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

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

TAN LAI TING

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
in partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

MARCH 2015

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.

DR. HAZILAWATI BINTI HJ. HAMZAH

DVM (UPM), MVSc (UPM), PhD (MURDOCH)

Senior Lecturer

Department of Veterinary Pathology and Microbiology

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

PROF. DR. NOORDIN MOHAMED MUSTAPHA

DVM (UPM), MSc (UPM), PhD (Murdoch)

Professor

Department of Veterinary Pathology and Microbiology

Faculty of Veterinary Medicine

(Co-Supervisor)

DR. MOHD ROSLY BIN SHAARI

DVM (UPM), MSc (UPM)

Deputy Director

Strategic Livestock Research Institute

Malaysian Agricultural Research and Developmental Institute (MARDI)

Serdang, Selangor

(Co-Supervisor)

DEDICATION

To the alcohol drinkers, my father and cat owners.

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Hazilawati binti Hj. Hamzah for the continuous support of my research, for her patience, motivation, enthusiasm and knowledge. Her guidance helped me in all the time of research and writing of this thesis.

Besides, I would like to thank my co-supervisors, Prof. Dr. Noordin Mohamed Mustapha and Dr. Mohd Rosly Shaari for their encouragement, guidance and insightful comments. And I would like to thank Prof. Ariff, for his guidance and advises in statistics.

I would like to express my deepest appreciation to Sia Bang Wen, Rachel @ Tan Wei Miao and Jessie Ho Si Wai for helping me throughout my research. This project cannot be completed without any one of you.

My sincere thanks also goes to Mr. Subramaniam a/l Kunziraman and Mr. Siva for helping me in Mardi Experimental Animal House.

To all the staff of clinical pathology lab and histopathology lab who share their valuable knowledge with me.

To the experimental rats for their sacrifice in this research.

Not to forget, friends who had help and motivate me throughout this research.

Last but not least, to my beloved mother and family members who had support and advise me throughout my life.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVATIONS

ADH	**alcohol dehydrogenase**
ALD	**alcoholic liver disease**
ALP	**alkaline phosphatase**
ALT	**alanine aminotransferase**
ASH	**alcoholic steatohepatitis**
AST	**aspartate aminotransferase**
CDT	**carbohydrate deficient transferring**
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	***Phyllanthus niruri***
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 KECEDERAAN HATI YANG DIARUH OLEH ALKOHOL DAN MAKANAN BERKOLESTEROL TINNGI PADA TIKUS

Oleh

Tan Lai Ting

2015

Penyelia: Dr. Hazilawati Binti Hj. Hamzah

Penyelia bersama: Prof. Dr. Noordin Mohamed Mustapha, Dr. Mohd Rosly Shaari

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 hepatik ke steatohepatitis dan akhirnya sirosis hepatik 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). Lipoprotin 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

Tan Lai Ting

2015

Supervisor: Dr. Hazilawati Binti Hj. Hamzah

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 ±0.11mmol/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*

2.0 LITERATURE REVIEW

2.1 Alcoholic liver disease

Prolonged and excessive alcohol consumption is a brain-centered addictive behavioral disorder that crosses all boundaries of gender, race, age and economies strata (Gramenzi *et al*., 2006). Alcoholic liver disease (ALD) remains to be one of the most common etiologies of liver disease and is a major killer among the alcoholics. Progression of ALD is well-characterized and is actually a spectrum of liver diseases which ranges initially from simple steatosis (fat accumulation in hepatocytes), to inflammation and necrosis (steatohepatitis), to fibrosis and cirrhosis, chronically (Arteel, 2010). Alcoholic steatohepatitis (ASH), the second and rate-limiting step in the progression of ALD, is characterized by hepatic fat accumulation, hepatocellular necrosis, the presence of Mallory bodies, and surrounding infiltrated with polymorphonuclear leukocytes (Samuhasaneeto, Thong-Ngam & Klaikeaw, 2007). With prolonged alcohol abuse, it will progress to fibrosis and cirrhosis which typically characterized by sinusoidal and perivenular fibrosis that splits apart the parenchyma. Furthermore, sustained excessive alcohol intake favors the progression of other liver diseases, such as virus-related chronic hepatitis, also increasing the risk of hepatocellular carcinoma (Gramenzi *et al*., 2006)

2.2 Pathophysiology of alcohol-induced liver damage

The mechanism of alcohol-induced liver damage is uncertain and controversial. Moreover, the factors involved in ALD interact in complex ways (Jacquelyn, 1997). An

understanding of alcohol metabolism in liver provides the basis for understanding alcohol-induced liver damage (Jacquelyn, 1997). Most of the alcohol consumed is metabolized in the liver. There are two pathways that involved in alcohol metabolism. The major pathway is undertaken by enzyme alcohol dehydrogenase (ADH). This enzyme converts alcohol to acetaldehyde through a chemical process known as oxidation (Jacquelyn, 1997). Besides, there is another pathway known as microsomal enzyme oxidizing system (MEOS) that metabolize alcohol to by means of a cytochrome called P4502E1 or CYP2E1(Jacquelyn, 1997; Arus & Balasubramanian, 2011). The major factors that cause alcohol-induced liver damage include oxidative stress and hypoxia of liver parenchyma (Jacquelyn, 1997). Oxidative stress is defined as the imbalance between the prooxidant and antioxidant status (Rajagopalan *et al.*, 2010). It is mainly due to excessive production of reactive oxygen species or reduced levels of antioxidant defense in tissue or organ (Rajagopalan *et al.*, 2010). Small amount of free radicals are produced as normal by-products of various metabolic processes. Chronic alcohol consumption or substantial increased in alcohol consumption (binge drinking) subsequently increase alcohol metabolism and produces excessive free radicals. One common result of free radical attack is degradation of cellular membrane by a process known as lipid peroxidation (Jacquelyn, 1997). The lost of integrity of cellular membrane will cause leakage of preformed hepatic enzymes such as aspartate aminotransferase (AST), alkaline phosphatase(ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) which is expected to be elevated in this study.

In normal circumstances, free radicals are quickly scavenged by endogenous antioxidants. Chronic alcohol consumption reduces the levels of these antioxidants and renders liver cell more susceptible to free radical-induced injury (Jacquelyn, 1997; Arun & Balasubramanian, 2011). One important antioxidant that is affected by alcohol is glutathione (Jacquelyn, 1997). Alcohol interferes the transportation of glutathione from place of production to mitochondria (Jacquelyn, 1997). The resulting glutathione deficiency causes mitochondrial damage and cell death due to unimpeded lipid peroxidation (Jacquelyn, 1997).

Alcohol metabolism increases oxygen utilization by liver cells, thereby reducing availability of oxygen for other important cellular function especially in zone 3 of liver lobules (Jacquelyn, 1997). The unidirectional transport of blood within lobules from peripheral vessels to central vein causes zone 3 of liver lobules to be the least supplied with oxygen. Therefore, alcoholic liver damage is tends to concentrate in zone 3.

Adverse effect of alcohol is further aggravated by intake of high fat diet (Rajagopalan *et al.*, 2010). Many alcoholics used to take fried food items after a heavy binge of alcohol (Rajagopalan *at al.*, 2010). As described by Samuhasaneeto, Thong-Ngam and Klaikeaw (2007), alcohol requires a nutritional co-factor to exhibit hepatotoxicty. The fat increases the CYP2E1 activity and simultaneously alters membrane composition, making the membrane more susceptible to peroxidation (Nanji *et al.*, 1994; Jacquelyn, 1997).

2.3 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is defined when there is macrovesicular steatosis inside liver cells exceeding 5% of the liver, in the absence of significant ethanol consumption (Lik Fai, 2010). It comprises of a disease spectrum just like ALD, ranging from benign hepatic steatosis to non-alcoholic steatohepatitis (NASH) with inflammation and liver cirrhosis (Reddy, 2005). The causes of NAFLD depend on its nature, either primary or secondary. In primary NAFLD, it is always associated with at least one feature of the metabolic syndrome (obesity, glucose intolerance, diabetes mellitus, hypertension and hyperlipidemia). Prevalence of disease increased with increase in severity and number of metabolic disorder (Hui-Hui, 2010). Secondary causes of NAFLD include drugs, toxin exposure, parental nutrition, hypothyroidism, jejunoileal bypass surgery, *etc* (Hui-Hui, 2010).

Obesity (body mass index [BMI] > 30kg/m^2) has increased likelihood of developing NAFLD (Hui-Hui, 2010). NAFLD has been documented in 10-15% in normal individual and up to 70-80% of obese individuals (Hui-Hui, 2010). In the United States, NAFLD is the most common cause of chronic liver disease, with an estimated prevalence of 20-30% and an estimated prevalence of 3.5-5% for NASH (Hui-Hui, 2010).

2.4 Diagnosis of ALD and NAFLD

Most patients with moderate forms of ALD or NAFLD are asymptomatic and it is usually suspected when there is accidental elevation of liver enzymes during routine

blood test (European Association for the Study of the Liver [EASL] Clinical Practice Guidelines: Management of Alcoholic Liver Disease, 2013). It is especially challenging to differentiate ALD and NAFLD, as not all patients are honest about their alcohol intake and there is inadequate diagnostic difference between two diseases. Therefore, the diagnosis of ALD is enhanced when there is history of excess alcohol consumption which is more than 30 g per day in man and 20 g per day in women (European Association for the Study of the Liver [EASL] Clinical Practice Guidelines: Management of Alcoholic Liver Disease, 2013; Reddy, 2005). Although there is no single laboratory technique available to establish chronic alcohol consumption, carbohydrate deficient transferring (CDT) and gamma glutamyl transferase (GGT) are common to be used as markers to detect previous alcohol consumption (European Association for the Study of the Liver [EASL] Clinical Practice Guidelines: Management of Alcoholic Liver Disease, 2013). Besides, when ALD or NAFLD is suspected, it is important to rule out other diseases that cause elevated liver enzymes such as hepatobiliary obstructions or tumours.

Blood test is the initial diagnostic method undertaken in normal circumstances. Routine blood test which includes gamma glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) can indicate early ALD or NAFLD whereas advanced disease can be suspected when there is decreased albumin, prolonged prothrombin time and increased bilirubin level (European Association for the Study of the Liver [EASL] Clinical Practice Guidelines: Management of Alcoholic Liver Disease, 2013). Generally, when ALT level

measured at two different occasions are two times higher than the upper limit of normal (ULN) justified further diagnostic work up includes ultrasonography, computerized tomography (CT) scan, magnetic resonance imaging and liver biopsy (Schreuder, 2008).

Ultrasonography is the common and least expensive technique among the three diagnostic imaging. However, it only can detect moderate to severe hepatic steatosis (Schreuder, 2008). Although diagnostic capacity enhanced by increased severity of hepatic steatosis, accurate measurement of hepatic steatohepatitis is quite impossible.

Computerized tomography (CT) scan can be used to detect and quantify hepatic steatosis. The quantification is based on measurement of grey scales (representing the amount radiation absorbed) of the liver and spleen and expressed in Houndfield units (HU). This measurement correlates well with the percentage of hepatocytes with fatty infiltration (Schreuder, 2008).

Nuclear magnetic resonance (NMR) is the most accurate method for detection and quantification of hepatic steatosis (Schreuder, 2008). The main advantage of this technique is the possibility of acquiring in-phase (water) and opposed-phase (fat) images in one breath hold, thereby reducing the influence of breathing movements and contrast absorption (Schreuder, 2008). On T1-weighted images, a shorter relaxation time represents higher signal intensity (SI). In a healthy individual, SI of liver is higher than spleen. In a patient with mild steatosis, the SI of liver will drop and equal to that of spleen. Furthermore, in patient with moderate to severe hepatic steatosis, the SI of liver will be lower than SI of spleen (Schreuder, 2008).

Lastly, liver biopsy is another option to diagnose ALD or NAFLD. Nowadays, liver biopsy is not only a diagnostic tool and it also helps in prognosis assessment and making therapeutic management decision. However, it is not commonly used to diagnose ALD and NAFLD due to its invasiveness.

2.5 Feline hepatic lipidosis

Similar to NAFLD and ALD in human, feline hepatic lipidosis (FHL) is characterized mainly by hepatic steatosis associated with obesity (Mazaki-Tovi *et al.*, 2013). However, necrosis and inflammation is not routinely present in FHL unless the primary hepatic disease is present (Cynthia & Scott, 2010). FHL is an acquired, potentially fatal feline disease that usually happened secondary to other primary diseases or rarely environmental stress. It is prevalent in cat which is old age, obese and had gone through some stress which has led to reduce or lack of appetite. The primary underlying cause induces stress in the cat and it seems to 'trigger' FHL. Anorexia, usually the first sign of FHL causes extensive peripheral fat mobilization exceeding the hepatic capacity to either redistribute or use fat for β-oxidation (producing energy) leads to profound hepatocyte expansion with triglycerides stores (Mazaki-Tovi *et al.*, 2013). Rarely, FHL is triggered by environmental stress such as forced weight loss programmes with unacceptable food substitutions, moving the new household, boarding, accidental confinement and loss of pets or family members. Hepatic lipidosis can only be termed as 'idiopathic' when an underlying disease condition or event leading to inappetence cannot be identified.

Clinical signs of FHL are varied but usually include dramatic weight loss, depression, vomiting, ptyalism, pallor, hepatomegaly, jaundice, gastroperesis and intestinal ileus (Cynthia & Scott, 2010). Diarrhea is common in FHL with inflammatory bowel disease or enteric lymphoma as primary disease processes. Sometimes, bleeding tendencies can be observed in FHL and it resolves after vitamin K1 supplementation (Cynthia & Scott, 2010).

Diagnosis of FHL is based on history, physical examination findings, laboratory results, abdominal ultrasonography and hepatic aspiration cytology. Liver biopsy is not necessary unless hepatic lymphoma is suspected. Laboratory results reflect FHL as well as the underlying primary disease. In most cases, a non-regenerative anaemia with poikilocytosis and elevated red blood cell (RBC) Heinz bodies can be observed (Cynthia & Scott, 2010). Besides, hyperbilirubinemia, mild to marked increases in ALT and AST, and marked increase in ALP are normally reflected in blood biochemical analysis (Cynthia & Scott, 2010). The white blood cell (WBC) count is variable and is depending on the primary underlying cause. If the primary cause involves the biliary structures, GGT activity will be markedly increased exceeding the fold increase in ALT. In other condition that causing FHL, GGT remains normal or only modestly increased. Hence, the GGT to ALP ratio relationship is useful in discerning underlying cholangitis or cholangiohepatitis and other diseases involving the biliary structures (Cynthia & Scott, 2010).

Abdominal ultrasonography reveals homogenous, hyperecchoic hepatic parenchyma and hepatomegaly although it is subjective. Besides, ultrasonography should carefully assess the entire abdomen for evidence of an underlying disease process. In addition, cytology examination reveals vacuolar distention of hepatocytes involving more than 80% of hepatocytes. Canalicular cholestasis is commonly observed in FHL due to compression secondary to hepatocyte triglycerides vacuolar distention (Cynthia & Scott, 2010).

Prognosis for FHL is good with early diagnosis, full treatment support, and control of the underlying disease. The treatment of FHL is aimed at correcting fluid, electrolyte, and metabolic deficits and initiating food intake (Cynthia & Scott, 2010).

2.6 *Phyllanthus niruri*

The plant *Phyllanthus niruri* (PN) is widely distributed throughout the tropical and subtropical regions of both hemispheres. *Phyllanthus* means 'leaf and flower' because the flower, as well as the fruit, seems to become one with the leaf (Paithankar *et al*., 2011). It can be found along the road sides, in street corners, and dumps of building materials. Taxonomically, *Phyllanthus niruri* belongs to the family *Euphorbiaceae* of the order Malpighiales under class Magnoliopsida (Paithankar *et al*., 2011). The *Phyllanthus* genus contains over 600 species of shrubs, trees and herbs (Paithankar *et al*., 2011). The plant has different names in different languages. In malay culture, it is known as *Dukung Anak.* However, in Hindi and Tamil, it is known as *Chalmeri* and *Aru*, respectively.

The plant is 30 - 60 cm tall, quite glabrous, stem often branched at the base. The plant's leaves are simple, alternate or opposite, flowers are very small and diclinous, they cluster in cup-shaped structures, greenish, often with glands (Paithankar *et al*., 2011). The male flowers are one to three in numbers, while the female flowers are solitary in nature.

Phyllanthus niruri is well-known for its liver healing properties, commonly used in Chinese medicine for treatment of liver diseases. The active phytochemicals; flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts of the plant (Harish & Shivanandappa, 2006). The plant is believed to be effective against dysentery, influenza, tumors, diabetes, diuretics, jaundice, kidney stones, hepatitis and bacterial infection (Rajagopalan *et al.*, 2010)

This plant is chosen for this research due to its well-known hepatoprotective potential and it is widely available in Malaysia. There is no published research in Malaysia that justifies its hepatoprotective effect on alcohol and high cholesterol diet-induced liver damage in rats. There are several researches that demonstrate its liver protective characteristics. For example in previous research done by Harrish and Shivanandappa (2006), they have shown *Phyllanthus niruri* plant extract inhibit CCl_4-induced liver injury in rats. Besides, Rajagopalan *et al.* (2010) reported that the crude extract of *Phyllanthus niruri* effectively decreased the oxidative stress and damage.

3.0 MATERIALS AND METHODS

3.1 *Phyllanthus niruri* ethanolic leaf extract

Phyllanthus niruri ethanolic leaf extract was obtained from Mardi, Serdang. It was stored in a refrigerator before used. The extract was diluted with 10% dimethyl sulfoxide (DMSO) before it was given to the rats.

3.2 High cholesterol diet preparation

Commercial pellet was ground and mixed with 2.5% cholesterol (3β-Hydroxy-5-cholestene, Sigma Life Science, Japan). The commercial pellet diet comprised of 21% crude protein, 5% crude fiber, 3% crude fat, 13% moisture, 8% ash, 0.8% calcium and 0.4% phosphorus (Gold Coin-mouse pellet feed).

3.3 Experimental animal

A total of 20 male Sprague Dawley rats were obtained from a local supplier (Chenur Supplies Sdn. Bhd). The experiment was performed in the Experimental Animal House located at MARDI, Serdang. The rats were housed in separated plastic cages. Rats were given water *ad-libitum*. The rats were acclimatized for two weeks before the experiment was started. The experiment protocol was approved by the Institutional Animal Care and Use Committee (IACUC), UPM (UPM/IACUC/FYP – 2014/FPV.034).

3.4 Experimental design

All rats were weighed on day 0 and weekly throughout 19 days study period, with a digital weighing balance (AND GF-3000, A&D Company, Japan) and randomly divided into five groups, with four rats in each group (Onwuli, Brown & Ozoani, 2014). It comprised of two control groups and three treatment groups. The experimental design is tabulated in Table 1.

Table 1: Experimental design

Group	Alcohol	*Phyllanthus niruri* ethanolic extract	Diet
A	Normal saline	-	Commercial diet
B	20% ethanol	-	Commercial diet + 2.5% cholesterol
C	20% ethanol	75 mg/kg of body weight	Commercial diet + 2.5% cholesterol
D	20% ethanol	150 mg/kg of body weight	Commercial diet + 2.5% cholesterol
E	20% ethanol	250 mg/kg of body weight	Commercial diet + 2.5% cholesterol

Note: Alcohol (1 mL/kg of body weight) and *Phyllanthus niruri* ethanolic extract were given daily via oral gavage.

3.5 Serum biochemistry analysis

At the end of experimental period, all the rats were sacrificed via complete exsanguinations under anaesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were collected and centrifuged at 3000 rpm for 10 minutes with a portable centrifuge machine (Hettich EBA20, DJB Labcare, UK) to obtain serum. The serum samples were analysed for aspartate aminotransferase (AST), alkaline

phosphatase(ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine kinase (CK), blood urea nitrogen (BUN), creatinine, triglycerides, total cholesterol, LDL, HDL, total protein and albumin using dry biochemistry machine (Hitachi 902 Automatic Chemistry Analyzer, USA). Globulin levels were calculated by subtracting the albumin from the total protein.

3.6 Histopathology

After the animals were euthanized, liver samples were collected and relative liver weight was calculated based on formula in figure 1. The liver samples were fixed in 10% buffered formalin. The samples were processed with standard method followed by tissues were embedded in paraffin, sectioned into 3 μm thick, and stained with haematoxylin and eosin (H&E) staining. The liver tissues were examined and graded for steatosis and inflammation according to the modified lesion scoring described by Korourian *et al*. (1999) criteria (Tables 2 and 3).

Figure 3: Formula for relative liver weight calculation

$$\text{Relative Liver Weight} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}}$$

Table 2: Steatosis grading

Percentage of liver parenchyma containing fat (%)	Score
None	0
<25%	1
25-50%	2
50-75%	3
>75%	4

Adapted from Korourian *et al.,* (1999).

Table 3: Inflammation grading

Infiltration of polymorphonuclear leukocytes and mononuclear cells	Score
None	1
Occasional foci of inflammatory cells	2
Widely dispersed, organized foci of inflammatory cells	3
Frequently occurring, large foci of inflammatory cells	4

Adapted from Korourian *et al.,* (1999).

3.7 Statistical analysis

Selected serum biochemistry data were analyzed by one way ANOVA followed by Tukey test to detect inter-group differences where P-values < 0.05 were considered statistically significant. The cholesterol and HDL values were analyzed using Kruskal-wallis test. The liver histopathological lesion scores were analyzed using Kruskal-wallis test. All statistical analyses were done on SPSS Statistics software (IBM SPSS Statistics, IBM Corporation, U.S.A).

4.0 RESULT

4.1 Relative liver weight

The SPSS analysis of the relative liver weights showed in Figure 2 and Appendix 1. The mean relative liver weight of alcohol and high cholesterol diet group, group B was significantly higher than groups C, D and E which treated with low, moderate and high dose of *Phyllanthus niruri* ethanolic extract, respectively (Figure 2). However, the mean relative liver weight of group B is higher than group A with p-value of 0.056.

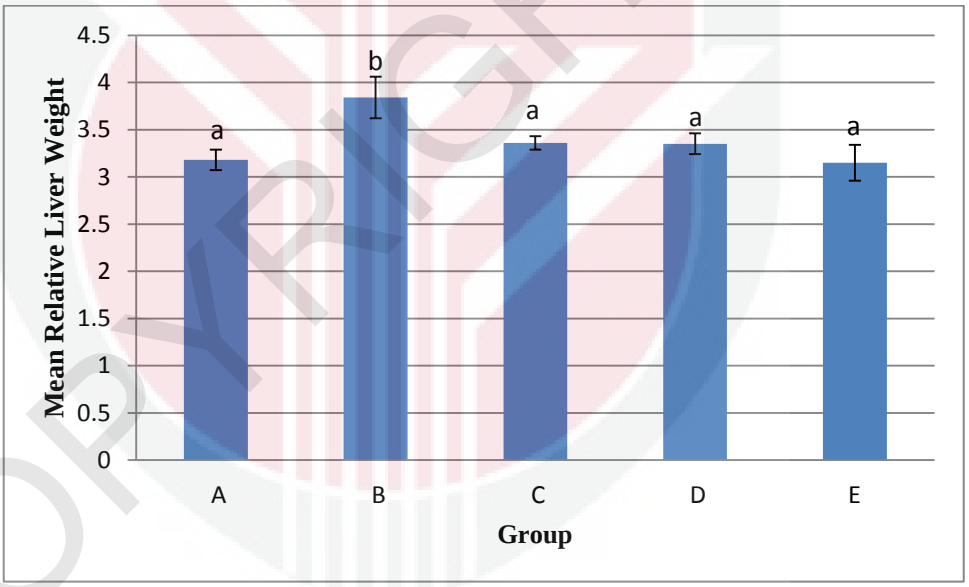

Figure 4: Comparison of mean relative liver weight between groups. Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg. Values with different superscript were significantly different at $p < 0.05$.

4.2 Serum biochemistry analysis

The effects of *Phyllanthus niruri* on selected blood biochemistry parameters are shown in Figures 3, 4 and 5, and Appendices 2 and 3.

Results showed the mean LDL of group B was significantly higher than groups A, D and E ($p<0.05$) (Figure 3 and Appendix 3).

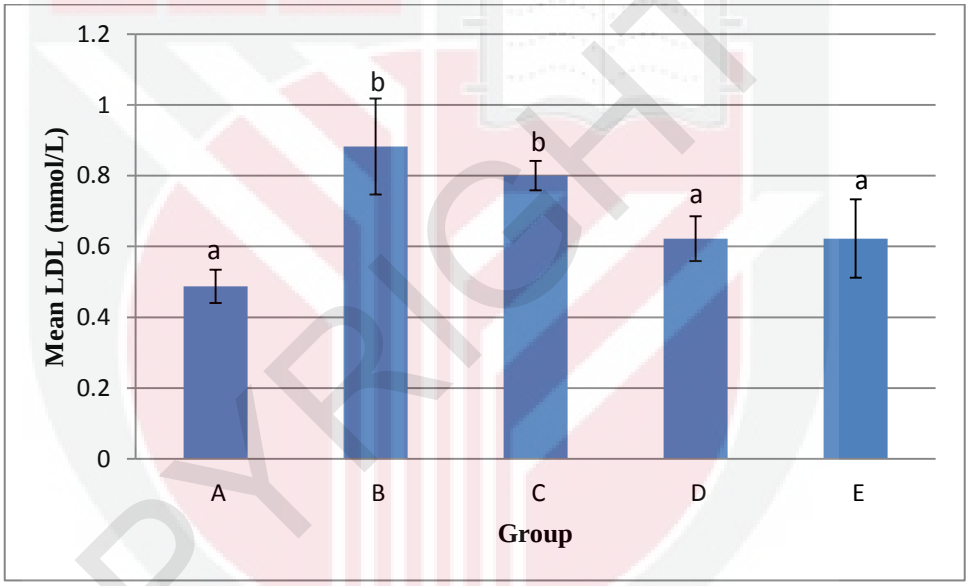

Figure 5: Comparison of mean LDL (mmol/L) between groups. Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg. Values with different superscript were significantly different at $p< 0.05$.

There were no significant ($p<0.05$) differences between groups for the liver, protein and lipid parameters (Figures 4 and 5, Appendices 2 and 3). The creatinine kinase (CK)

values of all rats were higher than normal, while urea and creatinine values were normal in all groups (Appendix 5).

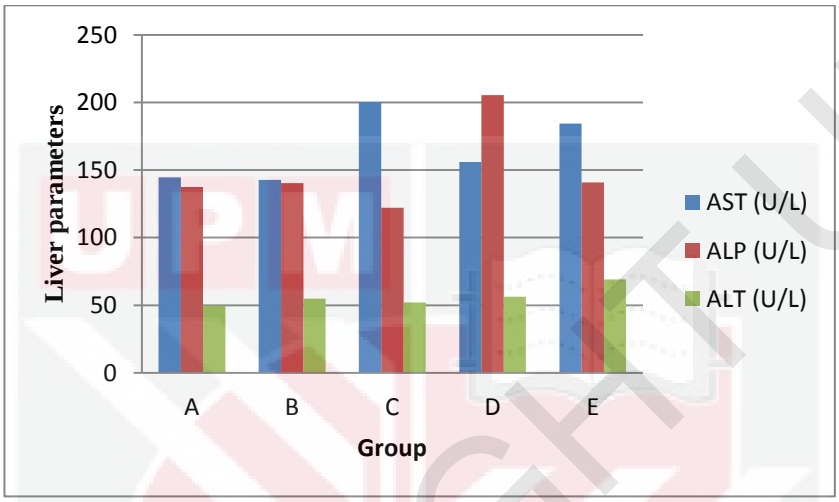

Figure 6: Comparison of AST, ALP and ALT (U/L) between groups. Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg. No significant differences ($p>0.05$) were observed between groups.

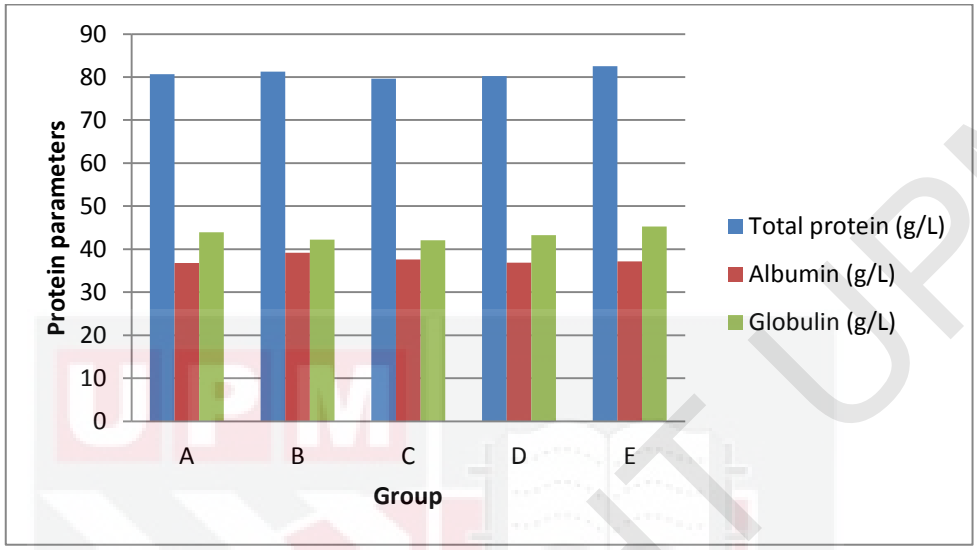

Figure 7: Comparison of protein parameters (g/L) between groups. Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg. No significant differences ($p>0.05$) were observed between groups.

4.3 Hepatic histopathology lesion scores

Results of the histopathological lesion (hepatic steatosis) scores are shown in Table 4, Table 5 and Figure 8. Microphotographs of livers of the rats in all groups are shown in Figures 9, 10 and 11.

A Kruskal-Wallis H test showed that there was a significant ($p<0.05$) difference in hepatic steatosis histopathological lesion grades between groups, Chi-Square= 15.709, $p= 0.003$, with mean ranks as tabulated in Table 4. No foci of inflammatory cells were observed in liver samples. Mann-Whitney U test showed that groups B, C and D had

significant higher score of the hepatic steatosis compared to groups A and E (Table 5 and Figure 8).

Table 4: Hepatic steatosis histopathological lesion scores between groups.

Group	Mean ±S.E.M	Mean ranks
A	0.50 ±0.29	3.50
B	2.75 ±0.25	16.00
C	2.50 ±0.29	14.50
D	2.25 ±0.25	13.00
E	1.00 ±0.22	5.50

Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg.

The inter-group difference in term of hepatic steatosis grading was analyzed using Mann-Whitney U test where P-value < 0.05 was considered statistically significant. The result is showed in Table 5 and Figure 6.

Table 5: Comparison between groups for the hepatic steatosis scoring using Mann-Whitney U test.

Group	Mann-Whitney U value	P-value	Interpretation
A and B	0.000	0.017*	group B > group A
A and C	0.000	0.018*	group C > group A
A and D	0.000	0.017*	group D > group A
A and E	4.000	0.127	No significant difference
B and C	6.000	0.495	No significant difference
B and D	4.000	0.186	No significant difference
B and E	0.000	0.011*	group B > group E
C and D	6.000	0.495	No significant different
C and E	0.000	0.011*	group C > group E
D and E	0.000	0.013*	group D > group E

Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg.

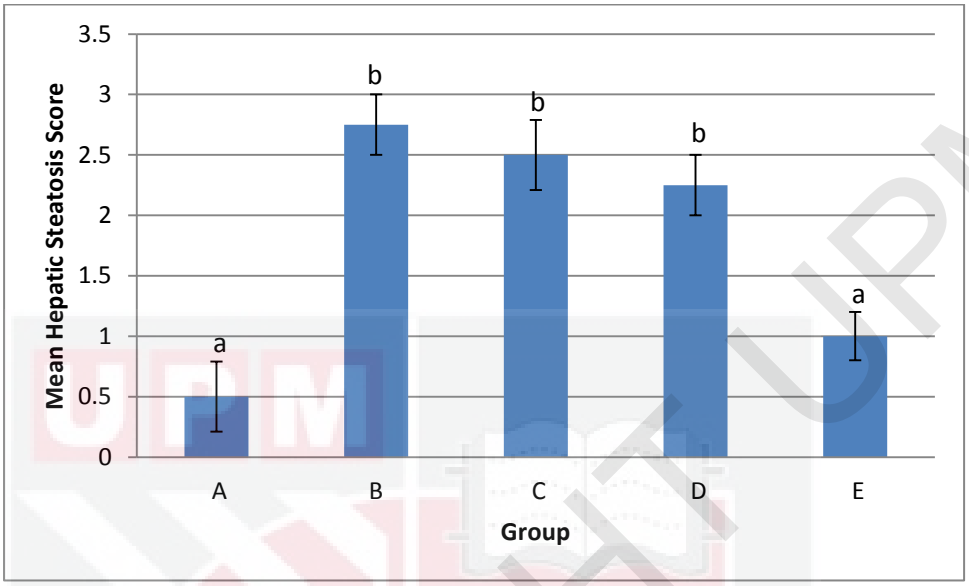

Figure 8: Comparison of hepatic steatosis histopathological lesion scores between groups. Group A: Normal saline (control); Group B: Alcohol+high cholesterol diet; Group C: Alcohol+high cholesterol diet+PN extract at 75 mg/kg; Group D: Alcohol+high cholesterol diet+PN extract at 150 mg/kg; Group E: Alcohol+high cholesterol diet+PN extract at 250 mg/kg. Values with different superscript were significantly different at $p < 0.05$.

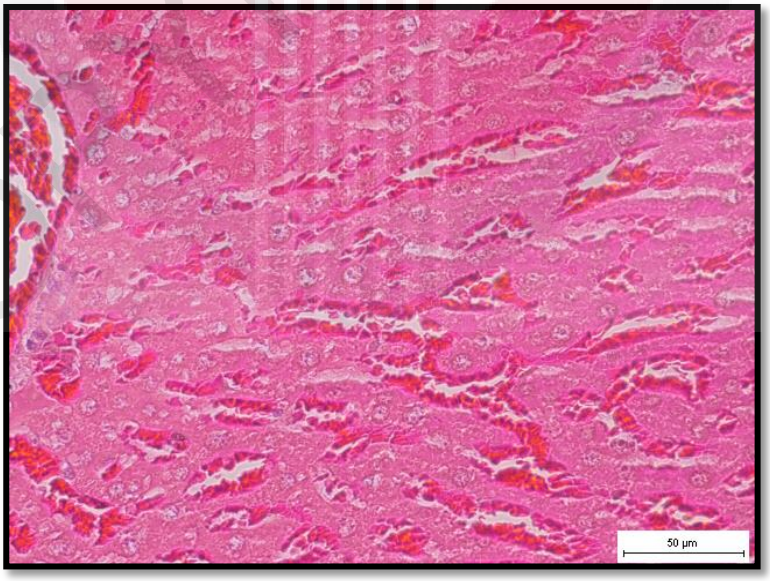

Figure 9: Light microphotograph of H&E-stained section (400X) of the formalin fixed liver of group A (control) showing normal hepatocytes.

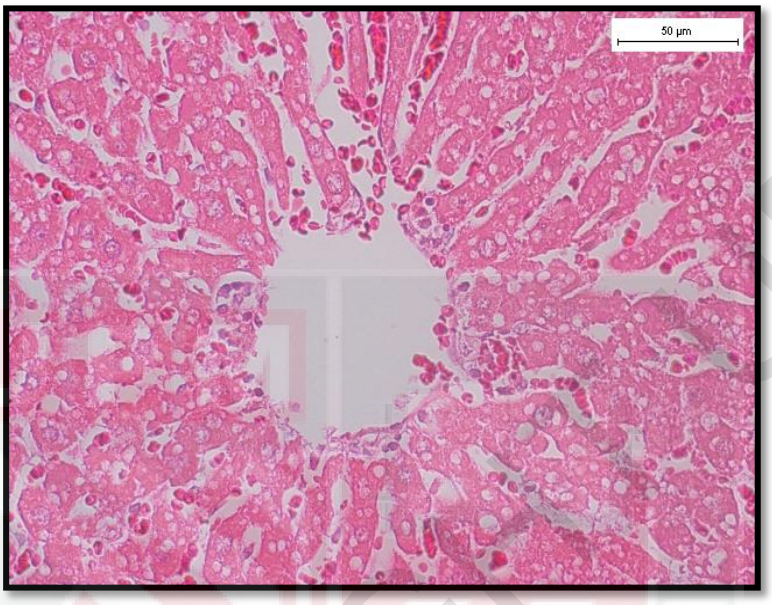

Figure 10: Light microphotograph of H&E-stained section (400X) of the formalin fixed liver of group B (alcohol + high cholesterol diet) showing extensive vacuolation within cytoplasm of hepatocytes.

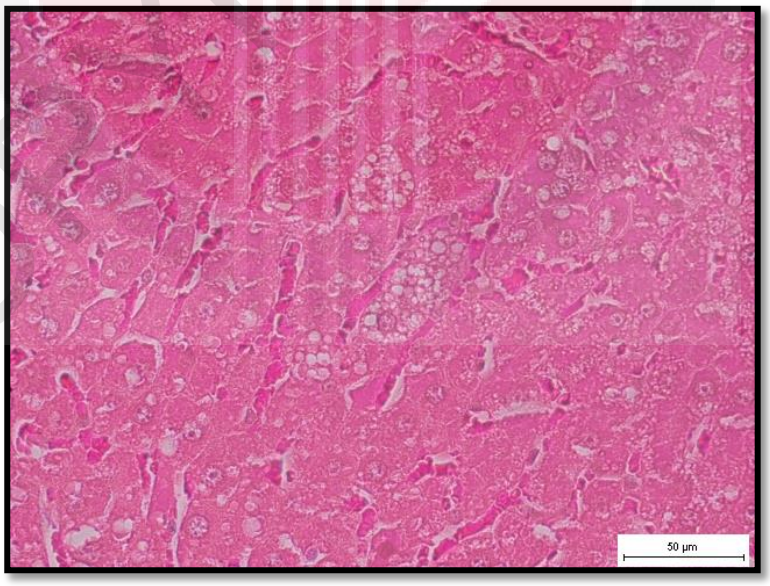

Figure 11: Light microphotograph of H&E-stained section (400X) of the formalin fixed liver of group C (alcohol + high cholesterol diet+ 75 mg/kg PN extract) showing extensive vacuolation within hepatocytes.

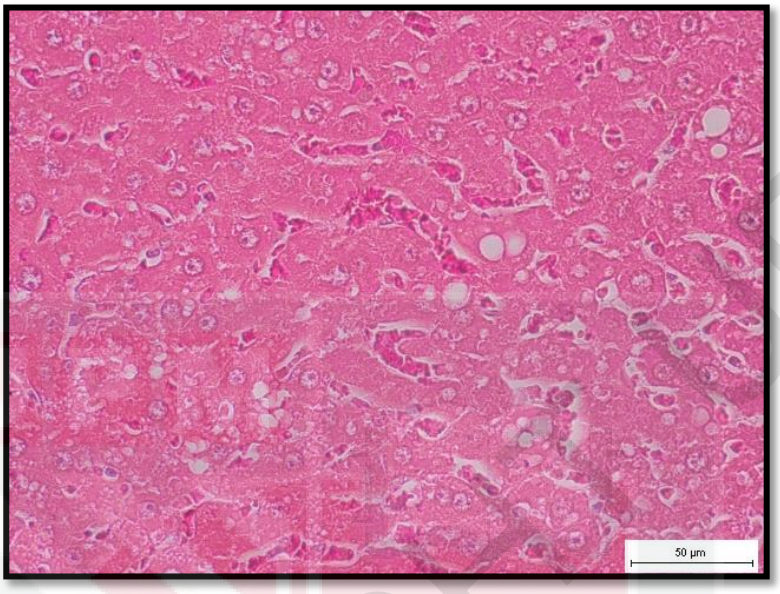

Figure 12: Light microphotograph of H&E-stained section (400X) of the formalin fixed liver of group D (alcohol + high cholesterol diet+ 150 mg/kg PN extract) showing vacuolation within hepatocytes.

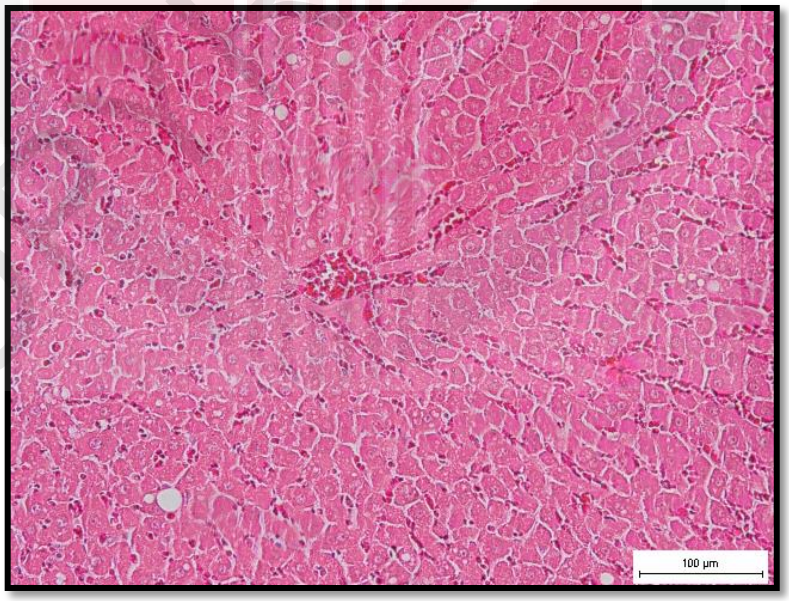

Figure 13: Light microphotograph of H&E-stained section (200X) of the formalin fixed liver of group E (alcohol + high cholesterol diet+ 250 mg/kg PN extract) showing mild vacuolation within hepatocytes.

5.0 DISCUSSION

5.1 Relative liver weight

The mean relative liver weight of group B (alcohol and high cholesterol diet group) was significantly ($p<0.05$) higher than groups C, D and E suggests that alcohol consumption and high dietary cholesterol causes hepatomegaly. This finding was supported by a study by Korourian *et al.* (1999), the mean liver weight of the ethanol-treated group was significantly greater than that of the control group suggesting that chronic alcohol consumption causes hepatomegaly and lipid accumulation which leads to increased liver mass and volume. However, in that study they use ethanol and carbohydrate deficient diet to induce liver injury. Besides, they have also proven low dietary intake of carbohydrates may be an important factor in the rapid development of alcohol-induced liver damage (Korourian *et al.,* 1999). The mechanism by which ethanol-induced liver injury develops in the carbohydrate deficient model appears to be associated with an enhanced cytochrome (CYP2E1) induction (Korourian *et al.*, 1999).

Furthermore, in a research done by Lodhi *et al.*, (2014), they suggest the increase in the liver weight may be due to hepatomegaly or hepatotrophy which is attributed to the fact that chronic alcohol consumption causes accumulation of lipids and proteins in hepatocyte. Water is retained in the cytoplasm of hepatocyte leading to enlargement of liver cells resulting in increased total liver mass and volume (Lodhi *et al*., 2014).

Based on the relative liver weight results, supplementation of *Phyllanthus niruri* extract reduced hepatomegaly induced by consumption of alcohol and high cholesterol diet.

The mean relative liver weight of group B is higher than group A with p-value of 0.056. This might be due to small sample size in our group because most of the previous researches were using at least more than 6 rats in each group (Manjrekar *et al.,* 2008; Rajagopalan *et al.*, 2010; Arun & Balasubramanian, 2011).

5.2 Serum biochemistry analysis

The mean low density lipoprotein (LDL) showed significant ($p<0.05$) difference between groups in which group B was significantly ($p<0.05$) higher than groups A, D and E. This finding suggests that feeding high cholesterol diet to rat model could increase the serum LDL level and supplementation of *Phyllanthus niruri* ethanolic extract at dosage of 150 mg/kg and 250 mg/kg are effective to prevent serum LDL elevation.

The serum LDL of group B was significantly ($p<0.05$) higher than group A suggests that dietary cholesterol could increase serum LDL levels. As in a research done by Cole *et al.*, (1984), addition of 2% cholesterol to the 5% fat diet produced significant increase in plasma cholesterol. Increased serum LDL levels in alcohol and high cholesterol diet group without *Phyllanthus niruri* supplementation (groups B) also suggests that chronic alcohol consumption will lead to increased LDL through the mechanism of down regulation of LDL receptor and enhancement of cholesterol biosynthesis pathway in the liver. Long-term excessive alcohol feeding to rats caused fatty liver and liver injury, which was associated with disrupted cholesterol homeostasis, characterized by increased hepatic cholesterol levels and hypercholesterolemia (Zhigang, Tong & Zhenyuan, 2010).

This can be explained by enhanced rate-limiting enzymes (SREBP-2 and HMG-CoA reductase) for cholesterol *de novo* synthesis, indicating enhanced cholesterol biosynthesis accompanied by decreased LDL receptor levels in the liver which leads to more LDL to be retained in circulation (Zhigang, Tong & Zhenyuan, 2010). However, this was associated with marked increased cholesterol enrichment in both HDL and LDL (Zhigang, Tong & Zhenyuan, 2010).

Supplementation of *Phyllanthus niruri* extract had prevented LDL elevation, which suggests that *Phyllanthus niruri* has lipid lowering activity. This finding is consistent with a study by Khanna, Rizvi and Chander (2002), the *Phylanthus niruri* extract significantly lowered the lipid levels of VLDL (very low density lipid proteins) and LDL in experimental animals. The mechanism of lipid lowering activity includes inhibition of cholesterol biosynthesis, increased plasma lecithin, increased fecal bile acids excretion and cholesterol acyltransferase activity.

The liver parameters were not increased in this study could be due to the short duration of study and low dosage of alcohol which is insufficient to cause elevation in liver parameters. ALD is a result of dose- and time-dependent consumption of ethanol (Enomoto *et al.*, 1999). In a study done by Enomoto *et al.*, (1999), ethanol caused steatosis, inflammation and necrosis in only a few weeks but aspartate transaminase (AST) level was only doubled in 8 weeks time.

In a study of ALD in rats, they have successfully induced liver injury in rats with marked elevation of liver parameters using 3.76 mL/kg of 40% alcohol for 25 days

(Kumar *et al.*, 2013). Lower dosage of alcohol (1 mL/kg, 20% ethanol) was used in our study because the maximum volume of ethanol that can be fully administered to the rats through oral gavage per administration is 0.5 mL. By reducing the dosage to half, we add in high cholesterol diet was added to enhance the liver injury caused by the ethanol. This was supported by a study done by Rajagopalan *et al.*, (2010), showed that administering 20% ethanol with 15% heated sunflower oil enhanced liver injury through enhancement of biomembranes unsaturation and lipid peroxidation.

The CK values were elevated in all rats can be due to handling during oral gavages which were carried out daily throughout the experiment period. The urea and creatinine values were within normal range (Appendix 5).

5.3 Hepatic histopathology lesion scores

Based on the mean rank values tabulated in Table 4, group B showed highest mean rank followed by groups C, D, E and the least is from group A. This showed that hepatic steatosis is the most severe in group B followed by groups C, D, E and the least in group A. Based on Figure 6, group A and group E were belongs to the same subset which indicate that *Phyllanthus niruri* ethanolic extract at 250 mg/kg was effective in preventing liver injury induced by alcohol and high cholesterol diet as in groups B, C and D. However, groups C and D were found to be ineffective may be is due to the dosage of *Phyllanthus niruri* ethanolic extract is too low to be sufficient to eliminate lesions established. In overall, *Phyllanthus niruri* ethanolic extract was effective in preventing ALD at the dosage of 250 mg/kg which is equivalent to 36 mg/kg in human.

A research done Rajagopalan *et al.,* (2010) demonstrated that fibrosis can be observed in liver treated with alcohol and heated polyunsaturated fatty acid (PUFA) and *Phyllanthus niruri* treated rats only showed sinusoidal dilatation.

6.0 CONCLUSION

The present study demonstrated that the ethanolic extract of *Phyllanthus niruri* has hepatoprotective effect against alcohol and high cholesterol diet-induced liver damage in rats at the dosage of 250 mg/kg through the reduction of mean relative liver weight, LDL level and hepatic steatosis scoring as shown in this study.

7.0 RECOMMENDATION

Higher dosage of ethanol and longer period of study are recommended to induce liver damage in rat model, aiming to observe elevation in liver parameters and steatohepatitis in histopathology examination. Different route of ethanol administration is recommended to overcome the restriction in oral gavage.

This study is preventive study whereby alcohol and plant extract were given simultaneously to the rats for a period of time. It is recommended that curative study can be done in future research to determine the potential of *Phyllanthus niruri* in curing hepatic lipidosis in cats.

REFERENCES

Arteel, G. (2010). Animal Models of Alcoholic Liver Disease. *Digestive Diseases*, *28*(6), 729-736. doi:10.1159/000324280

Arun, K., & Balasubramanian, U. (2011). Comapartive Study on Hepatoprotective activity of Phyllanthus amarus and Eclipta prostrata against alcohol induced in albino rats. *International Journal Of Environmental Sciences*, *2*(1), 361-373.

Baskaran, M., Periyasamy, L., & Rajagopalan, R. (2010). Effect Of Phyllanthus niruri on Alcohol and Polyunsaturated Fatty Acid induced Oxidative Stress in Liver. *International Journal Of Pharmacy And Pharmaceutical Sciences*, *2*(4), 58-62.

Cole, T., Kuisk, I., Patsch, W., & Schonfeld, G. (1984). Effects of high cholesterol diets on rat plasma lipoproteins and lipoprotein-cell interactions. *Journal Of Lipid Research*, *25*, 593-602.

Cynthia, M. & Scott, L. (2010). *The Merck Veterinary Manual Tenth Edition* (10th ed., pp. 420-423). New Jersey: MERCK & CO., INC.

European Association for the Study of the Liver (EASL) Clinical Practice Guidelines: Management of Alcoholic Liver Disease. (2013). *Clinical And Molecular Hepatology*, *19*(3), 216. doi:10.3350/cmh.2013.19.3.216

Eswar Kumar, K., Harsha, K., Sudheer, V., & Giri babu, N. (2013). In vitro antioxidant activity and in vivo hepatoprotective activity of aqueous extract of Allium cepa bulb in ethanol induced liver damage in Wistar rats. *Food Science And Human Wellness*, *2*(3-4), 132-138. doi:10.1016/j.fshw.2013.10.001

Gramenzi, A., Caputo, F., Biselli, M., Kuria, F., Loggi, E., Andreone, P., & Bernandi, M. (2006). Review article: alcoholic liver disease? pathophysiological aspects and risk factors. *Aliment Pharmacol Ther*, *24*(8), 1151-1161. doi:10.1111/j.1365-2036.2006.03110.x

Hui-Hui, T. (2010). Non-alcoholic Fatty Liver Disease. *Proceedings Of Singapore Healthcare*, *19*(1), 36-45.

Jacquelyn, M. (1997). Exploring Alcohol's Effects on Liver Function. *Alcohol Health & Research World*, *21*(1), 5-12.

Khanna, A., Rizvi, F., & Chander, R. (2002). Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats. *Journal Of Ethnopharmacology*, *82*(1), 19-22. doi:10.1016/s0378-8741(02)00136-8

Korourian, S., Hakkak, R., Ronis, M., Shelnutt, S., Waldron, J., Ingelman-Sundberg, M., & Badger, T. (1999). Diet and risk of ethanol-induced hepatotoxicity: carbohydrate-fat relationships in rats.*Toxicological Sciences*, *47*(1), 110-117. doi:10.1093/toxsci/47.1.110

Lik Fai, C. (2010). Non-alcoholic fatty liver disease in Asia: a systematic review. Retrieved from http://hdl.handle.net/10722/132349

Lodhi, P., Tandan, N., Singh, N., & Kumar, D. (2014). Green Tea Extract Ameliorates Ethanol Induced Liver Injury in Albino Rats. *America Journal Of Phytomedicine And Clinical Therapeutics*, *2*(5), 603-608.

Manjrekar, A., Jisha, V., Bag, P., Adhikary, B., Hedge, A., & Nandini, M. (2008). Effect of Phyllanthus niruri Linn. treatment on liver, kidney and testes in CCl4 induced hepatotoxic rats. *Indian Journal Of Experimental Biology*, *46*, 514-520.

Mazaki-Tovi, M., Abood, S., Segev, G., & Schenck, P. (2013). Alterations in Adipokines in Feline Hepatic Lipidosis. *Journal Of Veterinary Internal Medicine*, *27*(2), 242-249. doi:10.1111/jvim.12055

Reddy, J. (2005). Lipid Metabolism and Liver Inflammation. II. Fatty liver disease and fatty acid oxidation. *AJP: Gastrointestinal And Liver Physiology*, *290*(5), G852-G858. doi:10.1152/ajpgi.00521.2005

Samuhasaneeto, S., Thong-Ngam, D., & Klaikeaw, N. (2007). Factors Influencing a Rat Model of Alcoholic Liver Disease for Research Study. *Thai Journal Of Physiological Sciences*, *20*(1).

Schreuder, T. (2008). Nonalcoholic fatty liver disease: An overview of current insights in pathogenesis, diagnosis and treatment. *WJG*, *14*(16), 2474. doi:10.3748/wjg.14.2474

Zhigang, W., Tong, Y. & Zhenyuan, S. (2010). Chronic Alcohol Consumption Disrupted Cholesterol Homeostasis in Rats: Down-Regulation of Low-Density Lipoprotein Receptor and Enhancement of Cholesterol Biosynthesis Pathway in the Liver. *Alcoholism: Clinical And Experimental Research*,*34*(3), 471-478. doi:10.1111/j.1530-0277.2009.01111.x

APPENDICES

Appendix 1: Effect of *Phyllanthus niruri* on relative liver weight (mean ± S.E.M.) between groups.

Group	A	B	C	D	E
Relative Liver weight	3.18 ±0.11[a]	3.84 ±0.22[b]	3.36 ±0.07[a]	3.35 ±0.11[a]	3.15 ±0.19[a]

Values with different superscripts were significantly different at $p<0.05$.

Appendix 2: Effect of *Phyllanthus niruri* on liver parameters (mean ± S.E.M.) between groups.

Group	A	B	C	D	E
ALT (U/L)	49.80 ±7.02	55.03 ±5.07	52.05 ±3.16	56.40 ±12.47	69.20 ±8.24
ALP (U/L)	137.50 ±12.09	140.25 ±1.89	122.00 ±21.89	205.50 ±64.57	140.75 ±15.61
AST (U/L)	144.58 ±6.39	142.83 ±12.21	200.20 ±41.4	156.00 ±16.2	184.33 ±17.0
Total protein (g/L)	80.70 ±1.27	81.33 ±1.2	79.63 ±1.11	80.15 ±0.86	82.48 ±1.69
Albumin (g/L)	36.78 ±0.66	39.18 ±1.37	37.58 ±1.83	36.88 ±3.4	37.15 ±1.78
Globulin (g/L)	43.93 ±1.16	42.15 ±1.15	42.05 ±1.15	43.28 ±3.3	45.33 ±3.12

Values with no superscript were not significantly different between groups at $p<0.05$.

Appendix 3: Effect of *Phyllanthus niruri* on selected lipid parameters (mean ± S.E.M.) between groups.

Group	A	B	C	D	E
Triglycerides (mmol/L)	0.41 ±0.05[a]	0.39 ±0.08[a]	0.36 ±0.06[a]	0.28 ±0.02[a]	0.40 ±0.09[a]
LDL (mmol/L)	0.49 ±0.05[a]	0.88 ±0.14[b]	0.80 ±0.04[b]	0.62 ±0.06[a]	0.62 ±0.11[a]

Values with different superscripts were significantly different at $p<0.05$.

Appendix 4: Effect of *Phyllanthus niruri* on cholesterol and high density lipoprotein (HDL) between groups.

Group	A	B	C	D	E	Chi-square
Cholesterol (mmol/L)	9.00	14.75	8.88	12.25	7.63	3.93
HDL (mmol/L)	13.50	11.38	7.88	12.5	7.25	3.57

Values with no superscript were not significantly different between groups at $p<0.05$.

Appendix 5: Effect of *Phyllanthus niruri* on creatinine kinase, urea and creatinine (mean ± S.E.M.) between groups.

Group	A	B	C	D	E
Creatinine kinase (u/L)	952.00 ±219.23	683.75 ±95.58	1129.75 ±236.89	1037.50 ±132.72	1111.50 ±263.59
Urea (mmol/L)	5.70 ±1.10	7.40 ±1.41	8.78 ±4.97	6.40 ±1.96	7.90 ±1.10
Creatinine (μmol/L)	79.25 ±4.60	78.25 ±4.23	85.50 ±13.07	79.00 ±6.99	69.00 ±2.27