# 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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## ABSTRACT



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 Spraque-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-l-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 staning |
| HepG2 | = | *Human liver hepatocellular carcinoma cell line* |
| HSC | = | Hepatic stellate cells |
| HHC | = | Hexahydrocurcumin |
| HPLC | = | High-performance liquid chromatography assay |
| h | = | Hours |
| $H_2O_2$ | = | Hydrogen peroxide |
| $OH^-$ | = | Hydroxyl radical |
| eNOS | = | Endothelial nitric oxide syntase |
| iNOS | = | Inducible nitric oxide syntase |
| IL-12 | = | Interleukin-12 |
| ICAM-1 | = | Intracellular cell adhesion molecule |
| LDH | = | Lactase dehydrogenase |
| LPS | = | Lipopolysacaride |
| LOX | = | Lipoxygenase |
| MCV | = | Mean corpuscular erythrocyte volume |
| MEOS | = | Microsomal ethanol oxidizing system |
| MDA | = | Molondialdehyde |
| 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 | = | Phosphatidylcholin |
| PPAR-γ | = | Peroxisome proliferator-activated receptors-γ |

# ABBREVIATIONS (CONT.)

| | | |
|---|---|---|
| NAD | = | Nicotinamide adenine dinucleotide |
| NADH | = | Nicotinamide adenine dinucleotide reduced from |
| $NO_2^-$ | = | Nitrite |
| $NO_3^-$ | = | Nitrate |
| $ONOO^-$ | = | Peroxynitrite |
| PTX | = | Pentoxifylline |
| RNS | = | Reactive nitrogen species |
| ROS | = | Reactive oxygen species |
| $O_2^{\cdot -}$ | = | 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-alfa |
| VCAM-1 | = | Vascular cell adhesion molecule-1 |
| VSMC | = | Vascular smooth muscle cell |
| MTT assay | = | 3-2, 3 Adenine-diphenyl tetrazolium bromide assay |
| cAMP | = | 3', 5'-cyclic monophosphate |
| HNE | = | 4-hydroxynonenal |
| IU/ml | = | Internal unit per milliter |
| 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 |