UNIVERSITI PUTRA MALAYSIA

HEPATOPROTECTIVE EFFECT OF *MORINGA OLEIFERA* LEAVES EXTRACT ON ACETAMINOPHEN-INDUCED LIVER DAMAGE IN RATS

UMA NANTHINI LINGGI GAUNDAR

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2008

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By

UMA NANTHINI LINGGI GAUNDAR

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

August 2008

This thesis is especially dedicated to:

My loving father, Mr Linggi Gaundar, my caring mother Mrs. Jaya Letchumy and family, who are infinitely precious to me,

&

Sri Vignes, who has filled my life with joy and happiness,

&

My friends, who were there for me!

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

HEPATOPROTECTIVE EFFECT OF *MORINGA OLEIFERA* LEAVES EXTRACT ON ACETAMINOPHEN-INDUCED LIVER DAMAGE IN RATS.

By

UMA NALTHINI LINGGI GAUNDAR

August 2008

Chairman : Sharida Fakurazi, PhD

Faculty : Medicine and Health Sciences

Moringa oleifera (MO) is reported to have various medicinal properties. The aim of this study is to evaluate the hepatoprotective effect of MO leaf extract against acetaminophen (APAP) induced liver damage in rats. A dose of 3g/kg APAP was selected to induce liver damage. Seventy male Sprague-Dawley rats (n=70) were divided into seven groups. Five groups of animals were given various oral pretreatments of 200mg/kg MO, 800mg/kg MO and 200mg/kg Silymarin (Sil) in distilled water at 3ml/day for fourteen days. Meanwhile, two groups served as hepatotoxicity (3g/kgAPAP) and vehicle (40% sucrose) control groups were given distilled water in the similar manner. On day 15, the animals were challenged with 3g/kg APAP in 40% sucrose except for rats in the vehicle (40% sucrose) and MO control groups which received 40% sucrose solution. After 24 and 48 hours blood was withdrawn and livers were harvested. Plasma was prepared and liver function was carried out to determine levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Liver samples were taken for histopathalogical examination, measurement of hepatic reduced glutathione

(GSH) content, glutathione-*S*-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) activities as well as determining malondialdehyde (MDA) levels. Statistical analysis was performed using analysis of variance (ANOVA) and Kruskall Wallis analysis of variance coupled with the Mann–Whitney U-test. APAP treatment caused significant elevation ($p<0.05$) of ALT, AST after 24 and 48 hours. Histopathological observations substantiated these findings showing significant ($p<0.05$) liver damage. APAP treatment caused marked reduction ($p<0.05$) in hepitic GSH content, GST and GPx. activities coupled with significant increase ($p< 0.05$) in lipid peroxidation index. The changes observed were time dependent with more changes were noted after 48 hours. Significant ($p<0.05$) elevation of ALP and significant ($p<0.05$) decline of GR activity was only noted after 48 hours compared to other groups. 200mg/kg and 800mg/kg MO extract equally showed a significant ($p<0.05$) amelioration of ALT, AST and ALP levels and a significant reduction ($p<0.05$) of pathological alteration in a manner similar to Sil. MO extracts showed no signs of toxicity up to a dose level of 800 mg/kg. MO alone significantly increased ($p<0.05$) GSH content and restored GSH level ($p<0.05$) in the groups given MO and challenged with APAP. MO alone showed insignificant increase of GST, Gap and GR activities. The significant increase ($p<0.05$) of these antioxidant enzymes observed in groups received MO extracts and challenged with APAP. Lipid peroxidation was significantly ($p<0.05$) inhibited by the extracts in dose independent manner. A significant ($p<0.05$) increase of GST activities by 200mg/kg and 800mg/kg MO extracts to the level higher than vehicle group were observed as early as 24 hours in comparison with rats given pretreatment of Silymarin. On the other hand, 200 mg/kg MO significantly ($p<0.05$) showed similar increase in GPx activity to the level higher than vehicle group in comparison with

groups that given 200mg/kg Sil and 800mg/kg MO pretreatment. Prevention of enzyme leakage, preservation of hepatocytes structural integrity, prevention of GSH depletion, restoration of antioxidant enzymes activity that is essential in accelerating detoxification and excretion of APAP toxic metabolites, as well inhibition of lipid peroxidative processes reveals that the extracts of MO leaves possesses potential hepatoprotective activity against APAP induced damage in rats.

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

KESAN HEPATOPROTEKTIF EKSTRAK DAUN *MORINGA OLEIFERA* TERHADAP KEROSAKAN HEPAR TIKUS CETUSAN ACETAMINOPHEN

Oleh

UMA NANTHINI LINGGI GAUNDAR

August 2008

Pengerusi: Sharida Fakurazi, PhD

Fakulti : Perubatan dan Sains Kesihatan

Moringa oleifera (MO) dilaporkan mengandungi pelbagai nilai perubatan. Tujuan kajian ini dijalankan adalah untuk melihat kesan hepatoprotektif ekstrak daun MO terhadap kerosakan hepar tikus yang dicetuskan oleh acetaminophen (APAP). Dalam kajian ini, dos sebanyak 3g/kg APAP telah dipilih untuk mecetuskan kerosakan hepar. Tikus jantan Sprague dawley (n=70) telah dibahagikan kepada 7 kumpulan. Lima kumpulan menerima pelbagai jenis rawatan seperti 200mg/kg MO, 800mg/kg MO, 200mg/kg silymarin (Sil) dalam air suling secara oral pada 3ml setiap hari untuk empat belas hari. Tikus dalam kumpulan kawalan hepatotoxicity (3g/kg APAP) dan sukrosa 40% hanya diberi air suling dengan cara yang sama. Pada hari ke-15 tikus diberi 3g/kg APAP dalam 40% sukrosa kecuali kumpulan tikus kawalan dalam sukrosa dan MO yang menerima 40% sukrosa. Selepas 24 dan 48 jam, sampel darah diambil dan hepar dikeluarkan. Plasma disediakan untuk ujian fungsi hati yang merangkumi alanine aminotranferase (ALT), aspartate aminotransferase (AST) dan alkaline phosphatase (ALP). Sampel hepar diambil untuk kajian histopatologi,

penentuan aras glutathione (GSH) hepar, analisis aktiviti glutathione-*S*–transferase (GST), glutathione peroxidase (GPx), dan glutathione reductase (GR) serta aras malondialdehyde (MDA). Data dianalisis menggunakan analisi varians (ANOVA) dan Kruskall Wallis analisi varians dengan Mann–Whitney U-test. Hasil menunjukan bahawa rawatan APAP menyebabkan peningkatan aras ALT dan AST yang signifikan ($p<0.05$) selepas 24 dan 48 jam. Kajian histopatologi menyokong penemuan tersebut yang menunjukkan kerosakan hati ($p<0.05$). APAP menyebabkan penurunan signifikan ($p<0.05$) aras GSH, aktiviti GST dan GPx. serta menunjukkan peningkatan ($p<0.05$) dalam peroksidasi lemak. Perubahan yang diperhatikan adalah bergantung pada masa dengan lebih ketara ($p<0.05$) selepas 48 jam. Peningkatan ALP dan pengurangan aktiviti GR hanya signifikan ($p<0.05$) selepas 48 jam berbanding kumpulan lain. Kedua-dua dos MO ekstrak menunjukkan penurunan signifikan ($p<0.05$) yang setara dalam pemulihan aras AST, ALT dan ALP serta pengurangan perubahan patologi sepertimana diperhatikan pada Sil. Rawatan dengan ekstrak MO tidak menunjukkan kesan toksik setakat dos 800mg/kg. Ekstrak MO sahaja telah meningkatkan aras glutathione hepar ($p<0.05$) dan MO yang diberi bersama rawatan APAP menunjukkan pemeliharaan signifikan ($p<0.05$) aras GSH. Penigkatan aktiviti GST, GPx dan GR oleh MO sahaja adalah tidak signifikan. MO bersama cabaran APAP telah merangsangkan aktiviti-aktiviti enzim antioksidan tersebut. Peroksidasi lemak telah direncatkan secara signifikan ($p<0.05$) oleh MO tanpa dipengaruhi oleh dos. Dos 200mg/kg MO menujukkan keupayaan dalam rangsangan aktiviti GST yang signifikan ($p<0.05$) berbanding silymarin apabila menunjukan aktiviti yang tinggi melebihi kumpulan sukrosa seawal 24 jam. Manakala 200mg/kg MO juga merangsangkan aktitiviti GPx dengan lebih ketara ($p<0.05$) berbanding dengan kumpulan 800mg/kg MO dan Sil, dengan menunjukan

aktiviti melebihi kumpulan sukrosa seawal 24 jam. Pemulihan aras enzim-enzim yang menunjukan fungsi normal hati, pemeliharaan struktur cell hepar, pemeliharaan aras glutathione hati serta pemulihan aktiviti-aktiviti enzim antioksida yang memainkan peranan dalam penyahtoksikan metabolit APAP dan perencatan proksidasi lemak menunjukan bahawa MO menjanjikan aktiviti hepatoprotektif terhadap kerosakan hepar cetusan APAP.

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I certify that an Examination Committee has met on 6th August 2008 to conduct the final examination of **Uma Nanthini Linggi Gaundar** on her Master of Science thesis entitled "**Hepatoprotective Effect of *Moringa oleifera* Leaves Extract on Acetaminophen Induced Liver Damage in Rats**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Daud Ahmad Israf Ali, PhD
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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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Professor and Dean
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Date: 11 September 2008

DECLARATION

I hereby 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 or concurrently submitted for any other degree at UPM or other institutions.

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UMA NANTHINI LINGGI GAUNDAR

Date: 12/8/08

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