UNIVERSITI PUTRA MALAYSIA

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

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

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

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Cannabis is classified as a hallucinogen, 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 (Δ⁹-THC), also known as tetrahydrocannabinol, is the main psychoactive substance derived from Cannabis sp.. Δ⁹-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. Δ⁹-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 Δ⁹-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 Δ⁹-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 inveigated 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 Δ⁹-THC. 1.5 mg/kg of Δ⁹-THC gave significant differences at $p < 0.001$ when compared to control, 0.75, and 3.0 mg/kg of Δ⁹-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 Δ⁹-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 Δ⁹-THC and control showed no significant difference at $p > 0.05$. From these results, it can be concluded that 1.5 mg/kg of Δ⁹-THC improved the level of neurogenesis and neuronal plasticity when compared to control, 0.75 and 3.0 mg/kg of Δ⁹-THC. Δ⁹-THC at all dosages used was observed to give neuroprotective function against stress. Treatment of Δ⁹-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 ruberalis. Delta-9-tetrahidrocanabinol (Δ⁹-THC), juga dikenali sebagai tetrahidrocanabinol, merupakan bahan utama psikoaktif yang diperoleh daripada Cannabis sp.. Δ⁹-THC digunakan dalam merawat kesakitan, asma dan batuk, juga bertindak sebagai agen sedatif. Ia memberikan kesan pengurangan dalam pelbagai aspek utama termasuklah mendorong hipotermia, antinosiseptif, dan perubahan pada aktiviti lokomotor, panggilan segara ingatan, pengkalan data dan juga ingatan semasa dan jangka pendek. Δ⁹-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 ini dijalankan untuk mengetahui kesan Δ⁹-THC menggunakan dos yang lebih rendah ke atas genesis and plastisiti neuron merangkumi aspek nosiseptif dan kognitif pada tempoh rawatan akut (7 hari) dan kronik (21 hari) ke atas tikus Sprague dawley. Tiga dos Δ⁹-THC digunakan; 0.75, 1.5, dan 3.0 mg.kg Δ⁹-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 fibrially 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 Δ⁹-THC. 1.5 mg / kg Δ⁹-THC memberi perbezaan-perbezaan yang ketara pada p < 0.001 apabila dibandingkan dengan kawalan, 0.75 and 3.0 mg/kg Δ⁹-THC pada fungsi genesis neuron, kognitif dan nosiseptif. Pemerhatian-pemerhatian boleh diterimakapai oleh kedua-dua rawatan akut dan kronik melalui perubahan tingkahlaku dan molekular. Sementara itu, semua dos Δ⁹-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 Δ⁹-THC dan kawalan tidak menunjukkan perbezaan pada p > 0.05. Dari hasil ujian, disimpulkan bahawa 1.5 mg/kg Δ⁹-THC dapat meningkatkan kadar genesis dan plastisiti neuron berbanding kawalan, 0.75, and 3.0 mg/kg Δ⁹-THC. Δ⁹-THC memberi fungsi neuroprotektif terhadap stress. Rawatan Δ⁹-THC menunjukkan tiada kesan ketoksikan pada buah pinggang dan hati.
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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

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxiii</td>
</tr>
</tbody>
</table>

## CHAPTER 1

### 1. INTRODUCTION

1.

## CHAPTER 2

### 2. LITERATURE REVIEW

7

Cannabis 7

Delta-9-tetrahydrocannabinol (\(\Delta^9\)-THC) 10

Properties of \(\Delta^9\)-THC 10

\(\Delta^9\)-THC in neurogenesis 13

\(\Delta^9\)-THC in cognitive 15

\(\Delta^9\)-THC in nociception 19

Toxicity, neurotoxic, and neuroprotective properties of \(\Delta^9\)-THC 21

Tolerance of \(\Delta^9\)-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

\(C\)-fos in nociception 48

Toxicity 50

Neuronal cell death (NCD) 50

Toxicity 52

Kidney 53

Liver 55
Materials and Methods
Preparation of treatment 153
Treatment on Sprague 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