INVESTIGATION INTO THE EFFECTS OF INTERLEUKIN-17 AND LAURIC ACID ON PPARγ EXPRESSION IN HUMAN HEPG2 CELLS

By

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ABSTRACT

INVESTIGATION INTO THE EFFECTS OF INTERLEUKIN-17 AND LAURIC ACID ON PPARγ EXPRESSION IN HUMAN HEPG2 CELLS

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Peroxisome proliferator-activated receptors gamma (PPARγ) is a transcription factor with pivotal role in the regulation of inflammatory response. Lauric acid is a 12-carbon saturated fatty acid and a major constituent in coconut oil that has demonstrated anti-inflammatory properties. Interleukin-17 (IL-17) is a pro-inflammatory cytokine that promotes inflammation. The objective of the study was to determine the effects of lauric acid on PPARγ gene expression in human HepG2 cells co-treated with IL-17. The HepG2 cells were treated with different concentrations of IL-17 and lauric acid for 24 hours. Total cellular RNA and protein were extracted from the treated HepG2 cells using Tri-Reagent® LS. The integrity and purity of RNA samples were assessed using 2% (v/v) bleach 1% (v/v) agarose gel electrophoresis and spectrophotometric measurement, respectively. Subsequently, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed to quantify the mRNA expression of PPARγ by normalising to the expression of the housekeeping gene, glyceraldehyde-3-

phosphate dehydrogenase (GAPDH). The concentrations of protein samples were measured using Bio-Rad DC protein assay and the protein was separated according to size using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to Western blot analysis. Both qRT-PCR and Western blot analysis showed that IL-17 suppressed PPARγ expression in dose-dependent manner, with 100 ng/mL IL-17 showed the strongest suppression on PPARγ gene expression. Subsequent co-treatment of IL-17 with increasing concentrations of lauric acid showed dose-dependent up-regulation of PPARγ expression in HepG2 cells. The PPARγ gene expression was significantly up-regulated in HepG2 cells treated with 20 μM lauric acid and 10 ng/mL IL-17. These findings suggest that lauric acid displayed anti-inflammatory properties and it is able to abolish the pro-inflammatory effect of IL-17 on HepG2 cells. Both PPARγ mRNA and protein expression showed similar patterns with each other.

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Last but not least, I would like to express appreciation to my supportive and loving family. Words fail to describe my warmest gratitude to them.

DECLARATION

I hereby declare that the project report is based on my original work except for 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.

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NG HIN FUNG

APPROVAL SHEET

The project report entitled "**INVESTIGATION INTO THE EFFECTS OF INTERLEUKIN-17 AND LAURIC ACID ON PPARγ EXPRESSION IN HUMAN HEPG2 CELLS**" was prepared by NG HIN FUNG and submitted as partial fulfilment of the requirements for the Degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

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(Assoc. Prof. Dr CHEW CHOY HOONG) DATE: ….……...……

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PERMISSION SHEET

It is hereby certified that **NG HIN FUNG** (ID No: **14ADB01628**) has completed this final year project entitled "**INVESTIGATION INTO THE EFFECTS OF INTERLEUKIN-17 AND LAURIC ACID ON PPARγ EXPRESSION IN HUMAN HEPG2 CELLS**" under the supervision of Dr Chew Choy Hoong from the Department of Biomedical Science, Faculty of Science.

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

Yours truly,

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(NG HIN FUNG)

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-d(RFU)/dT	Rate of change in fluorescence unit with time
9-HODE	9-hydroxy-10, 12-octadecaienoic acid
12C	12-carbon
13-HODE	13-hydroxy-9, 11-octadecadienoic acid
15d-PGJ2	15-deoxy-Δ12, 14-prostaglandin J2
A_{260}	Absorbance read at 260 nm
A_{280}	Absorbance read at 280 nm
Acetyl-CoA	Acetyl coenzyme A
AF	Activation function
aP	Adipocyte protein
AP	Activator protein
APS	Ammonium persulphate
ATCC	American Type Culture Collection
BCP	1-bromo-3-chloropropane
bp	Base pair
BSA	Bovine serum albumin
CBP	CREB-binding protein
CCL	Chemokine (C-C motif) ligand
CD	Fatty acid translocase
cDNA	Complementary deoxyribonucleic acid

C/EBP	CCAAT enhancer binding protein
CO_2	Carbon dioxide
COX	Cyclooxygenase
Cq	Quantification cycle
DBD	DNA binding domain
DEPC	Diethyl pyrocarbonate
DHA	Docosahexaenoic acid
DNase	Deoxyribonuclease
EBF	Early B-cell factor
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EG	Ethanol: Glycerol
EPA	Eicosapentaenoic acid
ERK	Extracellular signal-regulated kinase
FBS	Foetal Bovine Serum
FFA	Free fatty acid
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GATA	GATA binding protein
GEG	Guanidine hydrochloride: Ethanol: Glycerol
GLUT	Glucose transporter
HDL	High density lipoprotein
HepG2	Hepatocarcinoma cell line
HETE	Hydroxyeicosatetraenoic acid

HRP	Horseradish peroxidase
ICAM	Intercellular adhesion molecule
IFN	Interferon
IκB	Inhibitor of κB
IL	Interleukin
IL-17R	IL-17 receptor
iNOS	Inducible nitric oxide synthase
IRS	Insulin receptor substrate
JNK	c-Jun N-terminal kinase
kDA	Kilodalton
KLF	Krüppel-like factor
Krox20	Early growth response-2
LBD	Ligand binding domain
LDL	Low density lipoprotein
LOX	Lipoxygenase
LPL	Lipoprotein lipase
Lys	Lysine
MAPK	Mitogen-activated protein kinase
MCFA	Medium-chain fatty acid
MEM	Minimum essential medium
mRNA	Messenger ribonucleic acid
NCoR	Nuclear co-repressor
NF-κB	Nuclear factor-kappa B

Nods protein	Nucleotide-binding oligomerisation domain-containing (Nods) protein
NR	Nuclear receptor
PBS	Phosphate buffered saline
PEPCK	Phosphoenolpyruvate carboxykinase
RXR	Retinoid X receptor
PI3K	Phosphatidylinositol 3-kinase
PPARγ	Peroxisome proliferator-activated receptors gamma
PPRE	PPAR response element
PUFA	Polyunsaturated fatty acid
PVDF	Polyvinylidene fluoride
qRT-PCR	quantitative reverse transcription-polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
RORγt	retinoic orphan receptor γt
RFU	Relative fluorescence unit
rRNA	Ribosomal ribonucleic acid
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
Ser	Serine
siRNA	Small (or short) interfering RNA

SIRT1	Silencing information regulator-1 or Sirtuin 1
SMRT	Silencing mediator for retinoic acid and thyroid hormone receptors
SREBP-1c	Sterol regulatory element-binding protein 1c
STAT	Signal transducer and activator of transcription
T2DM	Type 2 diabetes mellitus
TBE	Tris/Borate/EDTA
TBST	Tris buffer saline-Tween® 20
TEMED	N,N,N',N'–Tetramethylethylenediamine
TGF-β	Transforming growth factor-beta
Th cells	T helper cells
TLR	Toll-like receptor
Tm	Melting temperature
TNFα	Tumour necrosis factor alpha
TZD	Thiazolidinedione
v/v	Volume per volume
VCAM	Vascular cell adhesion molecule
w/v	Weight per volume