ROLE OF HISTONE REGULATIONS AND MODIFICATIONS IN COGNITIVE FUNCTIONS OF MITRAGYNINE (A MAJOR INDOLE ALKALOID OF MITRAGYNA SPECIOSA)

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by

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LIST OF SYMBOLS

℃	Degree celsius
=	Equal to
<	Less than
µ	Micro
>	More than
*	significance
%	Percent
V	voltage

LIST OF ABBREVIATIONS

µg	Micro gram
α	alpha
β	beta
Amg	Amygdala
APS	Ammonium persulfate
ANOVA	Analysis of variance
ARASC	Animal Research and Service Centre
C	Celcius
cm	centimeter
CNS	central nervous system
DA	dopaminergic
dH_20	distilled water
DTT	dithiothreitol
GP	globus pallidus
h	hour
Hpc	hippocampus
HRP	Horseradish peroxidase
i/p	intraperitoneal
IACUC	Institutional Animal Care and Use Committee
IPS	Institut Pengajian Siswazah
JPEG	Joint photographic experts group
kg	kilogram
LDS	Lithium dodecyl sulfate
LTP	long term potentiation
mg	milligram
mins	minutes
mPFC	medial prefrontal cortex
MWM	Morris water maze
N	total number
n	number per group
NAc	nucleus accumbens

NIH	National Institute of Health
OD	optical density
PAGE	Polyacrylamide gel electrophoresis
PAT	Passive avoidance task
PBS	phosphate buffered saline
PFC	prefrontal cortex
PVDF	Polyvinylidende difluoride
rcf	Relative centrifugal field
rpm	Revolutions per minute
secs	Seconds
SDS	Sodium dodecyl sulphate
SN	substantia nigra
TBS	Tris-buffered saline
TBST	Tris-buffered saline Tween 20
TEMED	tetramethylethylenediamine
USM	Universiti Sains Malaysia
VTA	Ventral tegmental area
WB	Western blot

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PERANAN REGULASI AND MODIFIKASI HISTON DALAM FUNGSI KOGNITIF MITRAGININA (SATU KOMPONEN UTAMA ALKAKOID DALAM MITRAGYNA SPECIOSA)

ABSTRAK

Pengenalan: Penyelidikan sebelum ini mendedahkan bahawa ketagihan dadah mengubahsuai mekanisme kognitif di dalam diri manusia dan membawa perubahan pada tingkah laku kognitif. Kajian juga menunjukkan bahawa ketagihan terhadap dadah seperti heroin, kokain dan yang lain mempengaruhi keupayaan memori dan pembelajaran; dan seterusnya mengakibatkan kemerosotan kognitif. Keadaan ini boleh bertambah buruk jika pengambilan dadah dilakukan secara berterusan dan dalam dos yang tinggi. Dalam kajian ini, kita melihat bagaimana mitraginina, satu komponen utama dalam *Mitragyna speciosa* memberi kesan pada kebolehan kognitif dan perubahan protein histon di dalam otak. Pada masa ini, tiada kajian yang telah dilakukan untuk menghubungkaitkan mitraginina dan epigenetik.

Objektif: Kajian ini bertujuan untuk menyiasat fungsi kognitif dan modifikasi histon dalam tikus yang berada di bawah pengaruh mitraginina.

Metodologi: Experimen tingkah laku telah dijalankan ke atas tikus yang berada di bawah pengaruh mitraginina bagi tujuan menganalisa memori dan gangguan pembelajaran yang disebabkan oleh dos mitragynina (1, 10 dan 30mg/kg) pada masa yang berbeza. Experimen penghindaran pasif (PAT) dan protokol western blot (WB) digunakan untuk kajian tingkah laku kognitif dan penganalisaan protein.

Hasil: Kajian ini menunjukkan hasil yang ketara daripada segi penurunan kognitif pada masa pengekalan 1 jam, tetapi tidak pada 24 jam dan 7 hari dalam PAT tanpa sebarang pengesanan ekspresi protein histon H3K9.

Konklusi: Mitraginina mengakibatkan penurunan kognitif semasa peringkat awal metabolisme dadah di dalam tubuh tikus. Kesimpulan dapat dibuat bahawa mitraginina menyebabkan disfungsi kognitif dengan tiada perubahan pada ekspresi protein histon.

ROLE OF HISTONE REGULATIONS AND MODIFICATIONS IN COGNITIVE FUNCTIONS OF MITRAGYNINE (A MAJOR INDOLE ALKALOID OF MITRAGYNA SPECIOSA)

ABSTRACT

Introduction: Previous researchers reveal that drug addiction does alter cognitive mechanisms in humans and bring changes to cognitive behaviour. Experimental studies involving addiction of drugs like heroin, cocaine and many others have shown to have impact on memory and learning capabilities which results in cognitive decline. This condition can worsen if drug is constantly consumed in long run and high doses. In this study, we were looking at mitragynine, a main compound of *Mitragyna speciosa,* affects cognitive abilities and the changes of histone protein in the brain. Currently, there is no research that has been done to correlate mitragynine and epigenetics.

Objective: This study primarily aims to investigate the cognitive function and histone modifications in the mitragynine treated rats.

Methodology: Behavioural task was conducted in mitragynine-treated rats to analyse the memory and learning functions caused by different doses of mitragynine (1, 10 and 30mg/kg) at different time frames. Passive avoidance task (PAT) and western blot (WB) protocol were used for cognitive behavioural study and protein analysis, respectively.

Results: This study highlighted significant results of cognitive impairment at 1 hour retention time, but not at 24 hours and 7 days in PAT with no changes of histone H3K9 protein expression.

Conclusion: Mitragynine caused cognitive impairment during early stages of drug metabolism in the rat's body. This study concluded that mitragynine caused cognitive dysfunction with no changes of histone protein expression.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The psychological and physical inability to stop consuming a chemical, drug, activity, or substance despite knowing the negative harm it causes, is called addiction (Liu and Li, 2018). There are many types of addictions but in this study, the focus is dependence on a psychoactive substance. Addiction is said to be a "disease" that affects the brain and behavior (Gould, 2010). Resisting the urge when one is addicted to drug is difficult and the process is pain-staking. Chemically, the brain is wired to repeat experiences that brings the feel good sensation (Agrawal *et al.*, 2012). It triggers the motivation to do the action repeatedly. Neurotransmitters and the brain reward system (the limbic system) are involved in this chemical process.

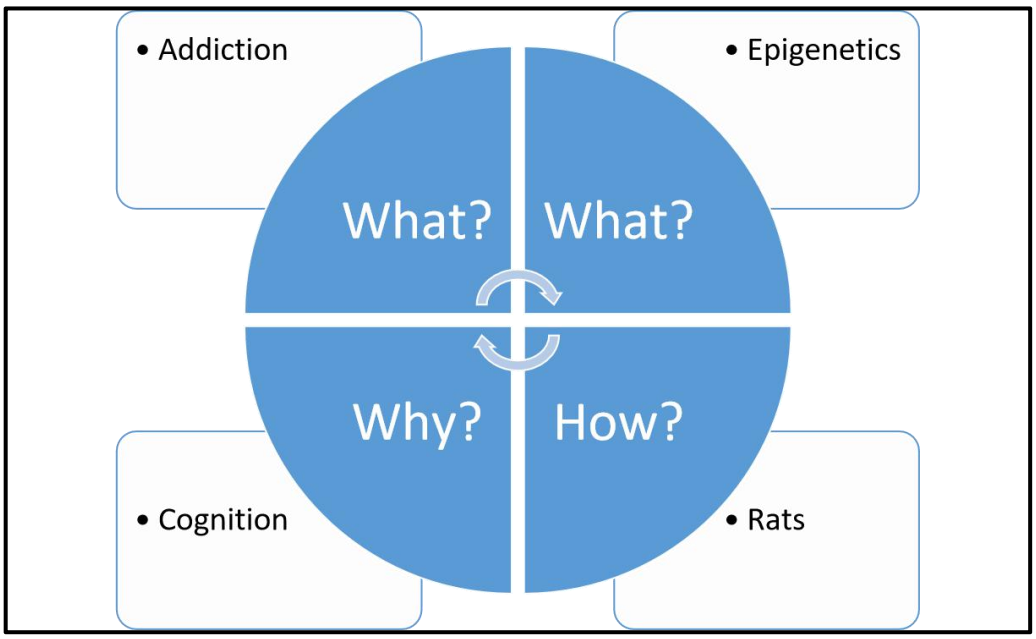

Figure 1.1 Conceptual Framework of this study

Effects of addictive drugs are targeting the brain's reward system (Gould, 2010). It floods the brain with a chemical called dopamine and this neurotransmitter acts to trigger a feeling of euphoria. If the drug is repeatedly taken at high dose, drug seeking ability is developed. Over time, the brain gets extra dopamine (Soremekun *et al.*, 2021). It takes the currently induced high dosage to give the same feeling as in low doses. Furthermore, other common enjoyable things tend to provide less pleasure. It is understood that when drug is consumed for quite some time, changes and disruptions occur in brain chemical circuits (Guindalini *et al.*, 2008). This can lead to poor executive functions such as judgment, decision making, problem solving, memory and ability to learn (Soremekun *et al.*, 2021). Chronic drug usage leads to addiction and exhibits a constant drug seeking behaviour pattern (Harun *et al.*, 2015). This behaviour indirectly changes the mechanisms in the brain of the addicted person (Agrawal *et al.*, 2012). Chemical changes happen in an addict's brain during the synapse process slowly modifies the chromatin structure (histone of DNA) (Ruzilawati *et al.*, 2019). These results in altered gene expression (Kreek *et al.*, 2012).

The reason why genetic is investigated in this study because genetics play a very important role in human system. Humans are a result of gene expression that had happened in the body. Diving deeper into genetics, epigenetics component is found to play a huge role in human biology mechanisms. Epigenetic is the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself (DNA sequence) (Nielsen *et al.*, 2012).

Types of epigenetics changes includes DNA methylation, acetylation, phosphorylation, ubiquitylation, and sumolyation (Heinbockel and Csoka, 2018). It basically alters the chemical or physical changes in chromatin. Factors contributing to these changes are diet, obesity, physical activity, tobacco smoking, alcohol

consumption, environmental pollutants, psychological stress and working on night shifts (Farris *et al.*, 2015).

In relation to this study, epigenetic factor will be mitragynine addiction. Mechanisms of this factor and how it alters the histone protein in the brain is further investigated and studied in this research. In biology, histones are regarded as highly alkaline proteins found in eukaryotic cell nuclei (Nielsen *et al.*, 2012). It functions to package and order the DNA into structural units called nucleosomes (Nielsen *et al.*, 2012). They are the chief protein components of chromatin, acting as ribbons around DNA winds and physiological form of genome (or epigenome) in all eukaryotic cells for gene regulation (Demers *et al.*, 2014; Heinbockel and Csoka, 2018). Chromatin is the substrate of many biological processes which regulates and transcribes gene, mitosis other protein level mechanisms (Burns and Gra, 2021). Since histones are extensively post-translationally modified (Biliński *et al.*, 2012), the identification of these covalent marks on canonical and variant histones is crucial for the understanding of their biological significance (Kreek *et al.*, 2012). Many studies have shown that histone modification can cause cognitive decline (Bridi, 2015; Burns and Gra, 2021; Gupta *et al.*, 2010; Peixoto and Abel, 2013)

Consuming drugs can affect the brain's limbic system (Gould, 2010). A wide range of drug-induced neurobiological modifications have been described in previous studies (Demers *et al.*, 2014; Hassan *et al.*, 2017). Some of these drugs can affect learning and memory functions. Stimulant drugs, like nicotine and amphetamine, improve cognitive function at low doses but impair memory performance at high doses (Flagel *et al.*, 2016). Depressant drugs, like alcohol and benzodiazepines, can cause long-term effects on prefrontal cortex function, disrupting cognitive abilities (Quinn *et al.*, 2015). Someone who has had a drug addiction may also present a

decline in abstract reasoning and have a hard time when solving problems (Soremekun *et al.*, 2021). Drugs induce structural and functional changes in the brain (Squire and Dede, 2015). Several studies have suggested that the influence of addiction might be explained because of the shared neurobiological mechanisms involved in learning and memory processes. Anatomically, there is an important overlap between the neural substrates of learning and memory, and addiction (Demers *et al.*, 2014). Some of the areas that show overlapping include the cerebral cortex, hippocampus, amygdala and striatum; all of them being components of the mesolimbic dopaminergic system (Gould, 2010; Squire and Dede, 2015)

The compound of interest in this study is mitragynine. The major indole alkaloid of *Mitragyna speciosa* is known as mitragynine (Suhaimi *et al.*, 2016). The *Mitragyna speciosa* (ketum, kratom, or kratum) is a tropical tree that can either be deciduous or evergreen depending on the environment and climate of the region (Harun *et al.*, 2015). The tree belongs to the coffee family (Rubiaceae) and their leaves are green, heart-shaped with pointed tips. There are various types of kratom and the most commonly accessible is the red, white and green veins of kratom (Ilmie *et al.*, 2015). The tree was first formally identified by the Dutch botanist Pieter Korthals (1807-1892) (Yusoff *et al.*, 2016). Apparently the plant was named *Mitragyna* because the stigmas resembled a bishop's mitre (Singh *et al.*, 2018).

Figure 1.2 Kratom leaves and preparation

Kratom has been used widely by people around the world probably because of its beneficial effects. Kratom leaves are known for its medicinal value and functions such as a first aid to cure diarrhea, pain killer to chronic pain and other stomach disturbances (Hassan *et al.*, 2019). There are also negative effects of kratom. Misuse and abuse of kratom can lead to addiction and other effects (Hassan *et al.*, 2013). Dried or powdered ketum leaves are sold for between USD50 to USD60 per kg. Indonesia is making hundreds of millions of ringgits per year from exporting kratom leaves. Analgesic, antipyretic, anti-depressant, and anxiolytic symptoms are all possible with kratom (Trakulsrichai *et al.*, 2015). They can also boost the immune system, lower blood pressure, and reduce appetite by acting as an antiviral, antidiabetic, and appetite suppressant (Yusoff *et al.*, 2014). The demand of kratom leaves is high among Malay ethnic group and is associated with abuse of drug and addiction.

Combining the key concepts of this research which are addiction and epigenetics, two problem statements are posed here for this research project. Firstly, to detect for any cognitive decline due to changes in epigenetics as a result of consuming mitragynine? Next, does the severity of drug dosage change the gene expression that also causes a change in behaviour? The drug abuse disorders develop strong associations between the rewarding effects and cognitive functions (Pang and Lu, 2019). These associations create powerful impacts that could alter the behaviour because of modification in gene expression. Understanding mechanisms in the brain that underpin such connections could potentially offer new understanding of drug kratom and offer better opportunities for treatment.

1.2 Rationale of Study

There have been studies on many drugs and its effects in cognitive decline. Drugs like heroine, morphine and cocaine have shown to have impact on learning and memory mechanisms in rats and humans (Koob *et al.*, 1997). Mitragynine has been studied in rats extensively and its effect on cognition are well understood (Koob *et al.*, 1997). Mitragynine shows cognitive decline in many research instances. Interestingly, mitragynine exhibits both positive and negative effects on human brain (Hassan *et al.*, 2017). However, no study has been done involving mitragynine addicted rats relating to histone modifications in the brain. This study hopes to shed some light into that niche and offer new understanding about the relationship between epigenetics changes and kratom.