



UNIVERSITI PUTRA MALAYSIA

**ANTI-INFLAMMATORY AND ANTINOCICEPTIVE PROPERTIES
OF THREE LOCAL CHANNA SPECIES CRUDE EXTRACTS**

SOLIAH BT MOHD HASSAN

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**ANTI-INFLAMMATORY AND ANTINOCICEPTIVE PROPERTIES OF
THREE LOCAL *CHANNA* SPECIES CRUDE EXTRACTS**

By

SOLIAH BT MOHD HASSAN

**Thesis Submitted to the School of Graduates Studies, Universiti Putra Malaysia, In
Fulfilment of the Requirements for the Degree of Master of Science**

February 2005



DEDICATION

“Dedicated to my Supervisor Associate Professor Dr. Muhammad Nazrul Hakim Abdullah, my husband (Zolkornain), my father (Mohd Hassan), my mother (Noriah), my mother-in-law (Samsiah), my brother (Zainol Abidin), and my sisters (Zainab, Rodiah, Rokhiah and Siti Rahmah).”



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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Chairman : Associate Professor Muhammad Nazrul Hakim bin Abdullah, PhD

Faculty : Medicine and Health Sciences

Water extract and aqueous portion of chloroform:methanol extract of *Channa striatus* (haruan), *Channa micropeltes* (toman) and *Channa lucius* (bujuk) were tested for its anti-inflammatory and antinociceptive properties. The anti-inflammatory activity of the extracts was investigated by three chemical mediators induced rat paw edema (acute inflammation) and cotton pellet granuloma in rats (chronic inflammatory modulation testing). The antinociceptive or analgesic properties were investigated by abdominal constriction test and hot plate test. The results showed that the percentage edema inhibition increased with increased in time interval and was found to be maximal at 4 hours after prostaglandin D₂ injection. The effects of 60 mg/kg body weight aqueous portion of chloroform:methanol extract of *Channa lucius* and *Channa micropeltes*, piroxicam (10 mg/kg) and water extract of *Channa striatus* (60 mg/kg) exhibited 85.4%, 79.8%, 84.3% and 78.7% edema inhibition at 4 hours respectively. However, all three local *Channa spp.* extracts did not show significant inhibition ($p > 0.05$) on histamine



and bradykinin induced paw edema. In the cotton pellet induced granuloma, the percentage inhibition of anti-transudative was significantly different ($p < 0.05$) by the water extract of *Channa striatus* (38.1%) and *Channa micropeltes* (30.3%) compared to the water extract of *Channa lucius* (10.8%) in inhibiting wet weight of the cotton pellet-induced granuloma formation in rats. Piroxicam showed significant inhibition ($p < 0.05$) by 30.92% and mefenamic acid (13.28%) in inhibiting wet weight granuloma. The water extract of *Channa striatus* and *Channa micropeltes* exhibited anti-transudative activity but *Channa lucius* was devoid of this property. Both extracts of three local *Channa spp.* did not show anti-proliferative activity. The aqueous portion of chloroform:methanol extract of *Channa striatus* (60 mg/kg) and mefenamic acid (10 mg/kg) increased the serum albumin levels at 24.83 ± 4.09 g/dL and 24.83 ± 4.30 g/dL respectively. The water extract of *Channa striatus* (30 mg/kg) and the aqueous portion of chloroform:methanol extract *Channa lucius* (15 mg/kg) produced antinociceptive effect on acetic acid induced abdominal constriction test. However both extracts of three local *Channa spp.* failed to exhibit antinociceptive effect on hot plate test. As a reference drug, the administration of morphine (0.6 mg/kg) caused significant inhibition ($p < 0.05$) by 99.3% in acetic acid induced abdominal constriction and 5 mg/kg of morphine produced significant increased the latency time in hot plate test when compared to control and both extracts of three local *Channa spp.* In conclusion, this study showed that *Channa striatus* extracts has more potent the anti-inflammatory and antinociceptive activities compared to the other closely related snakehead fish *Channa micropeltes* and *Channa lucius*. The extracts from *Channa striatus* have the potential being the anti-inflammatory and antinociceptive agents.



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

**CIRI-CIRI ANTI-KERADANGAN DAN ANTINOSISEPTIF EKSTRAK KASAR
TIGA SPESIS *CHANNA* TEMPATAN**

Oleh

SOLIHAH BT MOHD HASSAN

Februari 2005

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Ekstrak air dan bahagian akues ekstrak kloroform:methanol *Channa striatus* (haruan), *Channa micropeltes* (toman) dan *Channa lucius* (bujuk) telah dikaji ke atas ciri-ciri anti-keradangan dan antinosiseptif. Aktiviti anti-keradangan tiga spesis *Channa* tempatan dilakukan ke atas tapak kaki tikus yang diaruh edema/bengkak dengan menggunakan tiga pengantara bahan kimia (keradangan akut) dan tikus yang diaruh granuloma pada badan tikus dengan menggunakan gentelan/gumpalan kapas. Ciri-ciri antinosiseptif atau analgesik telah dikaji dengan ujian pencerutan abdomen dan ujian plate panas. Keputusan menunjukkan bahawa, peratus perencatan edema meningkat dengan meningkatnya selang masa dan telah ditunjukkan nilai maksima selepas 4 jam disuntik prostaglandin D₂. Kesan daripada 60 mg/kg berat badan bahagian akues ekstrak kloroform:methanol *Channa lucius* dan *Channa micropeltes*, piroksikam (10 mg/kg), dan ekstrak air *Channa striatus* (60 mg/kg) mempamerkan 85.4%, 79.8%, 84.3% 78.7%

perencatan edema masing-masing selepas 4 jam. Kedua-dua ekstrak tiga spesis *Channa* tempatan tidak menunjukkan perencatan yang signifikan ($p > 0.05$) ke atas histamine dan bradikinin teraruh edema pada tapak kaki. Dalam ujian gumpalan kapas mengaruh granuloma menunjukkan perbezaan signifikan ($p < 0.05$) pada peratus perencatan aktiviti anti-transudatif ekstrak air *Channa striatus* (38.1%) dan *Channa micropeltes* (30.3%) dibandingkan dengan ekstrak air *Channa lucius* (10.8%) dalam merencat berat basah pada gumpalan kapas bergranuloma yang terbentuk dalam tikus. Piroksikam menunjukkan perencatan yang signifikan ($p < 0.05$) sebanyak 30.9% berbanding asid mefenamik (13.3%). Ekstrak air *Channa striatus* dan *Channa micropeltes* mempamerkan aktiviti anti-transudatif tetapi ekstrak air *Channa lucius* tidak menunjukkan aktiviti anti-transudatif. Kedua-dua ekstrak tiga spesis *Channa* tidak menunjukkan sebarang aktiviti anti-proliferatif. Bahagian akues ekstrak kloroform:methanol *Channa striatus* (60 mg/kg) dan asid mefenamik (10 mg/kg) meningkatkan paras serum albumin kepada 24.83 ± 4.09 g/dL dan 24.83 ± 4.30 g/dL masing-masing. Ekstrak air *Channa striatus* (30 mg/kg) dan bahagian akues ekstrak kloroform:methanol *Channa lucius* (15 mg/kg) menghasilkan kesan antinosiseptif ke atas ujian asid asetik teraruh pencerutan abdomen. Kedua-dua ekstrak daripada tiga spesis *Channa* gagal untuk mempamerkan kesan antinosiseptif ke atas ujian plate panas. Sebagai dadah rujukan, pemberian morfin (0.6 mg/kg) memberikan peratus yang signifikan ($p < 0.05$) sebanyak 99.3% dalam asid asetik teraruh pencerutan abdomen dan 5 mg/kg morfin meningkatkan masa latensi ke atas ujian plat panas dibandingkan dengan kumpulan kawalan dan kedua-dua ekstrak daripada tiga spesis *Channa*.

Kesimpulannya, *Channa striatus* telah menunjukkan ciri-ciri anti-keradangan dan antinosiseptif yang lebih poten dibandingkan dengan *Channa micropeltes* dan *Channa lucius*. Ekstrak-ekstrak daripada *Channa striatus* berpotensi sebagai agen anti-keradangan dan antinosiseptif.

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I certify that an Examination Committee met on 8th February 2005 to conduct the final examination of Solihah Mohd Hassan on her Master of Science thesis entitled “Anti-Inflammatory and Antinociceptive Properties of Three Local *C'hanna* species Crude Extracts” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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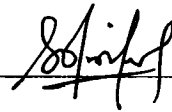
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



SOLIHAH MOHD HASSAN

Date: 22 JUNE 2005

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AD	Anno Domini
BC	Before Christ
CNS	Central Nervous System
COX	Cyclooxygenase
DHA	Docosahexanoic acid
EPA	Eicosapentanoic acid
HMWK	High Molecular Weight Kininogen
IASP	International Association for Study of Pain
IL-1	Interleukin-1
IL-6	Interleukin-6
IgE	Immunoglobulin E
LC	Locus ceruleus
NMDA	N-metyl-D-aspartate
NSAIDs	Non steroidal Anti inflammatory Drugs
PAGM	Periaqueductal gray matter
PGD ₂	Prostaglandin D ₂
PUFA	Polyunsaturated fatty acid
SPSS	Statistic Purposes for Social Science
TNF α	Tumour Necrosis Factor alfa
C:M	ratio of chloroform:methanol
μ	mu

$^{\circ}\text{C}$	degree celcius
e.g.	example gratis
etc	et cetra
i.e.	id est
i.p	intraperitoneal
mm	milimetre
kg	kilogram
mg	milligram
ml	mililitre
s.c	subcutaneous
β	beta
δ	delta
κ	kappa
σ	sigma
$\omega-3$	omega 3

CHAPTER 1

INTRODUCTION

Acute and chronic inflammatory diseases associated with pain are among the most common health problems in the world (Buyukokuroglu, 2002). Inflammatory diseases such as rheumatoid arthritis, cardiovascular diseases, asthma, osteoarthritis, allergic and non allergic rhinitis have been recently recognized as inflammatory in nature (Challem, 2003).

Steroid or nonsteroidal anti-inflammatory drugs are used for treatment of various inflammatory diseases. Though these drugs have potent activity, they have various and severe adverse effects such as diarrhea, nausea, fluid retention, gastrointestinal ulcer and bleeding. Therefore, agents of natural origin with very little side effects are required as substitute chemical therapeutic (Verpoorte, 1999). Since the available analgesic drugs exert a wide range of side effects and either too potent or too weak, the search for new analgesic compounds has been a priority to pharmacologist and pharmaceutical industries.

Many medicinal plants provide relief symptoms comparable to that obtained from allopathic medicines. Beside the medicinal plants, animal derived products are common



use in traditional remedies such as fish oil. Fish oil is widely consumed as health supplement among person with cardiovascular heart diseases, reduce cancer risk, asthma and inflammatory bowel disease (Challem, 2003).

Fish and fish oil fatty acid are currently under intense scientific investigation because of numerous health benefits attributed to them. A lot of investigation on fatty acid composition has been done on fatty acid composition of some Malaysian freshwater fish conducted by Suriah *et al.*, 1995 and fatty acid composition and cholesterol content of selected marine fish in Malaysian waters conducted by Osman *et al.*, 2001. Researchers have shown that freshwater fish generally contain lower proportions of ω -3 (n-3) polyunsaturated fatty acids (PUFA) than marine fish (Mohsen, 1985; Vlieg and Body, 1998). According to Leaf and Weber (1988), eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), which were found only in fish and other seafood, possess extremely beneficial properties for the prevention of human coronary artery disease.

Freshwater fish as a natural source have a high economic, nutritional and pharmaceuticals values. Because of these values, the researchers were attracted to investigate the medicinal benefit of freshwater fish. Since the Fishery Department in Malaysia is now encouraging and expanding the freshwater fish industry among agriculturist and fishermen to increase their income (Karim, 1990).

In the previous study, haruan or *Channa striatus* has been studied extensively and has been reported to possess antinociceptive, anti-eczema (Mat Jais *et al.*, 1997) and wound healing properties (Mat Jais *et al.*, 1994; Baie and Sheikh 2000). In Malaysia, *Channa striatus* is consumed as a remedy to help promoting healing after surgical intervention, childbirth or trauma (Mat Jais *et al.*, 1998). However, no previous study has been done for pharmacological benefits of the others two closely related snakehead fish *Channa micropeltes* (toman) and *Chana lucius* (bujuk).

Extract having antinociceptive or analgesic and wound healing properties may possess anti-inflammatory activity. The current study was carried out to evaluate and compare the properties of three snakehead fish extracts in anti-inflammatory and antinociceptive activities.

Objectives of The Study

- 1) To investigate the properties/effects of three local *Channa spp.* extracts: *Channa striatus*, *Channa micropeltes* and *Channa lucius* in modulating acute and chronic inflammation.
- 2) To investigate the antinociceptive properties of three local *Channa spp.* extracts: *Channa striatus*, *Channa micropeltes* and *Channa lucius*.

CHAPTER 2

LITERATURE REVIEW

2.1 Local *Channa spp.*

Channa spp. is snakehead fish indigenous to many tropical and subtropical countries including Malaysia (Mohsin and Ambak, 1983). It belongs to family Channidae. It is freshwater, air breather and carnivorous fish widely consumed in Malaysia and other Southeast Asian countries. There are many species of *Channa* such as *Channa striatus* (haruan), *Channa micropeltes* (toman) and *Channa lucius* (bujuk). Among the difference species belonging to Channidae family, *Channa striatus* largely used in traditional remedies and considered by most Malaysian as a good source of health food.

2.2 Description of *Channa spp.*

2.2.1 *Channa striatus*

Channa striatus (stripped snakehead fish) (Figure 1) is known as 'Haruan' among the locals in Malaysia. In other names, haruan is called 'Sang Yee', 'Aruan' and 'Toman paya'. It is found in rivers, lakes, swamps, paddy field, mining pools and in road side ditches. It had elongated body and head depressed. The length of haruan is 45 cm. It is carnivorous and feed on prawns, frog, worms and all kind of fish (Mohsin and Ambak, 1983).

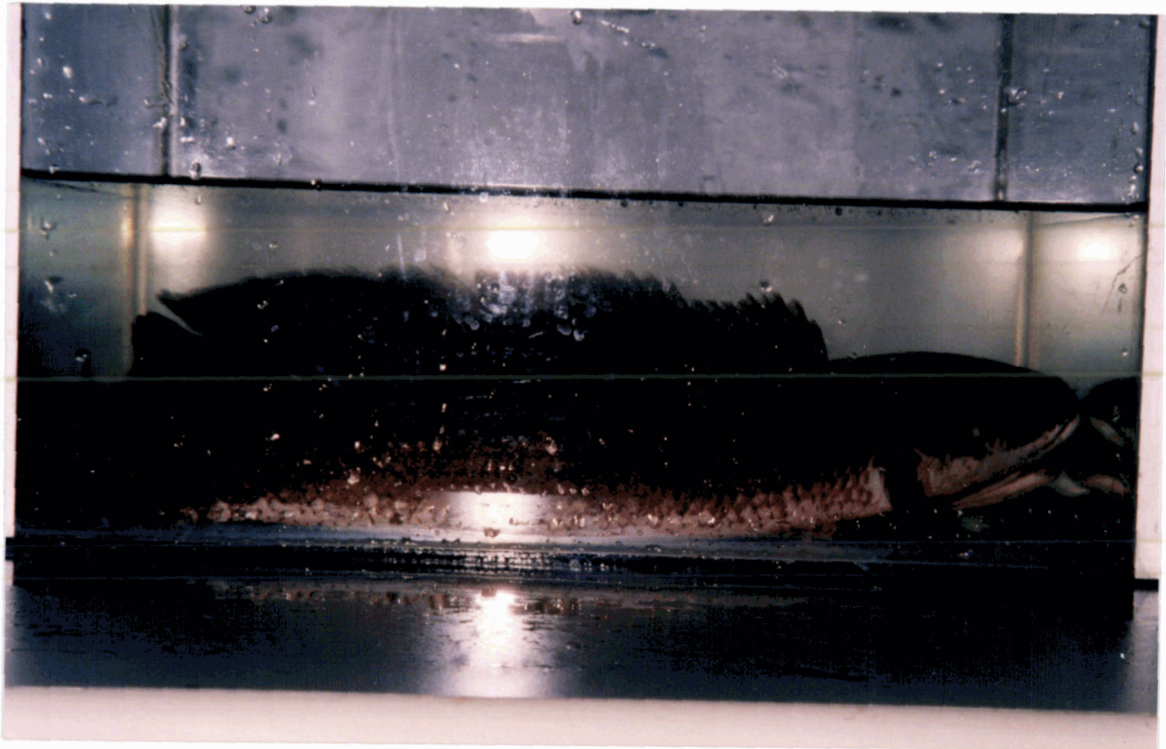


Figure 1: *Channa striatus* (Haruan)