EFFECTS OF STINGLESS BEE (*Heterotrigona itama*) HONEY ON PATHOGENESIS OF DEPRESSION IN CHRONIC UNPREDICTABLE MILD STRESS (CUMS) RAT MODEL

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by

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LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Degree Celsius
γ	Gamma
g	Gram
kg	Kilogram
µg/ml	Microgram/millilitre
µl	Microliter
mg	Milligram
mg/ml	Milligram/millilitre
ml	Millilitre
nm	Nanometre
%	Percentage
pg/ml	Picogram/millilitre
±	Plus–minus

LIST OF ABBREVIATIONS

3HK	3-Hydroxykynurenine
5-HMF	5-Hydroxymethlfurfural
5HT	Serotonin
ACTH	Adrenocorticotropic Hormone
AMPAR	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor
BBB	Blood-Brain Barrier
BDNF	Brain-Derived Neurotrophic Factor
BH4	Tetrahydrobiopterin
CA1	Cornu Ammonis 1
CA3	Cornu Ammonis 3
CNS	Central Nervous System
CREB	Camp Response Element-Binding Protein
CRL	Central Research Laboratory
CRS	Chronic Restrains Stress
CSDS	Chronic Social Defeat Stress
CUMS	Chronic Unpredictable Mild Stress
CUS	Chronic Unpredictable Stress
DA	Dopamine
ddH_2O	Double Distilled Water
DG	Dentate Gyrus
DMH	1,2-Dimethylhydrazine
DNA	Deoxyribonucleic Acid
DSM-5	Diagnostic And Statistical Manual of Mental Disorders, Fifth Edition
ELISA	Enzyme Linked Immunosorbent Assay
EPM	Elevated Plus Maze

ERK	Extracellular Signal-Regulated Protein Kinase
FST	Forced Swimming Test
GABA	Gamma-Aminobutyric Acid
GBA	Gut Brain Axis
GSK-3β	Glycogen Synthase Kinase-3 Beta
HPA axis	Hypothalamic–Pituitary–Adrenal Axis
IDO	Indoleamine 2, 3-Dioxygenase
IHC	Immunohistochemistry
ITPR1	Inositol 1,4,5-Trisphosphate Receptor Type 1
JAK2	Janus Kinase 2
KP	Kynurenine Pathway
L-DOPA	L-3,4-Dihydroxyphenylalanine
LPS	Lipopolysaccharides
LTD	Long-Term Depression
LTP	Long-Term Potentiation
MAO	Monoamine Oxidase
MAOIs	Monoamine Oxidase Inhibitors
MAPK	Mitogen-Activated Protein Kinase
MDD	Major Depressive Disorder
Mg	Magnesium
MIP	Macrophage Inflammatory Protein-1
mTOR	Mammalian Target of Rapamycin
mTORC1	mTOR Complex 1
MWM	Morris Water Maze
NA	Noradrenaline
NAc	Nucleus Accumbens
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells

NGF	Nerve Growth Factor
NMDA	N-Methyl-D-Aspartate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NSF	Novelty Suppressed Feeding Test
NRF2	NF-E2–related factor 2
NSPC	Neural Stem/Progenitor Cell
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
OFT	Open Field Test
p38 MAPK	p38 Mitogen-Activated Protein Kinases
PBS	Phosphate-Buffered Saline
PFC	Pre-Frontal Cortex
PFK	Phosphofructokinase
PI3K	Phosphoinositide 3-Kinase
PLCγ	Phospholipase Cγ
QA	Quinolinic Acid
RNS	Nitrogen Species
ROS	Reactive Oxygen Species
SBH	Stingless Bee Honey
SEM	Standard Error of the Mean
SERT	Serotonin Transporter
SGZ	Sub granular Zone
SN	Substantia Nigra
SNRIs	Serotonin and Noradrenaline Reuptake Inhibitors
SPT	Sucrose Preference Test
SSRIs	Selective Serotonin Reuptake Inhibitors

STAT5	Signal Transducer and Activator of Transcription 5
STAT6	Signal Transducer and Activator of Transcription 6
SVZ	Subventricular Zone
TCAs	Tricyclic Antidepressants
TDO	Tryptophan 2, 3-Dioxygenase
TRD	Treatment Resistance Depression
Tregs	Regulatory T cells
TrKB	Tropomyosin receptor Kinase B
TST	Tail Suspension Test
VTA	Ventral Tegmental Area
WHO	World Health Organization
Zn	Zinc

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KESAN MADU KELULUT (*Heterotrigona itama*) PADA PATOGENESIS DEPRESI DALAM MODEL TIKUS TEKANAN RINGAN TIDAK TERJANGKA (CUMS) KRONIK

ABSTRAK

Dalam kajian ini, kesan antidepresan madu lebah tanpa sengat (*Kelulut*) ke atas model tekanan ringan tidak terjangka *(CUMS)* kronik di dalam tikus *Sprague Dawley* jantan dan mekanisma tindakannya telah dikaji. Tikus telah didedahkan kepada CUMS selama 28 hari berturut-turut melalui tujuh jenis rangsangan stress berbeza bagi mencetuskan model kemurungan. Stress dicetuskan secara rawak untuk mengelakkan kesan pelaziman kepada sumber tekanan. Kajian ini merangkumi lima kumpulan berbeza; *sham*, DDH2O (1 ml/kg air suling berganda steril), PRX (10 mg/kg paroxetine), SBH D15 (SBH 2000 mg/kg) dan SBH D1 (SBH 2000 mg/kg). Setiap kumpulan terdiri daripada enam individu (n=6). DDH2O, PRX dan SBH D15 diberikan rawatan masing-masing sekali sehari bermula dari hari ke-15 hingga hari ke-28, manakala SBH D1 diberikan SBH dari hari pertama hingga hari ke-28 pendedahan CUMS. Pada penghujung protokol induksi CUMS, tikus tertakluk kepada ujian keutamaan sukrosa (SPT), ujian renang paksa (FST) dan *Morris water maze* (MWM), diikuti dengan pemeriksaan biokimia (serum) dan analisis histopatologi (tisu hipokampus). Semua kumpulan yang disebabkan oleh CUMS telah berjaya mendorong kepada tingkah laku seperti kemurungan dengan perilaku yang menunjukkan pengurangan pengambilan makanan, penambahan berat badan, peratusan pengambilan sukrosa dalam SPT, peningkatan waktu pegun dalam FST, waktu pencarian pelantar yang lebih pendek (MWM), masa pengekalan yang lebih lama dalam kuadran sasaran (MWM) berbanding kumpulan kawalan (*sham*). Kumpulan kawalan positif (PRX) dan kedua-dua kumpulan SBH (SBH D15 dan SBH D1) telah menunjukkan perilaku kemurungan yang kurang meningkat jika dibandingkan dengan kumpulan CUMS yang tidak dirawat (DDH2O). Kumpulan yang disebabkan oleh CUMS juga didapati mempunyai tahap dopamina (DA) yang lebih tinggi dan tahap serotonin (5HT) serta GABA yang lebih rendah berbanding kumpulan kawalan (*sham*). PRX dan kedua-dua

kumpulan SBH (SBH D15 dan SBH D1) telah menunjukkan perkembangan positif semua neurotransmiter yang telah dianalisis dalam kajian semasa, berbanding dengan kumpulan DDH2O. Di samping itu, serum dianalisis untuk lapan sitokin interleukin yang berbeza dan penanda keradangan IL-4, IL-5, IL-10, IL-13, IL-6, IL-2, IL-1β, TNF-α, dengan DDH2O menunjukkan tahap tinggi semua penanda berbanding kumpulan lain manakala, PRX dan kedua-dua kumpulan SBH (SBH D15 dan SBH D1) telah menunjukkan pengurangan keradangan. Tisu otak di kawasan hipokampus (cornu ammonis 1 (CA1), cornu ammonis 3 (CA3) and girus dentatus (DG)) dianalisis melalui ketumpatan optik bagi ekspresi imunohistokimia faktor neurotrofik yang dihasilkan otak (IHC BDNF), kumpulan DDH2O menunjukkan ekspresi ketumpatan optik terendah BDNF berbanding kumpulan lain manakala PRX dan kedua-dua kumpulan SBH (SBH D15 dan SBH D1) menunjukkan peningkatan ketumpatan optik yang positif bagi ekspresi BDNF. Sebagai kesimpulan, kedua-dua kumpulan SBH menunjukkan kemampuan dalam memperbaiki patogenesis kemurungan yang disebabkan paradigma CUMS selama 28 hari, berbanding kumpulan CUMS yang tidak dirawat (DDH2O). Semua keputusan menunjukkan bahawa SBH memberikan kesan sinergistik untuk meningkatkan parameter monoamina serta menurunkan sitokin keradangan yang akhirnya meningkatkan kesan pemulihan terhadap tingkah laku tikus yang tertekan.

EFFECTS OF STINGLESS BEE (*Heterotrigona itama*) HONEY ON PATHOGENESIS OF DEPRESSION IN CHRONIC UNPREDICTABLE MILD STRESS (CUMS) RAT MODEL

ABSTRACT

In this study, the antidepressant effect of stingless bee honey (SBH) on chronic unpredictable mild stress (CUMS) in male Sprague-Dawley rats and its mechanism of action were investigated. Rats were exposed to CUMS for 28 consecutive days by seven different stressors to induce depression. The stressor was triggered randomly to avoid habituation effects on the stressor. This study included five different groups; sham, DDH2O (1 ml/kg sterile double distilled water), PRX (10 mg/kg paroxetine), SBH D15 (SBH 2000 mg/kg), and SBH D1 (SBH 2000 mg/kg). Each of the groups comprised six individuals (n=6). DDH2O, PRX and SBH D15 were administered with their respective treatment once daily starting from day 15 to day 28, while SBH D1 was administered SBH from the first day to day 28 CUMS induction. At the end of the CUMS induction protocol, rats were subjected to a sucrose preference test (SPT), forced swimming test (FST), and Morris water maze (MWM), followed by biochemical examination (serum) and histopathological analysis (hippocampus brain). All CUMS-induced groups successfully induced depressive-like behaviour by showing a decrease in food intake, body weight gain, percentage of sucrose preference in SPT, increased immobility in the FST, shorter latency finding platform (MWM), and higher retention time spent in the target quadrant (MWM) when compared to the sham group. The positive control group (PRX) and both SBH groups (SBH D15 and SBH D1) were shown to attenuate depressive-like behaviour in comparison to the untreated group CUMS (DDH2O). The CUMS-induced groups were also found to have higher dopamine (DA) levels, lower serotonin (5HT) and lower GABA levels compared to the sham group. PRX and both SBH groups (SBH D15 and SBH D1) showed positive progression in all analyzed neurotransmitters in the current study, compared to the DDH2O group. In addition, serum was analysed for eight different interleukin cytokines and inflammatory markers (IL-4, IL-5, IL-10, IL-13, IL-6, IL-2, IL-1β,

and TNF-α). DDH2O showed high levels of all markers compared to the other groups. PRX and both SBH groups (SBH D15 and SBH D1) showed attenuation of inflammation. Brain tissues in the hippocampus regions (Cornu ammonis 1 (CA1), Cornu ammonis 3 (CA3) and Dentate Gyrus (DG)) for analysis of the optical density of IHC expression of BDNF, DDH2O group showed the lowest optical density expression of BDNF compared to other groups. Meanwhile, PRX and both SBH groups (SBH D15 and SBH D1) showed a positive elevation of the optical density of BDNF expression. In conclusion, both SBH groups showed an improvement in ameliorating the pathogenesis of depression after being induced with depression via CUMS paradigms for 28 days, compared to the DDH2O group. All results indicated that SBH provides synergistic effects to elevate the monoamine parameters as well as suppress the inflammatory cytokines that eventually improve the behaviour output of depressed rats.

CHAPTER 1

INTRODUCTION

1.1 Study Background

Depression is a psychiatric disorder characterized by psychological, behavioral, and physiological symptoms that include a persistent low mood, marked by the loss of pleasure in most activities, poor concentration, disruptions in appetite and sleeping patterns, cognitive impairments, a feeling of worthlessness, excessive guilt, and having suicidal thoughts (Knol et al., 2006). It is the leading cause of mental disability worldwide that poses a high emotional and financial burden (Mauskopf et al., 2009; Sousa et al., 2017). The World Health Organisation (WHO) estimates that depression will be declared a global burden by 2030, affecting an estimated 280 million people across all age categories (Chan et al., 2024; GBD 2019 Diseases and Injuries Collaborators, 2020). It has also been reported that suicides in the global population have increased by 3.8%, with a ratio of one death every 40 seconds in a person suffering from depression (Adaikkan et al., 2018; Adell, 2020).

Malaysia is no exception to the critical issue of depression. Even before the occurrence of the COVID-19 pandemic (Kader Maideen et al.,2014), depressive cases were already on the rise due to various factors such as relationship trauma, financial burdens, and more. Upon post-COVID-19, the situation became even more severe (Moy and Ng, 2021), with the emergence of unpredictable challenges, including the sudden loss of loved ones, financial hardships, prolonged movement restrictions, side effects of the virus, and increased consumption of unhealthy foods during lockdowns. In addition, the government-imposed lockdown had a significant impact on students in Malaysia (Moy and Ng, 2021; Wong et al., 2023), as their learning processes were disrupted, and they had to adapt to new methods of learning. All of these issues may contribute to elevated inflammation in the body (Zhong et al., 2019).

Depression covers various subtypes and etiologies (Cai et al., 2015) from monoamines to inflammatory and neurotrophic propositions. In the 1960s, the "catecholamine hypothesis" appeared as a popular monoamine hypothesis for explaining depression development. It suggested that serotonin (5HT) noradrenaline (NA) (Coppen, 1967; Schildkraut, 1965; Belmaker and Agam, 2008), dopamine (DA) (David et al., 2020), and gamma-aminobutyric acid (GABA) (Zhu et al., 2017; Ma et al., 2019) deficiency creates depression.

The inflammatory hypothesis proposes that depression is driven by interactions between inflammatory cytokines and the hypothalamic–pituitary–adrenal (HPA) axis, subsequently affecting the synthesis, release, and reuptake of neurotransmitters (Hayley et al., 2005; Hiles et al., 2012). These processes contribute to glucocorticoid resistance, glutamate excitotoxicity, and a reduction in brain-derived neurotrophic factor (BDNF) expression (Duman et al., 2021). Importantly, the inflammatory hypothesis is increasingly recognized as crucial to the pathogenesis of depression and has been identified as a major contributing factor to treatment-resistant depression (TRD) (Strawbridge et al., 2019; Halaris et al., 2021).

Since BDNF is reduced in the onset of depression, the neurotrophic hypothesis has become one of the critical etiologies of antidepressant progression. This hypothesis states that neurotrophic factors are essential to the development of neurons by promoting synaptic growth and maintaining neuronal survival. They play a crucial role in neuronal network formation and plasticity. On the contrary, the reduction of neurotrophic factors is implicated in the atrophy of stress-vulnerable hippocampus neurons, such as depression and cognitive disorder (Duman et al., 1997). This deficiency is believed to be reversed by antidepressant treatments that contribute to the resolution of depressive symptoms (Kharade et al., 2010).

Chronic unpredictable mild stress (CUMS) has been the most numerous depression model in rats used to study the pathogenesis of depression nowadays (Willner and Mitchell, 2002; Xu et al., 2018; Geng et al., 2020). This model includes different types of stressors (Gawali et al., 2017) that are exposed to subjects in a random manner to avoid habituation effects. Usually, the CUMS model was induced between 3 and 8 weeks depending on the researcher's preference and the aim of their studies (Wu et al., 2022; Liu et al., 2022a,b; Zhang et al., 2019a,b). This model is mostly used for studies investigating the efficacy of antidepressants, as it best represents the human condition that develops into depression (Zhang et al., 2019a).

Besides that, CUMS is also relevant as this model was having consistent results regarding the pathogenesis of depression such as neurotransmission alterations, depressive-like behaviour, inflammation and neuroendocrine disruptions (Antoniuk et al., 2019). Individuals may develop depression as a result of prolonged exposure to unpredictable daily stressors. When coping mechanisms become overwhelmed, the physiological response can lead to dysregulation, notably through the overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, as an attempt to maintain homeostatic balance (Belujon and Grace, 2015; Yang et al., 2017). This sustained HPA axis activation may contribute to the pathophysiology of depression.

Despite the availability of pharmacological interventions for depression, most patients continue to encounter suboptimal efficacy, delayed therapeutic onset, and undesirable side effects. These limitations underscore the necessity of investigating alternative therapeutic approaches or complementary approaches to cater to current limitations.

Stingless bee honey (SBH) is a unique type of honey that contains a significantly higher concentration of bioactive compounds (Mustafa et al., 2018). Its rich phytochemical profile has sparked growing interest in SBH as a promising candidate for alternative medicine. Among its constituents, phenolic compounds have been prominently highlighted as key contributors to its potent anti-inflammatory and antioxidant activities (Ibrahim et al., 2016). These properties are particularly compelling in the context of exploring SBH's potential as an alternative antidepressant, as the pathogenesis of depression and chronic stress has been strongly linked to oxidative stress and inflammation. Thus, SBH may offer a natural therapeutic approach to mitigate these underlying mechanisms.

1.2 Problem Statement

With the increasing cases of depression, the discovery of new treatments is imperative. At present, there is a vibrant demand for new treatment strategies as the flaws of conventional treatments are striking. For instance, many sources purport that antidepressants have a therapeutic delay onset, taking weeks rather than days to become effective (Delgado, 2004; Taylor et al., 2005). Prolonged exposure to antidepressant drugs imposes susceptibility to adverse side effects, such as interferences in sexual functioning, gastrointestinal disturbances, altered sleep patterns, and weight gain (Masand and Gupta, 2002; Schweitzer et al., 2009; Mihaljevićc-Peleš et al., 2011; Bet et al., 2013; Richelson, 2013; Sharma et al., 2016). Moreover, 30% of patients have been reported to be non-compliant with currently available treatments (Crisafulli et al., 2011; Ruhé et al., 2012; Chiu et al., 2018). Thus, there is a dire need for the development of new antidepressant treatments with better efficacy and are highly safer for patients (Manosso et al., 2015). This has a new opportunity for complementary and alternative medicines in treating depression (Ali et al., 2017; Gribkoff and Kaczmarek, 2017; Rodríguez-Landa et al., 2022).

Honey primarily contains a variety of active compounds that are beneficial for brain regulation, as well as in treating emotional and psychological disorders, including depression (Mijanur Rahman et al., 2014; Münstedt et al., 2015). It is one of the natural products that serves as an alternative medicine (Abd Wahab et al., 2017). Among the various types of honey, we focus on stingless bee honey (SBH). In Malaysia, SBH is well known as "madu kelulut" (Fatima et al., 2018). In addition to SBH, there are other honeys capable of treating several health problems named Tualang and Manuka (Biswa et al., 2017). However, in terms of nutritional composition, SBH contains a higher level of polyphenols (Özbalci et al., 2013; Kek et al., 2014; Biluca et al., 2014), an important active compound that participates in modulating signaling pathways, thus influencing neuronal survival and cell regeneration and development, which suffer detrimental effects after injury (Sun et al., 2017; Kanimozhi et al., 2017). The fact that the SBH industry continues to grow in Malaysia, there is an opportunity to set standards for the certification of high-quality SBH by integrating Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) principles, by which the honey can be further used in the clinical settings.

To date, limited studies are highlighting the potential of SBH as an alternative supplement to treat depression. Therefore, the current study discussed the effects of CUMS as a preclinical animal model for depression and investigated how the mechanism of action of SBH could act as a potential antidepressant to attenuate the pathogenesis of depression.

1.3 Hypothesis

H_0: SBH has no effects on the pathogenesis of depression in a preclinical CUMS model

H_1: SBH can attenuate the pathogenesis of depression in a preclinical CUMS model.

1.4 Objectives

1.4.1 General Objective

To evaluate the effects of SBH on the pathogenesis of depression in CUMS rat model.

1.4.2 Specific Objectives of Study

1. To observe the effects of food intake and body weight gain in CUMS model groups between non-treated and treated with SBH.
2. To observe the behaviour changes via sucrose preference test (SPT), forced swimming test (FST) and morris water maze (MWM) of CUMS model groups between non-treated and treated with SBH.
3. To determine the neurotransmitters level (5HT, DA and GABA) of CUMS model groups between non-treated and treated with SBH.
4. To measure the level of inflammatory markers (IL-4, IL-5, IL-10, IL-13, IL-6, IL-2, IL-1β, and TNF-α) CUMS model groups between non-treated and treated with SBH.
5. To determine the hippocampus (CA1, CA3, DG) BDNF protein expression of CUMS model groups between non-treated and treated with SBH.

1.5 Importance and Relevance of the Study

Depression is a complex and prevalent mental health disorder that affects millions worldwide. Despite the availability of pharmacological treatments, many patients experience limited treatment, delayed onset of action, and adverse side effects. This emphasizes the need to explore additional alternatives. The current study investigates the potential of SBH to attenuate the pathogenesis of depression using a CUMS model, which closely observed the behavior and neurobiological changes. Exploring SBH's effects in this model is crucial, as it could:

i. Offer novel insights into complementary therapies for depression.

ii. Provide scientific evidence for the neuroprotective or antidepressant properties of SBH.

iii. Contribute to the development of targeted therapies that may act on inflammatory pathways, oxidative stress, or neuroplasticity mechanisms often implicated in depression.

iv. Support the use of natural or functional food-based interventions with fewer side effects compared to synthetic drugs.

Moreover, this study has relevance in both neuropsychopharmacology and public health, encouraging translational research to connect traditional practices with contemporary scientific validation.

CHAPTER 2

LITERATURE REVIEW

2.1 Depression

According to the World Health Organization (WHO), approximately 300 million people worldwide suffer from depression, with the prevalence showing an increasing trend, highlighting it as one of the global life-threatening psychiatric disorders (Zhu et al., 2018). Depression is often triggered by prolonged exposure to stressful life events (Fries et al., 2023) and has been recognized by the WHO as a growing economic burden on nations (Roehrig, 2016). Although depression has been extensively studied for decades, its exact pathophysiological mechanisms remain unclear, particularly concerning its underlying biological pathways and the variability in treatment outcomes (Montejo et al., 2015; Yan et al., 2018). Individuals with depression are thought to be susceptible due to a combination of environmental, genetic, and epigenetic factors (Castrén & Monteggia, 2021). If left unaddressed, the global burden of depression is expected to rise, thus leading to an increase in individuals who are vulnerable to chronic stress and mood disorders (Roehrig, 2016).

The global prevalence of depression has surged dramatically in the wake of the COVID-19 pandemic, with Malaysia being no exception. Since 2019, the incidence of depression has been exacerbated by post-pandemic stressors, including the loss of loved ones, personal experiences with COVID-19 infection, economic instability (Moni et al., 2021), educational disruption (Moy and Ng, 2021), and the psychological toll of lockdown strategies implemented to contain the virus (Elengoe, 2020). In Malaysia, the third wave of COVID-19 was reported in October 2020 (Rampal and Liew, 2021), contributing to the escalation of mental health issues across multiple demographics.

Several Malaysian studies have documented the psychological impact of the pandemic on vulnerable populations, including university students (Wong et al., 2023), loss employment (Marzo et al., 2021) and others facing socioeconomic hardships. Notably, post-pandemic depression has also been associated with disturbances in the gut-brain axis, reinforcing the biological basis of mood disorders (Rentería et al., 2022). During the lockdown period, many individuals adopted unhealthy dietary habits consuming high-sugar "viral" foods as a coping mechanism. This shift contributed to long-term disturbances in gut microbiota, insulin regulation, systemic inflammation, and a heightened risk of developing metabolic syndromes.

Recent findings further highlight the bidirectional relationship between depression and metabolic diseases, such as obesity and diabetes (Chae et al., 2024; Zheng et al., 2024). This reinforces the need for integrative approaches that consider both physical and mental health. The inflammatory hypothesis of depression is gaining traction, particularly in light of increasing cases of TRD believed to be linked to chronic inflammation (Strawbridge et al., 2019; Halaris et al., 2021).

Beyond the effects of the pandemic, studies in Malaysia have also reported high rates of depression among adolescents. These cases are often associated with sociodemographic variables, risky behaviours, and childhood adversities such as family dysfunction, bullying, and other environmental stressors (Sahril et al., 2019; Kaur et al., 2014; Ishak et al., 2020; Latiff et al., 2016) which may contribute to long-term epigenetic changes (Yuan et al., 2023; Chen et al., 2024).

Depression is frequently comorbid with chronic diseases such as metabolic syndrome, cardiovascular disease (van der Kooy et al., 2007), diabetes (Nouwen et al., 2009), cancer (Penninx et al., 1998), and obesity (Schneider et al., 2012). These conditions collectively impair the quality of life and hinder national development efforts (Zuo et al., 2020; Jia et al., 2021; Lin et al., 2021). Numerous global health and economic projections suggest that depression will continue to impose a significant financial burden on healthcare systems by 2030 (Eshel & Roiser, 2010; Li et al., 2018b; Malhi & Mann, 2018; Zhao et al., 2020).

2.2 Treatment Resistance Depression (TRD)

As the global prevalence of depression continues to rise, the discovery and development of new treatment strategies have become imperative. Current conventional treatments, particularly antidepressant medications, present significant limitations. Notably, many antidepressants are associated with a delayed onset of therapeutic action, often requiring several weeks before noticeable effects are observed. Furthermore, approximately 40% of patients exhibit non-compliance due to limited efficacy, side effects, or lack of immediate relief (Korlatowicz et al., 2023). These shortcomings have opened new opportunities for exploring complementary and alternative medicine (CAM) as viable therapeutic options for depression.

TRD a severe subtype of depression that fails to respond to conventional therapies, has been closely associated with the inflammation hypothesis of depression (Raison et al., 2013; Ionescu & Papakostas, 2017). However, TRD is not solely attributed to inflammation; several other contributing factors include mineral imbalances (Mechlińska et al., 2022), gut microbiota dysbiosis (Hashimoto, 2023), and chronic stress-induced epigenetic changes (Cai et al., 2024). Thus, exploring alternative therapies that target these underlying mechanisms is crucial in mitigating TRD and improving treatment outcomes.

SBH has recently emerged as a promising natural product with the potential to address the multifactorial nature of TRD. It primarily possesses anti-inflammatory properties, rich in mineral profilings, and has a diverse range of beneficial probiotics. These bioactive components position SBH as a potential agent that could "complete the puzzle" in targeting the key pathophysiological mechanisms underlying TRD. Collectively, these features make SBH a strong candidate for further investigation as a complementary antidepressant, particularly for individuals who do not respond to conventional therapies.

TRD has also been linked to an increased risk of suicidal ideation and behavior, underscoring the urgent need for effective interventions (Adaikkan et al., 2018; Adell, 2020). It is estimated that only about 40% of individuals with TRD achieve remission with existing pharmacological treatments (Korlatowicz et al., 2023). The psychological and biological toll of untreated depression, including neuroinflammation and neurotransmitter dysregulation, significantly increases the risk of suicidal thoughts and long-term functional impairment (Zhong et al., 2019). According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), depression is characterized by symptoms such as anhedonia, persistent sadness, feelings of emptiness and despair, insomnia, appetite, and weight changes, all occurring for at least two weeks (Otte et al., 2016; Malhi & Mann, 2018; Villarroel & Terlizzi, 2020; Li et al., 2021).

Moreover, TRD has been linked to epigenetic modifications (Silva et al.2024), which may contribute to transgenerational susceptibility to depression and stress-related disorders if left unaddressed. This highlights the broader implication of untreated TRD not only on individual health but also on the genetic and psychological resilience of future generations. Ultimately, the long-term effects of TRD can impair the quality of life, reduce productivity, and pose a significant burden on the healthcare systems of developing nations, hindering national development goals. Therefore, advancing research into natural products such as SBH, with their multi-targeted mechanisms, may represent a promising and holistic approach to managing depression and addressing TRD.

2.3 Behavior

As aforementioned, behaviour alterations observed in depression due to disruption of neurotransmissions in the brain also refer to depressive-like behaviour. Depressive-like behaviour tools commonly investigated in the preclinical study are the sucrose preference test (SPT), forced swimming test (FST), Morris water maze (MWM), tail suspension test (TST), open field test (OFT), elevated plus maze (EPM) and novelty suppressed feeding test

(NSF) (Jia et al.,2021). All of these tests were proven to be reliable regarding the efficacy study of new antidepressant treatment.

The clinical study also highlighted the depressive-like behaviour from the guidelines DSM-5 to have all the depressive behaviour symptoms at least in two weeks as had been mentioned earlier. Depression patients usually affect behavior that is highly related to mood such as gloom, dysmnesia, inattention, irritability and anhedonia (Wang et al., 2020) which are crucial for diagnosis and treatment. The outcomes of depressive behaviour were heterogeneous between each sample and subject, as the effects of chronic stress were affected by the intensity, duration and rate of vulnerability to the subjects (Füzesi et al.,2016; McCarty, 2016; Castrén and Monteggia, 2021). Variation in subject response, such as over-activated and resilience, becomes a major challenge in finding the best treatment for depression.

2.4 Chronic Unpredictable Mild Stress (CUMS)

The pathogenesis of depression can be explored via clinical or preclinical studies. Rodent species have been used in numerous studies to explore the pathogenesis of depression as a comparison to the human condition. There are several preclinical models used to explore the pathogenesis of depression such as CUMS or also known as Chronic Unpredictable Stress (CUS) (Willner, 2005), Chronic Restraint Stress (CRS) (Radley et al., 2015), chronic social defeat stress (CSDS), (Venzala et al., 2012; Qiao et al., 2016; Yang et al., 2018b), sleep deprivation model (He et al., 2020) and also lipopolysaccharides (LPS) (Lee et al., 2018; Wang et al., 2019). All these types of models in preclinical studies have their advantages and limitations according to the aim of the mechanism interest in exploring the pathogenesis of depression.

CUMS model is the most common model and has great interest to explore the pathogenesis of depression (Willner, 2016). The model mimics the human condition to develop a depression state. Besides that, this experiment showed good characteristics to reproduce by other researchers and showed good predictive, face and construct validity that contributes to consistent results (Antoniuk et al., 2019). CUMS experiment paradigms, subjects will be exposed to a few different types of stressors and assigned randomly to avoid habituation effects on the subjects. The stressors that had been used in most studies had been selected according to the aim and available facilities for the experiment. Therefore, the CUMS model often reported different paradigms between researchers, even though they were stated CUMS models. The type of stressor and the duration of the CUMS also contribute to variable output in the different researchers.

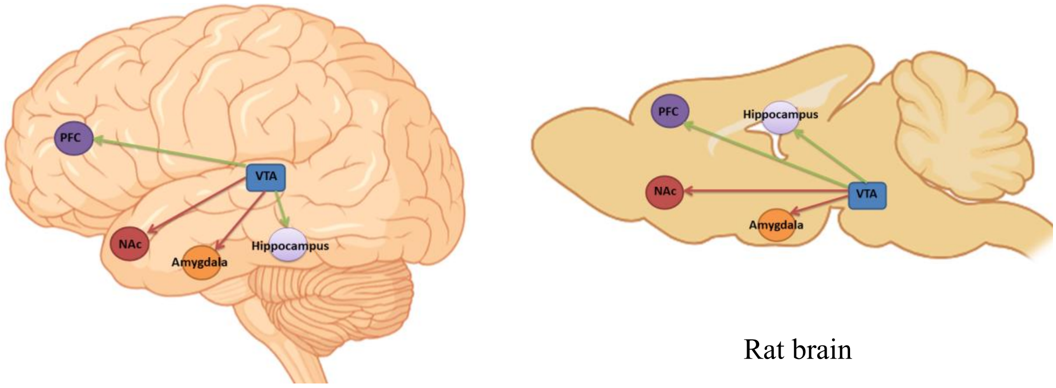
CUMS is also popular in the pathogenesis of depression studies in conjunction with the physiological changes that are represented in the human condition (Willner, 2017; Antoniuk et al., 2019). For example, multiple effects on neurotransmitters (5HT, DA, GABA), body weight changes, food intake patterns, psychological behaviour, inflammation, as well as the alteration of hippocampus function have been observed in this experimental model (Hill et al., 2012; Wang et al.,2018; Traslaviña et al.,2019). Inflammation has been reported widely in disease pathogenesis. Inflammation has been reported in TRD on current conventional pharmacotherapy (Akil et al., 2018). Therefore, the application of CUMS model in the preclinical study for the exploration of a new compound that can attenuate the inflammation signal will provide a huge impact on the pathogenesis of depression.

2.5 Brain

Depression is considered a mental health or neuropsychiatric disease where the alterations of brain condition highly contribute to depression problems (Khan et al.,2020). In short, all the hypotheses for the pathogenesis of depression come from the pathological changes that occur in brain homeostasis. The brain consists of several anatomical areas that have their specific roles in modulating overall body functions. Areas of the brain that are commonly studied in depression are the hippocampus (Liu et al., 2017), pre-frontal cortex (PFC) (Duman and Aghajanian, 2012; McEwen et al., 2016), amygdala (Prakash et al., 2020), and nucleus accumbens (NAc) (Ma et al., 2019), where the alterations of these areas contribute to disruption of normal brain operation.

The monoamine hypothesis is the earliest hypothesis that postulates the pathogenesis of depression (Gáll et al., 2020) and is related to the 5HT neurotransmitter. Therefore, serotonergic transmission in the brain is the main target for the treatment of depression. However, nowadays monoamine hypothesis is also related to dopaminergic, GABAergic, and glutamatergic neurotransmission. All of these neurotransmitters work synergistically with each other like an orchestra to support and modulate optimal homeostasis function in the brain. All these different types of neurotransmissions become crucial for the treatment of depression and anxiety (Patel et al., 2004; Hill et al., 2009; Linge et al., 2016; Bakas et al., 2017).

The ventral tegmental area (VTA) has also been reported in the pathogenesis of depression (Massaly et al., 2016). This pathway is known as a hub for dopaminergic circuitry. It is comprised of four different types of projection: mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular (Hong, 2013). Each of the projections has its specific function. This pathway not only consists of dopaminergic neurons (65%) but also GABAergic (30%) and glutamatergic neurons (5%) (Margolis et al., 2006; Nair-Roberts et al., 2008). It mainly correlates to one another in functioning the optimal conditions of the brain. From the four different types of VTA projection, the mesolimbic pathway has been numerously reported to have a linkage with depression as this pathway is related to reward, motivation, fear, memory and learning. These have become the core symptoms in the screening of depression subjects. This pathway involved in the brain includes the ventral tegmental area (VTA), prefrontal cortex (PFC), amygdala, hippocampus, NAc, anterior cingulate cortex, ventral pallidum, and orbitofrontal cortex (Der-Avakian and Markou, 2012: Lammel et al., 2011, 2012; Beier et al., 2015, Blaess et al., 2020).



Rat brain

Human brain

Figure 2.1. Mesolimbic pathway of human and rat brains. Image created with BioRender.com.

2.6 Hippocampus

The hippocampus has been the widely explored region in the brain region in correlation with the pathogenesis of depression. This region consists of different sub-regions: CA1, CA3, and DG. These sub-regions are related to each other to convey information signals from other brain areas. This region is important as an area that processes long-term memory in the brain. Therefore, to test whether this area is well functioning or has been affected by pathogenesis depression, the subject of the preclinical study is commonly assessed via the MWM behaviour test.

Furthermore, BDNF expression in the hippocampus has also been studied in antidepressant research (Dunham et al., 2009; Ray et al., 2011; Ray et al., 2014). BDNF variables are crucial in exploring antidepressant efficacy (Duman and Monteggia, 2006; Björkholm and Monteggia, 2016; Castrén and Kojima, 2017; Hing et al., 2018). This neurotropic factor is crucial in synaptic plasticity, dendrite and neurite function and branching, which are important to sustain optimal brain functioning, especially long-term potentiation (LTP) (Zagrebelsky and Korte, 2014).

2.6.1 CA1

CA1 is also important in describing the effects of antidepressant treatments, as this subfield of the hippocampus mainly regulates emotional responses (Sun et al., 2023). It is crucial to maintain the CA1 hippocampus in a good state, as this subregion has been reported as a novelty detector that can distinguish inconsistencies between input from the entorhinal cortex and the corresponding current situation (Murakami et al., 2005). Moreover, CA1 is functionalized in converting the short-term into long-term memory (Satrayasai et al., 2013).

However, some studies have also reported that this subfield was not affected by stress induction (Sousa et al., 2000; Magariños et al., 2011; Conrad et al., 2012). Previous studies reported that the dysregulation of CA1 outputs to the PFC, amygdala, NAc, and hypothalamus caused anxiety-like behaviours (Tannenholz et al., 2014; Loureiro et al., 2016; Kim and Cho, 2017, 2020; Ghasemi et al., 2022) and threaten memory processing (Nakamura et al., 2010). This could be due to the different types of stressor-induced model organisms, the duration, and intensity of which lead to different outcomes in terms of deleterious effects on the CA1 hippocampus. This is supported by the statement that the CA is crucial for encoding fear memory, short-term image contact, and image formation in both human and animal models, in addition to short- and medium-term spatial memory (Jeltsch et al., 2001; Hao et al., 2020). According to the order of information transmission in the hippocampus, CA1 is the last terminal (DG >CA3>CA1).

In addition, Liu et al. (2022a) experimented to investigate the effect of CUMS on mammalian target of rapamycin (mTOR) signalling in Sprague-Dawley. The mTOR signalling pathway is closely related to the BNDF signalling pathway (Costa-Mattioli and Monteggia, 2013; Watson and Baar, 2014; Duman et al., 2016). They found that the CA1 expression of postsynaptic density was reduced. This finding also supports a previous study reporting that the CA1 region of the hippocampus is associated with synaptic dysfunction in clinical patients (Duman and Aghajanian, 2012). Numerous studies reported the detrimental effects of stress in the CA1 hippocampus that further lead to neurological disease (Hu et al., 2020b).

The mTOR signalling pathway is a downstream pathway that is critical for regulating synaptic protein synthesis and contributes to synaptogenesis (Ignacio et al., 2016). Activation of mTOR complex 1 (mTORC1) causes phosphorylation and action of p70s6 kinase and eukaryotic translation, leading to the formation of new synapses (Duman et al., 2012; Tavares et al., 2018). Multiple studies have reported synaptic damage in hippocampal regions. In preclinical studies, CUMS was induced, increasing glutamate release that caused glutamate excitotoxicity (Sapolsky, 2000; Autry et al., 2011; Wippel et al., 2013). Glutamate excitotoxicity is enhanced by the increase in pro-inflammatory cytokines (Shultz and Zhong, 2017; Ramos-Chávez et al., 2018; Tehse and Taghibiglou, 2019). It has been reported that regulation of the mTOR signalling pathway alleviates depression symptoms and shows the effect of antidepressants.

In addition, the regulation of synaptic plasticity is also closely related to LTP. Previous studies have reported that the disruption of LTP in the CA1 hippocampus of rats is related to serotonergic mechanisms, where the 5HT depletion alters synaptic plasticity (Matsumoto et al., 2004; Lanfumey et al., 2008). Impairments of LTP in the CA1 subregion were also found, consequently decreasing the mean density of synaptophysin in the CA3 region of the hippocampus (Li et al., 2012). Moreover, the decreased density of dendritic spines and BDNF in CA1 and CA3 regions was accompanied by changes in synaptic plasticity in the CUMS-induced study over three weeks (Qiao et al., 2016).

2.6.2 CA 3

The CA3 region is known to play a central role in memory processing and susceptibility to seizures and neurodegeneration (Nasir et al., 2019). After DG has transmitted the information from EC, it forwards the information to the CA3 region to process the next response (Amaral and Witter, 1989; Sun et al., 2023). Memories are formed

(Aggleton and Brown, 1999) and stored (Mumby et al., 1999) in the hippocampus. For this reason, most clinical and preclinical models of stress showed detrimental effects on thinking, behaviour, emotions and physical well-being (Salmans, 1997). Chronic stress has been reported to cause degenerative changes by affecting the apical dendrites of the CA3 region in rats, tree shrews and monkeys (McEwen and Magarinos, 1997).

Synaptogenesis is known to be a key function in the mechanism of action of antidepressants (McEwen, 2007; Duman and Aghajanian, 2012; Yang et al., 2018a). A previous study reported that mice with heterozygous BDNF (+/-), whose BDNF levels were about 50% lower, had fewer branched dendrite trees in CA3 stress-induced models, indicating a reduction in dendritic branching in CA3, with BDNF playing a central role in dendritic spines in CA3 (Zhang et al., 2015). CA3 synaptogenesis is very important as it needs to receive information from DG, which relays information from EC.

2.6.3 DG

DG has been highlighted as a highly important region that maintains neurogenesis, as depletion of this subregion of the hippocampus produces an adverse behavioural response to antidepressants, while its elevation promotes the beneficial effects of antidepressants (Santarelli et al., 2003). Furthermore, alterations in neurogenesis have been shown to induce depression and anxiety-like behaviours in DG preclinical studies (Yun et al., 2018; Hill et al., 2015; Kim and Park, 2021).

The dopaminergic pathways of the VTA have been crucial for modulating emotional behaviours such as depression (Umschweif et al., 2021). The DG of the hippocampus is known as the ‘gateway’ as this region relays information from the cortical region (entorhinal cortex EC) to the CA3 and subsequently to the CA1 region (Basu and Siegelbaum, 2015; Sun et al., 2023). This uncovers why most antidepressants primarily focus on this region. DG played a central role in defining the effects of stress on hippocampal function (Gulyaeva, 2016). There is ample data on dopaminergic receptors in DG depression, which are important in mediating behaviour in response to the effects of antidepressants (Umschwert et al., 2021). DG Granule cells (GC) are known to be enriched with DA receptors such as D1 and D5, which play a crucial role in mediating the effects of antidepressants via dopaminergic projections from the VTA (Umschwert et al., 2021). This has also been confirmed by other studies in which overexpression of D1 receptors in the hippocampus DG has been associated with antidepressant behaviour (Shuto et al., 2018). The previous study mentioned that regulation of synaptic plasticity is facilitated by activation of the D1 in response to an antidepressant by a monoamine oxidase inhibitor (MAOI) (Ishikawa et al., 2019). Moreover, activation of dopaminergic receptors in the hippocampus DG resulted in increased plasticity of the perforant pathway in the GC in DG synapses that ultimately mediate memory encoding (Sarinana et al., 2014; Yang and Dani, 2014).

The preclinical study reported that the untreated CUMS-induced group showed the lowest expression compared to the control group (Chen et al., 2019). Previous studies demonstrated that treatments with selective serotonin reuptake inhibitors (SSRI) antidepressants led to an increase in BDNF compared to the stress-induced groups (Lu et al., 2018b). In a clinical study, the result of post-mortem tissue staining showed that the volume of DG increased by 68% in patients receiving conventional SSRI antidepressants compared to major depressive disorder (MDD) control groups (Boldrini et al., 2012).

Previous studies also reported that D1 receptors in the DG hippocampus were highly sensitive after chronic administration of the antidepressant SSRI (Kobayashi et al., 2012; Shuto et al., 2018). In addition, DG is also known for having receptacle spatial memory (Hao et al., 2020) as the effects of subjects retrieving any information or cues during the MWM test to plan the route and remember as soon as possible to find a platform in the target quadrant.

2.7 Neurotransmitters

The monoamine hypothesis is one of the earliest hypotheses that has been postulated in depression, as it is related to alterations of several neurotransmitters (Murrough et al., 2017). Several types of drugs have been produced that target this problem, ultimately. This includes SSRIs, tricyclic antidepressants (TCAs), MAOIs, 5HT and noradrenaline reuptake inhibitors (SNRIs) (Filho et al.,2016b). The 5HT neurotransmitter is the prime attention treated in the pathogenesis of depression (Kraus et al.,2017). This neurotransmitter is crucial to control mood and behaviour (Segi-Nishida, 2017). SSRIs are the conventional drugs used to treat depression subjects and are approved both in adult and pediatric patients (DeLucia et al., 2016). It is also known as first-line pharmacotherapy in the treatment of depression. These drug groups comprise paroxetine, fluoxetine, sertraline, citalopram, escitalopram, fluvoxamine and vilazodone (Chu and Wadhwa, 2023). The main mechanism of action of these conventional drugs is primarily functionalized via the inhibition of 5HT reuptake in the synaptic cleft, which acts on the serotonin transporter (SERT) of presynaptic neurons (Preskorn, 1997; Feighner, 1996; Xue et al., 2016). Therefore, 5HT levels will be elevated and achieve optimal physiological proses that are related to the 5HT neurotransmitter (Chu and Wadhwa, 2023).

Depression is a well-known psychiatric disorder that involves the dysregulation of the monoamine system, which leads to an imbalance of neurotransmitters, such as 5HT, DA, and noradrenaline (NA) (Nutt, 2008; Li et al., 2020). Monoamines are molecules involved in information transmission processes by connecting presynaptic to postsynaptic neurons (Südhof, 2021). They are classified according to their chemical structure and mechanism of action (Edmondson et al., 2004). With their distinctive nature of chemical structures, each monoamine is specific to its respective receptors (Südhof, 2021) and has a different function in the brain (Nutt, 2008; Kulkarni et al., 2009; Moret and Briley, 2011). For example, 5HT is a central nervous system monoamine that has a crucial role in regulating appetite, circadian cycle, anxiety, memory, and learning.

Apart from 5HT, DA is another important monoamine that fuels motivation and modulates pleasure, reward, and emotion. Additionally, NA is a key monoamine responsible for attentiveness, emotions, cognition, and social interactions. The monoamine hypothesis was formulated in the mid-1960s due to the underactivity of brain monoamines such as 5HT, DA, and NA (Freis, 1954). This hypothesis is based on antidepressant drug efficacy, such as SSRIs, norepinephrine–dopamine reuptake inhibitors (NDRIs), TCAs, and MAOIs (Pletscher, 1991; Radak et al.,2013). The mechanisms of action for this hypothesis with antidepressants are (1) inhibition of the reuptake of 5HT and/or NA, (2) antagonistic presynaptic inhibition of 5HT and/or NA, and (3) inhibition of monoamine oxidase (MAO) (Moret and Briley, 2011). Findings on these mechanisms of action showed that chronic treatment with antidepressants ultimately causes increased levels of monoamines. Apart from 5HT, DA, and NA, GABA is also reported to affect depression (Tunnicliff and Malatynska, 2003; Sequeira and Tureki, 2006; Lee et al., 2018). GABA plays a role in depression and anxiety through its interaction with inflammatory cytokines, NF-kB, and p38 MAPK signalling pathways (Sanacora and Saricicek, 2007).

2.8 Inflammation

Previous studies have reported that immune system function and the pathogenesis of depression have complex reciprocal communication between the CNS, endocrine system and immune system (Schiepers et al., 2005; Miller et al., 2009; Jolly and Verbeke, 2018). In addition to numerous theories and findings from previous findings, different or conflicting findings on the mechanisms of action of inflammation of the pathogenesis of depression have also been discussed about how the balance or communication between anti-inflammatory and pro-inflammatory factors shifts (Pedersen and Steensberg, 2002; Song et al., 2009; Schmidt et al., 2014).

The inflammation theory has also been linked to depression and has become a key point in the treatment direction for depression cases (Schmidt et al., 2016; Beurel et al., 2020; Jha et al., 2020; Schiweck et al 2020a, b; Obermanns et al., 2021). Believed to be fuelled by lifestyle, the inflammatory process is related to the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (Lee et al., 2011), a transcriptional factor that regulates various gene expressions. It is activated by extracellular stimuli, such as LPS administration, chronic stress (Wichers and Maes 2002; Oeckinghaus and Ghosh, 2009; Napetschnig and Wu, 2013), or by CUMS exposure (Song et al., 2018). The CUMS potentially activates this pathway via several mechanisms of action such as the HPA axis, leaky gut, oxidative stress as well as excitotoxicity (Liu et al., 2019), proposing its propensity to turned into haywire. Once it is activated, an inflammatory response takes place (FitzGerald, 2003; Yu et al., 2014). An inflammatory response includes the secretion of cytokines, which have a specific effect on the interactions and communications between cells (Munhoz et al., 2006; Ménard et al., 2016; Troubat et al., 2021).

Cytokines are signalling proteins secreted in response to the immune system's activation by stressors, such as injury, infection, or psychosocial factors (Miller et al., 2009). Moreover, the cytokines induce anti- or pro-inflammatory responses, whereby the anti-inflammatory cytokines are secreted to counteract the pro-inflammatory cytokines (Han and Yu, 2014; Llorens-Martin et al., 2014; Schmidt et al.,2016). Cytokines comprise lymphokine (cytokine made by lymphocytes), monokine (cytokine made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Part of them is recognized as IL-2, IFN-γ, IL-1β, TNF-α, IL-6, IL-12, IL-15 for pro-inflammatory functions (Koo and Duman, 2008; Dantzer et al., 2008; Norden et al., 2015) and as IL-4, IL-5, IL-13, IL-1Ra, IL-10 for anti-inflammatory action (Pérez-Sánchez et al., 2018; Köhler-Forsberg et al., 2019).

Inflammatory responses play a primary role in eliminating or inactivating harmful entities or damaged tissues in the body. However, over-activation of this system can cause detrimental effects, such as depressive-like behaviour (Kasai et al., 1997; Rubio-Perez and Morillas-Ruiz, 2012). Their alteration of the innate and adaptive immune system is undeniable to be related to the progression treatment of depression (Beurel et al., 2020; Schiweck et al., 2020a; Obermanns et al., 2021).

Previous findings have shown that depressed people have increased levels of inflammatory mediators, such as C-reactive protein (CRP) and pro-inflammatory cytokines (Dowlati et al., 2010; Leighton et al., 2018). In response to inflammation, the translocation of inflammatory mediators interferes with neuronal and glial well-being, resulting in cognitive and behavioural manifestations alongside synaptic plasticity that leads to neurodegeneration (Duman and Aghajanian, 2012). There are two major pathways for inflammatory cytokines that disrupt the synthesis of monoamine neurotransmitters, particularly 5HT, glutamate, and DA. They are pivotal for neurotransmitter regulation and ultimately affect mood regulation in depression, namely kynurenine and tetrahydrobiopterin (BH4) (Koo and Duman, 2008; Capuron and Miller, 2011; Haroon et al., 2016).

The activation of the kynurenine pathway (KP) within areas of the brain, such as the hippocampus, acts by causing alterations in emotional behaviours (Park et al., 2011; Dobos et al., 2012; Lawson et al., 2013). The KP primarily affects the most important neurotransmitter for the regulation of emotion, which is 5HT (Vancassel et al., 2018). When inflammation occurs, levels of indoleamine 2, 3-dioxygenase (IDO) and tryptophan 2, 3-dioxygenase (TDO) are elevated, and the tryptophan is used by the IDO and TDO in kynurenine production (Hestad et al., 2017). Hence, causing the depletion of the tryptophan level for 5HT production.

This has been proven in animal models and drug therapy in patients with interferon-α (O'Connor et al.,2009; Raison et al., 2010). IDO and TDO are induced by pro-inflammatory cytokines, such as IL-1, IL-2, IL-6 and IFN-γ (Hestad et al., 2017). KP causes the increased production of several harmful metabolites, such as 3-hydroxykynurenine (3HK) and quinolinic acid (QA), causing the over-activation of the N-methyl-D-aspartate (NMDA) receptor and inducing oxidative stress and kynurenic acid (Schwarcz and Stone, 2017). The linkage between inflammation and KP is evident through the increased number of astrocytes that are synthesized by kynurenic acid and the higher production of quinolinic acid by microglia (Vancassel et al.,2018).

Alongside kynurenine, the BH4 pathway is also significant due to the monoamine neurotransmitter synthesis that is disrupted in depression (Dantzer et al., 2008). The analysed SBH sample mainly identified organic bioactive compounds such as phenylalanine, alanine, tyrosine, valine, acetate, lactate, trigonelline, ethanol metabolites, glucose, fructose, sucrose, and maltose (Mustafa et al., 2019). Phenylalanine, which is consistently found in SBH, is converted to tyrosine, which simultaneously converts BH4 to 4a-Hydroxytetrahydrobiopterin and is catalysed by phenylalanine hydroxylase (Lu et al., 2018b). The BH4 acts as a cofactor for precursors of neurotransmitters, namely 5HT, DA, and NA (Haroon et al., 2016). For example, the serotonergic pathway biosynthesis of 5HT comes from tryptophan, whereas dopaminergic, noradrenergic, and adrenergic pathways are intermediated by the precursor L-3,4-dihydroxyphenylalanine (L-DOPA) for the synthesis of DA, adrenaline, and NA (Strasser et al., 2016; Froböse and Cools, 2018).

Inflammatory cytokines potentially disrupt BH4 production, which is crucial for neurotransmitter synthesis (Dantzer et al.,2008). Two mechanisms are involved in the disruption of BH4. Firstly, inflammatory cytokines stimulate nitric oxide synthase (NOS) to produce nitric oxide (NO). The elevated activity of NOS causes the increased utilization of BH4 that later on is converted to 7, 8-dihydrobiopterin (BH2). The conversion of arginine to NO by NOS is enhanced by BH4, which acts as an enzyme co-factor (Haroon et al., 2011). Furthermore, BH4 is very sensitive to oxidative stress. Inflammatory cytokines are known to increase oxidative stress through the production of both nitrogen and oxygen-free radicals. This causes the irreversible degradation of BH4 to dihydroxyanthopterin (Neurauter et al., 2008).