

UNIVERSITI TEKNOLOGI MARA

**THE MECHANISM OF ANDROGRAPHOLIDE AS
ANTIATHEROGENIC AGENT AGAINST
ATHEROSCLEROSIS INDUCED BY
PORPHYROMONAS GINGIVALIS - AN
EXPERIMENTAL STUDY IN RABBIT**

RAMI AL BATRAN

Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Dentistry

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a panel of examiners has met on 30th June 2014 to conduct the final examination of Rami Al Batran on his Doctor of Philosophy thesis entitled “The Mechanism of Andrographolide as Antiatherogenic Agent Against Atherosclerosis Induced by *Porphyromonas Gingivalis* - An Experimental Study in Rabbit” 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:

Mohamed Ibrahim Abu Hassan, PhD
Professor
Faculty of Dentistry
Universiti Teknologi MARA
(Chairman)

Kalavathy Ramasamy, PhD
Associate Professor
Faculty of Pharmacy
Universiti Teknologi MARA
(Internal Examiner)

Zubaidah Jubaidah Binti Haji Abdul Rahim, PhD
Professor
Faculty of Dentistry
Universiti Malaya
(External Examiner)

Khalid Almas, PhD
Professor
Faculty of Dentistry
University of Connecticut
(External Examiner)

SITI HALIJAH SHARIFF, PhD
Associate Professor
Dean
Institute of Graduates Studies
Universiti Teknologi MARA
Date: 18th July, 2014

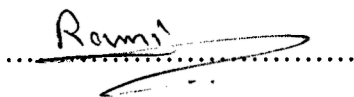
AUTHOR'S DECLARATION

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.

Name of Student : Rami Al Batran
Student ID No. : 2010788613
Programme : Doctor of Philosophy (DS990)
Faculty : Faculty of Dentistry

Thesis Title : The Mechanism of Andrographolide as
Antiatherogenic Agent Against
Atherosclerosis Induced by *Porphyromonas*
Gingivalis - An Experimental Study in Rabbit

Signature of Student : 
Date : July 2014

ABSTRACT

Atherosclerosis has been widely accepted as an inflammatory disease of the vascular system. An association between atherosclerosis and *Porphyromonas gingivalis* (*Pg*), a major periodontopathogen, has been shown. The aim of the present study was to evaluate the mechanism of the anti-atherogenic effect of andrographolide (AND) on atherosclerosis induced by *Pg* in male white New Zealand rabbits. The level of acute toxicity of AND has been assessed in *Sprague Dawley* (SD) rats and no sign of toxicity either through clinical or histopathological examination. Thirty rabbits were used and divided into five groups (six rabbits for each group) as follows: Group 1 stand as normal group; Groups 2-5 were orally challenged with *Pg* ATCC 33277 (0.2 mL of 1.5×10^{12} bacterial cells/mL in 2% CMC with PBS) five times; Group 2 stand as control group; Group 3 received atorvastatin (AV, 5 mg/kg), and Groups 4-5 received 10 and 20 mg/kg of AND, respectively, over 12 weeks. Rabbits in the control group (G2) were challenged only with *Pg* over 12 weeks developed a significant progression of atherosclerosis compared with the normal group (G1). Rabbits treated with AV and AND had significantly lower ($p < 0.05$) LDL and total cholesterol (TC) compared with the control group (G2). Meanwhile high-density lipoprotein (HDL) showed a significant increase ($p < 0.05$) compared to the control group (G2). The study also showed a significant reduction in lipid peroxidation index indicated by a low TBARs-MDA level ($p < 0.05$) in the groups treated with AV and AND compared to the control group (G2). The histopathology analysis of rabbits' aorta presented with thick foam cell formation in the control group (G2). However, there were fewer foam cell formations in the group treated with AV and AND. The kidney and liver analyses showed a lesser infiltration of inflammatory cells in the groups treated with AV and AND. On the other hand, AND improved the enzymatic activity of (SOD, CAT, GPx and GSH) in the groups treated with AV and AND compared to the control group (G2) due to its potent antioxidant activity. Further, AND reduced TNF α , IL-1 β , IL-6 and CRP levels in treated groups compared to the control group (G2). *Pg* 16S ribosomal DNA was used to detect *Pg* DNA in the rabbits' aorta and the results showed that *Pg* DNA amplification was higher in the control group (G2), while mild DNA amplification was seen in the groups treated with AV and AND. Protein expression (α -SMA) of the aortas of the groups treated with AV and AND showed mild expression of α -SMA protein compared to the control group (G2). This was supported by immunohistochemical examination of α -SMA protein. In conclusion, the feeding of 10 or 20 mg/kg of AND was able to inhibit and reduce the progression of atherosclerotic plaque development induced by *Pg*. That could be due to two main mechanisms: first, the anti-inflammatory mechanism involved in the reduction of inflammatory cytokines: and second, the potent antioxidant properties of AND.

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