UNIVERSITI TEKNOLOGI MARA

EFFECTS OF THEAFLAVINS RICH FRACTION ON PRO-INFLAMMATORY MARKERS AND OXIDATIVE STRESS IN STIMULATED ENDOTHELIAL CELLS

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of the requirements for the degree of
Master of Science

Faculty of Medicine

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a penel of examiners has met on 21st Jan 2016 to conduct the final examination of Remee Binti Awang Jalil on her Master of Science thesis entitled "Effects Of Theaflavins Rich Fraction On Pro-Inflammatory Markers And Oxidative Stress In Stimulated Endothelial Cells" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The panel of Examiners recommends that the student be awarded the relevant degree. The panel of examiners was as follows:

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Activation of endothelial cells (ECs) occurs early in atherogenesis leading to increased pro-inflammatory environment in the vessels. Oxidative stress also has been linked to atherogenesis, by promoting inflammatory environment and reduced nitric oxide availability in endothelium. Theaflavins rich fractions (TFs-RF) from black tea, *Camellia sinensis* are believed to promote cardio protective benefits. However, their potential therapeutic roles as anti-inflammatory and anti-oxidative in atherogenesis are not well established. The objectives of this study were to investigate (i) the anti-inflammatory effects of TF-RFs on cytokines [Interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α)] , cyclooxygenase-2 (COX-2), endothelial nitric oxide synthase (eNOS) and nitric oxide (NO), and (ii) the anti-oxidative effects of TFs-RF on reactive oxygen species (ROS) activity in stimulated human ECs. Cytotoxicity of TFs-RF (1.6 – 200 μg/ml) towards Human umbilical vein endothelial cells (HUVECs) were assessed by using 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-tetrazolium (MTS) assay. Confluent HUVECs were incubated with 10, 20, 30, 40 and 50 μg/ml of TFs-RF together with lipopolysaccharides(LPS) for 16 hours. Culture medium were collected and measured for IL-6, TNF-α, COX-2 and eNOS protein expression and NO level by using Enzyme-linked immunosorbent assay (ELISA) and colorimetric assay respectively. Cell pellets were extracted for measurement of mRNA expression of IL-6, TNF-α, COX-2 and eNOS by using quantitative Real-Time polymerase chain reaction (qRT-PCR). Culture medium from HUVECs that was incubated with hydrogen peroxide (H_2O_2) and various concentrations of TFs-RF for 16 hours were collected for measured the ROS activity by using intracellular ROS assay kit. TFs-RF ≤50μg/ml showed ≥95% cell viability in MTS assay. In LPS-stimulated HUVECs: TFs-RF 40 and 50 μg/ml significantly reduced IL-6 protein expression; TFs-RF 20 μg/ml significantly reduced gene expression of IL-6 and COX-2; TFs-RF 50 μg/ml significantly reduces protein expression of COX-2; TFs-RF failed to reduce TNF-α expression both in gene and protein level; TFs-RF 10-50 μg/ml significantly increases the protein expression of eNOS; TFs-RF 30 and 50 μg/ml significantly increase eNOS gene expressions; TFs-RF 20-50 μg/ml significantly increase NO production. While in H_2O_2-stimulated HUVECs, TFs-RF 10-50 μg/ml significantly decreases the ROS activity. This study suggests that TFs-RF may prove to be potent therapeutic agent in the treatment of atherosclerosis or in reducing the risk of coronary artery disease (CAD) at inflammation level.

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