



*GARCINIA ATROVIRIDIS* FRUIT EXTRACT  
MODULATES LIPID ACCUMULATION IN 3T3-L1  
ADIPOCYTE CELLS

BY

HANISUHANA HAMIDON

A thesis submitted in fulfilment of the requirement for the  
degree of Master in Pharmaceutical Sciences  
(Pharmaceutical Technology)

Kulliyyah of Pharmacy  
International Islamic University Malaysia

DECEMBER 2017

## ABSTRACT

Obesity is the accumulation of excess body fat to the extent it may have an adverse effect on health. It is undeniably a complex disease with risk factors combination of genetics, lifestyle, nutrition, increasing age, and lack of physical activity. Alarming increase of obesity prevalence since past few years brings the concern to explore effective therapy against obesity. The hydroxycitric acid, HCA, is a potential compound in weight management that possesses a high market demand. It was commonly reported to be originated from *Garcinia cambogia*. There is little attempt to study the potential of our locally available underexplored *G. atroviridis* on its HCA content. This also has opened the gap to study the extract lipid alteration potential the on 3T3-L1 cell culture model. This present study aims to quantify the amount of HCA available in the fruit extract as well as to investigate its lipid modulation properties in 3T3-L1 cell culture model. The fresh fruit was macerated using ethanol and freeze-dried. Then, the HCA was screened and quantified using FTIR and HPLC analysis, respectively. The extract was further tested in vitro for its lipid modulation potential. Firstly, the cell viability assay was conducted to assess the possible extract's cytotoxic effect on the cell line. Then, the cell was induced to differentiate using differentiation medium in the presence of the extract. Differentiated adipocytes were analysed qualitatively and quantitatively using Oil Red O stain. The treatment's effect on the adipogenesis-related proteins, PPAR $\gamma$  and C/EBP $\alpha$  were also investigated by means of western blot. The lipid content in the differentiated adipocytes was also confirmed with leptin ELISA assay. Finally, the adipolysis assay was conducted to identify the extract's effect on the breakdown of lipid. From the FTIR spectroscopy, the presence of HCA in the extract was confirmed based on the significant functional groups of hydroxyl, carbonyl, and lactone. Later, the HPLC analysis quantified about  $11.35 \pm 0.55$  % (w/w) of HCA in the extract with retention time at 0.9 minute, corresponding to the standard. The extract was no cytotoxic on the cell line since the treatment did not cause any 50 % reduction in the cell viability, hence concentrations of 10, 45, and 60  $\mu\text{g/mL}$  were chosen for subsequent assays. In adipogenesis assay, it was observed that increased co-treatment concentration inhibited the formation of intracellular lipid. This was further confirmed with spectroscopic absorbance of Oil Red O stain and absorbance value measured shows reduction. Co-treatment at 60  $\mu\text{g/mL}$  significantly ( $p < 0.05$ ) inhibited the adipogenesis. From the western blot analysis, the treatment only affects the expression of C/EBP $\alpha$  protein significantly ( $p < 0.05$ ). It was concluded that the treatment suppressed adipogenesis by inhibiting the C/EBP $\alpha$  expression. Meanwhile, the leptin assay finding was also in agreement with adipogenesis analysis since the leptin released was significantly reduced ( $p < 0.05$ ) at increased treatment concentration. Finally, the extract enhanced the adipocytes breakdown (adipolysis) significantly ( $p < 0.05$ ) at 10 and 45  $\mu\text{g/mL}$  of extract. Taken together, the findings indicated that the fruit extract of *G. atroviridis* plays a role in alteration of lipid accumulation with the intervention of protein associated with adipogenesis and enhancing the adipolysis. Understanding adipogenesis and its molecular mechanisms may provide clues for future strategies development in preventing and managing obesity. Hence, the *G. atroviridis* fruit can be proposed as one of weight management agents due to its lipid modulation properties.

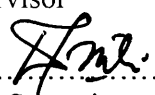
## خلاصة البحث

البدانة هي عبارة عن تجمع الدهون في الجسم الى درجة مضرّة للصحة. تعتبر البدانة مرض معقد هنالك مزيج من العوامل المسببه للمرض منها العوامل الوراثية و طريقة الحياة و التغذية بالإضافة الى الزيادة بالعمر و انعدام اللياقة البدنية. هنالك زيادات كبيرة لمؤشرات البدانة في السنين القليلة الماضية هذه الزيادات تفرض الاهتمام لايجاد علاج فعال ضد البدانة. يعتبر الهيدروكسيستركاسد (ه س أ) مركب من المحتمل ان يساعد في تنظيم الوزن؛ يعتقد ان هذا المركب مستخلص من نبات الجارسينيا كامبوجيا. هنالك القليل من الدراسات حول وجود مركب ال (ه س أ) في هذا النبات. و هذا قد اعطى فرصه لدراسه قابلية هذه النبتة لتغير الدهون في خلايا ال ٣-١. هذه الدراسة الحالية تهدف الى تحديد كمية ال ه س أ الناتج من مستخلص النبات بالإضافة الى معرفة مقدرته على تحوير الدهون في خلايا ال ٣-١. تأخذ الفاكه الطازجة من النبات ليم استخلاصها ثم تجميدها بجفاف؛ بعد ذلك قمنا بفحص و تحديد ال ه س أ بواسطة اجهزة ال اف تي اى ا ر و ال ه ي ال سي. تم اختبار خاصيه تحوير الدهون للمستخلص في المختبر حيث بدأنا من اختبار بقاء الخلايا لمعرفة ما اذا كان المستخلص سام للخلايا ؛ بعد ذلك قمنا بتمييز الخلايا باستخدام وسط للتمايز. تم تحليل الخلايا الدهنية المتمايزة نوعيا و رقميا باستخدام صبغة الدهن الاحمر و . تم ايضا اختبار المستخلص على البروتينات المرتبطة بالخلايا الدهنية باستخدام اختبار لطفة وسترن. تم تأكيد محتوى الدهون في الخلايا الدهنية المتمايزة من خلال فحص ال لبتين اليسا. اخيرا اجري فحص الخلايا الدهنية لمعرفة تأثير المستخلص على تحطيم الدهون. أكدت نتيجة التحليل الضوئي اف تي اى ا ر وجود مركب ال ه س أ في مستخلص النبات بالاعتماد على مجاميع وظيفية كبيرة من الهيدروكسيل و الكاربونيل و اللكتون. تحليل ال ه ي ال سي اظهر كيه من ال ه س أ ما يقارب ١١, ٣٥ ± ٠,٥٥% بوقت ٠,٩ دقائق. مستخلص النبات لم يؤثر على بقاء /مايكروغرام كانت التركيزات المختاره الخلايا في الخلايا الدهنية و قبل الدهنيه؛ ١٠ و ٤٥ و ٦٠ مليلتر للمستخلص. في اختبار تكوين الشحوم كان من الملاحظ مع زيادة تركيز العلاج قلة نسبة الدهون داخل الخلايا ؛ قد تم تأكيد هذه النتائج باختبارات امتصاص التحليل الضوئي لصبغة الدهن الحمراء وقياس القيمة الامتصاصيه التي اظهرت قيمة قليلة من الدهون ؛ العلاج المصاحب بتركيز ٦٠ مايكروغرام/ مل اظهر نتائج تثبيطيه بشكل ملحوظ (ب اقل من ٠,٥٠). نتائج لطفة وسترن اظهرت ان العلاج اثر في س/ي ب ب أ بروتين فقط بشكل ملحوظ (ب اقل من ٠,٥٠). نتائج تحليل اللبتين كانت توافق نتائج تحليل تكوين الدهون. كل النتائج و الموجودات اظهرت ان مستخلص النبات من الفاكه يعمل بدور كبير على تغيير تراكم الدهون مع تدخل البروتين المرتبط بتكوين الدهون و تحسين تحليل الدهون بشكل ملحوظ (ب اقل من ٠,٥٠). فهم طريقة تكوين الدهون و الاليه الجزيئية يمكنها ان تقدم دلائل لاليات تطوير في منع و تنظيم البدانة في المستقبل. نبات ال ج. اتروفيريديس يعتبر كاحد النباتات المقترحة لتنظيم الوزن بسبب خصائص تحوير الدهون التي تحتوي عليها.

## APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....  
Muhammad Taher  
Supervisor

  
.....  
Deny Susanti  
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

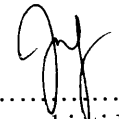
.....  
Wan Mohd Azizi bin Wan Sulaiman  
Internal Examiner

.....  
Hasmah binti Abdullah  
External Examiner

This thesis was submitted to the Department of Pharmaceutical Technology and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

.....  
Mohd Rushdi bin Abu Bakar  
Head, Department of Pharmaceutical  
Technology

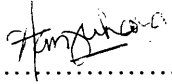
This thesis was submitted to the Kulliyah of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

  
.....  
Juliana binti Md Jaffri  
Dean, Kulliyah of Pharmacy

## DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Hanisuhana Hamidon

Signature .....  .....

Date ..... 14/12/2017 .....

**INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA**

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF  
FAIR USE OF UNPUBLISHED RESEARCH**

***GARCINIA ATROVIRIDIS* FRUIT EXTRACT MODULATES  
LIPID ACCUMULATION IN 3T3-L1 ADIPOCYTE CELLS**

I declare that the copyright holder of this thesis are jointly owned by the student and  
IIUM

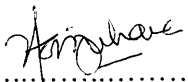
Copyright © 2017 Hanisuhana Hamidon and International Islamic University Malaysia.  
All rights reserved.

No part of this unpublished research may be reproduced, stored in a retrieval system,  
or transmitted, in any form or by any means, electronic, mechanical, photocopying,  
recording or otherwise without prior written permission of the copyright holder except  
as provided below

1. Any material contained in or derived from this unpublished research may be used by others in their writing with due acknowledgement.
2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purpose.
3. The IIUM library will have the right to make, store in a retrieval system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialisation policy.

Affirmed by Hanisuhana Hamidon

  
.....  
Signature

14/12/2017  
.....  
Date

## ACKNOWLEDGEMENTS

First and foremost, thanks to Allah SWT, through His blessing, I was able to complete this research and thesis. Without His help and blessing, it is definitely impossible for me to accomplish it. It was indeed a great experience throughout the process. All hardships and challenges that I faced through have given me courage to keep on trying, to always have faith in Him, and eventually becomes the better version of me.

My utmost and infinite appreciation to my dear parents, Mariam Omar and Hamidon Hj Salleh as well as my siblings, Hanisjasmah, Haniif Syaamil, and Hanissyazwani for their endless support, motivation and encouragement from a far. The unconditional love given had given me confidence and strength for me to keep on going and trying during difficult times. Thank you for always be there for me through my ups and downs.

Next, my gratitude goes to Dr. Muhammad Taher and Dr. Deny Susanti for their willingness to supervise me throughout this project. Thank you for the trust and all the valuable opinions on the research. Thank you also to Dr. Muhamad Rusdi and Dr. Solachuddin for the share of knowledge and fruitful discussion that indirectly help me with this project.

My sincere thanks also to science officers and laboratory staffs of Department of Pharmaceutical Technology and Basic Medical Science (Kulliyah of Pharmacy), Integrated Centre for Animal Research, Care, and Use (ICRACU) IIUM, Department of Pathology (Kulliyah of Medicine), as well as Department of Chemistry (Kulliyah of Science) for ease of access to the laboratories and instruments.

To my postgraduate colleagues; Maryam Saadah, Nurlaili Najmie, Nur Syafinaz, Nurul Adilah, Wastuti Hidayati, Sama Nazih, Asween Rowena, Ahmad Fahmi, Salahuddin, and Anugerah, thank you very much for the friendship that I shall treasured it forever. This journey is more meaningful with your presence. Thank you for the help, time, laugh, and tears that we shared together.

I would also like to thank International Islamic University Malaysia for financial support through IIUM Research Initiative Grant Scheme (RIGS15-122-0122) and MyBrain15 from Ministry of Higher Education Malaysia for MyMaster scholarship. Last but not least, thanks also to all who involved directly or indirectly in the completion of this project. May Allah bless all of you, here and hereafter.

## TABLE OF CONTENTS

Abstract .....	ii
Abstract in Arabic.....	iii
Approval Page.....	iv
Declaration .....	v
Copyright Page.....	vi
Acknowledgements.....	vii
Table of Contents .....	viii
List of Tables .....	xi
List of Figures .....	xii
List of Equations .....	xiv
List of Abbreviations .....	xv
<b>CHAPTER ONE: INTRODUCTION .....</b>	<b>1</b>
1.1 Research Background.....	1
1.2 Research Problem.....	2
1.3 Objectives of the Research.....	3
1.4 Hypothesis of the Research.....	3
1.5 Significance of the Study .....	3
1.6 Experimental Design.....	4
<b>CHAPTER TWO: LITERATURE REVIEW .....</b>	<b>6</b>
2.1 General Overview .....	6
2.2 Obesity .....	8
2.2.1 Pathogenesis of Obesity .....	9
2.2.2 Risk Factors and Treatment for Obesity.....	11
2.3 Natural Products for Antiobesity .....	13
2.4 <i>Garcinia atroviridis</i> .....	17
2.4.1 Phytochemical Content of <i>G. atroviridis</i> .....	18
2.4.2 Bioactivities of <i>G. atroviridis</i> .....	23
2.4.2.1 Antioxidant Activity .....	23
2.4.2.2 Antimicrobial Activity .....	24
2.4.2.3 Anti-inflammatory Activity .....	26
2.4.2.4 Cytotoxic Activity.....	27
2.4.2.5 Antihyperlipidaemic/antihypercholesteromic/ antiobesity Activities .....	28
2.4.2.6 Other Bioactivity Studies.....	29
2.4.3 Clinical Study of <i>G. atroviridis</i> .....	29
2.5 Review on Hydroxycitric Acid .....	30
2.5.1 The Biological Effect of HCA.....	31
2.5.2 Clinical Study of HCA .....	33
2.6 3T3-L1 Mouse Preadipocyte Cell Line.....	35
2.7 Adipocyte Differentiation .....	38
2.7.1 Transcriptional Regulation of Adipogenesis .....	39
2.7.2 Targeting Adipocyte Differentiation .....	43
2.8 Adipocyte Breakdown.....	44



2.9 Adipocytes and the Study of Obesity.....	45
<b>CHAPTER THREE: MATERIALS AND METHODS .....</b>	<b>47</b>
3.1 Materials.....	47
3.1.1 Chemicals .....	47
3.1.2 Instruments .....	48
3.1.3 Disposable Materials .....	48
3.1.4 Cell Line .....	49
3.2 Experimental Procedures .....	49
3.2.1 Plant Material and Crude Extract Preparation.....	49
3.2.2 Analysis of <i>G. atroviridis</i> Fruit Extract.....	50
3.2.2.1 Screening of HCA in Extract using ATR-FTIR Spectroscopy .....	50
3.2.2.2 Quantification of HCA in Extract using HPLC .....	50
3.2.3 General Cell Culture Protocol .....	51
3.2.3.1 Sample Preparation .....	51
3.2.3.2 Cell Thawing.....	51
3.2.3.3 Cell Maintenance .....	52
3.2.3.4 Cell Trypsinization.....	52
3.2.3.5 Cell Counting .....	52
3.2.3.6 Cell Cryopreservation .....	53
3.2.3.7 Cell Viability Assay .....	54
3.2.4 Lipid Modulation Study .....	55
3.2.4.1 Preadipocyte Differentiation Assay (Adipogenesis).....	55
3.2.4.2 Oil Red O Staining.....	56
3.2.4.3 Leptin Measurement Assay.....	57
3.2.4.4 Adipolysis Assay .....	58
3.2.5 Protein Expression Study .....	59
3.2.5.1 Cell Culture and Total Protein Extraction.....	59
3.2.5.2 Total Protein Quantification .....	60
3.2.5.3 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).....	60
3.2.5.4 Commasie Blue Staining.....	61
3.2.5.5 Protein Transfer (Western Blot).....	61
3.2.5.6 Ponceau Staining.....	61
3.2.5.7 Primary and Secondary Antibody Incubation.....	61
3.2.5.8 Chemiluminescence Detection.....	62
3.2.5.9 Protein Density Determination.....	62
3.2.6 Data Analysis.....	62
<b>CHAPTER FOUR: RESULTS .....</b>	<b>63</b>
4.1 The Analysis of <i>G. atroviridis</i> Fruit Extract.....	63
4.1.1 Physical Properties and Yield of Extract.....	63
4.1.2 Screening of HCA in Extract.....	63
4.1.3 Quantification of HCA in Extract.....	65
4.2 GAE Alters In Vitro Lipid Accumulation in Cell Line Used .....	67
4.2.1 The Effect of GAE on 3T3-L1 Cell Viability .....	67
4.2.2 The Effect of GAE on Adipogenesis, Proteins Expression, and Leptin Secretion.....	69

4.2.3 The Effect of GAE on Adipolysis .....	80
<b>CHAPTER FIVE: DISCUSSION</b> .....	<b>82</b>
5.1 The HCA Content in <i>G. atroviridis</i> Fruit Extract .....	82
5.2 GAE Alters In Vitro Lipid Accumulation in Cell Line Used .....	83
<b>CHAPTER SIX: CONCLUSION AND FUTURE WORKS</b> .....	<b>91</b>
6.1 General Conclusion.....	91
6.2 Future Works.....	92
<b>REFERENCES</b> .....	<b>93</b>
<b>APPENDIX A: REAGENTS AND SOLVENTS FOR WESTERN BLOT</b> .....	<b>108</b>
<b>APPENDIX B: ADDITIONAL EXPERIMENT RESULTS</b> .....	<b>112</b>
<b>APPENDIX C: CONFERENCES AND PRESENTATION</b> .....	<b>114</b>
<b>APPENDIX D: PUBLICATION</b> .....	<b>119</b>

## LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
2.1	Current FDA-approved antiobesity drugs	12
2.2	Natural pancreatic lipase inhibitor	14
2.3	Natural products that inhibit adipocyte differentiation	15
2.4	Natural products that promote lipid metabolism	16
2.5	Cell model and adipogenesis inducer	39
2.6	Hormones and cytokines released by the adipocytes and their involvement in physiological function	46
3.1	Medium composition for adipogenesis	56
4.1	Functional group present in GAE	65

## LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
1.1	Flowchart of experimental design	5
2.1	The vicious cycle of obesity and diabetes	10
2.2	The various parts of <i>G. atroviridis</i> plant	18
2.3	Structure of compounds isolated from <i>G. atroviridis</i>	22
2.4	Structures of HCA isomers	31
2.5	The inhibition of HCA on ATP-citrate lyase enzyme	32
2.6	The 3T3-L1 mouse preadipocyte cell line captured using inverted microscope	36
2.7	The differentiated 3T3-L1 mouse adipocyte cells captured using inverted microscope	37
2.8	Overview of stages in adipogenesis	41
4.1	Physical appearance of crude extract	63
4.2	FTIR spectrum of HCA standard	64
4.3	FTIR spectrum of GAE	64
4.4	The calibration curve of HCA standard	66
4.5	HPLC chromatogram of HCA standard	66
4.6	HPLC chromatogram of GAE	67
4.7	Effect of different treatment concentrations of GAE on percentage of 3T3-L1 preadipocyte cell viability	68
4.8	Effect of different treatment concentrations of GAE on percentage of 3T3-L1 adipocyte cell viability	68
4.9	Morphological changes in 3T3-L1 cells differentiation. From induction day, Day 0 to staining day, Day 8	70

4.10	The effect of 10 µg/mL of GAE on preadipocytes differentiation	71
4.11	The effect of 45 µg/mL of GAE on preadipocytes differentiation	72
4.12	The effect of 60 µg/mL of GAE on preadipocytes differentiation	73
4.13	The effect of HCA on preadipocytes differentiation	74
4.14	The effect of MDI on preadipocytes differentiation	75
4.15	Morphology of untreated (non-induced) cells after 8 days of differentiation induction	76
4.16	Effect of different treatment concentrations of GAE on adipogenesis	77
4.17	Relative protein expression of PPAR $\gamma$ . Band intensity was analysed and normalised with loading control of $\beta$ -actin	78
4.18	Relative protein expression of C/EBP $\alpha$ . Band intensity was analysed and normalised with loading control of $\beta$ -actin	79
4.19	Leptin levels observed in culture supernatant post preadipocytes differentiation	80
4.20	Effect of different treatment concentrations of GAE on adipolysis	81

## LIST OF EQUATIONS

<u>Equations No.</u>		<u>Page No.</u>
3.1	<i>% Cell Viability =</i> $(Viable\ cells\ count / Total\ cells\ count) \times 100$	53
3.2	<i>Cell density =</i> $Average\ cells\ counted\ per\ square \times Dilution\ factor$ $\times 10^4$	53
4.1	<i>Viability of cells (%) =</i> $\left[ \frac{(A_{sample} - A_{blank})}{(A_{untreated} - A_{blank})} \right] \times 100$	67

## LIST OF ABBREVIATIONS

AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
ap2	adipocyte protein2
APS	Ammonium persulfate
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
BMI	Body mass index
BSA	Bovine serum albumin
C/EBPs	CCAAT-enhancer-binding proteins
C/EBP $\alpha$	CCAAT/enhancer-binding protein $\alpha$
CAMP	Cyclic adenosine monophosphate
CO <sub>2</sub>	Carbon dioxide
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CPT 1	Carnitine palmitoyltransferase I
DEX	Dexamethasone
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EBV	Epstein Barr virus
ELISA	Enzyme-linked immunosorbent assay
FAS	Fatty acid synthase
FBS	Foetal bovine serum
FRAP	Ferric reducing antioxidant power
FTC	Ferric thiocyanate
FTIR	Fourier transform infrared spectroscopy
GAE	<i>G. atroviridis</i> extract
GLUT4	Glucose transporter 4
HCA	Hydroxycitric acid
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IBMX	3-isobutyl-1-methyl-xantine
ICRACU	Integrated Centre for Animal Research, Care and Use
IFN- $\gamma$	Interferon- $\gamma$
IL	Interleukin
IUPAC	International Union of Pure and Applied Chemistry

LDL	Low density lipoprotein
LPS	Lipopolysaccharides
MDI	Mixture of dexamethasone, IBMX, and insulin
mRNA	Messenger RNA
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl	Sodium chloride
NF- $\kappa$ B	Nuclear factor-kappa B
PBS	Phosphate buffered saline
PPAR	Peroxisome proliferator-activated receptor
PPAR $\gamma$	Peroxisome proliferator-activated receptor- $\gamma$
ppm	Parts per million
PVDF	Polyvinylidene fluoride
rpm	Rotation per minute
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TBA	Thiobarbituric acid
TBS	Tris-buffered saline
TEMED	N,N,N',N'-Tetramethylethylenediamine
TLC	Thin layer chromatography
TNF- $\alpha$	Tumour necrosis factor $\alpha$
TPC	Total phenolic content
TXB <sub>2</sub>	Thromboxane-B2
UNTX	Untreated



## **CHAPTER ONE**

### **INTRODUCTION**

#### **1.1 RESEARCH BACKGROUND**

Obesity is a global public health problem. Malaysia has been rated as the highest among Asian countries for obesity, thus “winning” the title of “The Fattest Country in Southeast Asia”. In Malaysia, according to National Health and Morbidity Survey in 2011, 2.5 million of Malaysian adults were obese. The prevalence has increased dramatically to 15.1 % in 2011 from 4.4 % in 1996 (Ministry of Health Malaysia, 2014). Meanwhile, the worldwide obesity prevalence has been increasing since past few years and according to World Health Organisation (WHO) (2015), in 2014 more than 1.9 billion adults worldwide were overweight and among the figure, 600 million were obese. Such alarming figure brings major concern on the need to explore effective therapy against obesity since many studies have been reported that obesity is a major risk factor for diabetes, cardiovascular, several types of cancer (such as endometrial, breast, colon and prostate), pulmonary, osteoarticular, and metabolic diseases (Reaven, 2011; Huang et al., 2014).

Up till today, natural product continues to play essential roles in healthcare. In 1985, the WHO reported that approximately 65 % of the world population depends on the plant-derived traditional medicine for their primary health care, meanwhile among the remaining population of developed countries, plant products still play indirect role in the healthcare system (Cragg & Newman, 2013). Herbal remedies are also being accepted in many developed countries. It is believed that herbal remedies promote healthier living and viewed as holistic and balanced approach of healing (Ekor, 2014).

Due to this, natural product contributes an important role in finding of the new chemical entities for drug discovery. Natural source-derived drugs served as leads that suitable for optimisation by synthetic means. It was estimated that about one quarter of all drugs in current pharmacopeias were derived from plants (Efferth & Koch, 2011). The natural product becomes the interest in drug development due to its structural diversity as well as their ability to possess highly selective and specific biological activity based on mechanism of action (Cragg & Newman, 2013).

Traditional herbal medicines may have some potential in managing obesity. The fruit rind of *Garcinia atroviridis*, also known as Asam Gelugor was reported as one of unique sources of hydroxycitric acid (HCA). The Asam Gelugor exhibits a distinct sour taste and has been safely used for centuries for cooking flavourings. HCA is a compound that have the potential as a weight management agent and possesses high demand. However, the exact mechanism of the compound in targeting obesity is not well elucidated and this opened the gap to understand the HCA content in the fruit of *G. atroviridis* as well as its lipid modulation potential in vitro.

## **1.2 RESEARCH PROBLEM**

Obesity is the accumulation of excess body fat to the extent it may have an adverse effect on health. It is undeniably a complex disease with risk factors combination of genetics, lifestyle, nutrition, increasing age, and lack of physical activity. Alarming increase of obesity prevalence since past few years brings the concern to explore effective therapy against obesity. In terms of medication approach for obesity, the approach only provides short-term solution and other problems such as its effectiveness and safety. The HCA has been proven beneficial in the treatment of obesity-related complications such as inflammation, oxidative stress, and insulin

resistance. It was commonly reported to be originated from *G. cambogia*. There was little attempt to study the potential of our locally available underexplored *G. atroviridis* on its HCA content as well as to understand the HCA effect on 3T3-L1 adipocyte cell line, especially its ability to exhibit lipid modulation properties.

### **1.3 OBJECTIVES OF THE RESEARCH**

The general objective of this research was to assess the lipid modulation potential of *G. atroviridis* fruit extract in vitro using 3T3-L1 adipocyte cells. Meanwhile the specific objectives of this research were:

1. To extract and quantify the HCA content in the fruit extract of *G. atroviridis*.
2. To determine the effect of the extract on the lipid accumulation and break down in 3T3-L1 adipocyte cells.
3. To quantify the expression of protein involved in adipogenesis (PPAR $\gamma$  and C/EBP $\alpha$ ) in 3T3-L1 adipocyte cells.

### **1.4 HYPOTHESIS OF THE RESEARCH**

The HCA rich extract from *G. atroviridis* fruit has the ability to modulate lipid accumulation in differentiated 3T3-L1 cell line without any toxic effect as well as affecting the protein expression of the adipogenesis-related proteins, PPAR $\gamma$  and C/EBP $\alpha$ .

### **1.5 SIGNIFICANCE OF THE STUDY**

Since Malaysia is the fattest country in Southeast Asia, this study is relevant to country in terms of managing obesity using natural resources. Obesity is implicated in development many chronic diseases, and the government's spending on health care

services is increasing in order to meet the rising demand for curative care for chronic disease patients. Thus, the finding is hoped to suggest novel source of treatment and management for obesity, which later leads to increment of quality of life among Malaysians and eventually decreases the burden of health care services together with reducing the government expenditure.

## **1.6 EXPERIMENTAL DESIGN**

In this study, the investigation was divided into two parts. The first part was the HCA screening and quantification. Meanwhile, the second part involved the cell culture investigation using 3T3-L1 cell line. Figure 1.1 shows the flowchart of the experimental design of this research.

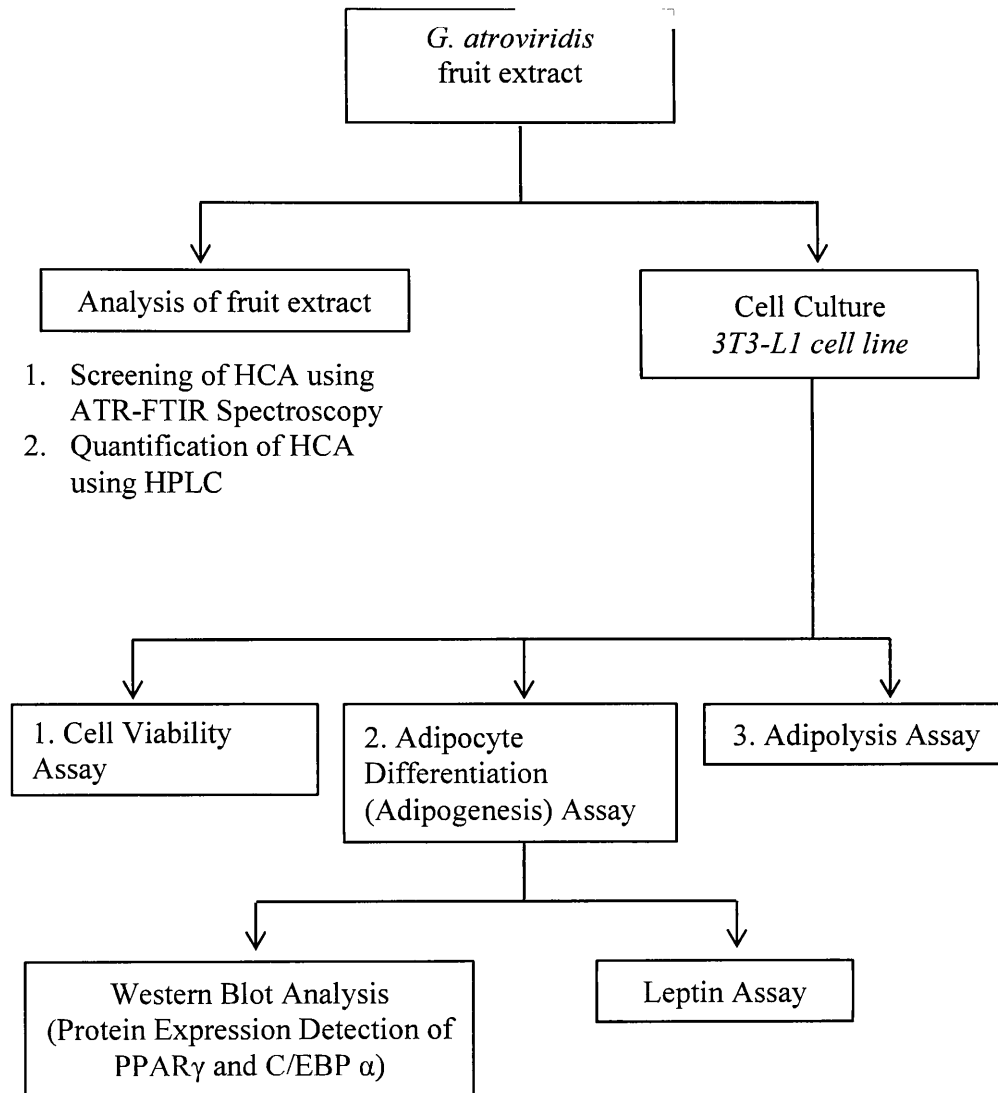


Figure 1.1 Flowchart of experimental design

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 GENERAL OVERVIEW**

Obesity is the accumulation of excess body fat to the extent it may have an adverse effect on health, resulting in reduced life expectancy, and/or increased health problems (Mohamed, Ibrahim, Salah, & Dine, 2014). Alarming increase in obesity prevalence brings major concern on the need to explore effective therapy against obesity since many studies have been reported that obesity is a major risk factor for diabetes, cardiovascular, several types of cancer (such as endometrial, breast, colon and prostate), pulmonary, osteoarticular, and metabolic diseases (Reaven, 2011; Huang et al, 2014). Current treatment for obesity that available in market involves two mechanisms of action which are reducing intestinal fat absorption through inhibition of pancreatic lipase and appetite suppressant (Yun, 2010).

There are a few numbers of drugs available in the market to ameliorate or prevent obesity, but other consideration must be taken such as the costs, efficacy, and side effects. In finding the effective therapy against obesity, the adipose tissue has become an important target and understanding the process of adipogenesis has become important. Adipogenesis is an important driver of the expansion of adipose tissue mass during the state of excess calories intake that later links to obesity. Antiobesity agent suppresses lipid accumulation during adipogenesis in 3T3-L1 preadipocytes, associated with a decrease in peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ), CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) and fatty acid synthase (FAS) protein expression (Cheong et al., 2015).

Phytochemicals dietary supplements usually contain a complex mixture of phytochemicals which have additive or synergistic interactions. Garcinia extract has been used in the traditional Ayurvedic medicine (Lim, 2012). Garcinia is a plant under the family of Clusiaceae that is commonly used as a flavouring agent. Different types of phytochemicals including flavonoids and organic acids have been identified from this plant. Among all types of organic acids, hydroxycitric acid has been identified as a potential supplement for weight reducing and management agent. The HCA originated from *G. cambogia* able to reduce lipid accumulation in 3T3-L1 adipocyte (Kim, Kim, Kwon, & Park, 2004). HCA could be a promising antiobesity agent since it has the ability to inhibit adipocyte differentiation, reduce fatty acid synthesis, and epididymal fat accumulation through reducing ATP-citrate lyase activity, and suppress the appetite (Mohamed et al., 2014). In vivo studies meanwhile have contributed to the understanding of the antiobesity effects of HCA via regulation of serotonin level and glucose uptake (Chuah, Ho, & Beh, 2013). In term of safety, there is no significant adverse effects of HCA have been reported and it demonstrates the safety, bioavailability, and efficacy of HCA in weight management (Preuss et al., 2004).

Previous hypothesis stated that the abundant amount of fat leads to health risk. Hence, the idea of targeting the process of lipid formation to treat obesity has emerged. Targeting adipogenesis would be a direct approach to reduce the amount of fat present (Nawroki & Scherer, 2005). The process of adipogenesis involves series of transcriptional processes. Molecular interactions of CCAAT-enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptor (PPAR) families were suggested to be involved in the process of differentiation (Nicholson et al., 2007; Niemela, Miettinen, Sarkanen, & Ashammakhi, 2008; Moreno-Navarrete & Fernández-Real, 2012). The peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) is

a transcription factor that expressed mainly in adipose tissue (Cornelius, MacDougald, & Lane, 1994). It is the master regulator of adipogenesis and absolutely necessary and sufficient for adipogenesis. There are two major isoforms of PPAR $\gamma$  which are PPAR $\gamma$ 1 and PPAR $\gamma$ 2 (Rosen, Eguchi, & Xu, 2009). Recent studies showed that PPAR $\gamma$ 2 affects adipogenesis mainly since it was observed in adipocytes of morbidly obese individuals (Sarjeant & Stephens, 2012). Other than that, PPAR $\gamma$ 2 is also required for adipocyte differentiation and lipid metabolism (Rosen et al., 2009). During adipocyte differentiation, PPAR $\gamma$ 2 is involved in the sequential mRNA expression of adipocyte phenotypic markers, such as adipocyte protein2 (ap2) and fatty acid synthase (FAS) (Tontonoz, Hu, & Spiegelman, 1994).

Based on the above mentioned facts, this study is anticipated since there is no study has been documented on the effect of HCA from *G. atroviridis* on adipocytes cell. Findings from this study could help in understanding the mechanism of action of HCA as a natural source for lipid formation inhibitor as well as providing new insights towards the discovery of new natural and safe antiobesity agent.

## **2.2 OBESITY**

Overweight and obesity are closely associated with each other, with the definition of the abnormal or excessive fat accumulation that may impair health. Obesity is characterised with the increase in number or size of adipocytes, where adipocytes play a vital role in monitoring lipid and glucose metabolism (Guilherme, Virbasius, Puri, & Czech, 2008).

According to WHO (2015), the body mass index (BMI) value of greater or equal to 25 is described as overweight. Meanwhile, the BMI value of greater than or equal to 30 is the indication for obesity. Recently, obesity has become a serious public