EFFECTS OF MALAYSIAN COCOA POWDER CONSUMPTION ON BIOCHEMICAL PARAMETERS STATUS OF HEALTHY SUBJECTS AT UNIVERSITI PUTRA MALAYSIA, SERDANG

SURYATI MUHAMMAD ALINAFIAH

FPSK(m) 2011 29
EFFECTS OF MALAYSIAN COCOA POWDER CONSUMPTION ON BIOCHEMICAL PARAMETERS STATUS OF HEALTHY SUBJECTS AT UNIVERSITI PUTRA MALAYSIA, SERDANG

By

SURYATI MUHAMMAD ALINAFIAH

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirements for the degree of Master Degree

March 2011
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFECTS OF MALAYSIAN COCOA POWDER CONSUMPTION ON BIOCHEMICAL PARAMETERS STATUS OF HEALTHY SUBJECTS AT UNIVERSITI PUTRA MALAYSIA, SERDANG

By

SURYATI MUHAMMAD ALINAFIAH

March 2011

Chairman: Professor Amin Ismail, PhD

Faculty: Medicine and Health Sciences

In most of developing countries, cardiovascular disease (CVD) will be the leading cause of death worldwide. By the year of 2030, almost 23.6 million people will die from CVD, mainly heart diseases and stroke, which also representing 29% of all global health. Dietary intervention revealed that the plants-derived food is negatively correlated with the risk of CVD. The consumption of cocoa (Theobroma cacao L) and its products has been often hypothesized to reduce the cardiovascular risk due to their polyphenols and antioxidant content. Polyphenols is a phytochemical that could play important role in attenuating the development of non-communicable diseases such as diabetes, atherosclerosis, cardiovascular disease, rheumatoid arthritis, ageing and certain cancers. Cocoa powder is rich in polyphenols compound compared to other food products. Malaysian cocoa
beans have been reported to contain the highest phenolic content compared to Sulawesian, Ghanaian and Ivorian Coast beans. Hence this study may contribute to the crucial knowledge on the health benefits of Malaysian cocoa, which believed to have positive effects on delaying the risk of cardiovascular diseases. This cross over study was conducted to investigate the effect of Malaysian cocoa beverage (CB) consumption on biochemical parameters among healthy subjects in UPM. There were 37 subjects who consisted of 20 women and 17 men aged between 25-45 years consisted of UPM staff volunteered for the study. This study was carried out for ninth weeks and the subjects were randomized into two groups; Group 1 (n=19) and Group 2 (n=18). In the first four weeks, Group 1 was a treatment group whereas Group 2 was a control group. Subjects in treatment group were assigned to receive 18 g CB (contained 1260 mg polyphenols) daily for four weeks whereas the subjects in control group did not receive any beverage to be consumed. After the washout period (1 week), Group 2 was a treatment group and Group 1 acted a control group. Ten milliliters of fasting blood of the subjects was taken to measure cardiovascular biomarkers (plasma lipid profiles, antioxidant status and enzymes, glucose level, high sensitive-CRP and malondialdehyde). Other parameters (bodyweight changes and blood pressure levels changes) were also measured. The present study observed an improvement of lipid profiles levels and antioxidant enzymes of subjects after the consumption of Malaysian cocoa powder for 4 weeks. The total cholesterol (TC) concentrations reduced significantly by 7.29% (p<0.05).
The low density lipoprotein (LDL-C) and triglycerides (TG) and high density lipoprotein (HDL-C) had improved but not with significant changes at the end of the study. The present study also showed improvement in antioxidant status and enzymes of the subjects. The levels of glutathione peroxidase (GPx) increased significantly by 68.64% (p<0.05). The level of total antioxidant status (TAS) and Superoxidase Dismutase (SOD) also improved but not with significant changes between the two groups. There were no significant changes on high sensitive-CRP (hs-CRP) and melondialdehyde (MDA) of the subjects at the end of the study. Consumption of 18 g CB daily for 4 weeks had also improved blood pressure levels and caused no toxicity effects in healthy subjects. In conclusion, several improvements in biomarkers associated with cardiovascular health following consumption of CB were observed in healthy individuals involved in the study. Malaysian CB may possibly use as functional foods to help in preventing the promotion of oxidative stress linked diseases.
Penyakit kardiovaskular merupakan penyebab kematian utama di kebanyakan negara membangun di dunia. Menjelang tahun 2030, hampir 23.6 juta penduduk dunia akan mati disebabkan penyakit kardiovaskular terutamanya jantung dan strok, iaitu mewakili 29% jumlah kematian di dunia. Intervensi pemakanan menunjukkan bahawa makanan berasaskan tumbuhan adalah berkait secara negatif dengan risiko kardiovaskular.

Pengambilan koko (Theobroma Cocoa L) and produknya lazimnya dihipotesiskan dapat mengurangkan risiko kardiovaskular disebabkan kandungan polifenol dan antioksidannya. Polifenol adalah bahan fitokimia yang memainkan peranan penting dalam mengurangkan perkembangan penyakit tidak berjangkit seperti kencing manis, atherosklerosis, penyakit kardiovaskular, rheumatoid artritis, penuaan dan kanser. Serbuk koko mengandungi kompaun polifenol yang tinggi berbanding dengan produk
makanan lain. Kajian menunjukkan biji koko Malaysia mengandungi fenolik yang tinggi jika dibandingkan dengan biji koko yang terdapat di Sulawesi, Ghana dan Ivory Coast. Oleh itu, kajian ini diharapkan dapat menyumbangkan maklumat penting berkenaan kebaikan produk koko Malaysia yang dipercayai mempunyai kesan positif dalam mengurangkan risiko penyakit kardiovaskular. Kajian ini adalah kajian silang yang dijalankan bagi menentukan kesaan pengambilan minuman koko Malaysia ke atas paras biokimia yang terpilih subjek sihat di UPM. Subjek yang sihat berumur 25-45 tahun (20 wanita dan 17 lelaki), terdiri daripada staf UPM telah dipilih sebagai sampel kajian. Kajian ini dijalankan selama 9 minggu dan subjek telah dibahagikan secara rawak kepada dua kumpulan; Kumpulan 1 (n=19) dan (n=18) pada awal kajian. Pada 4 minggu di awal kajian, Kumpulan 1 bertindak sebagai subjek kumpulan rawatan manakala Kumpulan 2 menjadi kumpulan kawalan. Subjek kumpulan rawatan telah diberi 18 g CB (mengandungi 1260 mg polifenol) untuk diminum setiap hari selama 4 minggu manakala kumpulan kawalan tidak menerima sebarang produk kajian. Selepas fasa ‘washout’ selama 1 minggu, Kumpulan 2 telah menjadi kumpulan rawatan manakala Kumpulan 1 telah menjadi kumpulan kawalan. 10 mililiter sampel darah berpuasa telah diambil pada minggu 1, 4, 6 dan 9 bagi mengukur perubahan paras biokimia darah subjek (profil lipid, status antioksidan dan enzim, malondialdehyde, highly sensitive-CRP dan paras gula dalam darah). Selain dari itu, kesan perubahan berat badan dan paras tekanan darah subjek juga diukur. Kajian
ini didapati menunjukkan kesan positif ke atas perubahan profil lipid dan paras enzim antioksidan dalam darah subjek selepas mengambil minuman koko selama 4 minggu. Paras total kolesterol (TC) turun secara signifikan sebanyak 7.29% (p<0.05). Paras lipoprotein berketumpatan rendah (LDL-C), trigliserida (TG) dan paras lipoprotein berketumpatan tinggi (HDL-C) mengalami perubahan yang baik tetapi tidak signifikan pada akhir kajian. Kajian ini juga menunjukkan perubahan baik bagi paras status antioksidan dan enzim subjek. Pada akhir kajian, paras enzim antioksidan dalam darah subjek rawatan, Glutathione Peroxsidase mengalami kenaikan signifikan sebanyak 68.64% (p<0.05). Paras status antioksidan (TAS) dan Superoxidase Dismutase (SOD) mengalami perubahan yang baik tetapi tidak signifikan di antara kumpulan rawatan dan kawalan tanpa menyebabkan perubahan signifikan ke atas berat badan dan paras gula dalam darah subjek. Tiada perubahan signifikan diperhatikan bagi paras high sensitive-CRP (hs-CRP) dan melondialdehyde (MDA) bagi kedua-dua kumpulan rawatan dan kawalan kajian. Pengambilan CB selama 4 minggu didapati berjaya memperbaiki paras tekanan darah dan tidak menyebabkan kesan toksik ke atas subjek yang terlibat dalam kajian. Kesimpulannnya, pengambilan minuman koko Malaysia (CB) selama 4 minggu ke atas subjek yang sihat telah memberi kesan positif ke atas beberapa parameter biokimia yang berkait dengan penyakit kardiovaskular. Minuman koko Malaysia (CB) berpotensi sebagai makanan berfungsi bagi membantu mengelakkan penyakit yang berkait dengan oksidatif stress.
ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt gratitude to ALLAH the AL MIGHTY for the strength given to me to complete the study. My deepest thanks go to my research Supervisor, Prof Dr Amin Ismail for his support, guidance, patience and advice throughout the study. My special thanks go to my co-supervisors, Prof Dr Zarida Hambali, Assoc Prof Dr Rokiah Mohd Yusof and Dr Hazizi Abu Saad for their guidance and assistance during the study. I would like to convey my appreciation to Head of Department, Department of Nutrition & Dietetics, Faculty of Medicine and Health Sciences, UPM for his support and guidance. Thank you for permitting me to pursue my study. I would like to thank my family members (especially my parents) for their kindness and support. My highest gratitude goes to my beloved husband, Muhamad Shafei Jamahuri for his love, support and patience. I would also like to express my endless gratitude for the tremendous help, support and contribution to Department’s laboratory staff for their big hands during the research. I am sincerely grateful to Malaysian Cocoa Board (MCB) for the sponsorship and not to forget, POLAR group members (especially Mr Muhammad Ibrahim) for their help. My gratitude also goes to staff of Pathology Laboratory, FPSK for their helps in biochemical analysis. Last but not least, to my friends (Safarina, Sarina, Nisak, Siti Zuraini, Norbaizura & K Zack) thanks for your kindness and supports.
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 Science. The members of the Supervisory Committee were as follows:

**Amin bin Ismail, PhD**  
Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Zarida Hambali, MD**  
Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Rokiah Mohd Yusof, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Hazizi Abu Saad, PhD**  
Senior Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

---

**HASANAH MOHD. GHAZALI, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
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 at any other institution.

_______________________
SURYATI MUHD ALINAFIAH
Date: 30 March 2011
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
</tr>
<tr>
<td></td>
<td>2.1. Cocoa and cocoa based products</td>
</tr>
<tr>
<td></td>
<td>2.2. Medicinal uses of cocoa</td>
</tr>
<tr>
<td></td>
<td>2.3. Composition of cocoa beans</td>
</tr>
<tr>
<td></td>
<td>2.4. Cocoa polyphenols</td>
</tr>
<tr>
<td></td>
<td>2.5. Effect of cocoa polyphenols on cardiovascular disease</td>
</tr>
<tr>
<td></td>
<td>2.6. Antioxidants properties of polyphenols</td>
</tr>
<tr>
<td></td>
<td>2.7. Human trials on polyphenols rich food</td>
</tr>
<tr>
<td>3</td>
<td>MATERIAL AND METHODOLOGY</td>
</tr>
<tr>
<td></td>
<td>3.1. Location of the study</td>
</tr>
<tr>
<td></td>
<td>3.2. Design of the study</td>
</tr>
<tr>
<td></td>
<td>3.3. Pre test procedures</td>
</tr>
<tr>
<td></td>
<td>3.3.1. Screening of subjects</td>
</tr>
<tr>
<td></td>
<td>3.3.2. Clinical screening examination</td>
</tr>
<tr>
<td></td>
<td>3.3.3. Recruitment of subjects</td>
</tr>
<tr>
<td></td>
<td>3.3.4. Ethical clearance</td>
</tr>
<tr>
<td></td>
<td>3.4. Baseline data on recruited subjects</td>
</tr>
</tbody>
</table>
3.5 Preparation of cocoa beverage powder
   3.5.1 Source of cocoa powder
   3.5.2 Preparation of cocoa beverage powder
   3.5.3 Determination of total phenolic content of product
   3.5.4 Chemicals analysis
3.6 Data collection
   3.6.1 Treatment and control groups
   3.6.2 Blood collections
3.7 Determination of plasma lipid profiles
3.8 Determination of plasma lipid peroxidation
3.9 Determination of plasma glucose level
3.10 Determination of antioxidant enzymes activities
   3.10.1 Whole blood antioxidant enzymes
3.11 Determination of total antioxidant status (TAS)
3.12 Determination of high sensitive c-reactive protein (hs-CRP)
3.13 Toxicity test
3.14 Anthropometric and blood pressure measurements
3.15 Nutritional status assessment
3.16 Socio-demographic data questionnaire
3.17 Assessment of physical activity
3.18 Statistical Analysis

4 RESULTS AND DISCUSSIONS
4.1 Subjects of the study
4.2 Baseline: characteristics of subjects
   4.2.1 Demographic and socioeconomic background
4.3 Medical backgrounds and lifestyle habits of studied subjects
4.4 Cocoa beverage consumption effects on subjects
   4.4.1 Dietary intakes of subjects in treatment and control group
   4.4.2 Physical activities
4.5 Effect of cocoa beverage (CB) supplementation on
biochemical parameters

4.5.1 Effect of CB on bodyweight 72
4.5.2 Effect of CB on blood pressure 74
4.5.3 Effect of CB on lipid profiles 76
4.5.4 Effect of CB on total antioxidant status 83
4.5.5 Effect CB on antioxidant enzymes 85
4.5.6 Effect of CB on plasma glucose 87
4.5.7 Effect of CB on highly sensitive c-reactive protein and malondialdehyde 88
4.5.8 Effect of CB on toxicity 91
4.5.9 Effect of CB on total protein and albumin 92

5 RESEARCH SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH 100

REFERENCES 106
APPENDICES 128
BIODATA OF STUDENT 145