



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF COCOA POLYPHENOL-RICH EXTRACT ON THE  
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA  
EXPRESSION IN ADIPOSE AND SKELETAL MUSCLE TISSUE OF OBESE  
DIABETIC RATS**

**FARHANA BINTI AMINUDDIN**

**FPSK(m) 2012 23**

**EFFECTS OF COCOA POLYPHENOL-RICH EXTRACT ON THE  
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA  
EXPRESSION IN ADIPOSE AND SKELETAL MUSCLE TISSUE OF OBESE  
DIABETIC RATS**



**FARHANA BINTI AMINUDDIN**

**MASTER OF SCIENCE  
UNIVERSITI PUTRA MALAYSIA**

**2012**

**EFFECTS OF COCOA POLYPHENOL-RICH EXTRACT ON THE  
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA  
EXPRESSION IN ADIPOSE AND SKELETAL MUSCLE TISSUE OF OBESE  
DIABETIC RATS**

By

**FARHANA BINTI AMINUDDIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

**December 2012**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**EFFECTS OF COCOA POLYPHENOL-RICH EXTRACT ON THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA EXPRESSION IN ADIPOSE AND SKELETAL MUSCLE TISSUE OF OBESE DIABETIC RATS**

By

**FARHANA BINTI AMINUDDIN**

**December 2012**

**Chair: Professor Amin Ismail, PhD**

**Faculty: Medicine and Health Sciences**

*Theobroma cacao* bean is known to have potential anti-obesity and anti-diabetic activities because of its bioactive phytochemicals and their antioxidant capacities. An oral administration of cocoa polyphenols-rich extract (CoPE) (600 mg/kg daily) containing high phenolic ( $129.877 \pm 0.06$  mg/ g cocoa extract) and flavonoid ( $118.92 \pm 0.01$  mg/g cocoa extract) contents was given to obese-diabetic (Ob-db) induced rats tend to minimize type 2 diabetic condition in 8-weeks of time. As compared to the obese-diabetic (Ob-db) group, increment in body weight and plasma glucose were significantly suppressed for Ob-db with CoPE supplementation. Improvement in lipid profile parameters was also observed with a decrease in total cholesterol (TC), triglyceride (TAG), low density lipoprotein cholesterol (LDL-c), and an elevation in high density lipoprotein cholesterol (HDL-c) levels. However, there was no significant difference in insulin level after 8-weeks of CoPE administration. Oral glucose tolerance test revealed that cocoa supplementation to Ob-db rats significantly

reduced plasma glucose at 60 min by 18% compared to unsupplemented Ob-db rats ( $p < 0.05$ ).

The effects of CoPE on the expression of PPAR- $\gamma$  in adipose tissues and skeletal muscle of Ob-db rats were also evaluated. PPAR- $\gamma$  known as a key gene regulator for diabetes, and became our interest in improving the status of diabetic condition in Ob-db rat's model supplemented with cocoa bean extract. The regulation of target genes by PPAR- $\gamma$  activation induces glucose homeostasis, lipid metabolism and adipogenesis. Results from Immunoblotting protein expression showed an overexpression of PPAR- $\gamma$  in both adipose tissue and skeletal muscle of Ob-db rats supplemented with CoPE. In addition, qRT-PCR results demonstrated that CoPE enhanced PPAR- $\gamma$  mRNA expression in both adipose tissue and skeletal muscle by 10-fold and 6-fold respectively. However, Ob-db group also showed an increased expression of protein and mRNA PPAR- $\gamma$  because of the high levels of free-fatty acids presence that forming a natural PPAR- $\gamma$  ligand without improved any status of diabetic condition.

The results suggest that the anti-diabetic activity of CoPE may result from improvement of glucose level, lipid profiles and weight gain. Insulin sensitivity also been improved as assessed by HOMA-IR status. To gain insight, major polyphenol compounds found in CoPE (catechin, epicatechin, caffeine, theobromine and theophylline) might form an active PPAR- $\gamma$  ligand binding. Thus, the findings may provide a scientific rationale through potential mechanism for the natural anti-diabetic action of CoPE through PPAR- $\gamma$  activation. In addition, PPAR- $\gamma$  activation by CoPE could play a central role in lipid and glucose homeostasis. Thus, the

findings indicated that PPAR- $\gamma$  is one of a molecular target for CoPE by revealing the mechanism of action in the treatment of type 2 diabetes mellitus, hence suggest that CoPE would be effective in preventing and/or ameliorating the metabolic syndrome.



Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**KESAN EKSTRAK KOKO YANG KAYA DENGAN POLIFENOL  
TERHADAP EKSPRESI PPAR GAMMA DALAM TISU LEMAK DAN  
OTOT TIKUS GEMUK YANG MENGHIDAP KENCING MANIS**

Oleh

**FARHANA BINTI AMINUDDIN**

**Disember 2012**

**Pengerusi: Profesor Amin Ismail, PhD**

**Fakulti: Perubatan dan Sains Kesihatan**

Biji *Theobroma cacao* dipercayai mempunyai potensi ke atas aktiviti anti-obesiti dan anti-diabetes kerana fitokimia bioaktif dan kapasiti antioksidan mereka. Pengambilan ekstrak koko yang kaya dengan polifenol (CoPE) (600 mg / kg setiap hari) yang tinggi dengan kandungan fenolik ( $129.877 \pm 0.06$  mg / g koko ekstrak) dan flavonoid ( $118.92 \pm 0.01$  mg / g koko ekstrak) oleh tikus gemuk yang menghidap diabetes (Ob-db) telah mengurangkan kesan kencing manis jenis 2 dalam masa 8 minggu. Jika dibandingkan dengan kumpulan obes-diabetes (Ob-db), kenaikan berat badan dan paras glukosa darah adalah ketara dalam tikus Ob-db yang mendapat rawatan CoPE. Perubahan ke atas profil lipid juga telah dikenalpasti dengan penurunan jumlah kolesterol (TC), trigliserida (TAG), kolesterol lipoprotein ketumpatan rendah (LDL-c), serta peningkatan dalam kolesterol lipoprotein ketumpatan tinggi (HDL-c). Walau bagaimanapun, terdapat perbezaan yang ketara pada tahap insulin selepas 8-minggu pengambilan CoPE. Ujian toleransi glukosa oral membuktikan bahawa pengambilan CoPE ke atas tikus Ob-db telah menurunkan paras glukosa darah pada 60 min

sebanyak 18% berbanding dengan tikus Ob-db yang tidak dirawat ( $p < 0.05$ ).

Kesan CoPE pada ungkapan PPAR  $\gamma$  ke atas tisu adipos dan otot rangka tikus Ob-db juga dikaji. PPAR- $\gamma$  dikenali sebagai pengatur gen utama untuk penyakit kencing manis, dan menjadi kepentingan untuk meningkatkan status penyakit diabetes mellitus dalam tikus Ob-db yang dirawat dengan ekstrak biji koko. Pengaktifan PPAR- $\gamma$  mendorong aktiviti homeostasis glukosa, metabolisme lipid dan adipogenesis. Keputusan dari Immunoblotting ungkapan protein menunjukkan peningkatan ekspresi PPAR- $\gamma$  dalam kedua-dua tisu adipos dan otot rangka tikus Ob-db yang dirawat dengan CoPE. Di samping itu, keputusan qRT-PCR menunjukkan bahawa CoPE meningkatkan ungkapan PPAR- $\gamma$  mRNA dalam kedua-dua tisu adipos dan otot rangka dengan masing-masing 10 dan 6-kali ganda masing-masing. Walau bagaimanapun, kumpulan Ob-db juga menunjukkan peningkatan ungkapan PPAR- $\gamma$  protein dan mRNA disebabkan oleh kehadiran asid lemak bebas yang tinggi yang membentuk pelengkap semulajadi kepada PPAR- $\gamma$  tanpa memperbaiki status penyakit diabetes mellitus jenis 2.

Hasil kajian mencadangkan bahawa aktiviti anti-diabetes oleh CoPE meningkatkan paras glukosa, profil lipid dan berat badan. Sensitiviti insulin juga telah menunjukkan pertambahan yang baik seperti yang dinilai oleh status HOMA-IR. Sebatian polifenol yang ditemui dalam CoPE (catechins, epicatechin, kafein, teobromina dan teofilin) mungkin boleh membentuk PPAR- $\gamma$  pelengkap yang aktif untuk mengikat. Oleh itu, penemuan kajian boleh menyediakan rasional saintifik melalui mekanisme yang berpotensi untuk tindakan semulajadi anti-diabetes melalui pengaktifan PPAR- $\gamma$ . Di samping itu, pengaktifan PPAR- $\gamma$  oleh CoPE boleh memainkan peranan penting



terhadap homeostasis lipid dan glukosa. Oleh itu, kajian menunjukkan bahawa PPAR- $\gamma$  adalah satu sasaran molekular untuk CoPE dengan membuktikan tindakan mekanisme dalam rawatan diabetes mellitus jenis 2, dan dengan itu mencadangkan bahawa CoPE berkesan dalam mencegah dan / atau memperbaiki sindrom metabolik.



## ACKNOWLEDGEMENTS

All praises and thanks to almighty ALLAH SWT, the most merciful, for the blessing and strength to complete this study.

I would like to express my sincere gratitude to my supervisor, Prof. Dr. Amin Ismail for his invaluable advice, support, encouragement, patience and understanding that made this study possible. Not forgetting, supervisory committee members, Assoc. Prof. Dr. Chong Pei Pei and Assoc. Prof. Dr. Muhajir Hamid for their constructive comments during the on-going research work and the preparation of this thesis.

I am sincerely grateful to the financial support by Ministry of Science, Technology and Innovation of Malaysia (National Science Foundation). Special thanks are extended to all members of the Laboratory of Nutrition at Faculty of Medicine and Health Sciences, UPM, Mr. Hasbullah, Mdm. Che Maznah, Mr. Simon and Mr Ramli for their assistance and tremendous help in laboratory work. Not to forget, Dr Cheah Yoke Kqueen for his guidance while doing a RNA work and Ms. Azreen for her guidance in doing protein and RNA analysis.

My heartfelt thanks are also extended to my friends Nurul Nadirah, and Nurul Zarith for their help and support towards the completion of this thesis. And a million thanks to my father, mother, brothers and sister for their love, support and inspiration throughout the period of my study in UPM and my entire life. Lastly, a thousand thanks to all member in POLAR group.

I certify that an Examination Committee has met on 3 December 2012 to conduct the final examination of Farhana Binti Aminuddin on her thesis entitled “Effects of Cocoa Polyphenol-rich Extract on the Peroxisome Proliferator-Activated Receptor Gamma Expression in Adipose and Skeletal Muscle of Obese Diabetic Rats” in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee are as follows:

**Asmah binti Rahmat, PhD**

Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Loh Su Peng, PhD**

Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Norhaizan binti Mohd Esa, PhD**

Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Wan Rosli bin Wan Ishak@Wan Ahmad, PhD**

Professor  
School of Health Sciences  
Universiti Sains Malaysia  
(External Examiner)

---

**SEOW HENG FONG, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 21 March 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Amin Ismail, PhD**

Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Muhajir Hamid, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**Chong Pei Pei, PhD**

Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



**FARHANA BINTI AMINUDDIN**

Date: 3 December 2012

## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	viii
<b>APPROVAL</b>	ix
<b>DECLARATION</b>	xi
<b>LIST OF TABLES</b>	xv
<b>LIST OF FIGURES</b>	xvi
<b>LIST OF ABBREVIATIONS/ANNOTATIONS</b>	xviii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	
1.1 Research Background	1
1.2 Statements of Problem	5
1.3 Significance of the Study	6
1.4 Objectives	8
<b>2 LITERATURE REVIEW</b>	
2.1 Diabetes mellitus	9
2.1.1 History of diabetes mellitus	10
2.1.2 Classification of diabetes	11
2.1.3 Risk factors of developing diabetes	16
2.1.4 Signs and symptoms of diabetes	17
2.1.5 Diagnosis of diabetes	18
2.1.6 Medication of diabetes	22
2.2 Cocoa	
2.2.1 Background of cocoa	24
2.2.2 Health benefits of cocoa and cocoa products	25
2.2.3 Bioactive compounds in cocoa and cocoa products	26
2.3 Peroxisome proliferator-activated receptor (PPAR)	
2.3.1 PPARs and their expression	30
2.3.2 Role of PPAR- $\gamma$ in fighting diseases	33
2.3.3 Novel therapeutic application of TZDs as PPAR- $\gamma$ agonist	35
2.3.4 Natural sources as PPAR- $\gamma$ agonist	37
2.3.5 PPAR- $\gamma$ protein and mRNA expression	38
2.3.6 Detection of PPAR- $\gamma$ gene expression by RT-PCR	39
2.4 Association of PPAR-gamma and Diabetes Mellitus	
2.4.1 Gene variants	41
2.4.2 Genetic basis of Type 2 Diabetes Mellitus	43
2.4.3 PPAR- $\gamma$ and insulin resistance	44
2.4.4 Gene profiling of PPAR- $\gamma$ ligands	45
2.4.5 Potential impact on public health and other Implications.	47

<b>3</b>	<b>MATERIALS AND METHODS</b>	
3.1	Properties of cocoa bean extract and HPLC analysis	
3.1.1	Preparation of cocoa extract	48
3.1.2	Determination of bioactive compounds	49
3.1.3	Determination of total phenolic content	50
3.1.4	Determination of total flavonoid content	50
3.1.5	Ferric reducing antioxidant power assay	51
3.1.6	2,2-diphenyl-2-picrylhydrazyl radical scavenging assay	51
3.2	Effects of cocoa polyphenols-rich extract Supplementation on blood glucose and lipid profiles of Ob-db rats model	
3.2.1	Animal study	52
3.2.2	Oral glucose tolerance test	57
3.2.3	Blood collection for biochemical analysis	57
3.2.4	Determination of lipid profiles, plasma glucose, insulin levels	58
3.2.5	Homeostasis model assessment of insulin resistance index	58
3.3	Expression of PPAR- $\gamma$ protein and mrna on adipose tissue and skeletal muscle of Ob-db rats supplemented with CoPE	
3.3.1	Protein extraction	59
3.3.2	Bicinchoninic acid assay	59
3.3.2	SDS-PAGE electrophoresis and western blot	60
3.3.3	Total RNA extraction	61
3.3.4	RNA quality assessment	62
3.3.5	Quantitative real-time PCR	62
3.4	Statistical analysis	63
<b>4</b>	<b>RESULTS AND DISCUSSIONS</b>	
4.1.1	Bioactive compounds in cocoa extract	64
4.1.2	Total phenolics and flavonoid content	66
4.1.3	Ferric reducing activity based on FRAP assay	68
4.1.4	Scavenging activity on 2,2-diphenyl-2-picrylhydrazyl radical	73
4.2.1	Effects of Cocoa Polyphenols-Rich Extract in Body weight, BMI and food intake of experimental rats	75
4.2.2	Oral glucose tolerance test	78
4.2.3	Glucometabolism parameters and lipid profiles	82
4.3.1	Expression of PPAR- $\gamma$ protein in adipose tissue	91
4.3.2	RNA integrity	97
4.3.3	Expression of PPAR- $\gamma$ mRNA in adipose tissue and skeletal muscle	100

<b>5</b>	<b>SUMMARY, GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH</b>	111
5.1	Summary and General Conclusions	111
5.2	Limitation of Study and recommendations of future research	114
	<b>REFERENCES</b>	116
	<b>APPENDICES</b>	139
	<b>BIODATA OF STUDENT</b>	146
	<b>LIST OF PUBLICATIONS</b>	147

