## **UNIVERSITI TEKNOLOGI MARA**

# EXPLORING POTENTIAL NEUROPROTECTIVE PROPERTIES OF *Centella asiatica* AQUEOUS EXTRACT ON CHRONIC STRESS-INDUCED RATS

### MUHAMAD ZULHUSNI BIN ABDUL WAHAB

Thesis submitted in fulfillment of the requirements for the degree of Master of Science (Physiology)

**Faculty of Health Sciences** 

May 2019

#### ABSTRACT

*Centella asiatica* is one of the traditional herbs consumed by various communities due to its versatility and wide range of applications such as treatment for Parkinsonism, promoting memory enhancement and preventing oxidative stress. This study investigates the neuroprotective potential of Centella asiatica extract (CAE) against neurodegeneration induced by chronic stress. Forty adult Wistar rats were divided into five groups: Normal (NC), positive control (PC), CAE 200 (200 mg/kg/day), CAE 400 (400 mg/kg/day) and CAE 800 (800 mg/kg/day). Rats from respective groups were administered accordingly to respective dosages for a period of 21 days along with exposure to chronic stress by restrainer and forced swimming. The administration of CAE over a period of 21 days showed no apparent toxicity or morbidity in chronic stress-induced rats. The blood serum biochemical and haematological parameters showed no significant changes in groups supplemented with CAE with comparison to normal group (p>0.05) and the values were within normal physiological range. The administration of CAE at three different dosages showed significant neurogenesis activities through apparent thickening of dentate gyrus, improved neuroproliferation and reduced neuronal cell death (p < 0.05). The significant improvement in neurogenesis activities was reflected by significant elevation of c-fos protein expression in hippocampus of rats administered with CAE (p < 0.05). The neuroprotective potential of CAE was further assessed through metabolic patterns in blood serum, which demonstrated a significant elevation of lactate, isoleucine, proline, methionine, valine, leucine and glutamine (p < 0.05). The chronic stress-induced rats showed apparent distinction between CAE-administered groups and PC through PCA and PLS-DA analyses, as well as recovery shifting pattern towards normal group. Overall, these results suggested that CAE possess positive neuroprotective potential that can promote neurogenesis activities and reduce neuronal cell damage in chronic stress-induced rats.

### ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and Most Merciful. Thousands of praises and thanks to Him, for He has provided me with good health, courage and knowledge to complete this study. Peace and blessing of Allah be upon prophet Muhammad S.A.W. I am eternally grateful for his endless blessings.

The path towards this thesis has been circuitous. Its completion is thanks in large part to the special people who challenged, supported and stuck with me along the way. I am tremendously fortunate to have Mrs. Fatin Nadzirah binti Zakaria as my supervisor and Prof. Dr. Zulkhairi bin Amom as my co-supervisor. They have brought a depth of knowledge that few could match. I gratefully thank them for supporting my master study and giving such thoughtful feedback, always aimed at moving me forward. Though it was daunting to embark into postgraduate study, their generosity and kindness spurred me to keep on moving forward. Thank you for stepping up to save the day despite their overwhelming schedule.

I am highly indebted and thoroughly grateful to my mother for her tremendous support, both financially and emotionally. My postgraduate study would not have been possible without her wise words of encouragement, valuable advices and incessant support. This master's degree is a gift to you and to my late father.

I would like to extend my sincere gratitude to Ms Awin and Mrs Halimatul for exceptional mentoring and great help in molecular works and metabolomics analysis, which was pivotal in the thesis's development. I also owe a deep sense of gratitude to Ikhtiar as his guidance during my stay at UPM have formed and transformed my understanding of what technical dexterity and tolerance can do in the world. I thank profusely to all the staffs of Faculty of Health Sciences, UiTM, Faculty of Medicine and Health Sciences, UPM and AuRIns, UiTM for their endless support and cooperation throughout my study.

Last but not least, it is my privilege to be surrounded with supportive and whimsical friends, whom have shared merriment, despondency and dejection with me throughout my master study. In no particular partiality, Amanda Low, Norawit Suwannakarn and Dhani, thank you for saving the day.

## **TABLE OF CONTENT**

ii
iiii
iv
V
vi
Х
xi
xii
xiv
XV

CHA	APTER ONE: INTRODUCTION			
1.1	Background	1		
	1.1.1 Centella asiatica	1		
	1.1.2 Chronic Stress	2		
1.2	Problem Statement	3		
1.3	Research Objectives	5		
	1.3.1 General Objective	5		
	1.3.2 Specific Objectives	5		
1.4	Significance of the Study			
1.5	Hypothesis	7		
1.6	Scope and Limitations of the Study	7		
CHA	8			
2.1	Centella asiatica	8		
2.2	Botanical Description	9		
2.3	Chemical Composition of Centella asiatica	11		
	2.3.1 Active Constituents	13		
	•			

2.4	Medic	inal Benefits of Centella asiatica	13
	2.4.1	Wound Healing	14
	2.4.2	Antidepressant	15
	2.4.3	Anticonvulsant	15
	2.4.4	Antioxidant	16
	2.4.5	Memory Enhancement	17
	2.4.6	Neurotoxicity and Brain Injuries	17
2.5	Anator	nical Structure of Hippocampus	18
	2.5.1	Hippocampal Formation	18
	2.5.2	Hippocampus Proper	19
	2.5.3	Dentate Gyrus	20
2.6	Physio	logical Role of Hippocampus	22
	2.6.1	Memory	23
	2.6.2	Learning	24
2.7	Neurog	genesis	25
2.8	c-Fos		27
2.9	Stress		27
	2.9.1	Overview of Stress	28
	2.9.2	Effects of Acute and Chronic Stress	29
	2.9.3	Vulnerability of Hippocampus Following Chronic Stress	29
2.10	Neuroj	protectivity and Neuroregenerative Properties of Centella asiatica	30
	2.10.1	In Vitro Studies on Neuroprotection and Neuroregeneration of	
		Centella asiatica	31
	2.10.2	In Vivo Studies on Neuroprotection and Neuroregeneration of	
		Centella asiatica	32
2.11	Metab	olomics	33
	2.11.1	Metabolic Profiling	33
	2.11.2	Metabolites Associated with Neuroprotectivity	34
CHAI	PTER T	<b>'HREE: MATERIALS AND METHOD</b>	35
3.1	l Materials		
3.2	Centella asiatica Extract		
3.3	Source	e of Animals	39
3.4	Anima	l Trials	39
		V11	