UNIVERSITI PUTRA MALAYSIA

HEPATOPROTECTIVE EFFECT OF Muntingia calabura L. LEAF EXTRACTS

NUR DIYANA BINTI MAHMOOD

FPSK(M) 2016 25

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By

NUR DIYANA BINTI MAHMOOD

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

July 2016

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

HEPATOPROTECTIVE EFFECT OF *Muntingia calabura* L. LEAF EXTRACTS

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NUR DIYANA BINTI MAHMOOD

July 2016

Chairman: **Associate ProfessorZainulAmiruddinZakaria, PhD**
Faculty: **Medicine and Health Sciences**

Muntingia calabura is a plant of the family Elaeocarpaceae, a tropical species and known to the Malays as 'Kerukup Siam'. Traditionally, the Peruvian believed that *M. calabura*'sleaves could reduce gastric ulcers and swelling of prostate gland as well as alleviate headache and cold. Therefore, the objective of this study was to determine the hepatoprotective activity of methanolic extract of *M.calabura* leaves (MEMC) and its partitions using rat models. The hepatoprotective potential of MEMC was investigated using paracetamol (PCM)- and carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Briefly, male Sprague Dawley rats (n = 6 per group) were divided into groups and administered orally once daily with 10% dimethyl sulfoxide (DMSO) (negative control), 50 mg/kg N-acetylcysteine (NAC) (positive control), or MEMC (50, 250, and 500 mg/kg) for 7 consecutive days, followed by hepatotoxicity induction using PCM or CCl_4.MEMC was laterpartitioned into 3 fractions: petroleum ether extract (PEMC), ethyl acetate extract (EAMC) and aqueous extract (AQMC).The protective effect of PEMC, EAMC and AQMC were tested on PCM-induced hepatotoxicity rat model. Blood samples were subjected to biochemical analysis to evaluate alanine transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) levels; some parts of the liver were subjected to microscopic analysis. Fresh samples of liver tissueswere used to determine the superoxide dismutase (SOD) and catalase (CAT) activities. All extracts (MEMC, PEMC, EAMCand AQMC) were tested for antioxidant activity study using the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), superoxide dismutase scavenging activity assay (SOD), and oxygen radical absorbance capacity assay (ORAC), and anti-inflammatory study using xanthine oxidase (XO) and lipoxygenase (LOX) assays. Total phenolic content (TPC), phytochemical screening, and high-performance liquid chromatography (HPLC) analysis were also performed. From the histological observation, lymphocyte infiltration and marked necrosis were observed in the DMSO-treated group (negative control). MEMC at 500 mg/kg significantly reduce the toxic effect of PCM and CCl_4 on the liver by reducing the weight of the liver; histological observation demonstrated normalization of the histopathological changes, preserving hepatocytes structure, causing a significant decline in ALT and AST levels ($P< 0.05$) and increase in the SOD and CAT activities. Among the partitions, AQMC at 250 mg/kg showed significant reduction in the toxic effect of PCM by causing significant decline in the ALT and AST levels. AQMC were then tested at doses of 50 mg/kg and 500 mg/kg and the result

demonstrated reduction in the liver enzymes in a dose-dependent manner with augmentation of SOD and CAT activities. MEMC had the highest TPC value, followed by EAMC, PEMC, and AQMC. MEMC and AQMC demonstrated strong free radical scavenging activity in the DPPH and SOD assays. AQMC showed the highest ORAC value while MEMC was the lowest among the extracts. All extracts in the present study demonstrated strong anti-inflammatory activity via inhibition of LOX. Phytochemical screening of the extracts showed that MEMC, PEMC and EAMC contained flavonoids, tannins, and steroids. Only PEMC showed the presence of triterpenes. However, the phytochemical screening showed that AQMC contained fewer compounds. HPLC analysis suggests that MEMC and AQMC containedflavonoid-based compounds. In conclusion, MEMC exerted potential hepatoprotective activity that can be attributed to its antioxidant activity, and AQMC was considered to have the best activity among other partitions, which warrants further investigation.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN HEPATOPROTEKTIF OLEH EKSTRAK DAUN *Muntingia calabura* L.

Oleh

NUR DIYANA BINTI MAHMOOD

Julai 2016

Pengerusi: **Profesor Madya Zainul Amiruddin Zakaria, PhD**
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Muntingia calabura ialah sejenis tumbuhan tropikal dari keluarga Elaeocarpaceae dan dikenali sebagai Kerukup Siam oleh masyarakat Melayu. Secara tradisi, masyarakat Peru percaya bahawa daun *M. calabura* mampu mengurangkan ulser gastrik dan bengkak pada kelenjar prostat termasuk juga meredakan sakit kepala dan selsema. Oleh itu, objektif kajian ini adalah untuk menentukan aktiviti hepatoprotektif ekstrak methanol daripada daun *M. calabura* dan pecahannya dengan menggunakan model tikus. Potensi hepatoprotektif dari ekstrak metanol daun *M. calabura* (MEMC) telah diuji menggunakan rangsangan hepatotoksisiti paracetamol (PCM)- dan karbon tetraklorida (CCl_4) pada tikus. Secara ringkas, tikus 'Sprague Dawley' jantan (n = 6 bagi setiap kumpulan) dibahagikan kepada beberapa kumpulan dan diberi makan secara oral sekali sehari dengan 10% dimetil sulfoxide (DMSO) (kawalan negatif), 50 mg/kg N-acetylcysteine (NAC) (kawalan positif), atau MEMC (50, 250, dan 500 mg / kg) selama 7 hari, diikuti dengan aruhan hepatotoksisiti menggunakan PCM atau CCl_4. MEMC seterusnya dibahagikan kepada 3 pecahan: ekstrak petroleum eter (PEMC), ekstrak etil asetat (EAMC) dan ekstrak air (AQMC). Kesan perlindungan oleh PEMC, EAMC dan AQMC telah diuji dengan model aruhan PCM ke atas tikus. Sampel darah yang telah diambil dibuat kajian biokimia untuk menganalisis paras enzim seperti alanine transferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Manakala sebahagian daripada sampel hati pula diuji secara mikroskopik. Sampel hati yang segar pula diuji untuk aktiviti superoxide dismutase (SOD) dan catalase (CAT). Semua ekstrak (MEMC, EAMC dan AQMC) diuji untuk kajian antioksidan menggunakan cerakin 2, 2-difenil-1-picrylhydrazyl radikal (DPPH), pengujian perangkap aktiviti superoxide dismutase (SOD), dan cerakin penyerapan oksigen radikal kapasiti (ORAC), dan kajian anti-radang menggunakan analisis aktiviti xanthine oxidase (XO) dan lipoxygenase (LOX). Kandungan jumlah fenol (TPC), pemeriksaan fitokimia, dan analisa kromatografi cecair berprestasi tinggi (HPLC) juga telah dilaksanakan. Dari segi pemerhatian histologi penyusupan limfosit dan nekrosis diperhatikan dalam kumpulan rawatan DMSO (kawalan negatif). MEMC pada dos 500 mg/kg menunjukkan pengurangan yang signifikan kepada kesan toksik oleh PCM dan CCl_4 ke atas hati, dengan menyebabkan penurunan berat hati, pemantauan histology menunjukkan pemulihan struktur sel-sel hati, dan menyebabkan penurunan paras ALT dan AST secara signifikan (P<0.05) dan peningkatan aktiviti SOD dan CAT. Antara semua pecahan, AQMC pada dos 250 mg/kg menunjukkan pengurangan yang

signifikan ke atas kesan toksik oleh PCM yang dengan menurunkan paras ALT dan AST secara signifikan. AQMC diuji pada dos 50 mg/kg dan 500 mg/kg dan keputusan menunjukkan penurunan paras enzim hati dengan kebergantungan pada peningkatan dos dan juga peningkatan aktiviti enzim SOD dan CAT. MEMC mempunyai nilai TPC paling tinggi diikuti oleh EAMC, PEMC, dan AQMC. MEMC dan AQMC menunjukkan aktiviti yang paling tinggi dalam pengujian perangkap DPPH dan SOD. AQMC menunjukkan nilai ORAC yang tinggi manakala MEMC menunjukkan nilai yang paling rendah berbanding ekstrak lain. Semua ekstrak menunjukkan anti radang yang kuat dalam menghalang LOX. Pemeriksaan fitokimia ekstrak menunjukkan MEMC, PEMC dan EAMC mempunyai flavonoid, tannin, dan steroid. Hanya PEMC yang mempunyai triterpene. Walau bagaimana, AQMC hanya mempunyai sedikit sehaja sebatian. Analisa HPLC mencadangkan bahawa MEMC dan AQMC mempunyai sebatian daripada flavonoid. Kesimpulannya, MEMC mempunyai potensi sebagai agen hepatoprotektif yang juga bergantung kepada aktiviti antioksidan, dan AQMC dianggap mempunyai aktiviti yang terbaik di antara pecahan ekstrak, yang memerlukan siasatan lanjut.

ACKNOWLEDGEMENT

Alhamdulillah, All Praises are for Allah subhanahu wa ta`ala for helping me in coordinating my life.

I wish to extend my deepest appreciation to my supervisor, Associate Professor Dr. Zainul Amiruddin Zakaria, for his guidance, valuable advice, patience, support and continuous supervision throughout the course of this project. I truly thank him for giving me the opportunity to be his postgraduate student.

I would like to express my gratitude and appreciation to my co-supervisor, Associate Professor Dr Norhafizah Mohtarrudin, for her kindness to teach and guide me in histological study that is definitely important in order to complete this project.

My sincere appreciation goes to the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, for giving me the opportunity to carry out this project.

My special dedication to Encik Ramli bin Suhaimi, staff of Pharmacology Laboratory and of Histology Laboratory for their kind cooperation, excellent facilities and support, which helped me to perform the analyses smoothly. I would like to thank all my fellow friends, Farah Hidayah bt Kamisan, Mohammad Fauzi Fahmi bin Kamarolzaman, Siti Syariah bt Mamat, Roihana bt Rodzi, Tavamani Balan, Farhana bte Yahya, Nur Liana bt Md Nasir, NoorSyaza Eddrina bt Kamsani, Noor Wahida bt Suhaimy, Mohd Khusairi bin Azmi, Mohd Hijaz bin Sani, and Salahuddin bin Mumtaz Ahmad for their kindness, patience and cooperation in completing this project successfully.

Words are not enough to express my gratitude to my beloved family, especially my dearest parents, Mahmood bin Ibrahim and Fatimah binti Mamat for their love, comfort, encouragement, support and advice that truly motivated me to accomplish my Master's degree successfully. I owe them a depth gratitude which can never be repaid. May Allah shower His countless blessings upon them.

Thank you so much.

I certify that a Thesis Examination Committee has met on 18 July 2016 to conduct the final examination of Nur Diyana binti Mahmood on her thesis entitled "Hepatoprotective Effect of *Muntingia calabura* L. Leaf Extracts" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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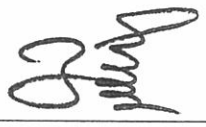

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Member of
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LIST OF ABBREVIATIONS

AAPH	2,2'-azobis-2-methyl-propanimidamide, dihydrochloride
AQMC	Aqueous extract of *Muntingia calabura*
CYP450	Cytochrome P450
CYP2E1	Cytochrome P450 2E1
EAMC	Ethyl acetate extract of *Muntingia calabura*
IC_{50}	Median inhibitory concentration
IL-1	interleukin-1
MEMC	Methanol extract of *Muntingia calabura*
NSAIDs	Non-steroidal anti-inflammatory drugs
ORAC	Oxygen radical absorbance capacity
PCM	Paracetamol
PEMC	Petroleum ether extract of *Muntingia calabura*
PPAR-α	Peroxisome proliferator-activated receptor alpha
TNF	Tumor necrosis factor
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Research background

Liver is a vital organ in the body that plays a role in regulation of diverse processes including metabolism, secretion, storage, and detoxification of waste metabolites (endogenous) and toxic compounds (exogenous) (Madrigal-Santillán *et al*., 2014). Continual damage to the liver by acute liver insult will eventually result in the development of hepatic fibrogenesis, which promotes the abnormal structural changes to the tissue known as cirrhosis (Qua and Goh, 2011). The increasing incidence of mortality due to the liver diseases has been reported to be the tenth leading cause of death in the United States of America (Jiaquan *et al*., 2016). Meanwhile, the prevalence of liver cirrhosis in Malaysia is 15 in every 10,000 population, and the distributions of underlying etiology vary regionally with viral hepatitis being much higher compared to the European countries (Ng *et al*., 2011).

For millennia, natural products have been used traditionally to treat and/or prevent various liver diseases in many parts of the world. Regardless of the abundance of the number of modern drugs in the pharmaceutical market, natural products have begun to gain importance and popularity in the world for promoting health care as well as disease prevention, and been used as conventional or complementary medicines for treatable and incurable diseases (Zhang *et al*., 2013). The World Health Organization (WHO) estimated that up to four billion people representing 80% of developing societies of the world's population consider on natural products as their most preferred healthcare option (Amiri *et al*., 2012; Ekor, 2014).

Natural products become an interest in drug discovery as it contributes unique structural diversity, which provides the opportunities for discovering mainly novel low molecular weight lead compounds that have desirable properties (Dias *et al*., 2012). An analysis from 1981 to 2010 by Newman and Cragg (2012) showed that more than two-thirds of the drug active compounds recently introduced are likely derived from natural sources, and only about 30% are completely synthetic origin (Newman and Cragg, 2012).

Since less than 10% of the 250,000 species of the world's biodiversity has been evaluated for medicinal purposes (Ramasamy *et al*., 2011; McChesny *et al*., 2007), many more species await to be discovered for therapeutic benefits. Malaysia, a well known tropical country, has been acknowledged worldwide as a land of floral and faunal prosperity, and is believed to be a reservoir of a large collection of potential medicinal plants.

Muntingia calabura from the family of Elaeocarpaceae, is one of the native plants in Malaysia that has been widely tested and documented for its promising

pharmacological properties. Numerous investigations showed that the leaves possess various pharmacological activities, including antiulcer, antipyretic, antinociceptive and anti-inflammatory activities along with antioxidant and antiproliferative properties (Mahmood *et al*., 2014). However, hepatoprotective properties of this plant have not yet been explored. As such, further research on this activity is significant to nominate another plant to the list of potential medicinal hepatoprotective plant-based products.

1.2 Problem statement

Liver diseases remain one of the major threats to community health and a worldwide burden. Particularly, drug-induced hepatotoxicity is a major cause of hepatic damage or dysfunction. Paracetamol (PCM), a mild analgesic and antipyretic drug developed in the past few decades, causes severe liver injuries in human and experimental animals when taken overdose. Liver cirrhosis develops from excessive alcohol consumption is the common alcoholic liver disease in western countries (Byass, 2014). Zain *et al* (2006) reported that in Singapore, PCM represented 55% of overdosing cases in year 1999 among patients admitted to the hospital for intentional self-poisoning. In spite of remarkable advances in modern medicine, there are hardly reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cells. Due to adverse effects of drug, a great deal of research has been conducted to explore natural products from plants as promising hepatoprotective agents. It is known that agents possessing anti-inflammatory activity have the potential to act as an antioxidant (Yanpallewar *et al*., 2002). Previous studies on *M. calabura* reported the presence of antioxidant and anti-inflammatory activity that is relevant to hepatoprotective activity. Considering these reports, the antioxidant and anti-inflammatory activity indicate different pathways assisting the hepatoprotective effect. ROS generated from drug or chemical metabolism appear to be the fundamental mechanisms underlying most human ailments. The antioxidant and anti-inflammatory properties of plants facilitate the free radical scavenging process and regulate the inflammatory response, respectively, which are believed to initiate their damaging effects on the liver. Therefore, this study is expected to discover the capacity of *M. calabura* for hepatoprotective activity.

1.3 Objective(s)

1.3.1 General objective

To determine the hepatoprotective activity of methanol extract of *Muntingia calabura* (MEMC) leaves and its partitions using rat models.

1.3.2 Specific objectives

- To determine the hepatoprotective effect of MEMC against paracetamol (PCM) and carbon tetrachloride (CCl_4)-induced liver toxicity in rats,
- To determine the most effective partition of MEMC (petroleum ether, ethyl acetate and aqueous extracts) on liver toxicity study,
- To elucidate the possible mechanisms of hepatoprotection of MEMC and the most effective partition

1.4 Research hypotheses

1. It is hypothesize that methanol extracts of *M. calabura* leaves (MEMC) will exhibit a hepatoprotective activity in paracetamol (PCM)- and carbon tetrachloride (CCl_4)- induced liver toxicity assays.

2. AQMC is expected to have good hepatoprotective activity in PCM-induced liver toxicity.

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