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

UMA NANTHINI LINGGI GAUNDAR

FPSK(M) 2008 2
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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

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

By

UMA NALTHINI LINGGI GAUN DAR

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/kg APAP) 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 After 24 and 48 hours blood was withdrawn and livers were harvested.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 histopathological 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 hepatic 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

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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 aminotransferase (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 menunjukan 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 sepetimana 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 antioksidan yang memainkan peranan dalam penyahtoksikan metabolit APAP dan perencatan proksidasi lemak menunjukan bahawa MO menjanjikan aktiviti hepatoprotektif terhadap kerosakan hepar cetusan APAP.
ACKNOWLEDGEMENTS

I would like to take this opportunity to thank all those who gave great support to me while doing the project. My sincere praises and thanks giving to God Almighty for his unfailing love and grace in guiding me to complete my master project. I would like to express my utmost gratitude to my project supervisor Dr. Sharida Fakurazi from the Department of Human Anatomy, Faculty of Medicine and Health Science, for the continuous guidance, encouragement, support, advice and assistance throughout the course of this thesis as partial fulfillment of the requirement for the degree of Master’s Science (Pharmacology). Deep obligation and sincere appreciation also goes to my co-supervisor, Associate Prof. Dr. Hairuszah Ithnin for her kindness, advices and support in helping me to complete this work as expected.

I would also like to express my deepest appreciation and sincere gratitude to Associate Prof Dr. Johnson Stanslas who has offered insightful advice and suggestions as well as for allowing me to use the Biochemistry Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. A special acknowledgement is owed to Dr. Abdah binti Md. Akim who has generously provided advice and improvements of methodology of enzymatic study.

I would like to express my gratitude for tremendous help and contribution of staff in the Department of Nutrition and Dietetic, Department of Human Anatomy, Department of Biomedical Sciences and Laboratory of Molecular Biomedicine, Institute Bioscience, Universiti Putra Malaysia for the technical assistance and advice as well as material provision. My sincere appreciation is also extended to staff in
Laboratory of Haematology and Clinical Biochemistry, Faculty of Veterinary Medicine, Universiti Putra Malaysia, for providing technical guidance invaluable and skillful help in handling laboratory equipments. Without them, this project paper may not have materialized. I have greatly benefited from all of them who have been the essences in completing this project.

My heartfelt gratitude goes to my fiancé and friends, for their endless guidance, support, and help throughout the completion of this research project. Last but not least, the warmest appreciation and sincere gratitude goes to my beloved family especially father and mother for their endless support and love. May God bless them all.
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:

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Date:
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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Universiti Putra Malaysia

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.

UMA NANTHINI LINGGI GAUNDAR

Date: 12/8/08
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4.2.14 Photomicrograph shows liver section of rats challenged with 2g/kg APAP and sacrificed after 24 hours. The section demonstrated well preserved architecture of hepatocytes, around portal tract area (PT). Magnification: 200x
4.2.15 Photomicrograph shows liver section from rat treated with 2g/kg APAP sacrificed 48 hours post-treatment. The section reveals diffuse infiltration of lymphocytes and neutrophils (N) presence of diffuse microvesiculation (M), focal individual necrotic debris (NC) around perivenular area (PV). Magnification: 200x

4.2.16 Photomicrograph shows liver section from rat treated with 2g/kg APAP sacrificed 48 hours post-treatment. The section reveals disintegration of hepatocytes around perivenular area (PV) with diffuse infiltration of lymphocytes (L), neutrophils (Ne), necrotic debris (ND) and prominent microvesiculation (M). Hepatocytes around portal tract (PT) was well preserved. Magnification: 100x

4.2.17 Photomicrograph shows liver section of rats challenged with 2g/kg APAP and sacrificed after 72 hours. The section indicates cluster of lymphocytes (Æ) around some perivenular (PV) area Magnification:100x

4.2.18 Photomicrograph shows liver section of rats challenged with 2g/kg APAP and sacrificed after 72 hours. The section indicates presence of microvesiculation (Æ) around some perivenular (PV) area Magnification:200x

4.2.19 Photomicrograph shows liver section of rats challenged with 2g/kg APAP and sacrificed after 72 hours. The section demonstrated well preserved architecture of hepatocytes, around portal tract area (PT). Magnification: 200x

4.3.1 The effect of Moringa oleifera pretreatment on plasma Alanine Aminotransferase (ALT) level, following APAP administration after 24 and 48 hours. Results are expressed as means ± SEM. a Groups are compared with 40% sucrose control. b Groups compared with group 3g/kg APAP at 24 hours. c Groups compared with group 3g/kg APAP at 48hours All values are statistically different (p<0.05).

4.3.2 The effect of Moringa oleifera pretreatment on plasma Aspartate Aminotransferase (AST) level, following APAP administration after 24 and 48 hours. Results are expressed as means ± SEM. a Groups are compared with 40% sucrose control. b Groups compared with group 3g/kg APAP at 24 hours. c Groups compared with group 3g/kg APAP at 48hours. All values are statistically different (p<0.05).