UNIVERSITI PUTRA MALAYSIA

ANTIDEPRESSANT ACTIVITY OF Channa striatus Bloch EXTRACTS IN RODENTS AND THEIR POSSIBLE MECHANISM

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Chair: Associate Professor Mohamad Taufik Hidayat Bin Baharuldin, PhD
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Depression is a major mood disorder. Despite the availability of effective pharmacotherapy, around 30% of patients do not respond to current treatments. Several natural products exhibited antidepressant activity in previous studies. *Channa striatus* (Malay: Haruan) exhibited antinociceptive effect in mice through serotonergic system. Dysfunction of serotonergic system has been associated with depression and many antidepressant drugs act through it. Hence, this study evaluated the antidepressant activity of extracts of *C. striatus* in rodents and their mechanism of action. Aqueous and lipid extracts were prepared from the fillet of *C. striatus* and their individual components were quantified. Both extracts were tested in forced swimming test (FST), tail suspension test (TST) and open field test (OFT) in male ICR mice. The mechanism of action was evaluated by pretreatment with selected monoamine antagonists. The lipid extract was tested in postpartum depression (PPD) model in female Sprague-Dawley rats and chronic unpredictable mild stress model (CUMS) in male Sprague-Dawley rats through FST and OFT. The plasma levels of corticosterone, oxytocin, brain prefrontal cortex and hippocampal levels of monoamines, brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6) and nuclear factor-kappa B (NF-κB) were determined by ELISA in PPD and CUMS experiments. Additionally, body weight and sucrose preference were measured at every week during CUMS study. Analysis of variance followed by appropriate post hoc test was used as the statistical test with significance considered at $p < 0.05$. The aqueous extract produced significant ($p < 0.001$) antidepressant activity in FST and TST and its mechanism was found to be acting through the serotonergic system and noradrenergic system. In another experiment, aqueous and lipid extracts, prepared by a different method, produced significant ($p < 0.05$) antidepressant activity in FST and TST through serotonergic and noradrenergic systems. The lipid extract was found to contain oleic acid, palmitic acid as major fatty acids along with docosahexaenoic acid. The aqueous extract was found to contain aspartic acid and glutamic acid as major amino acids. The lipid extract produced significant ($p < 0.001$) antidepressant effect in PPD model, with the mechanism mediated through the decrease in corticosterone, increase in

oxytocin and decrease in NF-κB in prefrontal cortex. The lipid extract produced significant ($p < 0.001$) antidepressant effect in FST in CUMS model, with the mechanism mediated through the decrease in corticosterone, increase in serotonin level in prefrontal cortex, increase in dopamine and noradrenaline levels in hippocampus and prefrontal cortex, increase in BDNF level in hippocampus and prefrontal cortex, and decrease in IL-6 and NF-κB levels in prefrontal cortex. The lipid extract also significantly ($p < 0.05$) reversed the effects of stress on body weight of animals and sucrose preference in CUMS model. In conclusion, the aqueous and lipid extracts of *C. striatus* produced significant antidepressant effect in animal models of depression with the common mechanism of action through monoaminergic systems. These findings may be useful to explore further the clinical utility and molecular mechanism of action of these extracts.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

AKTIVITI ANTIDEPRESAN OLEH EKSTRAK *Channa striatus* Bloch KE ATAS TIKUS DAN KEMUNGKINAN MEKANISMANYA

Oleh

MOHAMED SALEEM ABDUL SHUKKOOR

November 2016

Pengerusi: Professor Madya Mohamad Taufik Hidayat Bin Baharuldin, PhD
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Kemurungan adalah gangguan emosi yang serius. Walaupun terdapat ubat-ubatan yang berkesan, sekitar 30% daripada pesakit tidak bertindak balas kepada ubat-ubatan yang didapati kini. Di dalam beberapa kajian yang lepas, terdapat beberapa produk semula jadi yang mempunyai kesan antidepresi. *Channa striatus* (Melayu: Haruan) menunjukkan kesan antinosiseptif kepada mencit melalui sistem serotonergik yang mana terdapat banyak ubatan antidepresi yang bertindak melalui sistem ini. Oleh itu, kajian ini menilai aktiviti antidepresi oleh ekstrak *C.striatus* terhadap mencit dan tikus, dan mekanisma tindakannya. Ekstrak akuas dan lipid disedia menggunakan filet *C.striatus* dan komponen setiap ekstrak telah dipastikan. Kedua-dua ekstrak diuji menggunakan ujian paksaan renang (FST), ujian penggantungan ekor (TST) dan ujian lapangan terbuka (OFT) terhadap mencit ICR jantan. Mekanisma tindakan dinilai menggunakan pra-rawatan oleh antagonis monoamin yang terpilih. Ekstrak lipid telah digunakan dalam model kemurungan selepas bersalin (PPD) pada tikus Sprague-Dawley betina dan model tekanan ringan tidak menentu kronik (CUMS) pada tikus Sprague-Dawley jantan melalui FST dan OFT. Paras kortikosteron dan oksitosin dalam plasma, monoamin dari prefontal korteks dan hippokampal, faktor neurotropik yang dikawal oleh otak (BDNF), interleukin-6 (IL-6) dan faktor-kappa nuklear B (NF-κB) ditentukan menggunakan ELISA didalam eksperimen PPD dan CUMS. Berat badan dan kecenderungan terhadap glukosa diukur setiap minggu sewaktu ujian CUMS. Analisa varian dan *post hoc* yang sesuai digunakan sebagai ujian statistik dan jika $p < 0.05$, data dianggap signifikan. Ekstrak akuas menunjukkan aktiviti antidepresi yang signifikan ($p < 0.001$) didalam ujian FST dan TST. Mekanisma tindakannya adalah melalui sistem serotonergik dan noradrenergik. Didalam ujian lain, ekstrak akuas dan lipid yang disediakan menggunakan kaedah lain, menunjukkan aktiviti antidepresi yang signifikan ($p < 0.05$) didalam ujian FST dan TST melalui sistem serotonergik dan noradrenergik. Ekstrak lipid didapati mengandungi asid oleik, asid palmitik sebagai asid lemak utama bersama-sama dengan asid dokosaheksanoik. Ekstrak akues didapati mengandungi asid aspartik dan asid glutamik sebagai asid amino utama. Ekstrak lipid menunjukkan kesan antidepresi

yang signifikan ($p < 0.001$) terhadap model PPD, dengan mekanisme tindakannya dikawal melalui penurunan kortikosteron plasma, peningkatan oksitosin plasma dan penurunan dalam NF-κB pada prefontal korteks. Ekstrak lipid mempamerkan kesan antidepresi yang signifikan ($p < 0.001$) di dalam model CUMS yang menggunakan FST. Mekanisme ini, atau sebahagian daripadanya, dikawal melalui penurunan kortikosteron plasma, peningkatan kandungan serotonin di korteks prefontal, peningkatan dopamin dan noradrenalin dalam hipokampus dan korteks prefontal, peningkatan BDNF dalam hipokampus dan korteks prefontal. Ekstrak lipid juga mempunyai kesan yang signifikan ($p < 0.05$) dalam mengembalikan berat badan haiwan dan kecenderungan terhadap glukosa didalam model CUMS. Kesimpulannya, ekstrak akuas dan lipid C. *striatus* menghasilkan kesan antidepresi yang signifikan didalam model haiwan dengan mekanisma tindakan melalui system monoaminergik. Dapatan ini berguna untuk meneroka kegunaan klinikal mekanisme tindakannya pada peringkat molekul oleh ekstrak-ekstrak ini.

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I certify that a Thesis Examination Committee has met on 23-11-2016 to conduct the final examination of Mohamed Saleem Abdul Shukkoor on his thesis entitled "Antidepressant Activity of *Channa striatus* Bloch Extracts in Rodents and Their Possible Mechanism" 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 Doctor of Philosophy.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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5-HT	5-hydroxy tryptamine
AA	Arachidonic acid
ACTH	Adrenocorticotrophic hormone
ADH	Antidiuretic hormone
AECSF	Aqueous extract of *C. striatus* fillets
ALA	α-linolenic acid
ANOVA	Analysis of variance
APA	American Psychiatric Association
AVP	Arginine vasopressin
BDNF	Brain-derived neurotrophic factor
BMI	Body Mass Index
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CREB	cAMP response element-binding protein
CRF	Corticotrophin releasing factor
CRH	Corticotrophin releasing hormone
CRH R1	Corticotrophin releasing hormone receptor 1
CRH R2	Corticotrophin releasing hormone receptor 2
CUMS	Chronic unpredictable mild stress
CYP2D6	Cytochrome P4502D6
DALY	Disability-adjusted life year
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DSM-5	Diagnostic and Statistical Manual of Mental Disorders-5
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
EPA	Eicosapentaenoic acid
FDA	Food and Drug Administration (US)
FST	Forced swimming test
GABA	Gamma amino butyric acid
GC	Gas chromatography
GPR	G-protein coupled receptors
GSK-3	Glycogen synthase kinase 3
GSK-3β	Glycogen synthase kinase-3β
HCl	Hydrochloric acid
HPA	Hypothalamo-pituitary-adrenal
HPLC	High performance liquid chromatography
HSP	Hormone-simulated pregnancy
IFN-γ	Interferon gamma
IL-10	Interleukin-10
IL-1β	Interleukin-1β
IL-6	Interleukin-6
iNOS	inducible nitric oxide synthase

IκB	I kappa B kinase
LA	Linoleic acid
LC	Locus coeruleus
LD_{50}	Lethal dose 50
L-DOPA	L- dihydroxy phenyl alanine
L-NAME	L-N^G-nitroarginine methyl ester
MAO-A	Monoamine oxidase A
MAO-B	Monoamine oxidase B
MAOI	Monoamine oxidase inhibitor
MDA	Malondialdehyde
MDD	Major depressive disorder
NaCl	Sodium chloride
NDRI	Noradrenaline dopamine reuptake inhibitor
nNOS	neuronal nitric oxide synthase
NO	Nitric oxide
NF-κB	Nuclear factor-kappa B
Nrf2	Nuclear factor-like 2
NRI	Noradrenaline reuptake inhibitor
OFT	Open field test
PBS	Phosphate buffer saline
PCPA	*p*-chlorophenylalanine methyl ester
PFC	Prefrontal cortex
PGE2	Prostaglandin E2
PVN	Paraventricular nucleus
RNA	Ribonucleic acid
S. E. M.	Standard error of mean
SNRI	Serotonin noradrenaline reuptake inhibitor
SON	Supraoptic nucleus
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressants
TNF-α	Tumor necrosis factor-alpha
Trk	Tropomyosin receptor kinase
TST	Tail suspension test
WHO	World Health Organization
YLD	Years lived with disability

CHAPTER 1

INTRODUCTION

Depression is one of the major mood disorders which affect human beings (Daly, 2009). More than 350 million people of all ages suffer from depression, all over the world (WHO, 2015). According to WHO report on global burden of disease 2010, depression is the leading cause of disability, and is a major contributor to the global burden of disease (Ferrari et al., 2013). Depression is characterized with main symptoms of persistent low mood and inability to experience happiness (Fava and Kendler, 2000; Association, 2013). Stressful life events are associated with onset of major depression (Kendler et al., 1999). People may experience depression following extremely stressful situations such as loss of affectionate persons, death of immediate family members, severe accidents, financial loss, and bankruptcy. Furthermore, depression is reported to occur more frequently in people with physical disability (Turner and Noh, 1988; Psarra and Kleftaras, 2013).

Depression could disable and decrease quality of life in affected individuals if it persists for chronic periods (Fava and Kendler, 2000; Bylund and Reed, 2007). Depression may lead to suicide and in fact, it is one of the major causative factors of suicide and suicidal attempts (Carlson and Cantwell, 1982; Blair-West et al., 1999; Fava and Kendler, 2000). Depression affects approximately two to five percentage of population worldwide irrespective of race, ethnicity and location with a lifetime prevalence of 15% (Bylund and Reed, 2007). In the United States, the prevalence of major depressive disorder was found to be approximately 7% (Association, 2013). In Malaysia, in the National Health and Morbidity Survey IV (NHMS IV) report published in 2011, the prevalence of lifetime depression was 2.4% and current incidence of depression was 1.8% (IPH, 2011; Guan, 2014). Depressed individuals are reported to cause loss of billions of dollars in productivity in industrialized countries (Greenberg et al., 2003). Depressed individuals carry higher risk for diabetes (Knol et al., 2006) and coronary artery disease (Ford et al., 1998). Depression also affects daily social activities and sense of belonging (Steger and Kashdan, 2009). Therefore, therapeutic intervention is necessary to improve the quality of life in affected individuals. Depression is treated by psychotherapy and antidepressant drugs in current clinical practice (Pampallona et al., 2004). Cognitive behavioral therapy is also reported to be efficacious against depression (Tolin, 2010). In addition, several natural products and dietary supplements also have been consumed by patients to improve the mood and sense of wellbeing (Muszynska et al., 2015). These natural products and dietary supplements are sold in pharmacies, health stores and online stores without doctor's prescription (Morris and Avorn, 2003).

1.1 Problem Statements

Currently, several USFDA (U.S. Food and Drug Administration) approved antidepressant drugs are available in the market to treat depression (Delgado, 2004).

They include tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, dopamine reuptake inhibitor, noradrenaline reuptake inhibitors and other classes of drugs. These drugs primarily work by increasing the synaptic concentrations of monoamine neurotransmitters such as serotonin, dopamine and noradrenaline in brain (Delgado, 2004; Taylor et al., 2005; Bylund and Reed, 2007). These antidepressant drugs produce several adverse effects such as fatigue, weight gain and sexual problems which significantly limit patient's adherence to therapy (Ashton et al., 2005).

The current pharmacotherapy offers better therapeutic options than before and is relatively safe. The advantages include improvement in mood, behavior and quality of life (Williams et al., 2000; Pampallona et al., 2004). However, there are several disadvantages associated with these drugs. The onset of antidepressant effect takes approximately 4-6 week time (Delgado, 2004; Taylor et al., 2005). Around 30% of patients do not respond at all to these drugs (Crisafulli et al., 2011). Some patients especially children, adolescents and young adults who are treated with antidepressant drugs experience suicidal thoughts as side effect (Coupland et al., 2015; Sharma et al., 2016) and few others experience sexual dysfunction as a major side effect (Montejo et al., 2001; Baldwin and Mayers, 2003; Schweitzer et al., 2009; Bijlsma et al., 2014). The long term side effects of antidepressant drugs include weight gain, withdrawal effects, drug interactions and sexual dysfunction (Masand and Gupta, 2002). The slow onset of action, unresponsiveness to existing drugs and development of major adverse effects have significant impact on quality of life of patients and decrease patient's compliance to therapy (Ashton et al., 2005). Hence, development of new therapeutic interventions with better efficacy and safety is required.

Natural products have been used for many ailments throughout the history. They have been used for prevention and cure of psychological and central nervous system disorders (Fugh-Berman and Cott, 1999; Clement et al., 2004). Natural products contributed to the development of several modern drugs both directly and indirectly (Harvey, 2008). Approximately 49% of the currently existing anticancer drugs have been derived from natural products (Newman and Cragg, 2012). Several natural products have exhibited antidepressant-like effect in animals. Few natural products exhibited antidepressant effect in human studies. St John's wort (Butterweck and Schmidt, 2007; Zhai et al., 2015), ginseng (Dang et al., 2009; Yamada et al., 2011; Chen et al., 2014), turmeric (Xu et al., 2006; Xia et al., 2007; Kulkarni et al., 2008; Jiang et al., 2013), Piperine (Lee et al., 2005; Li et al., 2007), *Centella asiatica* (Sakina and Dandiya, 1990) and *Bacopa monneiri* (Zhou et al., 2007) showed antidepressant-like effect in animal models of depression. Omega-3 fatty acids (Parker et al., 2006; Sinclair et al., 2007; Stahl et al., 2008; Colangelo et al., 2009; Liperoti et al., 2009; Grosso et al., 2014), curcumin (Lopresti et al., 2014) and St John's wort (Nathan, 2001; Whiskey et al., 2001) exhibited antidepressant effect in clinical studies in humans. Hence, it is worth exploring other natural products for potential antidepressant-like effects in animal models to develop safe and efficacious drugs for human beings.

Channa striatus Bloch (Malay: Haruan), is a popular fresh-water fish (Wee, 1982), consumed in Malaysia and Indonesia for its beneficial effects during the post-partum period (Jais et al., 1994; Jais et al., 1997; Baie and Sheikh, 2000b; Zakaria et al., 2004). It exhibited antinociceptive activity (Jais et al., 1997; Dambisya et al., 1999; Zakaria et al., 2008), wound healing activity (Jais et al., 1994; Baie and Sheikh, 2000a; Laila et al., 2011) and activity against osteoarthritis (Michelle et al., 2004) in animal models. The mechanism of antinociceptive activity of aqueous supernatant of *C. striatus* extract was found to act through muscarinic, $GABA_A$, alpha-adrenergic, serotonergic receptor systems (Zakaria et al., 2005a) and through nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway (Zakaria et al., 2005b) in a mouse abdominal constriction model. The serotonergic (Fuller, 1991; Baldwin and Rudge, 1995), adrenergic (Delgado and Moreno, 2000), GABAergic (Monteleone et al., 1990) and nitrergic systems (Dhir and Kulkarni, 2011) are involved in the regulation of mood and behavior. The imbalance in one or more of these neurotransmitter systems is linked to the development of depression (Elhwuegi, 2004).

1.2 Hypothesis

Based on the evidence that extract of *C. striatus* was acting through alpha-adrenergic and serotonergic receptor systems (Zakaria et al., 2005a), and the involvement of adrenergic (Delgado and Moreno, 2000) and serotonergic systems (Fuller, 1991; Baldwin and Rudge, 1995) in depression, it was hypothesized that the extract of *C. striatus* may possess antidepressant-like effect in animal models.

Hence, the antidepressant-like effect of extracts of *Channa striatus* in animal models of depression was explored in this study.

1.3 Rationale

To the best of our knowledge, there are no reports on the antidepressant activity of *C. striatus* extracts. Hence, this study aimed to evaluate the antidepressant-like effect of *C. striatus* extract in selected animal models of depression. Furthermore, *C. striatus* was reported to contain amino acids and fatty acids (Jais et al., 1994; Jais et al., 1998; Zakaria et al., 2007; Muhamad and Mohamad, 2012). Although several studies were conducted on the molecular mechanisms of polyunsaturated fatty acids in animal models of neurological disorders and *in vitro* studies (Calviello et al., 2013), not many studies have been reported on the effect of unsaturated fatty acids, especially the fatty acids from fish extracts, on the inflammatory markers such as interleukin-6 and nuclear factor-kappa B in the animal models of depression. Therefore, this study aimed to explore the effect of *C. striatus* extracts on inflammatory markers such as interleukin-6 and nuclear factor-kappa B in animal models of depression.

1.4 Objectives

The general objective of this study was:

To evaluate the antidepressant-like effect of extracts of *C. striatus* in animal models of depression

The specific objectives of this study were:

1. To prepare the aqueous extract and lipid extract from fillets of *Channa striatus* and quantify their chemical components
2. To determine the LD_{50} of aqueous and lipid extracts of fillets of *Channa striatus*
3. To evaluate the antidepressant-like effect of aqueous and lipid extracts of fillets of *Channa striatus* in forced swimming test and tail suspension test in mice
4. To explore the possible mechanism of action of the observed antidepressant-like effect of aqueous and lipid extracts of fillets of *Channa striatus* in mice
5. To evaluate the antidepressant-like effect of most potent extract of fillets of *Channa striatus* in animal model of postpartum depression in female rats and to explore its possible mechanism of action
6. To evaluate the antidepressant-like effect of most potent extract of fillets of *Channa striatus* in chronic unpredictable mild stress model of depression in male rats and to explore its possible mechanism of action

1.5 Significance of the Study

No previous research has been reported on the evaluation of antidepressant effect of any extract of *C. striatus* in animal models or in human clinical studies. Therefore, the findings of this study was expected to be the first report on the antidepressant effect of extracts of *C. striatus* in animal models. Furthermore, this study aimed to explore the pathways of mechanism of action of antidepressant-like effect of fish extracts that contain fatty acids and amino acids in animal models of depression. Such exploration study, especially on the level of inflammatory markers such as interleukin-6 and nuclear factor-kappa B, was expected to give insights on the role of these fatty acids and amino acids in the molecular mechanism underlying the neurobiology of depression and its treatment. In addition, the chemical composition of freshwater fishes is slightly different than the marine fishes particularly with respect to their lipid composition (Muhamad and Mohamad, 2012). Although many studies have been reported on the effect of marine fish oils on depression (Chalon et al., 1998; Su, 2009; Vines et al., 2012), the effect of extracts of freshwater fish such as *C. striatus* on depression is unknown. Hence, the results of this study was expected to provide novel findings in the area of research on depression. Since *C. striatus* is consumed by local population, the positive findings of this study was expected to influence the consumption pattern of *C. striatus* amongst the local population.

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BIODATA OF STUDENT

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