EFFECTS OF "JIN BATU" (STROBLANTHES CRISPUS) EXTRACT ON SERUM LIPID PROFILE AND ANTIOXIDANT STATUS OF HYPERCHOLESTEROLEMIA-INDUCED RABBITS

NURHAFZAN ANIS BINTI ISMAIL

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2007
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By

NURHAFZAN ANIS BINTI ISMAIL

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

February 2007
To my beloved husband, mummy and papa with love.
Hypocholesterolemic, anti-atherogenic, toxicity effects and changes in the antioxidant status of *Strobilanthes crispus* extract (SCE) in atherogenically induced animal model were studied. As much as, 58.05 ± 1.08% total dietary fiber (TDF), 54.61 ± 3.92% insoluble dietary fiber (IDF) and 6.01 ± 0.82% soluble dietary fibers (SDF) was found in the *Strobilanthes crispus* (SC) ground, dried leaves (AOAC method). At concentration of 0.32-5.12 mg/ml, SCE showed 3.76 ± 7.45% to 56.72 ± 2.49% scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals with EC$_{50}$ of 2.21 mg/ml. The total flavonoids content in SC fresh leaves and ground, dried leaves were 0.99 ± 0.04 % and 0.46 ± 0.06 %, respectively. Quercetin was found highest in SC ground, dried leaves, followed by kaempferol, luteolin and rutin while kaempferol was found highest in SCE, followed by quercetin, luteolin and rutin (HPLC method). Twenty-four adult female New Zealand white rabbits (1.8-2.5 kg) were randomly assigned into four groups (n=6/group) and fed with normal diet; negative control (NC),
0.25% high cholesterol diet (HCD); positive control (PC), 0.25% HCD + SCE (0.2% of diet); SC and 0.25% HCD + simvastatin (SV) (20 mg/kg body weight); SV for 12 weeks. Food and water were given ad libitum. Blood samples were biweekly drawn for serum lipid profiles, alanine amino transferase (ALT), gamma glutamic transpeptidase (GGT), urea, creatinine, conjugated diene (CD), malondialdehyde (MDA) levels of serum, liver, kidney, heart and brains. After being killed, ascending aorta, liver, kidney and heart tissue specimens were excised immediately and prepared for the histopathological studies. PC group showed significantly increased (p<0.05) TC, LDL and HDL levels, HDL/TC ratio, LDL/HDL ratio, atherogenic index, CD levels, serum, heart, liver and kidney MDA levels, and relative liver weight. Significantly (p<0.05) elevated HDL and reduced kidney MDA levels were observed in SC group, while significantly reduced (p<0.05) TC, LDL, heart, liver and serum MDA levels found in SV group. Massive macrophages, foam cells and atheroma plaque formation were detected, which slightly increased intima to media ratio and thickened the PC group’s aorta. Moderate to fairly intense lymphocyte infiltration, slight macrovesicular lipid droplets, edematous hepatocytes, inconsistent binucleated cells and vacuolated cytoplasm were found in PC group’s liver. Significantly prevented (p<0.05) atheroma plaque formation, both treatment groups showed lesser aortas’ thickening, irregular mild edematous hepatocytes, binucleated cells, vacuolated cytoplasm and lipid droplets inhibition. Though mild lymphocyte infiltration was found in SV, such changes were almost invisible in SC group. Briefly, SC plant may possess the hypocholesterolemic, anti-atherogenic and hepatoprotective effect due to the antioxidative properties of flavonoids, i.e. quercetin, kaempferol, luteolin, rutin and catechin.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN EKSTRAK JIN BATU (STROBLANTHES CRISPUS) TERHADAP PROFIL LIPID DAN STATUS ANTIOKSIDAN SERUM ARNAB TERARUH HIPERKOLESTEROLEMIA**

Oleh

**NURHAZFAN ANIS BINTI ISMAIL**

**Februari 2007**

**Pengerusi: Profesor Maznah Ismail, PhD**

**Fakulti**: Perubatan dan Sains Kesihatan

Kesan hipokolesterolemik, anti-aterogenik, ketoksikan dan perubahan status antioksidan ekstrak *Strobilanthes crispus* (SCE) terhadap model haiwan diaruh aterogenik dikaji. Sebanyak 58.05 ± 1.08% serat diet jumlah (TDF), 54.61 ± 3.92% serat diet tak larut (IDF) dan 6.01 ± 0.82% serat diet larut (SDF) telah diperolehi di dalam daun kering SC terkisar (kaedah AOAC). Pada kepekatan 0.32-5.12 mg/mL, SCE telah menunjukkan sebanyak 3.76 ± 7.45% hingga 56.72 ± 2.49% kesan perencatan terhadap radikal 1,1-difenil-2-pirrilhidrazil (DPPH) dengan EC50=2.21 mg/mL. Jumlah kandungan flavonoid yang terdapat di dalam daun *Strobilanthes crispus* (SC) segar dan kering terkisar ialah masing-masing sebanyak 0.99 ± 0.04% dan 0.46 ± 0.06%. Kuersetin didapati tertinggi di dalam daun kering SC terkisar, diikuti oleh kemferol, luteolin dan rutin, manakala kemferol didapati tertinggi di dalam ekstrak kasar SC diikuti oleh kuersetin, luteolin dan rutin (kaedah HPLC). Sebanyak 24 ekor arnab putih betina dewasa New Zealand (1.8-2.5 kg) telah dibahagikan secara rawak kepada empat kumpulan (n=6/kumpulan); dan
diberikan diet normal; kawalan negatif (NC), 0.25% diet berkolesterol tinggi (HCD); kawalan positif (PC), 0.25% HCD + SCE (0.2% daripada diet); SC dan 0.25% HCD + simvastatin (20 mg/kg berat badan); SV, selama 12 minggu. Arnab-arnab tersebut diberi makanan dan minuman tanpa halangan. Sampel darah diambil pada setiap dua minggu bagi analisis profil lipid, alanin aminotransferase (ALT), gamma glutamil transpeptidase (GGT), urea, kreatinin, diene terkonjugat (CD) serum dan malondialdehid (MDA) dalam serum, hepar, ginjal, jantung dan otak. Setelah dibunuh, spesimen tisu aorta, hepar, ginjal dan jantung diambil dengan segera dan disediakan bagi kajian histopatologi. Kumpulan PC menunjukkan peningkatan kolesterol jumlah (TC), lipoprotein berketumpatan rendah (LDL) dan nisbah lipoprotein berketumpatan tinggi kepada kolesterol jumlah (HTR), nisbah LDL/HDL, indeks aterogenik, kepekatan MDA serum, jantung, hepar dan ginjal, kepekatan CD serum serta berat hepar relatif yang signifikan (p<0.05). Peningkatan paras lipoprotein berketumpatan tinggi (HDL) dan juga pengurangan paras MDA ginjal secara signifikan (p<0.05) telah didapati oleh kumpulan SC. Manakala kumpulan SV menunjukkan kesan penurunan paras TC, LDL, MDA jantung, hepar dan serum secara signifikan (p<0.05). Sejumlah besar makrofaj, sel buih dan lesi aterosklerosis telah dikekan, yang meningkatkan nisbah intima terhadap media dan menebalkan aorta kumpulan PC. Penyerapan limfosit dari yang sederhana ke agak padat, titisan kecil lipid makrovesikular, hepatosit beredema, kehadiran sel dengan dua nukleus dan sitoplasma bervakuol yang tidak konsisten dilihat pada kumpulan PC. Dengan perncatan pembentukan plak ateroma yang signifikan (p<0.05), kedua-dua kumpulan rawatan menunjukkan aorta yang kurang menebal, sedikit hepatosit beredema, sel dengan dua nukleus dan sitoplasma bervakuol yang tidak
konsisten dan perencatan titisan lipid. Walaupun penyerapan limfosit yang rendah telah dikesan di dalam kumpulan SV, perubahan tersebut hampir tidak kelihatan di dalam kumpulan SC. Kesimpulannya, tumbuhan SC mungkin memiliki ciri-ciri hipokolesterolemik, anti-aterogenik dan perlindungan hepar disebabkan ciri-ciri antioksidatif flavonoid, contohnya kuersetin, kemferol, luteolin dan rutin serta katekin.
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I certify that an Examination Committee has met on 15th February, 2007 to conduct the final examination of Nurhafzan Anis binti Ismail on her Master of Science thesis entitled "Effects of "Jin Batu" (Strobilanthes crispus) Extract on Serum Lipid Profile and Antioxidant Status of Hypercholesterolemia-Induced Rabbits" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby 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 or concurrently submitted for any other degree at UPM or other institutions.

NURHAFZAN ANIS BINTI ISMAIL

Date: 27 APRIL 2007
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>ix</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xx</td>
</tr>
</tbody>
</table>

## CHAPTERS

### 1 INTRODUCTION

1.1 Research background 1
2.1 Objectives 6
3.1 Contribution of the study 7

### 2 LITERATURE REVIEW

2.1 Introduction to lipid 8
2.2 Lipoproteins 10
   2.2.1 Chylomicrons 12
   2.2.2 Very Low Density Lipoprotein (VLDL) 13
   2.2.3 Low Density Lipoprotein (LDL) 13
   2.2.4 High Density Lipoprotein (HDL) 14
2.3 Cholesterol 15
2.4 Bile acids 17
2.5 Atherosclerosis 19
   2.5.1 Atherosclerosis risk factors 20
   2.5.2 Development of atherosclerosis 24
2.6 Hypercholesterolemia and toxicity 27
   2.6.1 Alanine Amino Transferase (ALT) 27
   2.6.2 Gamma-Glutamyl Transpeptidase (GGT) 29
   2.6.3 Urea 30
   2.6.4 Creatinine 32
2.7 Free radicals and lipid peroxidation 33
2.8 Hypercholesterolemia and antioxidants 36
   2.8.1 Endogenous antioxidant 37
   2.8.2 Dietary antioxidant 39
   2.8.3 Flavonoids 40
2.9 3-Hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors 42
2.9.1 Relationship with atherosclerosis 43
2.10 Atherosclerosis and experimental animals 46
2.11 Strobilanthes Crispus 48

3 MATERIALS AND METHODS 51
3.1 Materials 51
3.1.1 Strobilanthes Crispus 51
3.1.2 Chloroform:Methanol (2:1) extraction 51
3.2 Chemicals and reagents 52
3.2.1 Quantification of Total Dietary Fiber (TDF) 52
3.2.2 Quantification of the scavenging effect of Strobilanthes Crispus extract on DPPH radical 52
3.2.3 Quantification of the flavonoids by using HPLC method 53
3.2.4 Quantification the Total Flavonoids by using two complimentary method 53
3.2.5 In vivo study 54
3.3 Quantification of Total Dietary Fiber (TDF) 55
3.4 Quantification of the scavenging effect of Strobilanthes Crispus extract on DPPH radical 55
3.5 Quantification of the flavonoids by using HPLC method 56
3.6 Quantification the Total Flavonoids by using two complimentary method 57
3.6.1 Aluminium Chloride Colorimetric method 58
3.6.2 2,4-Dinitrophenylhydrazine Colorimetric method 58
3.7 In vivo study 59
3.7.1 Animals 59
3.7.2 Ethical approval 59
3.7.3 Experimental design 60
3.7.4 Preparation of experimental diets 63
3.7.5 Lipid profile analysis 63
3.7.6 Total Antioxidant Status Analysis (TAS) 67
3.7.7 Toxicity profile 67
3.7.8 Lipid peroxidation 71
3.7.9 Evaluation of atheroslerotic plaque 73
3.7.10 Histological analysis by light microscopy 74
3.8 Statistical analysis 77

4 RESULTS AND DISCUSSION 78
4.1 Total Dietary Fiber (TDF) 78
4.2 Scavenging effect of Strobilanthes Crispus extracts on DPPH radicals 80
4.3 Flavonoids content by HPLC 84
4.4 Total flavonoids content 91
4.5  *In vivo* study

4.5.1  Daily food consumption

4.5.2  Weekly body weight

4.5.3  Relative brain, heart, kidney and liver weight

4.5.4  Lipid profile

4.5.5  Total Antioxidant Status (TAS) level

4.5.6  Toxicity profile

4.5.7  Lipid peroxidation

4.5.8  Atherosclerosis plaque evaluation

4.5.9  Aortic histological quantitative and qualitative assessment

4.5.10  Liver, heart and kidney histological qualitative assessment

5  CONCLUSION AND RECOMMENDATIONS

REFERENCES  184

APPENDICES  212

BIODATA OF THE AUTHOR  224
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Nutritional composition of normal rabbit pellet</td>
<td>60</td>
</tr>
<tr>
<td>3.2</td>
<td>Tissue dehydration in a tissue processor machine (TP1020)</td>
<td>75</td>
</tr>
<tr>
<td>3.3</td>
<td>Colouration with Hematoxyline and Eosin (H&amp;E)</td>
<td>76</td>
</tr>
<tr>
<td>4.1</td>
<td>Fiber content of <em>Strobilanthes crispus</em> leaves (%)</td>
<td>78</td>
</tr>
<tr>
<td>4.2</td>
<td>The Effective Concentration (EC$_{50}$) values of all tested samples</td>
<td>83</td>
</tr>
<tr>
<td>4.3</td>
<td>The amount of flavonoids in both <em>Strobilanthes crispus</em> ground, dried leaves and <em>Strobilanthes crispus</em> crude extracts</td>
<td>87</td>
</tr>
<tr>
<td>4.4</td>
<td>Comparison of Flavonoid Content (%) of fresh and dried leaves using aluminium chloride (AlCl$_3$) colorimetric method and 2,4-dinitrophenylhydrazine (2,4-D) colorimetric method</td>
<td>94</td>
</tr>
<tr>
<td>4.5</td>
<td>Relative Organ Weight (ROW)</td>
<td>101</td>
</tr>
<tr>
<td>4.6</td>
<td>The extent of atherosclerosis of the thoracic aorta as indicated by the mean percentage of the lesion area</td>
<td>140</td>
</tr>
<tr>
<td>4.7</td>
<td>Histology analysis of the rabbits' aorta</td>
<td>145</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The structure of lipoprotein</td>
<td>11</td>
</tr>
<tr>
<td>2.2</td>
<td>Model for lipoprotein transport in humans</td>
<td>11</td>
</tr>
<tr>
<td>2.3</td>
<td>Molecular structure of cholesterol</td>
<td>17</td>
</tr>
<tr>
<td>2.4</td>
<td>Molecular structure of cholic acid</td>
<td>18</td>
</tr>
<tr>
<td>2.5</td>
<td>Molecular structure of chenodeoxycholic acid</td>
<td>18</td>
</tr>
<tr>
<td>2.6</td>
<td>Molecular structure of deoxycholic acid</td>
<td>19</td>
</tr>
<tr>
<td>2.7</td>
<td>Molecular structure of lithocolic acid</td>
<td>19</td>
</tr>
<tr>
<td>2.8</td>
<td>Various stages of atherosclerotic lesions in comparison with the normal artery wall</td>
<td>26</td>
</tr>
<tr>
<td>2.9</td>
<td>Mechanism of lipid autoxidation</td>
<td>34</td>
</tr>
<tr>
<td>2.10</td>
<td>Molecular structure of flavonoids</td>
<td>41</td>
</tr>
<tr>
<td>2.11</td>
<td>Molecular structure of flavans</td>
<td>41</td>
</tr>
<tr>
<td>3.1</td>
<td>Experimental design of in vivo study</td>
<td>61</td>
</tr>
<tr>
<td>4.1</td>
<td>Scavenging effect of methanolic extracts from Strobilanthes crispus on DPPH radicals with different concentrations</td>
<td>81</td>
</tr>
<tr>
<td>4.2</td>
<td>Calibration curve of quercetin (peak area)</td>
<td>84</td>
</tr>
<tr>
<td>4.3</td>
<td>Calibration curve of rutin (peak area)</td>
<td>85</td>
</tr>
<tr>
<td>4.4</td>
<td>Calibration curve of kaempferol (peak area)</td>
<td>85</td>
</tr>
<tr>
<td>4.5</td>
<td>Calibration curve of luteolin (peak area)</td>
<td>86</td>
</tr>
<tr>
<td>4.6</td>
<td>Molecular structure of quercetin</td>
<td>88</td>
</tr>
<tr>
<td>4.7</td>
<td>Molecular structure of rutin</td>
<td>89</td>
</tr>
</tbody>
</table>
4.8 Molecular structure of kaempferol 90
4.9 Molecular structure of luteolin 91
4.10 Calibration curve of quercetin (absorbance) 93
4.11 Calibration curve of naringenin (absorbance) 93
4.12 Average food consumption during treatment 96
4.13 Rabbits weekly body weight (Kg) 98
4.14 Total Cholesterol (TC) level for each group 102
4.15 HDL level for each group 104
4.16 LDL level for each group 106
4.17 TG level for each group 107
4.18 LDL/HDL ratio for each group 109
4.19 HDL/TC ratio for each group 110
4.20 Atherogenic Index (AI) for each group 112
4.21 TAS level in each group 117
4.22 ALT level in each group 120
4.23 GGT level in each group 121
4.24 Urea level for each group 125
4.25 Creatinine level for each group 127
4.26 Malondialdehyde (MDA) level in rabbits serum 131
4.27 MDA level in the heart, liver, brain and kidney for each group 133
4.28 Conjugated Diene (CD) level in rabbits serum 135
4.29 Representative photograph of initial surface of the thoracic aortas from NC and PC groups 142
4.53 Cardiac tissue of a PC group 166
4.54 Cardiac tissue of an SC group 167
4.55 Cardiac tissue of an SC group 168
4.56 Cardiac tissue of an SV group 169
4.57 Cardiac tissue of an SV group 170
4.58 An NC group rabbit’s renal cortex 171
4.59 An NC group rabbit’s renal medulla 172
4.60 A PC group rabbit’s renal cortex 173
4.61 A PC group rabbit’s renal medulla 174
4.62 A PC group rabbit’s renal cortex 175
4.63 An SC group rabbit’s renal cortex 176
4.64 An SC group rabbit’s renal medulla 177
4.65 An SV group rabbit’s renal cortex 178
4.66 An SV group rabbit’s renal medulla 179
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxyl toluene</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>CD</td>
<td>Conjugated Diene</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma Glutamyltranspeptidase</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl Coenzyme A</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HTR</td>
<td>High Density Lipoprotein to Total Cholesterol Ratio</td>
</tr>
<tr>
<td>IDF</td>
<td>Insoluble Dietary Fiber</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>LM</td>
<td>Light Microscopy</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>NC</td>
<td>Negative Control</td>
</tr>
<tr>
<td>PC</td>
<td>Positive Control</td>
</tr>
<tr>
<td>SDF</td>
<td>Soluble Dietary Fiber</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>SV</td>
<td>Simvastatin</td>
</tr>
<tr>
<td>SC</td>
<td><em>Strobilanthes crispus</em></td>
</tr>
<tr>
<td>TAS</td>
<td>Total Antioxidant Status</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric Acid</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric Acid Reactive Substances</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>TDF</td>
<td>Total Dietary Fiber</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Research Background

The World Health Organization (WHO) attributes 12 million deaths a year worldwide to cardiovascular diseases (CVD). The disease involves disorders of the blood circulation system and pathological changes in blood vessels associated mainly with the heart and brain (Cruez, 2005). Among the specific cardiovascular disease, ischaemic heart disease (IHD), often called coronary heart disease (CHD), and ischaemic stroke (insufficient blood flow to a region of the brain) accounted for the main causes of mortality.

According to a study by Khoo et al. (1991), in 1950 CVD was the third biggest killer in Malaysia. Twenty years later, the disease has emerged as the number one killer in Malaysia. In this new millennium era, CVD remains as prevalent as ever. The disease has been reported as the most deadly disease in developing countries (WHO Monica Project, 1988). The dietary pattern in these countries have become westernized after rapid growth of their economies. For instance, according to a data from the Nutrition Society of Malaysia, in 1961 Malaysians consumed 70% vegetables and 30% animal products. However, in 1997 Malaysians were eating 45% vegetables and 55% animal products. There has been an increase in the consumption of refined carbohydrates such as simple sugars in coffee, cookies, cakes and increase in fats in the diet, especially
saturated fats from animal sources. As a result, the number of people suffering from diet-related diseases such as obesity, diabetes, hypertension, CVD and various cancers has increased significantly (Krauss et al., 1998; Kritchevsky, 1995; Sabaratnam, 2003).

Atherosclerosis is the underlying disorder in the majority of patients with CVD. Atherosclerosis refers to the build up of fatty material in the arterial wall, which leads to narrowing of an artery with potential blockage. Elevated levels of plasma or serum low density lipoprotein (LDL) cholesterol is associated with atherosclerosis (Linder, 1991; Leys et al., 2002). It is now well established that statins (also known as HMG-CoA Reductase inhibitors) are potent and effective but expensive drugs for treating hypercholesterolemia. According to the Health Ministry of Malaysia (MOH), expenditure to treat heart diseases increased from RM 226 millions in 1996 to RM 751 millions in 2003. The use of statins in the primary prevention of atherosclerosis alone cost nearly RM 10 millions annually (Cruez, 2005). Apart from this, subsidy payments for National Heart Institute (IJN) to treat civil servants and poor patients rose from RM 31.3 million between September 1992 and August 1993 to RM 144.5 million between September 2003 and August 2004. These statistics reflect the financial burden borne by the Government due to CVD.

Coronary heart diseases increased from 27% of total cardiovascular deaths in 1985 to 30.5% in 2002 (Cruez, 2005). In 2003, cardiovascular diseases accounted for 120,295 admissions in government hospital or 7% of total admissions. From this, 5,162 were terminal cases representing 14.2% of all terminal cases (MOH, 2003).
Recently, the trend of going back to nature is getting popular in Malaysia and globally. Demand for natural health supplements (e.g.: vitamins and herbal remedies) is increasing since modern medicine and synthetic drugs have not been totally successful in solving health problems such as cancer and heart disease. Despite the beneficial LDL cholesterol lowering potential of statins, liver function test monitoring is required with the statins treatment since statins is related with possible hepatic dysfunction, myopathy together with muscle pain, tenderness or weakness. Therefore, the growing public alarm about the hazards associated with excessive use of synthetic drugs has revived the interest in the use of herbal medicines.

More Malaysians are taking active measures off illnesses and maintain good health by taking supplements. There has also been an increasing worldwide recognition of the important role of traditional herbal medicine. For example, the WHO promotes the use of herbal medicines for certain conditions such as arthritis, asthma, diabetes, stroke and vaginitis (Natila, 2002). Locally, the Forest Research Institute of Malaysia (FRIM), together with a private company is helping to upgrade the traditional medicine industry and preserve trees and herbal plants with medicinal values.

The current global trend shows that herbal therapy enters the mainstream medicine as being observed from 1970 to 2000 (Wazir, 2003). Herbs and medicinal plants are mainly used for flavours and fragrance, biopesticides, pharmaceutical and nutraceuticals. Most of Malaysian pharmaceutical products are mainly analgesics, antacids, diuretics, antibiotics and anti-histamines in the form of tablets, capsules, drops,