UNIVERSITI PUTRA MALAYSIA

TOXICITY EVALAUTIONS OF ETHANOLIC EXTRACT OF Christia vespertilionis (L. F.) BAKH. F. IN MALE SPRAGUE DAWLEY RATS

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By

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Chairman : Associate Professor Hazilawati binti Hamzah, PhD
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The term Butterfly tea refers to the decoction of *Christia vespertilionis* (L.f.) Bakh. f. leaves which is widely consumed by cancer patients throughout Malaysia, and it has gained a huge popularity among researchers yearning to discover the real potential of this plant. Herein, this study is aimed at evaluating possible toxicity in a 14-day acute, 28-day subacute and 90-day subchronic oral toxicity of the ethanolic extract *Christia vespertilionis* (L.f.) Bakh. f. in male Sprague Dawley rats. The 14-day acute toxicity study was conducted to detect lethal dose 50 (LD_{50}) *Christia vespertilionis* (L.f.) Bakh. f. while the 28-day subacute and 90-day subchronic toxicity studies are to detect the non-observed-adverse-effect level (NOAEL). In the acute toxicity study, rats were divided into control, 5% DMSO (vehicle) and 2000 mg/kg groups. The extract was administered orally on day 1 and observed for 14 days. Meanwhile, in the subacute and subchronic toxicity studies, a total of 30 rats were divided into control, 5% DMSO (vehicle), low dose (75 mg/kg), medium dose (125 mg/kg) and high dose (250 mg/kg) groups. The extract was administered daily from day 1 until day 28 for subacute and day 90 for subchronic. Standard toxicology parameters including mortality, behavioural changes, motor-neuronal abnormalities, feed-water consumption pattern and body weight were measured. The haematological, serum biochemical parameters and histopathological assessment of kidney and liver functions were also carried out. Results of acute oral toxicity showed that single dose (2000 mg/kg) of ethanol extract of *Christia vespertilionis* (L.f.) Bakh. f. leaves induced no treatment-related signs of toxicity or mortality in male Sprague Dawley rats. The haematological results also showed no changes in the control and treated groups in all 3 studies. However, serum biochemistry results for acute study, showed a significant decrease in the CK and AST level when compared with the control and treated groups. Meanwhile results for serum biochemistry in subacute and subchronic showed no changes in the control and treated groups for both studies. Organs to body weights ratio after euthanisation in all 3 studies showed no significant differences when comparing treated and control groups. On histopathological analysis, acute study showed significant differences ($p<0.05$) of

lesions observed on hepatic necrosis (mild to moderate) and degeneration (very mild) in the treated group (2000 mg/kg). Meanwhile, subacute study showed significant differences ($p<0.05$) of lesions observed on high dose, medium dose and low dose groups has mild to moderate, mild and very mild lesion of hepatic necrosis and very mild hepatic degeneration and hepatitis were scored in all three groups in subacute study. Besides, for subchronic study showed significantly differences ($p<0.05$) in hepatic necrosis and activated kupffer cells. Hepatic necrosis was observed mild to moderate in both high dose and medium dose groups, while low dose group only had mild lesion in subchronic study. On the other hand, the number of activated kuffer cells was significantly ($p<0.05$) higher in low and medium dose groups compared to the high dose group. On the other hand, all three studies, there were no significant ($p>0.05$) on lesion for renal toxicity were scored.

In conclusion, for the acute toxicity result, lethal dose 50 (LD_{50}) of *Christia vespertilionis* (L.f.) Bakh. f. is greater than 2000 mg/kg and both subacute and subchronic study showed induces dose-dependent oral hepatotoxicity in rats. As hepatic necrosis was predominantly seen compared to hepatic necrosis and hepatic degeneration in subacute toxicity study, it is suggested that subchronic toxicity study of *Christia vespertilionis* (L.f.) Bakh. f. extract induces more permanent damage to the hepatocytes.

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

ANALISIS TOKSISITI DARIPADA EKSTRAK ETANOL DAUN *Christia vespertilionis* (L.F.) BAKH. F. PADA TIKUS JANTAN SPRAGUE DAWLEY

Oleh

NURUL SYAHIRAH BINTI AHMAD SAYUTI

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Pengerusi : Profesor Madya Hazilawati binti Hamzah, PhD
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Teh Rerama merujuk kepada rebusan daun *Christia vespertilionis* (L.f.) Bakh. f. yang di konsumi oleh pesakit kanser di Malaysia dan telah menjadi popular di Malaysia bukan sahaja dikalangan pesakit kanser tetapi juga kepada penyelidik untuk mengkaji pontensi sebenar pokok ini. Bagi kajian ini, tujuan kajian ini adalah untuk menilai aktiviti toksisiti 14-hari akut, 28-hari subakut dan 90-hari subkronik ekstrak etanol *Christia vespertilionis* (L.f.) Bakh. f. ke atas tikus jantan spesis Sprague *Dawley*. Akut 14-hari aktiviti toksisiti dijalankan untuk mengenalpasti dos *Christia vespertilionis* (L.f.) Bakh. f. yang menyebabkan 50% kematian (LD_{50}) dan diikuti kajian 28-hari subakut dan subkronik toksisiti untuk mengenalpasti dos yang tidak menyebabkan kesan sampingan (NOAEL). Untuk kajian toksisiti akut, tikus dibahagikan kepada kumpulan kawalan normal, 5% DMSO (transportasi) dan kumpulan dos 2000mg/kg. Ekstrak diberi secara oral pada hari pertama (1) dan diperhati selama 14 hari. Manakala, untuk subakut dan subkronik kajian toksisiti, sebanyak 30 ekor tikus dibahagikan kepada kumpulan kawalan normal, 5% DMSO (transporatasi), dos rendah (75 mg/kg), dos sederhana (125 mg/kg) dan kumpulan dos tinggi (250 mg/kg). Ekstrak diberikan setiap hari selama 28 hari subakut dan 90 hari subkronik. Aktiviti toksisiti dinilai melalui parameter-parameter seperti kadar kematian, perubahan perangai, ketidaknormalan motor dan neuron, berat badan dan polar makanan dan air yang dikonsumi. Selain itu, analisis darah, serum biokimia dan histopatologi buah pinggang dan hati telah dijalankan. Keputusan untuk kajian akut oral toksisiti menunjukkan satu dos 2000mg/kg etanol ekstrak daun *Christia vespertilionis* (L.f.) Bakh. f. tidak menyebabkan kematian terhadap tikus jantan *spesis* Sprague Dawley. Keputusan analisis darah untuk ketiga-tiga kajian juga tidak menunjukkan perubahan yang ketara di antara kumpulan kawalan normal dan kumpulan yang diberi ekstrak. Keputusan analisis serum biokimia dalam kajian akut toksisiti menunjukkan paras CK dan AST signifikan ($p<0.05$) bila dibandingkan dengan kumpulan kawalan normal dan kumpulan yang diberikan ekstrak, walaubagaimanapun, keputusan analisis serum biokimia untuk subakut dan subkronik menunjukkan tiada perubahan di antara

kumpulan kawalan normal dan kumpulan yang diberikan ekstrak untuk kedua-dua kajian. Kadar perubahan berat organ ratio kepada berat badan untuk ketiga-tiga kajian juga menunjukkan tiada signifikan perubahan di antara kumpulan kawalan normal dan kumpulan yang diberikan ekstrak. Histopatologi analisis, kajian akut toksisiti menunjukkan signifikan perubahan skor nekrosis sel hepar (sedikit ke sederhana) dan degenerasi (sangat sedikit) dalam kumpulan dos 2000mg/kg. manakala kajian subakut menunjukkan signifikasi perubahan skor ($p<0.05$) pada kumpulan dos tinggi, dos sederhana dan dos rendah adalah sedikit ke sederhana, sedikit dan sangat sedikit pada perubahan skor nekrosis sel hepar dan skor degenerasi dan hepatitis sangat sedikit untuk ketiga-tiga dos. Disamping itu, kajian subkronik menunjukkan perubahan signifikan ($p<0.05$) pada skor nekrosis sel hepar dan bilangan sel kuffer yang aktif. Nekrosis sel hepar dinilai sedikit ke sederhana untuk kedua-dua dos tinggi dan dos sederhana, manakala untuk dos rendah menunjukkan sedikit perubahan skor sahaja. Bilangan sel kuffer yang aktif adalah signifikan tinggi dalam dos rendah dan dos sederhana jika dibandingkan dengan dos tinggi. Sebaliknya, ketiga-tiga kajian menunjukkan tiada signifikan ($p>0.05$) perubahan skor pada toksisiti buah pinggang.

Secara kesimpulannya, merujuk kepada keputusan kajian akut, dos yang menyebabkan kematian 50 peratus (LD_{50}) ekstrak *Christia vespertilionis* (L.f.) Bakh. f. adalah lebih tinggi daripada 2000 mg/kg dan kedua-dua kajian subakut dan subkronik menunjukkan kebergantungan dos ke atas hepatotoksisiti tikus. Nekrosis sel hepar sebahagian besarnya dilihat dalam kajian subkronik jika dibandingkan dengan nekrosis sel hepar dan degenrasi sel hepar dalam kajian subakut, ia menunjukkan kajian subkronik toksisiti ekstrak *Christia vespertilionis* (L.f.) Bakh. f. menyebabkan lebih kerosokan kekal kepada sel hepar.

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The 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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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CK	Creatinine kinase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
IV	Intravascular
LD_{50}	Lethal dose
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MTD	Maximum tolerated dose
NOAEL	Non-adverse-effect level
PCV	Packed cell volume
RBC	Red blood cell
WBC	White blood cell

CHAPTER 1

INTRODUCTION

1.1 Background

Herbal remedies, particularly those used for therapeutic purposes, are widely used in many cultures for thousands of years. It is universally popular in primary healthcare, predominantly in developing countries such as Malaysia. The wide usage of these so-called “natural remedies” or “medicinal herbs" for self-medication is a result of the fact that the general public believes them to be safe and do not have any compromising health effects (Obici *et al.,* 2008). However, overtime there have been numerous warnings issued regarding the potential toxicity of these therapies, which further suggests the constant need for practitioners to keep abreast of reported incidence for renal and hepatic toxicity caused by ingestion of medicinal herbs and for investigational studies to be done on it.

In recent times, focus on plant research has increased all over the world and many evidences have been collected to show immense potential of medicinal plants used in various traditions. The wide and largely untapped field of traditional medicines still remains as an unique source for the discovery of bioactive compounds (Chen *et al.,* 2008; Kahumba *et al.*, 2014). Nearly, 80% of African and Asian population depends on traditional medicines for their primary healthcare (WHO, 2009; Karnataka Medicinal Plant Authority, 2009) and reported as high as 37% in Australia use herbal remedies as lifetime prevalence (Thomson *et al.,* 2012).

It is also estimated that about 25% of the drugs prescribed worldwide are derived from plants, with about 121 active compounds in use (Sahoo *et al.,* 2010). Between the years 2005 and 2007, 13 drugs derived from natural products were approved in the United States. More than 100 natural product-based drugs are in clinical studies (Li and Vederas 2009), and of the total 252 drugs in the World Health Organization’s (WHO) essential medicine list, 11% are exclusively of plant origin (Sahoo *et al.* 2010). Besides, several clinical studies of traditional Chinese herbal medicines were undertaken, and others are still ongoing (Fu *et al.,* 2013 and Liu *et al.,* 2013).

Herbs and plants can be processed and be taken in many different forms and ways. These can be in the form of a whole herb, tea, syrup, essential oils, ointments, salves, rubs, capsules or even tablets that contains grounded or powdered form of the raw herb or its dried extract. Plants and herbs extract vary in the solvent based on its extraction, temperature extraction time, and inclusions of substances such as alcoholic extracts, vinegars extracts, hot water extract, long-term boiled extract roots or bark, and cold infusion of plants (macerates). There is no standardization, and components of an herbal extract or a product are likely to vary significantly between batches and producers (Sissi and Iris, 2011).

***Christia vespertilionis* (L.f.) Bakh. f.** also known as the Rerama leaf has recently gained attention on its supposed potential to cure cancer. Various parts of this plant (mainly leaves) are widely used in traditional medicine for the treatment of numerous disorders. A decoction of the plant leaves is commonly used in remedies treating snake bites, tuberculosis, healing of bone fractures, bronchitis and cold and to increase blood circulation (Brach and Song, 2006), anti-plasmodium and high cytotoxicity against Hela and MRC54 (Nguyen *et al.,* 2007), inhibit neuroendocrine tumours (Hofer *et al.,* 2013) and inhibit growth of S180 tumour and H22 tumour cells (Wu *et al.,* 2012). Although several pharmacological studies (as antiplasmodial, anti-tumour) have been carried out on this plant, there is no experimental evidence on its toxicity. Hence, in this present study, the main aim is to evaluate its toxicity effects. This study was designed to investigate the toxicological assessment of acute, subacute and subchronic ethanolic leaf extract of ***Christia vespertilionis* (L.f.) Bakh. f.** on the blood, liver and kidney tissues of male Sprague Dawley rats.

1.2 Problem statement

The increased interest recently in herbal medicines accelerated on the belief that these medicines are natural and have been traditionally used for centuries and are therefore assumed as safe and harmless. Nevertheless, their natural origin is not a guaranteed safety especially when there are risks associated with the use of herbal products that have been noted (Whiting *et al.,* 2002). Hence, gathering scientific information regarding the safety of consumption of this plants for use as an alternative and/or complementary medicine is very important before it is further developed into a new medicinal herbal therapy. In accord to this, a study was in need to be conducted to determine whether the use of ***Christia vespertilionis* (L.f.) Bakh. f.** as a plant base herbal drink that is widely distributed and consumed in Malaysia especially by cancer patients in the form of a tea bags, safe. At present time, there is no known published research on the toxicity study of this plant. Therefore, this study concentrates on the toxicity study for *M. christia verpertilionis* in male Sprague Dawley rats especially in determining the lethal dose (LD_{50}) and the non-observe-effect level (NOEL) of the extract.

1.3 Hypotheses

a) Null hypotheses: The median lethal dose (LD50) of ***Christia vespertilionis* (L.f.) Bakh. f.** is lesser than 2000 mg/kg.
b) Null hypotheses**:** NOAEL of ***Christia vespertilionis* (L.f.) Bakh. f.** for both subacute and subchronic toxicity studies is lesser than 250 mg/kg.

1.4 Objective

This study is conducted to evaluate acute oral toxicity study (14-day), subacute oral toxicity study (28-day) and subchronic oral toxicity study (90-day) of ethanolic extracts of ***Christia vespertilionis*** **(L.f.) Bakh. f.** in male Sprague Dawley rats.

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