

ผลของสารสกัดมาตรฐานบัวบก (ECa 233) ต่อเอนไซม์ไซโตโครมพี 450 ของมนุษย์และเอนไซม์ที่เกี่ยวข้องกับ
เมแทบอลิซึมของยาในเฟส 2 ในหนูแรท

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EFFECTS OF THE STANDARDIZED EXTRACT OF *CENTELLA ASIATICA*
(ECa 233) ON HUMAN CYTOCHROME P450 ENZYMES AND PHASE II DRUG
METABOLIZING ENZYMES IN RATS

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พิจณาการศึกษา : ผลของสารสกัดมาตรฐานบัวบก (Eca 233) ต่อเอนไซม์ไซโตโครมพี 450 ของมนุษย์และเอนไซม์ที่เกี่ยวข้องกับเมแทบอลิซึมของยาในเฟส 2 ในหนูแรท (EFFECTS OF THE STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* (Eca 233) ON HUMAN CYTOCHROME P450 ENZYMES AND PHASE II DRUG METABOLIZING ENZYMES IN RATS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. พ.ต.ท.หญิงสมทรง ลาวัณย์ประเสริฐ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. นवलศรี นีวัตศิษฎ์, ผศ. ดร. วราภรณ์ วารีน้อยเจริญ, 137 หน้า.

สารสกัดมาตรฐานบัวบก (Eca 233) มีฤทธิ์ในการกระตุ้นการเรียนรู้และความจำ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดนี้ต่อเอนไซม์ไซโตโครม พี450 (CYP) ในตับของมนุษย์โดยใช้รีคอมบิแนนท์เอนไซม์ไซโตโครม พี450 ของมนุษย์ในการศึกษาแบบนอกกาย รวมทั้งศึกษาผลของสารสกัดนี้ต่อเอนไซม์ที่เกี่ยวข้องกับการเมแทบอลิซึมของยาในเฟส 2 ซึ่งได้แก่ UDP-glucuronosyltransferase (UDPGT), sulfotransferase (SULT), glutathione S-transferase (GST), and NAD(P)H quinone oxidoreductase (NQOR) แบบในกายของหนูแรท ผลการศึกษาแบบนอกกาย พบว่า Eca 233 มีฤทธิ์ยับยั้งสมรรถนะของเอนไซม์ CYP3A4 ($IC_{50} = 210.98 \mu\text{g/ml}$), CYP2C19 ($IC_{50} = 365.18 \mu\text{g/ml}$) และ CYP2B6 ($IC_{50} = 871.14 \mu\text{g/ml}$) สารสกัดนี้ไม่มีผลต่อเอนไซม์ CYP1A2 และ CYP2D6 ($IC_{50} > 1,000 \mu\text{g/ml}$) รวมทั้งเอนไซม์ CYP2C9 ($IC_{50} > 750 \mu\text{g/ml}$) Eca 233 ไม่มีผลยับยั้ง CYP2E1 ที่ความเข้มข้นสูงถึง $250 \mu\text{g/ml}$ ผลการศึกษานี้ให้ข้อมูลว่า Eca 233 มีโอกาสในการเกิดอันตรกิริยาระหว่างยาเมื่อได้รับสารสกัดนี้พร้อมกับยาอื่น ๆ ที่ถูกเปลี่ยนแปลงด้วยเอนไซม์ CYP3A4, CYP2C19 และ CYP2B6 แต่จะมีผลทำให้เกิดอันตรกิริยาระหว่างยาอย่างมีนัยสำคัญทางคลินิกหรือไม่ ควรมีการศึกษาต่อไป การศึกษาผลของ Eca 233 ต่อเอนไซม์ที่เกี่ยวข้องกับการเมแทบอลิซึมของยาในเฟส 2 จากการป้อนสารสกัด ขนาด 10, 100 และ 1,000 มก/กก/วัน ทางปากเป็นเวลา 90 วัน ในหนูแรทสายพันธุ์วิสตาร์ทั้งเพศผู้และเพศเมีย ผลการทดลองพบว่าสารสกัดนี้ทุกขนาดความเข้มข้น ไม่มีผลต่อสมรรถนะของเอนไซม์ UDPGT, GST และ NQOR ทั้งในหนูเพศผู้และหนูเพศเมียเมื่อเทียบกับกลุ่มควบคุม แต่มีผลยับยั้งสมรรถนะของเอนไซม์ SULT ในหนูแรทเพศผู้โดยไม่มีผลในหนูเพศเมียเมื่อเทียบกับกลุ่มควบคุม ผลการศึกษานี้เป็นข้อมูลของ Eca 233 ต่อเอนไซม์ที่เกี่ยวข้องกับเมแทบอลิซึมของยาในเฟส 2 ในสัตว์ทดลอง การแปลผลไปยังมนุษย์ต้องมีการศึกษาเพิ่มเติม

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PITCHAYAPA SEEKA: EFFECTS OF THE STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* (ECa 233) ON HUMAN CYTOCHROME P450 ENZYMES AND PHASE II DRUG METABOLIZING ENZYMES IN RATS. THESIS PRINCIPAL ADVISOR : ASSOC. PROF. POL. LT. COL SOMSONG LAWANPRASERT Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. NUANSRI NIWATTISAIWONG, ASST. PROF. WARANGKANA WARISNOICHAROEN Ph.D., 137 pp.

The standardized extract of *C. asiatica* (ECa 233) was shown to exhibit learning and memory enhancing effects. The purpose of this study was to investigate effects ECa 233 on major human drug-metabolizing CYPs by using recombinant human CYPs in the *in vitro* study. Effects of this extract on phase II drug metabolizing enzymes including UDP-glucuronosyltransferase (UDPGT), sulfotransferase (SULT), glutathione *S*-transferase (GST) and NAD(P)H quinone oxidoreductase (NQOR) were also studied *in vivo* in rats. The results of the *in vitro* study showed that ECa 233 inhibited CYP3A4 ($IC_{50} = 210.98 \mu\text{g/ml}$), CYP2C19 ($IC_{50} = 365.18 \mu\text{g/ml}$) and CYP2B6 ($IC_{50} = 871.14 \mu\text{g/ml}$). ECa 233 did not affect CYP1A2 and CYP2D6 ($IC_{50} > 1,000 \mu\text{g/ml}$), and CYP2C9 ($IC_{50} > 750 \mu\text{g/ml}$). No inhibition of ECa 233 on CYP2E1 was observed at the concentration up to 250 $\mu\text{g/ml}$. This finding provided a preliminary information that ECa 233 possessed a potential effect to have an interaction with other medicines that were metabolized by CYP3A4, CYP2C19 and CYP2B6. Whether or not, this effect was clinically significant need to be further studied. To investigate effects of ECa 233 on the activities of phase II drug metabolizing enzymes. All doses (10, 100 and 1,000 mg/kg/day) of ECa 233 were orally given to both male and female Wistar rats for 90 days. The results showed that all dosage regimens of ECa 233 did not affect the activities of UDPGT, GST and NQOR as compared to the control group in both male and female rats. The activity of SULT was significantly decreased in male rats treated with ECa 233 at all doses as compared to the control group, whereas this enzyme was not changed in female rats. This study provided the information of effects of ECa 233 on phase II drug-metabolizing enzymes in animals, thus interpretation of these data from animal to human should be further studied.

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LIST OF ABBREVIATIONS

β	beta
$^{\circ}\text{C}$	degree celcius
μg	microgram
μl	microlitre
μmol	micromole
μM	micromolar
α	alpha
A	Alpha
APS	adenosine-5'-phosphosulfate
ATP	adenosine triphosphate
BROD	benzyloxyresorufin <i>o</i> -dealkylation
BHA	1,4-dialkylphenol
BHT	butylated hydroxytoluene
BOMCC	7-benzyloxymethyloxy-3-cyanocoumarian
BSA	bovine serum albumin
CDNB	1-chloro-2,4-dinitrobenzene
CHC	3-cyano-7-hydroxycumarin
cm	centimeter
CYP	cytochrome P450
DCNB	1,2-dichloro-4-nitrobenzene
DCPIP	2,6-dichlorophenol-indophenol
DHEA	dehydroepiandrosterone
DMSO	dimethylsulfoxide
DMXAA	dimethylxanthenone-4-acetic acid
DNA	deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	ethylenediaminetetraacetic acid
EOMCC	ethyloxymethyloxy-3-cyanocoumarian
e.g.	example gratia

et al.	et alii
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
FXR	farnesoid-X-receptor
g	gram
g	gravity
G6P	glucose 6-phosphate
G6PD	glucose 6-phosphate dehydrogenase
GSH	glutathione reduced form
GST	glutathione <i>S</i> -transferase
GST-P	GST <i>pi</i>
IC ₅₀	median inhibitory concentration
i.e.	id est (that is)
i.p.	intraperitonium
kg	kilogram
L	litre
LTB ₄	leukotriene B ₄
M	molar
M	Mu
mEq	milliequivalent
MFO	mixed-function oxidase
min	minute
mg	milligram
mg/kg	milligram per kilogram body weight
ml	millilitre
mm	millimeter
mM	millimolar
mmole	millimole
MW	molecular weight
NADP	nicotinamide adenine dinucleotide phosphate

NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NBC	<i>p</i> -nitrobenzylchloride
NNK	4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone
NO	nitric oxide
nm	nanometer
nmol	nanomole
NQO1	NAD(P)H quinine oxidoreductase 1
NQO2	NAD(P)H quinine oxidoreductase 2
NQO3	NAD(P)H quinine oxidoreductase 3
NQO4	NAD(P)H quinine oxidoreductase 4
NQOR	NAD(P)H quinine oxidoreductase
P	Pi
<i>p</i>	para
PAH	polycyclic aromatic hydrocarbon
PAPS	3'-phosphoadenosine-5'-phosphosulfate
PAPSS	PAPS synthetase
pH	potential of hydrogen
p.p.	post partum
PROD	pentoxyresorufin <i>o</i> -dealkylation
PUFA	polyunsaturated fatty acid
R ²	correlation coefficient
RFU	relative fluorescence unit or fluorescent intensity
S	Sigma
SEM	standard error of the mean
SSRI	selective serotonin reuptake inhibitor
SULT	Sulfotranferase
T	Theta
TCA	trichloroacetic acid
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
THA	2,4,6-trihydroacetophenone

TNF- α	tumor necrosis factor- α
Tris	tris (hydroxymethyl) aminomethane
U	unit
UDPGA	UDP-glucuronic acid
UDPGT	UDP-glucuronosyltransferase
viz.	videlicet
vs	versus
v/v	volume by volume
w/v	weight by volume
w/w	weight by weight