UNIVERSITI PUTRA MALAYSIA

EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF ACMELLA ULIGINOSA (SW.) CASS. METHANOLIC CRUDE EXTRACT IN MICE

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FPSK(m) 2013 39

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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

EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF *ACMELLA ULIGINOSA* (SW.) CASS. METHANOLIC CRUDE EXTRACT IN MICE

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October 2013

Chairman: Prof. Mohd Roslan Sulaiman, PhD

Faculty: Medicine and Health Sciences

In Malaysia, there are various plants being used as a remedy to overcome many types of pain for centuries. However, the actual mechanisms and compounds of these medicinal plants against nociception are yet to be investigated. The present study examined the potential antinociceptive activity of *Acmella uliginosa* (Sw.) Cass. methanolic crude extract (MEAU) by using both chemicals and thermal models of nociception in mice. The antinociceptive activity of the extract was investigated using acetic acid-induced abdominal constriction test, formalin-induced paw licking test and hot plate test. Then the possible mechanisms of its antinociception through capsaicin, glutamatergic, opioidergic, dopaminergic, serotoninergic, noradrenergic, adenosinergic, nitric oxide-cGMP-PKC pathways and potassium channels systems were studied. Mice that were pretreated with the extract (100 mg/kg, p.o.) were also subjected to the rota-rod test to evaluate the possible non-specific sedative effects by using Ugo Basile, model 47600. Evaluation of acute and chronic toxicity of MEAU were also carried out to determine its safety in oral consumption. It was demonstrated in the present study that MEAU (p.o.) at doses of 3, 10, 30 and 100 mg/kg produced significant dose-dependent inhibition in acetic acid-induced abdominal constriction test, hot plate test, formalin-, capsaicin- and glutamate-induced paw licking test as compared to control. Furthermore, the antinociception caused by the MEAU (100 mg/kg, p.o.) in the acetic acid-induced abdominal constriction test was significantly attenuated by intraperitoneal (i.p.) treatment of mice with naloxone (opioid receptor antagonist, 5 mg/kg), pindolol (a 5-$HT_{1A/1B}$ receptor antagonist, 1 mg/kg) and WAY100635 (a 5-HT_{1A} receptor antagonist, 0.7 mg/kg). It is also worth to mention that MEAU had greatly reversed its antinociception in α_2-noradrenergic system (yohimbine, α_2-adrenoreceptor antagonist). At the same time, MEAU was found to inhibit pain in the acetic acid-induced abdominal constriction test through nitric oxide pathway by deactivating the L-arginine-NO-cGMP-PKC pathways as well as potassium channels. In contrast, MEAU neither participate in the attenuation of antinociception in the dopaminergic, adenosinergic nor noradrenergic (prazosin, α_1 receptor antagonist) systems. MEAU was not associated with non-specific effects such as muscle relaxation or sedation. In addition, MEAU at the dosage of 300 mg/kg (p.o.) did not cause occurrence of death or abnormal behaviour during the period of observation, Together, these results indicate that the methanolic crude extract of *A. uliginosa* (Sw.) Cass. produced dose-related antinociception in several models of chemical and thermal pain through mechanisms that involve an interaction with opioid system, serotoninergic system

(i.e., through 5-$HT_{1A/1B}$ and 5-HT_{1A} receptors), adrenergic system (i.e., through α_2 receptor), nitric oxide-cGMP-PKC pathways and potassium channels.

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

PENILAIAN AKTIVITI ANTINOSISEPTIF EKSTRAK KASAR METANOL *ACMELLA ULIGINOSA* (SW.) CASS PADA MENCIT

Oleh

ONG HUI MING

Oktober 2013

Pengerusi: Prof. Mohd Roslan Sulaiman, PhD

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Di Malaysia, terdapat pelbagai tumbuh-tumbuhan yang digunakan sebagai ubat untuk mengatasi pelbagai jenis kesakitan selama berabad-abad. Walau bagaimanapun, mekanisme sebenar sebatian tumbuh-tumbuhan tersebut terhadap kesakitan masih belum diselidik. Penyelidikan ini bertujuan untuk mengkaji aktiviti antinosiseptif ekstrak kasar metanol *Acmella uliginosa* (Sw.) Cass. (MEAU) pada mencit ICR. Kegiatan antinosiseptif ekstrak ini diuji dengan ujian penggeliatan perut mencit, ujian penjilatan tapak kaki mencit dan ujian plat panas. Selain itu, mekanisme antinosiseptif MEAU juga dikaji melalui model eksperimen capsaicin, glutamate, sistem opioid, sistem dopaminergik, sistem serotoninergik, sistem noradrenergik, sistem adenosinergik, sistem nitrik oksida-cGMP-PKC dan juga saluran K^+. Tikus yang dirawat dengan ekstrak kasar metanol *A. uliginosa* (Sw.) Cass. (100 mg/kg, po) turut diuji dengan model eksperimen rod berputar untuk menilai kesan tumbuhan ini ke atas sistem motor dengan menggunakan mesin Ugo Basile, model 47600. Ujian toksik MEAU juga dilakukan untuk menentukan keselamatan penggunaan tumbuhan ini. Kajian ini menunjukkan ekstrak *A. uliginosa* (Sw.) Cass. ini (p.o.) pada dos 3, 10, 30 dan 100 mg/kg telah menghasilkan penghambatan secara signifikan dalam ujian penggeliatan perut mencit, ujian penjilatan tapak kaki mencit, ujian plat panas, ujian capsaicin dan ujian glutamate. Selain itu, MEAU (100 mg/kg, p.o.) telah menggurangkan kesakitan secara signifikan apabila dicabar dengan naloxone, pindolol dan WAY100635. MEAU juga menunjukkan bahawa ia melibatkan sistem noradrenergik-α_2 dalam aktiviti antinosiseptifnya. Pada masa yang sama, MEAU juga didapati bahawa penghambatan kesakitannya melibatkan sistem L-arginina-NO-cGMP-PKC dan saluran K^+. Sebaliknya, MEAU tidak menggunakan sistem dopaminergik, adenosinergik dan noradrenergik-α_1. Penggunaan ekstrak metanol *A. uliginosa* (Sw.) Cass. ini juga tidak menjejaskan sistem motor. Selain itu, MEAU pada dos 300 mg/kg (p.o.) tidak menyebabkan kematian atau kelakuan tidak normal selama tempoh pemerhatian dalam ujian toksik. Sebagai kesimpulan, keputusan dari penyelidikan ini telah membuktikan bahawa ekstrak kasar metanol *A. uliginosa* (Sw.) Cass. dapat menghasilkan aktiviti antinosiseptif melalui mekanisme yang melibatkan sistem opioid, sistem serotoninergik (reseptor 5-$HT_{1A/1B}$ dan 5-HT_{1A}) dan sistem adrenergik (reseptor- α_2), sistem nitrik oksida-cGMP-PKC dan juga saluran K^+.

ACKNOWLEDGEMENTS

Praise to God Almighty for granting me grace and strength to persevere throughout my master study and to overcome all the challenges that I had gone through in the study.

First of all, I am very grateful to my supervisor, Prof. Dr. Mohd Roslan Sulaiman, for his unrelenting guidance, understanding, and support throughout this study. I truly thank him for giving me an opportunity to be his postgraduate student without any hesitation.

A million thanks to my co-supervisors, Prof. Dr. Daud Ali Israf, Prof. Dr. Nordin Lajis and Dr. Zainul Amiruddin Zakaria particularly for their kindness to provide me the invaluable advice and motivation. My greatest gratitude to Ms. Siti Nurulhuda Mastuki who had kindly carried out extraction of the plant for this study.

My sincere appreciation dedicates to the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for giving me the opportunity to carry out this project.

My special dedication to all the physiology labmates, especially Dr. Azam, Dr. Akira, Dr. Enoch, Anib, Mimi, Azyyati, Ming Tatt, Jac, Dilla, Ina, Izzati, Nasier and Yatie Shamsul for their constant companion, inspiration and assistance throughout the study. I must also thank the cell signalling labmates, Revathee, Ayien, Chau Ling, Choi Yi, Omar, Sally and Asma for their friendship and care towards me.

The deepest gratitude from my heart to Encik Ramli (animal house), Kak Yatie (physio lab), Kak Ngah (physio lab), Anas (physio lab), Ayien (cell signalling lab), Encik Zul (cell signalling lab), Kak Juita (histopath lab) and Encik Akhir (Euroscience) for their assistance and patience.

Last but not least, to my beloved Ong family, especially my dearest parents, thanks for their unwavering love, encouragement and comfort that helped me to accomplish my master study successfully. I must thank my dearest father in personal for being the most loyal supporter in my master study.

I certify that a Thesis Examination Committee has met on **4th October 2013** to conduct the final examination of **Ong Hui Ming** on her thesis entitled "**Evaluation of antinociceptive activity of *Acmella uliginosa* (Sw.) Cass. methanolic crude extract in mice**" 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.

Members of the Thesis Examination Committee were as follows:

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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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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

ONG HUI MING

Date: 4th October 2013

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LIST OF ABBREVIATIONS

MEAU	*Acmella uliginosa* (Sw.) Cass. methanolic crude extract
NO	Nitric oxide
cGMP	Cyclic Guanosine Monophosphate
PKC	Protein Kinase C
µg	Microgram
mg	Milligram
g	Gram
kg	Kilogram
ml	Milliliter
µl	Microliter
p.o.	Orally
i.p.	Intraperitoneally
s.c.	Subcutaneously
i. pl.	Intraplantarly
µg	Microgram
TRPV1	Transient receptor potential cation channel subfamily V member 1
Na^+	Sodium ion
K^+	Potassium ion
Ca^{2+}	Calcium ion
LC50	Lethal concentration, 50%
ppm	Parts per million
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
s	second (s)

min	Minute (s)
h	Hour (s)
mmol	Millimoles
µmol	Micromoles
PBS	Phosphate buffered saline
AST	Amino Transferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphate
ASA	Acetylsalicylic acid
ODQ	1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one
PMA	Phorbol 12-myristate 13-acetate
L-NAME	L-NG-Nitroarginine Methyl Ester
USA	United States of America
EDTA	Anticoagulant ethylenediaminetetraacetic acid
ATP	Adenosine-5'-triphosphate
rpm	Revolutions per minute
S.E.M.	Standard error of mean
p	P-value
ANOVA	Analysis of variance
H & E	Hematoxylin and eosin stain
NMDA	N-methyl-d-aspartic acid receptors
AC	Adenylyl cyclase
AR	Adrenoreceptor

CHAPTER 1

INTRODUCTION

Pain is the most common reason for any individual to seek for health care (Okuse, 2007). The number of people who require the treatment for pain from back disorders, degenerative joint diseases, rheumatologic conditions, visceral diseases and cancer is expected to increase as the population ages (Brookoff, 2000). Thus, relief of pain has always been the ultimate aim in medicines (Melzack et al., 1992). The International Association for the Study of Pain (IASP) defined pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage''(Merskey and Bugduk, 1994). Drugs with predominant pain-killing action are generally referred as analgesic or antinociceptive drugs and they can be categorised as narcotic and non-narcotic drugs (Eddy and May, 1973). Although some analgesic drugs like morphine still remains as the most effective narcotic analgesic over the years to treat severe and chronic pain (Lasagna, 1964), prolonged usage of these drugs may present a wide range of side effects while treating pain. In fact, continuous and heavy applications of these analgesic drugs have often led to severe health problems like gastropathy, kidney failure and liver damage. These adverse effects of the contemporary painkillers have accelerated the studies of searching for other antinociceptive compounds with equivalent effects yet limited side effects.

Finding healing powers in plants is an ancient idea (Cowan, 1999). Natural constituents from plants may give a new source of antinociceptive agents with possible novel mechanisms of action in antinociception. Plants have always been a rich source of biochemical compounds. Many of these biochemical compounds are useful drugs in themselves and others have been the basis for synthetic drugs. These herbal plants can be the potential antinociceptive drugs and they are totally natural. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Mothana and Lindequist, 2005). In Malaysia, there are various plants being used as a remedy to overcome many types of pain for centuries. However, the actual mechanisms and compounds of these medicinal plants against nociception are yet to be investigated.

Acmella uliginosa (Sw.) Cass. is one of the frequently used plants in Malaysia to treat pain. It is a perennial herbaceous plant belonging to the daisy family, Asteraceae, which is indigenous and widely distributed in the tropics and sub-tropics especially in the West Indies, Venezuela, Brazil, Africa, Indonesia and Malaysia (Pandey et al., 2007). In Peninsular Malaysia, it is popularly known as 'Subang Nenek' or 'Butang Baju Siti Fatimah'. It grows in abundance as a naturalized weed on open hillsides and the rocky shores of rivers. When consumed, its flowers and leaves have a pungent taste that accompanied by tingling and numbness. In Malaysia, *A. uliginosa* (Sw.) Cass. has been generally used as a traditional herbal medicine for its analgesic and antispasmodic properties. The use of the flowers of *A. uliginosa* (Sw.) Cass. in particular, are more common than other parts of the plant, and are widely used as a remedy for the relief of pain especially in mouth ulcers, toothache, sore throat and stomach ache. The flowers and/or the leaves are crushed and its paste

is topically applied to the affected areas caused by insect bites to alleviate itch, redness and swelling.

Objectives of study

The general objectives of this study were to evaluate:

1. The antinociceptive avtivity of *A. uliginosa* methanolic crude extracts (MEAU) in mice
2. The possible mechanisms of action involved in MEAU

The specific objectives of this study were to evaluate:

1. The peripheral and central antinociceptive activities of MEAU
2. The sedative effect of MEAU
3. The involvement of capsaicin system in MEAU's antinociceptive activity
4. The involvement of glutamatergic system in MEAU's antinociceptive activity
5. The involvement of opioid system in MEAU's antinociceptive activity
6. The involvement of dopaminergic system in MEAU's antinociceptive activity
7. The involvement of serotoninergic system in MEAU's antinociceptive activity
8. The involvement of noradrenergic system in MEAU's antinociceptive activity
9. The involvement of adenosinergic system in MEAU's antinociceptive activity
10. The involvement of nitric oxide-cGMP-PKC pathways in MEAU's antinociceptive activity
11. The involvement of potassium channels in MEAU's antinociceptive activity

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