STUDIES OF MITRAGYNINE, THE PRINCIPLE ALKALOID OF *MITRAGYNA SPECIOSA KORTH* (KRATOM) ON ITS ABUSE AND ADDICTIVE PROPERTIES IN RAT BEHAVIOURAL MODELS

NORSYIFA BINTI HARUN

UNIVERSITI SAINS MALAYSIA

2016
STUDIES OF MITRAGYNINE, THE PRINCIPLE ALKALOID OF MITRAGYNA SPECIOSA KORTH (KRATOM) ON ITS ABUSE AND ADDICTIVE PROPERTIES IN RAT BEHAVIOURAL MODELS

by

NORSYIFA BINTI HARUN

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

March 2016
ACKNOWLEDGEMENT

First and foremost, I am grateful to The Mighty God for enabling me to complete this challenging PhD journey. This thesis would not have been possible to be completed without His blessing and the help and support of these kind people around me.

I would like to express my gratitude to my principal supervisor Dr Zurina Hassan for her indispensible guidance and support. I am also extremely indebted to both my co-supervisors Professor Dr Sharif Mahsufi Mansor and Professor Emeritus Dato’ Dr Visweswaran Navaratnam for their continuous support and unsurpassed knowledge of natural products as well as in initiation of the research. My deepest appreciation goes to my field supervisor, Dr Mohammed Shoaib from Institute of Neuroscience, Newcastle University, United Kingdom for his helpful and constructive advice, kindness and continuous encouragement during the execution of this research project. As part of collaboration between Universiti Sains Malaysia and Newcastle University, Dr Mohammed Shoaib has provided me with four months training of operant behaviour techniques in his own lab before these techniques could be transferred to our laboratory in Centre for Drug Research.

My gratitude extends to all the staffs and students of Centre for Drug Research, Universiti Sains Malaysia, specifically the Neurobehavioral group for the help and support given to finish this research project.
I would also like to express my sincere thanks to my husband, Muhammad Syazwan bin Hashim for his continual encouragement and sustained support. I would like to thank my little daughter, Safiyyah binti Muhammad Syazwan who has inspired me to complete this thesis. This acknowledgement also goes to my parents; Haji Harun bin Sidik and Hajjah Sabariah binti Mohd Akib, the whole family and family in laws for their unequivocal moral support throughout this PhD journey.

Last but not least, I would like to acknowledge all these funding resources for financially supporting my research work; Higher Education Centre of Excellence (HiCoE) special funding, USM Research University Grant (RUT), International Research Collaboration Fund and MyBrain15 Scholarship from Ministry of Higher Education.
TABLE OF CONTENTS

Acknowledgement ........................................................................................................ ii
Table of Contents ........................................................................................................... iii
List of Tables ................................................................................................................ x
List of Figures ................................................................................................................ xi
List of Abbreviations ..................................................................................................... xvi
List of Symbols ............................................................................................................. xviii
List of Appendices ......................................................................................................... xix
Abstrak ........................................................................................................................... xx
Abstract ......................................................................................................................... xxii

CHAPTER 1 – INTRODUCTION .................................................................................... 1

1.1 Problem statements ................................................................................................. 4
1.2 Objectives of study .................................................................................................. 6

CHAPTER 2 – LITERATURE REVIEW ......................................................................... 8

2.1 *Mitragyna speciosa* Korth (kratom)..................................................................... 8
   2.1.1 Botanical origin ................................................................................................. 9
   2.1.2 Plant preparation and consumption ................................................................. 10
   2.1.3 Kratom consumption in humans ..................................................................... 13
   2.1.4 Phytochemistry ................................................................................................ 15
2.4 Drug discrimination ................................................................. 45
  2.4.1 Background ................................................................. 45
  2.4.2 Operant behaviour of drug discrimination .............................. 46
  2.4.3 Discrimination training ................................................... 47
  2.4.4 Stimulus generalisation or substitution test ............................. 48
  2.4.5 Relevance of drug discrimination model in abuse liability assessment 50
  2.4.6 Advantages and disadvantages of drug discrimination model .......... 50

2.5 Drug addiction ...................................................................... 52
  2.5.1 Stages of addiction ........................................................... 52
  2.5.2 Neurobiology of addiction ............................................... 53
  2.5.3 Putative addictive properties of kratom ................................ 55

2.6 Drug self-administration ....................................................... 57
  2.6.1 Background ................................................................. 57
  2.6.2 Operant behaviour of intravenous drug self-administration .......... 58
  2.6.3 Substitution procedure .................................................... 61
  2.6.4 Relevance of intravenous drug self-administration model in addiction research as well as abuse liability assessment ....................... 62
  2.6.5 Advantages and disadvantages of intravenous drug self-administration model .......................................................... 63

2.7 Summary ............................................................................. 64

CHAPTER 3 – METHODOLOGY .................................................... 66

3.1 Discriminative stimulus properties of mitragynine ........................ 66
3.1.1 Animals ................................................................. 66
3.1.2 Apparatus .............................................................. 67
3.1.3 Drugs ................................................................. 69
3.1.4 Drug discrimination procedure .................................. 70
  3.1.4.1 Lever-press training session ............................ 70
  3.1.4.2 Discrimination training session .......................... 72
  3.1.4.3 Stimulus control test ....................................... 77
  3.1.4.4 Stimulus substitution or generalisation test session .... 78
3.1.5 Experimental designs ............................................. 82
  3.1.5.1 Dose response evaluations of mitragynine and morphine associated discrimination ........................................... 82
  3.1.5.2 Effect of substitution of morphine on mitragynine associated discrimination ....................................................... 82
  3.1.5.3 Effect of substitution of mitragynine on morphine associated discrimination ...................................................... 83
  3.1.5.4 Effect of substitution of 7-hydroxymitragyne on morphine associated discrimination ............................................... 83
  3.1.5.5 Effect of substitution of cocaine (psychostimulant) on mitragynine associated discrimination ..................................... 84
  3.1.5.6 Effect of substitution of cocaine (psychostimulant) on morphine associated discrimination ..................................... 84
  3.1.5.7 Effect of naloxone pre-treatment on the discriminative stimulus effect of mitragynine and morphine .............................. 85
  3.1.5.8 Effect of naloxone pre-treatment on 7-hydroxymitragynine substitution on morphine associated discrimination ............. 86
3.1.6 Parameters for the assessment of discriminative stimulus effect ........ 86
3.1.7 Statistical analysis ........................................................................... 87

3.2 Effects of mitragynine in rat model of fentanyl dependence ................. 88

3.2.1 Animals ......................................................................................... 88
3.2.2 Drugs .............................................................................................. 89
3.2.3 Apparatus ....................................................................................... 89
3.2.4 The microrenathane catheter preparation ............................................ 91
   3.2.4.1 Catheter assembly ................................................................. 91
3.2.5 Catheter accessories ........................................................................ 95
   3.2.5.1 The flushing syringe .............................................................. 95
   3.2.5.2 The stylet cap and protective cover ....................................... 97
3.2.6 Intravenous catheter implantation surgery ......................................... 98
   3.2.6.1 Catheterisation procedures ................................................... 99
   3.2.6.2 Head mounted cannula connector implantation procedures 104
   3.2.6.3 Cathether evaluation, failure and maintenance ...................... 108
3.2.7 Drug infusion system ...................................................................... 109
3.2.8 Preparation of the operant self-administration chamber ..................... 111
3.2.9 Acquisition of fentanyl self-administration ....................................... 113
3.2.10 Saline substitution test .................................................................. 116
3.2.11 Vehicle (20% Tween 20 solution) substitution test ......................... 116
3.2.12 Cross-substitution tests with mitragynine ..................................... 117
3.2.13 Statistical analysis ....................................................................... 117
4.1 Discriminative stimulus properties of mitragynine ........................................ 119

4.1.1 Acquisition of mitragynine and morphine discriminative stimuli ..... 119

4.1.2 Mitragynine and morphine produced discriminative stimulating effects ................................................................................................................................. 123

4.1.3 Generalisation tests with graded doses of mitragynine and morphine ................................................................................................................................. 125

4.1.4 Morphine substitution tests in mitragynine-trained rats ................. 127

4.1.5 Mitragynine substitution tests in morphine-trained rats ............... 129

4.1.6 7-Hydroxymitragynine substitution tests in morphine-trained rats ... 131

4.1.7 Effects of 7-hydroxymitragynine compared to morphine and mitragynine in substitution for morphine discriminative stimulus .......... 133

4.1.8 Cocaine substitution tests for mitragynine-trained rats ............... 135

4.1.9 Cocaine substitution tests for morphine-trained rats ................. 135

4.1.10 Effect of naloxone pre-treatment on discriminative stimulus effects of mitragynine and morphine ................................................................. 137

4.1.11 Effect of naloxone pre-treatment on discriminative stimulus effects of 7-hydroxymitragynine .................................................................................. 140

4.2 Effects of mitragynine in rat model of fentanyl dependence .................. 142

4.2.1 Acquisition of fentanyl self-administration ........................................... 142

4.2.2 Maintenance of fentanyl self-administration ....................................... 144

4.2.3 Saline substitution (extinction) test ....................................................... 147

4.2.4 Vehicle (20% Tween 20 solution) substitution test ............................ 150
4.2.5 0.3 mg/kg/infusion of mitragynine substitution ................................ 152
4.2.6 1.0 mg/kg/infusion of mitragynine substitution .............................. 154
4.2.7 3.0 mg/kg/infusion of mitragynine substitution .............................. 156
4.2.8 Summary of mitragynine profile in rat model of fentanyl dependence
............................................................................................................. 158

CHAPTER 5 – DISCUSSION ........................................................................... 159

CHAPTER 6 – CONCLUSION ....................................................................... 190

Study limitations .......................................................................................... 192
Future studies .................................................................................................. 193
References ...................................................................................................... 194
List of publications ......................................................................................... 221
Appendices ..................................................................................................... 222
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Reasons for using kratom in specific population at Northern Malaysia</td>
<td>15</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Structures and potential effects of alkaloids extracted from kratom</td>
<td>18</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Potency of 7-hydroxymitragynine in relative to morphine and mitragynine in substitution for morphine discriminative stimulus demonstrated by this study</td>
<td>134</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>The experimental design demonstrating the assessment of kratom abuse and addictive potential using two behavioural models</td>
<td>7</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>The plant of <em>Mitragyna speciosa</em> Korth (1) Leaves of the plant, (2) naturally occurring trees, (3) and (4) cultivated plants</td>
<td>10</td>
</tr>
<tr>
<td>Figure 2.2(A)</td>
<td>A kratom user chewing the kratom leaves</td>
<td>12</td>
</tr>
<tr>
<td>Figure 2.2(B)</td>
<td>Products of kratom sold in the market</td>
<td>12</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Molecular structures of mitragynine, 7-hydroxymitragynine, morphine and cocaine</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Schematic illustration of drug discrimination training session utilising two-lever operant conditioning approach</td>
<td>48</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Schematic illustration of stimulus generalisation or substitution test session utilising two-lever operant conditioning approach</td>
<td>49</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Schematic illustration of operant conditioning chamber for intravenous self-administration in rats</td>
<td>60</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Components of operant behavioural package for rat</td>
<td>69</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>A standard two-lever operant conditioning chamber of drug discrimination study in rats</td>
<td>76</td>
</tr>
</tbody>
</table>
Figure 3.3  The schematic illustration of drug discrimination procedures

Figure 3.4(A)  The experimental design demonstrating the test session schedules for rats trained with mitragynine

Figure 3.4(B)  The experimental design demonstrating the test session schedules for rats trained with morphine

Figure 3.5  A complete assembled microrenathane catheter with a bump made of silicon sealant

Figure 3.6  A flushing syringe

Figure 3.7(A)  A stylet cap

Figure 3.7(B)  A threaded protective cover

Figure 3.8  Steps of catheterisation procedures

Figure 3.9  Steps of head mounted cannula connector implantation procedures

Figure 3.10  Materials for setting up a drug infusion system

Figure 3.11(A)  A Syringe pump with drug solution attached to swivel system via PVC tubing

Figure 3.11(B)  Intravenous self-administration operant conditioning chamber

Figure 3.12  The experimental design demonstrating the phases of cross-substitution tests
Figure 4.1(A) Training data for acquisition of mitragynine as discriminative stimulus 122

Figure 4.1(B) Training data for acquisition of morphine as discriminative stimulus 122

Figure 4.2 Stimulus control tests under extinction conditions of both mitragynine and morphine 124

Figure 4.3(A) Dose-response curve in rats trained to discriminate mitragynine from vehicle 126

Figure 4.3(B) Dose-response curve in rats trained to discriminate morphine from vehicle 126

Figure 4.4 Morphine substitution maintained discriminative behaviour in rats previously treated with mitragynine 128

Figure 4.5 Mitragynine substitution maintained drug discriminative behaviour in rats previously trained with morphine 130

Figure 4.6 7-Hydroxymitragynine substitution maintained drug discriminative behaviour in rats previously treated with morphine 132

Figure 4.7(A) Cocaine substitution maintained drug discriminative behaviour in rats previously treated with mitragynine 136

Figure 4.7(B) Cocaine substitution did not maintain drug discriminative behaviour in rats previously treated with morphine 136

Figure 4.8(A) Effect of pre-treatment of naloxone doses on discriminative stimulus effects of morphine 139
Figure 4.8(B)  Effect of pre-treatment of naloxone doses on discriminative stimulus effects of mitragynine 139

Figure 4.9  Effect of naloxone pre-treatment on of 3.0 mg/kg of morphine-like effects of 7-hydroxymitragynine 141

Figure 4.10  Acquisition profile of rats trained to self-administer fentanyl 143

Figure 4.11(A)  Number of infusions earned across incremental FR session in rats responding for fentanyl 146

Figure 4.11(B)  Total average of active and inactive lever responses of fentanyl self-administration across incremental FR session 146

Figure 4.12(A)  The profile of extinction when saline was substituted for fentanyl 149

Figure 4.12(B)  Total average of active and inactive responses when saline was substituted for fentanyl 149

Figure 4.13(A)  The number of infusions of vehicle (20% Tween-20 solution) self-administered over the three test sessions 151

Figure 4.13(B)  Total average of active and inactive lever responses when vehicle (20% Tween-20 solution) was substituted for fentanyl 151

Figure 4.14(A)  The number of infusions of 0.3 mg/kg mitragynine self-administered over the three test sessions 153
Figure 4.14(B) Total average of active and inactive lever responses when 0.3 mg/kg/infusion of mitragynine was substituted for fentanyl

Figure 4.15(A) The number of infusions of 1.0 mg/kg mitragynine self-administered over the three test sessions

Figure 4.15(B) Total average of active and inactive lever responses when 1.0 mg/kg/infusion of mitragynine was substituted for fentanyl

Figure 4.16(A) The number of infusions of 3.0 mg/kg mitragynine self-administered over the three test sessions

Figure 4.16(B) Total average of active and inactive lever responses when 3.0 mg/kg/infusion of mitragynine was substituted for fentanyl
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>Intracerebroventricular</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>i.e.</td>
<td>That is</td>
</tr>
<tr>
<td>e.g.</td>
<td>Example</td>
</tr>
<tr>
<td>etc.</td>
<td>Et cetera (and other things)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximal reaction concentration</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximal reaction time</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median lethal dose</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Effective dose at 50% of activity</td>
</tr>
<tr>
<td>95% CI</td>
<td>95% of confidence interval</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error mean</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
</tbody>
</table>

xvi
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>min</td>
<td>Minute (s)</td>
</tr>
<tr>
<td>h</td>
<td>Hour (s)</td>
</tr>
<tr>
<td>sec</td>
<td>Sec (s)</td>
</tr>
<tr>
<td>µg/ml</td>
<td>Microgram per millilitre</td>
</tr>
<tr>
<td>mg/ml</td>
<td>Milligram per millilitre</td>
</tr>
<tr>
<td>ml/kg</td>
<td>Millilitre per kilogram</td>
</tr>
<tr>
<td>mg/kg</td>
<td>Milligram per kilogram</td>
</tr>
<tr>
<td>ng/ml</td>
<td>Nanogram per millilitre</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume over volume</td>
</tr>
<tr>
<td>IU/ml</td>
<td>International unit per millilitre</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
</tbody>
</table>
LIST OF SYMBOLS

%  Percentage
µ  Mu
δ  Delta
κ  Kappa
α  Alpha
±  Plus minus
°  Degree
ºC  Degree Celsius
n  Number of animals
=  Equal to
>  Greater than
≥  Greater than or equal to
≤  Less than or equal to
™  Trade mark
©  Copyright
®  Registered
LIST OF APPENDICES

Appendix A  Animal ethical clearance letters
Appendix B  Fentanyl calculation of unit dose per infusion
Appendix C  Mitragynine calculation of unit dose per infusion
Appendix D  Non-linear regression plot for mitragynine substitution to morphine discriminative stimulus
Appendix E  Non-linear regression plot for morphine substitution to mitragynine discriminative stimulus
Appendix F  Non-linear regression plot for 7-hydroxymitragynine substitution to morphine discriminative stimulus
Appendix G  Non-linear regression plot for morphine generalisation curve
Appendix H  Publications
Appendix I  Poster presentation
KAJIAN TENTANG MITRAGININA, ALKALOID UTAMA DARIPADA
MITRAGYNA SPECIOSA KORTH (KRATOM) KE ATAS SIFAT-SIFAT
PENYALAHGUNAAN DAN KETAGIHAN DALAM MODEL-MODEL
TINGKAH LAKU TIKUS

ABSTRAK

Mitraginina adalah alkaloid aktif utama yang dipencil daripada daun
Mitragyna speciosa atau kratom. Sementara mitraginina atau kratom menghasilkan
kesan-kesan analgesik, anti-radang dan relaksi otot, namun sedikit yang telah
diketahui tentang sifat-sifat penyalahgunaan dan penagihannya. Diberikan
kemungkinan kepentingan sistem opioid dalam perantaraan kesan-kesan farmakologi
mitraginina, kajian ini bertujuan meninjau kesan-kesan tingkah laku daripada
mitraginina menggunakan tatacara pendiskriminasian drug dan pemberian sendiri
drug. Tikus jantan jenis Sprague Dawley telah dilatih untuk mendiskriminasi antara
mitraginina dan pembawa menggunakan tatacara pendiskriminasian drug dua tuil di
bawah pembolehubah selang secara tandem (VI-60 saat) dan nisbah tetap (FR-10)
dalam jadual pengukuhan makanan. Tikus-tikus itu telah berjaya memperoleh
pendiskriminasian mitraginina (15.0 mg/kg, i.p.) yang mirip dengan kumpulan tikus
yang satu lagi dengan pendiskriminasian morfina (5.0 mg/kg, i.p.) Untuk
memperoleh pemahaman yang lebih baik tentang penyerupaan farmakologi yang
dikongsi antara mitraginina dan morfina, siri-siri ujian penukargantian yang serupa
telah dijalankan pada tikus yang dilatih untuk mendiskriminasi morfina daripada
pembawa. Mitraginina telah ditukargantikan sepenuhnya kepada rangsangan
diskriminatif morfina dan sebaliknya, mencadangkan penyerupaan farmakologi di
antara kedua-dua jenis drug. Mekanisme farmakologi daripada derivatif mitraginina, 7-hidroksimitraginina dengan fokus yang spesifik kepada penglibatan reseptor opioid juga telah diperiksa pada tikus-tikus yang dilatih untuk mendiskriminasi morfina daripada pembawa. Pemberian derivatif 7-hidroksimitraginina pada dos 3.0 mg/kg (i.p.) mengakibatkan penukargantian penuh kepada rangsangan diskriminatif morfina. Dalam usaha mengkaji kesan tindakan dual mitraginina, kesan penukargantian kokaina terhadap rangsangan diskriminatif mitraginina telah dijalankan pada tikus-tikus yang telah dilatih dengan mitraginina. Rangsangan mitraginina menunjukkan penukargantian separa ke atas rangsangan kokaina (10.0 mg/kg, i.p.) manakala kesan ini tidak dapat dilihat dalam tikus-tikus yang dilatih untuk mendiskriminasi morfina daripada pembawa. Oleh kerana sifat-sifat subjektif yang menyerupai opioid dikesan melalui tatacara pendiskriminasian drug, kajian selanjutnya bertujuan untuk menilai profil mitraginina dalam model tikus yang menunjukkan kebergantungan kepada fentanil (agonis µ-opioid) menggunakan tatacara dua tuil ‘pilihan’ pemberian sendiri drug. Mitraginina dalam tiga unit dos (0.3, 1.0 dan 3.0 mg/kg/infusi) telah mengekalkan gerak balas tekan tuil daripada pemberian sendiri fentanil (2.0 µg/kg/infusi). Profil sepanjang ujian saling penukargantian mitraginina berbeza daripada aras-aras garis dasar yang diperlihatkan dalam ujian-ujian pemupusan, lalu mencadangkan mitraginina mungkin mempunyai kesan-kesan pengukuhan. Keseluruhananya, kesan rangsangan diskriminatif dan potensi penghasilan kebergantungan yang ditunjukkan oleh mitraginina memberikan sebahagian bukti kepada potensi penyalahgunaan dan ketagihannya yang boleh menyokong pengelasan mitraginina atau kratom sebagai dadah yang berbahaya.
STUDIES OF MITRAGYNINE, THE PRINCIPLE ALKALOID OF
MITRAGYNA SPECIOSA KORTH (KRATOM) ON ITS ABUSE AND
ADDICTIVE PROPERTIES IN RAT BEHAVIOURAL MODELS

ABSTRACT

Mitragynine is the main active alkaloid isolated from the leaves of Mitragyna
speciosa or kratom. While mitragynine or kratom provides analgesic, anti-
inflammatory and muscle relaxant effects, little is known about its abuse and
addictive properties. Given the likely importance of opioid system in mediating the
pharmacological effects of mitragynine, the present study aims to explore the
behavioural effects of mitragynine using drug discrimination and drug self-
administration procedures. Male Sprague Dawley rats were trained to discriminate
between mitragynine and vehicle in two-lever drug discrimination procedure under a
tandem variable-interval (VI-60 sec) and fixed-ratio (FR-10) schedule of food
reinforcement. The rats successfully acquired mitragynine discrimination (15.0
mg/kg, i.p.) which was similar to another group of rats with morphine discrimination
(5.0 mg/kg, i.p.). To gain a better understanding on the pharmacological similarities
shared between mitragynine and morphine, a similar series of substitution tests were
conducted in rats trained to discriminate morphine from vehicle. Mitragynine was
fully substituted to the morphine discriminative stimulus and vice versa, suggesting
pharmacological similarities between the two drugs. The pharmacological
mechanism of mitragynine derivative, 7-hydroxymitragynine with a specific focus on
opioid receptor involvement was also examined in rats trained to discriminate
morphine from vehicle. The administration of 7-hydroxymitragynine derivative at
3.0 mg/kg (i.p.) dose engendered full substitution to the morphine discriminative stimulus. In order to study the dual actions of mitragynine, the effect of cocaine substitution to the mitragynine discriminative stimulus was performed in mitragynine-trained rats. The mitragynine stimulus was partially substituted to cocaine (10.0 mg/kg, i.p.) stimulus while this effect was not observed in rats trained to discriminate morphine from vehicle. Due to the ‘opioid-like’ subjective properties detected in drug discrimination model, further studies assessed the profile of mitragynine in rat model of fentanyl (i.e. μ-opioid agonist) dependence using two-lever ‘choice’ of self-administration procedure. Mitragynine at three unit doses (0.3, 1.0 and 3.0 mg/kg/infusion) maintained the lever-pressing responses of the fentanyl (2.0 μg/kg/infusion) self-administration. The profile over the mitragynine cross-substitution tests was different from baseline levels observed during extinction tests, suggesting that mitragynine may have its reinforcing effects. Altogether, the discriminative stimulus effect and dependence-producing potential exhibited by mitragynine provide some evidence for its abuse and addictive potential, which may support the classification of mitragynine or kratom as a harmful drug.
CHAPTER 1

INTRODUCTION

It has been documented that natural products have contributed to development of new psychoactive drugs primarily due to pain relieving effects. Recently, new natural products appear with more than doubled over the period of 2009 to 2013 on the drug markets (UNODC, 2014). The emerging search of new psychoactive drugs brings forward the long traditional use of the products to the global markets (Rosenbaum et al., 2012). Regardless of the efficacy of the products which are yet to be proven in controlled clinical trials, such products are being used extensively. Although they are considered safe due to their natural origins (Kronstrand et al., 2011), animal studies and clinical reports such as toxicity, adverse effects, as well as abuse-and addictive-related effects may indicate their potential risks to humans.

One natural product that appears on the drug scene and gains widespread popularity in Malaysia is *Mitragyna speciosa* Korth or kratom (Adkins et al., 2011; Hassan et al., 2013). Mitragynine is the main active alkaloid extracted from the leaves of *M. speciosa* Korth (Shellard, 1974; Ponglux et al., 1994). For this reason, mitragynine is assumed to be the major chemical responsible for the effects of the plant. There are also other constituents of *M. speciosa* that may contribute to the effects which may involve 7-hydroxymitragynine, a minor constituent of the leaves extracts (Ponglux et al., 1994). Many scientific reports have indicated that mitragynine produces a variety of pharmacologic effects which include the induction
of analgesic effects (Matsumoto et al., 1996a; Stolt et al., 2014), inhibition of ileum motility (Watanabe et al., 1997) and vas deferens contractions of smooth muscle (Matsumoto et al., 2005b), as well as the inhibition of gastric acid secretion (Tsuchiya et al., 2002), which is consistent with the actions of morphine. In addition, studies with opioid receptor antagonists indicate that the effects are primarily mediated by the actions on opioid receptors (Matsumoto et al., 2005b; Watanabe et al., 1997).

However, the psychoactive properties of *M. speciosa* appear to be contradictory as there are reports claiming it possesses both narcotic and stimulant properties (Assanangkornchai et al., 2007; Macko et al., 1972; Ward et al., 2011; Grewal, 1932a; Grewal, 1932b; Suwanlert, 1975, Reanmongkol et al., 2007). This suggests that kratom produces an unusual combination of opioid- and psychostimulant-like effects. Nevertheless, the pharmacological basis underlying the dual effects of mitragynine is still unclear. The stimulant and euphoric effects exerted by kratom caused the plant to be extensively misused as a herbal drug. Due to easy availability and cheap herbal preparation, kratom abuse has gained significant attention in Malaysia and Thailand (Chan et al., 2005). Furthermore, the wide availability of kratom to purchase through the internet reflects an increasing demand of misusing this herbal product globally (Boyer et al., 2008).

The likelihood that a drug will be abused is generally based on the subjective and reinforcing effects exerted by the drug (Moser et al., 2011b). This has provided the framework for animal models of drug of abuse. Drug discrimination is used as a model to study the subjective effects of psychoactive drugs since the action of a drug’s discriminative stimulus in animals is closely related to its subjective effects in
human (Overton, 1988). Given that the action of mitragynine resembles morphine-like (opioid) effects, it was hypothesised that mitragynine may share discriminative stimulus properties with morphine. Intravenous drug self-administration model is used to assess the reinforcing effects of drugs to reveal the reasons why humans abuse and become addicted to drugs (Shoaib, 2006). A cross-substitution experiment in which rats previously trained to self-administer intravenous injections of opioid will be presented with various doses of mitragynine. The dependence-producing potential of mitragynine from rat model of opioid dependence provides information on its potential reinforcing effects.

The research to date has tended to focus on the effects of kratom on health, psychological, cognitive, behavioural and social impact (Macko et al., 1972; Moklas et al., 2008; Apryani et al., 2010; Idayu et al., 2011; Ahmad and Aziz, 2012; Sabetghadam et al., 2013a; Sabetghadam et al., 2013b; Yusoff et al., 2014) with little attention towards the assessment of abuse and addictive potential of the plant extracts or its active compounds. The present study addresses the assessment of abuse and addictive properties of the naturally occurring compound mitragynine, the major psychoactive constituent of *M. speciosa* Korth or kratom using rat behavioural models.
1.1 Problem statements

Kratom has been widely used for decades for both medical and non-medical purposes. Even though the efficacies are yet to be scientifically proven in humans, kratom is continued to be used extensively. The available human reports on kratom abuse and addictive properties have heightened the need for the assessment of its abuse and addictive potential.

As a major constituent of *M. speciosa*, mitragynine is known as a psychoactive compound that produces major pharmacologic effects at opioid receptors. Most studies in the past decades have shown the resemblance of mitragynine towards morphine-like pharmacological characteristics. Thus, there is an increasing concern that mitragynine is being disadvantaged to the similar extent as the established opioid (*i.e.* morphine). Since drugs of abuse in humans produce subjective effects in experimental animals, there is a paucity of research whether mitragynine produces its subjective effects in rat behavioural models.

In addition, dual properties of opium-like (opioid) and coca-like (psychostimulant) effects demonstrated by mitragynine and kratom extracts represent the complexity in understanding their mechanisms of action. This situation raises an interest in elucidating the pharmacological similarity between mitragynine and opioid (*i.e.* morphine) and between mitragynine and psychostimulant (*i.e.* cocaine).

Besides mitragynine, 7-hydroxymitragynine also becomes another alkaloid of interest when it is reported as a minor psychoactive compound present in the plant. 7-Hydroxymitragynine has been thought as another key component that contributes
towards the opium-like properties. Thus, it is suggested that the compound may also associate with the opioid discriminative stimulus effects.

In addition, drug abuse and addiction are often characterised by their reinforcing effects. The elucidation of mitragynine dependence-producing potential in opioid-dependent animals is warranted to provide information on its reinforcing effect.

Last but not least, striking increases in the number of opioid users have expanded the need for pharmacotherapeutic interventions. Even though human reports and pre-clinical studies suggested potential valuable therapeutic properties of kratom, the neurobiological mechanism of using kratom as a substitution treatment amongst opioid addicts is still unclear. In fact, there is lack of scientific data to support the use of kratom as a potential substitution treatment.
1.2 Objectives of study

Six specific objectives are constructed in this study in order to address the abovementioned problem statements:

(i) To demonstrate the induction of mitragynine discrimination in rats

(ii) To evaluate the substitutability of mitragynine to morphine and cocaine

(iii) To evaluate the substitutability of morphine to mitragynine and cocaine

(iv) To evaluate the effect of 7-hydroxymitragynine derivative on discriminative stimulus effect of morphine

(v) To investigate the effect of naloxone, an opioid antagonist on discriminative stimulus effect of mitragynine, morphine and 7-hydroxymitragynine substituted responses

(vi) To assess the dependence profile of mitragynine in a rat model of fentanyl self-administration
Figure 1.1: The experimental design demonstrating the assessment of kratom abuse and addictive potential using two behavioural models.
CHAPTER 2

LITERATURE REVIEW

2.1 *Mitragyna speciosa* Korth (kratom)

*Mitragyna speciosa* Korth is a tropical plant indigenous to Southeast Asia region particularly in Peninsular of Malaysia and Thailand (Jansen and Prast 1988a; Hassan et al., 2013). The plant is known as ‘biak-biak’ or ‘ketum’ in Malaysia and as ‘kratom’ in Thailand (Jansen and Prast, 1988a; Adkins et al., 2011). It has been used as herbal stimulant by labourers and farmers in increasing work productivity during harsh conditions (Grewal, 1932a; Suwanlert, 1975; Hassan et al., 2013). The plant is known for decades as traditional herbal medicine for common illnesses such as diarrhoea, coughing, fever, malaria, muscle pain, as well as to improve blood circulation, sexual performance and to treat diabetes (Watanabe et al., 1997; Chan et al., 2005; Assanangkornchai et al., 2007; Kumarnsit et al., 2007a; Chittrakarn et al., 2010; Hassan et al., 2013).

Apart from the energising and medicinal properties, kratom has been used as a substitute for opiate addiction due to the capability of suppressing opioid withdrawal symptoms (Wray, 1907; Suwanlert, 1975; Vicknasingam et al., 2010; Ahmad and Aziz, 2012). The earliest use of kratom substitution for opium was reported in Malaysia as early as 1895 (Wray, 1907; Burkill, 1935).
2.1.1 Botanical origin

*Mitragyna speciosa* Korth, as shown in Figure 2.1 belongs to the *Rubiaceae* family. The plants grow freely in swampy areas, fertile soil with sufficient sun exposure in regions that are protected from the strong winds (Shellard and Lees, 1965; Macko et al., 1972; Hassan et al., 2013). They are large trunk trees which can grow up 4 to 9 metres height and 5 metres wide. The dark yellow flowers are globular in shape bearing up to 120 florets attached to the leaf axils on long stalks. The dark glossy green colour leaves normally grow to over 18 cm long and 10 cm wide with an ovate acuminate shape and tapered ends (Shellard and Lees, 1965; Hassan et al., 2013). The leaves will normally fall abundantly during hot season and grow intensely during rainy season (Macko et al., 1972). From all parts of the plant, the leaves are generally consumed (Jansen and Prast, 1988b; Kumarnsit et al., 2006; Hassan et al., 2013; Saingam et al., 2013) for their effects.
2.1.2 Plant preparation and consumption

There are various ways to prepare kratom leaves. Some users simply chew the fresh leaves (Wray, 1907; Jansen and Prast, 1988a; Hassan et al., 2013) while some prefer kratom in the form of dried powder, in which they can smoke, swallow or prepare as tea for ingestion (Wray, 1907; Jansen and Prast, 1988a; Babu et al., 2008). Locals will usually dry the leaves when kratom is abundance and use it as tea when there is a shortage of supply. The kratom users claim to experience no difference between the effects of fresh and dried leaves (Saingam et al., 2013; Sakaran et al., 2014). To make it easier for consumption, kratom is sold as drinks with a cheap price. In Malaysia, a small packet of the drink is sold at RM 1 (RM 1 = 0.30 USD) (Chan et al., 2005; Ramanathan and Mansor, 2014). Sometimes, the drinks are consumed together with sugar or honey to mask their bitter taste. Lemon juice and salt are also added to facilitate the extraction of kratom leaves and prevent
constipation respectively (Jansen and Prast, 1988a; Tanguay, 2011; Hassan et al., 2013). In Southern Thailand, kratom is sometimes mixed with caffeine-containing soft drinks and codeine- or diphenhydramine-containing cough syrup to give the anxiolytic, antidepressant and analgesic effects (Tanguay, 2011; Hassan et al., 2013). Recently, kratom drink is mixed with some other toxics or addictive substances (i.e. cough medication, rat poison and mosquito coils) to increase the euphoric effects and give extra flavours to the drink (Saingam et al., 2013; Ramanathan and Mansor, 2014).

Nevertheless, many users tend to believe that kratom is not as harmful as other illicit drugs like heroin, morphine, cocaine, methamphetamine and etc. This may explain the increasing demand for kratom which not only observed in Malaysia and Southeast Asia countries but also in other parts of the world. In recent years, kratom has spread to Europe and United States where its products are readily available via internet, ranging from dried powder leaves to capsules, tablets and concentrated extracts (Boyer et al., 2007; Boyer and Wines, 2008). Kratom is also popular as a herbal blend krypton when it is mixed with O-desmethyltramadol, an active metabolite of a commonly prescribed analgesic (i.e. tramadol) to give more powerful effects (Backstrom et al., 2010; Kronstrand et al., 2011). The easy availability and cheap price of kratom compared to other illicit drugs reflect the wide spread of misusing the natural product (Boyer et al., 2007; McWhirter and Morris, 2010; Adkins et al., 2011; Ramanathan and Mansor, 2014). The pictures of kratom consumption and several kratom products are shown in Figure 2.2A and Figure21.2B respectively.
Figure 2.2: (A) A kratom user chewing the kratom leaves. Picture is adapted from Matsumoto, (2006) (B) Products of kratom sold in the market. Pictures are adopted from Prozialeck et al. (2012).
2.1.3 Kratom consumption in humans

Typically, kratom is popular among elderly who find it beneficial for enhancing their physical energy and increasing work efficiency (Suwanlert, 1975; Vicknasingam et al., 2010; Saingam et al., 2013). Thus, people who consume kratom are regarded as better than those who abuse illicit drugs because they will become energetic while illicit drugs users usually feel lazy (Saingam et al., 2013). Besides, kratom users believe that their kratom practice is harmless and socially accepted as they do not disturb anyone which is opposite to the effects of illicit drugs (Assanangkornchai et al., 2007; Saingam et al., 2013). In fact, 99% of the kratom users still take a good care of their families (Ahmad and Aziz, 2012).

In addition, kratom is also used as traditional herbal medication for various health disorders such as diabetes, hypertension, coughing, diarrhoea and muscle pain which enabling them for self-treatment (Suwanlert, 1975; Saingam et al., 2013). The knowledge of kratom as herbal medication is passed from generation to generation, keeping such treatment available to be practised (Saingam et al., 2013). Interestingly, the beneficial use of kratom is supported by recent pharmacological studies when kratom and its active constituents have demonstrated analgesic, antidiabetic and antidiarrheal effects, the morphine-like action on gastric acid secretion and reduction of morphine withdrawal symptoms in animal studies (Matsumoto et al., 1996b; Tsuchiya et al., 2002; Kumanasit et al., 2007a; Reanmongkol et al., 2007; Khor et al., 2011).

The Northern states of Malaysia (i.e. Kedah and Perlis), which are located at the border of Thailand have become a ‘redspot’ for kratom (Chan et al., 2005; Singh
et al., 2014). This leads to a cross-sectional survey done by Singh et al. (2014) to reveal various reasons of kratom use and the result is shown in Table 2.1. Majority of the respondents with 28% consumed kratom to increase their physical energy. There are about 15% of the respondents who consumed kratom to abstain from the effects of illicit drugs. Meanwhile, 13% of them consumed kratom as self-treatment for their medical problems. A small percentage (i.e. 6%) of the respondent claimed that kratom improved their mood, making them happy and relax. This finding coincides with previous reports when kratom users described the effects of ‘well-being’ or euphoria induction following kratom consumption (Jansen and Prast, 1988a; Jansen and Prast, 1988b; Assanangkornchai et al., 2007). Similar reasons of using kratom amongst the users were also reported in Thailand (Assanangkornchai et al., 2007; Saingam et al., 2013).  

Despite of its effects, about 21% of the respondents reported that they were using kratom due to curiosity and peer influence. This survey aligns with Thailand study when kratom users reported their initial use was due to curiosity and peer-pressure (Assanangkornchai et al., 2007). This finding is supported by Saingam et al. (2013) where kratom is known amongst young people for fun or relaxation purposes.
Table 2.1: Reasons for using kratom in specific population at Northern Malaysia

<table>
<thead>
<tr>
<th>Reason</th>
<th>Respondent (n)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>To enhance physical energy</td>
<td>83</td>
<td>28</td>
</tr>
<tr>
<td>Curiosity</td>
<td>61</td>
<td>21</td>
</tr>
<tr>
<td>Peer influence</td>
<td>46</td>
<td>16</td>
</tr>
<tr>
<td>To abstain from illicit drugs/alcohol</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>Self-treatment</td>
<td>38</td>
<td>13</td>
</tr>
<tr>
<td>To improve mood/ease boredom</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>To relieve fatigue</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>293</td>
<td>100</td>
</tr>
</tbody>
</table>

* Adopted from Singh et al. (2014)

2.1.4 Phytochemistry

The chemistry of *M. speciosa* has been continuously studied for years. The investigation began in the 1920s when Hooper and Field first reported the isolation of mitragynine compound from *M. speciosa* leaves extracts (Field, 1921; Jansen and Prast, 1988a). However, the chemical structure of mitragynine was fully determined only almost 30 years later by Zacharias et al. (1965). A study by Shellard et al. (1974) later resulted in the isolation of over 25 alkaloids from various samples of *M. speciosa* leaves extracts. The main alkaloids present in the leaves of *M. speciosa* are mitragynine (12 to 66%) and its analogues that include paynantheine (9%), speciogynine (7%) and speciociliatine (1%) (Shellard, 1974; Hassan et al., 2013). A new analogue, 7-hydroxymitragynine was subsequently isolated as a minor
constituent (2%) (Ponglux et al., 1994). Kratom leaves contain approximately 0.2% mitragynine by weight of the total kratom leaves (Grewal, 1932a; Suwanlert, 1975). However, the mitragynine content was reported to vary by geographical region and season (Shellard, 1974; Hassan et al., 2013) as report shows that higher percentage of mitragynine (66%) of total alkaloid extracts of M. speciosa leaves was extracted from the leaves produced in Thailand compared to only 12% from the leaves extracted in Malaysia (Takayama, 2004).

As the major constituent, mitragynine is assumed to be mostly responsible for the pharmacological effects of the plant extracts and this has led to an increased interest in characterising its chemical, toxicological and pharmacological properties in recent years (Macko et al., 1972; Shellard, 1974; Matsumoto et al., 1996a; Takayama et al., 2002; Takayama, 2004; Matsumoto et al., 2005b; Reanmongkol et al., 2007; Azizi et al., 2010; Taufik Hidayat et al., 2010; Sabetghadam et al., 2013a). Structurally, mitragynine or 9-methoxy-corynantheidine (C\textsubscript{23}H\textsubscript{30}N\textsubscript{2}O\textsubscript{4}) with a molecular weight of 398.5 g/mol (Kumarnsit et al., 2007b) consists of a methoxy group at C19 position and C9 position of the indole ring (Beckett et al., 1966a; Beckett et al., 1966b; Shellard, 1974).

Previous studies have reported that the antinociceptive effect of pure mitragynine was less potent than that of the crude extract of the plant (Watanabe et al., 1997; Yamamoto et al., 1999; Matsumoto et al., 2006) which suggested the presence of other active compounds in the total alkaloid extracts. This may involve 7-hydroxymitragynine, the minor constituent of the leaves extracts. 7-Hydroxymitragynine is the compound derived from mitragynine with the introduction of a hydroxyl group at the C7 position of the mitragynine skeleton.
This claim is supported by evidence that the antinociceptive effect of 7-hydroxymitragynine is relatively more potent than mitragynine (Matsumoto et al., 2004; Takayama, 2004). Thus, mitragynine and 7-hydroxymitragynine are reported to be the most pharmacologically active, although the other constituents may also possess the pharmacological effects as listed in Table 2.2.
Table 2.2: Structures and potential effects of alkaloids extracted from kratom

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage</th>
<th>Structures</th>
<th>Structures and pharmacological activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitragynine</td>
<td>12-66%</td>
<td><img src="attachment" alt="OCH₃" /></td>
<td>Structurally similar to yohimbine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Analgesic, antitussive, anti-diarrheal, adrenergic, antimalarial effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Activity on µ, δ and κ receptors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Activates descending noradrenergic and serotonergic pathways in spinal cord</td>
</tr>
<tr>
<td>Paynantheine</td>
<td>9%</td>
<td><img src="attachment" alt="OCH₃" /></td>
<td>Smooth muscle relaxant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibits twitch contraction without naloxone blocking effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibits muscarinic receptors on smooth muscle</td>
</tr>
<tr>
<td>Speciogynine</td>
<td>7%</td>
<td><img src="attachment" alt="OCH₃" /></td>
<td>Smooth muscle relaxant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibits twitch contraction without naloxone blocking effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibits muscarinic receptors on smooth muscle</td>
</tr>
<tr>
<td>Speciociliatine</td>
<td>1%</td>
<td><img src="attachment" alt="OCH₃" /></td>
<td>C3 stereoisomer of mitragynine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Possess other than stimulation on opioid receptors i.e. may inhibit acetylcholine release from presynaptic nerve</td>
</tr>
<tr>
<td>7-hydroxy mitragynine</td>
<td>2%</td>
<td><img src="attachment" alt="OCH₃" /></td>
<td>Possess –OH group on C7 position</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Analgesic, antitussive, anti-diarrheal effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Higher opioid agonist potency than mitragynine and morphine</td>
</tr>
</tbody>
</table>

*Note: Percentage denotes the percentages (%) of the compound in the alkaloid fraction/extract

*Adopted from Hassan et al. (2013)
2.1.5 Pharmacokinetics

The quantification analyses of kratom alkaloids in the biological systems are fundamental in order to provide better understanding of the pharmacological activity of its extracts. The pharmacokinetic of mitragynine was well characterized in rat plasma following oral and intravenous administrations. Oral administration of 40 mg/kg of mitragynine in rats led to a peak plasma concentration ($C_{\text{max}}$) of 0.63 μg/ml after $T_{\text{max}}$ of 1.83 h (Janchawee et al., 2007) using a High Performance Liquid Chromatography-Ultra Violet (HPLC/UV) method. In the following study using HPLC and tandem mass spectrometry (LC-MS/MS), mitragynine was reported to be rapidly absorbed with a maximum concentration ($C_{\text{max}}$) of 423.68 ng/ml and a $T_{\text{max}}$ of 1.26 h after oral administration of 20 mg/kg mitragynine. The elimination half-life ($t_{1/2}$) was shown to be 3.85 h (de Moraes et al., 2009). A pharmacokinetic study done by Parthasarathy et al. (2010) using solid-phase extraction and rapid HPLC-UV analysis reported that oral administration of 50 mg/kg dose of mitragynine resulted in the $C_{\text{max}}$ of 0.7±0.21 μg/ml and the $T_{\text{max}}$ of 4.5 ± 3.6 h. The elimination half-life ($t_{1/2}$) was 2.9 ± 2.1 h. The variations of the pharmacokinetic profiles observed from these studies could be attributed by poor oral absorption of mitragynine.

Meanwhile, following intravenous administration of 1.5 mg/kg, the $C_{\text{max}}$ was reported to be 2.3 ± 1.2 μg/ml with $T_{\text{max}}$ of 1.2 ± 1.1 h and elimination half-life ($t_{1/2}$) was determined at 2.9 ± 2.1 h (Parthasarathy et al., 2010). In contrast, a more recent pharmacokinetic study using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) reported the $C_{\text{max}}$ of 3.9 ± 0.7 μg/ml at 1 min following the administration of 5 mg/kg mitragynine while the elimination half-life ($t_{1/2}$) was 2.6 ±
0.6 h (Vuppala et al., 2011). However, it is important to note that the pharmacokinetic on multiple dose study of mitragynine has yet to be reported.

2.1.6 Toxicity studies in animals

The earliest acute toxicity report of mitragynine by Macko et al. (1972) found no absolute evidence of toxicity, whereby no tremors or convulsions were observed at the doses as high as 920 mg/kg in mice. However, a recent study by Janchawee et al. (2007) demonstrated lethal effects after an oral administration of 200 mg/kg mitragynine in rats. The similar fatal effect was also observed after administration of 200 mg/kg alkaloid extract of *M. speciosa* to rats (Azizi et al., 2010).

The earliest sub-acute toxicity study by Macko et al. (1972) demonstrated no side effects in rats after administration of 5 or 50 mg/kg/day of mitragynine for five days per week within six weeks duration. There was only a significant decrease in liver and kidney weights that were observed at higher mitragynine dose. Similarly in dogs, no side effects were observed following 20 mg/kg of mitragynine administration per day for three weeks duration. However, hematological findings including leukopenia, lymphocytosis, granulocytopenia, monocytosis and immature lymphocytes were observed in dogs from day 22 when the dose was increased to 40 mg/kg/day for six days per week. In fact, these changes were reversed after the drug was withdrawn. A most recent sub-chronic study by Sabetghadam et al. (2013b) demonstrated no mortality or toxic effects in mice after administered at low or intermediate dose (*i.e.* 1 and 10 mg/kg) of mitragynine. However, decreases in food intake and body weight were observed at the highest mitragynine dose (*i.e.* 100
mg/kg). Overall, the previous reports suggested that mitragynine is relatively safe at low sub-chronic doses (i.e. 1 to 10 mg/kg), but exhibits toxicity at higher sub-chronic dose (i.e. 100 mg/kg at 28 days) (Sabetghadam et al., 2013b). Nevertheless, information about the dose level that could be translated from animal data to human perspectives is still lacking.

2.1.7 Toxicity and adverse effects in humans

Several human studies and case reports also emerged to provide evidence of long term toxicity and adverse effects of kratom preparations. The first study of mitragynine in humans was reported by Grewal (1932b) when some of the human subjects developed nausea, vomiting, slide tremors of the hand and tongue, giddiness, feeling of haziness and slight flushing of the face following mitragynine administration. It was reported that a man who tried to abstain from kratom had difficulty to sleep, wriggling sensation in the shoulders and the back, dragging sensation in the hips, bitemporal headache, became extremely weak and also could hardly walk (Thuan, 1957). A recent study by Vicknasingam and colleagues (2010) also revealed that kratom produced mild side effects such as loss of weight, dehydration, constipation but no other medical problems were reported. However, prolonged use of kratom was reported to cause adverse effects which include nausea, diarrhoea, vomiting, hallucinations, psychosis, agitation, dizziness, itching, sweating, dry mouth, respiratory depression, constipation, anorexia, increased urination, palpitations and weight loss (Suwanlert, 1975; Jansen and Prast, 1988a; Babu et al., 2008; Adkins et al., 2011; Prozialeck et al., 2012).
Other than adverse effects, there are no reports of mortalities following mitragynine or kratom consumption alone, even after chronic and high dosage consumption (Ramanathan and Mansor, 2014). However, the combination of kratom with other drugs (i.e. krypton) (Backstrom et al., 2010; Hassan et al., 2013) had contributed to unintended death (Kronstrand et al., 2011). A similar fatal case was also reported following the co-ingestion of propylhexedrine and mitragynine (Holler et al., 2011). Even though the contribution of kratom or its active compound, mitragynine to the unintended deaths remain unclear, the adulterated form of kratom products could contribute to its multidrug actions. To date, little is known about the clinical effects and the potential toxicities of mitragynine or kratom in humans. The available reports in humans may suggest the potential adverse effects at extremely high dosage of mitragynine or kratom consumption. However, there are no precise studies on mitragynine or kratom dose ranges that are responsible for those effects at present.

2.1.8 Metabolism

Mitragynine is extensively metabolised in rats and humans. This mechanism involved the hydrolysis of the methylester in position C16, O-demethylation of the 9-methoxy group and of the 17-methoxy group followed by oxidation and reduction reactions from the intermediate aldehydes to form carboxylic acids and alcohol, respectively. Finally, four phase 1 metabolites were conjugated to form four glucuronides and one sulphate in rats while the conjugation resulted in three glucuronides and three sulphates in humans (Philipp et al., 2009). Previous report has demonstrated that M. speciosa extract showed the most potent inhibition on
cytochrome P450 enzyme (i.e. CYP 2D6) with IC$_{50}$ value of 3.6 ± 0.1 µg/ml suggesting that the consumption of the extract together with other drugs that have the same metabolic pathway may contribute to herb-drug interactions (Hanapi et al., 2010). This finding possibly suggests that the mixture of kratom preparations with other psychoactive drugs may lead to overdose and adverse effects.

2.1.9 Opioid agonist property

Opioid is any chemicals that possessed opiate-like pharmacological effects. An opiate is a plant-derived material product from opium poppy (Papaver somniferum) (Van Ree et al., 1999). The seed capsule of the poppy contains morphine, codeine and other alkaloids. The clinical benefits of opioid as a pain-relief agent led to the development of synthetic opioid medications such as methadone, heroin and fentanyl (Linneman et al., 2000; Walwyn et al., 2010). Thus, the term opiates arise in describing any of the psychoactive opioid compounds with opioid-agonistic function (Van Ree et al., 1999). Opioids exert their effects by binding to opioid receptor subtypes (i.e. µ-, δ- and κ-receptors) that are distributed in the central and peripheral nervous systems (Quock et al., 1999).

The opioid-like properties of mitragynine was assessed using the twitch contraction of guinea pig ileum induced by electrical stimulation (Watanabe et al., 1997). The inhibitory effect of mitragynine on gastric acid secretion was well demonstrated in rats when injection of mitragynine into the fourth cerebroventricle inhibited the 2-deoxy-D-glucose-stimulated gastric acid secretion. Interestingly, this inhibitory effect was reversed by naloxone, suggesting the inhibitory effects
possessed by mitragynine were mediated by the opioid system which was similar to morphine (Tsuchiya et al., 2002). Besides, the opioid receptor agonistic effect of mitragynine was also reported in other studies (Matsumoto et al., 1996b; Watanabe et al., 1997; Yamamoto et al., 1999; Boyer et al., 2008; Taufik Hidayat et al., 2010). In addition, mitragynine was also reported to produce antinociceptive activity through supraspinal opioid receptors when its antinociceptive activities following intraperitoneal (i.p.) and intracerebroventrical (i.c.v.) administrations were antagonised by i.c.v. naloxone in both tail-pin chase and hot-plate tests (Matsumoto et al., 1996b).

Besides, competitive binding studies showed the involvement of three types of opioid receptors in mitragynine effect, which include μ-, κ- and δ- opioid receptors (Yamamoto et al., 1999; Boyer et al., 2008; Taufik Hidayat et al., 2010). From the three receptor subtypes, mitragynine was shown to exert most of its effects via μ-opioid receptor (Matsumoto et al., 1996b; Thongpradichote et al., 1998; Shamima et al., 2012; Stolt et al., 2014). In addition, receptor binding assays also demonstrated that 7-hydroxymitragynine bound preferentially to μ-opioid receptors, suggesting its full agonistic properties (Takayama et al., 2002; Matsumoto et al., 2004). Taken together, mitragynine and its 7-hydroxymitragynine derivative are reported to act on the central nervous system (CNS) and exhibit high affinities toward μ-opioid receptor (Yamamoto et al., 1999; Matsumoto et al., 2006).