



UNIVERSITI PUTRA MALAYSIA

***TOXICITY STUDY OF ERYTHROXYLUM CUNEATUM KURZ, AND ITS  
EFFECTS ON RODENT NEUROBEHAVIOURAL PROPERTIES***

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

**TOXICITY STUDY OF *ERYTHROXYLUM CUNEATUM* Kurz. AND ITS  
EFFECTS ON RODENT NEUROBEHAVIOURAL PROPERTIES**

By

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**October 2015**

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Therapeutic and new alternative potential compounds of natural product have been widely used in pharmaceutical industry. Interest on alternative efforts to search for new compounds with less or no adverse effects has led to the discovery of new potential plants in the treatment of diseases. *Erythroxylum cuneatum*, also known as 'Chinta Mula' plant among Malaysians has been discovered and recently proven to possess antiwithdrawal properties with a potential to be explored as a source of new drugs for the treatment of addiction. *Erythroxylum cuneatum* can be found in primary and secondary forests. Due to less information on this plant, this elusive plant was investigated advancedly to understand its roles and potential as a new source of pharmacologically in the pharmaceutical industry. Thus, further scientific exploration was made to understand the mechanisms of action involving toxicity and neurobehavioral properties were proposed. Three different extracts were produced from the leaves of *Erythroxylum cuneatum* which were aqueous, methanol and alkaloid extracts and three semi pure compounds were fractionized from the crude alkaloid extracts by different solvent systems. There is no toxicity profile on this plant, thus, their toxicity on cell lines, brine shrimp and mice were determined. In the cytotoxicity and Brine Shrimp Lethality Assay (BSLA) studies, alkaloid extract was found to be toxic to the cells and brine shrimp compared to the aqueous extract. Indeed, in the BSLA study, aqueous extract seems to give no toxic effect to the shrimp at the high dose as compared to the alkaloid extract. However, for the *in-vivo* toxicity study, aqueous extract showed almost similar toxic effect as alkaloid extract. The presence of alkaloid compounds that is typically associated with the central nervous system (CNS) has directed the study of behavioral profiles. Locomotor activity in Open-Field Test (OFT), anxiety study using elevated-plus maze (EPM) and working memory task using Novel Object Discrimination test (NOD) were performed for the determination of neurobehavioral profiles. Sixty four male Wistar rats were used in the study

and divided into eight groups which were normal group, control group, three groups of positive drug control and three groups of three different doses of alkaloid extracts (5, 25 and 50 mg/kg). All doses were chronically administrated intraperitoneally to the test group for 21 consecutive days and evaluated on day 21 after the last dose. The findings showed that locomotor activity was not significantly increased ( $P>0.05$ ) in all doses of alkaloid extracts. In anxiety study, two parameters were performed on each rat including time spent in open arms and open arm entries frequency. All doses did not increase in both parameters as compared to the standard drug Diazepam. In the Novel Object Discrimination test, alkaloid treated rats did not show any significant discrimination between the old and new object ( $P>0.05$ ) thus, it can be interpreted as a memory deficit. After the completion of behavioral study, rats were sacrificed and their kidney and liver were collected to evaluate any morphological changes. Normal to mild changes were noted in both liver and kidney of treated rats with 5 and 25 mg/kg of alkaloid extract while the highest dose (50 mg/kg) did not show any morphological changes. From this data, it is clear that *Erythroxylum cuneatum* does not affect behavioral properties. In conclusion, *Erythroxylum cuneatum* could be a scientist new hope in the process of developing new anti withdrawal drugs as *Erythroxylum cuneatum* has a low toxic effects and does not produce any behavioural changes.

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

**KAJIAN TOKSISITI *ERYTHROXYLUM CUNEATUM* Kurz. DAN KESANNYA  
TERHADAP TINGKAH LAKU NEURO PADA TIKUS**

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Sebatian terapeutik dan potensi alternatif baru daripada sumber semula jadi telah digunakan secara meluas dalam industri farmaseutikal. Keinginan atas usaha alternatif untuk mencari pengganti baru sebatian semulajadi dengan kesan buruk yang sedikit atau tiada kesan buruk telah membawa kepada penemuan tumbuhan baru yang berpotensi dalam rawatan perubatan moden. *Erythroxylum cuneatum* (EC), yang juga dikenali sebagai pokok 'Chinta Mula' di Malaysia telah ditemui baru-baru ini dan terbukti memiliki sifat anti-ketagihan dan ia berpotensi untuk diteroka sebagai sumber ubatan baru untuk merawat ketagihan. *Erythroxylum cuneatum* boleh ditemui di hutan primer dan sekunder. Maklumat yang kurang mengenai tumbuhan ini mendorong kajian lebih lanjut dilakukan untuk memahami peranan dan potensinya sebagai komponen baru dalam industri farmaseutikal. Oleh itu, penerokaan lanjut secara saintifik diusulkan untuk memahami mekanisme yang melibatkan ciri-ciri toksisiti dan neuro perlakuan. Tiga ekstrak yang berbeza telah dihasilkan dari daun EC iaitu akueus, metanol dan ekstrak alkaloid bersama tiga sebatian separa tulen iaitu pecahan dari ekstrak alkaloid mentah itu sendiri melalui sistem pelarut yang berbeza. Masih tiada profil ketoksikan pada tumbuhan ini, oleh itu ketoksikannya pada sel, 'brine shrimp' dan tikus dikaji. Dalam kajian sitotoksik dan *brine shrimp lethality assay* (BSLA), ekstrak alkaloid didapati lebih toksik kepada sel dan 'brine shrimp' berbanding dengan ekstrak akueus. Malah, dalam kajian BSLA itu, ekstrak akueus seolah-olah tidak memberi kesan toksik kepada 'brine shrimp' walaupun dalam dos yang tinggi berbanding dengan ekstrak alkaloid. Berbeza sedikit dengan kajian ketoksikan *in-vivo*, di mana ekstrak akueus menunjukkan kesan toksik hampir menyamai ekstrak alkaloid. Kehadiran sebatian alkaloid yang dikaitkan dengan tindakan sistem saraf tunjang telah mendorong kajian ini kepada kajian profil tingkah laku. Aktiviti lokomotor dalam 'Open Field Test' (OFT), kajian keresahan menggunakan 'Elevated-Plus Maze' (EPM) dan kajian ingatan kerja

menggunakan ujian ‘Novel Object Discrimination’ (NOD) telah dijalankan bagi menentukan profil tingkah laku. Enam puluh empat ekor tikus Wistar jantan telah digunakan dalam kajian ini dan dibahagikan kepada lapan kumpulan iaitu kumpulan normal, kumpulan kawalan, tiga kumpulan kawalan positif dadah dan tiga kumpulan dos yang berbeza daripada ekstrak alkaloid (5, 25 dan 50 mg/kg). Semua dos diberi secara kronik menerusi suntikan intraperitoneal (IP) kepada setiap tikus dalam kumpulan selama 21 hari berturut-turut dan diuji pada hari ke-21 selepas rawatan dos terakhir. Hasil kajian menunjukkan bahawa aktiviti lokomotor secara statistik tidak meningkat dengan ketara ( $P>0.05$ ) dalam semua dos ekstrak alkaloid. Dalam kajian kerasahan, dua parameter telah dinilai dalam setiap tikus termasuklah masa yang diambil berada dalam lapangan terbuka dan kemasukan ke lapangan terbuka. Kedua-dua parameter tidak meningkat dalam semua dos berbanding dadah piawai Diazepam. Dalam ujian ‘Novel Object Discrimination’, tikus yang dirawat dengan alkaloid tidak menunjukkan sebarang diskriminasi yang signifikan di antara objek yang dikenali dan objek baru ( $P>0.05$ ) sekaligus ianya ditafsirkan sebagai kemerosotan ingatan. Selepas tamat kajian tingkah laku, tikus dimatiakan dan ginjal beserta hati diambil untuk menilai sebarang perubahan morfologi. Tiada perubahan kepada perubahan sederhana diperhatikan dalam kedua-dua hati dan buah pinggang tikus yang dirawat dengan 5 dan 25 mg/kg manakala dos tertinggi alkaloid 50 mg/kg tidak menunjukkan sebarang perubahan morfologi. Melalui data yang diperolehi ianya jelas menunjukkan bahawa *Erythroxylum cuneatum* tidak memberi kesan kepada cirri-ciri tingkah laku. Sebagai kesimpulannya, *Erythroxylum cuneatum* boleh memberi harapan kepada ahli sains dalam proses membangunkan ubatan anti ketagihan baru kerana *Erythroxylum cuneatum* mempunyai kesan toksik yang rendah dan tidak memberi perubahan dalam profil tingkah laku.

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I certify that a Thesis Examination Committee has met on 20 October 2015 to conduct the final examination of Mohamad Syahmi bin Shahril on his thesis entitled "Neurobehavioural properties and toxicity study of *Erythroxylum cuneatum* Kurz. in rodents" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science (Anatomi).

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## LIST OF ABBREVIATIONS

AMP	Amphetamine
ASW	Artificial sea water
UV	Ultra violet
BSLA	Brine Shrimp Lethality Assay
BZ	Benzodiazepines
CBR	Central-type benzodiazepine
CNS	Central Nervous System
DZ	Diazepam
EPM	Elevated Plus Maze
GABA	Gamma-aminobutyric acid
IACUC	Institutional Animal Care and Use Committee
<b>IC<sub>50</sub></b>	Half Maximal Inhibition Concentration
<b>LC<sub>50</sub></b>	Half Maximal Lethal Concentration
<b>LD<sub>50</sub></b>	Half Maximal Lethal Dose
LTM	Long Term Memory
NOD	Novel Object Discrimination
OFT	Open Field Test
Rf	Retention factor
STM	Short Term Memory
TLC	Thin Layer Chromatography
V/V	Volume/Volume
WK	Working Memory

## CHAPTER 1

### INTRODUCTION

#### 1.1 Research Background

Malaysia is rich in biodiversity and a habitat for variety species of herbs and plants that have been used by our ancestors as a local medicine in their daily life. Reported by McCann (2003), herbs in biomedical antidotes nowadays have a significant value. At least one active ingredient prescribed today comes from plants and the rest are chemically laboratory produced. For a long period, scientists have been developing many drugs and herbal medicine from natural products based on previous local claims. Since that, nowadays many therapeutic and new alternative potential compounds of natural product have widely been used in pharmaceutical industry. In consumption of some pharmaceutical products, many diseases have decreased in their mortality and morbidity, yet the effectiveness is often overshadowed with many adverse effects (Rates, 2001). Aware with all the adverse effects of synthetic products, there is an increase in interest on alternative effort to search for new naturally occurring compounds with lesser or no adverse effects.

*Erythroxylum cuneatum* which belongs to the family Erythroxylaceae has been recently discovered and proven to possess many beneficial effects such as antioxidant, anti-inflammatory and antinociceptive properties (Saleh et al., 2012). In Malaysia, this plant is also known as 'Chinta Mula' and according to Sosef et al. (1998), it can be found scattered in most types of the forest. There was a report by Burkhill (1923) stated that in the Philippines, the leaves were used as a poison for fish and might be used as miscarriage tonics in Pahang. In Thailand, the leaves of this plant were used in their Thai traditional medicine as anti-fever and anti-inflammatory agents (Kanchanapoom et al., 2005). According to Payens (1955), the leaves were pounded and applied on the forehead of woman after miscarriage in Terengganu. Meanwhile in Buguran, Indonesia, the leaves are reported to be used in a vegetable soup (sajur). It is evident that plants serve as an alternative source of new molecule. However, there are no studies regarding its toxicity and neurobehavioral properties. Thus, this study aimed to understand the mechanisms of action involving toxicity and neurobehavioral properties and to isolate the responsible bioactive compound from *Erythroxylum cuneatum*.

## **1.2 Hypothesis**

In Malaysia drug abuse has been a serious public health problem. In consumption of modern drugs, people become addicted to it and the recent treatment compounds have an unwanted adverse effect and there is no data on the success of it. From previous study, *Erythroxylum cuneatum* has been proven in treating drugs addiction. In this study, we aim to prove that the administration of *Erythroxylum cuneatum* does not induce behavioral changes and toxic level. Thus, save to be developed as a new anti withdrawal natural product.

## **1.3 Objectives**

### **1.3.1 General Objectives**

The aim of this study is to evaluate the neuropharmacological and toxicity properties of *Erythroxylum cuneatum*.

### **1.3.2 Specific Objectives**

1. To produce an aqueous, methanol and alkaloid extracts of *Erythroxylum cuneatum* leaves.
2. To determine the effects of *Erythroxylum cuneatum* extract on locomotor activity, anxiety level and working memory in rats.
3. To evaluate the toxicity effect of *Erythroxylum cuneatum* leaves extract in SK-N-SH cell lines, *Artemia salina* L. (Artemiidae) and in mice.
4. To evaluate the chronic effects of *Erythroxylum cuneatum* extract on histopathological changes of the liver and kidney in rats.

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## **LIST OF PUBLICATIONS**

1. Mohamad Syahmi S, Mohamad Aris M.M, Mohamad Taufik Hidayat B, Rusliza B, Mohd Ilham A, Mohd Fadzly Amar J, Che Norma M. T, Noor Azuin S, Muhammad Amin Z. (2016). *In Vivo Toxicity Study of Erythroxylum cuneatum Leaves Extract and its Effects on Working Memory of Rats*. Journal of pharmacological and toxicological investigations, 2 (1), 1-4.

## **PROCEEDING/ CONFERENCE:**

1. Shahril MS, Moklas MAM, Taufik Hidayat M, Zaki MAA, Mohd Ilham A, Jamil MFA, Mat Taib CN, Suliman NA. Toxicity study of *Erythroxylum cuneatum* on SK-N-SH cell lines, *Artemia salina* L. and ICR mice. 5<sup>th</sup> International Neuroscience Symposium, Monash University Malaysia 27<sup>th</sup> September 2014.
2. Zaki MAA, Moklas MAM, Mat Taib CN, Adenan MI, Taufik Hidayat M, Jamil MFA, Shahril MS. Anti-withdrawal properties of *Erythroxylum cuneatum* (EC) in SK-N-SH neuroblastoma cell. 5<sup>th</sup> International Neuroscience Symposium, Monash University Malaysia 27<sup>th</sup> September 2014.
3. Mohamad Syahmi Shahril, Mohamad Aris Mohd Moklas, Mohamad Taufik Hidayat Baharuldin, Mohd Ilham Adenan, Mohd Fadzly Amar Jamil, Che Norma Mat Taib. The effect of *Erythroxylum cuneatum* leaves extract on anxiety level in rats. Malaysian Journal of Microscopy. 2015, Vol 11(suppl. 1). Pg 28.
4. Mohamad Syahmi S, Mohamad Aris M.M, Mohamad Taufik Hidayat B, Rusliza B, Mohd Ilham A, Mohd Fadzly Amar J, Che Norma M. T, Noor Azuin S, Muhammad Amin Z. the determination of *Erythroxylum cuneatum* leaves extract effects on memory in an novel object recognition task in rats. 30<sup>th</sup> Scientific Meeting of Malaysian Society of Pharmacology and Physiology (MSPP), Putrajaya Shangri-La Hotel, Malaysia, 15-16<sup>th</sup> August 2016.