ANTIOXIDATIVE AND ANTI-INFLAMMATORY EFFECT OF GAJUS
(ANACARDIUM OCCIDENTALE L.) LEAVES EXTRACT ON
ENDOTHELIAL DYSFUNCTION IN EARLY STAGE OF
ATHEROSCLEROSIS

MOHD KAMAL BIN NIK HASAN

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MASTER OF SCIENCE
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By
MOHD KAMAL BIN NIK HASAN

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

August 2010
DEDICATION

This thesis is especially dedicated to my late father, Nik Hasan bin Nik Yahya, may the blessing of Allah lay upon him. Not forgetting my mother and siblings; thanks for their support and inspiration. My special thanks are due to my extraordinary supervisor, Associate Professor Dr. Zulkhairi bin Hj Amom for giving me the chance to join his research group and enabling me to complete my master’s project. Dr. Zulkhairi’s guidance and dedication will keep inspiring me throughout my life. This work is also dedicated to my lovely cell culture group members; Daryl and Ihsan. Last but not least, I would like to express my heartiest appreciation to my research group members; Sakinah, Fazali, Khairun Nur, Amalina, Hafipah and all colleagues who were involved in this project.
ANTIOXIDATIVE AND ANTI-INFLAMMATORY EFFECT OF GAJUS (ANACARDIUM OCCIDENTALE L.) LEAVES EXTRACT ON ENDOTHELIAL DYSFUNCTION IN EARLY STAGE OF ATHEROSCLEROSIS

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MOHD KAMAL BIN NIK HASAN

August 2010

Chairman: Zulkhairi bin Hj Amom, PhD

Faculty: Medicine and Health Sciences

Anacardium occidentale (A. occidentale), also known as Gajus among Malaysians, is a member of the Anacardiaceae plant family which is widespread in Malaysia. In this study, the effects of A. occidentale leaves aqueous extract (AOE) as antioxidative and anti-inflammatory agent on endothelial dysfunction in the early stage of atherogenesis development was investigated using human vein endothelial cells (HUVECs) and isolated human low density lipoprotein particles (LDL) as models. The inhibitory concentration (IC\textsubscript{50}) of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and the effective concentration (EC\textsubscript{50}) of AOE in preventing H\textsubscript{2}O\textsubscript{2}–induced cell injury were assessed using the MTT assay in order to evaluate cell viability. It was observed that 250 µM of H\textsubscript{2}O\textsubscript{2} reduced cell viability by 50% (IC\textsubscript{50}). Cytotoxic assessment of AOE was performed by exposing the HUVECs to AOE at concentrations ranging from 50 to 700 µg/mL for 24 hr with complete medium. Anacardium occidentale extract was found to be non-toxic to the cells as no IC\textsubscript{50} was obtained. The cells were pretreated with AOE at different
concentrations within the range of 50-700µg/mL for 30 mins followed by 24-hour incubation with \( \text{H}_2\text{O}_2 \) (250 µM). The EC\(_{50}\) of AOE that protected against \( \text{H}_2\text{O}_2 \)-induced cell injury was found to be 180 µg/mL.

The antioxidative and anti-inflammatory effects of AOE on \( \text{H}_2\text{O}_2 \)-induced cell injury were further carried out by seeding HUVECs in 6-well plates and divided them into three groups; positive control, negative control, and treated groups. In the positive control (PC) group, HUVECs were exposed to either 250 µM \( \text{H}_2\text{O}_2 \) or 10 ng/mL TNF-α alone, whereas in the treated groups HUVECs were treated with various concentrations of AOE (100, 180, 250 and 300 µg/mL) for 30 minutes prior to exposure to \( \text{H}_2\text{O}_2 \) (250 µM) or TNF-α (10 ng/mL). In the negative control (NC) groups, HUVECs were incubated with culture medium only. The cells were incubated for 24 hours at 37 °C with 5% CO\(_2\) supply for analysis of NO, NF-κB, VCAM-1, ICAM-1, MMP-9, MCP-1 and M-CSF.

The AOE doses within the concentration range 100-300 µg/mL protected against cellular damage and prevented microsomal lipid peroxidation in \( \text{H}_2\text{O}_2 \)-induced HUVECs as indicated by low MDA levels. The treatment with AOE at concentrations ranging from 250 to 300 µg/mL caused significant reduction in the anti-oxidative enzyme (SOD, GPx and Catalase) activities (p<0.05/p<0.01) with concomitant reduction of NO production in comparison with the PC. Besides that, the expressions of VCAM-1, ICAM-1, MMP-9, MCP-1 and M-CSF in the AOE-treated groups were lowered (p<0.05/p<0.01) whereas NF-κB was inactivated in comparison with the respective expressions in the non-treated counterparts. Furthermore, application of AOE at
concentrations within the range of 300 to 500 µg/mL to the isolated LDL particles prevented the lipid peroxidation processes and protected against LDL oxidation as was indicated by low MDA formation. These findings suggest that AOE possesses antioxidative and anti-inflammatory properties and that it attenuates the initial stage of atherogenesis in vitro. Inhibition of NF-κB activation could be the possible underlying mechanism in modulating early events of atherogenesis.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ANTIOKSIDAN DAN ANTI-INFLAMASI EKSTRAK DAUN GAJUS (ANACARDIUM OCCIDENTALE L.) KE ATAS DISFUNGSI ENDOTELIUM PADA PERINGKAT AWAL ATEROSKLEROSIS

Oleh

MOHD KAMAL BIN Nik Hasan

Ogos 2010

Pengerusi: Zulkhairi Haji Amom, PhD

Fakulti: Perubatan dan Sains Kesihatan

Anacardium occidentale (A. occidentale) atau dikenali sebagai Gajus di kalangan masyarakat Malaysia adalah sejenis tumbuhan dari famili Anacardiaceae yang mana didapati tumbuh di Malaysia. Dalam kajian ini, efek kawalan ekstrak akuas daun A. occidentale (AOE) sebagai agen antioksida dan antiinflamasi dalam aterosklerosis peringkat awal telah dilakukan dengan menggunakan sel endotelial vena uri manusia (HUVEC) dan partikel terpencil lipoprotein berketumpatan rendah (LDL) sebagai model. Ujian pendahuluan ke atas potensi sitotoksik AOE dan kepekatan merencat (IC_{50}) hidrogen peroksida (H_{2}O_{2}) telah digunakan dalam keseluruhan eksprimen dan kepekatan efektif (EC_{50}) AOE untuk mengelak kerosakan sel disebabkan aruhan H_{2}O_{2} di lakukan dengan menggunakan asai MTT untuk mengukur sel yang hidup. HUVEC sebanyak 1 x 10^{6} dihidupkan di dalam plat 96-telaga dan diaruhkan dengan pelbagai kepekatan H_{2}O_{2} (0-700 uM) selama 24 jam. Didapati 250 uM H_{2}O_{2} menyebabkan kematian sebanyak 50 % (IC_{50}). Ujian sitotoksik AOE telah dilakukan dengan mengaruhkan HUVEC dengan AOE pada pelbagai kepekatan berjulat 50 hingga
700 μg/mL selama 24 jam bersama-sama medium komplit. Ekstrak *A. occidentale* didapati tidak toksik kepada sel tersebut kerana tidak didapati bacaan IC₅₀. Dengan menggunakan IC₅₀ H₂O₂ sebagai kontrol positif, sel HUVEC telah di rawat dengan AOE pada kepekatan pelbagai (50-700μg/ml) selama 30 min, seterusnya dieram bersama-sama pengaruhan H₂O₂ (250 μM). Hasil eksperimen didapati EC₅₀ AOE yang mengelak kerosakan sel aruhan H₂O₂ adalah 180 μg/m.

Ujikaji kesan anti-oksidatif dan anti-inflamasi AOE ke atas kerosakan sel aruhan H₂O₂ diteruskan dengan sel HUVEC dihidupkan dalam plat 6- telaga seperti sebelumnya dan dibahagikan kepada tiga kumpulan. Kumpulan kawalan positif diaruhkan dengan 250 μM H₂O₂ ataupun 10 ng/ml TNF-α sendirian manakala kumpulan rawatan AOE telah dimasukkan AOE (100 μg/ml, 180 μg/ml, 250 μg/ml dan 300 μg/ml) selama 30 minit kemudiannya sel didedahkan dengan 250 μM H₂O₂ ataupun 10 ng/ml TNF-α. Kumpulan kawalan negatif hanya dimasukkan medium komplit. Sel itu kemudiannya dimasukkan ke dalam inkubator selama 24 jam pada suhu 37°C dengan bekalan 5% CO₂ untuk analisis NO, NF-κB, VCAM-1, ICAM-1, MMP-9, MCP-1 dan M-CSF. Ekstrak akuas *A. occidentale* (100-300μg/ml) mampu mengelakkan kerosakan sel dan peroksidasi lipid mikrosom di dalam eksperimen HUVEC aruhan- H₂O₂ dengan menunjukkan bacaan MDA rendah. Rawatan 250 to 300 μg/ml AOE menyebabkan aktiviti enzim antioksida menurun (SOD, GPx and Catalase) (p<0.05/p<0.01) bersama-sama kekurangan penghasilan NO dengan signifikan berbanding kawalan positif. Selain itu, pengurangan secara signifikan ekspresi VCAM-1, ICAM-1, MMP-9, MCP-1 dan M-CSF (p<0.05) telah dikesan dalam kumpulan rawatan AOE berbanding kumpulan kawalan positif manakala NF-κB didapati telah dinyahaktif di dalam kumpulan yang
dirawat oleh AOE berbanding kumpulan yang tidak dirawat. Seterusnya, rawatan AOE pada kepekatan 300 hingga 500 µg/ml boleh menyelamatkan partikel LDL terpencil daripada proses oksidasi sepetimana dilihat pada pengurangan pembentukan MDA. Data-data ini mengesyorkan bahawa AOE mengandungi bahan antioksida dan boleh melambatkan proses awal aterogenesis in vitro. Kemampuan AOE menghalang pengaktifan NF-κB adalah mekanisma yang mungkin terlibat dalam mengawalatur proses aterogenesis peringkat awal.
First and foremost, praise be to Allah for His blessing and compassion. I wish to show my gratitude to my supervisor, Associate Professor Dr Zulkhairi Hj Amom. Thank you for your guidance, encouragement, and support. I could not finish this project work without your great and brilliant suggestion. I would also like to thank my extraordinary co-supervisor, Dr. Abdah Md. Akim for her help and caring in helping me to do this thesis. Dr Abdah gave me lots of information in doing this project. No body can do such a hard work without any helps from the others. Here, I also want to say thank you to Professor Dr Daud for trusting me to use his laboratory in order to complete this project. A big gratitude to my great friend, Hafizah Abdul Hamid and her supervisor, Associate Professor Dr Zaiton for her courtesy in helping me to handle HUVEC. Last but not least, special thank you to all the members of Associate Professor Dr Zulkhairi’s group; Daryl, Ihsan, Amalina, Fazali, Khairun Nur Fairuz, Kamilah, Sakinah, Hasnah and all my friends who have supported me in completing this thesis.
I certify that an Examination Committee has met on 10 August 2010 to conduct the final examination of Mohd Kamal Bin Nik Hasan on his Master thesis entitle Antioxidative and Anti-inflammatory Effect of *Anacardium occidentale* Linn. Leaves Extract on Endothelial Dysfunction in Early Stage of Atherosclerosis in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the Master of Science.

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

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Date:
DECLARATION

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

MOHD KAMAL BIN NIK HASAN

Date: 10 August 2010
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ANACARDIUM OCCIDENTALE AQUEOUS EXTRACT ATTENUATES HYDROGEN PEROXIDE-INDUCED LIPID PEROXIDATION ON LOW DENSITY LIPOPROTEIN PARTICLES; A CONDITION THAT OCCURS IN THE INITIAL STAGE OF ATHEROGENESIS

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