



UNIVERSITI PUTRA MALAYSIA

**ROLE OF DELTA-9-TETRAHYDROCANNABINOL IN
NEUROGENESIS AND NEURONAL PLAST**

NOOR AZUIN BINTI SULIMAN

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AND NEURONAL PLAST**

By

NOOR AZUIN BINTI SULIMAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

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of the requirement for the degree of Master of Science

**ROLE OF DELTA-9-TETRAHYDROCANNABINOL IN NEUROGENESIS
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March 2013

Chair: Che Norma Binti Mat Taib, PhD

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Cannabis is classified as a halucinogen, is prepared as a bhang, ganja, hashish, and marijuana. Three putative varieties of cannabis are *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruberalis*. Delta-9-tetrahydrocannabinol (Δ^9 -THC), also known as tetrahydrocannabinol, is the main psychoactive substance derived from *Cannabis sp.*. Δ^9 -THC is used in treating pain, asthma and coughs, also acts as sedative agent. It was known to show impairment effects on variety of central effects including producing hypothermia, antinociception and changes of locomotor activity, immediate recall, memory retrieval, and also in working and short-term memory. Δ^9 -THC was known to alter the neurogenesis and neuronal plasticity observed in animal models. However, the doses used in experiments were high and even higher in cell culture method. The purposes of this study was to observe the effects of Δ^9 -THC at lower dose on neurogenesis and neuronal plasticity involving nociception and cognitive performances in acute (7 days) and chronic (21 days) treatments on

Sprague dawley rats. Three doses of Δ^9 -THC were used; 0.75, 1.5 and 3.0 mg/kg, and 0.9% normal saline with 3% ethanol was used as a control. Two types of behavioural tests were run; hot plate and novel-object discrimination (NOD) tests, for observing the nociception and cognitive performances, respectively. The brain samples were collected for further analyses with Western blot technique to determine the protein concentration of Doublecortin (DCX), *c-fos*, and downstream regulatory element modulator (DREAM) as a marker for cognitive and nociception, respectively. The brain was also investigated using immunohistochemistry (IHC) technique, in detecting the BrdU, DCX, nestin, Class III β -tubulin (TuJ-1), and glial fibrillary acidic protein (GFAP) as markers for neurogenesis. Cresyl violet stain was used for observing the neuronal cell death (NCD) present in the hippocampus of the brain. Liver and kidney were collected and further processed with hematoxylin and eosin stain in observing the toxicity effects of Δ^9 -THC. 1.5 mg/kg of Δ^9 -THC gave significant differences at $p < 0.001$ when compared to control, 0.75, and 3.0 mg/kg of Δ^9 -THC observed on the neurogenesis, cognitive function and nociceptive response. The observations were acceptable for both acute and chronic treatments, behavioural and molecularly. Meanwhile, all dosages of Δ^9 -THC showed significant differences at $p < 0.001$ as compared to control on toxicity test involving brain after giving stress. In studying the effect on liver and kidney, all dosages of Δ^9 -THC and control showed no significant difference at $p > 0.05$. From these results, it can be concluded that 1.5 mg/kg of Δ^9 -THC improved the level of neurogenesis and neuronal plasticity when compared to control, 0.75 and 3.0 mg/kg of Δ^9 -THC. Δ^9 -THC at all dosages used was observed to give neuroprotective function against stress. Treatment of Δ^9 -THC showed no toxic effect observed in the kidney and liver.

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

**PERANAN DELTA-9-TETRAHIDROCANABINOL KE ATAS GENESIS
DAN PLASTIK NEURON**

Oleh

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Canabis terdorong sebagai agen halusinasi, disediakan sebagai satu bhang, ganja, dadah, dan marijuana. Tiga jenis tumbuhan cannabis ialah *Cannabis sativa*, *Cannabis indica*, dan *Cannabis rubealis*. Delta-9-tetrahydrocannabinol (Δ^9 -THC), juga dikenali sebagai tetrahydrocannabinol, merupakan bahan utama psikoaktif yang diperoleh daripada *Cannabis sp.* Δ^9 -THC digunakan dalam merawat kesakitan, asma dan batuk, juga bertindak sebagai agen sedatif. Ia memberikan kesan pengurangan dalam pelbagai aspek utama termasuklah mendorong hipotermia, antinosisseptif, dan perubahan pada aktiviti lokomotor, panggilan segera ingatan, pengkalan data dan juga ingatan semasa dan jangka pendek. Δ^9 -THC dapat mempengaruhi genesis and plastisiti neuron dalam model haiwan. Walau bagaimanapun, dos yang digunakan dalam kajian adalah tinggi, malah ianya lebih tinggi dalam kaedah tisu kultur. Kajian in dijalankan untuk mengetahui kesan Δ^9 -THC menggunakan dos yang lebih rendah ke atas genesis and plastisiti neuron merangkumi aspek nosisseptif dan kognitif pada tempoh rawatan akut (7 hari) dan kronik (21 hari) ke atas tikus Sprague dawley. Tiga dos Δ^9 -THC digunakan; 0.75, 1.5, dan 3.0 mg.kg Δ^9 -THC serta 0.9% air garam

berserta 3% etanol sebagai kawalan. Dua ujian tingkahlaku dijalankan; plat panas dan diskriminasi objek-novel (NOD), untuk melihat kesan nosiseptif dan kognitif. Sampel otak diambil untuk dianalisis menggunakan teknik Western Blot bagi menentukan kepekatan protein Doublecortin (DCX), *c-fos*, dan *downstream regulatory element antagonistic modulator* (DREAM) kerana ia merupakan penanda untuk kognitif dan nosiseptif. Otak juga disiasat menggunakan teknik imunohistokimia (IHC) bagi mengesan BrdU, DCX, nestin, *Class III β -tubulin* (TuJ-1), dan *glial fibrillary acidic protein* (GFAP) sebagai penanda untuk genesis neuron. Pewarnaan Cresyl violet digunakan untuk mengenalpastikan sel neuron yang telah mati (NCD) pada hipokampus. Sampel hati dan ginjal diambil dan diproses menggunakan pewarnaan hematoxilin dan eosin bagi melihat kesan keracunan disebabkan oleh Δ^9 -THC. 1.5 mg / kg Δ^9 -THC memberi perbezaan-perbezaan yang ketara pada $p < 0.001$ apabila dibandingkan dengan kawalan, 0.75 and 3.0 mg/kg Δ^9 -THC pada fungsi genesis neuron, kognitif dan nosiseptif. Pemerhatian-pemerhatian boleh diterimapakai oleh kedua-dua rawatan akut dan kronik melalui perubahan tingkahlaku dan molekular. Sementara itu, semua dos Δ^9 -THC menunjukkan perbezaan pada $p < 0.001$ berbanding dengan kawalan dalam ujian ketoksikan melibatkan otak selepas diberikan tekanan. Dalam mengkaji kesan ke atas hati dan buah pinggang, semua dos Δ^9 -THC dan kawalan tidak menunjukkan perbezaan pada $p > 0.05$. Dari hasil ujian, disimpulkan bahawa 1.5 mg/kg Δ^9 -THC dapat meningkatkan kadar genesis dan plastisiti neuron berbanding kawalan, 0.75, and 3.0 mg/kg Δ^9 -THC. Δ^9 -THC memberi fungsi neuroprotektif terhadap stress. Rawatan Δ^9 -THC menunjukkan tiada kesan ketoksikan pada buah pinggang dan hati.

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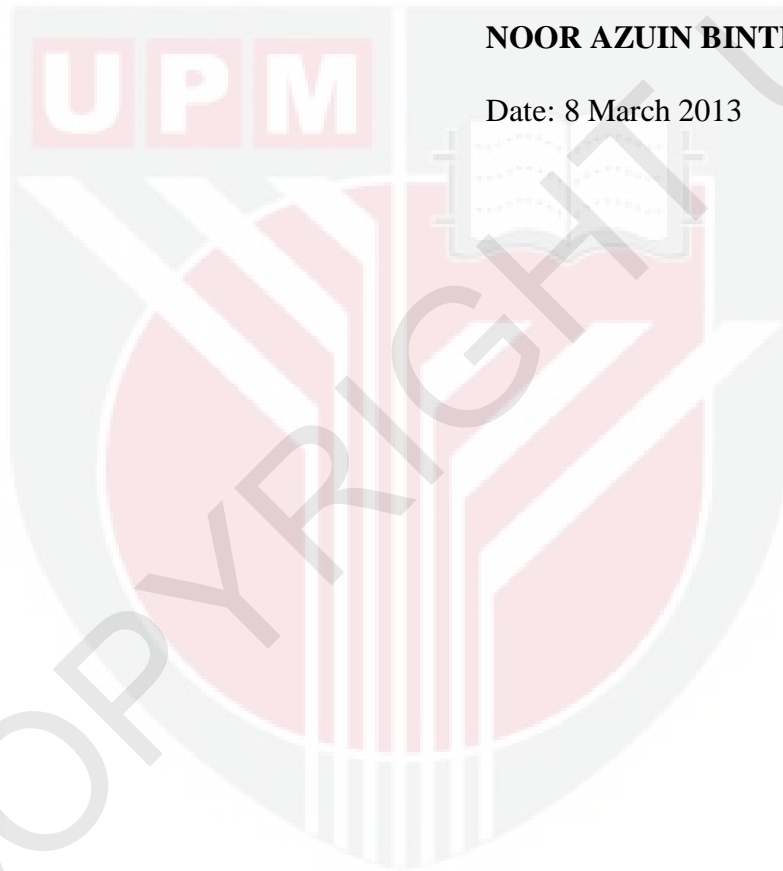
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DECLARATION

I declare that the thesis is my original work except for the 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.

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NOOR AZUIN BINTI SULIMAN

Date: 8 March 2013



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TABLE OF CONTENTS

		Page
	ABSTRACT	ii
	ABSTRAK	iv
	ACKNOWLEDGEMENTS	vi
	APPROVAL	vii
	DECLARATION	ix
	LIST OF TABLES	xiii
	LIST OF FIGURES	xv
	LIST OF APPENDICES	xxii
	LIST OF ABBREVIATIONS	xxiii
	CHAPTER	
1.	INTRODUCTION	1
2.	LITERATURE REVIEW	7
	Cannabis	7
	Delta-9-tetrahydrocannabinol (Δ^9 -THC)	10
	Properties of Δ^9 -THC	10
	Δ^9 -THC in neurogenesis	13
	Δ^9 -THC in cognitive	15
	Δ^9 -THC in nociception	19
	Toxicity, neurotoxic, and neuroprotective properties of Δ^9 -THC	21
	Tolerance of Δ^9 -THC	23
	Hippocampus	25
	Neurogenesis	27
	Introduction of neurogenesis	27
	Mechanism of neurogenesis	30
	Bromodeoxyuridine (BrdU) in neurogenesis	33
	Neuronal plasticity	34
	Cognition Performance	35
	Cognitive function	35
	Behavioural performance	37
	Doublecortin (DCX) expression and cognitive function	39
	Brain-derived neurotrophic factor (BDNF) and cognitive function	40
	Nociceptive Responses	42
	Nociception	42
	Behavioural performance	44
	Downstream regulatory element antagonistic modulator (DREAM) in nociception	46
	<i>C-fos</i> in nociception	48
	Toxicity	50
	Neuronal cell death (NCD)	50
	Toxicity	52
	Kidney	53
	Liver	55

	Summary	57
3.	Δ^9-THC ON NEUROGENESIS ON SPRAGUE DAWLEY	59
	Introduction	59
	Materials and Methods	60
	Preparation of treatment	60
	Treatment on Sprague dawley	61
	Treatment of BrdU	62
	Slide preparation	62
	Staining	63
	Scoring	64
	Analysis	66
	Results	66
	Discussion	86
	Conclusion	92
4.	Δ^9-THC ON COGNITION ON SPRAGUE DAWLEY	93
	Introduction	93
	Materials and Methods	94
	Preparation of treatment	94
	Treatment on Sprague dawley	95
	Behavioural test	95
	Protein determination	96
	ELISA technique	99
	Analysis	100
	Results	100
	Behavioural test	100
	Protein determination	112
	ELISA technique	116
	Discussion	118
	Conclusion	124
5.	Δ^9-THC ON NOCICEPTION ON SPRAGUE DAWLEY	126
	Introduction	126
	Materials and Methods	128
	Preparation of treatment	128
	Treatment on Sprague dawley	129
	Nociceptive test	129
	Protein determination	130
	Analysis	132
	Results	132
	Behavioural test	132
	Protein determination	135
	Discussion	143
	Conclusion	150
6.	TOXICITY OF Δ^9-THC ON SPRAGUE DAWLEY	151
	Introduction	151

Materials and Methods	153
Preparation of treatment	153
Treatment on Spraque dawley	153
Thermal-stress	153
Slide preparation	154
Staining	155
Scoring	156
Analysis	159
Results	159
Discussion	170
Conclusion	176
7. SUMMARY, GENERAL CONCLUSION, AND RECOMMENDATION FOR FUTURE RESEARCH	177
REFERENCES	180
APPENDICES	212
BIODATA OF STUDENT	224