UNIVERSITI PUTRA MALAYSIA

NEUROPROTECTIVE AND NEUROREGENERATIVE PROPERTIES OF HARUAN (CHANNA STRIATUS) TRADITIONAL FORMULATION

MOHD AFFENDI BIN MOHD SHAFRI

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NEUROPROTECTIVE AND NEUROREGENERATIVE PROPERTIES OF HARUAN (*CHANNA STRIATUS*) TRADITIONAL FORMULATION

MOHD AFFENDI BIN MOHD SHAFRI

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

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NEUROPROTECTIVE AND NEUROREGENERATIVE PROPERTIES OF HARUAN (*CHANNA STRIATUS*) TRADITIONAL FORMULATION

By

MOHD AFFENDI BIN MOHD SHAFRI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

August 2012

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor in Philosophy in Physiology

NEUROPROTECTIVE AND NEUROREGENERATIVE PROPERTIES OF HARUAN (*CHANNA STRIATUS)* TRADITIONAL FORMULATION
By

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August 2012

Chair: Prof. Abdul Manan Mat Jais, PhD

Faculty: Fakulti Perubatan dan Sains Kesihatan

Neurodegenerative conditions continue to affect a large number of people. Despite numerous studies carried out in the last few decades, no effective treatment has been found and current management of neurodegenerative conditions are not effective, marred by side-effects, costly and could only provide symptomatic alleviations. Haruan or *Channa striatus,* is rich in many important amino acids and fatty acids, which may act as suitable pharmacological modulators to neuron cells as they have potential to cross the blood-brain barrier efficiently and have anti-oxidative action and may trigger neurite growth receptor on neuron cell's surface. The neuroprotective and neuroregenerative effects of haruan traditional formulation (HTF) on PC12 cell line, an established cell line used for studying neurite outgrowth, was first studied to see its effect of cell growth behaviour, morphology and neurite outgrowth. From the study, HTF appears to influence neurite outgrowth, cell morphology and growth behaviour in PC12 cells in concentration dependent manner. It was found that HTF at 100 µL in the serum rich assay was most effective in providing protection against cell death as well as in

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stimulating greatest neurite extension ($p < 0.001$, one way ANOVA with Tukey's post hoc test). Next, in *in vivo* experiment using Sprague Dawley rats, the effect of HTF on rats' nose-dipping and rearing behaviours in neuroprotective and neuroregenerative assays, in which two neurodegenerative agents, ketamine and methamphetamine given intraperitoneally, 4 four times a day at 2 hour interval at different doses were used, was studied using a hole board maze. It was found that HTF could provide some neuroprotective ($p < 0.01$ for the nose dip and $p < 0.05$ for rearing; one way ANOVA with Tukey's post hoc analysis) and neuroregenerative ($p < 0.001$ for both nose dip and rearing; one way ANOVA with Tukey's post hoc analysis) effects on rats behaviour for the LEK group only. Consequent change in hippocampus was assessed by further analyses of the hippocampus CA3 region in terms of live neuron cell count, and pathological change in the overall structural integrity by staining the hippocampal sections using cresyl violet stains. Cell counting was done using Java-Installed Image J software, images were captured using a Nikon T*i* Inverted Fluorescent Microscope and Imaging software and data was statistically analysed using a Sigma Plot 11.0 for Windows. It was found that the best effect in term of preservation of structural integrity and regeneration of live cell number ($p < 0.001$ one way ANOVA with Tukey's post hoc analysis) was in the LEK. The HTF is less able to produce positive changes in the methamphetamine-treated groups which may be used to identify the mode of actions of HTF's neurorestorative mechanism in future research. In view of other results however correlation between functional, numerical and structural changes is not straightforward. Although there is evidence of neuroprotective and neuroregenerative effects, HTF must be studied further for more conclusive evidence.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah PhD Fisiologi

UPAYA PERLINDUNGAN DAN PENJANAAN SEMULA NEURON OLEH FORMULASI HARUAN (*CHANNA STRIATUS)* SECARA TRADISIONAL

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Keadaan degenerasi neuron terus memberi kesan kepada sebilangan besar penduduk dunia. Walaupun banyak kajian yang dijalankan dalam beberapa dekad yang lalu, tiada rawatan yang berkesan telah ditemui. Ubatan semasa mempunyai kesan-kesan sampingan, mahal dan hanya boleh mengurangkan gejala penyakit bukan merawatnya. Haruan atau *Channa striatus*, adalah kaya dengan asid amino dan asid lemak penting, yang boleh bertindak sebagai modular farmakologi yang sesuai untuk sel-sel neuron kerana mempunyai potensi untuk merentasi sempadan otak-sistem pembuluh darah secara cekap, mempunyai tindakan anti-oksidatif dan kebolehan untuk mencetuskan reseptor pertumbuhan neurite pada permukaan sel neuron. Kesan neuroprotektif dan neuroregenerasi formulasi tradisional haruan (HTF) diuji ke atas sel PC12, yang digunakan untuk mengkaji neurite untuk melihat kesan ke atas tingkah laku pertumbuhan sel, morfologi dan pertumbuhan neurite. Dari kajian tersebut, HTF muncul untuk mempengaruhi pertumbuhan neurite, morfologi sel dan tingkah laku pertumbuhan

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dalam PC12 sel-sel bergantung kepada kepekatan berbeza. HTF pada 100 μL dalam cerakin kaya serum adalah yang paling berkesan dalam menyediakan perlindungan terhadap kematian sel serta dalam merangsang pertumbuhan neurite ($p < 0.001$, ANOVA sehala dengan ujian post hoc Tukey's). Seterusnya, di dalam eksperimen in vivo menggunakan tikus Sprague Dawley, kesan HTF pada frekuensi haiwan itu memasukkan hidungnya ke dalam lubang dan berdiri di atas kaki belakangnya dalam cerakin neuroprotektif dan neuroregenerasi, di mana dua ejen neurodegenerasi, ketamin dan methamphetamine, diberikan secara intraperitoneal, empat kali sehari dengan 2 jam selang pada dos yang berbeza telah digunakan, dan dikaji menggunakan peralatan Papan Berlubang. Dapatan menunjukkan bahawa HTF boleh menyebabkan kesan neuroprotektif ($p < 0.01$ untuk frekuensi memasukkan hidung ke dalam lubang dan $p < 0.05$ untuk kelakuan berdiri di atas kaki belakang; one-way ANOVA dengan analisis post hoc Tukey's) dan neuroregenerasi ($p < 0.001$ untuk kelakuan memasukkan hidung ke dalam lubang dan berdiri di atas kaki belakang; one-way ANOVA dengan analysis post hoc Tukey's) untuk kumpulan LEK sahaja. Perubahan dalam rantau CA3 hippocampus telah dinilai selanjutnya dari segi kiraan sel neuron hidup, dan perubahan patologi dalam integriti keseluruhan struktur dengan menggunakan pewarna ungu cresyl. Pengiraan dilakukan dengan bantuan perisian Image J dengan Java, imej ditangkap menggunakan perisian T*i* Nikon Inverted Microscope Pendarfluor dan Pengimejan dan data telah dianalisis menggunakan Sigma Plot 11.0 untuk Windows. Kajian ini mendapati bahawa kesan terbaik dalam jangka pemeliharaan integriti struktur dan pertumbuhan semula bilangan sel hidup ($p < 0.001$ one-way ANOVA dengan analisis post hoc Tukey) adalah dalam LEK. Secara keseluruhannya, perubahan positif didapati berlaku kepada neurodegenerasi yang disebabkan oleh ketamine dan kesan positif

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terhadap perubahan neurodegenerasi methamphetamine adalah kurang dan ini boleh digunakan untuk mengenalpasti dengan jelas lagi mekanisma neurorestoratif HTF di dalam kajian mendatang. Dengan mengambil kira keputusan lain dalam kajian ini, hubungkait antara perubahan berfungsi, bilangan sel dan integriti struktur tidak mudah untuk dirungkai. Walaupun kajian ini menunjukkan terdapat bukti kesan neuroprotektif dan neuroregenerasi pada HTF, HTF perlu dikaji lagi untuk keterangan lebih muktamad.

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DECLARATION

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

MOHD AFFENDI BIN MOHD SHAFRI

Date: 8 August 2012

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LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
AMP	adenosine monophosphate
BDNF	brain-derived neurotrophic factor
BSE	bovine spongiform encephalopathy
Ca^{2+}	calcium
CA3	cornu ammonis
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CNS	central nervous system
COX	cyclooxygenase
CPK	creatine phosphokinase
CPK-MB	creatine phosphokinase-MB
CTF-II	cardiotoxic factor-II
DFPL	double fractionated palm olein
DHA	docosahexaenoic acid
DMSO	dimethyl sulphic oxide
DNA	deoxyribonucleic acid
EMEM	Eagle's Minimum Essential Medium
EPA	eicosapentaenoic acid
EPO	erythropoietin
ERK	extracellular-signal regulated kinase
FAMA	Federal Agricultural Marketing Agency
FBS	foetal bovine serum

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GABA	gamma-aminobutyric acid
H_2O_2	hydrogen peroxide
HTF	haruan traditional formulation
IFN-γ	interferon gamma
IL	interleukin
KMnO4	potassium permanganate
LA	left atrium
LEK	long-exposure ketamine
LEM	long-exposure methamphetamine
LV	left ventricle
Mn^{2+}	manganase
MPDV	methylenedioxypyrovalerone
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolinbromide
NADA	National Anti Drug Agency
NaOH	sodium hydroxide
NBT	neurobehavioural test
NGF	neuronal growth factor
NVM	non-vitamin/mineral
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
NR1	NMDA-receptor 1
PBS	phosphate buffer solution
PC12	phaechromocytoma 12
PDRM	Polis di-Raja Malaysia

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PG	prostaglandin
PKC	protein kinase C
PKG	protein kinase G
RA	right atrium
RBC	red blood cell
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
RV	right ventricle
SEK	short-exposure ketamine
SEM	short-exposure methamphetamine
SFSE	shol fish skin extract
SVZ	supraventricular zone
TNF- α	tumour necrosis factor-α
Trypsin-EDTA	trypsin-ethylenediaminetetraacetic acid
TIP	transferring-insulin-progesterone
VM	vitamin and mineral
WBC	white blood cell

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