

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E42125

**EFFECTS OF CURCUMINOIDS ON ETHANOL-INDUCED TOXICITY IN
HEPG2 CELLS AND RATS**

RUTTIYA THONGRUNG

**A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Sciences Degree
in Pharmacology and Biomolecular Sciences (International Program)
March 2012
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This thesis entitled “Effects of curcuminoids on ethanol-induced toxicity in HepG2 cells and rats” submitted by Ruttiya Thongrung in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) is hereby approved.

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ACKNOWLEDGEMENT

First of all, I would like to express my sincere gratitude to my advisor, Assistant Professor Dr. Sakonwun Praputbut for her valuable guidances, continuous supports and encouragements throughout my graduate study at Naresuan University. Her kind advices and attentions to my course works and experimental works, without her, the works would never have been undertaken.

I gratefully thank to my co-advisor Assistant Professor Dr. Nanteetip Limpeanchob for her helps, suggestions and taking care of. I also would like to thank the members of my committees: Assistant Professor Dr. Kornkanok Ingkaninan, and Assistant Professor Dr. Rataya Luechapudiporn for their valuable comments and suggestions and also special thank Assistant Professor Dr. Julintorn Somran for her valuable suggest and provide the materials in the part of liver tissue histological examination.

I would like to acknowledge the Center of Excellence for Innovation in Chemistry (PERCH-CIC), The Graduate School Naresuan University, The Thailand Research Fund (TRF), Ministry of Education and Faculty of Pharmaceutical Sciences, Naresuan University for the financial support of this study.

I also thank my parents who have listened and encouraged me from the beginning to the end of this thesis and my laboratory and student members of the program for encouragement and valuable friendships.

Finally, I would like to thank the others, who inspired me but may not be named in this acknowledgement.

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Title	EFFECTS OF CURCUMINOIDS ON ETHANOL-INDUCED TOXICITY IN HEPG2 CELLS AND RATS
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Advisor	Assistant Professor Sakonwun Praputbut, Ph.D
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Academic paper	Thesis M.S. in Pharmacology and Biomolecular Sciences (International Program), Naresuan University, 2011
Keywords	Alcoholic liver disease, ethanol, hepatoprotective agent, curcuminoids

ABSTRACT

E 42125

Excessive alcohol consumptions cause alcoholic liver disease (ALD). It is one major disease leading to mortality. Nowadays, there is no approved medicine for ALD treatment. Alcohol metabolisms in hepatocytes increase oxidative stress; reactive oxygen species, nitric oxide and lipid peroxidation. Turmeric, a local herbal plant has been used in gastrointestinal disorders. Curcuminoids, a complex compounds derived from the turmeric extracts have shown many pharmacological effect including an antioxidant activities. This study was aimed to evaluate the hepatoprotective effects of curcuminoids in an animal model and HepG2 cell culture. Male Sprague-Dawley rats were fed with 6 g/kg/day for 60 days for chronic ethanol-induced liver toxicity. The ethanol-induced toxicity rats were treated with various doses of curcuminoids (250, 500, 750 mg/kg/day), for another 45 days. Curcuminoids (500 and 750 mg/kg/day) significantly decreased serum liver function enzymes; aspartate aminotransferase and alanine aminotransferase in hepatotoxicity rats. From the liver histological examination showed that curcuminoids attenuate fatty livers and inflammation lesions. In addition, curcuminoids significantly decreased lipid peroxidation, however there was no changes of the hepatic superoxide dismutase antioxidant enzyme in the liver microsomal extractions in this study .

HepG2 cells were used to examine the antioxidant property of curcuminoids. The results confirmed that ethanol increases cytotoxicity in dose and time dependent manners. Curcuminoids at various concentrations (0.156, 0.313, 0.625, 1.25, 2.5, 5

µg/ml) decreased cytotoxicity in the ethanol-stimulated HepG2 cells. Curcuminoids also attenuated lipid peroxidations production in ethanol-stimulated hepatic cells.

Furthermore, from the oral acute toxicity test, we has demonstrated the safety of curcuminoids in both normal and hepatotoxicity rats. Therefore, the results from our study suggested that curcuminoids has potential benefit to use as a hepatoprotective agent in ethanol-induced hepatic toxicity.

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ABBREVIATIONS

AP-1	=	Activation protein-1
ADH	=	Alcohol dehydrogenase
AH	=	Alcoholic hepatitis
Alc	=	5.5% alcohol
ALT	=	Alanine aminotransferase
ALD	=	Alcoholic liver disease
ALDH	=	Aldehyde dehydrogenase
ASH	=	Alcoholic steatohepatitis
ALP	=	Alkaline phosphatase
AST	=	Aspartate aminotransferase
BAC	=	Bicinchoninic acid
CCl ₄	=	Carbon tetrachloride
CD-14	=	Cluster of differentiation-14
CMC	=	Carboxymethyl cellulose
C _{max}	=	The maximum (or peak) concentration
COX-2	=	Cyclooxygenase 2
°C	=	Degree Celsius
DAF-2DA	=	4,5 Diaminofluorescein diacetate
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
DMEM/F12	=	Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham
DMSO	=	Dimethyl sulfoxide
EDTA	=	Ethylenediaminetetraacetic acid
ErbB2	=	Epithelial growth factor receptor
FBS	=	Fetal bovine serum
FDA		Food and drug administration
GGT	=	Glutamyl transferase
GR	=	Glutathione reductase
GSH	=	Glutathione

ABBREVIATIONS (CONT.)

GSSG	=	Oxidized glutathione
GPx	=	Glutathione peroxidase
g	=	Gram
H&E	=	Hematoxylin and eosin staining
HepG2	=	<i>Human liver hepatocellular carcinoma cell line</i>
HSC	=	Hepatic stellate cells
HHC	=	Hexahydrocurcumin
HPLC	=	High-performance liquid chromatography assay
h	=	Hours
H ₂ O ₂	=	Hydrogen peroxide
OH [•]	=	Hydroxyl radical
eNOS	=	Endothelial nitric oxide synthase
iNOS	=	Inducible nitric oxide synthase
IL-12	=	Interleukin-12
ICAM-1	=	Intracellular cell adhesion molecule
LDH	=	Lactate dehydrogenase
LPS	=	Lipopolysaccharide
LOX	=	Lipoxygenase
MCV	=	Mean corpuscular erythrocyte volume
MEOS	=	Microsomal ethanol oxidizing system
MDA	=	Malondialdehyde
MRP 1,2	=	Multidrug resistance protein 1,2
NALD	=	Non-alcoholic liver disease
NAFLD	=	Non-alcoholic fatty liver disease
NASH	=	Non-alcoholic steatohepatitis
NO	=	Nitric oxide
NF-κB	=	Nuclear factor kappa B
PBMC	=	Peripheral blood mononuclear
PC	=	Phosphatidylcholine
PPAR-γ	=	Peroxisome proliferator-activated receptors-γ

ABBREVIATIONS (CONT.)

NAD	=	Nicotinamide adenine dinucleotide
NADH	=	Nicotinamide adenine dinucleotide reduced form
NO ₂ ⁻	=	Nitrite
NO ₃ ⁻	=	Nitrate
ONOO ⁻	=	Peroxynitrite
PTX	=	Pentoxifylline
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
O ₂ ^{•-}	=	Superoxide anion radical
SAMe	=	S-adenosylmethionine
SOD	=	Superoxide dismutase
t-BOOH	=	t-butyle hydroperoxide
THC	=	Tetrahydrocurcumin
STAT	=	Signal transducer adhesion molecule-1
TBA	=	Thiobarbituric acid
TBARs	=	Thiobarbituric acid reactive substance
TCA	=	Trichloroacetic acid
t _{max}	=	Time to peak concentration
TNF-α	=	Tumor necrosis factor-α
VCAM-1	=	Vascular cell adhesion molecule-1
VSMC	=	Vascular smooth muscle cell
MTT assay	=	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay
cAMP	=	3', 5'-cyclic monophosphate
HNE	=	4-hydroxynonenal
IU/ml	=	International unit per milliliter
m	=	Meter
mg	=	Milligram
mg/ml	=	Milligram/milliliter

ABBREVIATIONS (CONT.)

min	=	Minute(s)
µg/ml	=	Microgram/milliliter
% v/v	=	The percentage volume by volume
w/v	=	Weight by volume