



UNIVERSITI PUTRA MALAYSIA

PHARMACOLOGICAL PROPERTIES OF *CHANNA SPP.* EXTRACTS

AZLINA BINTI MOHD. JOHARI

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PHARMACOLOGICAL PROPERTIES OF *CHANNA SPP.* EXTRACTS

By

AZLINA BINTI MOHD. JOHARI

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

**ANTI-INFLAMMATORY, ANTINOCICEPTIVE AND ANTIPYRETIC
EFFECTS OF EXTRACTS FROM SNAKEHEAD FISH
(*Channa striatus* AND *C. lucius*)**

By

AZLINA BINTI MOHD. JOHARI

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Chairman: Associate Professor Arifah Abdul Kadir, PhD

Faculty : Veterinary Medicine

Channa spp. is a snakehead fish and widely consumed in Malaysia. A study was conducted to determine the effect of *Channa striatus* and *Channa lucius* extracts on carrageenan-induced synovitis in rabbits. The antinociceptive and antipyretic properties of both *Channa spp.* were also investigated in mice. The extracts of *C. striatus* and *C. lucius* were prepared as water and aqueous portion of chloroform: methanol extracts, respectively. Sixteen rabbits were randomly assigned into four groups. Each group of rabbits was treated orally 30 minutes before the induction of inflammation with *C. striatus* (60 mg/kg), *C. lucius* (60 mg/kg), ketoprofen (3 mg/kg) and saline solution (control), respectively. The right stifle joint was intra-articularly injected with 0.5 mL of 1% carrageenan. Whole blood was taken for serum thromboxane (TxB₂) assay before and at 1, 3, 6, 9, 12 and 24 hour (h) after the induction of inflammation. Synovial fluid and synovial membrane were collected during post-mortem for analysis and histopathology. The results indicated that TxB₂ synthesis was significantly ($p < 0.05$) inhibited for 12 h after oral dosing of ketoprofen in rabbits. Serum TxB₂ for *C. striatus* was lower than that of the control group at 1, 3, 6 and 12 h. As for *C. lucius*, TxB₂

synthesis was inhibited at 9 and 12 h. However, the inhibition of TxB₂ for both *Channa spp.* was small and not significant as compared to the control group. As for *C. striatus* and ketoprofen treated groups, the total white blood cell (WBC) count was reduced compared to the control group but was not significant different. Histopathological results indicated a mild infiltration of leucocytes in the synovial membrane of ketoprofen treated rabbits. However in the control, *C. striatus* and *C. lucius* treated groups showed massive leucocyte infiltration, congestion in the blood vessels and fibrin exudation. As for the analgesic activity of *Channa spp.*, twenty four mice were allocated equally into three treatment groups and one control group. The extracts of *C. striatus* (60 mg/kg), *C. lucius* (60 mg/kg) or ketoprofen (1 mg/kg) was administered intraperitoneally, 30 minutes before injection of acetic acid. Both extracts of the local *Channa spp.* and ketoprofen showed significant ($p < 0.05$) reductions in the number of abdominal constriction and hind limb stretching as compared to the control group. As for the antipyretic effect of the *Channa spp.* extracts, twenty four mice were equally divided into three treatment groups and one control group. Mice were injected with 30% (w/v) suspension of yeast in saline at the dosage of 10 mL/kg subcutaneously. The temperature was recorded 18 hour before and measured every half an hour for 5 hours after dosing. *C. striatus* (60 mg/kg) and *C. lucius* (60 mg/kg) reduced hyperthermia significantly ($p < 0.05$) at 2.5 to 5 h and 3 to 4 h, respectively. Ketoprofen (1 mg/kg) caused significant inhibition ($p < 0.05$) at 0.5, 1, 1.5, 2, 3 and 3.5 h after dosing. In summary, *C. striatus* and *C. lucius* extracts may possess antinociceptive and antipyretic in mice but at 60 mg/kg, both fish extracts did not produce anti-inflammatory activity in rabbits.

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

**CIRI-CIRI ANTI-KERADANGAN, ANTINOSISEPTIF DAN ANTIPIRETIK
DARIPADA EKSTRAK IKAN YANG KEPALANYA MENYERUPAI ULAR
(*Channa striatus* DAN *C. lucius*)**

Oleh

AZLINA BINTI MOHD. JOHARI

Mac 2008

Pengerusi : Profesor Madya Arifah Abdul Kadir, PhD

Fakulti : Perubatan Veterinar

Channa spp. adalah ikan yang kepalanya menyerupai ular dan secara amnya dimakan oleh masyarakat Malaysia. Satu kajian telah dijalankan untuk menentukan kesan ekstrak *Channa striatus* dan *Channa lucius* terhadap keradangan sinovial akibat karraginan pada arnab. Ciri-ciri antinosiseptif dan antipiretik daripada kedua-dua *Channa spp.* juga dikaji pada mencit. *Channa striatus* disediakan melalui pengekstrakan air manakala *C. lucius* melalui pengekstrakan bahagian akues menggunakan kloroform: metanol. Enam belas ekor arnab dibahagikan secara rawak kepada empat buah kumpulan. Setiap kumpulan arnab diberikan rawatan secara oral, iaitu *C. striatus* (60 mg/kg), *C. lucius* (60 mg/kg), ketoprofen (3 mg/kg) dan larutan saline (kawalan), masing-masing, 30 minit sebelum induksi keradangan dilakukan. Lutut kanan telah disuntik dengan 1% karraginan sebanyak 0.5 mL secara intra-rawan. Darah telah diambil untuk esei thromboxane B₂ (TxB₂) sebelum dan pada 1, 3, 6, 9, 12 dan 24 jam selepas induksi keradangan. Cecair sinovial dan membran sinovial



telah diambil ketika bedah siasat untuk analisis dan histopatologi. Keputusan menunjukkan penghasilan serum TxB_2 yang signifikan ($p < 0.05$) telah direncat selama 12 jam selepas ketoprofen diberikan secara oral kepada arnab. Perencatan penghasilan serum TxB_2 daripada *C. striatus* pula adalah lebih rendah daripada kumpulan kawalan pada jam 1, 3, 6 dan 12. Untuk *C. lucius* pula, perencatan penghasilan TxB_2 adalah pada jam 9 dan 12. Walaupun begitu, perencatan TxB_2 yang dihasilkan oleh kedua-dua *Channa spp.* adalah kecil dan tidak signifikan jika dibandingkan dengan kumpulan kawalan. Untuk kumpulan rawatan *C. striatus* dan ketoprofen, jumlah kiraan sel darah putih adalah lebih rendah berbanding dengan kumpulan kawalan tetapi tidak signifikan. Keputusan histopatologi bagi kumpulan rawatan yang telah diberikan ketoprofen menunjukkan sedikit penyebaran leukosit di dalam membrane sinovial. Walaupun begitu, pada kumpulan kawalan, *C. striatus* dan *C. lucius* dapat diperhatikan penghasilan leukosit yang banyak, kesesakan oleh sel-sel darah pada saluran darah dan fibrin. Untuk aktiviti analgesik yang dihasilkan oleh *Channa spp.*, dua puluh empat ekor mencit diagihkan sama rata kepada tiga kumpulan rawatan dan satu kumpulan kawalan. *Channa striatus* (60mg/kg), *C. lucius* (60mg/kg) dan ketoprofen (1 mg/kg) telah disuntik secara intra-peritoneal, 30 minit sebelum disuntik dengan asid asetik. Kedua-dua jenis *Channa spp.* tempatan dan ketoprofen menunjukkan penurunan yang signifikan ($p < 0.05$) kepada jumlah pencerutan abdomen dan keregangan dikedua-dua belah kaki belakang jika dibandingkan dengan kumpulan kawalan. Untuk aktiviti antipiretik yang dihasilkan oleh *Channa spp.*, dua puluh empat ekor mencit telah dibahagikan kepada tiga kumpulan rawatan dan satu kumpulan kawalan.

Mencit telah disuntik dengan 30% (w/v) larutan ragi sebanyak 10 mL/kg subkutis. Suhu telah direkodkan 18 jam sebelum dan setiap setengah jam selama lima jam selepas rawatan diberikan. *Channa striatus* (60 mg/kg) dan *C. lucius* (60 mg/kg) masing-masing telah dapat menurunkan suhu yang tinggi, secara signifikan ($p < 0.05$) pada jam ke 2.5 hingga jam ke 5 dan jam ke 3 hingga jam ke 4. Ketoprofen (1 mg/kg) menyebabkan perencatan secara signifikan ($p < 0.05$) pada jam 0.5, 1, 1.5, 2, 3 dan jam ke 3.5 selepas diberikan rawatan. Kesimpulannya, *C. striatus* dan *C. lucius* ekstrak telah menunjukkan ciri-ciri antinosiseptif dan antipiretik ke atas mencit tetapi pada 60mg/kg, kedua-dua jenis ikan tidak menunjukkan ciri-ciri anti-keradangan ke atas arnab.

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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:

ARIFAH ABDUL KADIR, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

MUHAMMAD NAZRUL HAKIM ABDULLAH, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

MOHD ZAMRI SAAD, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 August 2008



I certify that an Examination Committee has met on 14th March 2008 to conduct the final examination of Azlina Haji Mohd Johari on her Master of Science thesis entitled “Anti-Inflammatory, Antinociceptive and Antipyretic Effects of Extracts from Snakehead Fish (*Channa striatus* and *C. lucius*)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Mohamed Ali Rajion, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Zuraini Ahmad, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Roslida Abd. Hamid @ Abd. Razak, PhD

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Chung Lip Yong, PhD

Associate Professor
Department of Pharmacy
Faculty of Medicine
Universiti Malaya
(External Examiner)

HASANAH MOHD. GHAZALI, PhD

Professor and Deputy Dean
School and Graduate Studies
Universiti Putra Malaysia

Date:



DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledge. 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.

AZLINA MOHD JOHARI

Date: 13 July 2008



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LIST OF ABBREVIATIONS

°C	degree Celcius
AA	arachidonic acid
ANOVA	one-way analysis of variance
B ₀	blank
CNS	central nervous system
COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
CPM	count per minute
DHA	docosahexaenoic acid
DHETs	dihydroxyeicosatrienoic acids
ED ₂₀	dose of a drug required to produce 20% of the drug's maximal effect.
ED ₅₀	dose of a drug required to produce 50% of the drug's maximal effect.
ED ₈₀	dose of a drug required to produce 80% of the drug's maximal effect.
EETs	epoxyeicosatrienoic acids
EP	prostaglandin receptor
EPA	eicosapentaenoic acid
h	hour
HETEs	monohydroxy eicosatetraenoic acids



IL-1	interleukin-1
IL-6	interleukin-6
IP	prostacyclin receptor
LPS	lipopolysaccharide
mPGES-1	microsomal PGE synthase-1
NOS	nitric oxide synthase
NSAIDs	non-steroidal anti-inflammatory drugs
NSB	non specific binding
PGD ₂	prostaglandin D ₂
PGE ₂	prostaglandin E ₂
PGF _{2α}	prostaglandin F _{2α}
PGG ₂	prostaglandin G ₂
PGH ₂	prostaglandin H ₂
PGI ₂	prostacyclin
PKA	protein kinase A
PKC	protein kinase C
PMN	polymorphonuclear leucocytes
POPOP	[1,4-di-(2-(5-[phenyloxazoly benzene])
PPO	2,5-diphenyloxazole
PUFA	polyunsaturated fatty acid
QC-H	quality control at high concentration

QC-L	quality control at low concentration
RIA	radioimmunoassay
S.D	standard deviation
S.E.M	standard error mean
TC	total count
TNF	tumour necrosis factor
TxA ₂	thromboxane A ₂
TxB ₂	thromboxane B ₂
v	volume
v/v	volume/volume
VCAM-1	vascular adhesion molecule-1
w	weight
w/v	weight/volume
WBC	white blood cell
β	beta
μl	microlitre

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CHAPTER 1

INTRODUCTION

Inflammatory diseases which are associated with pain such as arthritis are among the most common health problem in the western countries (Horrocks and Yeo, 1999). In Malaysia, two million people have problems associated with synovitis and later it developed into arthritis, where 600,000 people are more than 60 years old (Najibah, 2002). Inflammation is part of the body's immediate response to infection or injury. It is represented by redness, swelling, heat and pain (Galbraith *et al.*, 2001). Inflammation can also contribute to the rise of temperature in the body and produce other symptoms such as weakness, unable to concentrate and decrease activity (Crocetti *et al.*, 2002). Furthermore, high fever can be harmful to the body and may trigger brain disturbance such as convulsion in infants (Griffith, 2006).

Acute synovial inflammation include increasing of synovial fluid with distension of synovial capsule, increasing skin temperature over the joint and hyperplasia of the synovial membrane. During synovitis, permeability of the synovial membrane increases and proteins accumulates in the joint. Eicosanoids and other inflammatory mediators contribute to the inflammatory response by increasing the permeability of synovial vessels and increasing the movement of leukocytes from the blood stream into the surrounding tissues (Owens *et al.*, 1996). The earliest cells appearing at inflamed sites are granulocytes, with

monocytes/macrophages and lymphocytes appearing later. Granulocytes and monocytes/macrophages are involved in pathogen killing, clearing up cellular and tissue debris and tissue repair (Calder, 2006).

Sensory and emotional experiences associated with injury or inflammation are known as pain (Almeida *et al.*, 2004). Pain can be divided into acute and chronic pain. Acute pain ceases when the injury recovered. As for chronic pain, it occurs for a longer time compared to acute pain and may cause mental depression and decrease motor activity (Shipton, 1999).

As with inflammation, there could be an increase in the body temperature. This is due to the production of cytokines such as interleukins, tumor necrosis factor and others (Werner *et al.*, 2006). These cytokines will trigger the production of prostaglandin E_2 in the thermoregulatory centre at the anterior of the hypothalamus to produce fever (Sehic *et al.*, 1996).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of inflammation, pain and fever. The main mechanism of action of these drugs is believed to be the inhibition of the cyclooxygenase enzymes and leads to the conversion of arachidonic acid to prostaglandins (Lee *et al.*, 2003). Even though some of the drugs have a good effect in reducing the symptoms of the diseases, they have various side effects such as gastrointestinal ulcer, bleeding and renal damage (Coppelli *et al.*, 2004). This leads to a major interest from the

