UNIVERSITI PUTRA MALAYSIA

ANTI-INFLAMMATORY AND ANTI-OXIDATIVE PROPERTIES OF COCOA ETHANOLIC EXTRACT RICH IN POLYPHENOLS

YAZAN RANNEH

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By

YAZAN RANNEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to both my parents. My father, Mr.KHALED RANNEH, has not only raised and nurtured me but also burdened himself dearly over the years for my education and intellectual development. My mother, Mrs.ZULIKHA AL-MASRI, has been a source of motivation and strength during moments of despair and discouragement. His and her kindhearted have been clearly shown in incredible ways recently. Finally, this thesis is dedicated to all those who believe in the richness of learning.

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

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Chairman: **Norhaizan Mohd Esa, Phd**

Faculty: **Medicine And Health Sciences**

This study aimed to investigate the anti-oxidative and anti-inflammatory properties of ethanolic cocoa extract-rich in polyphenols *in vitro*. It is hypothesized that cocoa extract polyphenols may be benefit for diseases-related to chronic inflammation. The accumulation of macrophage, during the inflammatory state, plays a potent role in releasing pro-inflammatory markers. Additionally, the accumulation of free radicals along with inflammatory state is considered to be the initiative of several diseases such as cancer, atherosclerosis, diabetes and arthritis. Finding a potent agent suppressing the free radicals and pro-inflammatory markers is still required. Polyphenols have been thought to own strong anti-oxidative and anti-inflammatory characteristics. To confirm this assumption, the phenolic compounds of cocoa bean powder were isolated and identified by column chromatography and HPLC. Polyphenolic compounds were then characterized by HPLC-UV-/ESI-MS-MS. Five phenolic compounds were detected namely, catechine, epicatechine, gallic acid, cholorgenic acid and protocatechuic acid. In this study, the scavenging or the TAC (%) (Total Antioxidant Capacity) of cocoa polyphenol (CP) extract that contain 114.0 mg/g of gallic acid ±equivalent phenolics and 94.3 mg/g catechin- equivalent flavonoids were also investigated. Their free radical-scavenging activity was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) DVVD\-carotene bleaching test, and XO (Xanthin Oxidase) inhibitory activity and compared with standard drugs (allopurinol) in different concentrations (5, 10, 20) mg/ml. TAC was further assessed by CP extract against the myoglobin-induced oxidation of ABTS (2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) and expressed as Trolox equivalent in different concentration (100, 200, 300) µM/TE g. CP extract had significantly ($P < 0.05$) potential antioxidant activities with various concentrations. On the other hand, the anti-inflammatory activity of CP extract was assessed with their ability to inhibit the pro-inflammatory enzyme 5-lipooxygenase (5-LOX) using synthetic substrate (Soybean lipoxygenase) and mediators prostaglandin E2 (PGE2), Reactive Oxygen Species (ROS), Nitric Oxide (NO) and Tumor necrosis factor-alpha (TNF-. using

RAW264.7 cells sensitized by lipopolysaccharide (LPS). Our findings indicated that cocoa extract significantly produced inhibition against 5-LOX activity (P< 0.05). In addition, CP extract, in a dose-dependent manner, exhibited a high ability in suppressing the production of ROS, NO, TNF-. DQG 3*(LQ 5 AW 264.7 cells. Conclusively, our *in vitro* study suggest that the improvement of anti-oxidative and inflammatory state is a pivotal action of dietary polyphenols-derived from cocoa beans which in turn provide a rationale application in clinical nutrition practice for treatment of chronic diseases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

CIRI-CIRI ANTI-KERADANGAN DAN ANTI-OKSIDATIF EKSTRAK ETANOL KOKO KAYA DENGAN POLIFENOL

Oleh

YAZAN RANNEH

Januari 2014

Pengerusi: **Norhaizan Mohd Esa, Phd**

Fakulti: **Perubatan Dan Sains Kesihatan**

Kajian ini bertujuan untuk menyelidik ciri-ciri antioksidatif dan anti-keradangan ekstrak etanol koko-kaya dengan polifenol *in vitro*. Polifenol ekstrak koko dihipotesis mempunyai manfaat terhadap penyakit berkait dengan keradangan kronik. Penumpukan makrofaj semasa peringkat keradangan, memainkan peranan penting dalam membebaskan penanda pro-keradangan. Tambahan pula penumpukan bersama-sama dengan radikal bebas di peringkat keradangan dianggap pencetus kepada beberapa penyakit seperti kanser, ateroskleorosis, diabetis dan artritis. Penemuan agen perencatan terhadap radikal bebas dan penanda pro-keradangan yang poten adalah amat diperlukan. Polifenol diandaikan mempunyai ciri-ciri anti-keradangan dan anti-oksidatif yang kuat. Untuk mengesahkan andaian ini, komponen fenolik di dalam serbuk koko, diasingkan dan diidentifikasi dengan menggunakan kromatografi turus dan kromatograpi cecair berkemampuan tinggi (HPLC). Sebatian polifenol kemudian dicirikan melalui HPLC-UV-/ESI-MS-MS. Lima sebatian fenolik dikesan iaitu katekin, epikatekin, asid galik, asid krologenik dan asid protokatuik. Dalam kajian ini, jumlah kapasiti antioksida (TAC) dan kebolehan memerangkap oleh ekstrak koko polifenol (CP) yang mengandungi 114.0 mg asid galik-setara fenolik dan 94.3 mg/g katekin-setara flavonoid ditentukan. Aktiviti pemerangkapan radikal bebas ditentukan melalui assai DPPH (*2,2-diphenyl-1-picrylhydrazyl*), ujian pelunturan -karotene dan aktiviti perencatan xantin oksidase (XO) dan dibandingkan dengan dadah kawalan (allopurinol) dalam kepekatan berbeza (5, 10, 20) mg/ml. TAC ekstrak CP kemudian dinilai lagi melalui keupayaannya dalam menentang pengoksidaan mioglobin yang diaruh oleh 2,2'-azino-di (*3-ethylbenzthiazoline-6-sulfonic acid*) (ABTS) dan dinyatakan sebagai setara Trolox dalam kepekatan berbeza (100, 200, 300) µM/TE g. Ekstrak CP mempunyai potensi aktiviti antioksidan yang signifikan dalam pelbagai kepekatan ($P < 0.05$). Disamping itu, aktiviti anti-keradangan ekstrak CP ditentukan dengan melihat kebolehannya untuk merencatkan enzim pro-keradangan, 5-lipooksigenase (5-LOX) menggunakan substrak sintetik (lipoksigenase kacang soya) dan mediator prostaglandin E2 (PGE2), spesies oksigen reaktif (ROS), oksida nitrik (NO) dan

Tumor necrosis factor-alpha (TNF-. menggunakan sel RAW264.7 yang disensitifkan oleh lipopolisakarida (LPS). Penemuan dari kajian ini menunjukkan bahawa ekstrak koko mampu menghasilkan aktiviti perencatan terhadap 5-LOX secara signifikan (P< 0.05). Tambahan lagi, ekstrak CP yang juga bergantung kepada dos, menunjukkan kebolehan yang tinggi dalam mencegah penghasilan ROS, NO, TNF-. dan PGE2 dalam sel RAW 264.7. Kesimpulannya, kajian *in vitro* ini mencadangkan bahawa dengan memperbaiki anti-oksidatif dan keradangan adalah satu tindakan penting dalam pemakanan polifenol-berasal dari koko yang seterusnya menyediakan aplikasi yang sesuai dalam amalan pemakanan klinikal untuk rawatan penyakit kronik.

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I certify that a Thesis Examination Committee has met on 28 January 2014 to conduct the final examination of YAZAN RANNEH Mattar on his thesis entitled "ANTI-INFLAMMATORY AND ANTI-OXIDATIVE PROPERTIES OF COCOA ETHANOLIC EXTRACT RICH IN POLYPHENOLS" in accordance with the 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 Master of Science.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science.
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DECLARATION

Declaration by the student

I hereby confirm that:

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Declaration by Members of Supervisory Committee

This is to confirm that:

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