



**UNIVERSITI PUTRA MALAYSIA**

***Channa striatus* AQUEOUS EXTRACT IMPROVED COGNITIVE  
FUNCTION IN RATS**

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FUNCTION IN RATS**

**By**

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**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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***Channa striatus* AQUEOUS EXTRACT IMPROVED COGNITIVE FUNCTION  
IN RATS**

By

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**September 2012**

**Chairman : Professor Abdul Manan Mat Jais, PhD**

**Faculty : Medicine and Health Sciences**

*Channa striatus* (Haruan) is a snakehead fish that is consumed traditionally in many Southeast Asian regions as a wound healing agent to relieve pain. The experiment was carried out to assess the effects of *C. striatus* aqueous extract on cognitive function in rats. The experiment involved a ten-week study to investigate the effects of *C. striatus* aqueous extract on spatial learning and memory in rats. Forty male Sprague-Dawley rats were randomly allocated into four treatment groups of 10 animals each. All rats were fed 15 g of standard pellet per rat per day. Treatment oil and extract were administered by oral gavages. Rats in the menhaden group (MHD group, positive control, n = 10) were gavaged with 10 ml/kg menhaden oil per rat per day. Similarly 10 ml/kg of the *C. striatus* aqueous extract were given daily as oral gavages to each rat in the Haruan group (HRN group, n = 10). The rats in butter group (BTR group, n = 10) were treated with 10 ml/kg butter and rats in the control group (CTL group, n = 10) were the untreated controls. The individual body weights were recorded weekly to calculate their body weight gain during the trial. The baseline observation for spatial learning and memory were acquired using the Morris Water Maze (MWM) test at week-0 and re-evaluated

after week 10. Blood collections were performed by cardiac puncture to determine the fatty acid composition in the plasma using gas chromatography after week-10. After ten weeks of treatment, there was a significant improvement ( $P < 0.05$ ) in distance and time to reach the hidden platform in the MWM test of the rats in MHD and HRN group. Rats in the MHD group followed a shorter distance followed with the rats in HRN group, BTR group and lastly the rats in the CTL group. The same pattern of results was also observed for the time to reach the hidden platform. The rats in MHD group were fed a high level of docosahexaenoic acid (DHA, 22:6n-3 polyunsaturated fatty acids) which led to the better performance to reach the hidden platform as well as the rats in HRN group. The plasma of the MHD and HRN animals also had significantly increased ( $P < 0.05$ ) levels of n-3 PUFAs, at the end of the trial compared to both BTR and CTL groups. The results indicated that *C. striatus* aqueous extract supplementation produced a better performance in the MWM test and tissue fatty acid composition compared to the unsupplemented control.

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

**EKSTRAK AKUES *Channa striatus* MEMPERBAIKI FUNGSI KOGNITIF  
DALAM TIKUS**

Oleh

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*Channa striatus* (Haruan) ialah ikan berkepala ular yang digunakan secara tradisional oleh penduduk kebanyakannya di kawasan Asia Tenggara sebagai agen pengubat luka untuk menahan sakit. Eksperimen ini telah dijalankan untuk mengkaji kesan ekstrak akues *C. striatus* terhadap fungsi kognitif pada tikus. Eksperimen ini melibatkan penyelidikan selama sepuluh minggu ini, dijalankan untuk menyiasat kesan ekstrak akues *C. striatus* dalam perkembangan pembelajaran berasaskan ruang dan memori dalam tikus baka Sprague-Dawley. Empat puluh tikus baka Sprague-Dawley jantan telah diagihkan secara rawak kepada empat kumpulan rawatan yang mengandungi sepuluh ekor tikus dalam setiap rawatan. Semua tikus diberi makan 15 g pellet tikus seekor sehari. Rawatan minyak dan ekstrak diberikan secara pemakanan paksa melalui mulut. Tikus dalam kumpulan menhaden (MHD, kawalan positif,  $n = 10$ ) telah diberi makan 10 ml/kg minyak ikan setiap seekor tikus sehari. 10ml/kg ekstrak akues *C. striatus* diberikan setiap hari kepada setiap ekor tikus di dalam kumpulan Haruan (kumpulan HRN,  $n = 10$ ). Tikus di dalam kumpulan mentega (kumpulan BTR,  $n = 10$ ) diberi rawatan 10 ml/kg mentega dan tikus di dalam kumpulan kawalan (kumpulan CTL,  $n =$

10) adalah kawalan yang tidak dirawat. Berat badan tikus dicatat secara individu sekali seminggu. Kemampuan pembelajaran berasaskan ruang dan ingatan (kebolehan mental) telah dinilai dengan menggunakan ujian Morris Water Maze (MWM) pada minggu awal dan dinilai sekali lagi pada minggu kesepuluh. Pensampelan darah dilakukan melalui jantung untuk menentukan kandungan asid lemak plasma menggunakan teknik kromatografi gas selepas minggu kesepuluh. Selepas rawatan selama sepuluh minggu, keputusan menunjukkan perbezaan bererti pada jarak laluan dan masa yang diambil untuk sampai ke platform untuk tikus dalam kumpulan MHD dan HRN. Tikus dalam kumpulan MHD menunjukkan jarak laluan yang pendek diikuti dengan tikus dalam kumpulan HRN, BTR dan yang terakhir tikus dalam kumpulan CTL. Keputusan untuk masa yang diambil oleh tikus untuk sampai ke platform juga menunjukkan corak keputusan yang sama. Subjek daripada kumpulan MHD telah diberi makan dengan minyak ikan yang kaya dengan asid dokosaheksanoic (DHA, 22:6n-3 lemak tak tepu) yang mencatatkan jarak laluan dan masa yang lebih baik untuk menuju ke platform, begitu juga tikus di dalam kumpulan HRN. Plasma dalam haiwan MHD dan HRN juga menunjukkan peningkatan tahap asid lemak politaktepu ( $P < 0.05$ ) di akhir eksperimen, berbanding kumpulan BTR dan CTL. Keputusan ini menunjukkan suplementasi ekstrak akues *C. striatus* menyumbang kepada prestasi lebih baik tikus dalam ujian MWM dan profil asid lemak tisu berbanding tikus yang tidak disuplementasi.

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## APPROVAL

I certify that an Examination Committee has met on 26<sup>th</sup> September 2012 to conduct the final examination of Muhamad Faris bin Ab Aziz of his Master of Science thesis entitled “*Channa striatus* Aqueous Extract Improved Cognitive Function in Rats” 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.

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## DECLARATION

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

The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. It features a red and white geometric design with a central vertical element and a large 'U' shape. The letters 'UPM' are prominently displayed in white on a red rectangular background at the top left of the shield.

**MUHAMAD FARIS BIN AB AZIZ**

Date: 26 SEPTEMBER 2012

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## LIST OF ABBREVIATIONS

ADHD	attention deficit hyperactivity disorder
ANOVA	analysis of variance
AA	Arachidonic acid
AD	Alzheimer's disease
°C	degree celcius
°C/min	degree celcius per minute
cAMP	cyclic adenosine monophosphate
CCL	chronic constant light
cm	centimeter
CNS	Central nervous system
DHA	Docosaehaenoic acid
DPA	Docosapentaenoic acid
EFA	essential fatty acid
EPA	Eicasopentaenoic acid
FA	Fatty acid
FAME	Fatty Acid Methyl Esters
FID	Flame Ionization Detector
g	gram
G	gravity force
h	hour
HTE	Haruan traditional extracts
IL	interleukin



kcal	kilo calories
kDa	kilo dalton
kg	kilogram
L	litre
LA	linoleic acid
LC-PUFA	long-chain polyunsaturated fatty acid
LTD	long-term depression
LTP	long-term potentiation
M	Molar
min	minute
μL	microliter
mL	millimeter
mL/min	millimeters per minute
MPC	membrane phospholipid composition
MUFA	monounsaturated fatty acids / Monoenoic fatty acids
MWM	Morris Water Maze
NMDA	<i>N</i> -methyl-D-aspartate
n-3:n-6 ratio	Total n-3 PUFA to Total n-6 PUFA
P:S ratio	Total PUFA to Total SFA ratio
PUFA	Polyunsaturated fatty acid
RAM	radial arm maze
SD	standard deviation
SE	standard error

sec	second
SFA	Saturated fatty acid
SPECT	Single photon emission computer tomography
TAG	triacylglycerol
UFA	unsaturated fatty acids
U:S ratio	total UFA to total SFA ratio

v	volume
w	weight

## CHAPTER 1

### INTRODUCTION

In Malaysia, fresh water and marine fish are very important components of the Malaysian diet, and fish constitute approximately 60–70% of the protein intake in Malaysia (Abd Rahman *et al.*, 1995). Apart from its nutritional values, fish is also recognized for its medicinal properties. The consumption of 300-600 g daily of freshwater fish showed positive effects in lowering blood pressure, lowering plasma lipid concentration and increased plasma concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Dahlan *et al.*, 2010).

Haruan *Channa striatus*, is a common snakehead fish that is found in many tropical and subtropical countries including Malaysia, Thailand, Brunei and Indonesia (Zakaria *et al.*, 2005, Mohsin and Ambak, 1983). The fish is traditionally consumed for wound healing especially for postnatal recovery and for post-circumcision care in the children, to alleviate post-operative pain and discomfort (Mat Jais *et al.*, 1994). Many studies have been carried out to evaluate the fatty acids and amino acids composition in the *C. striatus*. This healing process is also being promoted by the essential amino acids, especially glycine (Zakaria *et al.*, 2007; Mat Jais *et al.*, 1994) which responsible in the formation of collagen. The mucus extract and roe of *C. striatus* were reported to have unsaturated fatty acids (monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA)) particularly oleic acid and linoleic

acid (Mat Jais *et al.*, 1998b), arachidonic acid (AA), which is a precursor for prostaglandins and thromboxanes in the *C. striatus* (Mat Jais *et al.*, 1994).

Fatty acids are one of the most important components that are necessary for human health. They are essential components of the diet, sources of food energy and cognitive performance (Hafandi, 2007; Whalley *et al.*, 2004; Rudin and Felix, 1996). Fatty acids are also involved in early life development of the central nervous system, growth development and learning ability (Yamamoto *et al.*, 1987). The clinical signs related to essential fatty acid deficiency include cardiovascular disease, immune system and neurologic as well as brain development (Uauy and Dangour, 2006). These findings showed that the fatty acids are important for the development of the cognitive performance in mammals.

Two fatty acid families namely the n-3 and n-6 family, are essential in human, because they cannot be synthesized by the body. They are necessary as constituents of membrane phospholipids. Therefore the parent fatty acids of these families, alpha-linolenic acid and linoleic acid, respectively, have to be supplied by nutrition. Two of their long chain, highly unsaturated derivatives are docosahexaenoic acid (DHA) from n-3 family and arachidonic acid (AA) from n-6 family (Bakker *et al.*, 2003). The abundance sources of DHA found in fish oil from menhaden, salmon and sardines affects the cognitive function of the brain (Chen *et al.*, 2006; Fewtrell *et al.*, 2004; Suzuki *et al.*, 1998; Yonekubo *et al.*, 1993). The supplementation of n-3 PUFA in the diets can contribute to good health, especially on the cardiovascular system including for a healthy heart and eyes, and the brain function including the functioning of the cerebral membrane (McCann and Ames, 2005; Bakker *et al.*,

2003; Youdim *et al.*, 2000; Kalmijn *et al.*, 1997). The n-3 and 6 polyunsaturated fatty acids (PUFA) are essential for the growth and functional development of the infant in early life and showed positive effects on cardiovascular diseases and cancer (Horrocks and Yeo, 1999; Conner, 1997). In infants, the breastfeeding, which leads to higher DHA concentration in the brain, or supplementation of DHA, is related to a better cognitive performance at later ages (Agostoni *et al.*, 1995; Makrides *et al.*, 1994; Lauring *et al.*, 1994). A study by O'Brien *et al.* (1964) demonstrated that DHA and AA account up to 50% of the total fatty acids in the grey matter of the brain.

Many studies have been done on the medicinal properties of *C. striatus*, including antinociceptive (Mat Jais *et al.*, 1997), anti-inflammatory (Somchit *et al.*, 2004, Zakaria *et al.*, 2004) and antioxidant properties (Dahlan *et al.*, 2010). However no investigation has been carried on the cognitive or neuro-enhancing potential of the *C. striatus* in human population.

### **Hypothesis**

It was hypothesized that *C. striatus* aqueous extract will improve spatial learning and memory (cognitive function) in rats. The fatty acid composition particularly the docosahexaenoic acid (DHA) and the n-6: n-3 ratio in *C. striatus* aqueous extract will lead to a better cognitive performance in rats.

### **Objectives of this study**

- 1) To determine the fatty acid composition in the *C. striatus* aqueous extract
- 2) To investigate the effects of the *C. striatus* aqueous extract on the Morris (MWM) test for spatial learning and memory in rats
- 2) To determine the effects of the *C. striatus* aqueous extract supplementation on the plasma fatty acid composition in rats

### **Justification of this study**

The outcome of the study enabled an objective for evaluation of the neurotherapeutic properties of the *C. striatus* aqueous extract for the production and commercialization of the antidote for neuro-enhancing therapy (development of *C. striatus*-based drug).

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