ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF *Melalueca cajuputi* ESSENTIAL OIL IN CHRONIC IMMOBILISATION STRESS (CIS)-INDUCED MICE MODEL

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by

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AEA	Anandamide
ACC	Anterior cingulate cortex
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionicacid
α-MSH	Alpha melanocyte stimulating hormone
AANAT	Aralkylamine N acetyltransferase
ANOVA	Analysis of variance
BLA	Basolateral amygdala
BST	Brine shrimp test
BBB	Blood brain barrier
BDNF	Brain-derived neurotropic factor
BDI-2	Beck's depression inventory
BDI	Beck's anxiety inventory
BEO	Bergamot essential oil
BCL2	B - cell lymphoma 2
BAX	BCL - 2-associated X
cAMP	Cyclic adenosine monophosphate
CBD	Cannabidiol
COX-1	Cyclooxygenase 1
CNS	Central nervous system
CIS	Chronic immobilisation stress
CRH	Corticotrophin releasing hormone
CRH_2	Corticotropin-releasing hormone receptor 2

CA3	Cornu ammonis 3
CB_1	Cannabinoid receptor type 1
DSM 5	Diagnostic and statistical manual of mental disorder 5
DA	Dopamine
DG	Dentate gyrus
DMSO	Dimethyl sulfoxide
D2	Deiodinase enzyme type 2
D3	Deiodinase enzyme type 3
EO	Essential oil
EC_{50}	Effective concentration in 50% population
EPMT	Elevated plus maze test
ELISA	Enzyme-linked immunosorbent assay
FST	Force swimming test
FAAH	Fatty acid amide hydrolase
GCMS	Gas chromatography-mass spectrometry
GAD	Generalised anxiety disorder
GR	Glucocorticoid receptor
GABA	Gamma-aminobutyric acid
GSTP1	Glutathione S-transferase pi 1
GCL	Granular cell layer
HADS	Hospital anxiety depression scale
HIOMT	Hydroxyindole-O-methyltransferase
HPT	Hypothalamic-pituitary thyroid
H&E	Haematoxylin and eosin
HPA	Hypothalamus-pituitary-adrenal

HSEO	*Hypericum scabrum* essential oil
IL-6	Interleukin-6
IFN-c	Interferon- c
IFNs	Interferons
i.p	Intraperitoneal
INH	Inhalation
IDO	Indoleamine 2,3-dioxygenase
KYN	Kynureninase
KCC2	Potassium–chloride co-transporter 2
LEO	Lavender essential oil
LD_{50}	Lethal dose for 50% population
LARUSM	Laboratory animal research and service centre
MDD	Major depressive disorder
MC	*Melaleuca cajuputi*
MCEO	*Melalueca cajuputi* essential oil
MCAE	MC aqueous extract
MOA	Mechanism of action
MR	Mineralocorticoid receptor
mRNA	Messenger ribonucleic acid
MOIs	Monoamine oxidase inhibitors
MT_1	Melatonin receptor 1
MT_2	Melatonin receptor 2
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
NAS	*N*-acetylserotonin
NMDAR	N-methyl-D-aspartate receptor

NREM	Non-rapid eye movement
NHMS	National health & morbidity survey
NPY/AgRP	Neuropeptide Y/ Agouti-related protein
NKCC1	Sodium-potassium-chloride co-transporter 1
Nrf2	Nuclear factor erythroid 2- related factor 2
NE	Norepinephrine
OXT	Oxytocin
OS	Oxidative stress
OD	Optical density
PRL	Pre-limbic
PD	Panic disorder
PG	Prostaglandin
PHQ-9	Patient health questionnaire-9
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
PNS	Peripheral nervous system
PCL	Pyramidal cell layer
qPCR	Quantitative polymerase chain reaction
REM	Rapid eye movement
ROS	Reactive oxygen species
rT3	Reverse triiodothyronine
R^2	Regression value
ROW	Relative organ weights
RNS	Reactive nitrogen species
SAD	Separation anxiety disorder

SM	Selective mutism
SOD-1	Superoxide dismutase
STAI	State-trait anxiety inventory
5-HT	Serotonin
5-HT$_{1A}$	5-HT 1A receptor
5-HT$_{1AD}$	5-HT 1D receptor
5-HT$_{1A}$R	5-HT$_{1A}$ auto-receptors
5-HT$_{2A}$	5-HT 2A receptor
5-HT$_{2C}$	5-HT 2C receptor
SSRIs	Selective serotonin reuptake inhibitors
SNRIs	Serotonin noradrenaline reuptake inhibitors
SLC6A4	Solute carrier family 6 member 4
SM	Selective mutism
TRP	Tryptophan
TCA	Tricyclic antidepressants
T4	Thyroxine
T3	Triiodothyronine
THC	9-Tetra-hydrocannabinol
TSH	Thyroid-stimulating hormone
TRH	Thyrotropin-releasing hormone
TNF-a	Tumour necrosis factor alpha

LIST OF SYMBOLS

%	Percentage
ml	Mililitre
g	Gram
^0C	Degree celcius
cm	Centimetre
mm	Milimetre

KESAN ANTI KEMURUNGAN DAN ANTI KERESAHAN MINYAK PATI

Melalueca cajuputi KE ATAS MODEL MENCIT YANG DIARUH STRES

IMOBILASI KRONIK (CIS)

ABSTRAK

Kemurungan dan keresahan adalah masalah penyakit mental yang sering berlaku kerana ketidakseimbangan neurotransmiter monoamine. Rawatan yang sedia ada pada masa kini kurang berkesan dan menghasilkan beberapa kesan sampingan, maka memerlukan kaedah rawatan alternatif. Kajian awal menunjukkan bahan fitokimia dalam minyak pati *Melalueca cajuputi* (MCEO) mempunyai bahan kimia yang bersifat anti kemurungan (43.6%) dan anti keresahan (34.3%). Kajian ini dilakukan untuk menilai kesan anti kemurungan dan anti keresahan MCEO dalam mencit yang diaruh stres imobilasi kronik (CIS). Sebanyak 64 ekor mencit jenis *Swiss albino* jantan dibahagikan kepada lapan kumpulan (n=8) secara rawak yang mengandungi kumpulan kawalan dan kumpulan rawatan. Kumpulan kawalan terdiri daripada kawalan normal, kawalan negatif dan kawalan positif amitriptilin (20 mg/kg), manakala kumpulan rawatan dibahagikan mengikut lima kepekatan MCEO yang berbeza (1%, 2.5%, 5%, 7.5% dan 10% v /v). Kumpulan mencit tersebut kecuali kumpulan normal telah diaruh dengan CIS selama 2 jam setiap hari selama tempoh 15 hari berturut-turut. MCEO dilarutkan dengan 1% dimetil sulfoksida (DMSO) dan 0.9% saline, yang disediakan sejurus eksperimen dijalankan. Semua mencit dalam kumpulan rawatan dirawat dengan MCEO melalui penyedutan dan amitriptilin (20 mg/kg) secara intraperitonial pada hari ke-16 hingga hari ke-21. Kesan anti kemurungan dan anti keresahan dinilai menggunakan ujian "force swimming" dan

"elevated plus maze", perubahan luas permukaan pada lapisan sel granular (GCL) dan lapisan sel piramidal (PCL) serta tahap hormon pelepasan kortikotropin (CRH) pada hari ke-21. Keputusan ujian dianalisa menggunakan ANOVA sehala diikuti ujian post-hoc Dunnet yang dianggap signifikan pada tahap $p < 0.05$. MCEO pada kepekatan 1%, 2.5%, 5%, 7.5% dan 10% v/v dapat mengurangkan kesan kemurungan manakala pada kepekatan 5%, 7.5% dan 10% v/v mampu mengurangkan kesan keresahan pada mencit yang diaruh CIS berbanding dengan kumpulan kawalan negatif. Berat badan mencit meningkat secara signifikan ($p < 0.05$) dalam kumpulan rawatan MCEO 5%, 7.5% dan 10% v/v. Selain itu, MCEO meningkatkan luas permukaan GCL dan PCL secara signifikan ($p < 0.05$) pada kepekatan 7.5% dan 10% v/v. Tahap CRH dalam darah mencit didapati berkurangan secara signifikan pada semua kepekatan MCEO yang diuji. Kesimpulannya, MCEO pada kepekatan 7.5% v/v adalah kepekatan yang paling efektif yang memberi kesan anti kemurungan dan anti keresahan dalam mencit yang diaruh dengan CIS. MCEO menunjukkan kesan anti kemurungan dan anti keresahan kemungkinan disebabkan kandungan fitokimia yang mengawal atur CRH yang membawa kepada peningkatan luas permukaan GCL dan PCL. Data yang diperoleh daripada kajian ini dapat dijadikan panduan untuk kajian klinikal pada masa hadapan.

ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF *Melalueca cajuputi* ESSENTIAL OIL IN CHRONIC IMMOBILISATION STRESS (CIS) - INDUCED MICE MODEL

ABSTRACT

Depression and anxiety are common mental disorders that occurred due to monoamine neurotransmitter imbalance. Current available therapies are less effective and produce several side effects, thus requiring alternative treatment approaches. From the preliminary study, the phytochemical compounds in *Melalueca cajuputi* essential oil (MCEO) possessed antidepressant (43.6%) and anxiolytic (34.3%) properties. This study was conducted to evaluate antidepressant and anxiolytic effects of MCEO in chronic immobilisation stress (CIS) - induced mice. Sixty-four males *Swiss albino* mice were divided into eight groups randomly (n=8) that comprised of control and treatment groups. The control groups consisted of normal control, negative control and positive control amitriptyline (20 mg/kg), while treatment groups were divided according to five different MCEO concentrations (1%, 2.5%, 5%, 7.5% and 10% v/v). The mice groups except the normal group were induced with CIS procedure for 2 hours everyday for 15 consecutive days. Freshly prepared MCEO was dissolved in 1% dimethyl sulfoxide (DMSO) and 0.9% saline, before the experiment. All mice in the treatment group were treated with MCEO via inhalation and amitriptyline (20 mg/kg) via intraperitoneal route on day 16 until day 21. The antidepressant and anxiolytic effects were evaluated via forced swimming test, elevated plus maze test, changes of granular cell layer (GCL) and pyramidal cell layer (PCL) surface areas and corticotrophin releasing hormone (CRH) level on day 21 respectively. The test results

were analysed by using one-way ANOVA followed by Dunnet's post-hoc test with significant level at $p<0.05$. MCEO at 1% 2.5%, 5%, 7.5% and 10 % v/v significantly reduced depression-like behaviours while at 5%, 7.5% and 10 % v/v significantly reduced anxiety-like behaviours in the CIS-induced mice compared to the negative control group. The mice body weights were significantly increased ($p<0.05$) in the MCEO treatment groups of 5%, 7.5% and 10% v/v. Besides, there were significant increase ($p<0.05$) in the GCL and PCL surface areas at MCEO concentrations of 7.5% and 10% v/v. CRH blood level in the mice was found to be reduced significantly following treatment with MCEO at all tested concentrations. In conclusion, MCEO at concentration of 7.5% v/v is the most effective concentration that exhibits antidepressant and anxiolytic effects in mice that induced with CIS. MCEO exhibits antidepressant and anxiolytic effects probably due to phytochemical contents that regulate the CRH pathway that lead to increased of GCL and PCL surface areas. The data of this study can be used as guideline for future clinical study.

CHAPTER 1

INTRODUCTION

1.1   Study background

Melalueca cajuputi (MC) is the most common species, that can be found at the Papua New Guinea, Thailand, Vietnam, Cambodia, Indonesia, Malaysia, and northern Australia (Craven, 1999). The plant has been used to treat scabies, diarrhoea, intestinal worms, muscular pain and cholera in traditional medicine (Al-Abd *et al.*, 2016). Besides that, various of medicinal properties are already discovered in MC such as anti-dengue, anti-inflammatory, anticonvulsant activities, anti-microbial and anti-cancer (Daud *et al.*, 2015). The *Melaleuca* species is also one of the aromatic medical plant that is well known from the production of essential oils (Barbosa *et al.*, 2013). The flavour and odours are originated from complex mixture of compounds such as sesquiterpene, terpene, diterpene and oxygenated sesquiterpene (Ashokkumar *et al.*, 2018).

Apart of that, the volatile aromatic compounds are fat soluble, very clean and easily absorbed through skin (Lima *et al.*, 2013). Moreover, it has the ability to promote mental awareness in human and animal by exerted their effect on the brain via inhalation route (Lima *et al.*, 2013). Previous study reported that, a lot of aromatic essential oils from plants are already proven to alleviate stress and reducing anxiety such as *Acorus calamus*, *Cananga odorata*, *Citrus aurantium* and *Salvia sclare* essential oil (Cheng *et al.*, 2015). Moreover, MC essential oil is used in this study

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because of extensively used in traditional medicine to alleviate depression and anxiety, widely distributed especially at coastal region and produce a lot of essential oil compared to other MC species. The essential oil can be administered through inhalation, topical application and oral ingestion, but the common routes are through local application and inhalation (Woronuk *et al.*, 2011). The administration of essential oil via inhalation will be absorbed either by nasal mucosa and lungs or via olfactory neural pathway to reach central nervous system (CNS) and interact with neurotransmitter of the CNS (Chioca *et al.*, 2013). Inhalation is considered as the best administration route to reduce stress and anxiety because it offers the fastest effect to the CNS compared to oral ingestion and topical application (Zhang *et al*., 2020). Moreover, the inhalation route can be used in future clinical study due to its convenient administration compared to injection route that requires technical skill and may cause pain.

Next, the depression and anxiety syndromes are the most common mental disorders that can occur in adulthood, adolescence and even childhood (Bernaras *et al.*, 2019). Both of these disorders are diagnosed based on guidelines of Diagnostic and Statistical Manual of Mental Disorders 5, which prepared by the WHO (Bernaras *et al.*, 2019). Moreover, the causative effects of these disorders are taught to originate from endocrine disorders, sleep disorder, neurotransmitter alteration, genetic factors, elevation of inflammatory cytokines and oxidative stress (Nie *et al.*, 2017; Bernaras *et al.*, 2019; Kalkman, 2019; Li *et al.*, 2019; Vega-Rivera *et al.*, 2020). In this study, the corticotrophin releasing hormone is measured rather than cortisol because this hormone is responsible to initiate hypothalamic-pituitary-adrenal axis dysregulation

cascade in response to stress (Van tol *et al.*,2010). Besides that, female mice are not used in this study to avoid any hormonal changes during estrus cycle that can affect the mood state (Van Doeselaar *et al.*,2021).

1.2 Study rationale

Anxiety and depression are two common mental health problems that have high burden of diseases and economic (Shafiee *et al.*, 2018). Both of these disorders have high prevalent among childhood and adolescence (Bettis *et al.*, 2018). Previous study reported that the female patient has a greater tendency to develop anxiety disorder as compared to the male patient during childhood and adolescence (Albert, 2015; Essau *et al.*, 2018). This might be associated with biological and body changes related to puberty (Essau *et al.*, 2018). Based on WHO reports, about 350 million people of all ages suffer from depression and about 7.3% of people are affected by anxiety globally (Salehi-Abargouei *et al.*, 2018).

Overall, the prevalence of depression in Malaysia ranges between 3.9% to 46% while for anxiety is 8.2% (Mukhtar *et al.*, 2011; Kader *et al.*, 2015). Both of these disorders might occur at the same time that make the illness more severe, disabling, and resistant to treatment and have a greater risk of suicide (Mukhtar *et al.*, 2011; Kader *et al.*, 2015). The treatments for both of these disorders are focusing on the psychological and medical perspective (Malaysian Psychiatric Association, 2020). The psychological approaches help the patient to identify and respond with daily life's stresses, thus alter the negative thinking stigma towards positive patterns (Malaysian Psychiatric Association, 2020). The example of psychological approaches include

cognitive behaviour therapy, interpersonal therapy, behaviour therapy and psychodynamic therapy (Malaysian Psychiatric Association, 2020). Moreover, for medical treatment antidepressants and anxiolytic are usually prescribed when the condition is severe or not possible for psychological treatments (Malaysian Psychiatric Association, 2020). The example of antidepressants and anxiolytic agents are tricyclic antidepressants, serotonin reuptake inhibitors, monoamine oxidase inhibitors and serotonin noradrenaline reuptake inhibitors (Sartori *et al.*, 2019; Shmuts *et al.*, 2020; van Westrhenen *et al.*, 2020). Sometimes both of psychological and medical treatments are required if the condition is too severe (Malaysian Psychiatric Association, 2020).

However, the psychological treatments effectively are started to decline due to limited access towards adequate treatment mainly due to practical problems, required much longer time in each session together with low self-motivation to seek for help and treatment (Harvey *et al.*, 2015). Moreover, the antidepressants and anxiolytic agents able to cause adverse and side effects such as sexual dysfunction, miscarriage, fatigue, drowsiness, organ damage (Tareen *et al.*, 2017; Vengeliene *et al.*, 2018). Thus, development of safe and effective anti-depressant and anxiolytic agent might help government to reduce medical cost and health therapies incurred by government and prevent men power loss due to low of productivity. Besides that, it also coherent with mental health strategy that developed by Ministry of Health to overcome mental problem.

1.3   Study objectives

1.3.1   General objectives

- To evaluate the antidepressant and anxiolytic effects of MCEO in the chronic immobilisation stressed (CIS)-induced mice model

1.3.2   Specific objectives

1. To determine the chemical compositions of MCEO via gas chromatography - mass spectrometry (GCMS).

2. To determine the percentage of motility time (%) and percentages of time spent in open area (%) with effective concentration of MCEO by using force swimming test and elevated plus maze test.

3. To evaluate the effect of MCEO on body weight, relative organs weight and hippocampus (surface area of granular cell layer and pyramidal cell layer) of the treated mice.

4. To determine the blood level of corticotrophin releasing hormone by using hormonal assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Depression

2.1.1 Prevalence of depression

The worldwide depression prevalence was estimated to be approximately 12% (Patel *et al.*, 2019). Previous study indicated that up to 30% of depression cases showed chronic symptoms that could last at least 2 years (Nübel *et al.*, 2020). Chronic depression prevalence for 12 month and lifetime were both 1.5% and 3 to 6% (Nübel *et al.*, 2020). Children's Depression Inventory and Children's Depression Scale were used to evaluate depression prevalence rates. In Spain, about 4% have been observed followed by 6% in Finland, 8% in Greece, 10% in Australia and 25% in Colombia (Bernaras *et al.*, 2019). Previous study estimated that, about 85 % of patients get depression and tend to develop anxiety symptoms that occurred at any age (Möller *et al.*, 2016). Women are more susceptible to depression compared to men and a study reported that one third of women will experience a major depressive episode in their lifetime (Alshawwa *et al.*, 2019). According to National Health Morbidity Survey during 2015, the prevalence rate of depression in Malaysia was 1.8% with female was more affected than male (Malaysian Healthcare Performance Unit, 2016). Moreover, the highest prevalence rates of depression among adult was spotted on Indian ethnic while in children was spotted on Chinese ethnic (Malaysian Healthcare Performance Unit, 2016). Furthermore, among other differences state of Malaysia the highest prevalence rate were shown on Sabah and Wilayah Persekutuan Labuan (Malaysian

Healthcare Performance Unit, 2016). Apart from that, according to National Health and Morbidity Survey during 2017, one out of five adults in Malaysia suffered from depression and the highest rate of prevalence came from Indian ethnic (Institute for Public Health, 2017).

2.1.2   Types and diagnosis of depression

Depression is characterised by feelings of guilt, loss of pleasure, sadness, tiredness or sleep and appetite disturbance (WHO, 2017). It can be long-term or persistent which impaired daily life activities and in severe case will lead to suicide (WHO, 2017). This disorder affects about 300 million people all over the world and was marked as second common of death causes between ages of 15 and 29 (Bernaras *et al.*, 2019). Depressive symptoms can arise from different pathogenic pathways that associated with certain risk factors, comorbidity pattern and impairment levels (Hakulinen *et al.*, 2020). Previous study proposed a model which assumes that an individual that are suffering from depression can show different symptoms' presentation compared to an individual that only experience depressive symptoms but still does not pass the clinical diagnosis threshold for depression (Hakulinen *et al.*, 2020).

This model is called Diagnostic and Statistical Manual of Mental Disorders 5 (DSM 5) (Bernaras *et al.*, 2019). This diagnostic criteria is used as guidelines to make diagnosis followed with clinical judgment (Bernaras *et al.*, 2019). Based on DSM 5, all of depression disorders share one common characteristic such as sad, empty or

mood swing followed by cognitive and somatic changes that can affect individual's capacity function (Bernaras *et al.*, 2019). According to WHO, DSM 5 categorises depression into eight sections; major depressive disorder, persistent depressive disorder (dysthymia), disruptive mood dysregulation disorder, premenstrual dysphoric disorder, substance/medication-induced depressive disorder, depressive disorder due to another medical condition, and other specified and unspecified depressive disorder (Bernaras *et al.*, 2019).

Major depressive disorder (MDD) presented with depressed mood, loss of pleasure, insomnia, worthlessness feeling, weight gain or loss, fatigue, psychomotor retardation, loss ability to think properly and suicidal attempt thought which occurred whether among adolescents or children (Bernaras *et al.*, 2019). All of these symptoms presented almost every day and women were mostly affected nearly 1.5–3 times prevalence rates compared to male (Bernaras *et al.*, 2019). The depressed moods in adolescents or children can be resentful and last at least for one year (Bernaras *et al.*, 2019). Moreover, the symptoms also can be occurred once in two months (Bernaras *et al.*, 2019). This disorder can be diagnosed with minimum five symptoms such as anorexia or hyperphagia, insomnia , fatigue, unable to make wise decision, lack of self-esteem and despair feeling within two weeks period (Patel *et al.*, 2019; Nussbaum, 2020).

Furthermore, persistent depressive disorder is characterised with depressed mood nearly every day for at least 2 years (Nübel *et al.*, 2020). The patient must experience at least two of following six symptoms such as fatigue, lack of self-

confidence, insomnia, loss of appetite or overeating, difficult to make decisions and despair (Schramm *et al.*, 2020). Moreover, the patient symptoms must persisted for more than two months at one time (Schramm *et al.*, 2020). Besides that, the patient must not have hypomania or mania history and the symptoms produced not due to medical problems or physiological effects of certain substance and cause daily activities impairments (Schramm *et al.*, 2020). The disorder can be occurred in early onset (before 21 years old) and late onset (more than 21 years old) (Schramm *et al.*, 2020). The early onset might occur due to maltreatment during childhood or neuroendocrine dysregulation while late onset associated with stressful life events (Schramm *et al.*, 2020).

The disruptive mood dysregulation disorder is characterised with severe and persistent temper outburst that is manifested through verbally and behaviourally that last less than 5 minutes but more than one minute (Bruno *et al.*, 2019). The outburst is not consistent with developmental level, occurred almost every day without any symptom-free interval within three months or more and persisted at least for twelve months(Bruno *et al.*, 2019). This disorder should not be diagnosed before 6 years old or after 18 years old and the age onset is before 10 years (Bernaras *et al.*, 2019; Bruno *et al.*, 2019). Besides that, the symptoms produced, should not due to medical problems or physiological effects of certain drugs and are not better explained by other disorders (Bruno *et al.*, 2019). The symptoms should present at least two within three settings (peer group, school and home) and severe in one setting (Bruno *et al.*, 2019). The prevalent rate among male children and teenage boys are estimated to be about 2 and 5% which is higher compared to female (Bernaras *et al.*, 2019).

Besides that, premenstrual dysphoric disorder is characterised by physical, behavioural and emotional distress during late luteal menstrual phase that disrupt with a woman's daily activities (Mattina *et al.*, 2020). Common symptoms include irritability, overwhelm sense, breast tenderness, headaches, depressed mood and poor concentration (Hantsoo *et al.*, 2020). All of the symptoms will be experiences for a few days after menstruation (Bernaras *et al.*, 2019). Previous study reported that, most women experience the following symptoms but only 1.8% was reported to have no impairment while about 1.3% suffer due to impairment (Bernaras *et al.*, 2019). Moreover to be diagnosed with this disorder the women should met at least two of stated symptoms(Osborn *et al.*, 2020).

Medication or substances-induced depressive disorder presented with common depressive disorder symptoms such as changes of appetite, fatigue, hyposomnia or hypersomnia, despair and suicidal thoughts after consumption, injection, or inhalation of certain drugs or substances (Bernaras *et al.*, 2019; Givon, 2019). The symptoms were assumed to persist whether after the intoxication or physiological or withdrawal effects which have disappeared (Bernaras *et al.*, 2019). Moreover, a relevant depressive disorder should have developed within a month after consumption of the medication or certain substances followed with evidenced by physical examination, clinical history and laboratory findings (American Psychiatric Association, 2013).

In addition, medical problem - induced depression is marked with depressed mood and loss of pleasure in all activities that occurred due to certain illness (Bernaras *et al.*, 2019). The depressive symptoms must etiologically related to the certain illness with evidence from physical examination, medical history and laboratory findings (Virginia Commission on Youth, 2017). Furthermore, presence of a clear relationship between the remission, onset and remission of the disease and the depressive symptoms is helpful to make diagnosis and judgment (Virginia Commission on Youth, 2017).Various of medical problems are known to show depression symptoms as symptomatic manifestation such as neurological illness, endocrine disorders and cardiovascular disorders (Virginia Commission on Youth, 2017).

Apart from that, others specified depressive disorders, that appeared with depression disorder symptoms characteristic which caused significant impairment in occupational, social and other important areas, but did not comply with any of depression criteria (Virginia Commission on Youth, 2017; Bernaras *et al.*, 2019). This category is used when the clinician choose to focus on specific symptoms that present without any criteria that meet specific depressive disorder (Virginia Commission on Youth, 2017). This is done when others specified depressive disorder symptoms is observed and recorded followed by specific reason symptoms (Virginia Commission on Youth, 2017).

In other unspecified depressive disorder that appeared with depressive disorder symptoms characteristic that cause impairment in social, occupational and other important areas but failed to appear and do not comply with any of depression criteria

diagnostic class (Virginia Commission on Youth, 2017; Bernaras *et al.*, 2019). The symptoms usually present with insufficient information that lead the clinician towards more specific criteria (Virginia Commission on Youth, 2017). This disorder is used when the clinicians choose to focus not on specific reason and the criteria are not met with the specific depressive disorder (Virginia Commission on Youth, 2017).

Besides that, the severity of the depression is measured by using several scales such as patient health questionnaire-9 (PHQ-9), Beck's depression inventory (BDI-2) and Whooley's questions. In PHQ-9, the questionnaire is comprised of 9 items and all of the items are scored as stated in Table 2.1 (Sherina *et al.*, 2012; Levis *et al.*, 2019; Maroufizadeh *et al.*, 2019; Plemmons, 2019). The total score for all items are ranged from 0 to 27 (Sherina *et al.*, 2012; Levis *et al.*, 2019; Maroufizadeh *et al.*, 2019; Plemmons, 2019). Moreover, to use this questionnaire, the person must experience the depression symptoms for at least 2 weeks. Then, the depression is categorised as stated in Table 2.1 (Sherina *et al.*, 2012; Levis *et al.*, 2019; Maroufizadeh *et al.*, 2019; Plemmons, 2019). This test is considered as valid, reliable and positively worded instruments (Sherina *et al.*, 2012; Levis *et al.*, 2019; Maroufizadeh *et al.*, 2019; Plemmons, 2019). For this test, it is less specific to young patients compare to old patients. A repeated screening can lead to false-positive result for MDD that lead to unnecessary antidepressants treatment. (Sherina *et al.*, 2012; Levis *et al.*, 2019; Maroufizadeh *et al.*, 2019; Plemmons, 2019).

Table 2.1 The PHQ-9 score, total depression score and depression classification

Score	Total depression score
0 = Not present	20 = Severe depression
1= Several days	15 = Moderate severe depression
2 = More than half of the days	10 = Moderate depression
3 = Almost every day	5 = Mild depression

Apart from that, the BDI-2 is comprised of 21 items that can measure emotion, vegetative, somatic and affective depression symptoms (Wang *et al.*, 2013; Jackson *et al.*, 2016; Knaster *et al.*, 2016; Macedo *et al.*, 2018; The National Child Traumatic Stress Network, 2018) Table 2.2 Most of the items are equivalent with DSM criteria and all of the items are graded according to severity from 0 (not at present), 1 (several days), 2 (more than half of the days) and 3 (almost every day) (Wang *et al.*, 2013; Jackson *et al.*, 2016). Furthermore, the score for minimal depression is between 0 - 13, mild depression is between 14 – 19, moderate depression is between 20 – 28 and severe depression is between 29 – 63 (Wang *et al.*, 2013; Jackson *et al.*, 2016). Besides that, total scores are range from 0 to 63 points 63 (Wang *et al.*, 2013; Jackson *et al.*, 2016). This test has a good sensitivity and specificity to detect depression with high internal consistency ($\alpha = 91$) and retest reliability ($r = 0.93$). However, the self-reporting results are easy to be manipulated 63 (Wang *et al.*, 2013; Jackson *et al.*, 2016). Besides, the standard samples are small because the subgroups are divided based on background variables (Wang *et al.*, 2013; Jackson *et al.*, 2016).

Table 2.2 The BDI-2 score, total depression score and depression classification

Score	Total depression score
0 = Not present	29 – 63: Severe depression
1= Several days	20 – 28: Moderate depression
2 = More than half of the days	14 – 19: Mild depression
3= Almost every day	0 – 13: Minimal depression

Last but not least, Whooley's questions that only consisted of two-items that designed based on DSM criteria in order to diagnose major depression disorder (MDD) which is identified as loss of interest or low mood (Howard *et al.*, 2018; Fontein-Kuipers *et al.*, 2019). The questions are scored either 1 (yes) or 0 (no). Yes or no in one or both questions are indicated positive screen. The total score are ranged between 0 to 2. The sensitivity and specificity ranges are within 95% and 65% when they are compared with other test for both women and men. Currently, the predictive ability in pregnant women was ranged low to moderate, but it was still considered as a good screening instrument for maternal distress. However, it still has tendency to produce false positives.

2.2   Anxiety

2.2.1   Prevalence of anxiety

Anxiety is one of the mental health illnesses that impaired with individual life quality which can be manifested with pessimism, excessive worry thoughts or nervousness symptoms (Stubbs *et al.*, 2017). Globally in 2010, anxiety disorder caused 390 disabilities among 100,000 persons with no observable changes over time (Stubbs *et al.*, 2017). Moreover, in 2015, the prevalence rates of this disorder was estimated to be 3.6% and more common in females compared to males (WHO, 2017). The rates were not varying significantly between the age groups although the trends started to be lowered in older age groups. It was estimated that about 264 million were diagnosed with this disorder and reflected the increment about 14.9% since 2005 due to population ageing and growth (WHO, 2017). In Malaysia, according to National Health & Morbidity Survey (NHMS) two out of five adults were diagnosed with anxiety and more predominant in female (Institute for Public Health, 2017). The report also stated that Indian and Bumiputera Sabah ethnics were affected more and followed by Malay and Chinese due to stress, substances used, somatic and medical condition (Institute for Public Health, 2017). Moreover, Sabah state showed the highest prevalence rate of anxiety followed by Selangor and Pahang which occurred due to social economic factor such as employment, community safety, education and social problem (Institute for Public Health, 2017). This problems affect the ability to have good and affordable medical care to manage the stress.

2.2.2 Types and diagnosis of anxiety

Anxiety is defined as emotional response of an organism when facing certain threat or danger and it was considered as pathologic when it negatively affected daily life, maladaptive, permanent and cannot be controlled (Sartori *et al.*, 2019). This disorder often developed in childhood or adolescence that lead to chronic stage when persisted during later life. According to DSM 5, anxiety disorder is categorised into specific phobia, generalised anxiety disorder, panic disorder, separation anxiety disorder, social phobia, selective mutism, substance-induced anxiety disorder, anxiety disorder due to medical condition and agoraphobia (American Psychiatric Association, 2013).

According to DSM 5, generalised anxiety disorder (GAD) is known as anxiety that is very difficult to be controlled, excessive and persisted for at least 6 months (Robichaud *et al.*, 2019). GAD is diagnosed with at least three of six different somatic symptoms such as nervous, restlessness, easily fatigue, difficult to concentrate in work, insomnia (typically to fall and stayed asleep) and muscle tension (Center for Behavioral Health Statistics and Quality, 2016; Robichaud *et al.*, 2019). Besides that, the anxiety and physical symptoms must able to cause significant impairment within social or other important functioning areas (Center for Behavioral Health Statistics and Quality, 2016). Apart from that, individuals with GAD sometimes do not show any obvious behaviour but the symptoms manifestation marked the problem (Robichaud *et al.*, 2019). Moreover, the presented symptoms must

also not due to medical problems or physiological effect due to consumption of certain substances (Center for Behavioral Health Statistics and Quality, 2016). The age onset of this disorder is variable with median age of 30 years (Kessler *et al.*, 2012).

Panic disorder (PD) is characterized as sudden fear or intense distress that occur within minutes accompanied with certain symptoms (American Psychiatric Association, 2013). In order to be diagnosed with this disorder at least four symptoms should be presented such as shortness of breath, sweating, heat sensations, chest pain, dizzy heart pounding, abdominal pain, fear of dying, choking feeling, fear of losing control, numbness and shuddering (Kim *et al.*, 2018). Moreover, this disorder is also able to enhance anxiety sensitivity that is caused from stress life environment (Kim *et al.*, 2018). The median age onset of this disorder is 24 years old with women more common than man (Roy-byrne, 2018). Moreover, the prevalence of PD decreases after 60 years old (Roy-byrne, 2018).

At the meanwhile, social phobia or known as social anxiety disorder that occurred due to negative emotional experience, which marked with excessive fear towards one or more social situations such as eating, giving speech in front of a crowd or converse with unfamiliar people (American Psychiatric Association, 2013; Reinhorn *et al.*, 2020). These social situations almost always provoke the fear that make the individual perceived that he or she will be negatively evaluated by others and make them avoid the situations (American Psychiatric Association, 2013; Reinhorn *et al.*, 2020). Moreover, the fear is not due to actual threat that occurred within the social

situations or sociocultural context but able to cause impairment in social or other important functioning area (American Psychiatric Association, 2013). The fear or avoidance behaviour is typically persist for 6 months or more (American Psychiatric Association, 2013). Furthermore to be diagnose with this disorder the symptoms must not due to medical problems or physiological effects due to consumption of certain substances (American Psychiatric Association, 2013). The prevalence of this disorder is higher among women and younger individuals that have lower education and socioeconomic status (Reinhorn *et al.*, 2020). Apart from that this disorder show strong comorbidity among other disorders such as mood disorder, drug dependence and suicidal behaviour (Zhao *et al.*, 2020).

The specific phobia refers to excessive fear that always provoke immediately towards certain things or situation such as when seeing blood, insect, height or narrow place (Rowa *et al.*, 2018). (American Psychiatric Association, 2013; Rowa *et al.*, 2018). The phobias are categories in five which are natural environment (narrow space), situation (flying), animal (insects), blood-injection-injury (taking blood sample) and other (fear of disease) (Rowa *et al.*, 2018). The individual will always avoid both of object or situation with intense fear although they are not posed actual threat (American Psychiatric Association, 2013). This fear behaviour able to last for six months or more and able to cause impairment in certain functioning areas The lifetime prevalence estimated that about 12.5% adults have susceptible specific phobia and it is more common among women (Rowa *et al.*, 2018). This disorder might occur due to genetic, neurobiology, temperament, and parenting (Oar *et al.*, 2019).

By the way, agoraphobia refers to fear in certain situation that is perceived to be inescapable, difficult to solve or helplessness (Sewart *et al.*, 2018). Example of agoraphobia is fear of being outside of the house alone, fear of using public transport and being in a crowd and fear being in open or enclosed space (American Psychiatric Association, 2013; Sewart *et al.*, 2018). The individual will always avoid these agoraphobia situations although they not posed actual threats (American Psychiatric Association, 2013). The fear and avoidance behaviours are last for 6 months or more threats (American Psychiatric Association, 2013). Moreover, to be diagnosed with disorder, medical illness or drug abused must be excluded (American Psychiatric Association, 2013). This disorder is might due to genetics, neuroticism, anxiety sensitivity, body maintenance factor, abuse and medical illness history (Sewart *et al.*, 2018). In body maintenance factor, certain people belief that by achieving ideal weight or body shape able to improve mood, self-view and relationship (Bohon *et al*., 2009). So, they tend to have less confidence and always avoid any social interaction until they meet the ideal body weight and shape (Bohon *et al*., 2009).

In addition, separation anxiety disorder (SAD), is described as a developmental problem and extreme anxiety sensation that is concerned on unwillingness or refuse to separate from attachment figures (West *et al.*, 2020). The SAD may present with persistent distress when experiencing separation from home or attachment figure, recurrent and excessive worry toward himself or herself and the attachment figures safety that can cause separation, persistent refusing to go anywhere due to the fear of separation, persistent and excessive fear to be alone and sleep without the attachment figures, recurrent nightmare regarding separation theme and repeated complaints about

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somatic symptoms such as stomach aches and headaches when separated with attachment figures excluded (American Psychiatric Association, 2013; West *et al.*, 2020). The disturbances must able to cause impairment in daily activity and cannot be better explained by another criteria of mental disorder (American Psychiatric Association, 2013). The anxiety, fear and avoidance behaviours are recurrent, last six months or more in adults and four weeks in children and adolescents (American Psychiatric Association, 2013; Möller *et al.*, 2015). Previous study reported that, about 73.5% of children who are diagnosed with SAD tend to develop another psychopathology disorder during adulthood although the SAD is already treated and recovered (West *et al.*, 2020).

The selective mutism (SM) is defined as the consistent failure to speak and communicate effectively in certain social situation such as school, but able to talk normally in other settings such as home (American Psychiatric Association, 2013; Hua *et al.*, 2016). The selective speech absences are persisted for at least one month and it is interfered with daily activities and the symptoms are not due to language faltering, communication problems, development disorder, a lack of knowledge or face a new situation (American Psychiatric Association, 2013; Hua *et al.*, 2016). SM can be diagnosed since infant, childhood or adulthood but the symptoms onset in childhood or adulthood are uncommon so further evaluation should be conducted (Kristensen *et al.*, 2019). The causes of this disorder might due to temperament, family factors, traumatic life events, immigration and bilingualism (Kristensen *et al.*, 2019).

Moreover, substance-induced anxiety disorder, that develops due to withdrawal or intoxication effects of certain medications or substances abused (Revadigar *et al.*, 2020). The individual may present certain symptoms such as elevation of vital signs, diaphoresis, paranoia, confused mind, choreoathetosis (movement disorder), auditory hallucinations and bruxism (teeth grinding) (Hategan *et al.*, 2018). All of these symptoms are normally appeared during intoxication effect and last after the effects end, for example a patient that consumed methamphetamine only experiences the intoxication symptoms for 48 hours. According to DSM 5 criteria, this disorder is classified into three stages; mild, moderate and severe. Mild stage is only presented with 2 to 3 symptoms and moderate stage is presented with 4 to 5 symptoms while severe stage is presented with 6 or more symptoms that persisted for 12 month period (Hategan *et al.*, 2018). Moreover, if certain substances are consumed in large amounts and in long periods of time, the person may experience multiple unsuccessful or difficult to discontinue the substances, exacerbated effects (physical and psychological problem), withdrawal and tolerance effects (Hategan *et al.*, 2018).

Besides that, anxiety disorder due to another medical condition, which the anxiety symptoms produced are directly linked with certain illness (Niles *et al.*, 2015). The symptoms evaluation should be performed by a qualified health care to diagnose the impairment level, estimating the treatment need and evaluate the mental health care capacity (American Psychiatric Association, 2013). Certain disorders such as autoimmune, cardiovascular, neurodegenerative disease are able to induce anxiety condition (Niles *et al.*, 2015).

In the meanwhile, the severity of anxiety is measured via several rating scales such as state-trait anxiety inventory (STAI), Beck's anxiety inventory (BDI) and hospital anxiety depression scale (HADS). STAI comprises of as two sets of questionnaire that have 40 items for assessing trait anxiety (how much anxiety liability which affecting personal characteristic or anxiety propensity) and state anxiety (current patient emotion) (Mirbastegan *et al.*, 2016; Greene *et al.*, 2017; Sobanko *et al.*, 2017; Yang *et al.*, 2019). The items are rated with 1-4 point of scale which 4 is the highest score of anxiety. Moreover, the scores of anxiety will be calculated by taking 10 items from state anxiety scale and 11 items from trait anxiety scale and vice versa for non – anxiety scores. The lowest and highest score ranges within 20-80. This test is proved to be sufficient, reliable, valid and adequate in clinical settings in order to diagnose anxiety and to distinguish it from depressive syndromes. However, it shows poor discriminant validity and did not differentiate persons with and without anxiety disorders for elderly populations.

The BDI emphasises on somatic symptoms such as nervousness that develops to discriminate anxiety and depression (Julian, 2011). BDI comprises of 21 items, responds rate and the total scores as mentioned in Table 2.3. This test only takes about 5 – 10 minutes to be accomplished, easy to use and is able to score the anxiety level. BDI is able to detect changes within psychiatric and medical populations, provide good validity and reliability (Julian, 2011). But, the test validity may discriminate younger populations compared to older populations because it emphasises more on somatic symptoms (Julian, 2011). Moreover, other primary anxiety symptoms such as worry and other cognitive aspects are not assessed.