UNIVERSITI PUTRA MALAYSIA

ANTINOCICEPTIVE ACTIVITY OF HARUAN *(Channa striatus)* TRADITIONAL EXTRACT

ZULEEN DELINA FASYA BINTI ABDUL GHANI

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ANTINOCICEPTIVE ACTIVITY OF HARUAN *(Channa striatus)* TRADITIONAL EXTRACT

By

ZULEEN DELINA FASYA BINTI ABDUL GHANI

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

January 2010

DEDICATION

This thesis is dedicated to all of the following people who have inspired me in a very special way of my life:

My self – To try the best I can do.

Mak and Ayah – For your eternal love and supports.

Along and Adik – For reminding me not to give up.

My family and friends – For being there.

My supervisor and co-supervisor – For trusting me.

"Patience, persistence and perspiration make an unbeatable combination for success" – Napoleon Hill (1883-1970)

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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Chair: Professor Dr. Abdul Manan Mat Jais

Faculty: Medicine and Health Sciences

The objectives of this study were to determine the amino acid and fatty acid composition of Haruan Traditional Extract (HTE), to elucidate the mechanism responsible for its antinociceptive activity and to clarify the relationship between the presence of amino acids and fatty acids with the expected activity. The HTE was obtained by steaming fresh fillet of *Channa striatus* with pressure cooker at 100^{o}C, for 3 hrs after 1 hr of seasoning process. For the antinociceptive activities of HTE at different concentration, the extracts were purified, filtered and prepared in concentrations of 100 (stock solution), 50 and 10% dilution in distilled water and subjected to the antinociceptive studies. The antinociceptive activities of HTE was done on different

molecular weight, where some of the extract was subjected to the centrifugation-filtration process with Ultraspeed Centrifuge for 7k G in 3 hr using Vivaspin Polythersulfone Membrane with different pore size (3k, 5k, 10k and 30k NMWL) while part of it would be kept in non-filtered conditions / stock solution prior to the antinociceptive assays. Lastly, for the elucidation of the involvement of opioid receptor in HTE antinociceptive activities, the antinociceptive action of the extract with the highest antinociceptive activity resulted from the prior study were tested against naloxone in all antinociceptive assays in a bid to further elucidate probable mechanisms of antinociception. In all of the above-mentioned studies were involved the use of 0.6% acetic acid-induced abdominal constriction, 5% formalin-induced paw-licking, and 50^{o}C hot plate test in rodent to evaluate the HTE antinociceptive activities. For the determination of amino acid and fatty acid the stock solution HTE was run into the High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), respectively. All data obtained were analyzed using the One-Way Analysis of Variance (ANOVA) followed by Dunnet test with $P<0.05$ as the limit of significance.

From the data obtained, the HTE was found to contain major amino acid cysteine, histidine, glutamic acid, glycine and aspartic acid. In addition, the HTE was found to have a high myristic acid followed by palmitic, arachidonic, linolenic and oleic acid. The HTE exhibited concentration/dosage-dependent manner significant antinociceptive activity ($P<0.05$). On the other hand, at different molecular, HTE showed the size-dependent in abdominal constriction, hot plate and first phase of formalin test. HTE also were showed

to have an involvement of central antinociceptive mechanism when tested against the naloxone.

As a conclusion, the HTE contains all the important amino acids and fatty acids and possesses the antinociceptive activities. Thus, proved the traditional claimed for reducing pain, inflammation and promote wound healing properties.

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

AKTIVITI ANTINOSISEPTIF TERHADAP EKSTRAK TRADITIONAL HARUAN *(Channa striatus)*

Oleh

ZULEEN DELINA FASYA BINTI ABDUL GHANI

Januari 2010

Pengerusi: Professor Dr. Abdul Manan Mat Jais

Fakulti: Perubatan dan Sains Kesihatan

Objektif penyelidikan ini adalah untuk menentukan komposisi asid amino dan asid lemak Ekstrak Tradisional Haruan (HTE) bagi mengenalpasti mekanisma yang melibatkan aktiviti antinosiseptif serta menjelaskan hubungkait di antara kehadiran asid amino dan asid lemak dengan aktiviti antinosiseptive yang dijangkakan. HTE diperoleh melalui proses pengukusan isi ikan *Channa striatus* (haruan) segar dengan menggunakan periuk tekanan pada suhu 100^{o}C selama 3 jam, selepas proses perapan selama 1 jam. Ketika aktiviti antinosiseptif HTE dalam berlainan kepekatan, ekstrak perlu dibersihkan, disaring dan disediakan dalam kepekatan 100 (larutan stok), 50, 10% dengan dilarutkan menggunakan air suling sebagai bahan untuk kajian

antinosiseptif. Untuk aktiviti antinosiseptif HTE dalam berlainan berat molekul, sebahagian daripada ekstrak HTE akan melalui proses emparan – saringan dengan menggunakan pengempar berkelajuan Ultra (7k G) selama 3 jam bersama-sama membran Vivaspin Polythersulfone yang mempunyai pelbagai saiz liang (3k, 5k, 10k dan 30k NMWL). Manakala sebahagian lagi akan dibiarkan dalam keadaan asal iaitu tanpa saringan / larutan tulen untuk analisa antinosiseptif. Akhir sekali, untuk mengenalpasti kehadiran reseptor opioid dalam aktiviti antinosiseptif HTE, kesan ekstrak antinosiseptif dengan keputusan yang tertinggi daripada kajian terdahulu akan diuji bersama-sama naloxone dalam semua ujian antinosiseptif bagi menentukan kebarangkalian mekanisma antinosiseptif. Dalam semua ujian yang disebutkan di atas, sebanyak 0.6% asid asetik digunakan untuk merangsang pencerutan abdomen, 5% formalin bagi merangsang haiwan untuk menjilat tapak kaki dan ujian 50^{o}C kepanasan piring terhadap tikus digunakan bagi menentukan aktiviti antinosiseptif HTE. Untuk penentuan asid amino dan asid lemak, larutan stok / 100% HTE akan dianalisa dengan menggunakan High Performance Liquid Chromatography (HPLC) dan Gas Chromatography (GC). Kesemua data yang diperolehi akan dianalisis dengan menggunakan kaedah One-Way ANOVA diikuti ujian Dunnet dengan limit signifikasi $P<0.05$.

Daripada data yang diperolehi, HTE telah dikenalpasti mengandungi asid amino dengan komponen majornya adalah terdiri daripada asid sistin, histidin, glutamik, glisin dan asid aspartik. Selain itu, HTE juga dikenalpasti mengandungi kandungan asid myristic yang tinggi, diikuti asid palmitik,

arakidonik, linolenik, dan oleik. HTE menunjukkan pergantungan dos aktiviti dengan signifikasi ($P<0.05$). Ketika kajian aktiviti antinosiseptif HTE dalam berlainan saiz tapisan, keputusan menunjukkan bahawa saiz dipengaruhi oleh pencerutan abdomen, piring panas serta fasa pertama ujian Formalin. HTE juga menunjukkan penglibatan mekanisma pusat antisiseptif apabila diuji dengan naloxone.

Kesimpulannya, HTE mengandungi semua asid amino serta sebahagian asid lemak yang penting dan mempunyai aktiviti antinosiseptif. Oleh itu, dibuktikan bahawa secara tradisional untuk mengurangkan kesakitan, keradangan serta pemulihan kecederaan.

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All gratification is referred to ALLAH S.W.T."

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I certify that a Thesis Examination Committee has met on 6 January 2010 to conduct the final examination of Zuleen Delina Fasya Binti Abdul Ghani on her thesis entitled "Antinociceptive Activity of Haruan *(Channa striatus)* Traditional Extract" 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 degree of Master of Science.

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Date: 12 February 2010

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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Date: 17 March 2010

DECLARATION

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

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ZULEEN DELINA FASYA BINTI ABDUL GHANI

Date: 16 March 2010

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