

CONTENTS

	Page
Acknowledgement	c
Abstract in Thai	d
Abstract in English	f
List of Tables	l
List of Figures	n
List of Abbreviations	q
Chapter 1 Introduction	1
1.1 Statement and significance of problem	1
1.2 Literature reviews	2
1.2.1 Obesity and adipocyte	3
1.2.2 Adipocyte life cycle	4
1.2.2.1 Hormonal Inducers of Differentiation	6
1.2.2.2 The adipogenic transcription factors	7
1.2.3 Natural product inhibited adipogenesis	8
1.2.4 Obesity-induced chronic inflammation	10
1.2.5 Inflammation induced insulin resistance	12

1.2.6 Glutinous rice grains local varieties (<i>Oryza sativa</i> L.)	
1.2.6.1 Local Purple rice variety	13
1.2.6.2 Commercial white rice variety	16
1.2.7 Rice and active ingredients	16
1.3 Objective of the study	21
Chapter 2 Materials and method	22
2.1 Chemicals and materials	22
2.2 Preparation of rice extracts	22
2.3 Experimental design	23
2.4 Cell culture and differentiation	24
2.5 Effect of rice extracts on 3T3-L1 adipocyte cell viability	25
2.5.1 Cytotoxicity assay	26
2.6 Effect of rice extracts on adipogenesis of 3T3-L1 preadipocyte	27
2.6.1 Lipogenesis assay	27
2.6.2 Differentiation assay	28
2.6.3 Oil red O staining	29
2.7 Effect of rice extracts on the expression of master regulators of adipocyte differentiation	29
2.7.1 Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)	30
2.7.1.1 RNA Extraction	30
2.7.1.2 cDNA Synthesis by Reverse Transcription	31
2.7.1.3 Quantitative Polymerase Chain Reaction (qPCR)	32
2.8 Effect of rice extracts on TNF- α -induced insulin resistance in adipocyte	32
2.8.1 TNF- α -induced insulin resistance in adipocyte	32

2.8.2	Glucose uptake assay	33
2.8.3	Lipolysis assay	34
2.9	Statistical Analysis	35
Chapter 3	Results	36
3.1	Effect of rice extracts on 3T3-L1 adipocyte cell viability	36
3.2	Adipocyte differentiation	39
3.3	Effect of rice extracts on differentiation of 3T3-L1 preadipocyte	40
3.3.1	Effect of rice extracts on adipocyte differentiation	40
3.3.2	Effect of rice extracts on the expression of master regulators of adipocyte differentiation	43
3.4	Effect of rice extracts on lipid accumulation of preadipocyte Without hormone induction	48
3.5	Effect of rice extracts on TNF- α -induced insulin resistance in adipocyte	51
3.5.1	Effect of rice extracts on glucose uptake in TNF- α -induced insulin resistant adipocytes	51
3.5.2	Effect of rice extracts on lipolysis in mature adipocyte	54
Chapter 4	Discussion and conclusion	57
4.1	Biological effects of rice	57
4.2	Effect of purple rice extracts on 3T3-L1 adipocyte cell viability	58
4.3	Effect of purple rice extracts on adipogenesis of 3T3-L1 preadipocyte and the expression of master regulators of adipocyte differentiation	58
4.4	Effect of purple rice extracts on TNF- α -induced insulin resistance	61

References	65
Appendix	78
Appendix A	78
Appendix B	80
Appendix C	81
Appendix D	84
Curriculum Vitae	87



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF TABLES

	Page
Table 1.1 Hormones and differentiation factors influencing adipocyte differentiation	7
Table 1.2 Examples of natural products with anti-adipogenesis effect	9
Table 1.3 phenotype of purple rice in this study	14
Table 2.1 Primer Sequences for qPCR	32
Table 3.1 Effect of dichloromethane extracts of rice on adipocyte differentiation	41
Table 3.2 Effect of methanol extracts of rice on adipocyte differentiation	42
Table 3.3 Effect of dichloromethane extracts of rice on the expression of CEBP- α	44
Table 3.4 Effect of methanol extracts of rice on the expression of CEBP- α	45
Table 3.5 Effect of dichloromethane extracts of rice on the expression of PPAR γ	46
Table 3.6 Effect of methanol extracts of rice on the expression of PPAR γ	47
Table 3.7 Effect of dichloromethane extracts of rice on adipocyte lipogenesis	49
Table 3.8 Effect of methanol extracts of rice on adipocyte lipogenesis	50
Table 3.9 Effect of dichloromethane extracts of rice on glucose uptake in TNF- α -induced insulin resistant adipocytes	52
Table 3.10 Effect of methanol extracts of rice on glucose uptake in TNF- α -induced insulin resistant adipocytes	53

Table 3.11 Effect of dichloromethane extracts of rice on lipolysis in TNF- α –induced insulin resistant adipocytes	55
Table 3.12 Effect of methanol extracts of rice on lipolysis in TNF- α –induced insulin resistant adipocytes	56



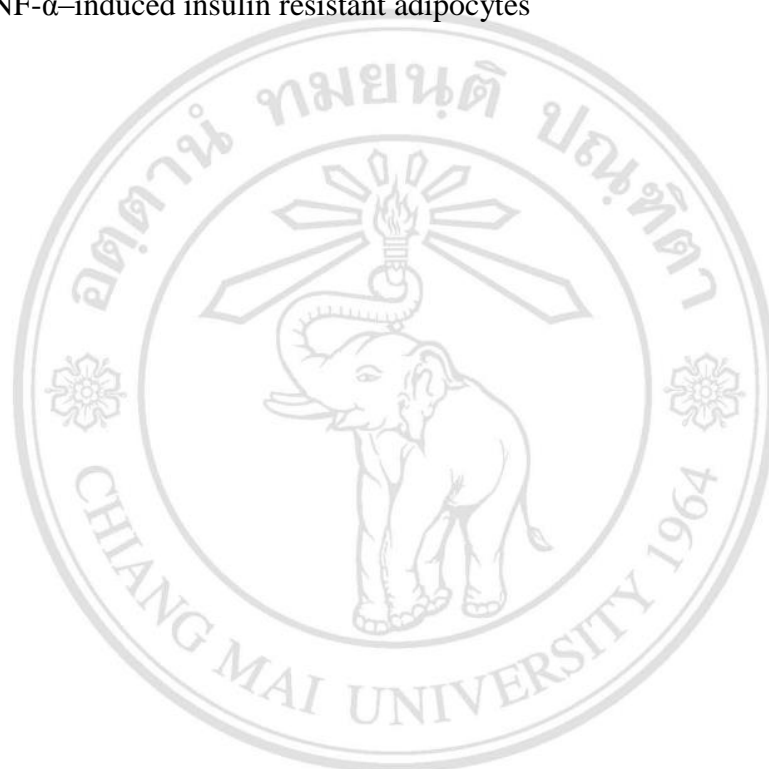
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF FIGURES

	Page
Figure 1.1 Prospective development of diabetes in the future worldwide	2
Figure 1.2 Life cycles of adipocytes	5
Figure 1.3 Obesity-induced chronic inflammations in adipocyte	11
Figure 1.4 Genetic distances clustering by using 5 sets of simple sequence repeat analysis showing genetic relationship among 25 varieties including glutinous purple rice and white rice used in this study	15
Figure 1.5 Distribution patterns of major constituents of brown rice	17
Figure 1.6 Composition of microcomponents including antioxidants of stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates	18
Figure 1.7 Group of phenolic compounds in foods	20
Figure 1.8 Structures of anthocyanin	20
Figure 2.1 A schematic diagram of rice extraction	23
Figure 2.2 Experimental designs to determine the anti-adipogenesis and anti-diabetic effect of rice extracts	24
Figure 2.3 A schematic diagram of cell culture and differentiation of 3T3-L1 adipocyte	25
Figure 2.4 A treatment diagram for 3T3-L1 adipocyte cell viability assay	26
Figure 2.5 Principle of WST-1 assay	27
Figure 2.6 A treatment diagram for 3T3-L1 preadipocyte lipogenesis assay	28
Figure 2.7 A treatment diagram for 3T3-L1 differentiation assay	29

Figure 2.8 A treatment diagram for the expression of master regulators of adipocyte differentiation	30
Figure 2.9 A treatment diagram of TNF- α -induced insulin resistance in adipocyte	33
Figure 2.10 2-[N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxy-glucose (2-NBDG) structure	34
Figure 2.11 Glycerol Assay Enzymatic Reaction	35
Figure 3.1 Effect of rice extracts on 3T3-L1 preadipocyte cell viability	37
Figure 3.2 Effect of rice extracts on 3T3-L1 mature adipocyte cell viability	38
Figure 3.3 The inverted microscope images of mature adipocyte on day 12 and mature adipocyte on day 12 with Oil red O staining	39
Figure 3.4 Effect of dichloromethane extracts of rice on adipocyte differentiation	41
Figure 3.5 Effect of methanol extracts of rice on adipocyte differentiation	42
Figure 3.6 Effect of dichloromethane extracts of rice on the expression of CEBP- α	44
Figure 3.7 Effect of methanol extracts of rice on the expression of CEBP- α	45
Figure 3.8 Effect of dichloromethane extracts of rice on the expression of PPAR γ	46
Figure 3.9 Effect of methanol extracts of rice on the expression of PPAR γ	47
Figure 3.10 Effect of dichloromethane extracts of rice on adipocyte lipogenesis	49
Figure 3.11 Effect of methanol extracts of rice on adipocyte lipogenesis	50
Figure 3.12 Effect of dichloromethane extracts of rice on glucose uptake in TNF- α -induced insulin resistant adipocytes	52

Figure 3.13 Effect of methanol extracts of rice on glucose uptake in TNF- α -induced insulin resistant adipocytes	53
Figure 3.14 Effect of dichloromethane extracts of rice on lipolysis in TNF- α -induced insulin resistant adipocytes	55
Figure 3.15 Effect of methanol extracts of rice on lipolysis in TNF- α -induced insulin resistant adipocytes	56



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF ABBREVIATIONS

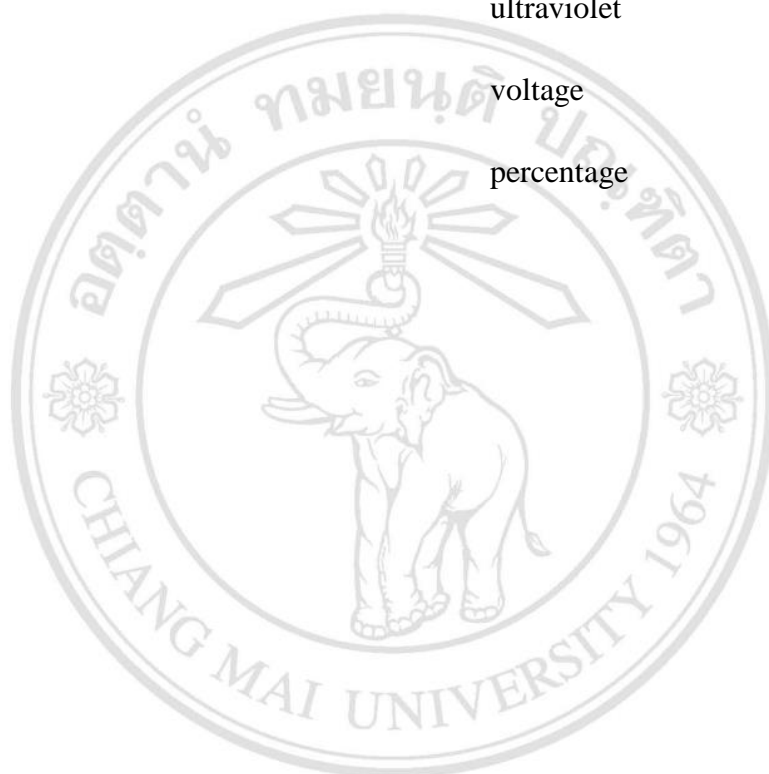
Abs	absorbance
bp	base pair
BSA	bovine serum albumin
°C	degree Celsius
CO ₂	carbon dioxide
DI	deionized water
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DW	distilled water
EDTA	ethylenediamine-N,N,N',N'-tetraacetic acid
EtOH	ethanol
FBS	fetal bovine serum
g	gram
g	gravity force
GABA	γ- aminobutyric acid
h	hour

HCl	hydrochloric acid
H ₂ O	water
HEPES	(4-(2-hydroxyethyl)-1-piperazineethaneulfonic acid)
KCl	potassium chloride
KH ₂ PO ₄	potassium dihydrogen phosphate
L	liter
M	molarity
mg	milligram
MgCl ₂	magnesium chloride
Min	minute
ml	milliliter
mM	millimolar
μg	microgram
μl	microliter
μM	micromolar
NaCl	sodium chloride
NaOH	sodium hydroxide
Na ₂ CO ₃	sodium carbonate
Na ₂ HPO ₄	disodium hydrogen phosphate
nm	nanometer



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright © by Chiang Mai University
 All rights reserved

No	number
PBS	phosphate buffer saline
RT	room temperature
SDS	sodium dodecyl sulfate
UV	ultraviolet
V	voltage
%	percentage



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved