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

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By

SYAZANA AKMAL SHARIFUDIN

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SYAZANA AKMAL BINTI SHARIFUDIN

December 2011

Chairman: Assoc. Prof. Sharida binti Fakurazi, PhD
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*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

SYAZANA AKMAL BINTI SHARIFUDIN

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Pengerusi : Prof. Madya. Sharida binti Fakurazi, PhD
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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 antioksida dan pelindung kerosakan hepar yang diberi bersama-sama dengan keracunan asetaminofen (APAP). Oleh itu, matlamat kajian ini adalah untuk mengkaji kesan antioksida 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 antioksida dijalankan dengan menggunakan 1,1-diphenyl-2-piclyhydrazyl (DPPH) penghapusan radikal bebas dan asai kuasa antioksida 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 kerosakan 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), superoksid 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 (R²=0.8765) dengan asai antioksida di mana ekstrak bunga-bunga juga menunjukkan kemampuan antioksida 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 antioksida dan bukan enzim antioksida 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.
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I certify that a Thesis Examination Committee has met on 15\textsuperscript{th} May 2012 to conduct final examination of Syazana Akmal bt Sharifudin on her thesis entitled Antioxidant properties of Leaf and Flower Extracts \textit{Moringa oleifera} Lam (Kelor) And Their Effects on Acetaminophen-induced Hepatotoxicity \textit{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)

Member of the Thesis Examination Committee were as follows:

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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’s of Science Thesis. The members of the supervisory Committee were as follows:

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Date:
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.

_______________________________
SYAZANA AKMAL SHARIFUDIN

Date:
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