[illegible] ANTI-INFLAMMATORY EFFECT OF STEVIOSIDE IN RELATION TO CARDIOVASCULAR RISK IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECs)

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Anti-inflammatory effect of stevioside in relation to cardiovascular risk in human umbilical vein endothelial cells (huvecs) / Leong Hui Min.

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ANTI-INFLAMMATORY EFFECT OF STEVIOSIDE IN RELATION TO CARDIOVASCULAR RISK IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECs)

By

Leong Hui Min

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Food Science (Food Service and Nutrition)

DEPARTMENT OF FOOD SCIENCE
FACULTY OF AGROTECHNOLOGY AND FOOD SCIENCE
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ENDORSEMENT

The project report entitled **Anti-inflammatory Effect of Stevioside in relation to Cardiovascular Risk in Human Umbilical Vein Endothelial Cells (HUVECs)** by ***Leong Hui Min***, *Matric No.* ***UK16612*** *has been reviewed and corrections have been* made according to the recommendation of examiners. This report is submitted to the Department of Food Science in partial fulfilment of the requirement of the degree of Bachelor of Food Science (Food Service and Nutrition), Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu.

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(DR. HAYATI BT MOHD YUSOF)

Main supervisor

Date: 29/1/2012

DECLARATION

I hereby declare that the work in this thesis in my own except for quotation and summaries which have been duly acknowledged.

Signature : .....

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Date : 29/1/2012

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ABSTRACT

Inflammation has been implicated in initiation and progression of cardiovascular disease risk. Expression of cell surface adhesion molecule plays an important role in adherence of monocytes or leukocytes which lead to pathogenesis of atherosclerosis. *Stevia rebaudiana* is a traditional medicinal plant in South America which also as regained popularity as natural sweetener. Stevioside is type of diterpenoid glycoside, a bioactive compound which has been reported to exhibit pharmacological effects *in-vivo*, *in-vitro* and human studies, in which include anti-inflammatory effect. In the present study, pre-incubation (prevention) and incubation (treatment) of stevioside on Lipopolysaccharide (LPS)-stimulated Human Umbilical Vein Endothelial cells (HUVECs) were explored its anti-inflammatory effect on the expression of inflammatory marker Vascular Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1(ICAM-1) further investigated. Cell Enzyme-linked Immunosorbent assay (ELISA) was used to assess the VCAM-1 and ICAM-1 expression. MTT cytotoxicity assay was implemented in order to determine the cytotoxicity effect of stevioside in normal primary cells, HUVECs. The present study demonstrated that the optimum LPS condition for expression of VCAM-1 and ICAM-1 were 1.25 μg/mL and 2.5 μg/mL, respectively at 3 hours incubation time. Pretreatment of stevioside at 100 μM for 24 hours pre-incubation time significantly attenuate VCAM-1 expression by 41.4% ($P<0.05$) and 100 μM at 12 hours preincubation time attenuate 28.7% ICAM-1 expression ($P> 0.05$). For treatment of stevioside at 50 μM and 12 hours incubation time attenuate 21.3% VCAM-1 expression ($P> 0.05$) and at 25 μM for 12 hours incubation time (significantly attenuate 36.4% ICAM-1 expression ; $P< 0.05$). Morover, it was found that pre-treatment of stevioside is more effective on VCAM-1 attenuation up to 41.4% compared with treatment. The present study also revealed that stevioside do not cause negative effect (cytotoxicity) in HUVECs. Further study could be carried out in others endothelial cells such as aortic and arterial endothelial cells. In addition, the antioxidant properties of stevioside can be further determined. For modulation of cardiovascular risk, the safety doses of stevioside should be verified in dietary supplement.

ABSTRAK

Kesan Anti-Inflamasi Stevioside berhubung dengan Penyakit Jantung dalam *Human Umbilical Vein Endothelial cells* (HUVECs).

Inflamasi telah terbabit dalam permulaan dan perkembangan kepada risiko penyakit jantung. Pengekpresan molekul protein pada sel permukaan memainkan peranan yang penting dalam pelekatan monosit dan leukosit, dimana menyebabkan patogenesis aterosklerosis. *Stevia rebaudiana* merupakan tumbuhan perubatan tradisional di Amerika Selatan, dimana juga muncul sebagai pemanis semula jadi. Stevioside adalah glycoside diterpenoid, sejenis sebatian bioaktif yang telah dilaporkan mempunyai kesan farmakologi *in-vivo, in-vitro* dan dalam kajian manusia, termasuk kesan anti-inflamasi. Dalam kajian ini, pra-pengeraman (pencegahan) dan inkubasi (rawatan) stevioside dalam HUVECs yang telah dirangsangkan dengan *lipopolysaccharide(*LPS), telah diteroka kesannya terhadap anti-inflamasi pada pengekpresan terhadap penanda inflamasi VCAM-1 dan ICAM-1. *Enzim linked Immunosorbent assay* (*ELISA*) telah digunakan untuk menentukan pengekpresan VCAM-1 dan ICAM-1. *MTT cytotoxicity assay* digunakan untuk menentukan kesan cytotoxicity stevioside dalam sel-sel HUVECs yang normal. Kajian ini menunjukkan bahawa keadaan optimum LPS untuk pengekpresan VCAM-1 dan ICAM-1 dalam HUVECs adalah 1.25 μg/ mL dan 2.5 μg/ mL, masing-masing pada 3 jam masa pengeraman. Pra-rawatan stevioside pada kepekatan 100 μM, 24 jam masa pra-pengeraman dapat mengurangkan pengekpresan VCAM-1 sebanyak 41.4% ($P < 0.05$) dan 100 μM pada 12 jam masa pra-pengeraman dapat mengurangkan 28.7% pengekpresan ICAM-1 ($P > 0.05$). Untuk rawatan stevioside pada kepekatan 50 μM dan 12 jam masa pengeraman dapat mengurangkan 21.3% pengekpresan VCAM-1 ($P > 0.05$) dan pada kepekatan 25 μM untuk 12 jam masa pengeraman (ketara dapat mengurangkan 36.4% pengekpresan ICAM-1; $P < 0.05$). Tambahan pula, didapati bahawa pra-rawatan stevioside adalah lebih berkesan untuk pengurangan pengekpresan VCAM-1 sehingga 41.4% berbanding dengan rawatan stevioside. Kajian ini turut melaporkan bahawa stevioside tidak menyebabkan kesan negative (*cytotoxicity*) dalam HUVECs. Kajian yang lanjut boleh dijalankan di sel-sel endothelial lain seperti sel-sel aortik dan sel-sel arteri endothelial. Di samping itu, sifat antioksidan stevioside boleh dikajikan. Dos stevioside yang selamat perlu ditentukan dalam suplemen untuk modulasi risiko penyakit jantung.