



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDANT PROPERTIES OF LEAF AND FLOWER EXTRACTS
OF MORINGA OLEIFERA LAM (KELOR) AND THEIR EFFECTS
ON ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN VIVO**

SYAZANA AKMAL SHARIFUDIN

FPSK(m) 2011 61

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By

SYAZANA AKMAL SHARIFUDIN

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

December 2011

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SYAZANA AKMAL SHARIFUDIN

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2011

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

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By

SYAZANA AKMAL BINTI SHARIFUDIN

December 2011

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Faculty : Medicine and Health Sciences

Moringa oleifera Lam (MO) is a tropical plant and an important food commodity. The plant has garnered enormous attention recently due to its nutritional value. Malaysian Indian community used this plant traditionally for various therapeutic purposes. Despite many studies conducted on its hepatoprotective action, no study has been designed to address the question whether MO could work as an antioxidant and protect the liver concurrently with acetaminophen (APAP) intoxication. Therefore the aim of this study is to investigate the antioxidant activity of different parts of *Moringa oleifera* tree and the action of flowers and leaves hydroethanolic extract respectively in protecting the liver following concurrent treatment of high doses of APAP.

Total phenolic content was conducted using Folin-Ciocalteu reagent and antioxidant assay was conducted using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay and ferric reducing antioxidant power (FRAP) assay of each part of the plant.

A preliminary study was carried out to obtain the optimum dose for hepatotoxicity and the results will determined the dose of APAP in subsequent studies. The doses used were 2.0g of APAP/kg body weight, 4.0g of APAP/kg body weight and 6.0g of APAP/kg body weight via oral route. The study measured liver and renal function as well as histological changes in the liver and kidney.

In hepatoprotective study, leaves and flowers hydroethanolic extract (200mg/kg body weight and 400mg/kg body weight, i.p) were administered an hour after APAP administration respectively. N-Acetylcysteine (NAC) was used as the positive control. Liver function tests, renal function tests, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) protein adduct were analysed and histological changes were compared between groups.

The total phenolic content was the highest in the flowers followed by leaves. The result correlates ($R^2=0.8765$) well with the antioxidant assay where flowers extract also showed the highest antioxidant capacity. Meanwhile, acetaminophen-induced hepatotoxicity increased the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Blood urea nitrogen (BUN) as well as serum creatinine was observed to be normal indicating no kidney damage. The level of GSH, SOD and CAT were significantly decreased and the MDA and 4-HNE protein adduct levels were elevated in groups treated with APAP. Histologically, the liver was observed to have severe inflammation and bridging necrosis.

When concurrent treatment was conducted with either flowers or leaves, the level of ALT and AST were significantly reduced. The results were consistent with histological observation. The liver sections obtained from rats treated with flowers and leaves hydroethanolic extract showed reduction in the severity of the liver damage. Serum BUN and creatinine were also decreased following plant extract treatment. The level of antioxidant enzymes such as SOD and CAT were found to have increased besides, GSH level was also enhanced. On the other hand, MDA and 4-HNE protein adduct were observed to be decreased.

These results had indicated the possible action of hydroethanolic extract of MO flowers and leaves in protecting liver against over dosage of APAP.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

**CIRI-CIRI ANTIOKSIDA EKSTRAK DEDAUN DAN BUNGA-BUNGA
MORINGA OLEIFERA LAM (KELOR) DAN KESAN TERHADAP
KEROSAKAN HEPAR AKIBAT HEPATOKSISITI ASETAMINOFEN *IN*
*VIVO***

Oleh

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Moringa oleifera Lam (MO) adalah tumbuhan tropikal dan komoditi makanan yang penting. Ia mendapat liputan meluas disebabkan nilai khasiatnya. Masyarakat India di Malaysia menggunakan tumbuhan ini secara tradisional untuk pelbagai tujuan perubatan. Walaupun banyak kajian telah dijalankan untuk mengkaji kesannya sebagai pelindung kerosakan hepar, tiada kajian dijalankan akan kesan antioksidan dan pelindung kerosakan hepar yang diberi bersama-sama dengan keracunan asetaminofen (APAP). Oleh itu, matlamat kajian ini adalah untuk mengkaji kesan antioksidan dari bahagian tumbuhan *Moringa oleifera* yang berlainan dan juga kesan ekstrak hidroetanolik bunga-bunga dan dedaun tumbuhan masing-masing dalam melindungi kerosakan hepar akibat dos APAP yang tinggi

Jumlah kandungan fenol, telah dijalankan menggunakan reagen Folin-Ciocalteu dan asai antioksidan dijalankan dengan menggunakan 1,1-diphenyl-2-picrylhydrazyl (DPPH) penghapusan radikal bebas dan asai kuasa antioksidan penurunan ferrik pada setiap bahagian tumbuhan tersebut.

Ujian saringan telah dijalankan bagi mendapatkan kesan optimum hepatoksisiti dan keputusan ujian tersebut akan digunakan pada kajian seterusnya. Dos yang diberikan 2.0g APAP /kg berat badan, 4.0g APAP/kg berat badan dan 6.0g APAP/kg berat badan adalah melalui mulut. Kajian ini mengukur ujian fungsi hepar dan ujian fungsi ginjal serta perubahan struktur histologi bahagian ini.

Dalam kajian pelindung kerosakkan hepar pula, ekstrak hidroetanolik bunga-bunga (200mg/kg dan 400mg/kg, i.p) dan dedaun (200mg/kg dan 400mg/kg, i.p) diberikan satu jam masing-masing selepas pemberian APAP. N-Acetylcysteine (NAC) digunakan sebagai kawal positif ujian. Ujian fungsi hepar, ujian fungsi ginjal, glutathione terturun (GSH), superoksida dismutase (SOD), katalase (CAT), malondialdehida (MDA), 4-hidroksinonenal (4-HNE) aduk protein dikaji dan perubahan struktur histologi dibandingkan mengikut kumpulan.

Kandungan fenol jumlah tertinggi terdapat pada ekstrak hidroetanolik bunga-bunga diikuti dengan ekstrak hidroetanolik dedaun. Keputusan juga mempunyai kaitan baik ($R^2=0.8765$) dengan asai antioksidasi di mana ekstrak bunga-bunga juga menunjukkan kemampuan antioksidasi yang tinggi. Sementara itu, kerosakan hepar disebabkan asetaminofen telah meningkatkan nilai alanine aminotransferase (ALT) dan aspartat aminotransferase (AST). Kandungan darah urea nitrogen (BUN) dan serum kreatinin didapati normal dan ini menandakan fungsi ginjal adalah baik.

Paras GSH, SOD dan CAT telah dilihat menurun secara signifikan ($p<0.05$) manakala MDA dan 4-HNE protein aduk meningkat pada kumpulan tikus-tikus yang

menerima APAP. Pemerhatian secara histologi, hepar didapati mempunyai keradangan teruk dan kematian sel-sel hepar yang berterusan

Apabila rawatan bersama bunga-bunga atau dedaun dijalankan, paras ALT dan AST menurun secara signifikan ($p < 0.05$). Keputusan adalah konsisten dengan pemerhatian histologi. Hirisan hepar daripada tikus yang dirawat dengan ekstrak hidroetanolik bunga-bunga atau dedaun menunjukkan kerosakan pada hepar dapat dikurangkan. Serum BUN dan kreatinin juga dilihat menurun selepas mendapat rawatan menggunakan tumbuhan tersebut. Paras enzim antioksidan dan bukan enzim antioksidan seperti SOD, CAT dan GSH telah meningkat. Manakala, paras MDA dan 4-HNE aduk protein didapati menurun.

Keputusan yang diperolehi menunjukkan kebolehan ekstrak hidroetanolik bunga-bunga dan dedaun MO dalam melindungi hepar daripada kerosakan akibat dos APAP yang tinggi.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Allah s.w.t Most Gracious and Most Merciful for His Blessing, and with that I am able to finish my Master of Science in Pharmacology and Toxicology. I would also like to express my most sincere gratitude to my supervisor Datin Assoc. Prof. Dr. Sharida Fakurazi, for her guidance, scientific advice, constructive ideas, and assistance throughout my research. I am grateful to Datin Dr. Sharida Fakurazi for introducing me to the field of pharmacology and toxicology, long-lasting encouragement, patience, scientific discussion as well as her efforts and dedication.

My sincere appreciation is also extended to two co-supervisor; Assoc. Prof. Dr. Hairuszah Ithnin and Dr Mohamad Aris Mohd Moklas, for their tremendous help, patience and kindness. I want to acknowledge them for their valuable comments and suggestion of the research.

I would also like to thank the officers and staff from Human Anatomy Research Laboratory, Cell Signalling Laboratory, Chemical Pathology Laboratory, Multipurpose Laboratory (centrifuge room), Animal House, Faculty of Medicine and Health Sciences and Department of Pathology, Hospital Kajang for being helpful and providing convenient working facilities. Pn Farhatani and Pn Rohaznida, both scientific officers have been very kind, generous and understanding in what I have gone through.

My warm thanks to my colleagues for creating a friendly and great atmosphere at Human Anatomy Research Laboratory, Faculty of Medicine and Health Sciences. Thank you very much Pn Shamima, Cik Amalina and Cik Fatin, for being there for me through ups and downs, giving constructive ideas and good advices. I would also like to extend my gratitude to my fellow friends, Dr Fayak and Dr Ajwad from Faculty of Veterinary Sciences for their help and valuable comments. I wish we can all continually support each other in many ways and have a long-lasting friendship.

I also wish to thank the Ministry of Science and Technology (MOSTI), Malaysia for providing NSF scholarship and Fundamental Research Grant Scheme (FRGS), UPM for research financial support. Both MOSTI and FRGS are gratefully acknowledged.

To my beloved parents and brothers, thank you very much for believing in me and supporting me in pursuing my studies. My family had contributed greatly towards my personal growth and continually giving inspiration to seek higher education. I'm very thankful to Allah to have such a wonderful family. I would also like to dedicate this thesis to my mother and father. God Bless.

I certify that a Thesis Examination Committee has met on 15th May 2012 to conduct final examination of Syazana Akmal bt Sharifudin on her thesis entitled Antioxidant properties of Leaf and Flower Extracts *Moringa oleifera* Lam (Kelor) And Their Effects on Acetaminophen-induced Hepatotoxicity *In Vivo* in accordance with Universities and Universities 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 in Science (Pharmacology & Toxicology)

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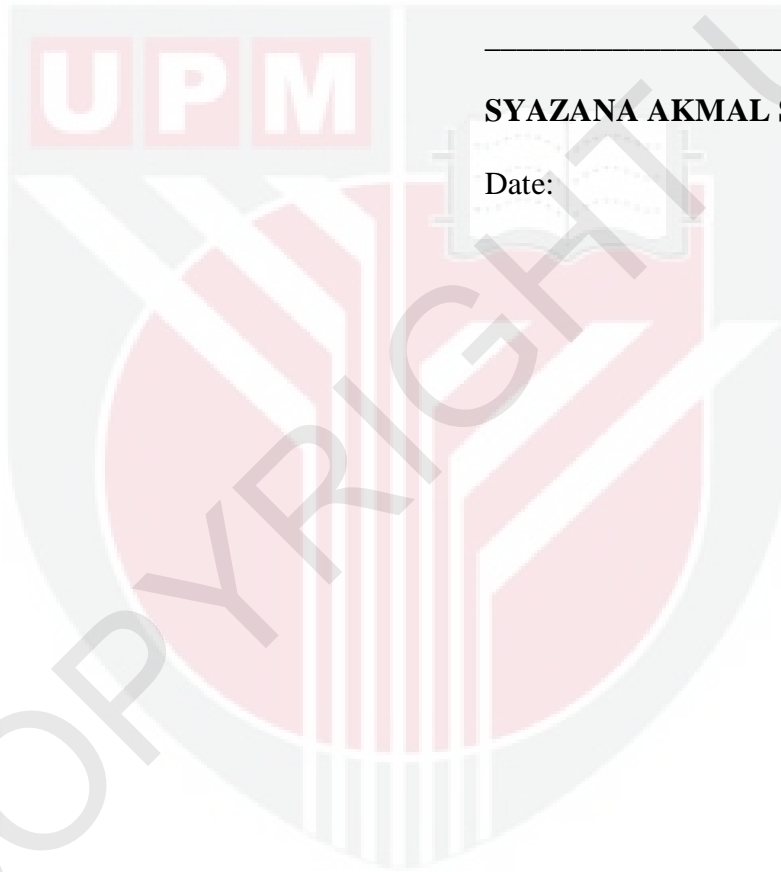
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DECLARATION

I declare that the thesis is based on my original work except for the 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 institution



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