UNIVERSITI PUTRA MALAYSIA

IN VITRO ANTI-ATHEROGENIC EFFECTS OF ASIATIC ACID IN HUMAN AORTIC ENDOTHELIAL CELLS

FONG LAI YEN

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

IN VITRO ANTI-ATHEROGENIC EFFECTS OF ASIATIC ACID IN HUMAN AORTIC ENDOTHELIAL CELLS

By

FONG LAI YEN

November 2016

Chair : **Zuraini Ahmad, PhD**

Faculty : **Medicine and Health Sciences**

In early pre-lesional stage of atherosclerosis, endothelial cell activation is characterized by increased endothelial permeability, increased expression of cell adhesion molecules (CAMs), leukocyte adhesion and migration across the endothelium. Asiatic acid is a major triterpene isolated from *Centella asiatica* (L.) Urban and it has been shown to possess anti-oxidant, hypolipidemia and anti-inflammatory activities. This study aimed to investigate protective effects of asiatic acid on TNF-α-induced early atherogenic events, in the context of endothelial cell activation, using human aortic endothelial cells (HAECs). Fluorescein isothiocyanate (FITC)-dextran permeability assay, U937 monocyte adhesion and migration assays were examined using fluorescence-based methods. The levels of soluble CAMs were measured using multiplex kits and flow cytometry. Localization of filamentous (F)-actin, diphosphorylated myosin light chain (diphospho-MLC), adherens junctions (AJs) and tight junctions (TJs) in cells were investigated using immunocytochemistry and confocal microscopy. Total protein expression of CAMs, diphospho-MLC, vascular endothelial (VE)-cadherin, β-catenin, occludin, zona occludens (ZO)-1 and phosphorylated inhibitors of κB (p-IκB-α) were determined using western blot analysis. The expression of AJ and TJ proteins in membrane, cytosolic and cytoskeleton fractions were also determined using western blot analysis. Asiatic acid significantly suppressed TNF-α-induced endothelial hyperpermeability, but did not reduce the increased monocyte adhesion and migration. Asiatic acid also inhibited VCAM-1 expression and production of soluble CAMs (sE-selectin, sICAM-1, sVCAM-1 and sPECAM-1). Besides, asiatic acid prevented TNF-α-induced redistribution of F-actin but failed to alter the increased F/G actin ratio. By using cytochalasin D, an actin depolymerizing agent, asiatic acid was demonstrated to stabilize peripheral F-actin filaments. Yet, asiatic acid did not improve cytochalasin D-induced increased permeability. Furthermore, asiatic acid localized diphospho-MLC filaments at the cell periphery and significantly augmented TNF-α-induced increased MLC diphosphorylation. Asiatic acid also prevented TNF-α-induced reticular AJ disruption by enhancing junctional areas covered by VE-cadherin and β-catenin. This protective effect was found to be independent of changes in either total amount or intracellular redistribution of VE-cadherin and β-catenin. For TJs, confocal imaging showed that asiatic acid opposed TNF-α-induced loss of ZO-1 from the cell borders.

However, asiatic acid did not alter both total occludin and ZO-1 expressions in whole cell lysate. Subcellular fractionation demonstrated that asiatic acid inhibited TNF-α-induced ZO-1 internalization from membrane to cytoplasm and occludin redistribution from cytoplasm to cytoskeleton. In addition, asiatic acid suppressed TNF-α-induced phosphorylation of IκB-α. These results suggest that asiatic acid protects against TNF-α-induced endothelial barrier disruption and reduces the release of soluble CAMs, which are important biomarkers for the risk prediction of cardiovascular diseases. Asiatic acid also stabilizes the cytoskeleton by localizing peripheral F-actin and diphospho-MLC filaments. However, the actin stabilization is not essential for barrier protective effect of asiatic acid. The barrier stabilizing effect of asiatic acid is found concomitant with enhancement of reticular AJ formation, inhibition of TJ redistribution and suppression of NF-κB activation. In conclusion, a novel protective role of asiatic acid in TNF-α-induced endothelial barrier dysfunction was demonstrated. This reveals new therapeutic usage of asiatic acid in the prevention of early atherosclerosis, which involves endothelial activation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN ANTI-ATEROGENIK ASIATIC ASID PADA SEL ENDOTELIAL AORTA MANUSIA *IN VITRO*

Oleh

Fong Lai Yen

November 2016

Pengerusi : Zuraini Ahmad, PhD

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Pada peringkat awal aterosklerosis di mana sebelum pembentukan lesi dimulakan, pengaktifan sel endotelial dicirikan oleh peningkatan ketelapan endotelial, peningkatan pengekspresan molekul-molekul lekatan dalam sel dan penambahan kelekatan serta penghijrahan leukosit melintasi lapisan endotelium. Asiatic asid adalah salah satu triterpena yang diasingkan daripada *Centella asiatica* (L.) Urban dan aktiviti-aktiviti anti-pengoksidaan, hipolipidemik dan anti-keradangannya telah dilaporkan. Kajian ini bertujuan untuk mengkajikan kesan perlindungan asiatic asid terhadap peristiwa awal aterogenik yang diaruhkan oleh TNF-α, dalam konteks pengaktifan sel endotelial, dengan menggunakan sel endotelial aorta manusia. Asai ketelapan fluoresein isotiosianat-dekstran, asai-asai kelekatan dan penghijrahan monosit U937 telah dinilaikan oleh keadah pendarfluor. Paras molekul-molekul lekatan dalam sel yang larut disukatkan oleh alat multipleks dan analisis sitometri. Penempatan F-aktin, rantai ringan miosin yang terfosforil, simpang adherens, simpang ketat dalam sel disiasatkan oleh teknik immunositokimia dan analisis mikroskop confocal. Analisis pemendapan Western digunakan untuk menentukan pengekspresan jumlah protein molekul-molekul lekatan dalam sel, rantai ringan miosin yang terfosforil, VE-cadherin, β-catenin, occludin, ZO-1 dan p-IκB-α. Analisis pemendapan western juga digunakan untuk menentukan pengekspresan protein simpang adherens dan simpang ketat di bahagian membran, sitosol dan sitoskeleton. Asiatic asid mengurangkan hiperketelapan endotelial yang diaruhkan oleh TNF-α dengan nyata sekali, tetapi ia tidak dapat menahankan kelekatan dan penghijrahan monosit yang diaruhkan oleh TNF-α. Asiatic asid juga mencegahkan pengekspresan VCAM-1 dan penghasilan molekul-molekul lekatan dalam sel yang larut (sE-selectin, sICAM-1, sVCAM-1 and sPECAM-1). Selain itu, asiatic asid mencegahkan penaburan semula F-aktin yang diaruhkan oleh TNF-α, tetapi ia gagal untuk mengubahkan peningkatan nisbah F/G-aktin. Dengan menggunakan sitokalasin D, salah satu ejen yang menyebabkan aktin depolimerisasi, ia telah menunjukkan bahawa asiatic asid menstabilkan filamen-filamen F-aktin di pinggiran sel. Akan tetapi, asiatic asid tidak mempertingkatkan hiperketelapan endotelial yang diaruhkan oleh sitokalasin D. Tambahan pula, asiatic asid mengekalkan filamen-filamen rantai ringan miosin yang terfosforil di pinggiran sel dan menambahkan lagi dipemfosforilan rantai ringan miosin yang diaruhkan oleh TNF-α.

Asiatic asid juga mencegahkan gangguan simpang retikuler yang diaruhkan oleh TNF-α dengan menambahkan kawasan simpang yang diliputi oleh VE-cadherin dan β-catenin. Ia didapati bahawa kesan perlindungan ini tidak bergantung kepada perubahan jumlah keseluruhan dan penaburan semula VE-cadherin dan β-catenin intrasel. Bagi simpang ketat, pengimejan sefokus menunjukkan bahawa asiatic asid menentangkan kehilangan ZO-1 dari sempadan-sempadan sel yang diaruhkan oleh TNF-α. Walau bagaimanapun, asiatic asid tidak mengubahkan pengekspresan jumlah protein ZO-1 dan occludin dalam lisat keseluruhan sel. Pemeringkatan protein subsel menunjukkan bahawa asiatic asid merencatkan penyebatian ZO-1 dari membran ke sitoplasma dan penaburan semula occludin dari sitoplasma ke sitoskeleton yang diaruhkan oleh TNF-α. Tambahan pula, asiatic asid menahankan pemfosforilan IκB-α yang diaruhkan oleh TNF-α. Data ini mencadangkan bahawa asiatic asid melindungi endotelium daripada gangguan sekatan endotelial yang diaruhkan oleh TNF-α dan mengurangkan penghasilan molekul-molekul lekatan dalam sel yang larut, di mana molekul-molekul tersebut adalah penanda biologi yang penting untuk meramalkan risiko penyakit-penyakit kardiovaskular. Asiatic asid juga menstabilkan sitoskeleton dengan mengekalkan F-aktin dan filamen rantai ringan miosin yang terfosforil di sempadan sel. Akan tetapi, penstabilan aktin oleh asiatic asid adalah tidak mustahak bagi kesan perlindungan sekatan endotelial-nya. Ia didapati bahawa kesan penstabilan sekatan endotelial asiatic acid adalah seiring dengan penambahbaikan pembentukan simpang retikuler, perencatan penaburan semula simpang ketat and pengurangan pengaktifan NF-κB. Kesimpulannya, satu peranan novel asiatic acid di mana ia melindungi endotelium daripada disfungsi sekatan endotelial yang diaruhkan oleh TNF-α telah ditunjukkan. Kajian ini mendedahkan penggunaan terapeutik asiatic asid yang baru dalam pencegahan peringkat awal aterosklerosis yang melibatkan pengaktifan endotelial.

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I certify that a Thesis Examination Committee has met on 23 November 2016 to conduct the final examination of Fong Lai Yen on her thesis entitled "*In Vitro* Anti-Atherogenic Effects of Asiatic Acid in Human Aortic Endothelial Cells" 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AJ	Adherens Junction
CAM	Cell Adhesion Molecule
diphospho-MLC	Diphosphorylated Myosin Light Chain
F-actin	Filamentous-Actin (Polymer Form)
G-actin	Globular-Actin (Monomer Form)
HAECs	Human Aortic Endothelial Cells
HUVECs	Human Umbilical Vein Endothelial Cells
ICAM-1	Intercellular Adhesion Molecule-1
JAM	Junctional Adhesion Molecule
MLC	Myosin Light Chain
NF-κB	Nuclear Factor Kappa B
PECAM-1	Platelet Endothelial Cell Adhesion Molecule-1
p-IκB-α	Phosphorylated Inhibitor Of κb
sE-selectin	Soluble E-Selectin
sICAM-1	Soluble Intercellular Adhesion Molecule-1
sPECAM-1	Soluble Platelet Endothelial Cell Adhesion Molecule-1
sVCAM-1	Soluble Vascular Cell Adhesion Molecule-1
TJ	Tight Junction
TNF-α	Tumour Necrosis Factor-alpha
TTFCA	Total Triterpenic Fraction Of *Centella asiatica*
VCAM-1	Vascular Cell Adhesion Molecule-1
VE-cadherin	Vascular Endothelial-Cadherin
ZO-1	Zona Occludens-1

CHAPTER 1

INTRODUCTION

1.1 Justification of the Study

Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and it serves as a primary approach used to prevent and treat atherosclerosis. However, long-term statin therapy causes various side effects including muscle weakness or pain, abnormal elevation of liver enzymes, acute renal failure and diabetes (Simic and Reiner, 2015; Alla *et al.*, 2013). Besides, patients undergoing statin therapy continue to experience recurrent cardiovascular events (Alla *et al.*, 2010). Owing to these adverse effects, the searching for novel anti-atherogenic compounds from plant-based sources could provide alternative therapeutic options for the treatment of cardiovascular diseases.

Despite that the anti-atherosclerotic effects of *Centella asiatica* (L.) Urban, a traditional medicinal herb, have been demonstrated in several clinical studies, the experimental studies intended to investigate the *in vitro* anti-atherosclerotic effect of *C. asiatica* remain widely lacking. Particularly, how each active constituent of *C. asiatica* would contribute to its anti-atherosclerotic effect and the mechanism of action remain unknown. Hence, detailed mechanistic study is needed in order to support the increasing use of *C. asiatica* as a potential anti-atherogenic agent.

1.2 Background of the Study

Ischaemic heart disease and cerebrovascular disease are the first and third leading causes of death, respectively, throughout the world. These cardiovascular diseases had a mortality rate of 247.9 deaths/100,000 persons, which accounts for 84.5 % of death from all types of cardiovascular diseases and 28.2% of global death in 2013 (Barquera *et al*., 2015; Collaborators., 2015). As a result, many approaches have been adopted to reduce the occurrence and severity of cardiovascular diseases, which include lifestyle modifications, advancement of surgical operations and introduction of new therapeutic agents. Atherosclerosis is the major cause leading to cardiovascular diseases and it is closely related to elevated plasma concentration of low density lipoprotein-cholesterol (Ramji and Davies, 2015). Atherosclerosis is characterized by build-up of plaques or lesions at the arterial wall, which gradually narrows the lumen of the blood vessel.

It is now widely accepted that atherosclerosis is a chronic inflammatory disease initiated by 'activation' of endothelial cells, which promotes the switch of endothelium to a pro-inflammatory phenotype (Funk *et al.*, 2012). The key features of endothelial cell activation include an increase in endothelial permeability, up regulation of cell adhesion molecules (CAMs) and recruitment of monocytes to adhere to the endothelium. Subsequently, the adhered monocytes migrate through the endothelium and accumulate at the arterial intima to form lipid-engulfing foam cells. Foam cell accumulation features the beginning of atherosclerotic lesion formation (Ramji and

Davies, 2015). Tumour necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, was detected in human atherosclerotic plaques, implying the crucial role of TNF-α in the progression of atherosclerosis (Mehta and Malik, 2006). Therefore, targeting TNF-α-activated inflammatory pathways might be a promising therapeutic strategy for atherosclerosis.

In physiological conditions, the endothelial permeability is tightly regulated by interendothelial junctions that connect the neighbouring endothelial cells together. Inflammatory stimuli trigger intercellular junctions to disassemble and disrupts the endothelial barrier integrity (Capaldo and Nusrat, 2009). Consequently, an increase in permeability occurs where passage of solutes and fluid across the endothelium is increased. Atherogenesis is initiated when endothelial hyperpermeability permits transport of low density lipoproteins into the intima. Therefore, improvement of the endothelial barrier function may serve as a novel therapeutic approach to prevent early pre-lesional stage of atherosclerosis.

The cell-cell junctions are anchored by actin cytoskeleton in the cytoplasm where the latter modulates stability of the intercellular junctions. Actin cytoskeleton is a dynamic structure undergoes continuous remodelling to regulate cell contraction, cell shape and motility. Phosphorylation of myosin light chain (MLC) promotes actin cytoskeleton to rearrange into stress fibers that are capable to generate contractile force and pull the intercellular junctions inward, causing disassembly of the cell-cell junctions (Bogatcheva and Verin, 2008). CAMs, such as intercellular adhesion molecule-(ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and platelet-endothelial cell adhesion molecule-1 (PECAM-1), also affect the formation of junctional complexes and are key determinants of the endothelial barrier integrity (Marcos-Ramiro *et al.*, 2014).

C. asiatica is a traditional medicinal herb that can be found in most tropical and subtropical countries. It is widely used in traditional Ayurvedic or Indian Medicine to enhance memory, heal wounds and alleviate various skin diseases such as leprosy and psoriasis (Gohil *et al.*, 2010). It is noteworthy to mention that *C. asiatica* has been shown to exhibit anti-atherosclerotic effects in several clinical studies (Belcaro *et al.*, 2015b; Belcaro *et al.*, 2014; Cesarone *et al.*, 2001a; Incandela *et al.*, 2001). Total triterpenic fraction of *C. asiatica* (TTFCA), a highly refined *C. asiatica* extract containing asiatic acid as a main component, has been shown to stabilize arterial plaques in atherosclerotic patients (Cesarone *et al.*, 2001a; Incandela *et al.*, 2001). Recently, researchers have reported that combined administration of a pine bark extract and TTFCA prevents the progression of atherosclerotic plaques in asymptomatic subjects and the anti-atherosclerotic effect is associated with reduction of oxidative stress (Belcaro *et al.*, 2015b; Belcaro *et al.*, 2014). Besides, *C. asiatica* has also been demonstrated to improve capillary permeability and prevent tissue edema in hypertensive patients (De Sanctis *et al.*, 2001; Belcaro *et al.*, 1990).

Asiatic acid is a major saponin triterpenoid derived from *C. asiatica*. It has been reported that asiatic acid possesses several biological activities including anti-inflammatory, wound healing, neuroprotective and hypolipidemic effects (Ramachandran *et al.*, 2014; Bian *et al.*, 2013; Huang *et al.*, 2011; Gohil *et al.*, 2010). Researchers have also shown that asiatic acid suppresses carrageenan-induced paw edema in mice (Huang *et al.*, 2011). However, the anti-atherogenic effect of asiatic acid *in vitro*, particularly in the context of endothelial activation has not been reported yet.

1.3 Hypotheses

Asiatic acid will possess protective effects against TNF-α-induced endothelial activation. Asiatic acid will inhibit TNF-α-induced disruption of endothelial barrier integrity and the increased interaction between leukocytes and endothelial cells. Asiatic acid will also inhibit TNF-α-induced increased expression of CAMs and cytoskeletal rearrangement. Besides, asiatic acid will also prevent TNF-α-induced disruption of cell-cell junctions. In addition, asiatic acid will suppress activation of signaling cascades stimulated by TNF-α.

1.4 Objectives

1.4.1 General Objective

The aim of this study was to evaluate effects of asiatic acid on TNF-α-induced activation of human aortic endothelial cells and the underlying mechanisms.

1.4.2 Specific Objectives

This study aimed to investigate effects of asiatic acid on TNF-α-induced

(i) endothelial hyperpermeability and increased leukocyte-endothelial cell interactions

(ii) increased expression of CAMs

(iii) cytoskeletal rearrangement

(iv) disassembly of interendothelial junctions

(v) activation of signaling pathway

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BIODATA OF STUDENT

Ms. Fong Lai Yen was born on 9th July 1988 in Kuala Lumpur, Malaysia. She received her primary education from Sekolah Rendah Jenis Kebangsaan (C) Chen Moh, Petaling Jaya, Selangor (1995 – 1999). Then, she continued her secondary education at Sekolah Menengah Jenis Kebangsaan Katholik, Petaling Jaya, Selangor (2000-2004). She also completed her Form Six (pre-university education) at the same high school.

She obtained her first degree in Bachelor of Science (Biomedical Science) from Universiti Putra Malaysia in 2011. She has developed an interest in biomedical research during her final year project, in which she gained her basic laboratory skills such as animal handling and performing bioassays. She decided to further her Doctor of Philosophy (PhD) study in Universiti Putra Malaysia, of which her research project is mainly focusing on prevention of early atherogenesis by using active compounds isolated from medicinal plants.

Ms. Fong Lai Yen was granted the MyBrain15 Scholarship (MyPhD) by the Ministry of Education Malaysia to pursue her PhD study. Besides, her research project was also granted by the Malaysia Toray Science Foundation (MTSF). She attended and presented her papers in several international and local conferences throughout her PhD study. She has also been working as a research assistant in Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for one year.

LIST OF PUBLICATIONS

Journals

Fong, L. Y., Ng, C. T., Cheok, Z. L., Mohd Moklas, M. A., Hakim, M. N. and Ahmad, Z. (2016). Barrier protective effect of asiatic acid in TNF-alpha-induced activation of human aortic endothelial cells. *Phytomedicine*, 23(2): 191-199.

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Proceedings/Conference Papers

LY Fong, CT Ng, Z Ahmad. (2015). Effects of asiatic acid on TNF-α-induced vascular inflammatory events in human aortic endothelial cells. Proceedings of the 8th Congress Federation of the Asian and Oceanian Physiological Societies (FAOPS), pg S-A106. 22th-25th of November, 2015, Bangkok, Thailand.

LY Fong, CT Ng, Z Ahmad. (2015). Asiaticoside reduces TNF-α-induced increased soluble platelet endothelial cell adhesion molecule -1 (sPECAM-1) levels in human aortic endothelial cells. Abstracts of 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA 2015), Budapest, Hungary. Published in *Planta medica* 81(16)-PW_115.

LY Fong, CT Ng, Z Ahmad. (2014). Asiaticoside inhibits tumor necrosis factor alpha (TNFα)-induced soluble adhesion molecules expression in human aortic endothelial cells (HAEC). Abstracts of 62nd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA 2014), Guimaraes, Portugal. Published in *Planta Medica* 80-P1L107.

CT Ng, LY Fong, Z Ahmad. (2014). Asiatic acid exhibits anti-inflammatory activities in human aortic endothelial cells. Abstracts of 62nd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Guimaraes, Portugal. Published in *Planta Medica* 80-P1L23.

LY Fong, CT Ng, Z Ahmad. (2014). Inhibitory Effect of Asiaticoside on Endothelial Barrier Dysfunction induced by Tumor necrosis factor-α (TNF-α). Abstracts of 6th Scientific Meeting of the Asian Society for Vascular Biology, Kuala Lumpur, Malaysia. Published in *Journal of Vascular Research* 51(Suppl 1):9.

LY Fong, CT Ng, Z Ahmad. (2013). *In vitro* anti-atherogenic effects of asiaticoside on human aortic endothelial cells. Abstracts of 12nd meeting of the Asia Pacific Federation of Pharmacologists, Shanghai, China. Published in *Acta Pharmacological Sinica*, 34 Supp:60

Conferences Attended

Effects of asiatic acid on TNF-α-induced vascular inflammatory events in human aortic endothelial cells (2015). Oral presentation at the 8th Congress Federation of the Asian and Oceanian Physiological Societies (FAOPS), 22-25th of November, held in Bangkok, Thailand.

Asiaticoside reduces TNF-α-induced increased soluble platelet endothelial cell adhesion molecule -1 (sPECAM-1) levels in human aortic endothelial cells (2015). Poster presentation at the 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2015), 23–27th of August, held in Budapest, Hungary.

Asiaticoside inhibits tumor necrosis factor alpha (TNFα)-induced soluble adhesion molecules expression in human aortic endothelial cells (2014). Poster presentation at the 62nd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2014), 31st of August–4th of September, held in Guimaraes, Portugal.

Inhibitory Effect of Asiaticoside on Endothelial Barrier Dysfunction induced by Tumor necrosis factor-α (2014). Poster presentation at the 6th Scientific Meeting of The Asian Society for Vascular Biology, 22-24th of August, held in Kuala Lumpur, Malaysia.

In vitro anti-atherogenic effects of asiaticoside in endothelial cells (2013). Poster presentation at the 27th Scientific Meeting of Malaysian Society of Pharmacology and Physiology (MSPP), 6-8th of September, held in Pahang, Malaysia.

In vitro anti-atherogenic effects of asiaticoside in endothelial cells (2013). Poster Presentation at the 12th meeting of Asia Pacific Federation of Pharmacologists (MAPFP), 9-13th of July, held in Shanghai, China.

Awards

Young scientist award from the 8th Congress Federation of the Asian and Oceanian Physiological Societies (FAOPS), 22-25th of November 2015, held in Bangkok, Thailand on 22-25th of November, 2015

Travel award from the Society for Medicinal Plant and Natural Product Research (GA) to attend the 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA 2015) held in Budapest, Hungary on 23–27th of August 2015.

Financial aid from Universiti Putra Malaysia to attend the 62nd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2014), held in Guimaraes, Portugal on 31st of August–4th of September, 2014

Travel award from Asia Pacific Federation of Pharmacologists (APFP) to attend the 12th meeting of APFP held in Shanghai, China on 9–13rd July 2013

Research Grants Received

Science & Technology Research Grant (by Malaysia Toray Science Foundation, 2012) - RM15,000

Research University Grant Scheme 2012 (by Universiti Putra Malaysia) – RM10,000