

ผลกึ่งเฉียบพลันของสารสกัดผลยอดต่อเอนไซม์ไซโตโครม พี450 ในตับ และค่าเคมีคลินิก
ในเลือดของหนูขาว



นางสาว อารมณ์ เจริญพิริยะะ

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SUBACUTE EFFECTS OF *MORINDA CITRIFOLIA* FRUIT EXTRACT ON HEPATIC
CYTOCHROME P450 AND CLINICAL BLOOD CHEMISTRY IN RATS

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ยอมีชื่อเรียกทางวิทยาศาสตร์ว่า *Morinda citrifolia* Linn. เป็นสมุนไพรที่มีรายงานว่ามีสรรพคุณในการรักษาโรคหลายชนิด การศึกษานี้มุ่งศึกษาถึงผลกึ่งเฉียบพลันของสารสกัดผลยอดต่อการทำงานของเอนไซม์ไซโตโครมพี 450 (cytochrome P450, CYP) ที่เกี่ยวข้องกับการกระตุ้นฤทธิ์ของสารก่อมะเร็ง/สารก่อการกลายพันธุ์ได้แก่ CYP1A1, CYP1A2, CYP2B1/2B2, CYP2E1 และ CYP3A นอกจากนี้ยังได้ศึกษาผลของสารสกัดนี้ต่อค่าเคมีคลินิกและโลหิตวิทยาด้วย การทดลองใช้หนูขาวเพศผู้พันธุ์สตาร์ โดยแบ่งหนูขาวแบบสุ่มเป็น 3 กลุ่ม กลุ่มละ 10 ตัว กลุ่มแรกเป็นกลุ่มควบคุมที่ได้รับน้ำกลั่น ขนาด 1 มิลลิลิตร/กิโลกรัม/วัน เป็นเวลา 30 วัน กลุ่มที่สองและสามเป็นกลุ่มที่ได้รับสารสกัดผลยอดความเข้มข้น 600 และ 1200 มิลลิกรัม/กิโลกรัม/วัน เป็นเวลา 30 วัน ตามลำดับ ระหว่างทำการทดลองบันทึกค่าน้ำหนักตัว ปริมาณอาหารและปริมาณน้ำที่หนูขาวดื่มทุก 5 วัน เมื่อครบระยะเวลาทำให้หนูหมดความรู้สึก เก็บตัวอย่างเลือดจากหัวใจเพื่อตรวจค่าโลหิตวิทยา และแยกซีรัมเพื่อตรวจค่าเคมีคลินิก นำตับมาเตรียมไมโครโซม เพื่อใช้ตรวจวิเคราะห์เอนไซม์ ผลการทดลองพบว่าสารสกัดผลยอดไม่มีผลต่อน้ำหนักตัว ปริมาณอาหาร และปริมาณน้ำดื่มของหนูขาว สารสกัดผลยอดขนาด 1200 มิลลิกรัม/กิโลกรัม/วัน มีผลลดสมรรถนะของ CYP1A1 อย่างมีนัยสำคัญแต่ไม่มีผลเมื่อให้ในขนาด 600 มิลลิกรัม/กิโลกรัม/วัน สารสกัดผลยอดทั้งสองขนาดไม่มีผลต่อสมรรถนะของ CYP1A2, CYP2B1/2B2, CYP2E1 และ CYP3A การที่สารสกัดผลยอดมีผลยับยั้ง CYP1A1 อาจมีส่วนใช้อธิบายผลของสารสกัดนี้ต่อการยับยั้งการก่อการกลายพันธุ์/สารก่อมะเร็งที่เหนี่ยวนำโดยสารเคมี สำหรับค่าเคมีคลินิกพบว่าสารสกัดผลยอดทั้งสองขนาดไม่มีผลต่อค่าเคมีคลินิกและโลหิตวิทยาต่าง ๆ ต่อไปนี้คือ SGOT, SGPT, ALP, total bilirubin, direct bilirubin, BUN, SCr, total cholesterol, TG, HDL-C, glucose, sodium, potassium, chloride, hemoglobin, hematocrit, platelet count, WBC count, % differential WBCs และ RBC morphology ผลจากการทดลองนี้แสดงให้เห็นว่าสารสกัดผลยอดไม่มีผลเปลี่ยนแปลงสมรรถนะของเอนไซม์ส่วนใหญ่ในเฟสหนึ่งที่มีบทบาทสำคัญในการกระตุ้นฤทธิ์ ยกเว้น CYP1A1 เมื่อให้สารสกัดในขนาดสูง นอกจากนี้พบว่า สารสกัดผลยอดไม่มีผลต่อการทำงานของอวัยวะหรือระบบของร่างกายที่สำคัญหลายอย่าง เช่น ตับ ไต ระบบเลือด อิเล็กโทรไลต์ รวมทั้งเมแทบอลิซึมของไขมันและคาร์โบไฮเดรต ควรมีการศึกษาเพิ่มเติมต่อไปถึงผลของสารสกัดผลยอดต่อการยับยั้ง CYP1A1

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APORN CHAROENPIRIYA: SUBACUTE EFFECTS OF *MORINDA CITRIFOLIA* FRUIT EXTRACT ON
HEPATIC CYTOCHROME P450 AND CLINICAL BLOOD CHEMISTRY IN RATS. THESIS ADVISOR:
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Morinda citrifolia Linn., called in Thai as "Yor" has been reported to have a broad range of therapeutic effects. This study examined subacute effects of *M. citrifolia* fruit extract on phase I hepatic cytochrome P450 (CYP) involving carcinogenic/mutagenic bioactivation such as CYP1A1, CYP1A2, CYP2B1/2B2, CYP2E1 and CYP3A in rats. Effects of this compound on clinical blood chemistry and hematology were also determined. Thirty male Wistar rats were randomly divided into 3 treatment groups. Each group comprised 10 rats. Rats in the first group were given distilled water 1 ml/kg/day orally for 30 days, serving as a control group. The other two groups of rats were given *M. citrifolia* fruit extract orally at dosages of 600 and 1200 mg/kg/day for 30 days. During the treatment period, body weight, food consumption and volume of drinking water were recorded at every five days. At the end of the treatment period, rats were anesthetized. Blood was collected by heart puncture and serum was prepared for measuring hematology and clinical blood chemistry, respectively. Microsomes were prepared from livers for enzyme assays. The results showed that there were no significant differences between control and treatment groups on body weight, food & water consumption. *M. citrifolia* significantly decreased CYP1A1 activity at a dosage of 1200 mg/kg/day but not at a dosage of 600 mg/kg/day. No changes of CYP1A2, CYP2B1/2B2, CYP2E1 and CYP3A activities were observed following both doses of the extract. The inhibitory effect of *M. citrifolia* fruit extract at 1200 mg/kg/day on CYP1A1 may partly explain its antimutagenic/anticarcinogenic effects of this plant on chemical-induced mutagenesis/carcinogenesis previously reported by other investigators. For clinical blood chemistry, rats received both dosage regimens of *M. citrifolia* demonstrated no changes of the following clinical blood chemistry and hematology: SGOT, SGPT, ALP, total bilirubin, direct bilirubin, BUN, SCr, total cholesterol, TG, HDL-C, glucose, sodium, potassium, chloride, hemoglobin, hematocrit, platelet count, WBC count, % differential WBCs and RBC morphology. This result illustrated that *M. citrifolia* fruit extract did not modulate activities of most of the phase I bioactivating enzymes except for CYP1A1 following high dose of the extract. In addition, no effect of this extract was shown on several important organs/systems such as liver, kidney, blood system, electrolytes as well as lipid and carbohydrate metabolism. Further studies to clarify the inhibition effects of *M. citrifolia* fruit extract on CYP1A1 were suggested.

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LIST OF ABBREVIATIONS

Adria	= adriamycin
ALP	= alkaline phosphatase
ANH	= aniline 4-hydroxylase
ANOVA	= a one way analysis of variance
AP-1	= transcription activator protein 1
B(a)P	= benzo(a)pyrene
BR	= benzyloxyresorufin
BROD	= benzyloxyresorufin O-dealkylase
BSA	= bovine serum albumin
BUN	= blood urea nitrogen
BW	= body weight
CDDP	= cisplatin
CD ₅₀	= median convulsant dose
cDNA	= complementary deoxyribonucleic acid
Cl-Ade	= 2-chloroadenosine
cm	= centimeter
CYP	= cytochrome P450
CYS-A	= cyclosporin A
dl	= deciliter
DMBA	= 7,12-dimethylbenz(a)anthracene
DMSO	= dimethyl sulfoxide
DNA	= deoxyribonucleic acid
ed	= editor
EDTA	= ethylenediaminetetraacetic acid
e.g.	= <i>exempli gratia</i>
EGF	= epidermal growth factor
ER	= ethoxyresorufin
EROD	= ethoxyresorufin O-dealkylase
<i>et al.</i>	= <i>et alii</i> (and other)

etc.	= and so on
EtOH-ppt	= Ethanol-precipitated fraction
EtOH-sol	= Ethanol-soluble fraction
5-FU	= 5-fluorouracil
g	= gram
<i>g</i>	= gravity
G6P	= glucose 6-phosphate
G6PD	= glucose 6-phosphate dehydrogenase
GI tract	= gastrointestinal tract
GS ⁻	= glutathione thiolate anion
GSP	= grape seed powder
GST	= glutathione S-transferase
Hb	= hemoglobin
Hct	= hematocrit
HDL-C	= high density lipoprotein cholesterol
IFN	= interferon
IL	= interleukin
ILS	= increase in life span
i.p.	= intraperitoneal
kg	= kilogram
L	= liter
LD ₅₀	= median lethal dose
LDL-C	= low density lipoprotein cholesterol
LLC	= lewis lung carcinoma
LPO	= lipidhydroperoxide
M	= molar (mole per liter)
mEq	= miliequivalent
MetOH-sol	= methanol-soluble fraction
MI	= metabolic intermediate
min	= minute
mg	= milligram

ml	= milliliter
mm	= millimeter
mM	= millimolar (millimole per liter)
mmol	= millimole
MR	= methoxyresorufin
MROD	= methoxyresorufin O-dealkylase
MST	= mean survival time
MTX	= methotrexate
MW	= molecular weight
NADP	= nicotinamide adenine dinucleotide phosphate
NADPH	= nicotinamide adenine dinucleotide phosphate (reduced form)
nm	= nanometer
nM	= nanomolar (nanomole per liter)
nmol	= nanomole
NO	= nitric oxide
PAH	= polycyclic aromatic hydrocarbon
pH	= potential of hydrogen
pmol	= picomole
PR	= pentoxyresorufin
PROD	= pentoxyresorufin O-dealkylase
PSA	= phenol-sulphuric acid
PYC	= pycnogenol
QD	= quaque die
QOD	= every day
RBC	= red blood cell
r.p.m.	= revolution per minute
SAR	= superoxide anion radicals
SCr	= serum creatinine
SD	= sprague dawley
SEM	= standard error of mean

sec	= second
SER	= smooth endoplasmic reticulum
SGOT	= serum glutamic oxaloacetic transaminase
SGPT	= serum glutamic pyruvic transaminase
spp.	= species
TCA	= trichloroacetic acid
TCDD	= 2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TG	= triglyceride
TNB	= tetrazolium nitroblue
TNF	= tumor necrosis factor
TPA	= 12- <i>O</i> -tetradecanoylphorbol-13-acetate
Tris	= Tris (hydroxymethyl) aminomethane
U	= unit
UDPGTs	= uridine diphosphoglucuronyltransferase
U.S. RDAs	= United States recommended dietary allowances
UV	= ultraviolet
VCR	= vincristine
vs.	= versus
v/v	= volume by volume
WBC	= white blood cell
w/v	= weight by volume
°C	= degree celsius
β	= beta
γ	= gamma
α	= alpha
μg	= microgram
μl	= microliter
μM	= micromolar (micromole per liter)