IN VIVO ANTIOXIDANT POTENTIAL AND CARDIOPROTECTIVE ACTIVITIES OF *BACCAUREA ANGULATA* FRUIT IN RELATION TO SUPPRESSED INFLAMMATORY RESPONSE

BY

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ABSTRACT

Cardiovascular diseases remain the single leading cause of deaths globally. Diet rich in fruits and vegetables is highly important in the maintenance of best possible cardiovascular health. Malaysia has a variety and large quantity of underutilized fruits which are rich in phenolic compounds. In the present study, cardiovascular protective effects of an underutilized *Baccaurea angulata* fruit were studied using a cholesterol-fed rabbit model. The study was divided into 5 parts. The objective of part one was to identify and quantify some phenolic compounds in the methanolic extracts of *B. angulata* whole fruit (WF), skin (SK) and pulp (PL). By using the ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC/MS-MS) based approach, a total of 17 compounds were detected and characterized on the basis of their chromatographic retention time, UV-vis spectra and mass spectra in the negative-ion mode and data from the literature. Part two was aimed at evaluating the effects of WF, SK and PL juices of *B. angulata* administered at a nutritional dose of 1 ml/kg/day on malondialdehyde (MDA) levels and antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) as well as on total antioxidant capacity (TAC) in rabbits fed with high-cholesterol diet for 12 weeks. The results showed that *B. angulata* fruit had modulating effects on lipid peroxidation and antioxidant enzyme activities in rabbits fed with high-cholesterol diet. It significantly ($p<0.05$) lowered the concentrations of plasma MDA. SOD, GPX, CAT and TAC decreased with cholesterol feeding and recovered with *B. angulata* juice administrations. The purpose of part three was to evaluate and compare the effects of three different *B. angulata* WF juice doses administered at nutritional doses of 0.5 ml/kg/day, 1 ml/kg/day and 1.5 ml/kg/day on serum, aorta & liver MDA levels, antioxidant enzymes (SOD, GPx and CAT) as well as on total antioxidant capacity in rabbits fed with cholesterol diet for 12 weeks. The results showed that higher doses (1 ml/kg/day and 1.5 ml/kg/day) of *B. angulata* WF juice exerted better antioxidant activity. Part four of the study was aimed at evaluating and comparing the effects of three different *B. angulata* WF juice concentrations administered at nutritional doses of 0.5 ml/kg/day, 1 ml/kg/day and 1.5 ml/kg/day on 12 serum, aorta & liver inflammatory biomarkers of atherosclerosis such as vascular cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), P-selectin, C-reactive protein (CRP), IL-1, IL-2, IL-6, IL-7, IL-8, IL-10, IL-18, and tumor necrosis factor α (TNF- α) in rabbits fed with cholesterol diet for 12 weeks. The results showed that *B. angulata* inhibited inflammatory biomarkers of atherosclerosis. Lastly, the aim of part five was to investigate the plaque-reducing activities of *B. angulata* fruit as a new anti-atherogenic plant in high-cholesterol fed rabbits for 12 weeks. Rabbits fed with cholesterol diet only successfully developed severe, extensive and more densely distributed human-like atherosclerotic lesions. However, supplementation of *B. angulata* fruit juice to rabbits fed with cholesterol diet significantly ($p<0.001$) reduced atherosclerotic lesions. In conclusion, *B. angulata* fruit was shown to enhance plasma antioxidant enzyme activities, inhibit inflammatory biomarkers and reduce progression of atherosclerotic plaque in cholesterol-fed rabbits. Thus, *B. angulata* fruit plays a favorable role on several mechanisms involved in combating atherosclerosis.

خلاصة البحث

لا تزال الأمراض القلبية الوعائية أن تكون السبب الرئيسي واحد من الوفيات في العالم. والنظام الغذائي الغني بالفاكهة والخضار مهمّ جدًّا في الحفاظ على أفضل صحة القلب والأوعية الدموية الممكنة. وفي ماليزيا أنوع وكمية كبيرة من الفواكه غير مستغلة الاستوائية وهي غنية في مركبات فينولية وفي هذه الدراسة أستكشف عن آثار فاكهة غير مستغلة الاستوائية ***Baccaurea angulata*** في وقاية القلب والأوعية الدموية، باستخدام نموذج تغذية الأرانب بالكولسترول. وقد تم تقسيم الدراسة إلى 5 أجزاء. وكان الهدف من الجزء الأول هو تحديد وقياس بعض المركبات الفينولية الموجودة في مستخلصات المثيلنول من الفاكهة بكاملها (WF)، والجلد (SK) واللب(PL) وباستخدام اللوني السائل فائقة عالية الأداء، مع قياس الطيف الكتلي (UHPLC / MS-MS) النهج القائم، وتم الكشف عن 17 مجموعة مركبات تابعة لفئات مختلفة من المركبات الفينولية، على أساس من الكروماتوغرافي والوقت الاحتفاظ، الأشعة فوق البنفسجية في ضوء الأطياف والأطياف الشامل في وضع سلبي ايون والبيانات من المراجع. وتهدف الجزء الثاني تقييم آثار عصير WF، SKو PL B. angulata في جرعة الغذائية لل 1مل /كغ / يوم على مستويات (MDA) malondialdehyde و CAT, SOD, (GPx) وكذلك على إجمالي القدرة المضادة للأكسدة (TAC) في الأرانب المغذية بالكولسترول لمدة 12 أسبوعا. وأظهرت النتيجة أن B. angulata قد تحور الآثار على بيروكسيد الدهون والأنشطة انزيم المضادة للأكسدة في الأرانب المغذية بالكوليسترول .ويمكن أن تخفض بشكل كبير (p<0.05) من تركيزات MDA البلازما .وانخفضت GPX, SOD، CAT و TAC في الدم مع التغذية الكولسترول بعصير B. angulata . وكان الغرض من الجزء الثالث من الدراسة هو مقارنة تأثير ثلاثة مختلفة B. angulata جرعات عصير WF في جرعات الغذائية من 0.5مل / كغ / يوم، 1مل /كغ / يوم و 1.5 مل / كغ / يوم على المصل ، ومستويات الشريان الأورطي والكبد TBARS،)و(SOD, GPx،CAT) وكذلك على إجمالي القدرة المضادة للأكسدة (TAC) في الأرانب المتغذية بالكولسترول. وأظهرت النتيجة أن الجرعات العالية 1)مل / كغ / يوم و 1.5 مل / كغ / يوم من عصير B. angulata WF تمارس النشاط بشكل أفضل مضادات الأكسدة وتهدف الجزء الرابع لتقييم ومقارنة آثار ثلاثة تركيزات مختلفة عصير WF B. angulata في جرعات الغذائية من 0.5مل / كغ / يوم، 1مل /كغ / يوم و 1.5 مل / كغ / يوم في 12 مصل، والشريان الأورطي الكبد المؤشرات الحيوية للالتهابات من تصلب الشرايين مثل الخلايا الوعائية جزيء التصاق (VCAM-1) 1، بين الخلايا جزيء التصاق الخلايا (ICAM-1) 1، فselectin ، بروتين سي التفاعلي (CRP)، IL-1، IL-2، IL -6، IL-7، IL-8، IL-10، IL-18، وعامل نخر الورم (TNF) في الأرانب المتغذية بالكولسترول. وأظهرت النتيجة أن B. angulata أمنعت الحيوية للالتهابات الشرايين. وأخيرا، تهدف الجزء الخامس للتحقيق في أنشطة B. angulata باعتبارها محطة ومكافحة جديدية تصلب الشرايين في الأرانب المتغذية بالكولسترول. في هذه الدراسة، الأرانب التي غذيت بالكوليسترول فقط لمدة 12 أسبوعا أظهرت بوضع شديد، وواسعة وأكثر كثافة توزيع آفات تصلب الشرايين مثل الإنسان. ومع ذلك، تغذية الأرانب بالفاكهة *B. angulata* للأرانب المتغذية بالكولسترول انخفض بحد كبير (p<0.001) آفات تصلب الشرايين. وفي الختام، وقد تبين بما سبق أنّ فاكهة *B. angulata* تستطيع تعزيز أنشطة إنزيم مضاد للأكسدة البلازما، وتمنع المؤشرات الحيوية للالتهابات وتقلل تطور وحة تصلب الشرايين في الأرانب المتغذية بالكولسترول. وهكذا, فالفواكه *B. angulata* يلعب دورا إيجابي على العديد من الآليات التي تشارك في مكافحة تصلب الشرايين.

APPROVAL PAGE

The thesis of Maryam Abimbola Mikail has been approved by the following:

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Maryam Abimbola Mikail

Signature…………………………. Date..……………………

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This project is dedicated to Allah and then His beloved Prophet and Messenger Muhammad (S.A.W).

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LIST OF ABBREVIATIONS

ApoB	Apolipoprotein B
CAT	Catalase
CRP	C-reactive protein
CSF	Colony-stimulating factors
CVD	Cardiovascular diseases
GPx	Glutathione peroxidase
GST	Glutathione-S-transferase
HDL	High-density lipoprotein
HUVEC	Human umbilical vein endothelial cell
ICAM-1	Intercellular adhesion molecule-1
IDLs	Intermediate-density lipoproteins
IFN	Interferons
IFNγ	Interferon-gamma
IL	Interleukin
LDL-C	Low-density lipoprotein cholesterol
MCP-1	Monocytes chemoattractant protein-1
M-CSF	Macrophage colony-stimulating factor
MDA	Malondialdehyde
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
Ox-LDL	Oxidized LDL
PGI_2	Prostaglandin

ROS	Reactive oxygen species
SMCs	Smooth muscle cells
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid
TGF	Transforming growth factors
TNF-α	Tumor necrosis factor α
UHPLC	Ultra high-performance liquid chromatography
VCAM-1	Vascular cell adhesion molecule-1
VLDLs	Very low-density lipoproteins
WHO	World health organization

LIST OF SYMBOLS

$	US Dollars
RM	Malaysian Ringgit
%	Percentage
m	Meter
mm Hg	Millimiter of Mercury
kg/m^2	Kilogram per meter square
kg	Kilogram
m^2	Metre square
mg	Milligram
g/day	Gram per day
g	Gram
ml	Milliliter
mmol/l	Millimole per liter
°C	Degree Celsius
rpm	Revolution per minute
µl	Microliter
µm	Micrometer
mm	Millimeter
mM	millimole
µl/minute	Microliter per minute
ml/minute	Milliliter per minute
±	Plus or minus
<	Less than
>	Greater than

R^2	Regression coefficients
mg/kg	Miligram per kilogram
ml/kg	Milliliter per kilogram
μM	Micromole
nm	Nanometre
U/L	Activity unit per litre
pg/ml	Picogram per milliliter
ng/ml	Nanogram per milliliter

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Infectious diseases pose comparatively fewer threats just at the start of the 21st century, while chronic diseases keep on afflicting a substantial proportion of the general population. Many drugs are used to fight acute diseases, but the biomedical model is restricted when handling the health catastrophe resulting from the long-lasting, life-threatening chronic diseases (Buttar, Li & Ravi, 2005). Among the most widespread chronic diseases that cut short the lives of far too many people in the world are cardiovascular diseases (CVDs), diabetes, some cancers, arthritis, osteoporosis, chronic obstructive pulmonary disease, obesity, inflammatory bowel disease and central nervous system degenerative diseases (Rezg, El-Fazaa, Gharbi & Mornagui, 2014).

Cardiovascular diseases continue to be the single leading cause of deaths globally from the aforementioned chronic diseases (Gerszten & Wang, 2008). World Health Organization (WHO) presented that, in 2008, an estimated 17.3 million people died from CVDs, and shockingly, nothing less than 3 million of these deaths occurred at ages below 60 (WHO, 2013). World Health Organization also presumes that if the existing fashion of lifestyle, stress, lack of exercise and smoking continue the way they are, and nothing much is done to improve cardiovascular health, by 2030, the number of deaths will rise to nearly 23.3 million (Mathers & Loncar, 2006).

Remarkably, a recent global report from WHO (2011) revealed that, over the past two decades and up to the minute, there are new developments in the incidence of

CVDs. There is a decrease in the incidence of cardiovascular disease (CVD) deaths in high-income countries, while CVD deaths and incidence rates have become greater than before in middle- and low-income countries.

In 1960s and 1970s, CVD was responsible for half of all deaths in the United States, at a time Asia was facing a totally dissimilar situation altogether. Communicable diseases were the top diseases in this part of the world, particularly for Malaysia and Singapore. The late 1990s and early 2000, however, experienced a rising trend of killer illnesses such as heart disease, stroke and cancer. Cardiovascular diseases most especially overtook communicable diseases as Asia’s biggest killer (Lu & Nordin, 2013). These life-altering diseases require complex and lifelong expensive treatments. Cardiovascular diseases are the subject of huge investment by the pharmaceutical and biotechnology industries. In Malaysia, the cost of treating CVDs is enormous, about $2.6 million (RM10 million) is estimated a year just on the use of statins in primary prevention of hypercholesterolemia, a major risk factor of atherosclerosis. Cardiovascular diseases cost the Singaporean Ministry of Health approximately $64 million (Singapore $110 million) annually (Dhanoa, 2004).

Africa even is not safe from this global tide of CVDs. Africa has experienced a boost urbanization and lifestyles shifting from the last four decades, factors which have, in turn, increased the occurrence of non-communicable chronic diseases, particularly CVDs (Kadiri, 2005). Therefore, in Africa, CVDs prevalence has reached near epidemic proportion. In the majority of African countries, after infectious diseases, CVD is now the second most regular cause of death. According to the World Health report, in 2001, CVDs roughly accounted for 9.2% of total deaths in the African region (WHO, 2002).

It is essential to know that the term “cardiovascular disease” refers to a number of conditions, which WHO sorts into 11 groups for the purpose of gathering mortality data. These groups include hypertension with or without renal disease, stroke, other diseases of arteries, arterioles and capillaries, and diseases of veins and lymphatics (WHO, 1993). In other words, CVDs are a collection of disorders of the blood vessels and the great heart (WHO, 2013). Most CVD patients are troubled with a condition known as atherosclerosis. Atherosclerosis is an illness of the innermost layer (intima) of the medium- and large-sized arteries that is more often known by the lay term as “hardening of the arteries,” characterized by the accumulation of lipid, inflammatory cells, connective tissues and vascular smooth muscle cells in the intima of the arterial wall (Davies & Woolf, 1993). The growth of this atheroma invades the innermost layer of the arterial wall, tightening it and extending into the lumen of the vessel, leading to lumen narrowing (stenosis), which may lead to clinical complications such as ischemia, thrombosis and infarction (Gotlieb, 2005).

Endothelial injury or dysfunction is the most basic stage of atherosclerosis, which stimulates the buildup and oxidation of low-density lipoprotein cholesterol (LDL-C) in the arterial wall. It has become abundantly clear over the last decade that endothelial injury causes oxidative modification of LDL-C (Brown & Goldstein, 1986; Steinberg, 1997). This modification then causes the migration of monocytes from the blood into the subendothelial intima. Monocytes change into macrophages and swallow up lipids (LDL-C) to form the lipid core called foam cells. Therefore, the grossly visible lesion from the foam cells is known as fatty streak (Douglas & Channon, 2010; Wang et al., 2012). In short, low-density lipoprotein (LDL) oxidation and chronic inflammation are the two clinical (pathological) hallmarks of atherosclerosis (Wang et al., 2012).

It has been widely accepted that inflammatory processes are vital to atherosclerotic lesion formation (Croce & Libby, 2007). In response to the activity of inflammatory cytokines and growth factors (e.g. tumor necrosis factor α and interleukin-1) secreted by damaged endothelial cells and foam cells, smooth muscle cells (SMCs) proliferate and migrate from the tunica media to the vascular intima (Libby, 2002). Soon after, apoptosis of SMCs turns out to become an acellular fibrous capsule covering the fatty streak. In the end, the fibrous capsule may possibly become weak and rupture, exposing the thrombogenic molecules within the lipid cores. These thrombogenic molecules cause arterial occlusion by rapidly slowing or stopping blood flow leading to the catastrophic events like myocardial infarction, ischemic stroke, or critical ischemia in peripheral tissues (Croce & Libby, 2007; Kruth, 2001).

Atherosclerosis is a multifactorial disease caused by risk factors such as high-cholesterol diet, tobacco smoking, dyslipoproteinemia, vitamin B_6 deficiency, obesity and diabetes mellitus. It usually develops many years before any clinical manifestation, or symptoms become visible (Fruchart, Nierman, Stroes, Kastelein & Duriez, 2004). High-cholesterol diet is the most crucial, of all these aforementioned factors, in the development of atherosclerosis. The dietary cholesterol-atherosclerosis relationship has gone from just a hypothesis to a widely accepted certainty (McNamara, 2000). Dietary fat is considered as an important environmental factor associated with the incidence of metabolic syndromes, such as CVD, hypertension and obesity (Hsu et al., 2011). Elevated LDL-C is the leading cause of CVDs in these sophisticated modern societies (Stocker & Keaney, 2004).

Healthy diet is highly important in the maintenance of the best possible cardiovascular health. Countless epidemiological studies have connected a diet rich in fruits and vegetables with decreased risk of CVD (Panagiotakos, Pitsavos, Arvaniti &