BEHAVIOURAL, BIOCHEMICAL, PROTEOMICS STUDIES AND REPLACEMENT THERAPY OF MORPHINE AND MITRAGYNINE WITHDRAWAL RAT MODELS

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by/oleh

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LIST OF SYMBOLS

%	Percentage
°C	degree Celsius
µg	microgram
µL	microliter
cm	centimeter
g	gram
g	earth's gravitational acceleration/relative centrifugal force
h	hour
L	liter
M	molar
mg	milligram
mg/kg	milligrams per kilogram
mL	milliliter
mM	millimolar
mm	millimeter
ng	nanogram
nm	nanometer
rpm	rotations per minute
V	volts
β	beta

LIST OF ABBREVIATIONS

ACN	Acetonitrile
ALP	Alkaline phosphatase
APS	Ammonium persulfate
AST	Aspartate amino transferase
BAR	Biorad Amplification Reagent
BioGRID	Biological General Repository for Interaction Datasets
BSA	Bovine serum albumin
CID	Collision-induced dissociation
CPP	Conditioned place preference
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DMSO	Dimethyl sulfoxide
DOP	Delta-opioid receptor
DTT	Dithiothreitol
ES	Electrospray
ESI	Electrospray ionization
FDA	Food and Dru*g* Administration
GO	Gene ontology
HCD	High-energy collision-induced dissociation
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HPRD	Human protein reference database
IAA	Iodoacetamide

ip	Intraperitoneal
IPA	Ingenuity pathway analysis
ITMS	Ion-trap mass spectrometry
KEGG	Kyoto Encyclopedia of Genes and Genomes
KOP	Kappa-opioid receptor
LC	Locus coeruleus
LC-MS	Liquid-chromatography mass spectrometry
MAT	Medication-assisted treatment
$MgCl_2$	Magnesium chloride
MINT	Molecular interaction
MOP	Mu-opioid receptor
MPIDB	Microbial protein interaction database
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry or mass spectrometry/mass spectrometry
NA	Noradrenaline
Na_3OV_4	Sodium orthovanadate
OTMS	Orbitrap mass spectrometry
OUD	Opioid use disorder
PANTHER	Protein analysis through Evolutionary Relationships
PD	Parkinson’s disease
PIPs	Human protein-protein interaction prediction
PTMs	Post-translational modifications
PVDF	Polyvinylidene fluoride
SDS	Sodium dodecyl sulfate

STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TAIR	The arabidopsis information resource
TBS	Tris buffered saline
TBST	Tris buffered saline/Tween 20
TFA	Trifluoroacetic acid
vs	Versus
WB	Western blotting
WHO	World Health Organization

LIST OF APPENDICES

KAJIAN TINGKAH LAKU, BIOKIMIA, PROTEOMIK, DAN TERAPI GANTIAN DALAM MODEL PENARIKAN TIKUS MORFINA DAN MITRAGININA

ABSTRAK

Ketagihan opiat adalah masalah kesihatan utama di kebanyakan negara. Dianggarkan 27 juta orang di seluruh dunia mengalami gangguan penggunaan opioid. Di Malaysia, opioid tetap menjadi bentuk utama ubat haram. Kesan buruk penyalahgunaan opioid bukan sahaja mempengaruhi penagih dadah, tetapi juga kepada masyarakat dan juga negara secara keseluruhan. Komponen penting dalam rawatan opiat adalah pengurusan tanda-tanda penarikan aversif, yang mana boleh menyebabkan pengambilan semula opiat. Dalam rawatan dadah gantian (MAT), metadon dan buprenorfina telah dilaksanakan sebagai ubat pengganti. Walaupun MAT berkesan, namun masih terdapat batasan dan kesan sampingan daripada penggunaan metadon dan buprenorfina. Salah satu farmakoterapi yang berpotensi adalah Mitragyna speciosa atau kratom, suatu tumbuhan yang digunakan secara meluas dalam mengurangkan keterukan penarikan opioid dan sebatian alkaloid utamanya adalah mitraginina. Walaupun terdapat kelebihannya, kratom juga telah dilaporkan menyebabkan kebergantungan dengan gejala penarikan yang kuat dan pada masa ini, tiada rutin rawatan rasmi yang tersedia untuk menangani kebergantungan terhadap kratom. Kajian ini dirancang untuk menguji kemampuan mitraginina untuk mengurangkan tanda-tanda tingkah laku penarikan morfina pada model tikus dibandingkan dengan metadon dan buprenorfina. Rawatan morfina selama enam hari dengan peningkatan dos morfina (10-50 mg/kg, ip) menyebabkan tanda penarikan

yang kuat selama 16 hari. Mitraginina (5-30 mg/kg; i.p.) secara signifikan mengurangkan tanda-tanda penarikan akut pada tikus yang bergantung kepada morfina. Ianya sekurang-kurangnya efektif seperti metadon (0.5-2 mg/kg; i.p.) dan buprenorfina (0.4-1.6 mg/kg; i.p.). Data ini mencadangkan bahawa mitraginina mungkin merupakan sebatian aktif dalam sediaan kratom yang mempengaruhi laluan opioid dan mungkin boleh dipertimbangkan sebagai ubat gantian alternatif untuk pengurusan penarikan opiat. Kajian kedua telah dirancang untuk mengukur kemampuan ubat pengganti sedia ada seperti metadon, buprenorfina dan clonidin untuk mengurangkan gejala penarikan mitraginina pada model tikus. Model penarikan mitraginina diberi 30 mg/kg mitraginina selama 14 hari dan menyebabkan tanda-tanda penarikan akut sehingga 9 hari. Rawatan 1.0 mg/kg metadon, 0.8 mg/kg buprenorfina dan 0.1 mg/kg klonidin secara signifikan mengurangkan tanda-tanda penarikan dalam model penarikan mitraginina, mencadangkan bahawa rawatan penggantian ini membantu memerangi kekerasan semasa penarikan mitraginina. Dalam kajian ketiga, kedua-dua model penarikan morfina dan mitraginina telah diteliti melalui pendekatan proteomik dengan menilai ekspresi protein daripada keseluruhan otak tikus dan biopenanda protein yang berpotensi semasa fasa penarikan. Daripada sejumlah 1762 protein yang dikenal pasti, 34 kawal atur menaik sedangkan 16 adalah ekspresi protein yang kawal atur menurun dalam model penarikan morfina. Sebaliknya, daripada 1524 protein yang dikenal pasti daripada model penarikan mitraginina, 31 protein kawal atur menaik, dan 3 protein adalah ekspresi protein yang kawal atur menurun. Rab35 ialah protein yang diatur ke atas dapat dikenal pasti pada kedua-dua model penarikan, oleh itu disahkan dengan lebih lanjut melalui teknik serap western. Oleh itu, adalah dicadangkan bahawa Rab35 dianggap sebagai biopenanda yang berpotensi semasa fasa penarikan pada kedua-dua model dan mungkin menjadi protein sasaran yang

berharga dalam pembangunan farmakoterapi baru di masa hadapan. Sesungguhnya, kajian ini hanyalah kajian permulaan, oleh itu, kajian lebih lanjut amat diperlukan, termasuk ujian klinikal yang terkawal, yang mana diperlukan untuk mengesahkan penemuan ini sebagai justifikasi penggunaannya untuk terapi penggantian opioid.

BEHAVIOURAL, BIOCHEMICAL, PROTEOMICS STUDIES AND REPLACEMENT THERAPY OF MORPHINE AND MITRAGYNINE WITHDRAWAL RAT MODELS

ABSTRACT

Opiate addiction is a major health problem in many countries. It is estimated that 27 million people worldwide experience opioid use disorder. In Malaysia, opioids remain the principal form of illegal drug. The adverse effects of opioid abuse not only affect drug addicts, but also on the society as well as the country as a whole. A crucial component of opioid treatment is the management of highly aversive opiate withdrawal signs, which may otherwise lead to resumption of drug taking. In a medication-assisted treatment (MAT), methadone and buprenorphine have been implemented as replacement drugs. Despite MAT effectiveness, there are still limitations and side effects of using methadone and buprenorphine. One of the potential pharmacotherapies may involve *Mitragyna speciosa* or kratom, a plant broadly used in mitigating the severity of opioid withdrawal and its major alkaloid compound, mitragynine. The current study was designed to test the ability of mitragynine to attenuate behavioural signs of morphine-withdrawal in a rat model in comparison to methadone and buprenorphine. A six-day treatment with escalating dose of morphine (10-50 mg/kg, i.p.) induced profound withdrawal signs for 16 days. Mitragynine (5-30 mg/kg; i.p.) significantly attenuated the acute withdrawal signs in morphine dependent rats. It was at least as effective as methadone (0.5- 2 mg/kg; i.p.) and buprenorphine (0.4-1.6 mg/kg; i.p.). These data suggest that mitragynine may be the active compound in kratom preparations that affect opioid pathway and might be

considered as an alternative replacement drug for the management of opiate withdrawal. The second study was designed to measure the capability of the common replacement drugs such as methadone, buprenorphine and clonidine to reduce mitragynine withdrawal syndrome in rat model. Mitragynine withdrawal model was given 30 mg/kg of mitragynine over a period of 14 days and induced acute withdrawal signs up to 9 days. 1.0 mg/kg methadone, 0.8 mg/kg buprenorphine and 0.1 mg/kg clonidine treatments significantly lessened the withdrawal signs in mitragynine withdrawal model, suggesting that these replacement treatments assist in combating the harshness during mitragynine withdrawal. In the third study, both morphine and mitragynine withdrawal models were examined through proteomics approach by evaluating the whole rats brain protein expression and potential protein biomarker during withdrawal phase. From a total of 1762 identified proteins, 34 were up-regulated whereas 16 were down-regulated proteins expression in morphine withdrawal model. On the other hand, of 1524 proteins identified from mitragynine withdrawal model, 31 proteins were up-regulated, and 3 proteins were down-regulated proteins expression. Up-regulated Rab35 could be identified in both withdrawal models, thus further validated via western blotting technique. Therefore, it is proposed that Rab35 might be considered as a potential biomarker during withdrawal phase in both models and might be valuable target protein in developing new pharmacotherapies in the future. Indeed, the present studies was only a prefatory study, thus, further research is required, including well-controlled clinical trials, will be necessary to confirm these findings as a justification of its use for opioid substitution therapy.

CHAPTER 1

INTRODUCTION

1.1 An overview

According to *World Drug Report 2019*, about 271 million people worldwide took drugs and almost 13 % suffered from drug use disorder, to a point where they may experience dependence and/or require treatment (The United Nations Office on Drugs and Crime, 2019). There are an estimated 27 million who experience opioid use disorder (the new terminology instead of addiction) (OUD) globally (World Health Organization, 2018; Harvard Health: Health Information and Medical Information, 2019) which remain as health challenges globally.

In Malaysia, opioid remain the main type of illicit substance, with estimated 187 771 opiate users in Malaysia (George et al., 2018). Illicit substance includes methamphetamine and Amphetamine-type Stimulants (ATS), kratom, cannabis, ketamine as well as MDMA (3,4methylenedioxy-N-methylamphetamine) or 'ecstasy' (Norliza et al., 2014; Satar, 2019). Drug trafficking, burglary, fraud, homicide, and suicide are the common types of crimes committed by drug addicts (Abd Rashid et al., 2008). These crimes affect not only the drug abuser, it would also affect their families, society and the country.

The early step in OUD treatment is detoxification or withdrawal management. 'Detoxification' or 'short-term treatments for relieving withdrawal syndrome' is the initial phase in recovery process, which is controlled and medically supervised withdrawal from drug. Detoxification method includes classic *'cold-turkey'* approach, opioid management such as methadone, buprenorphine and naltrexone as well as alpha-2 agonist such as clonidine (Salmond et al., 2019). However, the most successful

method in mitigating opioid withdrawal signs approach are methadone and buprenorphine. Generally, Clinical Opiate Withdrawal Scale (COWS) is used to monitor withdrawal management program. Transition from detoxification to medication-assisted treatment (MAT) in OUD will commence once the detoxification period was completed successfully (Salmond et al., 2019). This MAT or 'long-term pharmacotherapies' of opioid dependence and addiction aimed to reduce cravings and decrease the risk of relapse, thereby enhancing rehabilitation programs of psychological rehabilitation (Kosten and George, 2002). Three primary medications in MAT that were approved by Food and Drug Administration (FDA) are methadone, buprenorphine, and naltrexone (Hoffman et al., 2019; Salmond et al., 2019; Oesterle et al., 2019), with the methadone and buprenorphine are still having greater success rate than naltrexone. Long-term community-based resources such as counselling, behavioural therapies and vocational training should be offered in addition to MAT care to minimize the risk of relapse.

Methadone has generally been the primary drug used for opioid dependent patient during the detoxification phase. Whereas the alternative to methadone is buprenorphine. Methadone is a full agonist that acts at mu-opioid receptor (MOP), while buprenorphine is a partial agonist at MOP. Such two medications are advised in opioid pharmacotherapy according to their mechanisms of action in alleviating withdrawal signs (Dematteis et al., 2017). However, both have clinical limitations in their effectiveness and safety.

Generally, methadone therapy is considered as safe. It has a long half-life, making ambulatory management feasible. However, there have been some risk factors such as; i) the interaction of prescription medication with other medicines and ii) *torsade de pointes* (a life-threatening cardiac ventricular dysrhythmia) (Dinis-Oliveira,

2016). Methadone is metabolized by six different enzymes; CYP3A4, CYP2B6, CYP2C8, CYP2C19, CYP2D6, and CYP2C9 (Smith, 2009). Therefore, co-administration of methadone with other drugs that are substrates, inducers or inhibitors of these six enzymes may lead to a great deal of interaction potential. For example, the administration of CYP3A4 substrates or inhibitors can prolong and strengthen pharmacological effects and adverse effects, such as respiratory depression, while analgesic efficacy can decrease following administration of CYP3A4 inducers (Smith, 2009). Moreover, methadone has high inter-individual variance in efficacy due to pharmacokinetic and pharmacodynamic factors (Eap et al., 2002). In many patients, successful methadone half-life for analgesics does not represent a half-life without respiratory depression and cardiac adverse reactions, making methadone stable and safe dosing difficult (Whistler, 2012). This sometimes results in an overdose associated with significant adverse effects including a life-threatening respiratory failure. Methadone administration has more accidental death than other drugs (Trescot et al., 2008).

An alternative option to methadone is buprenorphine replacement therapy. It acts as mu-partial agonist at MOP and has 'ceiling effect' for respiratory depression. Thus, it offers an advantage in terms of safety as compared to methadone. Buprenorphine has been accepted as a safe maintenance treatment for opioid abuse in many nations, with decreased mortality rates in treating heroin abuse and especially reduce overdose risk for office-based treatment. However, several cases of buprenorphine asphyxia concomitant users have been reported in France with other drugs including benzodiazepines. Drug-drug association with buprenorphine may cause extreme respiratory depression (Mégarbane et al., 2006). Buprenorphine, however, has ceiling effects, which reduces the risk of respiratory depression.

Nonetheless, buprenorphine does not seem to have a ceiling effect on analgesia (Dahan et al., 2006). At high dose, it antagonizes the analgesic effects of other opioids, thereby complicating the treatment of pain in patients maintained at high-dose of buprenorphine (Heinzerling, 2019). Its antagonistic properties may also cause precipitation of acute withdrawal if given to a person who is physically dependent on opioids.

In addition to opioid detoxification, clonidine, an alpha-$_2$ adrenergic agonist is the most widely used non-opioid drug. Typically used as an anti-hypertensive drug, it is often used to manage opioid withdrawal syndrome (Ling and Compton, 2005; Strain, 2006). Alpha-$_2$ adrenergic agonist alleviate withdrawal effects by damping noradrenergic hyperactivity (Strain, 2006). However, it is not routinely recommended for use by National Institute for Clinical Excellence due to its effects such as rebound hypertension, hypotension, bradycardia and sedation. Due to these problems, it is rarely recommended for use in outpatient settings (Diaper et al., 2014; Jain et al., 2018). Thus, a need for better opioid agonist-replacement therapy with a lesser side effect is warranted.

Mitragynina speciosa Korth or kratom has long been traditionally used in South-East Asian nations, particularly Thailand and Malaysia for its pharmacological and narcotic effects. Kratom leaves have been claimed to have both stimulant- and opium-like effects. At low dose it acts as a stimulant, while being sedative at high dose (Jansen and Prast, 1988). Locals historically use the kratom to combat exhaustion and survive working under bright sunlight through its psychostimulant-like effect. Furthermore, kratom is also used to treat withdrawal syndrome and as a replacement of heroin and morphine (Beckett et al., 1965; Grundmann, 2017). Kratom leaves contain over 40 alkaloids, where mitragynine is the main indole alkaloid. Mitragynine

has a dual opioid and non-opioid pharmacology (Boyer et al., 2008; Kruegel et al., 2016; Hiranita et al., 2019; Obeng et al., 2020) which making this unique compound worth to be explored. Plus, even though mitragynine activated the G-protein–mediated signaling pathway much like traditional opioids, it did not "recruit" β-arrestin-2 (Varadi et al., 2016). Several studies indicate that MOP agonist that does not recruit ß-arrestin 2 cause less side effect such as constipation, tolerance progress and respiratory depression while it remains as a potent analgesic (Raehal et al., 2005; Bohn and Raehal, 2006). In kratom itself, no single case can be solely attributed to respiratory failure, a sharp contrast to other opioids where respiratory depression is the most common cause of death (Kruegel and Grundmann, 2017). Therefore, mitragynine might be serve as new molecular scaffolds for the development of centrally acting therapeutics drug with greater therapeutic index. Plus Hemby et al. (2019) also demonstrated that mitragynine is a good candidate for pharmacotherapies. In Malaysia, on average of RM300 is spent monthly on each drug addict, and RM300 million is spent annually on drug rehabilitation programs (Tam Cai and Foo Yie, 2013). Kratom plant is abundant and easily sourced in Malaysia. Therefore, mitragynine as an alternative for opioid-dependent management treatment could provide a more cost-effective program.

Proteomics is a high-performance technology for detecting changes in complex biological systems in global proteins. Proteomics approach has gained considerable attention in morphine withdrawal and addiction studies. For example, the proteomics methodology has been studied in the chronic morphine administration effects on mice hippocampal protein expression (Chen et al., 2007). Besides, the whole rat's brain protein expression alteration following morphine dependence has been disclosed by Bierczynska-Krzysik et al. (2006a) whereas Bierczynska-Krzysik et al. (2006b)

reported for certain brain regions such as cerebral cortex, hippocampus and striatum. Furthermore, study of morphine dependence and withdrawal in rats has been disclosed by Li et al. (2006) which focussed on, nucleus accumbens region, a key of mesolimbic reward system. Beside rodents, proteomics study in primates associated with morphine dependence and withdrawal also has been reported by Bu et al. (2012) which focused on nucleus accumbens region. Indeed, abrupt opiate discontinuation leads to the emergence of negative emotional states as well as somatic withdrawal syndrome which engage to many brain regions such as amygdala, forebrain, brainstem and other regions (Nestler, 2005). As opiate withdrawal involved in various brain regions and mechanisms (Koob and Volkow, 2010; Kosten and George, 2002), small number of findings for whole brain regions protein expression in proteomics analysis warrant further investigation in morphine withdrawal models in rats as well as in mitragynine withdrawal model. In addition, the potential protein biomarkers in both models within withdrawal phase might be crucial and valuable in developing new pharmacotherapies in future.

1.2 Problem statement and rationale of the study

Some limitations in available opioid replacements drugs, such as respiratory depression in methadone that could lead to accidental death and buprenorphine antagonistic effects that could lead to withdrawal of opioid-dependent individuals, have highlighted the need for a new potential active compound with lesser side effect. Currently, kratom has been used widely in management of opioid withdrawal. Kratom leaves contain over 40 alkaloids, and its major indole alkaloid is mitragynine. In the first part of the research, it is hypothesized that mitragynine might have a significant contribution in alleviating the withdrawal effects on opioid users. The present study was therefore aimed to evaluate the effectiveness of mitragynine in managing morphine withdrawal in the rat model in comparison with methadone and buprenorphine.

Whilst kratom have benefits, it also has been reported to cause dependence along long term consumption in human study. No available treatments were currently implemented. Singh et al. (2018c) also reported that kratom dependence patients also do not seek for medications as kratom withdrawal effect was mild which only lasted between one to three days. However, withdrawal is truly uncomfortable for some individuals which makes it hard to maintain abstinence (Swogger and Walsh, 2018). Several researchers have closely studied and concluded that kratom is not as risky as conventional opioids and that the dangers can be outweighed by the potential benefits (Prozialeck, 2016). Therefore, in the second part of the study, it is hypothesized that mitragynine might cause mild dependence in rat model and able to recover naturally in over a period of time. Thus, this study also aimed to develop the mitragynine withdrawal model in rat and evaluate its abstinence recovery without giving any medications. Furthermore, methadone, buprenorphine and clonidine will be used as

treatment for mitragynine withdrawal model rats. It is hypothesized that methadone, buprenorphine and clonidine would mitigate withdrawal syndrome caused by mitragynine dependence.

Proteomics study on morphine administration, dependence, withdrawal and addiction have been discovered in these recent years. However, currently, there is no proteome report on mitragynine. For this reason, this study aimed to investigate the proteomics study in mitragynine withdrawal model as well as in morphine withdrawal model.

1.3 Objectives of the study

The study was carried out with the following objectives:

1) To optimize the adopted withdrawal model in morphine and to develop the withdrawal model in mitragynine, respectively and to assess the withdrawal signs within 28 days of abstinence in both models.

2) To evaluate the effectiveness of mitragynine, methadone and buprenorphine in controlling withdrawal syndrome in morphine dependent rats.

3) To evaluate the effectiveness of methadone, buprenorphine and clonidine in controlling withdrawal syndrome in mitragynine dependent rats.

4) To determine the whole protein expression via quantification of protein-based mass spectrometry data analysis.

5) To predict the protein-protein interaction and its functional classifications via STRING and PANTHER databases, respectively in up- and down-regulated protein expressions of morphine and mitragynine withdrawal models.

1.4 Workflow

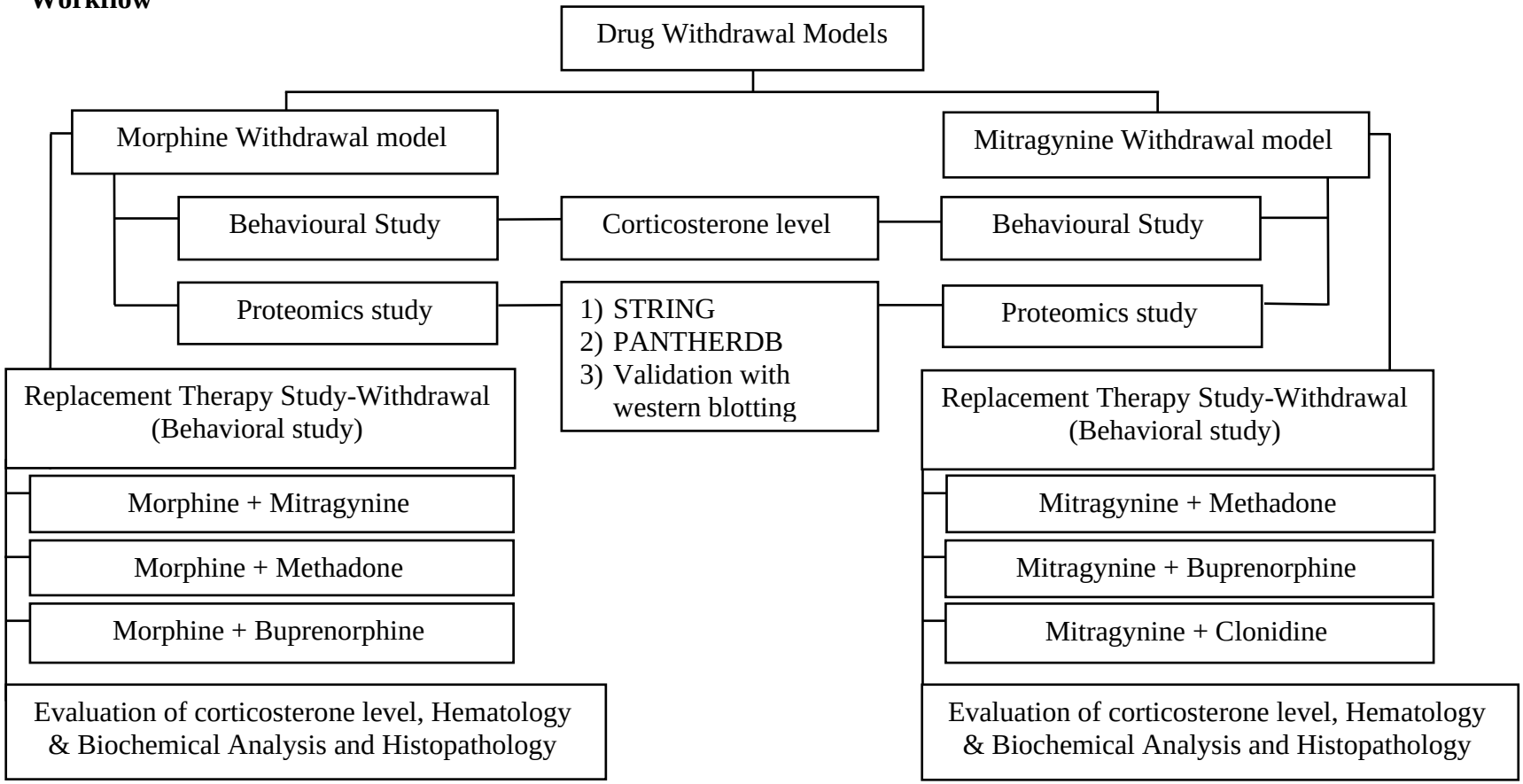

Figure 1.1 Flowchart of the study

CHAPTER 2

LITERATURE REVIEW

2.1 Drug Addiction

2.1.1 Definition of drug addiction

Drug addiction is defined as a chronically relapsing disorder, characterised by compulsion to seek and take the drug, loss of control in limited intake, and emergence of a negative emotional state when access to the drug is prevented (Koob and Volkow, 2010). Drug addiction involves the elements of impulsivity and compulsivity which caused recurrent addiction cycle which starts with intoxication, followed by withdrawal and lastly craving. Impulsivity refers to uncontrolled reactions to stimuli both internal and external with no regard to the consequences. Compulsivity on the other hand repetitions of inappropriate actions in excessive manner (Koob and Volkow, 2016). Figure 2.1 illustrated the stages of the addiction cycle.

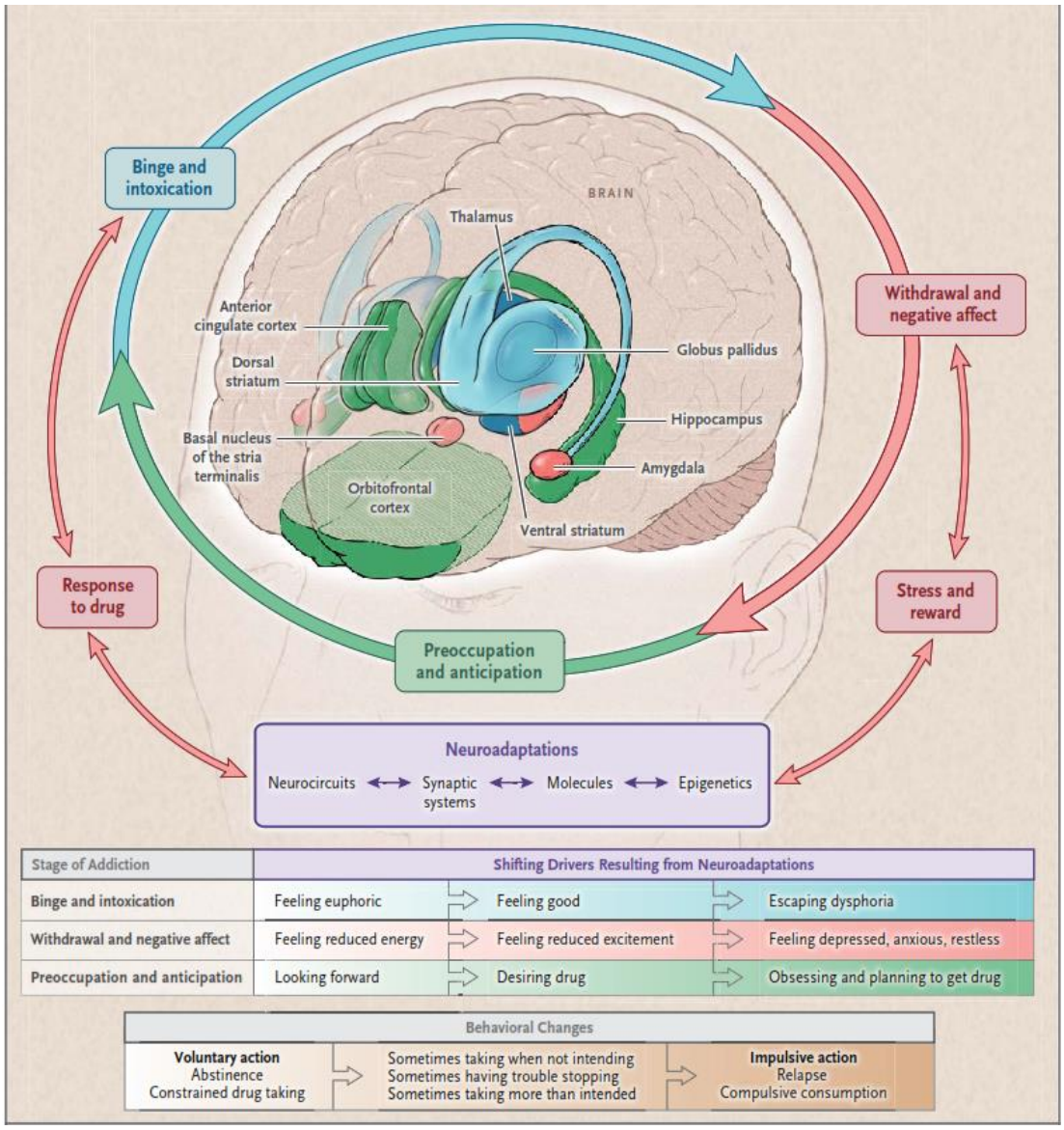

Figure 2.1 Stages of the addiction cycle (adapted with permission from Volkow et al. (2016)). During intoxication, drug-induced activation of the brain reward regions (in blue) is enhanced by conditioned cues in areas of increased sensitization (in green). During withdrawal, the activation of brain regions involved in emotions (in pink) results in negative mood and enhanced sensitivity to stress. During preoccupation, the decreased function of the prefrontal cortex leads to an inability to balance the strong desire for the drug with the will to abstain, which triggers relapse and reinitiates the cycle of addiction. The compromised neurocircuitry reflects the disruption of the dopamine and glutamate systems and the stress-control systems of the brain, which are affected by corticotropin-releasing factor and dynorphin. The behaviours during the three stages of addiction change as a person transitions from drug experimentation to addiction as a function of the progressive neuroadaptations that occur in the brain (Volkow et al., 2016).

2.1.2 Diagnostic criteria of addiction

In 2013, the American Psychological Association (APA) released Diagnostic and Statistical Manual of Mental Disorders, 5th ed. (DSM-5) in order to rank substance use disorder into mild, middle and severe. Rank is given based on amount of conditions met (max 11) in one year (Koob and Volkow, 2016; U.S. Department of Health and Human Services, 2016). Mild disorder has two-to-three conditions, moderate has four-to-five condition, while severe has equal or greater than six conditions (Table 2.1). The term 'addiction' in the DSM-5, is synonymous with the classification of severe substance-use disorder (Volkow et al., 2016).

Table 2.1 Criteria for diagnosing substance use disorders. Adapted from U.S. Department of Health and Human Services (2016).

Diagnostic Criteria for Substance Use Disorders
Using in larger amounts or for longer than intended
Wanting to cut down or stop using, but not managing to
Spending a lot of time to get, use, or recover from use
Craving
Inability to manage commitments due to use
Continuing to use, even when it causes problems in relationships
Giving up important activities because of use
Continuing to use, even when it puts you in danger
Continuing to use, even when physical or psychological problems may be made worse by use
Increasing tolerance
Withdrawal symptoms

Notes: Fewer than 2 symptoms = no disorder; 2-3 = mild disorder; 4-5 = moderate disorder; 6 or more = severe disorder.

2.2 Drug dependence and withdrawal

2.2.1 Definition of drug dependence

Drug dependence is defined as the state of mental and physical during which the person is compelled to take drug periodically or continuously in order to avoid physical discomfort or to achieve psychic effect such as euphoria (Gupta, 2018). Physical dependence would occur due to abstinence, antagonist treatment or reduction in dose taken (Polston and Wallace, 2017). Psychological dependence on the other hand govern drug seeking behaviour, which is the main cause of relapse and treatment is extremely complicated (Sun et al., 2009). The present study focused on physical dependence.

2.2.2 Withdrawal

2.2.2(a) Definition of withdrawal

Withdrawal is an illness that emerge when the body cannot function normally without the presence of the drug. The body has adjusted to the drug after a period of consumption and cessation or reduction in drug taken leads to withdrawal (Stephen et al., 2014). Not all drugs come with an identifiable syndrome of withdrawal. Withdrawal syndrome is the group of symptoms, that are vary among users and substance taken, but depression, anxiety, craving are common psychological withdrawal syndrome (Stephen et al., 2014). This dissertation focusses on the manifestation of physical withdrawal syndrome.

2.2.2(b) Withdrawal syndrome in opiate

The withdrawal syndrome usually develops from 6 to 48 hours after the last opioid dosage based on the half-life of the opioid used (WHO, 2009; Gupta, 2018). syndrome includes hyperthermia, diarrhoea, agitation pupillary dilation, hypertension, and hyperalgesia. Psychiatric disorder on the other hand includes dysphoria, anxiety, and depression (Kreek and Koob, 1998). Therefore, individuals are compelled to continue to use opioids to avoid this unpleasant somatic, autonomic, and psychiatric syndrome.

The withdrawal syndrome unmasked subsequently to the drug cessation that usually begins some time (typically within hours) after the drug administration ceases which includes, negative emotions and physical illness. This withdrawal syndrome will occur in all addictive substances, however, varies in terms of intensity and duration depending on types and severity of substance used (U.S. Department of Health and Human Services, 2016).

2.3 Drugs that cause addiction

There are several drugs that can cause drug addiction for examples barbiturates, cannabis, cocaine, amphetamine, methamphetamine and ecstasy (Verdejo-García et al., 2004; Zehra et al., 2018). Other than that, opioid also can cause addiction.

2.3.1 Opioid

Chemical, natural or synthetic compound that binds to the opioid receptor are termed opioid. Opiates on the other hand specifically refers to the naturally occurring alkaloids of poppy plant (Vallejo et al., 2011). Opium (also known as poppy tears), derived from the Greek term for 'juice,' is the brownish residue found following

desiccation of the poppy's juice (a dry latex), which is obtained from the poppy's unripe seed capsules, *Papaver somniferum*. Opium is the source of over 20 opiate alkaloids (Boström, 2007) and the major alkaloids derived from this poppy plant are morphine, codeine, thebaine, papaverine and narcotine (noscapine) (Chalise, 2015).

2.3.1(a) Opioid mechanism of actions and effect

Through interacting with opioid receptors, opioids develop their strongest pharmacological effects. Opioid receptor is a G protein-coupled receptors (GPCRs) with several subtypes such as mu-opioid receptor (MOP), kappa-opioid receptor (KOP), and delta-opioid receptor (DOP) while opioid receptor like-1 (ORL1) being the least characterized. All four opioid receptor subtypes share common pathways. Figure 2.2 demonstrated intracellular signal transduction mechanisms activated by opioid receptors.

For centuries, opiate and its derivatives were known in pain relieving. Morphine is example of opioids renowned for its analgesic property (Levy and Proudfit, 1979; Samii et al., 1981; Maher et al., 2020). Analgesic effect happens when opioid binds to the MOP. This would also trigger euphoric effect, since central dopamine is activated, which a key to the initiation of addiction. However, mu agonist usage has several drawbacks such as tolerance development, withdrawal, sedation, constipation, and respiratory depression (Table 2.2) (Al-Hasani and Bruchas, 2011).

While MOP, KOP and DOP subtypes activation induced analgesia, the activation also affects mood through central opioid signalling. Activation of MOP or DOP activation improves mood (Shippenberg et al., 2008; Filliol et al., 2000). KOP activation however caused dysphoria in humans and prodepressive behaviour in rodents (Lalanne et al., 2014) which limits KOP agonist usage in pain treatment.

Dynorphin/KOP is also more likely to induce stress behaviour in animal models for depression, anxiety, and drug seeking behaviours (Bruchas et al., 2010). Moreover, several studies pointed that blocking KOP might reduce response related to depression, stress and drug craving (Shippenberg, 2009). Therefore, agonist that partially bind to MOP but antagonist to KOP opioid is preferable as it gives analgesic effects without the dysphoric side effects.

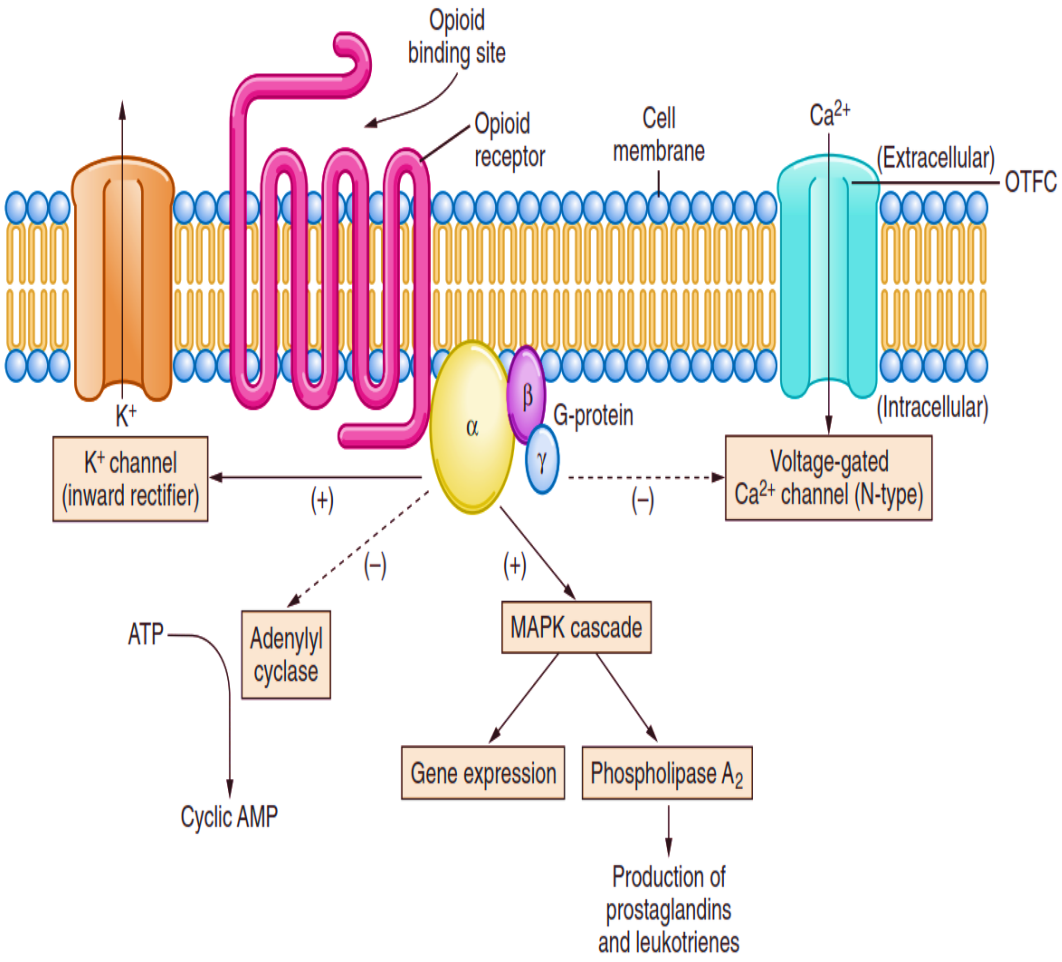

Figure 2.2 Opioid mechanisms of action (adapted with permission from Ogura and Egan (2019). The endogenous ligand or drug binds to the opioid receptor and activates the G protein (three distinct protein subunits), resulting in multiple effects that are primarily inhibitory. The activities of adenylate cyclase and the voltage-dependent calcium ion (Ca2+) channels are depressed. The inwardly rectifying potassium ion (K+) channels and mitogen-activated protein kinase (MAPK) cascade are activated. AMP, Adenosine monophosphate; ATP, adenosine triphosphate.

Table 2.2 Organ system effects of morphine. The actions summarized in this table are observed for all clinically available opioid agonists. Adapted from Al-Hasani and Bruchas (2011).

Organ Systems	Effects	
Central Nervous System	↑ Analgesia	↓ Cough reflex
	↑ Euphoria	↑ Miosis-Constriction of the pupils
	↑ Sedation	↑ Truncal rigidity
	↓ Rate of respiration	↑ Nausea and vomiting
Peripheral	**Gastrointestinal system**	
	↑ Constipation	↓ Peristaltic waves in the colon
	↓ Gastric motility	↑ Constriction of biliary smooth muscle
	↓ Digestion in the small intestine	↑ Oesophageal reflux
	Other smooth muscle	
	↑ Depression of renal function	
	↓ Uterine tone	
	↑ Urinary retention	
	Skin	
	↑ Itching and sweating	
	↑ Flushing of the face, neck and thorax	
	Cardiovascular system	
	↓ Blood pressure and heart rate if cardiovascular system is stressed	
	Immune System	
	↓ Formation of rosettes by human lymphocytes	
	↓ Cytotoxic activity of natural killer cells	
	Other	
	Behavioural restlessness	

2.3.2 Morphine: A classic opioid

Morphine is the chief alkaloid of opium, which has been extensively used over the past centuries as an antinociceptive agent. Morphine was used to compare potency between other opioid drugs (Gaertner et al., 2008) and to evaluate new opioid drugs (Ogura and Egan, 2019). Between 1803 and 1805, the German pharmacist Friedrich Sertürner discovered the active opium compound known as morphium after Morpheus, the Greek god of dreams, and several years later Gay-Lussac changed its name to morphine (Schmitz, 1985; Cruz Martin del Campo and Granados-Soto, 2015).

Morphine is a phenanthrene derivative and the chemical structure of morphine is illustrated in Figure 2.3. Morphine is a weak base with a pK_a value of 7.9. In acidic condition, morphine is highly soluble in water, while having poor solubility in lipid at physiological pH (7.4) (Christrup, 1997). It penetrates through the blood-brain barrier via paracellular route (De Gregori et al., 2012) which rely on morphine concentration. It is pumped out actively by P-glycoprotein (Boström et al., 2008). Thus, inhibition of the protein may increase morphine toxicity (Dewanjee et al., 2017) and coadministration with P-glycoprotein inhibitor should be avoided.

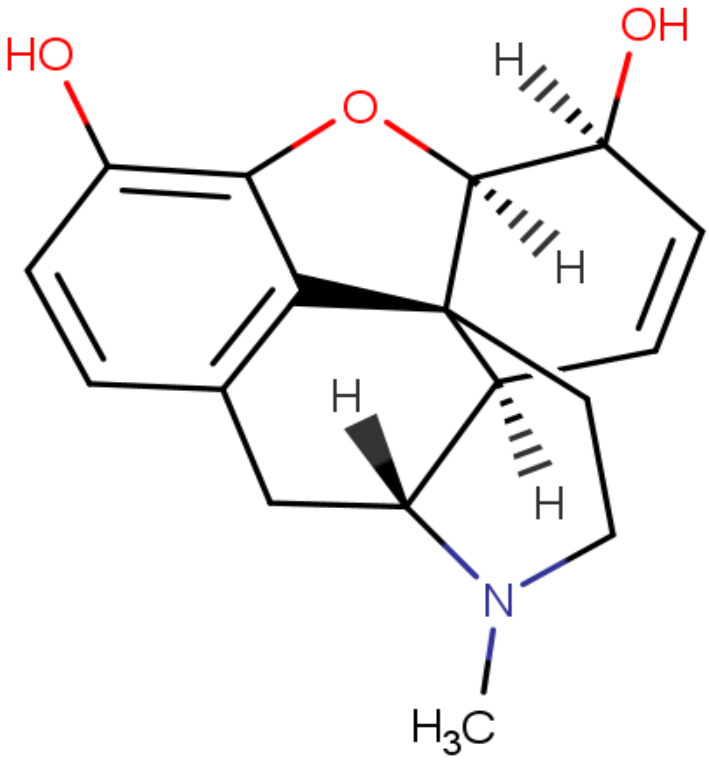

Figure 2.3 Chemical structures of morphine

2.3.2(a) Absorption, distribution, metabolism and excretion of morphine

Morphine have a good absorption through the gastrointestinal (GI) tract after oral administration (Christrup, 1997) with similar cumulative urinary excretion of morphine tagged with radioactivity between oral and intravenous adminisrations (Iwamoto and Klaassen, 1977). Even though morphine was completely absorbed via oral administration, 'first-pass effect' through intestinal and hepatic metabolisms reduced its systemic availability (Iwamoto and Klaassen, 1977). Peak level of morphine absorption via oral showed five to seven times lower than parenteral routes (Glare and Walsh, 1991). Nonetheless, study done by Iwamoto and Klaassen (1977) has compared the route of administrations of morphine in rats and revealed that morphine had no effect on route of administration which give the plasma half-life of about 115 minutes and 2-2.5 hours in humans (Glare and Walsh, 1991). In another study, a pharmacokinetic study in rats after intraperitoneal administration of morphine revealed that the elimination half-life of morphine was 46 ± 2.3 minutes (Zheng et al., 1998).

Furthermore, morphine is rapidly distributed to perfused tissues, such as liver, kidneys and lungs (Spector and Vesell, 1971). In human, almost thirthy five percent of morphine binds to albumin (Leow et al., 1993) whereas sixty percent is in rats (Boström, 2007). Drug half-life is affected by tissue binding since it acts as a storage point which gradually release the bound drug once the inequilibrium triggers due drug excretion or metabolism. A two-compartment model with a joint central compartment was done by Boström (2007) to describe the pharmacokinetics of morphine in blood and plasma which reported that the morphine concentrations in total brain tissue

declined with a half-life of 65 minutes, the half-life of unbound morphine in brain was 68 ± 18 minutes, which is longer than the half-life in blood of 38 ± 8 minutes.

In human, the bioavailability of morphine is between twenty to thirty percent due to first-pass metabolism effect which also varies between individuals (Hoskin et al., 1989). Hasselström and Säwe (1993) demonstrated oral bioavailability in humans was 29.2 ± 7.2%.

A fraction of morphine went into enterohepatic circulation (Hasselström and Säwe, 1993) and some was converted to glucuronides in bile (Hanks et al., 1988). Christrup (1997) has reported that most morphine is transferred from the intestine to the liver following oral administration and undergoes rapid metabolism into two main metabolites: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). The UGT2D7 enzyme, metabolizes morphine in a ratio of 1:9 (M6G/M3G) (De Gregori et al., 2012). Christrup (1997) also reported that morphine glucuronidation also occurs in the brain and kidney, despite low capacity compared to hepatic metabolism. Glucuronides are eliminated by bile and urine.

In human, M3G and M6G are morphine's main metabolites. Morphine clearance formed 57.3% M3G and 10.4% M6G respectively (Hasselström and Säwe, 1993). M6G is more inclined to bind MOP than for DOP and KOP which is similar to morphine. M6G is responsible for inducing most of the analgesic effect and its presence can be observed via the findings conducted by Smith et al. (1990) and Abbott and Palmour (1988). However, several studies also reported that M6G is not produced in rats (Coughtrie et al., 1989; Oguri et al., 1990; Kuo et al., 1991).

Generally, in rats, the main metabolite formed during the morphine elimination is M3G (Ekblom et al., 1993). M3G have no analgesic effect and does not bind to

opioid receptors (Ekblom et al., 1993). M3G has been found to antagonize morphine effects towards the antinociceptive effect of morphine and M6G, which explained tolerance development to the antinociceptive effect during administration of morphine (Smith et al., 1990). Moreover, M3G also reported to elicit hyperalgesia in mice (Roeckel et al., 2017) as well as activates the Toll-like 4 receptors initiating neurogenic inflammation in the central nervous system (Zylicz, 2018).

In addition, systemic plasma clearance of morphine was approximately 21.1 ± 3.4 mL/min/kg (1.27 ± 0.20 L/h/ kg) and volume of distribution was 2.9 ± 0.8 L/kg in human (Hasselström and Säwe, 1993). In rats, the total morphine plasma clearance was 66.1 ± 6.9 mL/min/kg after intravenous administration and 61.5 ± 4.2 mL/min/kg after systemic administration (Iwamoto and Klaassen, 1977).

2.4 Detoxification vs Medication-Assisted Treatment (MAT)

Detoxification is an initial treatment for patients seeking to stop illicit opioid misuse (Stein et al., 2020). On the other hand, medication-assisted treatment (MAT) is a long-term pharmacotherapy for opiate addiction. Methadone, buprenorphine, and naltrexone has been approved by FDA for this purpose (Bart, 2012).

2.4.1 Medication-Assisted Treatment (MAT) in Malaysia

Before MAT, the treatment and rehabilitation concept in Malaysia was the 'cold-turkey' detoxification approach, whose strategy is to rehabilitate drug dependents as effective members of society by severing their dependence on illicit drugs and preventing recurrence (Vicknasingam and Mahmud, 2008). MAT first emerged in Malaysia in 1995 with the advent of naltrexone, an opioid antagonist but not very effective due to precipitous withdrawal encounters with heroin (Ali et al., 2018).

Therefore, two alternative replacement drugs in MAT, buprenorphine and methadone were approved in 2002 and 2003 respectively (Singh et al., 2013) and showed good results (Reid et al., 2004). Finally, in 2005, syrup-shaped methadone began to be used in government clinics and hospitals to indirectly minimize HIV admission by treating patients with street drug problems including heroin and morphine (Ali et al., 2018). In 2010, the 'National Anti-Drug Agency (NADA)' plans to move from 'compulsory drug detention centers (CDDCs),' where at least two years of mandatory detention following CDDCs, to voluntary drug rehabilitation programs called 'Cure and Care (C&C) centers' which has been recognized by the international community as a major change in its withdrawal policy (Amon et al., 2014). The biggest difference with the network of mandatory rehabilitation centres is that these clinics mostly run on a voluntary basis. This policy promotes a much more holistic care plan with free facilities such as tuberculosis screening and opioid substitution without any legal ramifications if drug withdrawal is not achieved (Al-Darraji et al., 2014; Tanguay, 2011a).

2.4.2 Drugs used as replacement therapy in the present study

2.4.2(a) Methadone

Methadone is a synthetic opioid and act as a full agonist at MOP. Methadone is rapidly absorbed via oral administration with bioavailability between 70% to 80% (Bart, 2012). Approximately ninety percent of methadone bind to plasma proteins such as globulin fragments, α_1-acid-glycoprotein and albumin. The remaining ten percent is available for transport throughout tissues such as blood-brain barrier where the pharmacodynamic effects are mediated (Olsen, 1973). Methadone metabolism mainly occurred hepatically via the liver enzyme CYP 450 3A4 and 2B6 enzymes into inactive

compounds and eliminated through faecal and renal routes (Bart, 2012; Buchholz and Saxon, 2016).

Methadone is a schedule II medication which only licensed clinic could give treatment for opioid use disorder (McCarty et al., 2018). In Malaysia, methadone is classified as Schedule III under Poisons Act 1952 [Section 30] Psychotropic Substances (Pharmaceutical Services Programme; Ministry of Health Malaysia, 2020). Methadone has been used as opioid dependence treatment since early 1960s (Dole and Nyswander, 1965). Legal opioids substitution provides an opportunity to mitigate withdrawal and craving of patient while controlling illegal drug abuse. Because it is a long-acting opioid, it makes outpatient management feasible. Nevertheless, a shortage in the number of physicians qualified to prescribe methadone restricts access to methadone treatment. Therefore, daily pick up of methadone creates a barrier to individual living in remote location or jobs that require extensive travel schedule and daily dosing at clinic would be inconvenient (Burma et al., 2017).

Methadone safety is well documented and proven (Kreek, 1973). However, methadone could induce respiratory depression if taken above individual's tolerance (Bart, 2012). The respiratory depression could also be fatal in the event of overdose since there is no ceiling level to it (Mattick et al., 2008). In addition, unknown drug-drug interaction could also lead to death and records showed that methadone patients that abuse other illicit substance together with methadone usually triggers severe side effects (Mattick et al., 2008).

Indeed, unintentional deaths following methadone administration are much more common than with any other opioid (Trescot et al., 2008). Methadone fatality occurred not only among out-treatment patient but also for in-treatment patients.