EFFECTS OF A STANDARDIZED AQUEOUS EXTRACT OF *POLYGONUM MINUS* ON STRESS-INDUCED BEHAVIOR AND NEUROBIOLOGICAL CHANGES IN MICE

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UNIVERSITI SAINS MALAYSIA

2022

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

September 2022

ACKNOWLEDGEMENT

First of all, I am very thankful to Allah. Indeed, He is the most beneficial and merciful.

He made me able to complete this task. He said in Quran, “Recite: In the name of thy Lord who created man from a clot. Recite: And thy Lord is the Most Generous Who taught by the pen, taught man that which he knew not.” (Quran, 96:1-5)

I offer my respectful obeisance to my beloved Prophet Muhammad (PBUH), who is very dear to Allah. After that, I would like to thank my beloved father and mother for their vital support for me. I can never forget that my dear mother protected me, while she lost her life in a road accident. May Allah Almighty's blessings be upon my parents. I am also very thankful to my respected four elder brothers for their encouragement and prayers throughout my life.

I acknowledge the special participation of my respected main supervisor Dr Nur Hidayah Kaz Abdul Aziz, in my research career. I am very grateful to my supervisor for her precious time, attention to my research work, guidance in each step of my work, and her kind, professional behavior during my studies. She improved my skills and made me able to achieve my goals. She is my mentor. I wish to extend my sincere appreciation to respected Dr Dzul Azri Mohamed Noor for his expert teachings and kind guidance in my research work as a co-supervisor.

I also appreciate the respected Dean of the School of Pharmaceutical Sciences, Universiti Sains Malaysia, for providing us with a good research environment. I am thankful to the laboratory and admin staff of the school as well for their cooperation during my study.

I feel that it's an important to acknowledge the Institute of Postgraduate Studies (IPS) USM Malaysia for awarding me a Fellowship (scholarship) during my Doctoral studies. It was an outstanding award for me. It provided me with motivation for achieving my maximum goals.

Last but not least, I dedicate this thesis to my beloved late parents.

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LIST OF ABBREVIATIONS

AMI	Amitriptyline
ARG	Arginine
ALA	Alanine
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BW	Body weight
CUMS	Chronic Ultra Mild Stress
CORT	Corticosterone
CYS	Cystine
FDA	Food and Drug Administration
FST	Forced Swimming Test
GAD	Generalized anxiety disorder
GLY	Glycine
GLU	Glutamine
HPA	Hypothalamic-Pituitary-Adrenal Axis
5-HT	5-Hydroxytrptamine (serotonin)
ILE	Isoleucine
IACUC	Institutional Animal Care and Use Committee
KLF 11	Kruppel-like factor 11
LYS	Lysine
LEU	Leucine
MAO-A	Monoamine oxidase-A
MAOI	Monoamine oxidase inhibitor
MDD	Major Depressive Disorder

MOE	Molecular Operating Environment (Software)
MET	Methionine
NE	Norepinephrine
NET	Norepinephrine Transporter
P. minus	*Polygonum minus*
PHE	Phenylalanine
Q3G	Quercetin 3-glucuronide
SIRT1	Sirtuin (Silent mating type information regulation 2 homolog 1)
SPT	Sucrose Preference Test
SERT	Serotonin Transporter
SSRI	Selective Serotonin Reuptake Inhibitor
SNRI	Serotonin Norepinephrine Reuptake inhibitor
TCA	Tricyclic Antidepressant
TYR	Tyrosine
TRP	Tryptophan
USM	Universiti Sains Malaysia
WHO	World Health Organization

LIST OF APPENDICES

KESAN EKSTRAK AKUEUS TERPIAWAI *POLYGONUM MINUS* PADA PERUBAHAN TINGKAH LAKU DAN NEUROBIOLOGI YANG DIARUH OLEH TEKANAN PADA MENCIT

ABSTRAK

Tekanan adalah tindak balas badan terhadap pelbagai peristiwa fizikal atau emosi. Tekanan boleh menyebabkan tingkah laku seperti kemurungan, kebimbangan dan terjejas kognitif. Model tekanan ultra-ringan kronik (CUMS) digunakan untuk meniru gangguan kegelisaha dan kemurungan pada haiwan. Terdapat keperluan untuk mencari ubat-ubatan baharu bagi merawat gangguan tingkah laku dan mood dengan lebih berkesan dan baik. Ekstrak akueus piawai *Polygonum minus* telah digunakan dalam penyelidikan ini, yang mana kuersetin-3- glukuronida dan kuersitrin (kuersetin-3-rhamnosida) adalah komponen utamanya. Matlamat utama kajian ini adalah untuk menilai kesan ekstrak akueus *P. minus* terhadap tingkah laku dan neurobiologi yang disebabkan oleh tekanan ultra-ringan kronik (CUMS) dalam model mencit. Rawatan diberikan selama 8 minggu di mana mencit tertakluk kepada tekanan ultra- ringan kronik selama 6 minggu. Tingkah laku mencit disiasat melalui ujian keutamaan sukrosa (SPT), ujian renang paksa (FST), ujian medan terbuka (OFT), ujian pengambilan makanan dan ujian keutamaan makanan. Memori spatial telah diuji melalui ujian Barnes maze. Tambahan pula, tahap kortikosteron dalam serum, dan paras dalam tisu otak bagi yang berikut: faktor neurotropik terbitan otak (BDNF), serotonin (5-HT), norepinefrina (NE), enzim monoamina oksidase-A (MAO-A), faktor seperti Kruppel 11(KLF11) dan sirtuin (SIRT1) diukur. Dok molekul in silico kuersetin-3-glukuronide dan kuersitrin dengan struktur MAO-A juga dilakukan.

Keputusan menunjukkan bahawa rawatan ekstrak *P. minus* dengan ketara ($p < 0.05$) pemilihan mencit terhadap sukrosa di bawah aruhan CUMS, dan meningkatkan masa imobiliti dalam ujian rangan paksa dengan ketara ($p < 0.05$). Ia juga meningkatkan defisit ingatan yang disebabkan oleh CUMS dengan ketara ($p <0.05$). Ekstrak akueus *P. minus* mengurangkan dengan ketara ($p < 0.05$) anhedonia dan anoreksia aruhan tekanan. Pemberian ekstrak akueus *P. minus* secara ketara mengurangkan ($p < 0.05$) paras kortikosteron serum. Ia meningkatkan paras serotonin dan norepinefrina dalam hipokampus dengan ketara ($p <0.05$). Ia juga meningkatkan tahap BDNF dalam korteks prefrontal dan hipokampus dengan ketara ($p <0.05$). Rawatan ekstrak akueus *P. minus* menurunkan paras MAO-A dalam hipokampus dengan ketara ($p < 0.05$). *P. minus* juga menurunkan tahap KLF11 dan SIRT1 dalam hipokampus dengan ketara ($p <0.05$). Tambahan pula, kuersetin-3-glukuronida dan kuersitrin juga menunjukkan pertalian pengikatan yang lebih tinggi dengan struktur MAO-A melalui dok molekul in silico berbanding dengan perencat MAO-A standard. Kajian ini mencadangkan bahawa ekstrak akueus *P. minus* memperbaiki tingkah laku seperti kemurungan dan meningkatkan memori spatial di samping dengan mengurangkan tahap MAO-A dan kortikosteron, serta meningkatkan tahap BDNF dan monoamina (serotonin dan norepinefrina) dalam mencit.

EFFECTS OF A STANDARDIZED AQUEOUS EXTRACT OF *POLYGONUM MINUS* ON STRESS-INDUCED BEHAVIOR AND NEUROBIOLOGICAL CHANGES IN MICE

ABSTRACT

Stress is a reaction of the body against various physical or emotional events. Stress can induce the depression, anxiety and as well as impairs cognition. Chronic ultra-mild stress (CUMS) model is used to mimic the anxiety and depressive like disorders in animals. There is a need for new medicinal agents to treat the anxiety and mood disorders more adequately. A standardized aqueous extract of *Polygonum minus* was used in this research, in which quercetin-3- glucuronide and quercitrin (quercetin 3-rhamnoside) were the major constituents. The main aim of this study was to evaluate the effects of *P. minus* aqueous extract on chronic ultra-mild stress (CUMS) induced behavioral and neurobiological change in mice model. Treatment was given for 8 weeks in which the mice were subjected to chronic ultra-mild stress for 6 weeks. The behavior of subjects was investigated through the sucrose preference test (SPT), forced swimming tests (FST), open field test (OFT), food consumption and food preference tests. Spatial memory was examined through the Barnes maze assay. Furthermore, levels of serum corticosterone, and levels of the following from the brain tissue: brain derived neurotrophic factor (BDNF), serotonin (5-HT), norepinephrine (NE), monoamine oxidase-A (MAO-A) enzyme, Kruppel-like factor 11(KLF11) and sirtuin (SIRT1) were measured. *In-silico* molecular docking of quercetin-3-glucuronide and quercitrin with MAO-A structure was also performed. The results showed that *P. minus* extract treatment significantly ($p < 0.05$) reversed the CUMS-induced decreased

sucrose preference and significantly ($p < 0.05$) increased the immobility time of FST. It also improved the memory deficit developed under influence of CUMS significantly ($p < 0.05$). *P. minus* aqueous extract significantly ($p < 0.05$) reduced the stressed-induced anhedonia and anorexia as well. *P. minus* aqueous extract administration significantly ($p < 0.05$) reduced the serum corticosterone level. It increased the hippocampal serotonin and norepinephrine significantly ($p < 0.05$). It also increased the BDNF level in the prefrontal cortex and hippocampus significantly ($p < 0.05$). *P. minus* aqueous extract treatment decreased the MAO-A level in hippocampus significantly ($p < 0.05$). *P. minus* also decreased the KLF11 and SIRT1 level in the hippocampus significantly ($p < 0.05$). Furthermore, quercetin-3-glucuronide and quercitrin also showed a higher binding affinity with MAO-A structure through *in-silico* molecular docking compared to standard MAO-A inhibitors. The current study suggests that the *P. minus* aqueous extract ameliorated the depressive-like behavior and improved spatial memory along with reduced the MAO-A and corticosterone levels, as well as increased the BDNF and monoamines (serotonin and norepinephrine) level in mice.

CHAPTER 1

INTRODUCTION

1.1 General background

Stress constitutes a state of threatened homeostasis triggered by intrinsic or extrinsic adverse forces (stressors) (Salami et al., 2020). Stress can disturb the physiological homeostasis and causes neurobehavioral alteration. There are various neuropsychiatric problems such as anxiety, cognitive dysfunction, depression etc., are generally associated with stress. (Kumar et al., 2010). According to World Health Organization's latest survey, depression is predicted to affect 322 million individuals and anxiety disorders impact more than 260 million individuals worldwide by 2030 (Friedrich, 2017).

Anxiety can be defined as feeling of fear, dread, and uneasiness. It can be a normal reaction to stress. Anxiety disorders are conditions in which you have anxiety that does not go away and can get worse over time (Vanin, 2008). Anxiety includes social phobias, panic disorder, generalized anxiety disorder (GAD), obsessive-compulsive disorder and post-traumatic stress disorder (Mancini, 2021).

Depression can be defined as mood disorder that causes a persistent feeling of sadness and loss of interest, it affects how an individual feels, thinks and behaves. It can lead to a variety of emotional and physical problems (Zhao et al., 2019). Sadness, lack of interest or pleasure, feelings of guilt or poor self-worth, sleep or food interruptions, tiredness, and reduced attention are symptoms of a depressive illness. Depression might last a long time or come and go. In its most severe form, depression can lead to suicide (Mancini, 2021).

There are some hypotheses about the pathophysiology of depression, such as the neurotrophic hypothesis (reduced brain-derived neurotrophic factor), theories regarding monoaminergic systems (serotonergic, noradrenergic, and dopaminergic systems), and HPA axis dysfunction (hypercortisolemia). Researchers have created a variety of antidepressant medications based on these assumptions (Fekadu et al., 2017). Neurotrophic factors are important for the growth and plasticity of neural networks. The brain-derived growth factor is required for cell proliferation and synaptic plasticity (changes in neuron connections) throughout life (Martinowich & Lu, 2008). The monoamine neurotransmitters serotonin and norepinephrine have broad distribution across the central nervous system. They have a part to play in physiologic processes, like pain perception, hunger management, aggression, and mood modulation. Mood and anxiety problems have been linked to monoamine system dysfunction (Fekadu et al., 2017).

Clinical evidence also shows that imbalance in neurotransmission of serotonin (5-HT) and norepinephrine (NE) induce depression in the central nervous system. There is evidence that NE has a role in depression, and new research on neuronal circuits and symptoms have highlighted the special significance of NE in this condition (Moret & Briley, 2011). Monoamine oxidase (MAO) is the enzyme responsible for monoamine breakdown. MAO inhibitors have also been proposed as antidepressants by inhibiting MAO-A (Ishikawa et al., 2019). Kruppel-like factor11 (KLF11) is a cytostatic protein member of the Specificity protein 1/ Kruppel like factor11 (Sp1/KLF11) transcription factor family. It acts as a transcriptional activator for MAO-A (Udemgba et al., 2014). KLF11 increases the brain's MAO-A expression by attaching on binding sites and play an important role in stress-related depressive disorders (Grunewald et al., 2012). A significantly ($p < 0.05$) increased level of KLF11 protein usually found in the

hippocampus, so no significant changes in KLF11 expression were detected in the striatum and hypothalamus in the previous research that suggested that KLF11 protein expression is selectively affected by chronic stress (Duncan et al., 2015). Sirtuin 1, a silent mating type information regulation 2 homolog 1 (SIRT1), belongs to the Sirtuin family. Some previous studies suggested that SIRT1 protein could affect monoamine transmitter levels in the brain by activating MAO-A transcription. Therefore, dysregulation of SIRT1 is also involved in depression (Cai et al., 2015; Libert et al., 2011).

A recent study showed that KLF11 and SIRT1 are the main transcriptional up-regulators and activators of MAO-A. KLF11 directly activates the MAO-A gene transcription while SIRT1 indirectly activates it. They also concluded that flavonoids from herbal extract could alter the KLF 11 and SIRT1 level in the brain. Mostly, they could downregulate them, and MAO-A expression was decreased (Wang et al., 2019).

According to HPA axis dysfunction, its abnormality is the cause of increased cortisol production, which is further responsible for many neurological changes that decrease the volume of various parts of the brain and lead to depression. Serotonergic and noradrenergic balanced systems regulate HPA axis normality through continuous neurotransmission (Barden, 2004). Whereas in mice and rats, HPA axis dysregulation showed overexpressing of corticosterone hormone (Groenink et al., 2002).

Chronic ultra-mild stress (CUMS) model was developed to mimic the anxiety and depressive like behavioral disorders in animals more than two decades ago. Important for this model is that after prolonged exposure of tested animals to a series of CUMS stressors, a condition like anhedonia develops, which is noticed in the majority of depressive like disorders (Mineur et al., 2006). CUMS model is used now-a-days in

numerous research related to the neurobiological and biochemical changes associated with depressive like disorders (Ma et al., 2021).

Continuous stress can influence all the above factors and lead to depressive like disorders. An animal model is used for chronic stress and progression of human-like depression (Wang et al., 2019).

Neurotransmitter transmission problems were discovered in the hippocampus, prefrontal cortex, striatum, hypothalamus, and amygdala of stressed mice. The hippocampus is considered the central part that disturbs many normal brain functions under depression (Akter et al., 2019; Y.-E. Lin et al., 2016).

Some pharmacotherapies that target glutamate and gama butyric acid (GABA) neurotransmission are being used for anxiety treatment. Glutamate systems interact with GABAergic, serotonin, dopamine, and other systems involved in the stress response, which implies a potential role in the pathophysiology of anxiety disorder (Nasir et al., 2020).

Herbal medicine has proven to be a viable option for treating mental illnesses such as anxiety, depression and dementia. Discoveries from medicinal plants are very important in depression treatment (Liu et al., 2015). St John's wort *(Hypericum perforatum),* is an example of herbal medicine which is frequently used as a remedy for mild depression, it is also an anxiolytic and has cognition enhancing property (Barnes et al., 2019).

Polygonum minus Huds, synonymous with *Pericardia minor,* is from the family Polygonaceae and is commonly referred to as kesum or laksa leaf in Malaysia. This herb is associated with antioxidant activities and has many beneficial effects

(Christapher et al., 2015). The leaves of this herb have been reported to be high in an antioxidant flavonoid named quercetin-3-glucuronide and quercitrin (quercetin 3-rhamnoside). Aqueous extract of this plant showed significant beneficial effects on memory and cognitive functions with 100mg/kg dose in mice (George et al., 2014).

This study investigates the effects of *P. minus* leaf extract on stress-induced depressive, anxiety-like behaviors, and neurobiological changes through various behavioral and experimental assays. *P. minus* effects on KLF-11-MAO-A and SIRT1-MAO-A pathways were also determined through this study. Furthermore, *in-silico* molecular docking had been performed with abundant flavonoids (quercetin-3-glucuronide and quercitrin) of *P. minus* and other standard drugs into MAO-A co-crystal structure.

1.2 Standardized aqueous extract of *P. minus*

The standardized aqueous extract of *P. minus* used in this study, Biokesum ™ was acquired from Biotropics Malaysia Sdn Bhd. It is a patented *P. minus* aqueous extract. It contains some compounds like quercetin-3-glucuronide, which is not less than 0.45%, and quercitrin which is not less than 0.15%, remaining are trace phenolic contents (Lau et al., 2020).

1.3 Problem statement

A standardized aqueous extract of *P. minus* has showed positive mood improved memory in previous studies on human subjects. This extract also showed an increased cognition in scopolamine induced memory deficit mice model which is different pathophysiology compared to stress induced memory impairment. There was lack of screening about effects of this extract on stress induced behavioral and neurobiological

changes. In previous studies, researchers did not explain its effects on some vital neurochemicals which are involved in pathophysiology of stress induced behavioral disorders (negative mood, impaired cognition, anxiety and depressive like disorders). Another gap in those studies was, lack of some important behavioral assays related to stress induced behavioral changes. In current study, animal model was used to elaborate the effects of *P. minus* aqueous extract on neurobiological changes after introducing the chronic ultra-mild stress. Present research also showed the effects of *P. minus* aqueous extract on those behavioral assays which were note reported in previous studies.

1.4 Research question

i. What are the effects of *P. minus* aqueous extract on behavioral tests related to stress-induced behavioral changes in mice model?

ii. What are the effects of *P. minus* aqueous extract on some neurobiological parameters (BDNF, serotonin, norepinephrine, MAO-A, KLF11, SIRT1) and serum corticosterone hormone level in stressed mice?

1.5 Hypothesis

It was hypothesized that *P. minus* aqueous extract increases the monoamines (like serotonin and norepinephrine) in the hippocampus of stressed subjects, which are associated with behavioral disorders. It also increases the plasticity of the brain through enhancement of BDNF level or reduction of corticosterone level. It reduces the stress-induced behavior changes. It can reduce the MAO-A enzyme or its transcriptional activators.

1.6 Objectives

General Aim of Study

To evaluate the effects of *P. minus* aqueous extract on stress-induced behavioral changes in mice and identify its mechanism of action through measurement of various neurochemicals.

Specific objectives of the study are

1. To evaluate the effects of *P. minus* aqueous extract on cognition, spatial memory, depressive- and anxiety-like behaviors in stressed mice.
2. To determine the *P. minus* effects on anorexia and anhedonia in stress-induced mice.
3. To measure the levels of selected monoamines, serum corticosterone and BDNF following administration of *P. minus* aqueous extract in mice.
4. To measure KLF 11 and SIRT1 proteins levels.
5. To determine the binding energy and amino acid residue interactions of two abundant flavonoids of *P. minus* aqueous extract (quercetin-3-glucuronide and quercitrin) into MAO-A enzyme structure through *in-silico* molecular docking.

CHAPTER 2

LITERATURE REVIEW

2.1 Stress

Stress is a condition of endangered hemodynamic equilibrium caused by various stressors, which might be inherent or external, real or imagined challenges or stimuli. The stress system is a highly complex mechanism developed in organisms to maintain this ideal hemodynamic condition within a physiologic range. Repeated, transitory, and motivating stress events cause adaptive responses and habituations. Inadequate, unpleasant, severe, or persistent stress may exceed the regulating capacity (Agorastos & Chrousos, 2021).

Stress raises the likelihood of developing disorders that cause long-term emotional and cognitive problems. The chronic unpredictable stress (CUS) in an animal model is used to examine the effects of stress on behavior. It approximates the psychological characteristics of chronicity, unpredictability, and uncontrollability that people face daily. This model has been used to depict stress-related diseases, including depression and memory loss (Natarajan et al., 2017).

2.2 Chronic ultra-mild stress (CUMS)

The chronic ultra-mild stress (CUMS) model is widely used to mimic the depressive-like behaviors in animals. Mice or rats are exposed to continuous unpredictable micro-stressors in this model for some weeks. This model can cause various behavioral changes, including reduced response to rewards, clinical basic symptoms of depression, anhedonia are correlated with behavioral changes. CUMS can cause an endogenous depression that is further linked with neuropsychiatric disorders,

including behavioral, biochemical and neurochemical derangements (Mineur et al., 2006).

CUMS animal model is now an important tool to investigate the neurobiological, behavioral and hormonal changes which lead to stress-induced psychopathology. It plays a very vital role in understanding the pathophysiological mechanisms of depression and efficacy of antidepressant therapy (Biala et al., 2017).

2.3 Stress-induced behavioral changes

Chronic stress can result in depression (a loss of motivation or hope), anhedonia (a loss of ability to enjoy pleasurable activities such as food, sex, or social interactions), anergia, irritability, difficulty concentrating, disrupted sleep, appetite, and cognition, and a proclivity to commit suicide in living organisms (Yang et al., 2015). Chronic stress can also cause considerable weight loss in the body (Jeong et al., 2013).

When a person has trouble remembering the information, learning new things, focusing or making judgments that influence their daily lives, they have cognitive impairment in this situation. Mild to severe cognitive impairment exists (Petersen, 2011). Chronic stress has been shown to impair the brain's ability to learn. Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, both of which contribute to the endocrine stress response (Palumbo et al., 2010). In moderate cognitive impairment and most dementias, depression is prevalent. It might be a precursor to dementia in the future (Potter & Steffens, 2007).

Long-term or chronic stress has been related to clinically diagnosed depression in humans and depressive-like behavior in animals. This syndrome is frequently reported as a comorbidity in various illnesses, including depression and cancer (Hunter

et al., 2021; Robinette et al., 2020). On the other hand, anhedonia is a vital sign of sorrow and is characterized as a lack of enjoyment from rewarding or enjoyable activities (Liu et al., 2018). Anhedonia is linked to anorexia because it causes weight loss due to a lack of interest in eating or any other reward in living creatures (Haynos et al., 2021). As a result, anorexia can cause severe nutritional insufficiency and, in some instances, emergencies in patients (Guinhut et al., 2021). According to the American Psychiatric Association, major depressive disorder (MDD) is prevalent comorbidity in eating disorders (EDs), with anorexia occurring in 50–75 per cent of patients (Shilton et al., 2020). According to various research, there is a relationship between eating disorders and depression. Anorexia and severe other mood disorders have been observed to have many depressive symptoms (Lenzo et al., 2020). Chronic stress has been related to various illnesses, including depression, anxiety, anorexia nervosa, and bulimia nervosa. Changes in hunger and weight are typical diagnostic criteria for major depressive illness, and over half of adult depression patients have these signs and symptoms (Simmons et al., 2016). Chronic or long-term stress has been linked to clinically diagnosed depression in humans and depressive-like behavior in animal models (Rincón & Grace, 2020). Chronic stress led to a condition with depression-like symptoms like anhedonia and physical changes, including weight loss. Anhedonia is typically reported in people with anorexia, according to previous studies (Eliwa et al., 2021; Mishra et al., 2021). Patients with anorexia and test volunteers under the effect of stressful situations have reported a slightly sweet sensation. Anhedonia may be easily detected in a mouse model by limiting sweet food consumption. It has been claimed that anhedonia may be linked to stress-induced anorexia since repeated and chronic stress reduces the sensitivity of sweet taste in rats (Kim et al., 2015a). Weight loss of a substantial magnitude is also linked to severe anorexia. During an

examination, a weight reduction is a predictor of anorexia (Garber et al., 2019). A recent study has reported significant weight loss in prolonged stress-induced anhedonia (Eliwa et al., 2021).

2.4 Stress-induced anatomical changes in brain.

A stress disorder is a psychiatric ailment caused by a traumatic occurrence or strong stress that exceeds bearing ability. Several studies have found that severe stress causes anatomical changes in the brain, not just in the white matter but also in the grey matter. Extreme stress and trauma are linked to increased cortisol levels, damaging the hippocampus and interfering with morphology, microstructure, and cell quantity. Chronic stress has also been associated with dendritic changes in the orbital frontal cortex (OFC) and the medial prefrontal cortex (Liston et al., 2006). A decreased left amygdala volume is related to more significant life stress in six months, regardless of depression. In comparison to other areas of the brain, the hippocampus is crucial in the pathophysiology of depression (Sublette et al., 2016), because hippocampus formation plays a vital role in regulating learning, memory, motivation, and emotion. Reduced hippocampal volume is found in various mental illnesses including schizophrenia, dementia and post-traumatic stress disorder (Frodl et al., 2006). Hippocampal shrinkage is a common neuroanatomical alteration in stress-related mood disorders, including depression and anxiety. Stress-induced changes in hippocampal volume indicate vulnerability to psychopathology after chronic stress exposure (Tse et al., 2014). The hippocampus was associated with spatial learning and episodic, declarative, and contextual memory in the past. Several studies have found chronic stress to decrease monoamines like norepinephrine and serotonin in the hippocampus, which might explain its shrinkage (Joca et al., 2007). Glucocorticoids and other neuroendocrine

hormones of the hypothalamic-pituitary-adrenal (HPA) axis are mediators of diverse stress effects on the hippocampus and contribute to stress-related psychopathologies (Kim et al., 2015b). Numerous studies on the hippocampus's response to stress and antidepressants give compelling evidence for the convergence of stress and monoamines in depressed animal models. Prolonged stress inhibits the brain-derived neurotrophic factor (BDNF) transcription, whereas chronic monoamine modulator therapy increases BDNF transcription. Chronic stress and monoamine modulator therapy have opposite effects on two forms of hippocampus plasticity (Dranovsky & Hen, 2006). Too much corticosterone can also cause volumetric changes in the hippocampus, as well as decreased neurotransmitter levels. It can lead same like stress-related behavior disorders (Neto et al., 2012).

2.5 Depression

Depression is a mood or emotional state that is marked by reduced ability to enjoy life, feelings of guilt and low self-worth. A person who is depressed usually experiences some symptoms like feelings of sadness, hopelessness, or pessimism; lowered self-esteem and heightened self-depreciation; a decrease or loss of ability to take pleasure in ordinary activities; reduced energy and vitality; slowness of thought or action; loss of appetite; and disturbed sleep or insomnia (Ahmed et al., 2021). Major depression is a prevalent mental illness that significantly impacts psychosocial functioning and overall quality of life. The World Health Organization (WHO) listed severe depression as the third most significant cause of sickness burden in 2008, it is expected to rise by 2030. Its identification, diagnosis and management can be challenging for doctors in practice soon due to unpredictable course and variable response of medication (Malhi & Mann, 2018). So far, researchers have investigated

biochemical, genetic, anatomical and environmental factors that may play a role in the genesis of depression's early symptoms (Gałecki et al., 2018).

The etiology of depression is mainly unknown still. However, pathophysiological theories for depression include altered neurotransmission, HPA axis abnormalities linked with chronic stress, inflammation, decreased neuroplasticity, and neurotransmission dysfunction. All of the hypothesized processes are intertwined and have bidirectional interactions. In addition, psychological factors have been shown to directly influence neurodevelopment, resulting in a biological predisposition to depression, whereas biological factors can also contribute to a psychological disorder. While there may be numerous discrete endophenotypes of depression with unique pathophysiological causes. The authors suggest that observing sadness as a unified disorder in which diverse systems interact like nodes in a matrix might be beneficial (Dean & Keshavan, 2017). Several clinical features have been linked to the severity levels of mood disorders like cognitive impairment, anxiety, insomnia, hopelessness, anhedonia, anorexia and reduced body weight (Borserio et al., 2021).

Untreated depression can lead to severe conditions like suicide. Previous comprehensive research showed that higher suicide rates result from a lack of treatment (Crowder & Kemmelmeier, 2014). Continuous stress and depression can reduce the volume of the hippocampus which further lead the various serious neurodegenerative disorders (Schoenfeld et al., 2017).

2.6 Anxiety

Generalized anxiety disorder, panic disorder/agoraphobia, social anxiety disorder are the most prevalent mental disorders (Bandelow et al., 2017a). Anxiety and

stress have behavioral and neurological foundations that are interwoven. Anxiety is described as a temporally distributed emotional state triggered by a potentially dangerous circumstance with a low or unclear likelihood of harm. The two types of anxiety are state anxiety and trait anxiety. Hypervigilance in anticipation of a hazard, which can be produced by acute stress, is classified as state anxiety. Its primary role is to avoid dangerous circumstances and to aid memory consolidation. An individual's inclination to display continual concern is known as trait anxiety, and it raises the likelihood of state anxiety in potentially harmful situations (Daviu et al., 2019). Anxiety disorders result from a mix of psychosocial factors such as early adversity, stress, or trauma, as well as an inherited susceptibility shown in neurobiological and cognitive dysfunctions (Bandelow et al., 2017b). GAD (generalized anxiety disorder) is a prevalent and debilitating condition that is frequently misdiagnosed and mistreated. Suicide, as well as cardiovascular events and death, are all increased hazards for GAD sufferers. The majority of patients may be diagnosed and treated by primary care physicians. Antidepressant therapies including psychotherapy (usually cognitive behavioral therapy) and pharmacology (selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors) are widely used in the treatment of anxiety (DeMartini et al., 2019).

Current medication for anxiety disorders involves anxiolytic and antidepressant drugs, which have various side effects and tolerability. This emphasizes the need for new and better pharmacological therapies (Sartori & Singewald, 2019). In treating anxiety disorders, traditional Asian medicine, particularly Chinese and Persian medicine, plays a crucial role (Shahrajabian et al., 2021).

Untreated anxiety disorders have severe implications for both the person and society, including disability, developing illnesses (such as diabetes and heart disease), decreased capacity to work, and a high chance of suicide (Kasper, 2006).

2.7 Role of chronic ultra-mild stress in depression development

The chronic unpredictable mild stress (CUMS) model is a classic, established model for researching depressive disorders and antidepressants. The chronic mild stress paradigm resembles the etiology of some human depression disorders (Zhao et al., 2018). The pathophysiology of major depressive disorder (MDD) has been studied using a variety of animal models. The chronic mild stress (CMS) model, established by Paul Willner more than 30 years ago, is one of the best-known and commonly used models. This model has been utilized in over 2000 published investigations to evaluate new drugs with potential antidepressant effectiveness. The majority of these studies looked at the behavioral impacts of stress and concurrent pharmacological treatment. CMS-induced neurological alterations were the subject of far fewer investigations. Stress-induced cellular and molecular changes, on the other hand, are essential because they might be used as translational biomarkers to assist us in better understanding the pathophysiology of depression at the molecular level (Khan et al., 2020). A previous study of several stress protocols found that chronic ultra-mild stress increased immobility and decreased sucrose preference in the rats more than other protocols, suggesting that chronic mild stress effectively induces depression in animals (Zhu et al., 2014). Some behavioral alterations like anhedonic behaviors were also generated by the chronic mild stress protocol (Schweizer et al., 2009). In a previous study, mice were subjected to various stressors and then assessed using a decision-making task, and it

was shown that continuous ultra-mild stress significantly reduced mice's ability to make judgments compared to control animals (Pardon et al., 2000).

CUMS-induced cognitive impairments have been linked to the brain serotonin (5HT) system, including 5HT-induced dysregulation of the hypothalamus-pituitary-adrenal stress axis, desensitized 5HT receptors, and reduced neuronal firing (Bambico et al., 2009).

2.8 The monoamine hypothesis of depression

The monoamine hypothesis of severe depression was proposed over half a century ago. According to the findings, clinical depression is caused by a lack of monoamine neurotransmitters like norepinephrine and/or serotonin. Although this idea arose mostly from the accidental discovery of the mechanism of action of antidepressant medicines, it resulted in the development of the selective serotonin reuptake inhibitors (SSRI) family of antidepressants, which have proven to be effective in the treatment of clinical depression (Marathe et al., 2018). This idea has long been the most common explanation for primary depressive illness due to its simplicity and understandability (MDD). Most presently used antidepressants are thought to work on the monoamine theory (Boku et al., 2018). Low monoamine concentrations in synaptic areas, according to the theory, play a critical role in the onset of depression, other monoamine-related illness states, and regulatory dysfunction (Hinz et al., 2012).

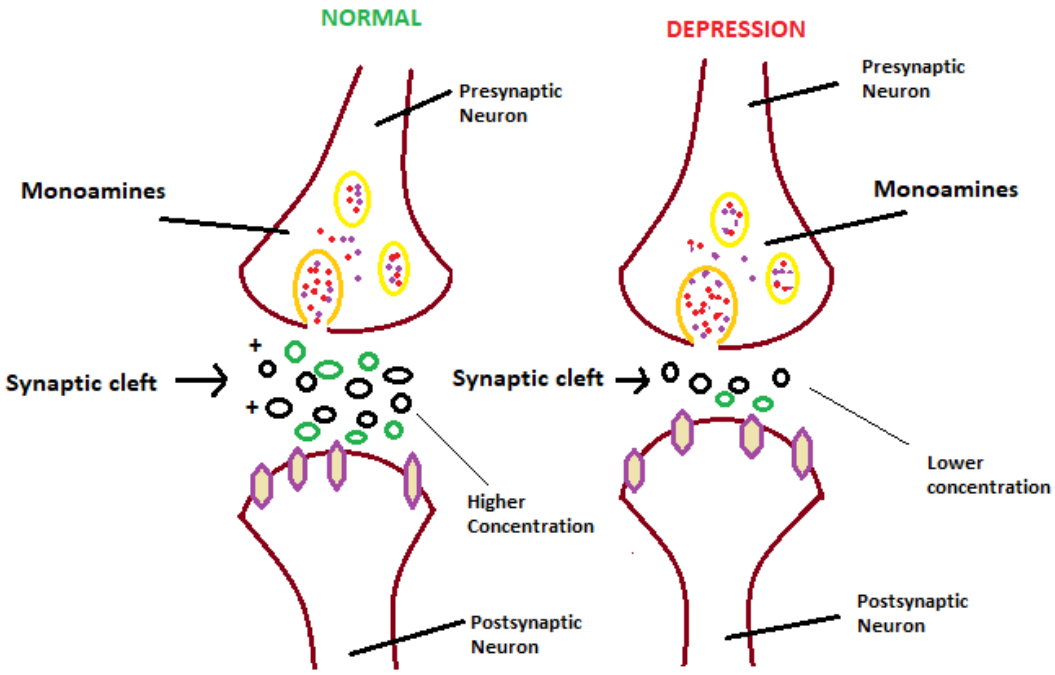

Figure 2.1 The monoamine hypothesis of depression

2.8.1 Serotonin

Serotonin is a neurotransmitter that has an impact on both the central and peripheral nervous systems. The neurotransmitter 5-hydroxytryptamine (5-HT), is often known as serotonin (Mohammad‐Zadeh et al., 2008). Serotonin regulates mood, happiness, and good emotions in the brain. This hormone performs several activities in the human body. It involves the transmission of nerve impulses act as a chemical messenger between nerve cells inside the brain (Rathore & Sarkar, 2021). Serotonin helps people to sleep, eat and digest food (Monti, 2011; Steiger, 2004). Drugs that target serotonin receptors are extensively employed in psychiatry and neurology because they alter brain activity and a wide variety of cognitive functions. The majority of serotonin

is present outside the brain, the 15 serotonin receptors are found both within and outside the brain (Berger et al., 2009).

2.8.2 Norepinephrine

Norepinephrine is also known as noradrenaline. Norepinephrine is a stress hormone and a neurotransmitter, naturally occurring molecule in the human body (a substance that sends signals between nerve cells). When the brain detects a stressful situation, a stress hormone is produced in the body (Lindau et al., 2016). Low NE neurotransmission is linked to low alertness, low energy, inattention, focus, and cognitive ability difficulties (Moret & Briley, 2011). Since the 1950s, the catecholamine neurotransmitter norepinephrine (NE) has been suspected of playing a role in depressive illnesses. The amino acid L-tyrosine, which is actively carried from the circulation, makes catecholamines like norepinephrine (NE) and epinephrine (EP). The metabolic pathway's rate-limiting enzyme tyrosine hydroxylase converts L-tyrosine to L-dihydroxyphenylacetic acid (L-dopa), Dopa-decarboxylase converts L-dihydroxyphenylacetic acid to dopamine (DA) which is an immediate precursor of NE and transports it into storage vesicles (Herrmann et al., 2004). Selective norepinephrine reuptake inhibitors seem to be the most effective treatment of depression which elevates the neurotransmission of norepinephrine in the synapse (Moret & Briley, 2011).

2.8.3 Monoamine oxidase-A (MAO-A)

Monoamine oxidases are enzymes that break down the monoamines' oxidation by cutting off their amine group with oxygen. Monoamine oxidase A and B have long been known as prospective therapeutic targets for depression and neurodegenerative diseases. Because of its role in serotonin and norepinephrine modulation, MAO-A is

frequently associated with depression (Mathew et al., 2016). The MAOs are a flavin-containing amine oxidoreductase protein family (Harismah & Mirzaei, 2020). MAOs have a vital role in the inactivation of monoamine neurotransmitters and the breakdown of monoamines present in food. They have been connected to various mental and neurological diseases, some of which can be treated with monoamine oxidase inhibitors (MAOIs), which prevent MAOs from performing their functions (Yeung et al., 2019).

2.9 Hypothalamic-pituitary-adrenal (HPA) axis hypothesis

The hypothalamic-pituitary-adrenal (HPA) axis is regulated by hypothalamic production of adrenocorticotrophic hormone-releasing factor (CRF) and arginine vasopressin (AVP), both of which drive pituitary secretion of adrenocorticotrophic hormone (ACTH). The adrenal cortex is then activated, causing it to release glucocorticoids (cortisol in humans and corticosterone in rodents) (Pariante & Lightman, 2008). Some brain signaling molecules, including serotonin, noradrenaline and neuropeptide Y, may activate the axis depending on the stress stimuli. When corticotropin-releasing hormone (CRH) is generated, it activates the corticotropin releasing hormone receptor 1 (CRHR1) and corticotropin releasing hormone receptor 2 (CRHR2) receptors, which interact with arginine vasopressin (AVP) and urocortin to govern the strength and duration of the stress response. As a result of this process, the glucocorticoid cortisol is synthesized and released. Cortisol may act on both mineralocorticoid and glucocorticoid receptors, and imbalances between them have been related to depression and maladaptive stress response (Schatzberg et al., 2014). When corticosterone levels are high, the volume of key brain areas such as the hippocampus, amygdala, and prefrontal cortex associated with depression, decreases (Johnson et al., 2006).

2.9.1 Corticosterone

Corticosterone, also known as 17deoxycortisol, is a corticosteroid hormone with 21 carbons generated in the adrenal gland cortex (Carlsson et al., 2021). Many animals, including amphibians, reptiles, rodents and birds have corticosterone (Narayan et al., 2013). Corticosterone is a primary glucocorticoid that regulates energy levels, immunological responses, and stress responses (Gong et al., 2015). On the other hand, cortisol is the most abundant glucocorticoid produced in the adrenal cortex's zona fasciculate and plays an essential role in depression development among humans. In experimentally applied stress paradigms, activation of the HPA axis and subsequent corticosterone levels were seen. Physical and psychological features of stressful stimuli may vary (Zhao et al., 2008). Corticosterone reduced BDNF protein (16.6 %) and BDNF mRNA in the CA3 (Cornu Ammonis 3) region of the hippocampus (19%). BDNF mRNA and protein levels in the frontal cortex, on the other hand, remained unchanged. Tissue levels of 5-HT and 5-HIAA were increased and lowered in the frontal brain and hippocampus, respectively (Jacobsen & Mørk, 2006). Long-term stress lowers the hippocampus volume and enhances the depression-like behaviors in mice, but the explanation is still uncertain (Zhang et al., 2015).

2.10 The neuroplasticity hypothesis

The neuroplasticity theory explains how structural changes in hippocampus neurons, such as dendritic shortening and reducing the number and density of spines, reduce hippocampal volume (Boku et al., 2018). Reduced hippocampus volume is one of the most prevalent findings in depressed people and prolonged depression episodes are linked to hippocampal volume changes. Adult hippocampal neurogenesis is thought to be hampered by variations in the amount of somatodendritic, axonal, synaptic and

glial cells. Hippocampal volume was reduced in post-mortem of animal models after stress in severe depression (D'Sa & Duman, 2002). The status of the brain as a static entity processing electrical and chemical information inside a fixed system has long been questioned. Neural circuits, brain nuclei, neurons, and synaptic connections undergo diverse lifetime alterations and adaptations due to environmental stimuli via neural plasticity processes (Serafini, 2012). Chronic stress inhibits the neuroplasticity, which can cause or worsen depression, but antidepressant therapy has the opposite impact and can improve neuroplasticity. Chronic stress affects the central nervous system in many ways, including neuroplasticity in brain regions that malfunction in severe depression (Pittenger & Duman, 2008).

2.11 The brain-derived neurotrophic factor (BDNF)

BDNF plasma levels are lower in persons with bipolar illness, manic depression and depression in several human research (Nay et al., 2021), which may have something to do with the canonical nerve growth factor. Both the brain and the peripheral nervous system produce neurotrophic factors. It is a crucial molecule in learning and memory-related plastic alterations (Yang et al., 2020). BDNF has been related to development, neuronal regeneration, synaptic transmission, synaptic plasticity and neurogenesis in both developing and adult mammalian brains. BDNF plasma levels were found to be lower in persons with bipolar disorder, manic depression, and depression in various human investigations (Yu & Chen, 2011). *In-vivo* animal models of BDNF depletion alter stress-related behaviors and BDNF is a frequent downstream intermediate due to environmental variables that exacerbate anxiety and depressive-like behavior (Notaras & van den Buuse, 2020).

In addition, BDNF is involved in the control of neural networks. Such a neuronal plastic alteration can have a good impact on mood or help people recover from depression. These changes in BDNF levels or neural plasticity in MDD patients can be assessed using blood or plasma BDNF concentrations before and after antidepressant therapy. Thus, BDNF levels can be valuable indications of clinical responsiveness or improvement in depression symptoms. The multistage pattern of BDNF synthesis and maturation allows BDNF isoforms to control activities that occur at various stages of brain development (Kowiański et al., 2018). Brain-derived neurotrophic factor (BDNF) is involved in various neurological processes in both animals and humans throughout development. BDNF is initially required for neurogenesis, neuronal survival, and proper brain growth and maturation. BDNF is critical for long-term memory consolidation as well as synaptic plasticity and dendritic development in adults. BDNF is a primary mediator of brain neuroplasticity (Grande et al., 2010).

2.12 Current antidepressant treatments

Individuals suffering from depression can be treated using a variety of approaches. Unfortunately, no therapy is 100% successful even chemicals and neuronal circuits are being targeted. The most often suggested first-line therapy for depression is drug therapy (Olfson & Marcus, 2009). Selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), norepinephrine reuptake inhibitors (NRI), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) are all antidepressants that target monoamine neurotransmission (Gadad et al., 2018; Marasine et al., 2021). Table 2.1 shows current classes of antidepressants and their drugs.

Table 2.1 Classes of antidepressants and drugs

Class of Antidepressants	Drugs
Selective serotonin reuptake inhibitors (SSRIs)	Citalopram, escitalopram, fluoxetine, paroxetine, sertraline, and fluvoxamine
Serotonin-norepinephrine reuptake inhibitors (SNRIs)	Venlafaxine (IR) and extended-release (XR), duloxetine, desvenlafaxine, milnacipran and levomilnacipran
Norepinephrine reuptake inhibitors (NRIs)	Atomoxetine, reboxetine, and viloxazine
Tricyclic Antidepressants (TCAs)	Amitriptyline, amoxapine, doxepin, desipramine, nortriptyline, protriptyline, imipramine and trimipramine
Monoamine oxidase inhibitors (MAOIs)	Clorgyline, moclobemide, isocarboxazid, phenelzine, selegiline and tranylcypromine

2.12.1 Selective serotonin reuptake inhibitors (SSRIs)

Selective serotonin reuptake inhibitors (SSRIs) are antidepressants that work by raising serotonin levels in the brain. Serotonin is a chemical messenger (neurotransmitter) that aids in communicating nerve cells in the brain (neurons) (Turkin et al., 2021). SSRIs inhibit serotonin from being reabsorbed (reuptake) into neurons by blocking the serotonin transporter (SERT), responsible for serotonin reuptake from the synapse to the presynaptic neuron. It improves neuronal communication by increasing the amount of serotonin accessible. This makes more serotonin available to improve the transmission of messages between neurons. The selective nature of SSRIs is because they only impact serotonin and no other neurotransmitters. SSRIs can be used to treat a variety of illnesses other than depression, such as anxiety disorders. It raises the quantity of serotonin to facilitate the neuronal communication (Khushboo & Sharma, 2017).

2.12.2 Serotonin-norepinephrine reuptake inhibitors (SNRIs)

By inhibiting both the serotonin transporter (SERT) and the norepinephrine transporter (NET), SNRIs prevent serotonin and norepinephrine from being reabsorbed (or reuptake) back into the nerve cells that produced them. This raises the concentrations of active neurotransmitters in the synaptic cleft (Raouf et al., 2017).

2.12.3 Norepinephrine reuptake inhibitors (NRIs)

By inhibiting the norepinephrine transporter, selective noradrenaline (norepinephrine) reuptake inhibitors (NRIs) restrict the absorption of main norepinephrine by presynaptic nerve terminals and enhance norepinephrine availability in the synaptic cleft (Jamkhande & Khawaja, 2016).

2.12.4 Tricyclic antidepressants

TCAs (tricyclic antidepressants) was first offered to the market as a pharmacological treatment for severe depression (Schneider et al., 2019). Tricyclic antidepressants (TCAs) are commonly recommended to treat depressive disorders because of their effectiveness in improving patients' moods, even in children, teens, and pregnant women (Pankajkumar-Patel et al., 2021). Additionally, they act as competitive antagonists on post-synaptic alpha cholinergic (alpha1 and alpha2), muscarinic, and histaminergic receptors (Elhwuegi, 2004; Lovatt, 2011).

2.12.5 Monoamine oxidase inhibitors (MAOIs)

The FDA authorized monoamine oxidase inhibitors (MAOIs) as one of the earliest pharmacological treatments for depression (Chamberlain & Baldwin, 2021). Monoamine oxidase inhibitors are medications that reduce the activity of one or both