



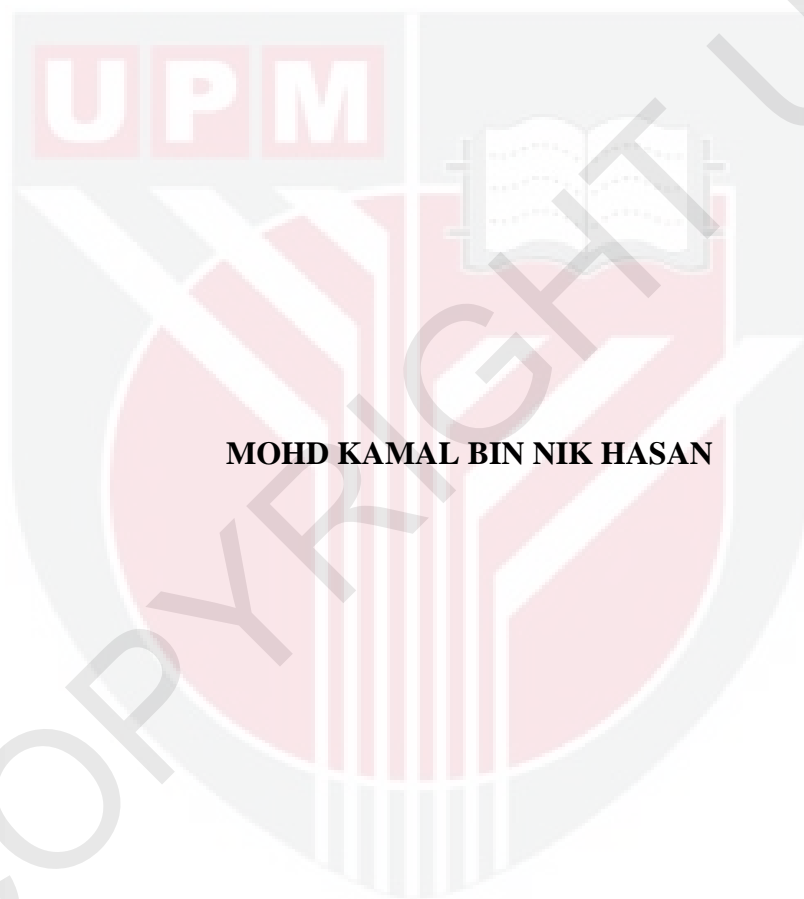
UNIVERSITI PUTRA MALAYSIA

**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

FPSK(m) 2010 19

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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.

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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MOHD KAMAL BIN NIK HASAN

August 2010

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Faculty: Medicine and Health Sciences

Anacardium occidentale (*A. occidentale*), also known as Gajus among Malaysian, 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_{50}) of hydrogen peroxide (H_2O_2) and the effective concentration (EC_{50}) of AOE in preventing H_2O_2 -induced cell injury were assessed using the MTT assay in order to evaluate cell viability. It was observed that 250 μM of H_2O_2 reduced cell viability by 50% (IC_{50}). Cytotoxic assessment of AOE was performed by exposing the HUVECs to AOE at concentrations ranging from 50 to 700 $\mu g/mL$ for 24 hr with complete medium. *Anacardium occidentale* extract was found to be non-toxic to the cells as no IC_{50} was obtained. The cells were pretreated with AOE at different

concentrations within the range of 50-700 μ g/mL for 30 mins followed by 24-hour incubation with H₂O₂ (250 μ M). The EC₅₀ of AOE that protected against H₂O₂- induced cell injury was found to be 180 μ g/mL.

The antioxidative and anti-inflammatory effects of AOE on H₂O₂-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 μ M H₂O₂ 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 μ g/mL) for 30 minutes prior to exposure to H₂O₂ (250 μ M) 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% CO₂ supply for analysis of NO, NF- κ B, VCAM-1, ICAM-1, MMP-9, MCP-1 and M-CSF.

The AOE doses within the concentration range 100-300 μ g/mL protected against cellular damage and prevented microsomal lipid peroxidation in H₂O₂-induced HUVECs as indicated by low MDA levels. The treatment with AOE at concentrations ranging from 250 to 300 μ g/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- κ B 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 $\mu\text{g/mL}$ 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- κ B 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

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Pengerusi: Zulkhairi Haji Amom, PhD

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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 antioksidan dan antiinflamasi dalam aterosklerosis peringkat awal telah dilakukan dengan menggunakan sel endotelial vena uri manusia (HUVEC) dan partikel terencil lipoprotein berketumpatan rendah (LDL) sebagai model. Ujian pendahuluan ke atas potensi sitotoksik AOE dan kepekatan merencat (IC_{50}) hidrogen peroksida (H_2O_2) telah digunakan di dalam keseluruhan eksperimen dan kepekatan efektif (EC_{50}) AOE untuk mengelak kerosakan sel disebabkan aruhan H_2O_2 dilakukan dengan menggunakan asai MTT untuk mengukur sel yang hidup. HUVEC sebanyak 1×10^6 dihidupkan di dalam plat 96-telaga dan diaruhkan dengan pelbagai kepekatan H_2O_2 (0-700 μM) selama 24 jam. Didapati 250 μM H_2O_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 pengaruh 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 antioksidasi 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 $\mu\text{g/ml}$ boleh menyelamatkan partikel LDL terpencil daripada proses oksidasi sepertimana dilihat pada pengurangan pembentukan MDA. Data-data ini mengesyorkan bahawa AOE mengandungi bahan antioksidan dan boleh melambatkan proses awal aterogenesis *in vitro*. Kemampuan AOE menghalang pengaktifan NF- κ B adalah mekanisme yang mungkin terlibat dalam mengawal proses aterogenesis peringkat awal.

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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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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.

The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. It features a red and white design with a central vertical element and a book at the top. The letters 'UPM' are prominently displayed in the upper left corner of the shield.

MOHD KAMAL BIN NIK HASAN

Date: 10 August 2010

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