# MODULATION OF DOPAMINERGIC SYSTEM BY MITRAGYNINE AND THE UNDERLYING MECHANISMS INSTIGATING IMPAIRMENT OF HIPPOCAMPAL SYNAPTIC PLASTICITY

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# MODULATION OF DOPAMINERGIC SYSTEM BY MITRAGYNINE AND THE UNDERLYING MECHANISMS INSTIGATING IMPAIRMENT OF HIPPOCAMPAL SYNAPTIC PLASTICITY

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| % | Percentage |
| > | More than |
| → | Until |
| °C | Degree Celsius |
| α | Alpha |
| β | Beta |
| γ | Gamma |
| δ | Delta |
| θ | Theta |
| σ | Sigma |
| µM | Micromolar |
| 1H-NMR | Proton nuclear magnetic resonance |
| AA | Amino acid |
| AC | Adenylate cyclase |
| ADHD | Attention defisit hyperactivity disorder |
| Ag/AgCl | Silver/Silver chloride |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| ANOVA | Analysis of variance |
| AP | Anterior posterior |
| ATD | Amino-terminal domain |
| BDNF | Brain-derived neurotrophic factor |
| CA | Cornu Ammonis |
| $Ca^{2+}$ | Calcium |
| CaMKII | Calmodulin-dependent protein kinase II |
| CAMKIIα | Calcium/calmodulin-dependent protein kinase type II subunit alpha |
| cAMP | Cyclic adenosine monophosphate |
| CB1R | Cannabinoid type 1 receptor |
| CFMs | Carbon fiber microelectrodes |
| cm/s | Centimeter per second |
| CNS | Central nervous system |

| | |
|---|---|
| CREB | cAMP response element-binding protein |
| CYPs | Cytochromes |
| D1R | Dopamine $D_1$ receptor |
| D2R | Dopamine $D_2$ receptor |
| DA | Dopamine |
| DAT | Dopamine transporter |
| DD | Dopamine- deficient |
| dd$H_2O$ | Double distilled water |
| DG | Dentate gyrus |
| DNA | Deoxyribonucleic acid |
| DSt | Dorsal striatum |
| DV | Dorsal ventral |
| EEG | Electroencephalogram |
| ELISA | Enzyme-linked immunosorbent assay |
| E-LTP | Early LTP |
| EPSP | Excitatory postsynaptic potential |
| ERK | Extracellular regulated kinase |
| fEPSP | Field excitatory postsynaptic potential |
| FFT | Fast Fourier transform |
| fMRI | Functional magnetic resonance imaging |
| g | Gram |
| GABA | Gamma aminobutyric acid |
| GPCRs | G protein-coupled receptors |
| HPLC | High performance liquid chromatography |
| Hz | Hertz |
| i.p | Intraperitoneal |
| I/O | Input-output |
| IACUC | Institutional Animal Care and Use Committee |
| I-LTP | Initial LTP |
| ISIs | Interstimulus |
| kg | Kilogram |
| LBD | Ligand or agonist binding domain |

| | |
|---|---|
| LCMS/MS | Liquid Chromatography with tandem mass spectrometry |
| LH | Lateral hypothalamus |
| L-LTP | Late LTP |
| LTD | Long-term depression |
| LTP | Long-term potentiation |
| MDMA | Methylenedioxymethamphetamine |
| mg | Milligram |
| $Mg^{2+}$ | Magnesium |
| MHz | Megahertz |
| ML | Medial lateral |
| mL | Milliliter |
| MOR | mu opioid receptor |
| mRNA | Messenger RNA |
| mV | Millivolt |
| NAc | Nucleus accumbens |
| NMDA | N-methyl-D-aspartate |
| OFT | Open field test |
| OUD | Opioid use disorder |
| OX1R | Orexin receptor 1 |
| OX2R | Orexin receptor 2 |
| pA | Picoampere |
| PBS | Primed-burst stimulation |
| PD | Parkinson’s disease |
| PFC | Prefrontal cortex |
| p-gp | P-glycoprotein |
| pH | Power of hydrogen |
| pKa | Acid dissociation constant |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PPD | O-phenylenediamine |
| PPF | Paired-pulse facilitation |
| Pt | Platinum-Iridium |

| | |
|---|---|
| SEA | Southeast Asia |
| SEM | Standard error of mean |
| SN | Substantia nigra |
| TAAR1 | Trace amine-associated receptor 1 |
| TBS | Theta-burst stimulation |
| TMD | Transmembrane domain |
| TORC | Transducers of regulated CREB |
| TrkB | Tyrosine kinase B |
| USM | Universiti Sains Malaysia |
| V | Voltage |
| VMAT2 | Vesicular monoamine transporter 2 |
| VTA | Ventral tegmental area |

## LIST OF APPENDICES


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**MODULASI SISTEM DOPAMINERGIK OLEH MITRAGININA DAN MEKANISME DISEBALIK PERMULAAN GANGGUAN KEPLASTIKAN SINAPTIK HIPOKAMPUS**

## ABSTRAK

Mitraginina adalah alkaloid indola primer yang terdapat pada pokok ketum (*M. speciosa* Korth) dan dianggap sebagai sebahagian dari komponen psikoaktif utama ketum. Selain menyumbang kepada kesan ketagihan, ia dikenali untuk merangsang kesan ganjaran dengan bertindak sebagai agonis ke atas reseptor opioid selain menyekat reseptor dopamina $D_2$. Selain itu, mitraginina menyebabkan defisit kognitif dan merosakkan keplastikan sinaptik hipokampus, tetapi mekanisma disebaliknya masih tidak diketahui. Pada awal kajian, modulasi sistem dopaminergik oleh mitraginina telah disiasat dalam tikus yang dirawat dengan mitraginina (1 dan 30 mg/kg) secara akut (rawatan 1 hari) dan berulang kali (rawatan 4 hari) melalui rakaman elektroensefalografi (EEG). Tahap dopamina diukur menggunakan biopenderia elektrokimia dan ELISA analsiss. Ekspressi pengangkut dopamina diukur menggunakan RT-qPCR. Keputusan menunjukkan bahawa pemberian mitraginina pada kedua-dua dos mencetuskan perubahan dalam spektrum frekuensi yang menunjukkan adaptasi sistem dopaminergik dalam tikus yang bebas bergerak. Pendedahan mitraginina secara berulang (1 dan 30 mg/kg) menyebabkan peningkatkan pelepasan dopamina selepas pemberian selama 4 hari, dimana pada dos yang rendah (1 mg/kg) mitraginina menunjukkan peningkatan pengeluaran yang tidak teratur. Pada dos yang tinggi (30 mg/kg) menunjukkan kesan pengeluaran dopamina yang stabil, tidak selepas pendedahan akut dalam rakaman masa nyata melalui biopenderia elektrokimia. Adaptasi sistem dopaminergik oleh mitraginina digandingkan dengan peningkatan expresi pengangkut dopamina di kawasan korteks pra-hadapan. Bahagian

kedua dalam kajian ini ialah untuk menyiasat mekanisme asas yang menyebabkan kerosakan keplastikan sinaptik hipokampus menggunakan teknik rakaman *in vivo* elektrofisiologi. Mitraginina menunjukkan modulasi potentsiasi jangka panjang (LTP) disebabkan pengelibatan pada saluran kalsium, reseptor ionotropik dan metabotropik. Tidak dijangka, mitraginina menerbalikkan kemerosotan LTP yang disebabkan oleh reseptor-reseptor NMDA, meyerupai D1 dan orexinergik. Secara keseluruhan, pemberian mitraginina mengaktifkan sistem ganjaran otak dengan meningkatkan tahap dopamina dan mengadaptasi neuron dopamina melalui regulasi neuron dopaminergik. Mitraginina menujukkan kemerosotan yang signifikan terhadap keplastikan sinaptik hipokampus di kawasan CA1. Akhir sekali, mitraginina menunjukkan potensi secara positif untuk menerbalikkan potensiasi jangka masa panjang yang disebabkan oleh penghalangan reseptor NMDA, D1 dan orexinergik. Sebagai kesimpulan, mekanisma mitraginina yang mencetuskan kemerosotan potensiasi jangka panjang di kawasan hipokampus dengan menyekat saluran kalsium yang mungkin mengurangkan kemasukan kalsium seterusnya menjejaskan keplastikan sinaptik. Penemuan semasa mendedahkan pemahaman yang lebih baik tentang kesan mitraginina dalam otak yang membawa kepada gangguan ingatan jangka panjang.

**MODULATION OF DOPAMINERGIC SYSTEM BY MITRAGYNINE AND THE UNDERLYING MECHANISMS INSTIGATING IMPAIRMENT OF HIPPOCAMPAL SYNAPTIC PLASTICITY**

## ABSTRACT

Mitragynine is the primary indole alkaloid of kratom (*M. speciosa*) Korth and is thought to be part of kratom's main psychoactive components. Besides contributing to the addiction, it is known to induce rewarding effects by acting as an agonist to opioid receptors and block the dopamine $D_2$ receptor. Moreover, mitragynine causes cognitive deficit and impairs the hippocampal synaptic plasticity, but the underlying mechanisms are still unknown. In the early part of the study, the modulation of dopaminergic system by mitragynine was investigated in rats treated with mitragynine (1 and 30 mg/kg) acutely (1-day treatment) and repeatedly (4-days treatment) through electroencephalography (EEG) recording. Level of dopamine release was quantified using electrochemical biosensor and ELISA kit analysis. The expression of dopamine transporter was measured using RT-qPCR. Results indicated that administration of mitragynine at both doses triggered changes in spectral frequencies demonstrating adaptations of dopaminergic system in freely moving rats. Repeated exposure of mitragynine (1 and 30 mg/kg) evoked increase of dopamine release after 4 days of administration, where at low dose (1 mg/kg) shows irregular increase of mitragynine released. High dose (30 mg/kg) shows a stable intensification of dopamine released, not after acute exposure in real time recording through an electrochemical biosensor. The adaptations of dopaminergic systems by mitragynine are coupled with increased expression of dopamine transporter in the prefrontal cortex. The second part of the study is to investigate the underlying mechanisms instigating impairment of hippocampal synaptic plasticity using *in vivo* electrophysiological recording

technique. Mitragynine demonstrates the modulation of long-term potentiation due to involvement of calcium channel, ionotropic and metabotropic receptors. Unexpectedly, mitragynine reversed the impairment of LTP caused by NMDA, D1-like and orexinergic receptors. Overall, the administration of mitragynine activates the brain reward system by increasing the dopamine levels through the regulation of dopaminergic neurons. Mitragynine showed significantly impaired long-term potentiation by blocking calcium channel at CA1 hippocampus region. Finally, mitragynine shows the potential to positively reversed the impairments of long-term potentiation caused by blockage of NMDA, D1-like and orexinergic receptors. As for the conclusion, the mechanism of mitragynine instigating the impairment of long-term potentiation in the hippocampus region by blocking calcium channel which might reduce calcium influx hence impaired the synaptic plasticity. The current finding revealed to better understanding on the effects of mitragynine in the brain leading to long-term memory impairment.

# CHAPTER ONE

# GENERAL INTRODUCTION

## 1.1 Overview

The use of kratom has been reported to no longer limited to the Southeast Asia population but broadened to western communities (Singh et al., 2019; Ramanathan and McCurdy, 2020). Kratom studies have been comprehensive (more than 450 journals), spanning the spectrum of work from botany to neuroscience. Although the argument for consumption differs slightly, studies focused on the established pharmacologically active compounds of mitragynine, 7-hydroxymitragynine, speciociliatine and corynanthidine out of of more than 40 identified kratom compounds (Eastlack et al., 2020).

In the past, traditional folk remedies have been using kratom for the treatment of various illnesses for over 150 years., e.g., relieve opiate withdrawal symptoms or wean oneself off of opiate dependence (Vicknasingam et al., 2010; Singh et al., 2019). Rural workers, in particular, rely on it for pain relief, euphoria, and a reduction in exhaustion from long days on their feet (Assanangkornchai et al., 2007). Concern about the potential negative effects associated with kratom use has prompted a large number of preclinical studies to be conducted on the topic. These studies have focused on issues such as the risk of developing an addiction to mitragynine and the possibility of abusing the substance (Eastlack et al., 2020). For instance, in the setting of prolonged mitragynine consumption, animal models (mice and rats) have shown addiction capacity (Yusoff et al., 2016; Hemby et al., 2019). In addition, addiction and toxicity appear to be linked to 7-OH mitragynine specifically, while mitragynine continues to be a minor risk (Sabetghadam et al., 2010; Hemby et al., 2019). Besides,

chronic usage has been linked to increased tolerance for punishment and reward-seeking behaviour (Ismail et al., 2017). According to the findings of Singh et al. (2014), effective kratom instrumentation does not rule out the possibility of prolonged drug use, which may, within certain conditions, undoubtedly lead to addiction. Ultimately, the concerns of dependency and addiction have always shadowed kratom users.

The correlation between the dopaminergic system and the development of addiction was studied extensively. Addictive substances could exhibit rewarding effects as they interfere with the dopaminergic system causing elevation of dopamine levels in the primary focal point of the brain neurocircuitry of reward (Volkow et al., 2011; Uhl et al., 2019). But even so, there remains a lack of data on the effects of mitragynine on the dopaminergic system. Boyer et al. (2008) reported that mitragynine also acts upon dopamine $D_2$ receptors. In the study, authors employed radioligand assay, but the strength of these affinities has not been reported (Kruegel and Grundmann, 2018). To close the gap, in the present study, effects of mitragynine on the modulation of dopaminergic system was carried out.

## 1.2 Problem statement

Beyond addiction concerns, studies focused the effects of mitragynine on the memory and learning processes. On long-term human kratom consumption impact, Singh et al. (2015) reported difficulties in focusing and recalling past events among long-term kratom consumers. Kratom users also have poor thinking and cognitive deficits (Assanangkornchai et al., 2007). High and large amounts (> 3 glasses/day, equivalent to 72.5–74.9 mg mitragynine) tend to affect cognitive function (Singh et al., 2019). Similarly, chronic administration of mitragynine in rodent models had an

effect on learning, as well as the consolidation and retrieval of memories (Apryani et al., 2010; Yusoff et al., 2016; Ismail et al., 2017). Although mitragynine may improve cognition, it has no effect on short-term memory and does not affect learning (Hazim et al., 2011), chronic exposure reported having an association with impairment in long-term potentiation (LTP) induction which could undermine long-term memory consolidation (Senik et al., 2012; Ilmie et al., 2015). Nonetheless, the studies were *in vitro* studies, and the mechanisms instigating the impairment of *in vivo* hippocampal synaptic plasticity were not reported elsewhere. Hence, in this study, effects of mitragynine on the dopaminergic systems and its mechanism of long-term potentiation impairment were investigated which may provide a significant value in the kratom research.

## 1.3 Hypothesis

Mitragynine from the leaves of *Mitragyna speciosa* have potential to modulate the dopaminergic system in the rats brain. In addition, the mechanism of mitragynine instigates impairment of long-term potentiation by blocking several brain systems includes calcium channel, glutamatergic, dopaminergic, cholinergic, GABAergic and orexinergic systems. Hence, the current study would contribute to a more comprehensive understanding of the modulation of the dopaminergic system by mitragynine and the possible underlying mechanisms instigating impairment of hippocampal synaptic plasticity.

## 1.4 Objectives

The general objective of this study is:

To investigate the modulation of the dopaminergic system by mitragynine (kratom) and the underlying mechanism instigating impairment of hippocampal synaptic plasticity.

The specific objectives of this study are:

1. To investigate the role of the dopaminergic system on spectral frequency changes in freely moving mitragynine-treated rats.
2. To examine the dopamine released evoked by mitragynine in real-time through the electrochemical sensor.
3. To quantify the molecular expression of dopamine and its transporter in rat brains treated with mitragynine.
5. To examine the effects of mitragynine on synaptic transmission in the CA1 region of the hippocampus.
6. To investigate the underlying mechanisms instigating changes in the synaptic transmission in the CA1 region of the hippocampus

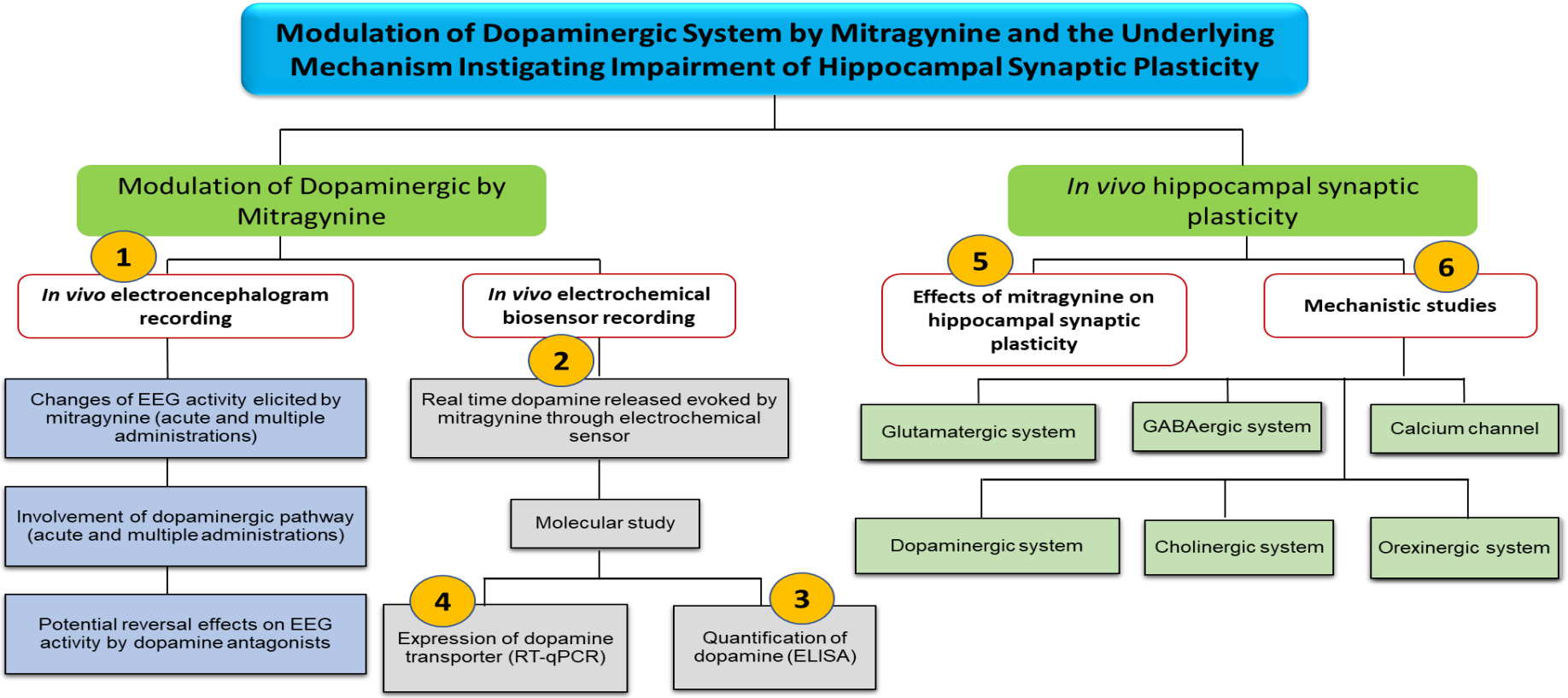


**Figure 1.1:** General outline of the study

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Drug addiction

Historically, addiction has been described as a hypothetically assumed behavioural or moral disorder of voluntary choice, the stigmatisation of individuals with substance abuse was focused on this presumption and continues until today (Volkow and Warren, 2014; Horseman and Meyer, 2019). Drug addiction throughout the scientific community is considered a persistent, recurrent condition marked by compulsive drug seeking, continuous consumption despite harmful consequences, and long-lasting brain changes. In Layman's concept, it is known as a fact or state of substance addiction (Fluyau and Charlton, 2019).

### 2.1.1 Theories of addiction

The Parsimonious theories attempted to describe how and why addiction occurs. One such theory stresses that overall life satisfaction and pleasure-seeking behaviours motivate drug abusers to increase the dose of drugs used to boost mood and manage physical with or without emotional pain. However, the coherent approach to explaining addiction is to consider its biological elements as hypothesised by incentive-sensitisation theory (Ouzir and Errami, 2016). Animal and human studies show that repetition in drug use alters the brain progressively, persistently and robustly (Robinson and Berridge, 2008). The incentive-sensitisation theory states that drugs reinforcing mechanisms are directly related to their subjective pleasurable effects causing brain changes (Robinson & Berridge, 1993; 2000).

### 2.1.2 Neurobiology of addiction

Neuroscience developments have given excellent insight and understanding of addiction neurobiology. Significant advancements in addiction neurobiology can be categorised into three stages: binge/intoxication, withdrawal / negative affect, and preoccupation/anticipation, which deteriorates with time due to synaptic plasticity modifications in the executive function, stress and reward systems of the brain (Koob and Le Moal, 1997; Goldstein and Volkow, 2002; Uhl et al., 2019). This paradigm is illustrated in Figure 2.1 is reinforced by several neuroadaptations in three areas: (1) improved motivation salience, (2) reduced brain reinforcement and increased stress, and (3) diminished executive function; and in three significant neurocircuits: basal ganglia, enlarged amygdala, and prefrontal cortex (Figure 2.1) (Uhl et al., 2019).

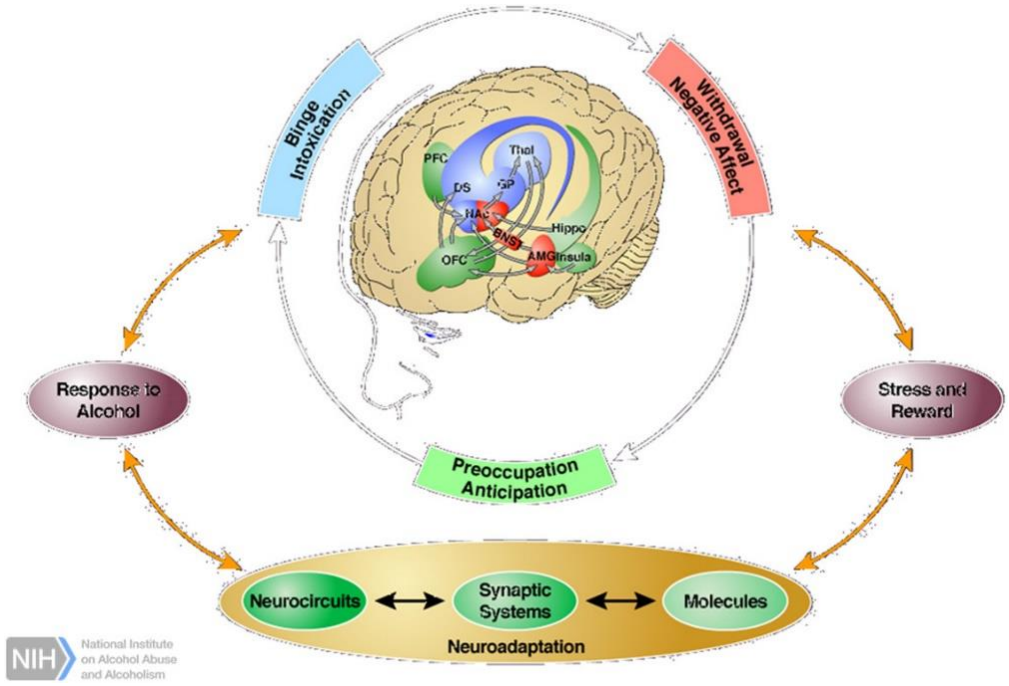


**Figure 2.1:** Conceptual framework for neurobiological bases of the transition to substance use disorders. PFC, prefrontal cortex; DS, dorsal striatum; GP, globus pallidus; NAc, nucleus accumbens; Hippo, hippocampus; Thal, thalamus; BNST, bed nucleus of the stria terminalis; AMG, amygdala; OFC, orbitofrontal cortex. (adapted from Uhl et al., 2019).

At the drug reward center, dopamine is a neurotransmitter and neuromodulator (Di Chiara and Imperato, 1988; Koob et al., 2014). Every substance that has the potential to become addictive raises dopamine (DA), influencing dopamine neurons in the ventral tegmental area (VTA) either directly or indirectly, which contributes to dopamine release in the nucleus accumbens (NAc) (Wise, 2008). Drugs with the potential for abuse elevate DA through various molecular targets based pharmacological effects, including additional neurotransmitters. These include endogenous cannabinoids or endogenous opioids, likewise, contribute to the reinforcing effects of drugs by modulating undesirable affective or hedonic responses (Mitchell et al., 2018) (Figure 2.2).

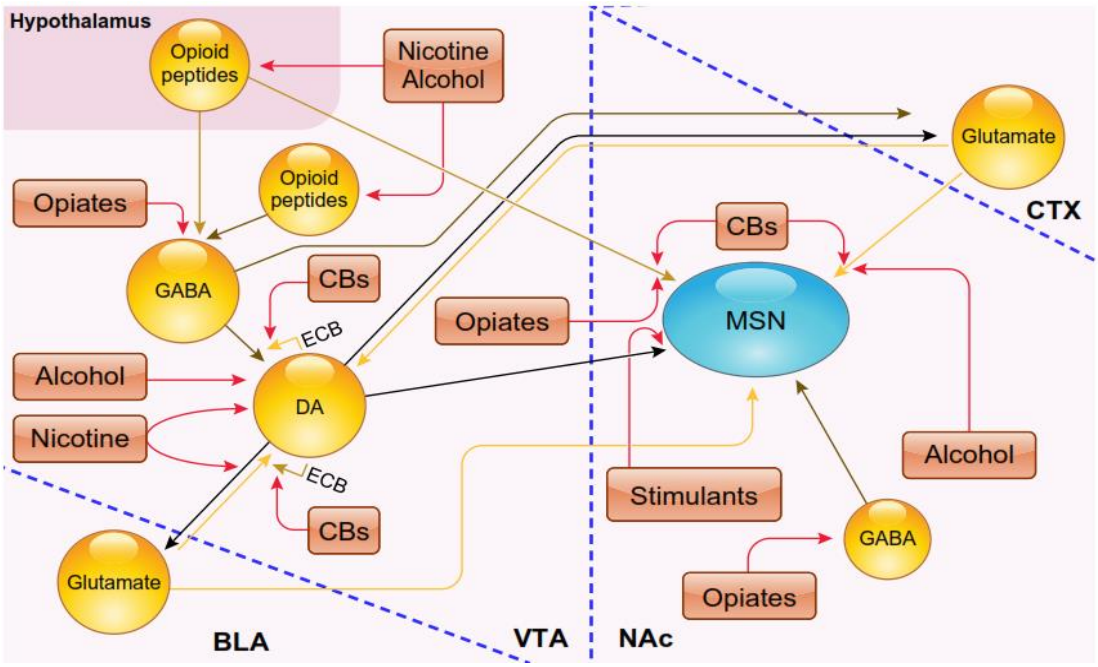


**Figure 2.2:** Schematic representation of crucial target sites for various drugs of abuse across the reward circuitry. CBs, cannabinoids; ECB, endocannabinoid; BLA, basolateral amygdala; VTA, ventral tegmental area; MSN, medium spiny neuron; NAc, nucleus accumbens. (adapted from Uhl et al., 2019).

Non-dopaminergic system's influence on reward processing was not widely studied, but its significance should be undervalued. Hnasko et al., (2005) showed that mice with dopamine-deficient (DD) demonstrated the conditioned place preference when provided with cocaine, which was shown to be regulated by DA neurons, presumably by releasing neuropeptides such as neurotensin and cholecystokinin or glutamate (Hnasko et al., 2007). Genetically engineered mice, on the other hand, demonstrated that mu-opioid receptors (MOR) are not the only major target for opiates and heroin. Eventually, rewarding properties for non-opioid drugs such as nicotine, alcohol and cocaine are also crucial (Herz, 1997; Charbogne et al., 2014).

Repetitive dopaminergic activation causes neuroadaptations of multiple neurotransmitter systems following repeated drug use, namely the glutamatergic pathway, synaptic excitation, and regulation of neuroplasticity (Scheefhals and MacGillavry, 2018), GABAergic system, by inhibiting transmission of action potential , opioid and endocannabinoid systems (Volkow et al., 2017; Mitchell et al., 2018; Wenzel and Cheer, 2018). Besides that. cholinergic (Lim et al., 2014; Dautan et al., 2016), noradrenergic and serotonergic (Borroto-Escuela et al., 2017) systems modulating the circuits in the brain responsible for affective, hedonic, and aversive responses. The neuroadaptations may initiate synaptic excitation or inhibition leading to regulation of neuroplasticity.

DA neurons in the midbrain and their connections to the NAc and dorsal striatum are involved in motivating and sustaining reinforced activities, which often include avoiding aversive stimuli or situations, in addition to the production of GABAergic neurons (Pignatelli and Bonci, 2015). The VTA's DA neurons travel to the NAc, and that is a primary focus of the reward pathway and the primary catalyst of target-driven activities susceptible to the current salience of a related purpose

(Salamone and Correa, 2012). At the same time, DA neurons in the substantia nigra (SN) dorsal striatum project and decode repeated reward signals into habitual actions. The actions are selected according to previous experience with the reinforcement relating with that action and gradually developed to actual or updated purpose values. Repetitive reward-related actions will eventually result in behaviour patterns (Everitt and Robbins, 2016). Furthermore, behavioural patterns may also arise from repeated drug exposure due to reduced attibutes into striatum from prefrontal cortex (PFC) contributing to a loss of influence over the initiation of action (Renteria et al., 2018).

Neurons of DA also project to the amygdala and hippocampus, facilitating self-regulation and salience attribution, all of which contribute to strengthening and conditioning chronic drug use. DA neurons obtained projections from different brain areas affecting tonic and phasic firing in VTA and SN. Recent studies have shown that variation within the VTA DA neuron population is significant due to afferent and efferent connectivity (Mateo et al., 2017), neurotransmitter co-release (glutamate or GABA or both), and presynaptic receptor expression at their terminals. These specifically regulate DA release when neurotransmitters like acetylcholine or GABA are present (Melchior et al., 2015).

DA neurons firing at a tonic rate (1 - 8 Hz) generally establish the contextual tone of the dopaminergic system, sufficient to strongly activate the DA D2 receptors (D2R). Responses to salient stimuli are encrypted by phasic firing (novel, unpredictable, aversive or rewarding) that can activate low-affinity DA D1 receptors (D1R) at 15 Hz (Garris et al., 1994). Tonic and phasic firing make it possible for drugs like cocaine to inhibit the reuptake of dopamine (DA) by the transporter back into the terminal, which leads to a greater accumulation of DA in extracellular space and also increases the regularity with which NAc release events take place (Aragona et al.,

2008). Both the D1R and the D2R can be activated when there is a high level of DA. Both the phasic firing of DA neurons and the activation of D1R are necessary for the experience of drug reward and the formation of conditioned associations. However, DA activation of D2R signals is combined with motivational commitment (Soares-Cunha et al., 2016). However, exposure to multiple alternate reinforcers can interfere with drug reinforcing effects. Notably, both D1R and D2R are simultaneously stimulated by positive reinforcement and maximum reward (Steinberg et al., 2014).

### 2.1.3 Neuroplasticity in addiction

Drugs cause long-term neuroplastic changes in DA neurons in the midbrain and their transitions through the NAc and dorsal striatum by a prolonged and repetitive dopaminergic stimulus, which was believed to be the foundation for addiction along with an enhanced incentive to drug signals and behavioural rigidity (Golden and Russo, 2012; Shan et al., 2014; Pignatelli and Bonci, 2015). Neuroplasticity or neuroadaptation arises through anatomical structure and physiological function of the neurons in response to environmental or internal stimuli. Brain neuroplasticity is modulated by addictive substances, resulting in an aberrant brain functioning pattern that helps generate and maintain addiction (Sampedro-Piquero et al., 2019). Following conditioning drug stimulus activation caused DA neurons to fire preceding drug exposure, indicating a future reward. Conditioning can be represented by a variety of cues, such as people and places associated with drug use, as well as mental states that prevailed at the time of drug use (bored, stressed, depressed or happy), all of which may later unconsciously rouse and motivate drug search (Schultz, 1998; Volkow et al., 2006).

The glutamatergic inputs that are heavily involved in the medium spiny neurons (MSNs) in NAc and the DA neurons in the VTA pave the way for neuroadaptations that lead to the subsequent behavioural changes in reward responsiveness and habituations that are known as addiction, including a constant likelihood of relapse making recovery a task (Kauer and Malenka, 2007). Few essential drug-induced changes, such as those to ionotropic glutamate receptors (AMPA and NMDA receptors) and dendritic morphology that result in long-term potential (LTP) and long-term depression, are comparable to synaptic changes brought on by learning (LTD) (Kauer and Malenka, 2007).

Synaptic strength is governed by pre- and post-synaptic. At the presynaptic site, the strength is governed by the glutamate release. The transmembrane glutamate ionotropic receptors (AMPA and NMDA) on the membrane surface are added or removed to control the synaptic strength at the postsynaptic site, and their subunit's structure can be changed to affect how effective they are (Uhl et al, 2019). In the presynaptic area, release of glutamate in NAc is deprived by triggering metabotropic glutamate receptors mGluA2/3, D2R (Higley and Sabatini, 2010), adenosine A1 (Borycz et al., 2007) or cannabinoid CB1R (Swanson et al., 2001; Robbe et al., 2002). Also, at the postsynaptic site D1R activation regulates AMPA and NMDA trafficking, facilitating the surface expression of AMPA. In the case of cocaine craving, the insertion of AMPA receptors which is highly permeable towards calcium is crucial for the development of the craving (Ferrario et al., 2011). Simultaneously, the increase in NMDA receptors is associated with neuroplasticity following chronic heroin and cocaine usage (Huang et al., 2009; Shen et al., 2011; Wang et al., 2018).

### 2.1.4 Drugs of abuse

Drug abuse was linked to substantial morbidity and mortality as a global problem. The primary contaminants include cocaine opiates, amphetamines, methamphetamines, cannabis and "designer drugs" (Quinn et al., 1997; Karch and Drummer, 2008; Büttner, 2014). Other than cardiovascular complications, psychological and neurological disorders represent the most common types of drug toxicity indicators (Cardoso and Jankovic, 1993; Neiman et al., 2000; Brust, 2004; Goforth et al., 2010; Mackesy-Amiti et al., 2012). Various changes in the central nervous system (CNS) were seen in drug users, despite the fact that current knowledge of drug effects comes from animal models (Büttner and Weis, 2006; Karch and Drummer, 2008; Büttner, 2011). Practically all abused drugs share a collective ability to stimulate rewarding effect or/and relieve negative states by activating dopaminergic systems (Feltenstein & See, 2008). Such drugs produce rewarding effects by directly influencing the dopaminergic neurons (opening or closing specific ion channels or influencing second messenger systems) or by indirectly modulating the dopaminergic neurons (Mathon et al., 2003).

Cocaine is very much an alkaloid found in Andes leaves that is extracted from *Erythroxylon coca*. Since more than a century ago, cocaine has been prescribed as medicine, primarily for local anaesthesia, vasoconstriction, and pupil dilation during nasal surgery (Langman and Snozek, 2019). Cocaine increases alertness and euphoric effects by highly stimulating the central nervous system. It acts by inhibiting dopamine and norepinephrine reuptake (Langman et al., 2018), raising the body temperature, blood pressure and heart rate. Cocaine's diverse mechanisms result from the nonenzymatic hydrolysis and enzymatic liver and plasma processing. The primary metabolites are ecgonine methyl ester and benzoylecgonine. These metabolites are

dormant and primarily formed in the liver. The cocaine half-life is very short (0.5 – 1.5 hours) while benzoylecgonine is long enough (47 hours) for screening (Langman et al., 2018). Because of this, benzoylecgonine is the main target of cocaine screening assays, and the antibodies used in these assays typically have excellent cross-reactivity with cocaine (la Porte et al., 2006; Rossi et al., 2006). The majority of validation assays show quantitation of the parent and metabolite. Cocaethylene, a cocaine metabolite that can only be produced when alcohol is present, makes alcohol and cocaine abuse extremely dangerous (Langman and Snozek, 2019).

Amphetamine-type stimulants are hallucinogens and stimulants sharing phenylethylamine's chemical structure. The majority of chiral sympathomimetic amines, amphetamines typically activate the central nervous system (CNS), whereas L-enantiomers have peripheral effects. Amphetamines are now classified as a schedule-controlled substance and were legally prescribed in the past to suppress appetite or depression (Langman and Snozek, 2019). Amphetamine was used to effectively halt depression, and the new use in therapy involves the management of attention deficit disorder and narcolepsy. Amphetamine works by elevating the synapse dopamine output, mainly by inducing presynaptic release rather than inhibiting reuptake. However, it increased dopamine amounts in the brain causing euphoria, leading to amphetamine misuse. Detection of amphetamine and methamphetamine targeting the amphetamine-type stimulant group covering a variety of so-called party products, namely methylenedioxymethamphetamine (ecstasy or MDMA). Regrettably, molecules like pseudoephedrine and psychoactive drugs demonstrated a cross-reaction with false-positive detection. Routine identification and confirmation assays do not differentiate between L-and D-isomers, while specially

designed chiral derivation processes do differentiate isomers (Langman and Snozek, 2019).

Opiates consist of natural or semi-synthetic opium-derived alkaloids from poppy seeds (*Papaver somniferum*). The main opiate found in nature is morphine, which is followed by heroin, hydromorphone, oxymorphone, hydrocodone, and levorphanol (Langman and Snozek, 2019). Opioid receptors are affected by opioids (mu, delta, and kappa). Mu receptors support physical dependence, euphoria, respiratory discomfort, gastrointestinal dysmotility, and analgesia. Mu receptors reduce the medullary response to hypercarbia and the respiratory response to hypoxia, which reduces the stimulation of breathing and promotes the development of apnea. Analgesia, diuresis, miosis, and dysphoria are all mediated by kappa receptors. Cough suppression, dopamine suppression, and analgesia have all been linked to delta receptors. Chronic use can contribute to tolerance and psychological dependence (Schiller et al., 2020). It is significant to note that opiate immunoassays do not consistently detect all members of the drug class, which is why pain management services frequently use urine drug testing to monitor compliance, misuse, or replacement of prescribed opiates. The majority of opiate testing assays concentrate on the drug morphine and show varying degrees of reactivity to codeine, hydrocodone, and hydromorphone. Poor cross-reactivity between oxycodone and oxymorphone makes opiate screening assays difficult to use reliably. Synthetic opioid abuse, like that of fentanyl, is quickly catching on in addition to the abuse of opiates that have long been abused like morphine and heroin. The majority of synthetic opioids simply cannot be classified using opiate assays, which creates an analytical challenge (Reisfield et al., 2007).

Natural cannabinoids are psychoactive substances that are extracted from the *Cannabis sativa* plant. These substances have been utilised for the purpose of inducing euphoria for over 4000 years, and they are the illicit drug that is used the most frequently all over the world. United Nation (UN) data show that 3.8 percent of the global total or 182 million people aged 15 to 64, use cannabis (Akerele & Olupona, 2017). Hashish and hash oil are made from distilled resin and lipid-soluble powder, respectively, whereas marijuana cigarettes are made from plant leaves and flowering tops. The 400 psychological-physical chemicals present in cannabis, of which delta-9-tetrahydrocannabinol (THC) is the most psychoactive, are collectively referred to as "cannabinoids" (Langman and Snozek, 2019). In addition to THC, smoking marijuana releases 150 additional compounds, many of which have physiological effects even though they lack psychoactivity (Langman and Snozek, 2019The central and peripheral nervous systems highlighted key cannabinoid receptors (CB1 and CB2) (Langman and Snozek, 2019). The most notable effects of CB receptor activation are euphoria and relaxation; users also experience feelings of well-being, grandiosity, and fleeting perception. The drug may cause memory impairment, somnolence, perceptual changes (such as visual inconsistencies), and decreased coordination. Cannabinoids are used in medical treatment because they have widespread side effects like a reduction in nausea, a reduction in intraocular pressure, and a reduction in chronic pain (Langman and Snozek, 2019). THC is quickly absorbed from the lungs into the bloodstream after cannabis is smoked, where it then effectively distributes to tissues. THC is widely metabolised, with 11nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) as its main inactive metabolite; THC-COOH results are typically reported (Langman and Snozek, 2019).

Novel drugs are novel synthetic illicit drugs with liability for abuse or analogues of established illicit drugs. Few of the novel drugs have higher potency and toughen identification and detection process compared to known and archived compounds. The availability of compounds and drugs causes the prevalence of these substances to fluctuate constantly. Despite the paucity of conclusive epidemiological evidence, novel and synthetic drugs pose a threat to the public health of adolescents (Wang and Hoyte, 2019). The use of novel drugs will result in significant medical, acute toxicity, and polydrug use, which are all linked to prominent morbidity and mortality (Ninneman et al., 2017; Palamar et al., 2017). This type of drug is commonly known as research substances, designer drugs, psychoactive or legal high.

Synthetic cannabinoids are chemically produced analogues of natural cannabinoids, not cannabis, and are among the new illicit products. The chemically created cannabinoids are sold as a powder or as a spray on dried marijuana herbs or other psychedelic goods. Currently, it is known as spice, K2 or Buddha and more than 200 synthetic cannabinoids have been reported worldwide. Synthetic cannabinoids act as agonists toward cannabinoid receptors. More than 11,000 emergencies were related to synthetic cannabinoids in 2010. Most cases were adolescents and young adults aged 12 to 29 (Bush and Woodwell, 2014). Now, teen use has declined from 11.4% to 3.5% since 2012 (Palamar and Acosta, 2015; Keyes et al., 2016; Johnston et al., 2019). Comorbidity associated with adolescent use of synthetic cannabinoids involves depressive symptoms and tobacco, drug and polysubstance usage (Ninneman et al., 2017; Palamar et al., 2017).

## 2.2 *M. speciosa* Korth and its primary alkaloids

Kratom, also known as *M. speciosa*, is a coffee family (Rubiaceae) member that is common in Southeast Asia (SEA) and has a long history of traditional use (Hassan et al., 2013, Singh et al., 2016). Oral consumption of the leaf or its extract is common for the alleviation of pain and a broad range of other ailments as well as to enhance the performance of manual and agricultural labour (Hassan et al., 2013; Singh et al., 2016). Because of how they are made, kratom and its alkaloids are considered to be rare opioids that are biologically different from morphine and other common opioids that come from the poppy family (Papaveraceae) (Raffa et al., 2018). Kratom arose as a natural substance that can be purchased online (Babu et al., 2008; Prozialeck, 2016) and was subsequently listed by the US Drug Enforcement Administration as a Drug of Concern. Studies show that people in the U.S. use kratom to help relieve and stop withdrawal from opioids (Boyer et al., 2007; Swogger et al. 2015; Grundmann, 2017; Smith and Lawson, 2017; Henningfield et al., 2018; Coe et al., 2019). Although inadequate empirical data to support therapeutic benefits found.

Kratom consists of at least 36 unique alkaloids, including mitragynine. In kratom leaves, primary alkaloids made up 66 percent of all alkaloids (Takayama, 2004; Kruegel and Grundmann, 2018). According to studies, mitragynine might just be a candidate for the creation of novel, efficient painkillers (Macko et al., 1972; Takayama, 2004; Kruegel and Grundmann, 2018), the effects are based on its acting on G-protein-based partial mu-opioid receptor agonists while neglecting recruitment of β-arrestin signalling pathways such as traditional opioids (Kruegel et al. 2016; Váradi et al., 2016). Results from animal studies show that kratom extracts and mitragynine have robust anti-nociceptive effects in both thermal and mechanical stimuli with slight respiratory depression (Macko et al., 1972; Matsumoto et al., 1996;

Sabetghadam et al., 2010; Hassan et al., 2013). However, mitragynine's abuse liability testing in rats showed that it lowers but does not maintain intravenous self-administration of morphine, indicating a low risk of abuse (Hemby et al., 2018). Although mitragynine itself is not self-administered, pretreatment with it has been shown to decrease heroin self-administration (Yue et al., 2018). Other than mitragynine, 7-hydroxymitragynine was well known to have pharmacological properties. In terms of yield, 7-hydroxymitragynine was presented only 2% from the leaves of Mitragyna speciosa. Although the quantity less than mitragynine, 7-hydroxymitragynine demonstrated mu-opioid receptor-mediated analgesic properties (Matsumoto et al., 2005), and it has demonstrated that it can replace morphine, stimulate and maintain intravenous self-administration in rats, and do so in a dose-dependent way (Hemby et al., 2018).

### 2.2.1 Plant description

The kratom trees can grow up to 15 - 50 meters high and can be up to 5 meters wide. The leaves are dark glossy green with an ovate-acuminated outline and tapered ends about 18 cm long and 10 cm wide. Its flowers grow through the colour of deep yellow carrying up to 120 florets in globular clusters attached to the leaf axils on long stubbles, forming winged seeds (Shellard and Lees, 1965; Shellard, 1974). The tree nurtures best in hot, humid, fertile soil with mild to maximum sun exposure in pieces surrounded by strong winds (Macko et al., 1972). The leaves shed throughout the year, mostly during the year's dry weather, then repeated during the rainy season. The parts used for use are its tiny leaf and roots. Gong et al. (2012) genetically defined and validity distinction between *Mitragyna* species is now feasible using internal transcribed spacer sequence analysis (Sukrong et al., 2007; Maruyama et al., 2009).

### 2.2.2 Current trend of usage and preparations

In Southeast Asia, kratom was widely used for various reasons, including but not restricted to its alleged medicinal and anti-fatigue properties. It was often drunk in Thai villages during religious ceremonies and as a leisure, drink to relax and socialize among people. Kratom leaves are commonly used as a household remedy for hypertension and diabetes (Burkill & Haniff, 1930; Burkill, 1935; Lee, 1957; Assanangkornchai et al., 2007; Saingam et al., 2013). Remarkably, the western population use of kratom focused more on chronic pain (Singh et al., 2016).

Over time, Vicknasingam et al. (2010) recorded that kratom use among Malaysians has moved to use kratom as an alternative (cost effective) to reduce their dependence on abused substances and control withdrawal symptoms. Youngsters in Thailand and Malaysia reveal worrying trends in urban use of kratom where it was used as an alcohol and drug replacement (Tungtananuwat & Lawanprasert, 2010; Singh et al., 2016).

### 2.2.3 Legality

Under the 1952 Poisons Act, kratom was illegalised in Malaysia and criminalised in Thailand since 1943. Cultivating kratom trees is illegal, under this act, both Malaysians and people of Thailand caught distributing or possessing processed kratom leaves may be fined up to RM 10,000 or 4 years in jail time (Vicknasingam, 2010). The plant is not only banned in Southeast Asia but also regulated by the many EU Member States such as Poland, Lithuania, Finland, Denmark, Romania and Sweden(Singh et al., 2016).

### 2.2.4 Kratom pharmacology (pharmacokinetic and pharmacodynamic)

Mitragynine was described as a weak base (pKa=8.1) and lipophilic (Log P= 1.73) (Ramanathan et al., 2015). It dissolved in aqueous media at pH 7 and 4 with concentration levels of 83 and 187 µM, respectively (Kong et al., 2017). Mitragynine is moderately stable at 37°C at neural pH but degrades approximately 3.5% after 3 hours and 26% degradation after 1-2 hours at pH 1.2 (Manda et al., 2014; Ramanathan et al., 2015).

#### 2.2.4 (a) Absorption

The absorption of mitragynine *in vitro* shows that mitragynine fluxes across the phospholipid bilayer at pH 4 and 7.4 were $0.23 \times 10^{-6}$ and $11 \times 10^{-6}$ cm/s, respectively (Kong et al., 2017). According to these findings, mitragynine permeated as the unionised form. Mitragynine's flux ratios were roughly equal to 1, and passive membrane diffusion demonstrated that it is not a P-glycoprotein substrate (P-gp). Captivatingly, mitragynine fluxes across MDR-MDCK cell monolayers which is comparable to propranolol, caffeine, atenolol, furosemide, carbamazepine and metoprolol. Across the gut epithelium, mitragynine permeability to the intact intestine *in situ* is $1.11 \times 10^{-4}$ cm/s in the absorption route (Jagabalan et al., 2019), which compared favourably with permeant atenolol ($0.41 \times 10{-}4$ cm/s) and propranolol ($1.12 \times 10{-}4$ cm/s). The P-gp inhibitors (azithromycin) or CYP3A4 (ciprofloxacin) shown to impact on mitragynine, proving that its absorption is rapid and passive.

#### 2.2.4 (b) Metabolism and excretion

Mitragynine metabolism included phase 1 and phase 2 which occurred mostly in the liver using a liver microsome or S9 fraction system (Manda et al., 2014; Kong et al., 2017). Philipp et al. (2009) identified 7 phase 1 mitragynine metabolites and five

conjugates. Besides, one sulphonate and four glucuronides were suspected to be phase 2 metabolites in studies involving urine samples of rats after an oral dose of 40 mg/kg mitragynine measured using LCMS / MS. While in human three sulphonates, and four glucuronidates were reported (Philipp et al., 2011). The effects of mitragynine on metabolic enzymes were reported at a great length. It enhances the expression of CYP2C9, CYP1A2, CYP2D6 and CYP3A4 (Hanapi et al., 2013; Lim et al., 2013; Manda et al., 2017). CYP1A2 and CYP3A4 were both weakly induced by mitragynine, as determined by protein and mRNA expressions (Lim et al., 2013). Considerably, the pregnane X receptor, which controls the transcription of P-gp and certain CYPs, was noticeably elevated. Nevertheless, aryl hydrocarbon receptor, a typical enzyme induces left unaffected, and mitragynine showed to only induced CYP1A2 (Manda et al., 2017). With regards to the action of mitragynine on P-gp, studies concluded that mitragynine might induce P-gp. Hence, co-administering mitragynine with drugs that act as a P-gp substrate can result in drug-herbal interaction. As protein binds free concentration of mitragynine together, extracellular levels fall below micromolar ranges (Ya et al., 2019).

### 2.2.4 (c) Pharmacodynamic

Kratom is not a specific, exclusive compound, but instead a combination of the plant's natural psychoactive alkaloids. Only four of these substances—mitragynine, 7-hydroxymitragynine (7-OH-mitragynine), corynantheidine and speciociliatine are regarded to be pharmacologically active out of the more than 40 that have been discovered so far (Takayama, 2004). The remaining alkaloids are not established to be pharmacologically active. Nonetheless, they can unknowingly contribute synergistically to the overall kratom effect. As kratom extracts contain a variety of alkaloids, each of which has unique potential pharmacologic qualities, the substance's

physiological effects are dynamic and dose-dependent, combining stimulant and opiate-like properties (small amounts, the effects are mostly stimulant, but at higher doses, the effects are mostly opioid) (Babu et al., 2008; Singh et al., 2016).

The binding affinities of mitragynine and 7-OH-mitragynine to opioid receptors varies significantly (Prozialeck et al., 2012). Despite extensive study, the exact nature in which kratom alkaloids function on each receptor appears unresolved. In extensive research, Takayama and colleagues propose both mitragynine and 7-OH-mitragynine function as agonists which bound more selectively on μ-and π- opioid receptors (Matsumoto et al., 2005). Contradictory results, however, give a distinct hypothesis; 7-OH-mitragynine and mitragynine seem to demonstrate dynamic receptor-dependent actions rather than operating as agonists. Specifically, the data show that mixed opioid receptor agonists/antagonists are both mitragynine and 7-OH-mitragynine, functioning as partial agonists on μ-receptors and competitive antagonists on κ-receptors, with negligible effects on κ-receptors (Kruegel et al., 2016).

Notably, the indole alkaloids found in kratom differ from their opioid counterparts in terms of pharmacodynamics and structural makeup, producing effects that somewhat overlap but are not the same. To distinguish these substances from morphine, semi-synthetic opioids, and endogenous ligands, they were given the name atypical opioids (Raffa et al., 2018). Like opioids, the indole alkaloids binding to opioid receptors initiates G - protein-coupled receptor (GPCR) signalling; but, unlike typical opioids, kratom's indole alkaloids do not activate the β-arrestin pathway (Varadi et al., 2016). Biased agonism or ligand-directed signalling is the term used to describe this circumstance, which selectively disengages the numerous signalling cascades connected to the receptor, allowing a single receptor to mediate multiple intracellular effects (Wisler et al., 2014). Ironically, β-arrestin is accountable for nearly

all opioid-related symptoms (e.g. constipation, sedation, respiratory depression) (Bohn et al., 2002; Raehal and Bohn, 2011). As a result, selective inhibition of β-arrestin is a required feature of an opioid, implying that mitragynine could be useful model for developing new opioids with less undesirable side effects (Eastlack et al.,2020).

Mitragynine frequently inhibits pain signalling via additional pathways in addition to its opioid-like analgesic effects, indicating a multimodal role in pain perception control. Mitragynine is structurally similar to yohimbine, another indole alkaloid with well-known adrenergic effects (Prozialeck et al., 2012). Like yohimbine, studies reveal that mitragynine modulates α-2 adrenergic postsynaptic receptors (Matsumoto et al., 1996). This is significant for the analgesic effects of mitragynine, as α-2 receptors are involved in modulatory "descending" pain pathways (Giovannitti et al., 2015). The significance of these mechanisms has only lately become evident and represents a significant development in pain's complex neurobiological comprehension (Ismail et al., 2019). A third anti-nociceptive mechanism was proposed with proof that mitragynine influences neuronal pain transmission by blocking $Ca^{2+}$ channels (Matsumoto et al., 2005). Furthermore, conditional analgesic properties were due to mitragynine's putative anti-inflammatory effects, which were secondary to the suppression of COX-2 and prostaglandin E2 mRNA expression (Mossadeq et al., 2009; Utar et al., 2011). The 5-HT2C and 5-HT7 serotonin receptors, D2 dopamine receptors, and A2A adenosine receptors are only a few of the receptors that mitragynine has some affinity for in the central nervous system. However, it is unknown what these correlations mean physiologically (Matsumoto et al., 2005; Eastlack et al., 2020).