ANTIHYPERGLYCAEMIC ACTIVITIES OF XANTHONE RICH EXTRACT OF MANGOSTEEN *(GARCINIA MANGOSTANA)*

BY

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ABSTRACT

Mangosteen (*Garcinia mangostana* Linn.) fruit pericarp has been used for centuries as a folk medicine. The study was conducted to evaluate the *in vitro* and *in vivo* antihyperglycaemic potential of *G. mangostana* extract (GME). The α-mangostin content in the extract was measured using HPLC and investigated for total phenolic and flavonoid contents. Antioxidant activities were measured by DPPH radical scavenging and reducing power assays whereas *in vitro* antidiabetic activities were evaluated by inhibition of α-glucosidase and α-amylase enzymes. Effects of GME on adipocyte cells were assessed through MTT assay, adipogenesis and glucose uptake measurements. In animal study, oral administration of GME1 (50 mg/kg), GME2 (100 mg/kg) and GME3 (200 mg/kg) to STZ-induced diabetic rats in single-dose (acute) and multiple-dose study (sub-acute) were examined. Serum biochemical parameters and histopathological alterations were evaluated and compared to standard hypoglycaemic drug, glibenclamide. The results showed that total phenolic and total flavonoid contents were 122.2±1.04 mg GAE/g and 72.8±1.75 mg QE/g of dry extract, respectively. DPPH radical scavenging activity and reducing power capacity reported with EC_{50} of 48.2 μg/ml and IC_{50} of 98.2 μg/ml, respectively. The enzymatic inhibition of α-glucosidase and α-amylase revealed higher percentage of inhibition comparable to acarbose with IC_{50} of 0.41 and 0.24 mg/ml, respectively. In cellular study, cytotoxicity assay reported that dose of less than 12.5 μg/ml does not affect cell viability. The differentiations of adipocytes were increased with higher GME concentration at 2.5 μg/ml, 5.0 μg/ml and 10.0 μg/ml. Glucose uptake measurements revealed a higher uptake of 2-deoxyglucose in GME2-treated cell (2243.3±232.3 cpm) as compared to GME1 (1864.0±146.3 cpm) and GME3 (1246.0±155.8 cpm). Oral administration of GME on diabetic rats indicated safe usage with absence of behavioural alterations, autonomic, neurological and toxic effects up to 2000 mg/kg. The results showed a significant reduction of glucose level in GME2 and GME3 ($p<0.001$) as compared to GME1. Total cholesterol, serum triglyceride, urea and creatinine were reduced in the treatment group while total protein contents were increased. Histological assessment of livers and kidneys revealed reduced lesions whereas mild regenerative activity of β-cell was observed in pancreas of diabetic rats. In conclusion, the findings demonstrated that GME could be a potential source in diabetes management owing to its antioxidant content, delayed carbohydrate digestion, induction of adipocyte differentiation, improvement in glucose uptake and antihyperglycaemic effect in diabetic rats.

ملخص البحث

قشرة فاكهة المانغوستين (غارسينيا مانغوستانا لين.) تمّ استخدامها لقرون في الطبّ الشعبي. الدراسة تمّ إجراءها لتقييم إمكانية مستخلص ج مانغوستانا كما تمّ التحقيق في كمية الفينولات HPLC محتوى ألفا-مانغوستين في المستخلص تمّ قياسه باستخدام تقنية لرصد الجذر الحرّ DPPH والفلافونويد الإجمالية. الخصائص المضادة للأكسدة تمّ قياسها باستخدام تقنية وكذا فحص قوة الإرجاع بينما تمّ تقييم الأنشطة ضد السكري عن طريق كبت إنزيميّ ألفا-غلوكوزيداز. MTT, Adipogenesis على الخلايا الدهنية تمّ تقييمه بواسطة تقنيات GME وألفا-أميلاز. تأثير مستخلص بتركيز 50 مغ/كغ GME1 وكذا قدرة امتصاص الغلوكوز. في الدراسة على الحيوان، تقديم مستخلص المستحثة بداء STZ بتركيز 200 مغ/كغ إلى فئران GME3 بتركيز 100 مغ/كغ ومستخلص GME2 السكري من خلال جرعة واحدة (حادّة) وجرعات متعدّدة (شبه حادة) تمّت دراستها. معلمات المصل البيوكيميائية والتعديلات المرضية تمّ تقييمها ومقارنتها مع معيار الدواء المضاد للسكري غليبنكلامايد./غ GAE النتائج أظهرت بأنّ كمية الفينولات والفلافونويدز الإجمالية كانت تعادل 122.2±1.04 مغ لرصد الجذر الحرّ وكذاDPPH/غرام من المستخلص الجاف على التوالي. تقنية QE و72.8± 1.75 مغ بمقدار 98.2 مكغ/مل على التوالي.IC50 بمقدار 48.2 مكغ/مل وEC_{50} قدرة قوة الإرجاع مع IC_{50} عملية كبت إنزيميّ ألفا-غلوكوزيداز وألفا-أميلاز أظهرت نسبة كبت عالية بالمقارنة مع أكاربوز ببمقدار 0.41 و 0.24 مغ/مل على التوالي. في الدراسة الخلوية، الفحص السمّي أظهر بأنّ جرعة أقل من 12.5 مكغ/مل لا تؤثر على قابلية حياة الخلايا. تفاضل الخلايا الدهنية كان في ارتفاع مع زيادة تركيز ال عند 2.5 مكغ/مل ، 5.0 مكغ/مل و10.0مكغ/مل. قياسات امتصاص الغلوكوز GME مستخلص GME2 في الخلايا التي تمت معالجتها بمستخلص 2-deoxyglucose أظهرت امتصاصا عاليا لـ(1864.0±146.3 cpm) ومستخلص GME1 بالمقارنة مع مستخلص (2243.3 ±232.3cpm) (إلى الفئران المصابة بالسكري أظهرGME تقديم مستخلص) 1246.0±155.8 cpm GME3 استخداما آمنا مع غياب التعديلات السلوكية، اللاإرادية، العصبية والآثار السامة إلى غاية تركيز قدره GME3 و 2000GME2 مغ/كغ. أظهرت النتائج انخفاضا في معدلات الغلوكوز في مستخلصات. معدل الكوليسترول الكلّي، ثلاثيات الغليسريد في المصل GME1 بالمقارنة مع مستخلص ($p<0.001$) اليوريا وكذا الكرياتينين تمّ تخفيضها في المجموعة الضابطة بينما ارتفع معدّل البروتينات الكليّة. التقييم النسيجي للكبد والكلية أظهر انخفاضا في الأضرار حيث نشاط الإصلاح المعتدل لخلايا بيتا تمّت ملاحظتها في البنكرياس الخاص بالفئران المصابة بالسكري. قد يكون مصدرا محتملا في التعامل مع مرض السكري GME كخلاصة، النتائج أظهرت بأنّ مستخلص نتيجة احتواءه على مضادات الأكسدة، القدرة المؤجلة لهضم الكربوهيدرات، استقراء تفاضل الخلايا الدهنية، تحسين قدرة امتصاص الغلوكوز وكذا القدرة على تخفيض مستوى السكر عند الفئران المصابة بداء السكري.

ABSTRAK

Kulit manggis (*Garcinia mangostana* Linn.) telah terbukti sejak dahulu lagi digunakan dalam perubatan tradisional. Kajian ini bermatlamat mengkaji potensi ekstrak *G. mangostana* (GME) terhadap penurunan glukosa dalam darah secara tabung uji dan keatas haiwan. Kandungan α-mangostin dalam ekstrak diukur melalui HPLC and diselidiki jumlah kandungan fenol dan flavonoidnya. Aktiviti antioksida diukur melalui cerakin hapus sisa radikal DPPH dan cerakin upaya penurunan kuasa manakala aktiviti antidiabetik secara tabung uji dinilai berdasarkan perencatan enzim α-glukosida and α-amilas. Kesan GME terhadap sel adiposit dinilai melalui cerakin MTT, induksi adipogenesis dan pengukuran pengambilan glukosa. Dalam ujian keatas haiwan, dos GME1 (50 mg/kg), GME2 (100 mg/kg) dan GME3 (200 mg/kg) secara oral kepada tikus diabetes STZ teraruh melalui kajian secara dos tunggal (akut) dan dos pelbagai (sub-akut) dinilai. Parameter serum biokimia dan perubahan histopatologi diselidik dan dibanding dengan dengan ubat standard diabetes, glibenclamide. Keputusan menunjukkan jumlah fenol dan flavonoid masing-masing adalah 122.2±1.04 mg GAE/g dan 72.8±1.75 mg QE/g daripada ekstrak kering. Aktiviti hapus sisa radikal DPPH dan cerakin upaya penurunan kuasa masing-masing melaporkan nilai EC_{50} 48.2 μg/ml dan IC_{50} 98.2 μg/ml. Melalui cerakin antidiabetik secara tabung uji, enzim α-glukosida dan α-amilas menunjukkan peratus perencatan yang tinggi berbanding akarbos dengan nilai IC_{50} masing-masing 0.41 dan 0.24 mg/ml. Dalam kajian sel, cerakin toksik sel menunjukkan dos kurang daripada 12.5 μg/ml tidak mempengaruhi hayat sel. Perubahan sel preadiposit kepada adiposit matang didapati bertambah dengan peningkatan konsentrasi GME pada 2.5 μg/ml, 5.0 μg/ml dan 10.0 μg/ml. Pengukuran pengambilan glukosa menunjukkan pengambilan 2-deoksiglukosa yang tinggi pada sel yang dirawat dengan GME2 (2243.3±232.3 cpm) berbanding GME1 (1864.0±146.3 cpm) dan GME3 (1246.0±155.8 cpm). Pemberian GME kepada tikus secara oral menunjukkan penggunaan ekstrak yang selamat dengan ketiadaan perubahan tingkahlaku, autonomik, neurologi dan kesan toksik sehingga dos 2000 mg/kg. Keputusan menunjukkan kadar penurunan glukosa yang signifikan dalam GME2 dan GME3 ($p<0.001$) berbanding GME1. Jumlah kolesterol, serum trigliserid, urea dan kreatinin didapati menurun dalam kumpulan yang dirawat sementara jumlah kandungan protin didapati bertambah. Penilaian histologi menunjukkan aktiviti regenerasi yang ringan pada sel β dalam tikus diabetes. Kesimpulannya, penemuan ini menunjukkan bahawa GME berupaya menjadi satu sumber berpotensi dalam mengatur diabetes merujuk kepada kandungan antioksidanya, berupaya melambatkan proses penghadaman karbohidrat, menggalakkan proses adipogenesis, pemulihan pengambilan glukosa serta kesan anti peningkatan glukosa dalam darah terhadap tikus diabetes.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Science (Pharmaceutical Technology)

……..
Muhammad Taher
Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Science (Pharmaceutical Technology)

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Abdul Razak Kasmuri
Examiner

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DECLARATION

I hereby declare that this thesis is the result of my own investigation, 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.

Tengku Muhamad Faris Syafiq bin Tengku Zakaria

Signature……..….. Date ……

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……..……..…… ……..

Signature Date

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ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ADD-1	Adipocyte determination and differentiation-dependent factor 1
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
C/EBPβ	CCAAT/enhancer-binding protein β
$CHCl_3$	Chloroform
cpm	Count per minute
DEX	Dexamethasone
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethylsulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
FBS	Fetal bovine serum
GLUT2	Glucose transporter-2
GLUT4	Glucose transporter-4
GME	Garcinia mangostana extract
GSK-3	Glycogen synthase kinase-3β
H_2SO_4	Sulphuric acid
IBMX	3-isobutyl-1-methylxanthine
IR	Insulin receptor
IS	Insulin
KRPH	Krebs-Ringer HEPES
MAPK	Mitogen-activated protein kinase
MCE	Mitotic clonal expansion
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAD^+	Nicotinamide adenine dinucleotide
OD	Optical density
ORO	Oil-Red-O
PBS	Phosphate buffer saline
PI3K/IRS-1	Phosphatidylinositol 3-kinase/Insulin receptor substrate-1
PIP2	Phosphatidylinositol (4,5)-bisphosphate
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PKB	Protein kinase B
PPARγ	Peroxisome proliferator-activated receptor gamma
PPH	Postprandial hyperglycaemia
PS	Penicillin-Streptomycin
qRT-PCR	Quantitative real-time polymerase chain reaction
R_f	Retention factor
ROS	Reactive oxygen species
Rt	Retention time
RXRα	Retinoid X receptor α
SREBP	Sterol regulatory element binding protein
STZ	Streptozotocin
TZD	Thiazolidinediones
T2D	Type 2 diabetes
v/v	Volume per volume

CHAPTER ONE

INTRODUCTION

1.1 GENERAL OVERVIEW

Research focused on phytochemicals purified from plant-derived natural products has recently increased all over the world. It is known that various plants may synthesize toxic chemicals to defend against hostile milieu as well as predators. Most of the bioactive compounds have broad range of properties and effects, from being acutely fatal to human to being curative in disease treatment. These secondary metabolites produced by the plants may exert various biochemical and pharmacological functions in animals and men (Acamovic & Brooker, 2005).

Special insight on medicinal plants in tropical countries has shifted researchers to find the lead compounds and pharmacologically viable derivatives for drug design and therapeutic purposes. It is worth noting that the application of scientific approach in modern medicine today has provided skeletons for constructing molecules from plant-derived compounds. On the other hand, crude plant extract also has paid a great attention owing to its properties for treating various ranges of ailments like cancer, heart diseases, diabetes mellitus and obesity (Eyre, Kahn, Robertson, Clark, Doyle & Gansler, 2004)

The recent applications of phytoconstituents were further expanded in areas such as nutraceuticals, agrochemicals and traditional medicines with additional focus as not only curative but delayed onset of complications as well as maintaining health. This has driven meticulous search to unravel underlying mechanisms and biological importance.

The most challenging part while conducting research involving natural products would be the unknown effects and complexity of plant extracts and presence of minute bioactive components in bioassays. It is known that the nature rich with unlimited sources of vital secondary metabolites, which might be of high pharmacological significance. According to World Health Organization, over 21,000 plant species were vastly utilized around the world mainly for medicinal purposes. Tropical countries enormously retained their unexplored medicinal plants and natural products which might contain novel biological activities (Trivedi, 2006).

Malaysia, among the 12 countries rich in biodiversity in the world is estimated to have 1,200 plants species in peninsular alone and 2,000 species in Sabah and Sarawak. Most of the plants are being collected for medicinal purposes or used in herbal preparations. The reserved rainforest of Malaysia offered great chances for research activity due to wide range of available species. Among 12,000 species of flowering plants reported, only 100 were recorded to exhibit medicinal value (Perry & Metzger, 1980). Hence, further investigation should be conducted to elucidate the potential bioactive compounds.

Certain secondary metabolites may hold vital functions in the living plants. For example, flavonoids able to eliminate free radicals produced during photosynthesis. Terpenoids may engage pollinators, as seed dispersers, or inhibit competing plants. Alkaloids usually protect from herbivore animals or insect attacks (phytoalexins). Other secondary metabolites function as cellular signaling molecules or responsible for some other functions in the plants (Mayer, 2004). The prevalence and severity of obesity, type 2-diabetes, and the resultant metabolic syndrome are rapidly increasing. As successful preventive and therapeutic strategies for these life-threatening health ailments often come with adverse side effects, nutritional elements are widely used in

many countries as preventive therapies to prevent or manage metabolic syndrome. Fruits are important dietary components, and contain various bioactive constituents. Many of these constituents have been proven to be useful to manage and treat various chronic diseases such as diabetes, obesity, cancer and cardiovascular diseases. Although exotic fruits are understudied throughout the world due to their limited regional presence, many studies reveal their potent ability to ameliorate metabolic derangements and the resultant conditions i.e. diabetes and obesity. The aim of this article is to review the role of exotic fruits and their constituents in the regulation of metabolic functions, which can beneficially alter diabetes and obesity pathophysiology.

1.2 SIGNIFICANCE OF THE STUDY

Reports concerning *Garcinia mangostana* Linn. are diversely documented for its health-promoting benefits and thus been classified as 'queen of fruit' (Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra & Pérez-Rojas, 2008). All parts of the plant such as leaves, heartwood, ripe fruits, stem barks and fruit hull (pericarp or rind) were reported to elicit significant biological properties. Despite numerous *in vitro* studies shown that the major compound xanthones, a family of tricyclic isoprenylated polyphenols which were extracted from pericarps possess anti-oxidant, anti-proliferative, pro-apoptotic, anti-inflammatory and anti-carcinogenic activities, there were few scientific reports on antihyperglycaemic effect of the plant. Thus, the present study was conducted primarily to ascertain that the crude extracted from pericarp could manage diabetic conditions via cell culture models (*in vitro*) and animal studies (*in vivo*).

1.3 GENERAL OBJECTIVES OF THE STUDY

1. To assess the phytochemical profile of *G. mangostana* pericarp extract (GME).
2. To determine the α-mangostin content in GME.
3. To evaluate the cellular effect of GME on 3T3-L1 adipocyte.
4. To examine the antihyperglycaemic effect of GME on streptozotocin-induced diabetic rats.
5. To perform toxicity study using GME.

1.4 RESEARCH PROBLEM

Diabetes is one of the major metabolic disorders that continue to present as a significant health problem worldwide and mostly associated with chronic hyperglycemic condition and disturbances in protein, carbohydrate and lipid metabolism (Rahimi, Nikfar, Larijani & Abdollahi, 2005). In 2013, a total of 381.8 million adults worldwide were affected with diabetes and estimated to reach 591.9 million in 2035 (Guariguata, Whiting, Hambleton, Beagley, Linnenkamp & Shaw, 2014). Hence, the search for alternative medicinal products is crucial to ameliorate this condition. The study highlighted the development of *G. mangostana* into a widely used nutraceutical. A comprehensive assessment of the biological activities of the extracts was performed using pharmaceutical approach such as phytochemical profiling, antioxidant capacities, enzymatic inhibitions, differentiation capabilities in adipocytes and reduction of blood glucose levels in diabetic rats. The study will provide preclinical evidence and elucidate the extent of pharmacological activities of this extracts as potentially relevant therapeutic drug in diabetes management. The experimental procedure was described in Figure 1.

1.5 HYPOTHESIS

High xanthones content of *G. mangostana* extract may potentiate a number of biological activities including antioxidant properties, inhibition of starch digestion, cellular response and antihyperglycaemic effect in experimental diabetic rats.

1.6 EXPERIMENTAL DESIGN

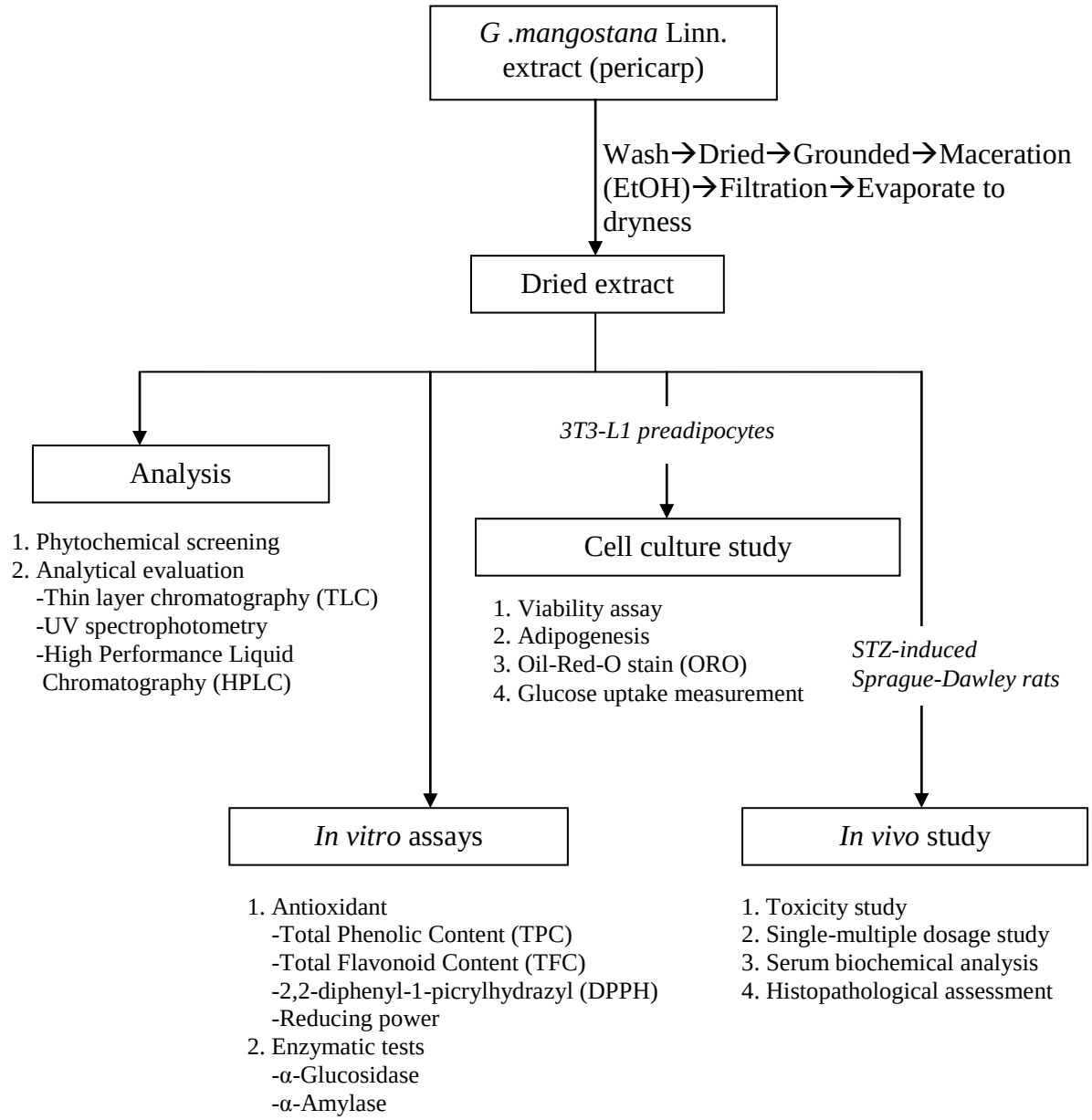

Figure 1. Flow chart of the study.