetinic acid as the internal standard. Then, samples were centrifuged for 10 min at 5,000×*g* to precipitate proteins. A volume of 150 µL of the supernatant was transferred into a sample vial and 10 µL of the supernatant was injected into the LC-MS/MS system. For the femur and tibia, decalcified bones were prepared by soaking in 10% EDTA solution for 2 weeks, and then the bones were chopped and processed in a similar manner to the other tissues.

For the determination of glucuronide conjugates of puerarin, 50 µL of plasma or urine were added to 50 µL of 0.1 M potassium phosphate buffer (pH 6.8) containing 1,500 units of β-glucuronidase. The mixture was incubated at 37°C for 15 min, and the reaction was stopped by adding 400 µL of methanol containing 20 ng of glycyrrhetinic acid as the internal standard. The mixture was centrifuged at 5,000×*g* for 10 min, the supernatant was collected, and 10 µL of the supernatant was injected into the LC-MS/MS system. For solid samples, 50 mg of tissue and faeces were chopped into small pieces, homogenised and mixed in 50 µL of 0.1 M potassium phosphate buffer (pH 6.8) containing 1,500 units of β-glucuronidase. These mixtures were then processed in a similar manner to the plasma and urine mixtures.

3.4 Method validation and quality control

Method validation was developed to verify the quality of puerarin measurement which is composed of:

- Lower limit of quantification (LLOQ) or sensitivity, defined as the lowest detectable concentration of analyte with a signal-to-noise ratio greater than 5.
- Linearity, defined as the range of analyzed concentrations that can be fitted with the calibration curve with $R^2 > 0.99$.
- Accuracy, determined by comparing the measured concentrations to the actual (low, medium and high) concentrations of quality control (QC) samples. .
- Precision, determined concurrently with accuracy by analyzing QC samples for intra-day (5 replicates within a day) and inter-day (once a day for 3 consecutive days).
- Recovery, calculated by comparing the peak-area of the prepared sample to that of the standard solution containing the same concentration.

3.5 LC-MS/MS analysis

The analytical method followed those of Li et al. [12] and Prasain et al. [18], with some modifications to allow for the analysis of puerarin with good linearity, precision and high accuracy. An Ultra LC 100 (Eksigent, Canada) system was equipped with a Synergi Fusion-RP C18 column as the stationary phase (Phenomenex, USA). The LC system used 100% methanol and 0.2% formic acid in water (pH 2.5) with a flow rate of 0.5 mL/min. The mobile phase was rinsed with 10% methanol for 0.5 min, then increased to 90% methanol from 1.5 to 3.5 min, and then decreased to 10% methanol from 4 to 4.5 min. The retention time of puerarin was 1.58 min, and that of the internal standard was 2.09 min. Detection was conducted in negative ionisation mode by monitoring precursor ion to product ion transitions with the mass

to charge ratios of 415/295 (puerarin) and 469/409 (glycyrrhetinic acid). The chromatograms were essentially free from endogenous interference (Figure 4). The limit of detection was estimated to be 0.16 µg/L, with a signal-to-noise ratio of 5. Calibration curves were constructed by the analysis of puerarin at 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/L, with good correlation coefficients (R^2 > 0.99) for all matrices.

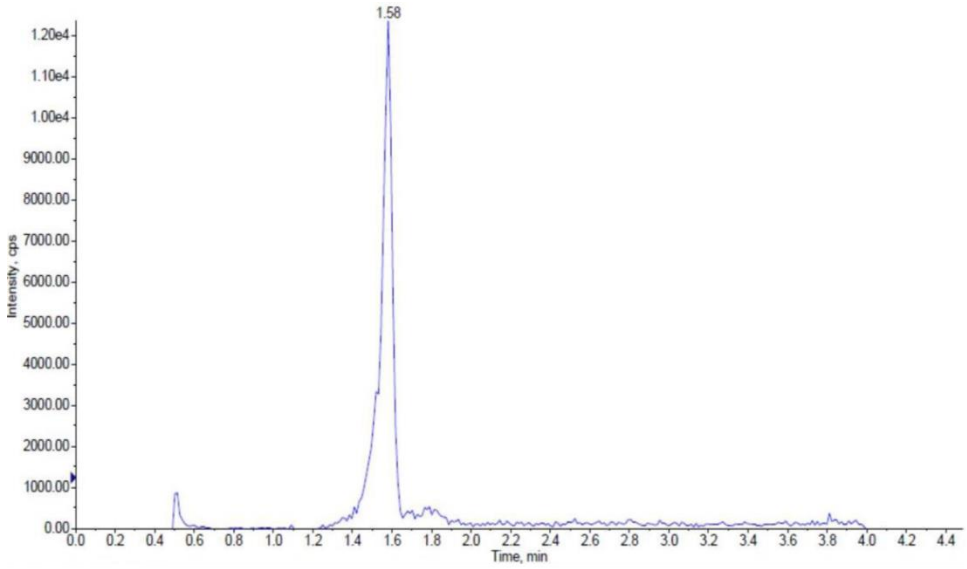

Figure 4 LC-MS/MS chromatogram of 100 µg/L of puerarin spiked into plasma.

3.6 Data analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis using PK Solutions 2.0 software (Summit Research Services, USA). The acquired results are reported as mean ± S.D. with a significance level of $p<0.05$. Statistical analysis was performed by non-parametric tests using SPSS ver. 16 (SPSS, USA). The following pharmacokinetic parameters are reported; maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the plasma concentration-time curve from zero to the last observed time (AUC_{0-t}) or infinity ($AUC_{0-\infty}$), the volume of distribution (Vd), total clearance (CL), mean residence time

(MRT) and elimination half-life ($T_{1/2}$). The absolute oral bioavailability of puerarin was calculated as ($AUC_{po}/dose_{po}$) divided by ($AUC_{iv}/dose_{iv}$). The tissue-to-plasma ratio of puerarin was calculated from the tissue concentration divided by the plasma concentration in each rat at the same time point. The percentage recovery of puerarin was calculated by dividing the puerarin level found in urine or faeces by the administered dose.

CHAPTER IV
RESULTS

4.1 Animal tolerability

Female rats that received puerarin at 1 mg/kg IV or 5 or 10 mg/kg PO had normal physiological and morphological appearance pre-dose and 24 h post-dose (Table 2). No significant changes in body weight or water or food intake were recorded for all rats. Liver biochemical profiles showed no significant changes in aspartate transaminase (AST) and alanine transaminase (ALT) levels at pre-dose and 24 h post-dose in all rats, although slight decreases in AST and ALT were observed at 24 h post dose. In the kidney biochemical profiles, blood creatinine showed no significant changes from pre-dose to 24 h post-dose after puerarin was given IV or PO.

Table 2 Physical appearance and plasma biochemical profiles before (0 h) and 24 h after administration of puerarin at 1 mg/kg IV and 5 and 10 mg/kg PO.

Parameters	Reference range	Puerarin					
		1 mg/kg IV		5 mg/kg PO		10 mg/kg PO	
		0 h	24 h	0 h	24 h	0 h	24 h
Physical appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal
AST (U/L)	45.70 – 80.80 U/L	61.67 ± 9.36	55.83 ± 2.90	57.67 ± 5.10	56.00 ± 6.84	55.60 ± 7.96	57.40 ± 5.12
ALT (U/L)	17.50 – 30.20 U/L	27.00 ± 26.18	11.20 ± 16.22	26.33 ± 18.78	13.17 ± 17.40	25.00 ± 13.92	12.17 ± 9.76
Creatinine (mg/dL)	0.20 – 0.80 mg/dL	0.20 ± 0.00	0.20 ± 0.00	0.21 ± 0.02	0.21 ± 0.02	0.21 ± 0.10	0.21 ± 0.08

Data are presented as mean ± S.D. (n=4).

4.2 Plasma concentration-time profile and oral bioavailability

Mean plasma concentration-time profiles of puerarin after IV and PO administration in rats are shown in Figure 5. Pharmacokinetic parameters of puerarin from non-compartmental analysis are summarised in Table 3. Following IV administration, the concentration declined rapidly and puerarin was cleared from the systemic circulation within 4 h, with C_{max} 621.96 ± 170.72 µg/L, $AUC_{0-\infty}$ 292.23 ± 108.93 µg.h/L, Vd 1.16 ± 0.56 L/kg, CL 5.43 ± 2.66 L/h/kg, MRT 0.24 ± 0.06 h, and $T_{1/2}$ 0.21 ± 0.06 h. After PO administration at 5 and 10 mg/kg, the maximum concentration was reached within 1 h, and then puerarin decreased to below the detection limit at 4-8 h post-dosing. C_{max} ranged from 140-230 µg/L and $AUC_{0-\infty}$ was 110-210 µg.h/L. The $T_{1/2}$ of puerarin ranged from 0.86-0.88 h, with no significant difference between doses. The absolute oral bioavailability of puerarin was approximately 7% at doses of 5 and 10 mg/kg.

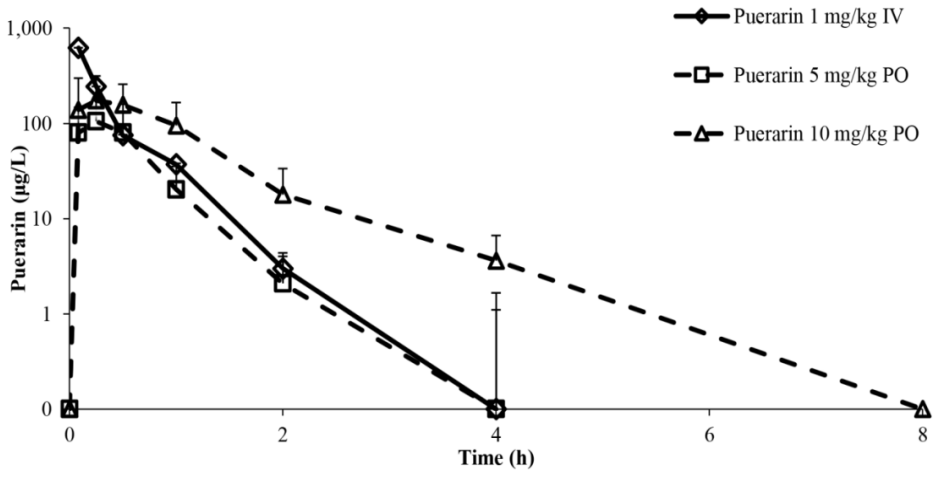

Figure 5 Plasma concentration-time profiles of puerarin after administration of puerarin at 1 mg/kg IV (◊), 5 mg/kg PO (□) and 10 mg/kg PO (Δ).
Data are shown as mean ± S.D. (n=4).

Table 3 Pharmacokinetic parameters of puerarin and puerarin glucuronide after administration of puerarin at 1 mg/kg IV and 5 and 10 mg/kg PO.

Parameters	**Puerarin**		
	1 mg/kg IV	**5 mg/kg PO**	**10 mg/kg PO**
Puerarin			
C_{max} (µg/L)	621.96 ± 170.72	145.47 ± 84.1	228.00 ± 164.84
T_{max} (h)	N/A	0.19 ± 0.09	0.33 ± 0.21
AUC_{0-t} (µg.h/L)	291.47 ± 108.05	109.45 ± 60.6	204.57 ± 157.47
$AUC_{0-\infty}$ (µg.h/L)	292.23 ± 108.93	109.58 ± 60.5	212.20 ± 157.50
Vd (L/kg)	1.16 ± 0.56	101.59 ± 119.5	90.17 ± 18.28
MRT (h)	0.24 ± 0.06	0.67 ± 0.21	0.88 ± 0.20
$T_{1/2}$ (h)	0.21 ± 0.06	0.88 ± 0.49	0.86 ± 0.56
CL (L/h/kg)	5.43 ± 2.66	65.06 ± 52.75	74.79 ± 0.17
Absolute bioavailability (%)	100	7.50	7.29
Puerarin glucuronide			
AUC_{0-t} (µg.h/L)	2,133.20	73.30	7,207.40
$AUC_{puerarin\ glucuronide}$ / $AUC_{puerarin}$	7.35	0.67	34.65

Data are presented as mean ± S.D. (n=4). C_{max}, maximum concentration; T_{max}, time to reach maximum concentration; AUC_{0-t}, area under concentration-time curve from time 0 to last observed time; $AUC_{0-\infty}$, area under concentration-time curve from time 0 to infinity; Vd, apparent volume of distribution; MRT, mean resident time; $T_{1/2,}$ half-life, CL; apparent clearance; N/A, not available.

4.3 Tissue distribution

Tissue-to-plasma ratios of puerarin in rats at 0.08, 1, 2 and 4 h after administration of puerarin at 1 mg/kg IV are shown in Figure 6. At 0.08 h, the highest levels of puerarin were found in the kidney, followed by the lung, stomach, liver, mammary gland and small intestine, all of which are highly perfused organs. The ratios in these organs were 10-100 at 0.08 h after IV dosing, and decreased to 1-10 at 1 h after dosing. At 2 h, puerarin was detected in most organs, including the hippocampus, heart, lung, stomach, liver, mammary gland, kidney, spleen and tibia, but not in the femur. The ratios in most organs continued to decrease up to 4 h, but there was an increase in poorly perfused tissue such as bone. Interestingly, the tissue-to-plasma ratios of puerarin in the hippocampus and brain increased significantly and continuously from 0.08 h to 1, 2 and 4 h after dosing.

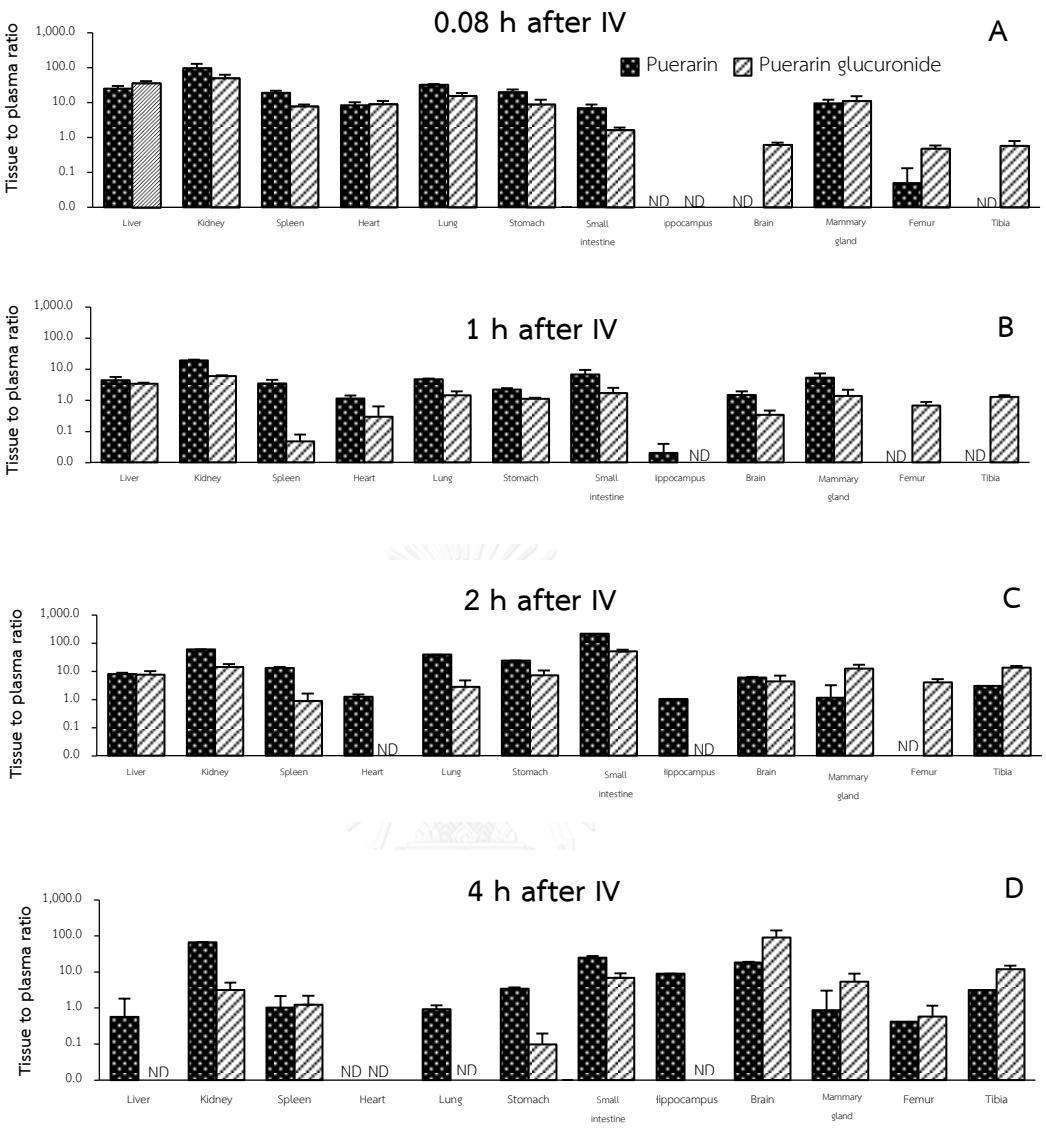

Figure 6 Tissue-to-plasma ratios of puerarin (black bars) and puerarin glucuronide (grey bars) in internal organs at 0.08 h (A), 1.0 h (B), 2.0 h (C) and 4.0 h (D) after administration of puerarin at 1 mg/kg IV.

Data are shown as mean ± S.D. (n = 4), ND = no detectable.

4.4 Metabolism

Mean plasma concentration-time profiles for puerarin glucuronide after administration of puerarin in rats are shown in Figure 7. The $AUC_{puerarin\ glucuronide}/AUC_{puerarin}$ ratio was about 7 after IV dosing. The concentration of puerarin glucuronide was higher than unchanged puerarin by about 30 times after administration of puerarin at 10 mg/kg PO. Regarding the tissue distribution, a high ratio of glucuronide metabolites was found in most tissues at 0.08 h after IV dosing (Figure 6). The tissue-to-plasma ratio of glucuronide metabolites decreased over time in most tissues, except for the brain and bone. However, the ratios of unchanged puerarin and puerarin glucuronide in the mammary gland remained at comparable levels for 4 h after dosing.

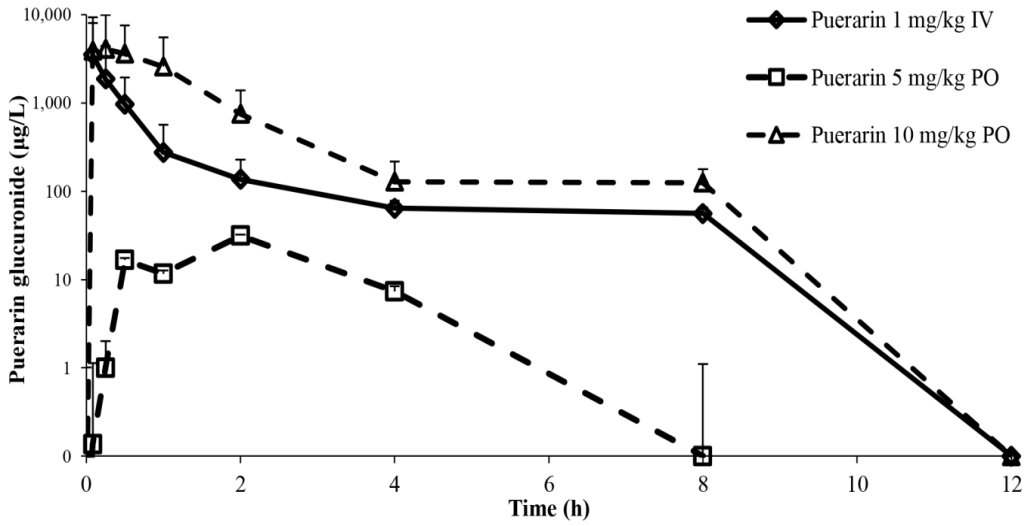

Figure 7 Plasma concentration-time profiles of puerarin glucuronide after administration of puerarin at 1 mg/kg IV (◊), 5 mg/kg PO (□) and 10 mg/kg PO (Δ). Data are shown as mean ± S.D. (n=4).

4.5 Excretion

The percentage recovery of unchanged puerarin and puerarin glucuronide in rat urine and feces after administration are shown in Table 4. Unchanged puerarin was present in urine and feces at a low level of less than 1% after PO and IV dosing. In the IV group, puerarin glucuronide was mainly excreted in urine in the first 24 h; this accounted to almost 50% of the administered dose. Approximately 15% of puerarin glucuronide was excreted in the feces within 72 h after IV dosing. At 10 mg/kg PO, approximately 10% of puerarin glucuronide was found in the urine in the 0-24 h period. A negligible amount of puerarin glucuronide was found in the feces from 0-72 h after PO dosing.

Table 4 Percent recovery of unchanged puerarin and puerarin glucuronide after administration of puerarin at 1 mg/kg IV and 5 and 10 mg/kg PO.

Recovery (%)	Puerarin		
	1 mg/kg IV	5 mg/kg PO	10 mg/kg PO
Puerarin			
Urine 0-24h	0.59 ± 5.70	0.08 ± 8.25	0.65 ± 8.47
Urine 24-48h	0.81 ± 1.15	0.14 ± 0.54	0.30 ± 3.75
Urine 48-72h	0.93 ± 2.45	0.06 ± 0.78	0.22 ± 0.44
Feces 0-24h	0.91 ± 9.25	0.09 ± 3.67	0.89 ± 1.57
Feces 24-48h	0.65 ± 0.55	0.04 ± 2.71	0.32 ± 0.15
Feces 48-72h	0.34 ± 2.03	0.04 ± 0.33	0.43 ± 0.01
Puerarin glucuronide			
Urine 0-24h	47.53 ± 51.37	0.56 ± 0.48	12.13 ± 13.01
Urine 24-48h	2.38 ± 0.97*	0.30 ± 3.05	0.08 ± 0.25
Urine 48-72h	1.89 ± 0.45*	0.22 ± 0.41	0.11 ± 0.44
Feces 0-24h	7.66 ± 2.51*	0.09 ± 1.57	1.94 ± 2.16
Feces 24-48h	3.55 ± 0.55*	0.23 ± 0.15	0.03 ± 3.70
Feces 48-72h	3.45 ± 0.80*	0.35 ± 0.02	0.04 ± 0.71

Data are presented as mean ± S.D. (n=4).

*$p<0.05$ among doses.

CHAPTER V
DISCUSSION AND CONCLUSION

5.1 Discussion

Puerarin has therapeutic effects on cardiovascular and cerebrovascular systems and bone [8]. Further understanding of these pharmacological activities requires information on the oral bioavailability and tissue distribution of puerarin. Therefore, this study was conducted to evaluate the ADME properties of puerarin at pharmacologically active sites in female rats. The rats could tolerate puerarin at 1 mg/kg IV and 5 and 10 mg/kg PO, which are pharmacologically active doses. All rats had a normal appearance without signs of toxicity after dosing. In addition, there were no significant changes in liver and kidney biomarkers within 24 h after dosing (Table 2). This suggests that puerarin is safe in rats in the pharmacologically active dose range, consistent with the findings in Chung et al. [36], in which male and female rats fed with puerarin for 28 days showed no toxicity at a dose up to 250 mg/kg per day.

The C_{max} of puerarin was reached within 0.08 h after PO dosing, and systemic clearance occurred until 8-12 h (Figure 5). Previous studies of PO administration of a puerarin suspension resulted in C_{max} at 0.45 to 5.00 h [12-16]. The wider range of T_{max} in those studies might be due to the use of a puerarin suspension formulation. The puerarin used in this study was prepared as a clear solution that was readily absorbed, resulting in a shorter T_{max} compared to that of the suspension. Biotransformation of puerarin via the formation of glucuronide conjugates was also clearly observed within the first time point after dosing for 0.08 h, indicating that the metabolism of puerarin through the glucuronidation pathway also occurs rapidly.

The amounts of plasma puerarin and glucuronide metabolites were investigated as a function of the oral dose to examine dose proportionality. The plasma concentration of puerarin propotionally increased from 5 to 10 mg/kg PO, but at higher doses of 20-100 mg/kg, the oral bioavailability of puerarin was found to be less than 1%. This may be because at the higher dose puerarin may be incompletely dissolved due to the precipitation *in vivo*. To maintain a consistent dose volume of 1 mL/kg, the concentration of puerarin was varied from 5-100 mg/mL in 20% DMSO in normal saline solution. Puerarin is in class IV of the Biopharmaceutical Classification System, indicating low solubility and low permeability, and is a weak acid that is ionised at high pH. Thus, it will have low solubility in the acid medium in the stomach of rats, which may cause precipitation [25]. Therefore, the lower bioavailability of puerarin at a higher oral dose may be strongly affected by the dissolution state of puerarin in GI fluid. Neither puerarin nor puerarin glucuronide was found in plasma, tissues, and urine after oral dosing at 20-100 mg/kg.

This is the first study on the tissue distribution of puerarin and puerarin glucuronide in the hippocampus, femur, tibia and mammary gland (Figure 4). Puerarin was widely distributed in several organs, and especially those with high porosity, consistent with its pharmacodynamic activities in these organs [18]. In addition, the volume of distribution of puerarin after IV dosing was approximately 1.16 L/kg, which implies a good tissue distribution. This correlates well with the physiochemical properties of puerarin including high lipophilicity (XlogP 1.95) and low water solubility (0.46 mg/mL). The tissue distribution in this study showed that puerarin at 1 mg/kg IV reached appropriate levels for pharmacodynamic activities in the hippocampus, femur, tibia and mammary gland related to the prevention of and therapy for neurodegenerative diseases osteoporosis and breast cancer [3, 8-10].

Glucuronide metabolites of puerarin were also detected in most tissues. The $AUC_{puerarin\ glucuronide}/AUC_{puerarin}$ ratios were 7 after puerarin IV dosing and 30 after puerarin at 10 mg/kg PO (Table 2). Glucuronide metabolites found in biological samples after puerarin administration are formed by UDP-glucuronosyltransferases [17], and puerarin was biotransformed to a glucuronide within 0.08 h in plasma (Figure 5). These results indicate that glucuronidation occurs rapidly and that most puerarin is converted into glucuronides. This first pass metabolism could reduce the amount of puerarin in the systemic circulation and may account for the relatively low oral bioavailability of puerarin.

The percentage of unchanged puerarin in urine and feces was less than 1% over 72 h after IV or PO dosing, indicating that puerarin is biotransformed before excretion via the bile or urine. Puerarin glucuronide in urine and feces was measured by using enzymatic hydrolysis of glucuronidase under optimised conditions. Puerarin was mainly excreted in the form of puerarin glucuronide, and approximately 50% of the administered dose of 1 mg/kg IV was detected in the urine as glucuronide metabolites during the first 24 h. After PO dosing, 1-10% of the administered dose was excreted in urine as the glucuronide conjugates. The difference in the percentage recovery between the different routes of administration (IV or PO) might be explained by distinct metabolic pathways between gut metabolism and systemic metabolism. Prasain et al. [18] reported that puerarin can be hydrolysed to daidzein by microbials in the GI tract, and then reduced to dihydrodaidzein and equol after oral dosing. In this study, daidzein and equol were also detected as minor metabolites in urine and feces at 72 h after dosing. However, the levels of daidzein and equol in urine and faeces accounted for only 1-2% of the administered dose.

5.2 Conclusion and recommendation

In conclusion, this study showed that puerarin has an oral bioavailability of approximately 7%. Puerarin was widely distributed to several organs related to diseases of aging, including the hippocampus, femur, tibia, and mammary gland. Glucuronides were the major metabolites of puerarin and were mainly excreted in the urine.

These results are beneficial development of puerarin and *Pueraria candollei* var. *mirifica* as phytopharmaceutical products for the prevention and treatment of the diseases of aging. The pharmacokinetics parameters data including absolute oral bioavailability, tissue distribution, metabolism and excretion of puerarin in female rats serve as guidance for further comparative pharmacokinetic study of pure puerarin and puerarin in standardised extract of *Pueraria candollei* var. *mirifica.*

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APPENDIX

APPENDIX A

Animal care and use protocol

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☑ Original ☐ Renew
Animal Use Protocol No. 15-33-001	**Approval No. 15 33 001**

Protocol Title
Pharmacokinetic Study of Puerarin from *Pueraria mirifica* Extracts in Rats

Principal Investigator

Phisit KHEMAWOOT, PhD

Certification of Institutional Animal Care and Use Committee (IACUC)

This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.

Date of Approval	Date of Expiration
April 22, 2015	April 21, 2017

Applicant Faculty/Institution
Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Road., Pathumwan
BKK THAILAND. 10330

Signature of Chairperson	Signature of Authorized Official
Name and Title **THONGCHAI SOOKSAWATE, Ph.D.** **Chairman**	Name and Title PORNCHAI ROJSITTHISAK, **Ph.D.** **Associate Dean (Research and Academic Service)**

The official signing above certifies that the information provided on this form is correct. The institution assumes that investigators will take responsibility, and follow university regulations and policies for the care and use of animals.

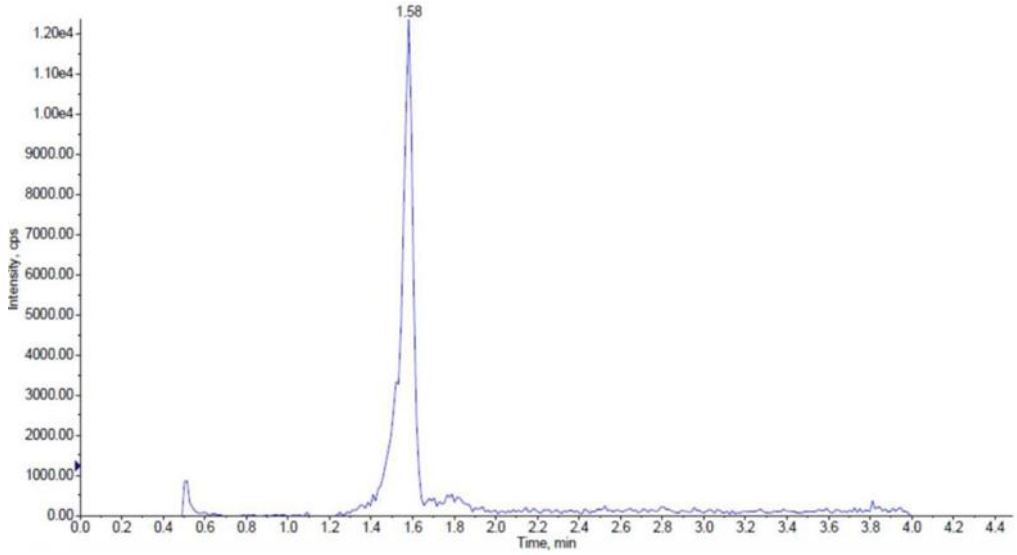
This approval is subjected to assurance given in the animal use protocol and may be required for future investigations and reviews.

APPENDIX B

LC-MS/MS chromatograms and conditions

LC-MS/MS chromatograms for puerarin

(a) 100 µg/L of puerarin spiked into plasma



Mass spectrometry parameters of standard chemicals used for measurement in biological samples.

MS Parameters	Puerarin	Glycyrrhetinic acid
Parent ion (m/z)	415.30	469.30
Daughter ion (m/z)	295.00	409.20
Declustering potential (Volt)	-60.00	-226.20
Entrance potential (Volt)	-5.00	-7.70
Collision energy (Volt)	-30.00	-59.36
Collision exit potential (Volt)	-36.00	-15.01

APPENDIX C

Tissue distribution

Table 1C Tissue-to-plasma ratios of puerarin and puerarin glucuronide in internal organs at 0.08 h after administration of puerarin at 1 mg/kg IV.

Organs	Compounds	
	Puerarin	Puerarin glucuronide
Liver	25.33 ± 8.76	36.07 ± 10.00
Kidney	98.76 ± 6.17	50.32 ± 2.46
Spleen	19.09 ± 5.48	7.73 ± 2.27
Heart	8.39 ± 3.70	9.08 ± 3.90
Lung	32.51 ± 3.01	15.64 ± 6.31
Stomach	19.96 ± 7.82	8.76 ± 6.81
Small intestine	6.91 ± 3.81	1.61 ± 0.60
Hippocampus	ND	ND
Brain	ND	0.61 ± 0.21
Mammary gland	9.53 ± 5.33	11.08 ± 8.13
Femur	0.05 ± 0.16	0.48 ± 0.23
Tibia	ND	0.58 ± 0.43

Data are shown as mean ± S.D. (n = 4), ND = no detectable.

Table 2C Tissue-to-plasma ratios of puerarin and puerarin glucuronide in internal organs at 1 h after administration of puerarin at 1 mg/kg IV.

Organs	Compounds	
	Puerarin	Puerarin glucuronide
Liver	4.41 ± 2.51	3.36 ± 0.73
Kidney	19.12 ± 2.55	5.97 ± 1.05
Spleen	3.47 ± 2.28	0.05 ± 0.07
Heart	1.15 ± 0.58	0.30 ± 0.70
Lung	4.77 ± 0.53	1.46 ± 1.02
Stomach	2.21 ± 0.59	1.12 ± 0.22
Small intestine	6.78 ± 5.74	1.72 ± 1.68
Hippocampus	0.02 ± 0.04	ND
Brain	1.47 ± 0.96	0.34 ± 0.26
Mammary gland	5.33 ± 4.27	1.39 ± 1.64
Femur	ND	0.68 ± 0.45
Tibia	ND	1.28 ± 0.37

Data are shown as mean ± S.D. (n = 4), ND = no detectable.

Table 3C Tissue-to-plasma ratios of puerarin and puerarin glucuronide in internal organs at 2 h after administration of puerarin at 1 mg/kg IV.

Organs	Compounds	
	Puerarin	Puerarin glucuronide
Liver	7.84 ± 5.64	7.56 ± 5.69
Kidney	60.27 ± 15.65	13.95 ± 8.51
Spleen	13.20 ± 3.72	0.88 ± 1.50
Heart	1.24 ± 1.35	ND
Lung	39.24 ± 31.35	2.82 ± 4.05
Stomach	23.93 ± 10.44	7.07 ± 7.79
Small intestine	213.395 ± 19.16	51.27 ± 37.37
Hippocampus	1.02 ± 0.84	ND
Brain	5.95 ± 1.71	4.38 ± 5.48
Mammary gland	1.14 ± 1.17	12.29 ± 10.36
Femur	ND	4.01 ± 2.81
Tibia	2.99 ± 3.46	13.52 ± 4.32

Data are shown as mean ± S.D. (n = 4), ND = no detectable.

Table 4C Tissue-to-plasma ratios of puerarin and puerarin glucuronide in internal organs at 4 h after administration of puerarin at 1 mg/kg IV.

Organs	Compounds	
	Puerarin	Puerarin glucuronidase
Liver	0.57 ± 1.13	ND
Kidney	66.19 ± 55.22	3.19 ± 3.68
Spleen	1.02 ± 1.51	1.25 ± 1.86
Heart	ND	ND
Lung	0.94 ± 1.25	ND
Stomach	3.41 ± 15.85	0.10 ± 0.20
Small intestine	25.01 ± 15.85	6.90 ± 4.62
Hippocampus	8.91 ± 7.67	ND
Brain	18.55 ± 21.18	90.31 ± 10.52
Mammary gland	0.88 ± 0.76	5.36 ± 7.31
Femur	0.42 ± 0.84	0.58 ± 1.16
Tibia	3.18 ± 3.71	11.99 ± 6.20

Data are shown as mean ± S.D. (n = 4), ND = no detectable.

VITA

Miss Pilaslak Poo was born on December 3, 1987 in Nakorn Sri Thammarat, Thailand. She graduted a Bachelor of Science in Pharmacy since 2011 from Khonkean University, Khonkean, Thailand. Currently, She works as hospital pharmacist at Samut Prakan hospital, Samut Prakan, Thailand.