UNIVERSITI PUTRA MALAYSIA

ANTI-ATHEROGENIC AND ANTIOXIDANT ACTIVITIES OF PATAWALI (TINOSPORA CRISPA) ON RABBITS FED WITH HIGH CHOLESTEROL DIET

HASNAH BAHARI

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By

HASNAH BAHARI

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

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DEDICATION

To my lovely parents, brother and sisters for giving inspiration and passion to pursue my study in this field. Special appreciation also to my mentor, Dr. Zulkhairi Amom who had contributed greatly towards my career development and personal growth. My longlife best friend, Miss Nursakinah Isemaail for all her effort and dedication in my journey to achieve Masters in Physiology. Last but not least, to myself the greatest motivator I have ever known.

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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July 2008

Chairman : Zulkhairi bin Haji Amom, PhD

Faculty :Medicine and Health Sciences

Tinospora crispa is a wild plant in Malaysia. Malay community used this plant traditionally for various therapeutic purposes. Despite many studies conducted on its antidiabetic and antipyretic effects, no study has been designed to address the question whether *T.crispa* could work as an antioxidant and lipid lowering agent. The aim of this experiment is to study the lipid lowering and antioxidant effects of *T.crispa* in rabbits fed with high cholesterol diet.

The *in vitro* study was conducted to determine the total antioxidant activity of *T.crispa* aqueous extract. The scavenging activity of *T.crispa* measured using DPPH analysis was 85.95±0.52%. The antioxidant activity of *T.crispa* measured via TBA test and FRAP assay was 39.20±2.97% and 0.89±0.03mmol/L respectively. The types of flavonoids found in *T.crispa* were catechin, luteolin, morin and rutin.

Thirty male New Zealand White rabbits were randomly divided into six groups for the *in vivo* studies. The normal control (NC) group was fed 100g/rabbit/day normal rabbit chow. Positive control (PC) and Simvastatin control (SC) groups were fed high cholesterol diet (HCD) (0.5% cholesterol). The SC group was supplemented 5 mg/kg/day of Simvastatin. The treatment groups (T150, T300 and T450) were fed with HCD and 150, 300 and 450 mg/kg/day of *T.crispa* extract respectively. The experimental period was designed for 10 weeks. Ear vein blood sampling were collected at week 0 (w0), week 5 (w5) and week 10 (w10) for plasma analysis. At the end of the experiment, the animals were sacrificed via exsanguinations, the aorta were excised and examined for histomorphometric analysis.

Through plasma analysis, the activity of gamma glutamyl transferase (GGT), aspartate amino transferase (AST) and alanine amino transferase (ALT) were significantly lower ($p<0.05$) in group T450 at w10 compared to group PC. Group T150 and T300 had a significantly lower ($p<0.05$) total cholesterol (TC) level compared to PC at w10. Group T450 had a significantly higher ($p<0.05$) TC level against PC. All groups supplemented with *T.crispa* had a significantly higher ($p<0.05$) in high density lipoprotein (HDL) level and significantly lower ($p<0.05$) LDL level compared to PC at w10. The TG level of T150 and T450 were significantly decreased ($p<0.05$) from w5 to w10. Activity of catalase (CAT) in T150, T300 and T450 were significantly lower ($p<0.05$) at w10 compared to PC. Group T450 had significantly higher ($p<0.05$) total antioxidant activity (TAA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities compared to PC at w10. No foam cell formation was visible in aorta of rabbits in group

NC, SC and T450. However, there were visible foam cell formation in the aorta of group PC, T150 and T300.

In conclusion, this study suggested that supplementation of 450 mg/kg of *T.crispa* extract would be able to reduce or retard the progression of atherosclerotic plaque development induced by dietary cholesterol. The enhanced serum HDL, increase in antioxidant status and flavonoids composition may be the possible underlying mechanisms of antiatherogenic effect of *T.crispa.*

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

KESAN ANTI-ATEROGENIK DAN ANTIOKSIDAN OLEH PATAWALI (TINOSPORA CRISPA) TERHADAP ARNAB YANG DIBERI DIET TINGGI KOLESTEROL

Oleh

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Tinospora crispa merupakan sejenis tumbuhan yang tumbuh meliar di Malaysia. Masyarakat Melayu menggunakan tumbuhan ini secara tradisional untuk pelbagai tujuan perubatan. Walaupun banyak kajian telah dijalankan untuk mengkaji kesannya sebagai antidiabetik dan antipiretik, tiada kajian terhadap kesannya sebagai antioksidan dan agen penurun lipid. Matlamat kajian ini dijalankan adalah untuk mengkaji kesan *Tinospora crispa* sebagai agen perendah lipid dan antioksidan pada arnab yang diberi diet tinggi kolesterol.

Kajian *in vitro* telah dilakukan untuk menentukan aktiviti antioksidan ekstrak akuas *T.crispa.* Keputusan DPPH menunjukkan penghapusan radikal bebas oleh *T.crispa* ialah 85.95±0.52%. Keputusan TBA dan FRAP masing-masing menunjukkan aktiviti antioksida *T.crispa* sebanyak 39.20±2.97% dan 0.89±0.03mmol/L. Catechin, luteolin, morin dan rutin merupakan jenis-jenis flavonoid yang dikenalpasti dalam *T.crispa*.

Untuk kajian *in vivo*, tiga puluh arnab jantan baka New Zealand dengan berat badan purata 2.5-3.0 kg dibahagi secara rawak kepada 6 kumpulan. Kumpulan NC, PC dan SC adalah kumpulan kawalan negatif dan positif yang diberi makanan standard arnab; diet tinggi kolesterol (makanan standard arnab dicampurkan dengan 0.5% kolesterol; dan diet tinggi kolesterol (HCD) dengan 5mg/kg simvastatin masing-masing. Kumpulan T150, T300 dan T450 bertindak sebagai kumpulan rawatan melalui pemberian diet tinggi kolesterol (HCD) bersama suplemen 150, 300 dan 450mg/kg ekstrak *T.crispa* masing-masing. Jangkamasa kajian adalah selama 10 minggu. Darah dikumpul melalui vein telinga pada minggu 0 (m0), 5 (m5) dan 10 (m10) dan plasma digunakan untuk analisis biokimia. Aorta diasingkan untuk penelitian selanjutnya

Aktiviti gamma glutamyl transferase (GGT), aspartate amino transferase (AST) dan alanine amino transferase (ALT) menunjukkan aras yang rendah secara signifikan ($p<0.05$) di dalam T450 pada m10 berbanding PC. Kumpulan T150 dan T300 menunjukkan aras TC yang rendah secara signifikan ($p<0.05$) berbanding PC pada m10. Manakala, pada m10, T450 menunjukkan peningkatan aras TC secara signifikan ($p<0.05$) berbanding PC. Kesemua kumpulan yang diberi *T.crispa* menunjukkan peningkatan aras kolesterol lipoprotein ketumpatan tinggi (HDL) dan penurunan aras LDL secara signifikan ($p<0.05$) berbanding PC pada m10. Kadar TG di dalam 150CTC dan 450CTC menurun secara signifikan ($p<0.05$) dari m5 hingga m10 berbanding PC. Aktiviti katalase (CAT) di dalam T150, T300 dan T450 adalah rendah secara signifikan ($p<0.05$) pada m10 berbanding PC. Kumpulan T450 menunjukkan peningkatan aktiviti total antioksida (TAA), gluthatione peroxidase (GSH-Px) dan superoxide dismutase

(SOD) secara signifikan ($p<0.05$) berbanding PC pada m10. Tiada pembentukan sel busa kelihatan pada aorta arnab dalam NC, SC dan 450CTC, tetapi ia kelihatan pada aorta arnab yang diberi diet tinggi kolesterol dan pada dua kumpulan rawatan yang diberi suplemen 150 dan 300mg/kg *T.crispa.*

Sebagai kesimpulan, kajian ini mencadangkan suplemen ekstrak *T.crispa* sebanyak 450mg/kg mungkin boleh mengurangkan atau menghentikan pembentukan plak aterosklerosis terhasil dari makanan mengandungi tinggi kolesterol. Peningkatan HDL, status antioksida dan komposisi flavonoid mungkin merupakan penyumbang kepada kesan *T.crispa* sebagai anti-aterogenik.

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In the name of ALLAH most gracious and most merciful, as through his blessings I have been able to complete this thesis successfully as required. This thesis is the end of my long journey in obtaining my Master of Science in Physiology.

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Finally, my special gratitude is due to my father, my mother, my sisters, my brother and their families for their loving support.

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

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Date: 13 November 2008

I certify that an Examination Committee has met on 9th July 2008 to conduct the final examination of Hasnah binti Bahari on his Master of Science thesis entitled "Anti-atherogenic and Antioxidant Effects of *Tinospora crispa* on Rabbits Fed With High Cholesterol Diet" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

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Date: 25 February 2008

DECLARATION

I declare that the thesis is based on 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 UPM or at any institution.

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HASNAH BINTI BAHARI

Date:

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LIST OF ABBREVIATIONS

%	Percentage
$^{\cdot}OH$	Hydroxyl Radical
<	Less than
=	Equal to
>	More than
±	Approximately or about
µm	Micrometer
µmol	Micromol
ANOVA	Analysis Of Variance
BHT	Butylated hydroxy toluene
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
et al.	Et alia
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
FRAP	Ferric Reducing Antioxidant Power
FRIM	Forest Research Institution Malaysia
FTC	Ferric Thiocyanate
GAE	Gallic Acid Equivalent
GSH-Px	Glutathione Peroxidase
H_2O_2	Hydrogen peroxide
HDL	High density lipoprotein
HPLC	High Performance Liquid Chromatography
IP	Percentage of Inhibition
LDL	Low density lipoproteins
M	Molar
MDA	Malondialdehyde
mg	Milligram
min	Minute
mL	Milliliter
mmol	Millimole
Mmol/L	Milimolar per Liter
nm	Nanometer
O_2	Oxygen molecule
$O_2^{\cdot -}$	Superoxide radical
ºC	Degree Celsius
OD	Optical density
OxLDL	Oxidized LDL
P	Probability
PBS	Phosphate Buffer Saline

ROS	Reactive Oxygen Species
Rpm	Rate per minute
SD	Standard deviation
SOD	Superoxide Dismutase
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric acid active compounds
TC	Total cholesterol
TCA	Trichloroacetic acid
TG	Triglyceride
ug/ul	Microgram per microlitre
VLDL	Very low density lipoprotein
α	Alpha
CAT	Catalase

CHAPTER 1

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease (Ross, 1993) and the major cause of cardiovascular problems (James, 2004). It is characterised by the accumulation of lipid in the artery wall. The common risk factors for atherosclerosis are hypercholesterolemia, hypertension and cigarette smoking (James, 2004). The risk of atherosclerosis increases with excessive rise in the concentration of the important class of lipoproteins, known as low density lipoprotein (LDL) (Franco and Cinzia, 2003).

Studies in hypercholesterolemic animal models indicate that oxidation of LDL is likely to play an important role in atherogenesis. High levels of cholesterol is one of the major risk factor for the development of cardiovascular diseases (CVD) including atherosclerosis, myocardial infarction, heart attacks and cerebral paralysis (Wald and Law, 1995). Decreased of cholesterol level in blood, particularly the atherogenic lipoproteins (very low density lipoprotein, VLDL and LDL) and an increase in protective high density lipoprotein (HDL) fraction can be considered as beneficial for cardiovascular disease prevention (Catherine *et al*., 2004). At present, there is a great deal of interest in the clinical benefit associated with raising high density lipoprotein (HDL) levels. This is partly because HDL has been shown to be a strong, independent and inverse risk factor for coronary heart disease (CHD) (Yusuf *et al.*, 2004).

Free radicals are defined as any molecules or atoms with one or more unpaired electrons. With the possession of the unpaired electrons, free radicals are usually