

**A STUDY ON THE EFFECTS OF LAURIC ACID ON ROS
FORMATION AND CYP2E1 mRNA EXPRESSION IN ALCOHOL-
INDUCED HEPG2 CELLS**

By

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A project report submitted to the Department of Biomedical Science

Faulty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Biomedical Science

September 2017

ABSTRACT

A STUDY ON THE EFFECTS OF LAURIC ACID ON ROS FORMATION AND CYP2E1 mRNA EXPRESSION IN ALCOHOL- INDUCED HEPG2 CELLS

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Cytochrome P450 2E1 (CYP2E1) is primarily involved in the alcohol metabolism known as microsomal ethanol oxidising system. During this process, massive reactive oxygen species (ROS) is generated, ultimately leading to oxidative stress and tissue damage. In this present study, lauric acid was investigated as a potential antioxidant against ethanol-mediated oxidative stress by evaluating its effect on CYP2E1 mRNA expression and ROS formation in alcohol-induced HepG2 cells. The results showed lauric acid was able to downregulate CYP2E1 expression under normal condition, suggesting it could act as negative transcription regulator of CYP2E1. Besides that, lauric acid downregulated alcohol-induced CYP2E1 expression from 3.83-fold to 1.14-fold, 1.04-fold, 0.98-fold, in the presence of 5 μ M, 10 μ M and 20 μ M of lauric acid respectively. In addition, in the presence of 5 μ M and 20 μ M of lauric acid, alcohol-induced ROS formation was reduced significantly. In both

assay, the suppression was greater in increasing dose of lauric acid, therefore suggesting lauric acid may work in a dose-dependent manner. By comparing the suppressive effect of resveratrol, a bioactive compound which is a well known antioxidant for alcoholic liver disease in phase 3 clinical trial, this study has shown the promising effect of lauric acid in reducing CYP2E1 mRNA expression and ROS formation. Therefore lauric acid has potential hepatoprotective properties against alcohol-mediated oxidative damage.

ACKNOWLEDGEMENT

First and foremost, I would like to thank the Department of Biomedical Science, Universiti Tunku Abdul Rahman for providing me an opportunity to get exposed with research work. I am grateful that it was completed on time with the assistance and support of all. First and foremost, I would like to express my sincere gratitude to my supervisor, Dr Chew Choy Hoong for her guidance, advice and tolerance throughout the project.

I would also like to thank my senior postgraduate students, Mr. Wong Hong Kin, Mr. Tham Yong Yao and Ms. Cheong Hui Ting for guiding me when I encounter difficulty in the experiment. Not to forget, I would like to thank my senior FYPs, Mr. Ng Hin Fung and Ms. Khoo Yie Woon who have guided me even during their FYP period. Besides that, I would like to convey my gratitude to all the lab officers for their assistance and tolerance especially Mr Tie Shin Wei, Mr. Gee Siew Meng, and Mr Saravanan A/L Sivasangaran. Sincere thanks to my benchmate, Ms Lim Hui Li and my friends, especially Ms. Tan Chui Ying for giving me emotional support throughout this project.

Last but not least, I would like to thank my beloved parents and siblings for their love and support that have given me much strength to overcome the hardship.

DECLARATION

I hereby declare that the project report is based on my original work except quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

ONG WEI WAH

APPROVAL SHEET

This project report entitled “**A STUDY ON THE EFFECTS OF LAURIC ACID ON ROS FORMATION AND CYP2E1 mRNA EXPRESSION IN ALCOHOL-INDUCED HEPG2 CELLS**” was prepared by ONG WEI WAH and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **ONG WEI WAH** (ID No: **14ADB07709**) has completed this final year project entitled “**A STUDY ON THE EFFECTS OF LAURIC ACID ON ROS FORMATION AND CYP2E1 mRNA EXPRESSION IN ALCOHOL-INDUCED HEPG2 CELLS**” under the supervision of Dr. Chew Choy Hoong (Supervisor) from the Department of Biomedical Science, Faculty of Science.

I hereby give permission to University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(ONG WEI WAH)

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LIST OF ABBREVIATIONS

A ₂₆₀	Absorbance at 260 nm wavelength
A ₂₈₀	Absorbance at 280 nm wavelength
Akt-p38 MAPK	Mitogen-activated protein kinases
ALD	Alcoholic liver disease
ARE	Antioxidant responsive element
ATP	Adenosine triphosphate
B-oxidation	Beta oxidation
BCP	1-Bromo-3-Chloropropane
bp	Base pair
CO ₂	Carbon dioxide
COX-I	Cyclooxygenase-I
COX-II	Cyclooxygenase-II
CYP450	Cytochrome P450
CYP1	Cytochrome P450 family 1
CYP2	Cytochrome P450 family 2
CYP3	Cytochrome P450 family 3
CYP2A6	CYP2 superfamily A member 6
CYP2B6	CYP2 superfamily B member 6
CYP2C8	CYP2 superfamily C member 8
CYP2C9	CYP2 superfamily C member 9
CYP2C19	CYP2 superfamily C member 19
CYP2D6	CYP2 superfamily D member 6
CYP2E1	CYP2 superfamily E member 1
CYP2J2	CYP2 superfamily J member 2

-d(RFU)/dT	Rate of change of the relative fluorescence units with time
DCP	Diethylchlorophosphate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
et al.	“et alia” (Italia word referring to ‘and other’)
etc	“et cetera”(to mean "and other similar things")
FBS	Foetal bovine serum
<i>g</i>	Acceleration of gravity (approximately 9.8 m/s ²)
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GATA4	GATA Binding Protein 4
H ₂ O ₂	Hydrogen peroxide
HepG2	Human hepatocellular carcinoma cell line
HepaRG	Human hepatocellular cholangiocarcinoma cell line
IFN- γ	Interferon gamma
I κ B α	Inhibitor of nuclear kappa B alpha
IL-1 β	Interleukin-1beta
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
KO	Knockout
LPS	Lipopolysaccharides
MDA	Malondialdehyde
MCFA	Medium chain fatty acid
mRNA	Messenger ribonucleic acid
NAD ⁺	Nicotinamide adenine dinucleotide (oxidised form)

NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate
NF- κ B	Nuclear factor kappa B
nM	Nanomolar
NR5A2	Nuclear receptor subfamily 5 group A member 2
Nrf2	Hepatic nucleic factor 2
PBS	Phosphate buffered saline
PI3K	Phosphatidylinositide 3-kinase
PKC	Protein kinase C
PPAR γ	Peroxisome proliferator-activated receptor γ
qPCR	Quantitative polymerase chain reaction
RFU	Relative fluorescence units
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
SP1	Specificity protein 1
SYBR	Synergy Brands, Inc. (stock symbol)
TBE	Tris-Borate-EDTA
TLR-4	Toll-like receptor-4
TGF- β 1	Transforming growth factor-beta 1
T _m	Melting temperature
TNF- α	Tumour necrosis factor- α
tRNA	Transfer ribonucleic acid
v/v	Volume/ volume
w/v	Weight/ volume
μ M	Micromolar

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