UNIVERSITI PUTRA MALAYSIA

ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY PROPERTIES OF MELICOPE PTELEFOLIA CHAMP EX BENTH ETHANOLIC EXTRACT

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FPSK(M) 2013 56

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October, 2013

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

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October, 2013

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Melicope ptelefolia, locally known as 'tenggek burung', is consumed for various purposes in folk medicine such as treating fever, pain, wounds and itches. In present study, investigation of anti-nociceptive and anti-inflammatory properties of MPEE was conducted in nociception and inflammation-induced murine models, respectively. The anti-nociceptive activity of the extract was assessed using acetic acid-induced abdominal writhing, hot plate and formalin-induced paw licking tests. Meanwhile, studies on inhibition of acute inflammation and underlying mechanisms were conducted via paw edema model, using 1% carrageenan and different inflammatory mediators as edemogen. Investigation on the potent anti-inflammatory effects was also conducted on cotton pellet induced granuloma test models. Toxicity analysis of MPEE was also conducted.

Oral administration of MPEE produced significant dose-dependent anti-nociceptive effects when tested in mice and rats using acetic acid-induced abdominal constriction

test and on the second phase of the formalin induced paw licking test, respectively. It was also demonstrated that MPEE had no effect on the response latency time to the heat stimulus in the thermal model of the hot-plate test. Additionally, MPEE anti-nociception was not reversed by pre-administration of naloxone. MPEE anti-nociception mechanism of action was demonstrated on the inhibition of glutamate induced paw licking test but not capsaicin. For anti-inflammatory activity, ethanol extract of *Melicope ptelefolia* significantly inhibited formation of edema throughout 390 minutes of edema formation. For inflammatory mediators - induced paw edema test, MPEE showed significant reduction in histamine (66.67%), serotonin (54.9%), arachidonic acid (65.63%), and prostaglandin (40%) - induced paw edema test. For chronic inflammatory test model, highest concentration of MPEE (300 mg/kg, p.o) inhibited both wet and dry weight of cotton pellet while not significantly elevating the value of three biochemical markers (ALT, ALP and total protein). Furthermore, oral administration of MPEE did not produce any significant effect on balance and motor coordination in rotarod test. In acute toxicity study, sign of toxicity was detected in liver tissue for dose of 1000 mg/kg MPEE. These results indicated that MPEE at all investigated doses exerted pronounced anti-nociceptive activity that acts peripherally in experimental animals and did not produce any effects on motor corrdination and balance. MPEE also is considered safe until the concentration of 300 mg/kg.

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

CIRI ANTI-NOSISEPTIF DAN ANTI-INFLAMATORI EKSTRAK ETANOL *MELICOPE PTELEFOLIA* CHAMP EX BENTH

Oleh

AZYYATI BINTI MOHD PADZIL

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Melicope ptelefolia atau lebih dikenali dalam kalangan masyarakat tempatan sebagai tenggek burung telah digunakan untuk pelbagai tujuan, contohnya merawat demam, mengurangkan rasa sakit, merawat luka dan mengurangkan rasa gatal. Kajian secara *in vivo* berkenaan sifat anti-nosiseptif , anti-inflamatori dan ketoksikan telah dijalankan dengan menggunakan ekstrak etanol *Melicope ptelefolia* (EEMP). Kajian anti-nosiseptif dilakukan dengan menggunakan kaedah kontraksi abdomen aruhan asid asetik, ujian plat panas, kaedah nyeri plantar aruhan formalin serta beberapa kajian ke atas mekanisma tindakan anti-nosiseptif menggunakan kaedah nyeri plantar, aruhan asid glutamat dan kapsaisin. Kemudian, kajian berkenaan anti-inflamatori dan kajian mekanisma tindakan anti-inflamasi dijalankan menggunakan model eksperimen aruhan pembengkakan kaki dengan menggunakan 1% Carrageenan dan beberapa perantara inflamatori sebagai pengaruh bengkak. Kajian kemudian diteruskan dengan model eksperimen inflamatori kronik yang menggunakan kaedah butiran kapas sebagai pengaruh penghasilan granuloma dan

diteruskan dengan analisis ketoksikan terhadap EEMP. Mengikut hasil ujian, administrasi EEMP secara oral memberikan kesan anti-nosiseptif yang signifikan dan selaras dengan dos pada mencit dan tikus melalui ujian kontraksi abdomen aruhan asid asetik, dan fasa kedua ujian nyeri plantar aruhan formalin. EEMP tidak menunjukkan kesan terhadap sebarang pengurangan terhadap masa latensi untuk memberikan respon masa ujian plat panas. Tambahan pula, kesan EEMP terhadap anti-nosiseptif tidak dapat dihalang dengan administrasi awal naloxone, manakala bagi ujian mekanisma tindakan, EEMP bertindak dalam menghalang mengurangkan kesan dalam ujian nyeri plantar aruhan asid glutamat tetapi tidak untuk kapsaisin. Untuk kesan anti-inflamatori yang diaruh oleh 1% carrageenan, MPEE telah memberikan kesan pengurangan yang signifikan sepanjang 390 minit waktu pembentukan bengkak. Untuk ujian perantara inflamatori yang mengaruh pembentukan bengkak, EEMP menunjukkan pengurangan yang signifikan pada bengkak yang diaruh oleh histamin (66.67%), serotonin (54.9%), asid arakidonik (65.63%), dan prostaglandin (40%). Bagi model ujian inflamatori yang kronik, dos EEPM tertinggi (300 mg/kg) telah menunjukkan pengurangan untuk berat basah dan berat kering butiran kapas, tetapi tidak memberi kesan signifkan terhadap tiga penanda aras biokima (ALT, ALP dan jumlah protein). Selain itu, administrasi EEPM tidak memberi sebarang kesan terhadap koordinasi motor dan imbangan semasa ujian rod berputar dijalankan. Semasa ujian ketoksikan secara akut dijalankan, kesan ketoksikan terhadap sel hati pada dos 1000 mg/kg telah dikesan. Dengan ini, keputusan ujian-ujian ini menunjukkan bahawa EEPM mempunyai kesan anti-nosiseptif dan anti-inflamatori secara periferi dalam haiwan ujian tanpa menunjukkan sebarang kesan pada koordinasi motor dan imbangan. EEMP juga selamat dari kesan ketoksikan sehingga dos 300 mg/kg.

ACKNOWLEDGEMENTS

"In the name of Allah S.W.T., the most benevolent and Merciful.
All gratification is referred to ALLAH S.W.T."

First and foremost, my warmest gratitude to the chairman of my supervisory committee, Prof. Dr. Mohd Roslan bin Sulaiman for his support, kindness and expert advice throughout my Masters research study. Thank you for being understanding and helpful supervisor.

Special thanks to Prof. Dr. Daud Ahmad bin Israf Ali, who always inspires me with his advice, unexpected opinion and experience, which encouraged me to appreciate knowledge in a different perspective.

My greatest appreciation and gratitude to Prof. Dr.Khozirah binti Shaari, the project manager for our research grant, for her trust, financial and moral support.

I would like to extend my endless gratitude to IPharm (Institute Pharmaceutical and Nutraceutical) Initiatives, Division of Biotechnology, Ministry of Science, Technology and Innovation for a supporting grant towards the success of this study.

To UPM staffs, En Nasrul, Pn Hasnijah, Pn Norhayati, En Ramli, En Salahuddin, Pn Juita, En Zul and Puan Nor Azren. To my dearest friends and colleagues, Lee Ka Heng, Nazri, Dr Seema, Dr Wan Mastura, Syamimi Khalid, Dr Azam, Dr Enoch, Lee Ming Tat, Ina, Adilla, Izzati, Nasir and all members of Physiology Lab, Cell Signalling Lab, Faculty of Medicine and Health Sciences as well as Natural Product

Lab and Drug Discovery Lab, Institute of Bioscience. Thank you for being helpful friends.

Last but not least, the utmost and distinctive gratitude to my beloved parents, Hj. Mohd Padzil bin Sukayat and Hjh. Marsidah binti Salim, for their prayers, love, support and advice, may Allah grant you with His greatest love and blessings.

Thank you !

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

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LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine
5-LOX	5-lipooxygenase
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
ANOVA	Analysis of Variance between groups
ASA	Acetylsalicylic acid
AST	Aspartate Transaminase
ATP	Adenine Triphosphate
B.C	Before Century
cAMP	Cyclic Adenosine Monophosphate
CGRP	Calcitonin Gene- Related Peptide
Cl^-	Chloride ion
COX	Cyclooxygenase
COXIB	Cyclooxygenase-2 Inhibitor
DNA	Deoxyribonucleic acid
ERK	Extracellular signal-regulated kinase
Fe	Ferum
GABA	γ -Aminobutyric acid
h	Hour
Hb	Haemoglobin
Hct	Hematocrit
His	Histidine

i.p	Intraparietonial
i.pl	Intraplantar
IASP	International Association of S Pain
ICR	*Imprinting Control Region*
IFN-γ	Interferon-γ
Ile	Isoleucine
IL-β	Interleukin-β
kg	Kilogram
LC-DAD-ESI-MS	Liquid chromatography-Diode array detector-Electrospray-Mass Spectrometry
LOX	Lipooxygenase
LPS	Lipopolysaccharide P
LT	Leukotriene
M	Molar
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligram
mL	Millilitre
MPEE	*Melicope ptelefolia* ethanolic extract
MPV	Mean platelet volume
NaCl	Sodium chloride
NGF	Nerve growth factor
NMDA	*N-Methyl-D-aspartate*
NO	Nitric oxide

NSAIDS	Non-Steroidal Anti Inflammatory Drugs
^{o}C	Degree Celsius
OECD	Organisation for Economic Co-operation and Development
p.o	From the Latin "*per os*", by mouth
PCR	Polymerase chain reaction
PDW	Platelet distribution width
PG	Prostaglandin
PI_3K	Phosphatidylinositol 3 kinase
PKA	Phosphokinase A
PKC_{ε}	Phosphokinase C_{ε}
PLA_2	Phospholipase A_2
PLC_{β}	Phospholipase C_{β}
RBC	Red Blood Cells/ Erythrocyte
RDW	Red distribution width
rpm	Rotation per minute
s.c	Subcutaneous
S.E.M	Standard Error Mean
tHGA	2,4,6-trihydroxy-3-geranylacetophenone
TNFα	Tumor Necrosis Factor α
TRPV1	Transient Receptor Protein V 1
TrK-A	Transforming Tyrosine kinase protein
TTX-R	Tetrodotoxin-resistant
TXA_2	Thromboxane
U	Unit
WBC	White Blood Cell

CHAPTER 1

INTRODUCTION

In the early years, even way before the independence of Malaysia, the use of natural products as medicine has been well documented (Burkill, 1966). In the year 1935, Malaysia was reported to have about 12,000 species of flowering plants of which 1,300 were said to be medicinal. For centuries, the habit of consuming raw plants in daily diet, have become a norm. However, the type of plants and the preference of taste vary from one region to another. Malaysia is endowed with precious tropical rainforest heritage with abundance of flora and fauna, in addition to this, the multiracial citizenship existing has enormously contributed to valuable cultural and custom diversity (Ab. Karim *et al.*, 2011). These two important sources have led us to diverse utilization of Malaysia rainforest product, from a daily personal care, health care supplements (nutraceuticals), cosmetics, fragrances, and even in the pesticides industry.

1.1 Natural Products in Treating Inflammation and Pain

Ample of studies regarding natural products in treating inflammation and pain have been done. Some of the researches have well extended into the pharmaceuticals industry, and some remain in nutraceuticals. Even from the year 1829, Henri Leroux, a French pharmacist discovered salicin in willow plants *(Spiraeaulmaria)* which gave rise to the pain reliever by inhibiting the production of prostaglandins through arachidonic acid

pathway. It was however continuously studied and worked upon. In the year 1915, the first Aspirin tablet was made (as cited in Fuster & Sweeny, 2011).

Another perfect example of natural products as a source of drug is morphine. In 1804, Friedrich Sertürner from Paderbon, Germany has first discovered morphine as the first alkaloid from opium poppy plant (*Papaver somniferum).* Acting as phenanthrene opioid receptor agonist, it was first marketed to the general public in 1817 as analgesic drug (as cited in Ebadi, 2006).

Through the years, abundance of bioactive compound were discovered and developed into analgesic drugs. They may inhibit different enzymes or receptors in a certain pathways, they may give different side effects in different length of time, but they will ultimately lead to a common goal of controlling the sense of pain. Those tiny little pills and injections have actually given us a tremendous impact in our lives especially in improving the quality of life. Scientist have never stopped to find and to improve more and better drugs which possibly could give better inhibition by eliminating unnecessary side effects.

1.2 *Melicope ptelefolia* Champ ex Benth

Using the same concept – "Learn from the nature", a Malaysian traditional salad *Melicope ptelefolia* Champ ex Benth locally known as tenggek burung or pauh pauh has become a subject matter in this current study of treating inflammation and pain (Shuib *et*

al., 2010). It originates from Rutaceae family, which can be recognized from the aromatic or lime-like smell from the broken twigs, or crushed leaves .It is widely used among Malays and is taken as 'ulam' and eaten along with rice, as an appetizer (Abas *et al.*, 2010) *Melicope ptelefolia* have been consumed for some medicinal properties, especially in treating fever, rheumatism, treatment for wound and itches, as well as aphrodisiac for men (Ab. Karim *et al*, 2011; Sulaiman *et al*., 2010). Through some distinguished claims and reports, *Melicope ptelefolia* has been reported to exhibit inhibiting response towards few experiments on inflammation (Sulaiman *et al*., 2010; Abas *et al*., 2006). Thus, it will be extremely important to study the behaviour of this remarkable species of flora towards inflammation and pain model, as well as the receptors and enzyme involved in the inhibition response.

1.3 Justification of Study

The current study was carried out to provide details of pharmacological prove in order to establish *Melicope ptelefolia* ethanolic extract (MPEE) for its anti-nociceptive and anti-inflammatory properties by using animal models through oral administration. MPEE's potential in anti-nociceptive and anti-inflammatory activity was assessed in central and peripheral system and its potential mechanism of action in both pain and inflammation. Toxicological evidence also included in this study. Besides providing scientifically proves and evidence for traditional claims in treatment of pain and inflammation, this study might also be useful for establishment for *Melicope ptelefolia* commercial products.

1.4 Objectives

The objectives of the study were to:

i) Evaluate anti-nociceptivity and anti-inflammatory activity of *Melicope ptelefolia* ethanolic extract.

ii) Elucidate possible mechanism of action involved in anti-nociceptivity and anti-inflammatory action of *Melicope ptelefolia* ethanolic extract.

iii) Evaluate toxicity effect of *Melicope ptelefolia* ethanolic extract following 28 days (acute toxicity) of continuous administration.

1.4.1 Specific objectives:

i) Evaluation and screening of anti-nociceptivity activity by chemical and thermal – induced nociception.

ii) Evaluation of anti- inflammatory activity by paw edema experimental model and cotton pellet-induced granuloma test.

iii) Elucidation of possible mechanism involved in anti-nociceptivity action of *Melicope ptelefolia* ethanolic extract in glutamate and capsaicin – linked pathway.

iv) Elucidation of possible mechanism involved in anti-inflammatory action of *Melicope ptelefolia* ethanolic extract in histamine, serotonin, bradykinin, phospholipase A_2, arachidonic acid and prostaglandin – induced paw edema model.

v) Evaluation of *Melicope ptelefolia* ethanolic extract following 28 days (acute toxicity) of continuous administration by behavioral observation, hematological & biochemical analyses and histological evaluation.

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