



UNIVERSITI PUTRA MALAYSIA

***HEPATOPROTECTIVE EFFECT OF *Phyllanthus niruri* ETHANOLIC
EXTRACT ON ALCOHOL AND HIGH CHOLESTEROL DIET-
INDUCED LIVER CELL DAMAGE IN RATS***

TAN LAI TING

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LIVER CELL DAMAGE IN RATS**

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A project paper submitted to the
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in partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia
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It is hereby certified that we have read this project paper entitled “Hepatoprotective Effect of *Phyllanthus niruri* Ethanolic Extract on Alcohol and High Cholesterol Diet-Induced Liver Cell Damage in Rats”, by Tan Lai Ting and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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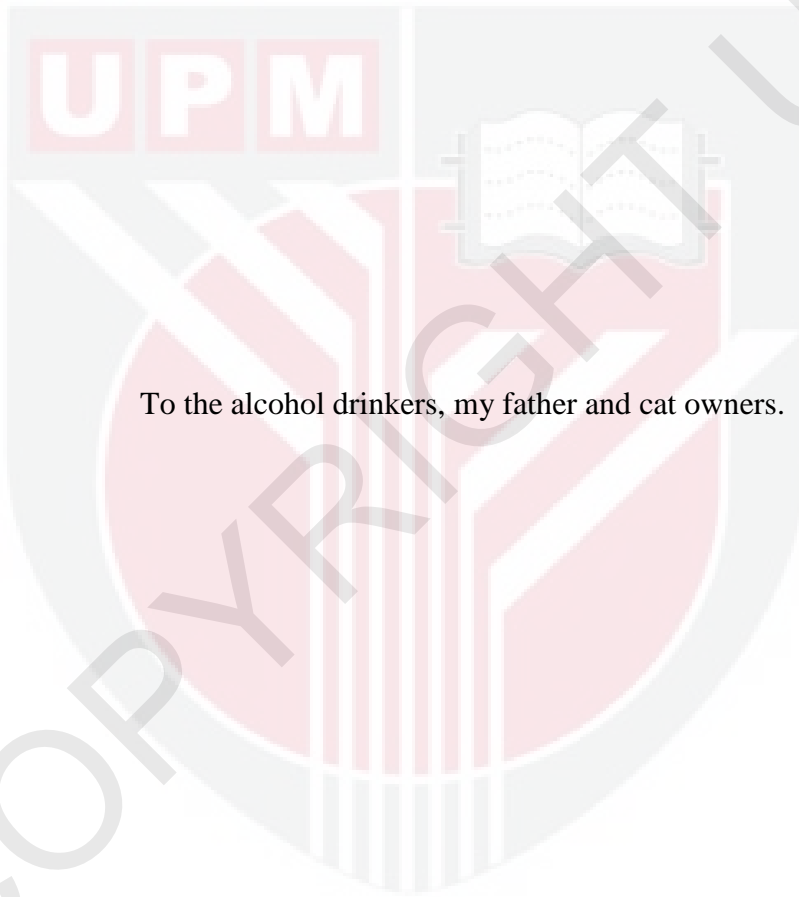
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DEDICATION

To the alcohol drinkers, my father and cat owners.



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LIST OF ABBREVIATIONS

ADH	alcohol dehydrogenase
ALD	alcoholic liver disease
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ASH	alcoholic steatohepatitis
AST	aspartate aminotransferase
CDT	carbohydrate deficient transferrin
CT	computed tomography
DMSO	dimethyl sulfoxide
FHL	feline hepatic lipidosis
GGT	gamma-glutamyl transferase
HDL	high density lipoprotein
HU	Houndfield units
LDL	low density lipoprotein
MEOS	microsomal enzyme oxidizing system
NAFLD	non-alcoholic fatty liver disease
NMR	nuclear magnetic resonance
PN	<i>Phyllanthus niruri</i>
ULN	upper limit of normal

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Akhir Tahun

**KESAN PERLINDUNGAN HATI BAGI EKSTRAK ETANOL *Phyllanthus niruri*
TERHADAP KECEDEeraan HATI YANG DIARUH OLEH ALKOHOL DAN
MAKANAN BERKOLESTEROL TINNGI PADA TIKUS**

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2015

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Phyllanthus niruri, atau dikenali sebagai “Dukung Anak” oleh masyarakat tempatan merupakan tumbuhan terkenal dengan kesan terapeutik untuk hepatitis, batu saluran kemih, disentri, influenza, penyakit kuning dan jangkitan bakteria. Penyakit hati alkohol merupakan satu penyakit hati berasal dari peminuman alkohol. Penyakit ini mempamerkan lesi yang bermula dari steatosis hepatic ke steatohepatitis dan akhirnya sirosis hepatic dalam kes kronik. Kajian ini telah menilai kesan perlindungan hati *Phyllanthus niruri* terhadap kecederaan hati yang diaruh oleh alkohol dan makanan berkolesterol tinggi pada tikus. Sejumlah 20 ekor tikus telah dibahagikan kepada 5

kumpulan iaitu kumpulan A (kawalan), kumpulan B (alkohol dan makanan berkolesterol tinggi), kumpulan C, D dan E (diberi alkohol dan makanan berkolesterol tinggi berikutan dengan ekstrak etanol *Phyllanthus niruri* pada dos 75 mg/kg, 150 mg/kg dan 250mg/kg masing-masing). Penilaian dijalankan selepas eksperimen telah tamat dengan analisa parameter hati dan lipid dalam serum, dan pemeriksaan histopatologi hati. Hasil kajian menunjukkan berat hati relatif kumpulan C (3.36 ± 0.07), D (3.35 ± 0.11) and E (3.15 ± 0.19) adalah lebih rendah dengan signifikan ($p < 0.05$) berbanding dengan kumpulan B (3.84 ± 0.22). Lipoprotein berketumpatan rendah (LDL) dalam kumpulan B (0.88 ± 0.14 mmol/L) adalah lebih tinggi dengan signifikan berbanding dengan kumpulan A (0.49 ± 0.05 mmol/L), D (0.62 ± 0.06 mmol/L) and E (0.62 ± 0.11 mmol/L). Parameter hati tidak mempamerkan perbezaan signifikan antara semua kumpulan. Penilaian histopatologi hati mendedahkan bahawa kumpulan B telah mempunyai skor steatosis tertinggi dan kedua-dua kumpulan A dan E telah mempunyai skor steatosis hampir sama. Kesimpulannya, ekstrak *Phyllanthus niruri* ethanolic mempunyai kesan perlindungan hati terhadap kecederaan hati yang diaruh oleh alkohol dan makanan berkolesterol tinggi pada tikus.

(Kata Kunci: alkohol, steatosis hepatik, berat hati relatif, LDL, *Phyllanthus niruri*)



ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 - Final Year Project

HEPATOPROTECTIVE EFFECT OF *Phyllanthus niruri* ETHANOLIC EXTRACT ON ALCOHOL AND HIGH CHOLESTEROL DIET-INDUCED LIVER CELL DAMAGE IN RATS.

By

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2015

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Co-supervisor: Prof. Dr. Noordin Mohamed Mustapha, Dr. Mohd Rosly Shaari

Phyllanthus niruri, locally known as “Dukung Anak” is well-known for its therapeutic effect against hepatitis, urolithiasis, dysentery, influenza, jaundice and bacterial infection. Alcoholic liver disease (ALD) is a liver disease in human caused by alcohol consumption. It is a spectrum of disease which started from hepatic steatosis to steatohepatitis and finally hepatic cirrhosis in chronic cases. In this study, the hepatoprotective effect of *Phyllanthus niruri* ethanolic extract was evaluated on alcohol and high cholesterol diet-induced liver cell damage in rats. A total of 20 rats were randomly divided into 5 groups comprised of group A (control), group B (alcohol and

high cholesterol diet), groups C, D and E (given alcohol and high cholesterol diet, and supplemented with *Phyllanthus niruri* ethanolic extract at 75 mg/kg, 150 mg/kg, and 250 mg/kg respectively. The evaluation was carried out through analysis of liver parameters, lipid parameters and histopathological examination of liver at the end of experiment. Result showed that the relative liver weight of group C (3.36 ± 0.07), D (3.35 ± 0.11) and E (3.15 ± 0.19) were significantly ($p < 0.05$) lower than group B (3.84 ± 0.22). The serum low density lipoprotein (LDL) level was significantly ($p < 0.05$) higher in group B (0.88 ± 0.14 mmol/L) compared to Group A (0.49 ± 0.05 mmol/L), D (0.62 ± 0.06 mmol/L) and E (0.62 ± 0.11 mmol/L). However, there were no significant differences on liver parameters in all groups. The histopathological evaluation of the liver revealed that group B was having the highest steatosis score and both group A and E were having nearly similar steatosis score. In conclusion, *Phyllanthus niruri* ethanolic extract has hepatoprotective effect against alcohol and high cholesterol diet-induced liver injury in rats.

Keywords: alcohol, hepatic steatosis, relative liver weight, LDL, *Phyllanthus niruri*

1.0 INTRODUCTION

In Malaysia, per capita consumption of alcohol in drinkers in year 2010 was 13.5L in males and 2.8L in females (World Health Organization, 2014). Although our country has relatively low per capita consumption of alcohol as compared to Europeans, study has shown that there are small segments of population that drinks heavily and experiences alcoholic liver disease (ALD) (World Health Organization, 2014). Based on a study by World Health Organization in year 2014, alcohol consumption has attributed the risk of 30.8% and 28.6% in male and female, respectively, for liver cirrhosis occurrence. ALD is a spectrum of disease which started from hepatic steatosis and slowly progress to steatohepatitis and hepatic cirrhosis, chronically. Feline hepatic lipidosis is a lethal disease that possesses the similar lesion as ALD which is fat infiltration in liver parenchyma.

Nowadays, there is a great interest in edible plants that contains medicinal value.

Phyllanthus niruri (Figures 1 and 2), which is commonly known as Dukung Anak in Malaysia, is a tropical plant that believed to be effective against hepatitis, urolithiasis, dysentery, influenza, tumours, diabetes, diuretics, jaundice, kidney stones, and bacterial infection. With its well-known medicinal value, several studies have been conducted to determine its hepatoprotective effect using different approaches to induce rats' hepatotoxicity such as on alcohol and polyunsaturated fatty acid-induced oxidative stress (Rajagopalan *et al.*, 2010) and carbon tetrachloride induced injury (Harish & Shivanandappa, 2006) in liver. However, there is no study conducted to determine the

hepatoprotective effect of *Phyllanthus niruri* on alcohol and high cholesterol diet induced liver injury in rats.

The aim of this study is to determine the hepatoprotective effect of *Phyllanthus niruri* ethanolic extract on alcohol and high cholesterol diet-induced liver cell damage in rats and extrapolate its potential as liver supplement to prevent alcoholic liver disease (ALD) in human and also to maintain good liver health in cats especially during stress events.

This study was undertaken:

1. to determine the *in vivo* hepatoprotective effect of *Phyllanthus niruri* ethanolic extract on alcohol and high cholesterol diet-induced liver cell damage through serum biochemistry analysis of liver and lipid parameters.
2. to evaluate the *in vivo* hepatoprotective effect of *Phyllanthus niruri* ethanolic extract on alcohol and high cholesterol diet-induced liver cell damage through gross and histopathological evaluation of liver.



Figure 1: *Phyllanthus niruri*



Figure 2: *Phyllanthus niruri*

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