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

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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

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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

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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 ± 0.06 mg/ g cocoa extract) and flavonoid (118.92 ± 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-γ in adipose tissues and skeletal muscle of Ob-db rats were also evaluated. PPAR-γ 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-γ activation induces glucose homeostasis, lipid metabolism and adipogenesis. Results from Immunoblotting protein expression showed an overexpression of PPAR-γ in both adipose tissue and skeletal muscle of Ob-db rats supplemented with CoPE. In addition, qRT-PCR results demonstrated that CoPE enhanced PPAR-γ 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-γ because of the high levels of free-fatty acids presence that forming a natural PPAR-γ 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-γ ligand binding. Thus, the findings may provide a scientific rationale through potential mechanism for the natural anti-diabetic action of CoPE through PPAR-γ activation. In addition, PPAR-γ activation by CoPE could play a central role in lipid and glucose homeostasis. Thus, the

findings indicated that PPAR-γ 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

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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 ± 0.06 mg / g koko ekstrak) dan flavonoid (118.92 ± 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 γ ke atas tisu adipos dan otot rangka tikus Ob-db juga dikaji. PPAR-γ 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-γ mendorong aktiviti homeostasis glukosa, metabolisme lipid dan adipogenesis. Keputusan dari Immunoblotting ungkapan protein menunjukkan peningkatan ekspresi PPAR-γ 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-γ 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-γ protein dan mRNA disebabkan oleh kehadiran asid lemak bebas yang tinggi yang membentuk pelengkap semulajadi kepada PPAR-γ 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-γ 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-γ. Di samping itu, pengaktifan PPAR-γ oleh CoPE boleh memainkan peranan penting

terhadap homeostasis lipid dan glukosa. Oleh itu, kajian menunjukkan bahawa PPAR-γ 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.

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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.

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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:

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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.

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FARHANA BINTI AMINUDDIN

Date: 3 December 2012

TABLE OF CONTENTS