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Effects Of *Cordia Myxa* Fruits Extract On Induced Animal Model Of Depression In Male Rats

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Summary

Depression is one of the most common mental illnesses, with significant rates of social function impairment and suicide, and it is the fourth leading cause of disability according to (WHO). Side effects, toxicity, and a delayed onset of action are all key concerns in current depression therapy. Herbal medications, such as *Withania somnifera* (ginseng), *Peony*, and *Epimedium*, have recently gained a lot of interest in the treatment of depression due to their safety, efficacy, and cost-effectiveness, So This study was aimed to evaluate the effects of *Cordia myxa* fruits extract on the model of chronic unpredictable mild stress (CUS) and brain antioxidant enzymes system and proinflammatory cytokines in the brains of male rats due to knowledge provided from previous studies about macrophage theory in the pathogenesis of depression and antioxidant properties of *Cordia myxa* fruit constituents.

Sixty male rats were divided into six groups. Group1 (control) was neither exposed to CUS nor received any treatment while group 2 was exposed to CUS for 24 days and received normal saline without treatment for 14 days, group 3 was exposed to CUS for 24 days and received 10 mg/kg fluoxetine daily on day 10 for 14 days, and group 4, 5 and 6 were exposed to CUS for 24 days and received *C. Myxa* extract (125,250 and 500 mg/kg respectively) on day 10 for 14 days. The antidepressant effect of fluoxetine and *C.Myxa* extract were evaluated by using forced swim test, open field test and a sucrose preference test.

At the end of the experiments, animals were sacrificed by decapitation, and antioxidant enzyme levels catalase (CAT) and superoxide dismutase (SOD) in addition to Interleukin-6 (IL-6) were determined by enzyme linked immunosorbent assays kits (Elisa) in rats brain tissues.

In group 3, group 4, group 5, and group 6 , the means of the immobility time of forced swim test on day 10 significantly increases (P-value < 0.05) as compared with day 0. While, in groups 3, 4, 5, and 6, the means of the immobility time on day 25 significantly decreased (P-value <0.05) as compared with day 10. The mean of the immobility time of group 2 significantly increased (P-*value* <0.05) as compared with

group 1, while in groups 3, 4,5, and 6 the means of the immobility time significantly decreased (*P-value* <0.05) as compared with group 2 on day 25.
Furthermore, in groups 3, 4, 5 and 6, the means of sucrose preference index (SPI) and Open field parameters on day 10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of SPI and open field parameters on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day10. In group 2, the mean of SPI and open field parameters significantly decreased (*P-value* <0.05) as compared with group 1, while in groups 3, 4, 5, and 6 the means of SPI significantly increased (*P-value* <0.05) as compared with group 2 on day 25.

In terms of the Interleukin-6 levels, The means of the IL-6 concentrations in brain tissue of group 2 significantly increased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of the IL-6 concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group 2. Antioxidant enzymes system levels show that in group 2, the means of the SOD & CAT concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group 1, while in groups 4, 5, and 6 the means of the SOD & CAT concentrations in brain tissue significantly increased (*P-value* <0.05) as compared with group 2. In group 3 there are no significant diffirences in the means of the SOD, CAT concentrations in brain tissue as compared with group 2 that (*P-value* > 0.05).

In conclusion, *C. myxa* has an antidepressant activity as found by behavioral tests results and biochemical tests results that reversed the stress induced higher levels of interleukin-6 in rat brain tissues, and increases antioxidant enzymes (SOD and CAT) in rat brain tissues that had been decreased due to chronic stress.

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Abbreviations	Meaning
5-HT	Serotonin
AMPA	3-amino-4-methyl-5-isoxasoe propionic acid
ANOVA	Analysis of Varience
APOE	Apolipoprotien E
ATP	Adenosine Triphosphate
BDNF	Brain Derived Neurotrophic Factor
C. Myxa	Cordia Myxa
CAT	Catalase
CBT	Cognitive Behavioral Therapy
CRH	Corticotropin releasing hormone
CUMS	Chronic Unpredictable Mild Stress
CUS	Chronic Unpredictable Stress
CYP	Cytochrome p
D.W.	Distilled water
DA	Dopamine
DSM-5	The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders
ELISA	Enzyme linked immunosorbent assays
FST	Forced swimming test
GABA	Gamma aminobutyric acid
GPX	Glutathione peroxidase
GR	Glutathione reductase
GRs	Glucocorticoid receptors
GSH	Glutathione

H	Histone
HPA	Hypothalamic-Pitutary_Adrenal
HRP	Horseradish Peroxidase
ICD	International Classification of Diseases
IL	Interleukin
INF	Interferon
LPS	Lipopolysaccharide
LTP	Long term potentiation
MAOIs	Monoamine Oxidase Inhibitors
MDA	Malondialdehyde
MDD	Major Depressive Disorder
MDE	Major depressive episode
MDE	Major Depressive Episode
MRs	Mineralocorticoid receptors
mtROS	Mitochondrial reactive oxygen species
NAC	Neucleus Accumbens
NADH	Nicotineamide Adenine Dineucleotide Hydrogen
NADPH	Nicotineamide Adenine Dineucleotide Phosphate
NASSA	Noradrenergic α2 -receptor antagonist with specific serotonergic receptors-2 and-3 antagonism
NDRIs	Norepinephrine Dopamine reuptake inhibitors
NE	Norepinephrine
NHS	National Health Service
NNT	Nicotinamide Nucleotide Transhydrogenase
NOS	Nitric Oxide Synthases

NRI	Norepinephrine Reuptake Inhibitors
NRISA	Norepinephrine reuptake inhibitor with serotonin receptors antagonism
OFT	Open Field Test
Oxphos	Oxidative Phosphorylation
PBS	Phosphate Buffer Saline
PPD	Post-partum Depression
P-Value	Probability Value
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPM	Round per minute
SAD	Seasonal Affective Depression
SARI	Serotonin receptors antagonist with serotonin reuptake inhibition
SNRI	Serotonin Norepinephrine Reuptake Inhibitors
SOD	Superoxide Dismutase
SPSS v23	Statistical Package of Social Sciences, version 23
SPT	Sucrose preference test
SSRI	Selective serotonin reuptake inhibitors
TCA	Tricyclic antidepressants
TGP	Total Glycoside Fraction of Peony
TNF	Tumer necrosis factor
TST	Tail suspension test
VTA	Ventral tegmental area
WHO	World Health Organization

1.1. Introduction

Depression is a serious and popular mental illness that affects about 350 million people of all ages across the world and it is the fourth major cause of disability according to the World Health Organization (WHO) (Kessler and Bromet, 2013). Major depression is the world's second-leading cause of sickness today. Approximately 1 in every 5 women and 1 in every 8 men will have a significant depressive episode at some point in their lives. (Muñoz *et al.*, 2010)

The annual cost of depression in the United States, including lost productivity and higher medical expenditures, is $83 billion, much surpassing the cost of the Afghan war. Depression costs the UK economy around £11 billion each year in missed earnings, demands on the healthcare system, and medication prescriptions. Depression-related disability costs the Australian economy $14.9 billion per year, while depression treatment costs the Australian society over $600 million per year. (Jesulola *et al.*, 2018)

Sadness, loss of interest or pleasure, feelings of guilt or low self-worth, interrupted sleep or food, weariness, and impaired concentration are all symptoms of depressive disorder. Depression, in its most extreme form, can lead to suicide and an increased risk of death. It is frequently a long-term condition (James *et al.*, 2018). Even in its mildest form, it has a negative impact on people's quality of life and performance. (Fu and Parahoo, 2009)

An episode of major depression is defined by the Diagnostic and Statistical Manual for Mental Disorders (DSM-5) (American Psychological Association, 2013) as two weeks or more of depressed mood, loss of interest or pleasure (anhedonia). The presence of a sad mood (dysphoria) and a loss of interest in previously enjoyable activities (anhedonia) for at least two weeks is the characteristic of major depressive

disorder (MDD). These symptoms must also be accompanied by at least four of the following manifestations such as changes in eating or weight, sleep patterns, changed psychomotor activity, feelings of worthlessness or guilt, trouble focusing or making choices, and recurring thoughts of death or suicide ideation. (Fekadu *et al.*, 2017)

In people with schizophrenia, depression and anxiety symptoms frequently occur. The majority of people with schizophrenia have had a depressive episode, with up to 80% of patients having depression in the early stages of their illness. While depression is common in the chronic stages of schizophrenia, it is thought to be more common during acute exacerbations, with depression rates ranging from 22 percent to 80 percent (Gannon *et al.*, 2019).

Because depression is a diverse illness with complicated phenomena and numerous probable aetiologies, the specific mechanism through which it develops is yet unknown. There are significant variations in the hypothalamic– pituitary –adrenal axis, as well as inadequacy levels of monoamine neurotransmitters, which appear to be caused by genetic, epigenetic, and environmental factors. Monoamine levels are important in the pathophysiology of depression, as seen by lower Dopamine DA levels in patients with significant depression and decreased cerebrospinal fluid monoamine metabolite levels in individuals who tried suicide and displayed depression. Furthermore, In people with depression, activation of the serotonergic system can boost the release of dopamine. So the serotonergic and dopaminergic systems may interact. (Shen *et al.*, 2019)

Antidepressants drugs are divided into several categories, including Tricyclic antidepressants TCAs , Selective serotonin reuptake inhibitors SSRIs and others.

For children and adolescents with depression, selective serotonin reuptake inhibitors are the first-line antidepressants. The Food and Drug Administration (FDA) has authorized fluoxetine for children aged 8 years and up. Escitalopram was authorized for those aged 12 years and up. Fluoxetine is the drug with the most evidence for

usage in children with depression. While sertraline and escitalopram are considered as first-line antidepressants for the initial treatment of major depression in adults. (Pearl and Mullen, 2018)

One of the major concerns of current depression treatments are side effects, increased risk of toxicity and delayed onset of action. So herbal medications have received a lot of attention because of their potential to treat mental illnesses. There has been an increase in their usage in depression treatment because of their effectiveness, safety, and inexpensive cost. (Shen *et al.*, 2019)

Cordia myxa is a species of the genus Cordia that is well-known for its effectiveness in treating lung and urinary infections. The chemical components such as phenolic, alkaloid, and terpenoids determine the efficacy of this plant for use as traditional medicine.

1.2. Aims of The Study

1- To measure the antidepressant effects of the *Cordia myxa* fruits extract in male rats.

2- To define the relationship between the depression and antioxidant enzyme system and inflammatory mediator (Interleukin 6) in the brain of the male rats and to define the effects of *Cordia myxa* fruit extract on them.

1.2. Depression

Depression is a chronic mental illness that affects one's emotions, thoughts, behavior, and physical well-being. It is a common but dangerous illness that may steal a person of their ability to enjoy life and cause a deterioration in their ability to do even the most basic daily chores. Depression is characterized by a variety of emotional symptoms, such as bouts of persistently low mood, a lack of interest in any activity, anhedonia, poor self-esteem, and cognitive impairment. In addition, several physical symptoms such as sleeplessness, restlessness, anorexia, and libido loss are common in patients. Recurrence of symptoms is also a typical sign of major depressive illness.(Pearl and Mullen, 2018)

Depression affects 12% of the world's population at some point in their lives. It is considered as one of the most prominent variables among neuropsychiatric disorders, which account for 11% of Disability Adjusted Life Years. Suicides linked to depression account for up to 16 per 100,000 deaths. When depressed individuals are given current conventional medication therapy, which primarily targets 5-HT receptors, only around two-thirds of them achieve remission. (Bajpai *et al.*, 2014)

1.2.1. Epidemiology

In recent years, the global incidence of depression and depressive symptoms has risen. Women's lifetime depression prevalence varies from 20% to 25%, whereas men's lifetime depression prevalence ranges from 7% to 12%. Depression is a major predictor of quality of life and survival, accounting for almost 50 percent of all psychiatric consultations and 12 percent of all hospital admissions. According to the World Mental Health Survey, 1 in 17 individuals experienced at least one episode of depression in 2016 year. Depression often starts at adolescent or young adult age, and it generally affects women more than men, with women of childbearing age worst

affected due to 10 – 20 % of women experiencing postpartum depression. (Jesulola *et al* ,. 2018)

Chronic medical diseases have the highest frequency of depression development and the percentage of occurrence of depression with chronic diseases can be summarized as follow: Asthma (27%), atopic dermatitis (5%), chronic obstructive pulmonary disease (24.6%), gouty arthritis (20%), rheumatoid arthritis (15%), systemic lupus erythematosus (22%) and stroke (30%) (Wang *et al.*, 2017). MDD is one of the most common mental illnesses, affecting 121 million individuals globally, with lifetime prevalence estimates ranging from 7% to 21% (De Zwart, Jeronimus, and De Jonge, 2019).

According to statistics made by the national health service NHS Business Services Authority about Covid-19 pandemic, Between October and December 2020, 20.5 million antidepressant medications were administered. In the previous quarter, there were 19.6 million things. In the same quarter in 2019/20, there were 19.3 million items. In the third quarter of 2020, 23 percent more patients received an antidepressant item.

1.2.2. Causes and risk factors of depression

Biological factors such as genetics and brain chemistry as well as certain medical disorders, stress, malnutrition, and abuse. A variety of genes have been linked to the onset of depression. Psychological or physical adversity might lead to depression in later life. Female sex, social isolation, the death of a loved one, being divorced or separated, unwanted pregnancy, poorer socioeconomic position, concomitant general medical problems, uncontrolled pain, sleeplessness, and cognitive or functional impairments are all risk factors for depression. (Maurer *et al.*, 2018)

Weak ties to family and friends, a lack of hope for the future, and diminished fundamental values such as love were the most significant factors contributing to depression. (Sapozhnikov, 2019)

A variety of endocrine system disorders have been recognized as potential factors to the genesis of depression. Changes in Growth hormone levels, anomalies in Thyroid hormone secretion and function, and dysfunctions in the Hypothalamus-Pituitary-Adrenal (HPA) axis are just a few examples. Various studies connecting chronic stress (a significant component in depression pathophysiology) with altered immune function have led to the hypothesis that cytokines may play a role in the development of depression. Several clinical conditions (e.g., Systemic Lupus Erythematosus, Traumatic Brain Injury, and Multiple Sclerosis) that cause significant neuroinflammation in the brain have been linked to a high prevalence rate of major depression, demonstrating the link between brain neuroinflammation and major depression. (Jesulola *et al.*, 2018)

1.2.3. DSM-V Classification of depression

There are many types of depressive disorders. Some forms of depression, such as dysthymic disorder and major depressive disorder (MDD), are thought to be affected by hereditary and biochemical factors, whereas others, such as mild depression and severe depressive episodes, are thought to be reactions to important life events. (Jesulola *et al.*, 2018)

1.2.3.1. Minor Depressive Disorder

This form of depressive illness is characterized by dysphoric mood and anhedonia, as well as physical changes such as weight loss or gain, increased or reduced hunger, sleep disturbances, and persistent tiredness. Lack of focus and clear thinking, as well

as a morbid preoccupation with ideas of death and suicide, are all signs of cognitive and executive dysfunction. (Benazzi, 2006)

1.2.3.2. Dysthymic Disorder

Persistent depressive disorder is another term for dysthymic disorder. Patients have a low mood or sorrow that lasts for the most time of the day in adults for at least two years and at least one year in children and adolescents. (Fekadu *et al.*, 2017)

1.2.3.3. Melancholic Depression

There is a near-complete lack of ability to enjoy pleasure. Early morning mood swings and worsening are more typical among the elderly, particularly in those suffering from more severe forms of depression and psychotic depression. (Benazzi, 2006)

1.2.3.4. Seasonal Affective Disorder (SAD)

Seasonal affective disorder (SAD) is a kind of depression that occurs in early winter. Low mood, feelings of shame and worthlessness, and increased irritability are all symptoms that are common to other depressive illnesses. Additionally, patients have an increase in appetite and a desire for high-carbohydrate meals, resulting in weight gain. (Fekadu *et al*., 2017)

1.2.3.5. Post-partum Depression (PPD)

Mothers are affected by this kind. The likelihood of a postpartum major depressive episode is increased by mood swings and anxiety symptoms during pregnancy, as well as the "baby blues". (Shibeshi *et al*., 2017)

1.2.3.6. Psychotic Depression

Psychotic depression is generally thought of as a mix of psychosis and depression that doesn't fit well into either category. Psychotic symptoms such as hallucinations or delusions are common. Psychotic depression is linked to a longer course, a poor response to existing medications, and a greater relapse rate, in addition to its severity. (Swartz C, 2007)

1.2.3.7. Major Depressive Disorder

The most researched kind of depression is defined as one or more major depressive episodes characterized by depressed mood or loss of interest (or both) for at least two weeks, with four associated depression symptoms (or three if both depressed mood and loss of interest are present) . Changes in appetite, weight, sleep, and psychomotor activity; decreased energy; feelings of worthlessness or guilt; difficulty thinking, concentrating, or making decisions; or recurrent thoughts of death or suicidal ideation, plans, or attempts are all symptoms that can appear in varying degrees of severity. It was differ from minor depressive disorder in the number of symptoms present. (Jesulola *et al.*, 2018)

1.2.4. Pathogenesis of Depression

In individuals with severe depressive illness, changes in numerous biological systems, including the neuroendocrine and immunological systems, have been repeatedly documented. As a result, various ideas concerning the etiology of this psychiatric illness have been proposed. (Baguley *et al.*, 2018)

1.2.4.1. Neurotrophic Hypothesis

Low levels of neurotrophic factors, such as Brain-derived neurotrophic factor BDNF, enhance stress sensitivity through their effects on nerve cells inside forebrain regions, according to the neurotrophic hypothesis of depression and antidepressant

treatments (Belardi and Lucertini, 2003). BDNF has attracted significant interest. Specifically, Preclinical research has found there are links between stress-induced depressive-like behaviors and decreased hippocampus BDNF levels, as well as increased BDNF expression after antidepressant therapy (Hasler, 2010).

1.2.4.2. Monoamine Hypothesis

Decrease in the quantity or function of cortical and limbic biogenic monoamines, such as serotonin (5HT), norepinephrine (NE), and/or dopamine, are linked to depression, The monoamine oxidases in the synaptic cleft degrade these neurotransmitters, resulting in their functional deficit. This hypothesis underpins the use of monoamine oxidase inhibitors as antidepressant medicines in the treatment of depression, intending to restore low monoamine neurotransmitter levels. (Ravichandran *et al.*, 2017)

Other explanations for neurotransmitter deficit can be found. Some neurotransmitters, including as glutamate, NE, and histamine, may interact to reduce the synthesis of Serotonin neurotransmitters as a result of the stress response produced by depression. Serotonin has been researched the most in depression. Studies employing tryptophan depletion, which lowers central serotonin production, provide the most direct evidence for abnormally decreased central serotonergic system function. In people who are predisposed to depression, such a decline causes them to acquire depressive symptoms (Neumeister A, Nugent AC, 2004) . So, in general, antidepressants appear to increase 5HT, norepinephrine, and/or dopamine synaptic availability.

1.2.4.3. Glutamatergic Hypothesis

A glutamatergic excitatory neurotransmitter system appears to have a role in the pathophysiology of depression. Several investigations have discovered higher glutamate concentrations in plasma and glutamine concentrations in the cerebral fluid of MDD patients. Furthermore, Serum and plasma glutamate concentrations, as well

as glutamine concentrations in the cerebrospinal fluid, have all been reported to be reduced by antidepressant medication therapy. Chronic antidepressant usage is linked to a decrease in glutamatergic neurotransmission mechanisms, such as presynaptic glutamate release in the hippocampus and cortical regions. Similarly, In experimental animal models, prolonged antidepressant treatment dramatically decreases depolarization-evoked glutamate release. (Ravichandran *et al.*, 2017)

1.2.4.4. Hypothalamic–Pituitary–Adrenal axis changes

The hypothalamic-pituitary-adrenal (HPA) axis (Figure 1.1) does not appear to be dysfunctional in most MDD patients (Hasler, 2010). Corticotropin-Releasing Hormone (CRH) appears to have a substantial role in the etiology of certain forms of depression, according to a growing body of research. Increased levels of CRH in the cerebrospinal fluid appear in some depressed subjects (Zunszain *et al.*, 2012)

The HPA axis regulates glucocorticoid production by stimulating forward and feedback inhibition loops involving the brain, pituitary, and adrenal glands. Cortisol is a hormone produced by the adrenal glands that bind strongly to mineralocorticoid receptors (MRs) in the brain and has a reduced affinity for glucocorticoid receptors (GRs). GR is found throughout the primate brain, whereas MR is mostly found in the hippocampus. Additionally, Glucocorticoid-responsive elements of numerous genes in the brain are also found in the regulatory regions. Cortisol's tonic effects are predominantly mediated by hippocampus MR, whereas feedback actions are handled by the pituitary, and activated brain areas like the amygdala are aided by GR. A dysregulation of MR and/or GR within the HPA system has been proposed as a cause of major depression. (Keller *et al.*, 2017)

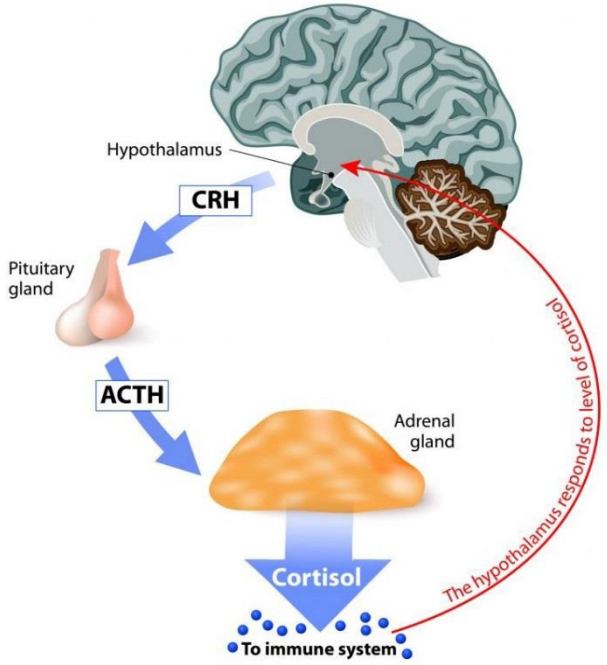


Figure 1.1. Explanation of CRH and ACTH production & target sites of action. (Guilliams and Edwards, 2010)

1.2.4.5. Immune system and inflammation (depression macrophage theory)

Changes in the peripheral immune system, as well as the activation of proinflammatory cytokines, as a result, have long been linked to mood disorders, leading to the development of the depression macrophage theory. Persistent stimulation of the peripheral immune system caused by cancer, systemic infection, or autoimmune illness, on the other hand, may encourage the development of significant depression in those who are exposed. Disorders of leukocyte function and/or the

number of leukocytes, as well as increased cytokine expression, have been proposed as potential biomarkers of depression. (Ménard, Hodes and Russo, 2016)

1.2.4.6 Structural and functional brain changes

Anomalies in brain areas responsible for mood modulation, reward response, and executive processes have been discovered through various structural and functional studies. Reduced volumes of grey matter and glial density in the prefrontal cortex and hippocampus, areas that drew the most focus in animal research on depression, were reported in post-mortem and neuroimaging investigations. Hypercortisolemia in depressive patients could be caused by a decrease in hippocampus activity, which is hypothesized to have an inhibitory influence on the hypothalamic-pituitary-adrenal (HPA) axis. The mesolimbic dopamine system, which includes the nucleus accumbens (NAC) and the ventral tegmental area (VTA) (Figure 1.2), is also thought to have a role in the pathophysiology of depression. (Fekadu *et al.*, 2017)

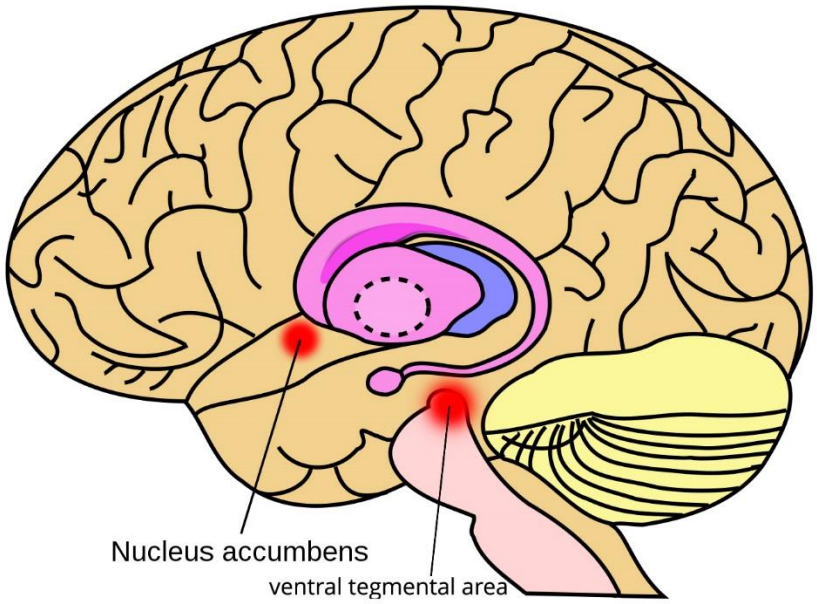


Figure 1.2. the location of Nucleus accumbens (NAC) & Ventral tegmental area (VTA) within Brain

1.2.4.7. Environmental factors

Depression risk varies by gender, age, occupation, and education, and the incidence and prevalence of depression exhibit varied patterns and trends among countries. Different physical conditions, such as cancer and cardiovascular diseases, have a high level of comorbidity. Poor social behaviors, such as economic instability and unemployment, have an impact on the developing brain, and they raise the likelihood of depression. Pollutants in the environment, which are all around us, are changeable and can alter human neurobiology. Air pollution, noise, heavy metals, poisonous compounds, and environmental radiation are examples of harmful situations. The impact of the environment on depression pathophysiology is a complicated web of interactions between many stimuli, each of which influences different brain structures and functions independently or interdependently. (van den Bosch and Meyer-Lindenberg, 2019).

1.2.4.8. Genetic factors

Although several genes have been linked to the development of depression, just a few genetic variants have been linked to MDD. Apolipoprotein E (APOE 2 and APOE 4), guanine nucleotide-binding protein (GND3), methylenetetrahydrofolate reductase (MTHFR 677T), dopamine transporter (SLC6A3), serotonin transporter (SLC6A4), and dopamine receptor gene are some of these genes (DRD 4). (Jesulola *et al.*, 2018)

1.2.4.9. Oxidants and Antioxidant system

Reactive oxygen and nitrogen species (ROS/RNS) are now widely acknowledged to have critical roles in a variety of biological processes ranging from bacterial growth to mammalian brain function (Cortese-Krott *et al.*, 2017).

ROS and RNS, such as superoxide (O2 -), hydrogen peroxide (H2O2), and nitric oxide (NO), are actively produced by mammalian cells and govern a wide range of

physiological functions, including metabolism, gene transcription/translation, motility, division, and differentiation. (Helmut Sies, Carsten Berndt, 2017).

The actions of endogenous antioxidant systems counterbalance the effects of ROS/RNS. The balance between oxidants and antioxidants is regarded to be critical for cellular function, fitness, and viability, and its disruption has been linked to diabetes, atherosclerosis, cancer, and neurodegenerative illnesses. Stress stimuli frequently affect the cell's redox processes, resulting in elevated oxidant levels, which can influence the cellular response to these stress assaults. (Benhar, 2020)

ROS and RNS affect cellular responses by a variety of methods, one of which is posttranslational protein function modulation, which is regarded to be a significant redox control mechanism in physiological and pathological processes.

Cysteine thiols undergo a range of reversible covalent changes in response to oxidative or nitrosative cues. RNS, such as NO, promote protein thiol nitrosylation (R–SNO), while certain ROS (such as H2O2) cause thiol sulfenylation (R–SOH). Further reactions of these oxidized thiol groups can result in thiol glutathionylation (R–SSG) or the formation of additional disulfide species (R–SSR). (Janssen-Heininger *et al.*, 2008)(Go, Chandler, 2015).

In mammalian cells, the antioxidant proteins thioredoxin (Trx), tripeptide glutathione (GSH), and their system components are among the most important defenses against ROS and RNS (Arnér and Holmgren, 2000). In addition, Trx and GSH also play a key role in reversing other thiol alterations, such as R–SNO, R–SSG, and R–SSH. (Benhar *et al.*, 2016)

1.2.5. DSM-V Criteria For Diagnosis of depression

The Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases (ICD) are the two most widely used diagnostic

classification systems (ICD). A Major Depressive Episode (MDE) is diagnosed when five or more symptoms occur during two weeks, according to the (DSM-5). A low mood or anhedonia, at the very least, would be one sign (loss of interest or enjoyment). Changes in appetite or weight, sleep difficulties (insomnia or hypersomnia), psychomotor discomfort or retardation, exhaustion or energy loss, decreased capacity to think or focus, feelings of worthlessness or excessive guilt, and suicidality are some of the secondary symptoms of MDD. All or none (0 or 1) are used to rate these symptoms. (Tolentino and Schmidt, 2018)

Symptoms of depression are known in common fashion by the mnemonic SIGECAPS: Sleep disturbance; Interest deficit (anhedonia); guilt (worthlessness, hopelessness, remorse); energy deficit; concentration deficit; appetite problem (either diminished or increased); psychomotor retardation or agitation; and suicidality. The two cardinal symptoms of depression are a depressed mood and anhedonia. When four SIGECAPS symptoms are present, as well as a low mood or anhedonia, depression is likely present, and further testing should be performed. (Maurer, Raymond and Davis, 2018)

1.2.6. Treatment of depression

For major depressive disorder, a variety of effective therapies are available. Depressive symptoms can be relieved by medication and brief psychotherapy (e.g., cognitive-behavioral therapy, interpersonal therapy). There is also evidence that brief psychotherapy (CBT) can help people avoid relapse. However, medication alone is insufficient treatment for children and adolescents. Furthermore, in all patient populations, a combination of medicine and psychotherapy produces the most rapid and long-lasting results. (Pearl and Mullen, 2018)

1.2.6.1. Psychotherapy

Despite significant advancements in major depression treatment and management measures, only around half of the patients respond to first-line antidepressant treatment or psychotherapy. As a result, psychological therapies can be used as a stand-alone or supplementary therapy for depressive disorders. For the treatment of various psychiatric illnesses, there are also a variety of psychological treatments. Psychological treatment is divided into four distinct cognitive and theoretical programs: psychoanalytic/dynamic, therapeutic, humanistic, and cognitive approaches. Therapists and patients collaborate in cognitive-behavioral therapy (CBT) to understand the relationship between emotions, feelings, and actions, as well as to recognize and change unhelpful thinking patterns, underlying beliefs, and idiosyncratic cognitive schemes about self, others, and the environment. (Van Bronswijk *et al.*, 2019)

1.2.6.2. Pharmacotherapy

For moderate-to-severe depression or depression that has not responded to a sufficient trial of psychotherapy, antidepressant drugs may be considered first-line treatment. Medications should not be used alone to treat depressive children, but rather in conjunction with psychotherapy. Both therapy modalities are used in clinical practice during an acute-care hospitalization, especially if the admission is for suicidal thoughts or attempts. (Pearl and Mullen, 2018)

1.2.6.3. Classes of Clinically Available Antidepressants (Trevor, 2015)

1. Tricyclic Antidepressants TCAs:

Tricyclic medicines have an immediate impact of inhibiting the reuptake mechanisms (transporters) responsible for the termination of NE and 5-HT synaptic activities in the

brain. This presumably causes their neurotransmitter effects to be potentiated at postsynaptic receptors.

2. Selective serotonin reuptake inhibitors SSRIs:

SSRIs have an immediate impact on the serotonin transporter that is extremely selective (SERT). SSRIs block the transporter allosterically, binding at a location different than serotonin's. They have minor inhibitory or blocking effects on the NE transporter and adrenergic and cholinergic receptors.

3. Serotonin norepinephrine reuptake inhibitors SNRIs:

SNRIs bind to both serotonin and NE transporters, increasing the effects of both neurotransmitters. Venlafaxine, unlike desvenlafaxine or duloxetine, has a lower affinity for the NE transporter. Unlike TCAs, SNRIs have no significant blocking effects on peripheral receptors such as histamine H1, muscarinic, or -adrenergic receptors.

4. Serotonin 5-HT2 Receptor Antagonists:

Blocking the 5-HT2A receptor, a G protein-coupled receptor found in multiple CNS areas including the neocortex, appears to be the main antidepressant activity of nefazodone and trazodone. The antianxiety and antidepressant effects of these medicines are linked to antagonistic activity of this receptor.

5. Other heterocyclic antidepressants:

Mirtazapine works by antagonizing presynaptic alpha2 adrenoceptors, which are involved in feedback inhibition, to enhance amine release from nerve terminals. The medication is also a serotonin 5-HT2 receptor antagonist. Bupropion's antidepressant mechanism is uncertain; the medication has no impact on 5-HT or NE receptors, nor on amine transporters.

6. Monoamine oxidase inhibitors MAOIs:

MAOIs raise brain amine levels by interfering with their metabolism in nerve terminals, resulting in an increase in NE and 5-HT vesicular storage. Increased levels of amines are produced when neural activity discharges the vesicles, probably boosting the activities of these neurotransmitters.

1.2.6.4. General Pharmacological Side Effects of Antidepressants

The presence of side effects are not always disadvantageous. Sedation, for example, is beneficial for patients who have insomnia as a symptom of their condition, and dry mouth with amitriptyline or imipramine might be a sign of an adequate dose. Some adverse effects, on the other hand, may result in complications or force the patient to stop taking the medication.

1. Dry mouth, constipation, urinary retention, impaired vision, cognitive impairment, exacerbated tardive dyskinesia, and tachycardia are among antimuscarinic adverse effects.
2. Sedation, cognitive impairment, and falls are all side effects of blocking histamine H-1. Central H1-blockade causes sensory impairment and poor sensory processing, notably in schizophrenia individuals. (Van Ruitenbeek *et al.*, 2009).
3. Blockade of noradrenaline alpha-1 Ejaculatory issues, postural hypotension
4. Nausea, diarrhea, decreased appetite, akathisia, anorgasmia, and serotonin syndrome are among symptoms of 5-HT reuptake inhibition.
5. 5-HT receptor blockade causes weight gain.
6. Stabilization of the membrane through blockage of voltage-gated sodium channels produce Asystole and cardiac dysrhythmia.
7. Fine tremor, sweating, myoclonus, incorrect ADH, epilepsy, and rapid-cycling bipolar illness are some of the more complex/unknown adverse effects. (Ramic *et al.*, 2020)

1.2.6.5. Herbal Antidepressants

The psychopharmacology of herbs has gotten a lot of attention in recent decades. A vast body of evidence suggests that herbs' complex psychotropic effects may aid in the treatment of depression. Many people are increasingly resorting to herbal therapy in the search for multi-target antidepressants with minimal toxicity. (Liu *et al.*, 2015)

Many of the psychotropic herbal medications such as Valerian (Valera officialis) accessible as "over-the-counter" psychiatric drugs are relatively safe and have less adverse effects than traditional pharmacotherapies like antidepressants (cholinergic symptoms, sexual dysfunction, insomnia, and withdrawal concerns) and benzodiazepines (somnolence, dependence, and withdrawal issues). Specific plant metabolites attach to neurotransmitter/neuromodulator receptors and affect neurotransmitter synthesis and general function are the primary mechanisms of action for herbal medications used to treat mental illnesses. In addition, the CNS may be stimulated or sedated, and the endocrine system may be regulated or supported in its healthy operation. (Sarris *et al.*, 2011)

Due to the monoamine theory of depression, several antidepressant medicines including herbal medicine have been developed to increase these neurotransmitters by inhibiting their reuptake in the brain. Meanwhile, The antidepressant properties of certain herbal medications are due to the stimulation of serotonin receptors or the inhibition of monoamine oxidases

For decades, traditional Chinese medicine has relied heavily on ginseng (Withania Somniferous) to enhance mood and keep people healthy in the West. Antidepressant properties of the ginseng was related to component 20(S)-protopanaxadiol. (Liu *et al.*, 2015)

Depressive disorders are treated with Peony, the processed roots of Paeonia lactiflora Pall (Ranunculaceaceae). Peony is an essential component in numerous Chinese medicinal formulations. (Mao *et al.*, 2012).

Peony's total glycoside fraction TGP has been shown in studies to alleviate depressive symptoms induced by persistent unexpected stress, and TGP's antidepressant-like action is thought to be mediated through monoamine oxidase inhibition and oxidative stress reduction in the mouse brain. (Mao *et al.*, 2009)

Icariin, a flavonoid found in Epimedium (a type of herb), significantly reduces the corticosterone rise generated by social defeat stress, implying that icariin's antidepressant action is mediated in part by restoring the normal expression and function of GR. (Wu *et al.*, 2011)

Perilla frutescens, a traditional Chinese plant, has been used to treat a variety of psychiatric diseases, including depression, for hundreds of years. In recent studies, the essential oil of Perilla frutescens (EOPF) was found to alleviate depressive symptoms in mouse models. (Yi *et al.*, 2013)

Radix Polygalae is made out of the dried root of Polygala tenuifolia, which has been used as a traditional herbal treatment in East Asian countries. In the forced swimming paradigm of rats and the tail suspension test, the extract of Radix Polygalae revealed antidepressant properties. Radix Polygalae extract has been shown to have fast-acting antidepressant action by modulating the AMPA receptor in the hippocampus. (Shin *et al.*, 2014)

1.2.7. Fluoxetine

Chemical formula : $C_{17}H_{18}F_3NO$
Molecular weight : 309.33 g/mol

Fluoxetine is approved by the FDA for major depressive disorder (age 8 years and older), obsessive-compulsive disorder (age 7 years and older), panic disorder, bulimia, binge eating disorder, premenstrual dysphoric disorder, bipolar depression (as an adjunct with olanzapine), and depression resistant to treatments when used in combination with olanzapine. (Sl *et al.*, 2019) (Dhenain and Francine, 2019)

Social anxiety disorder (social phobia), post-traumatic stress disorder in adults, borderline personality disorder, Raynaud phenomenon, and selective mutism are all non-FDA-approved applications for fluoxetine.(Li *et al.*, 2019)

Fluoxetine is a racemic SSRI, with the R(-) and S(+) enantiomers , with comparable efficacy as inhibitors of 5-hydroxytryptamine (5-HT) reabsorption in both in vitro and in vivo uptake tests. Furthermore, fluoxetine is converted to norfluoxetine, an active metabolite, by N-demethylation. Norfluoxetine works as an SSRI as well, although it has a higher potency than the parent molecule. This active metabolite is also available in an enantiomeric form, however unlike the fluoxetine enantiomers, S-norfluoxetine is almost 20 times more effective than the (R)-enantiomer at inhibiting 5-HT reuptake. (Sacre *et al.*, 2010)

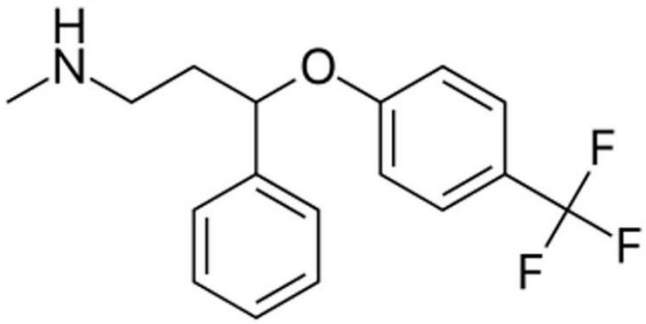


Figure 1.3. Two dimentional structure of fluoxetine (Makkonen *et al.*, 2011)

1.2.7.1. Mechanism of action

Fluoxetine works by inhibiting serotonin reuptake into presynaptic serotonin neurons by inhibiting the reuptake transporter protein in the presynaptic terminal. Desmethylfluoxetine, fluoxetine's primary metabolite, is likewise a powerful serotonin reuptake inhibitor, which could explain fluoxetine's lengthy duration of action.

Fluoxetine has a minor effect on the 5-HT2A and 5-HT2C receptors. Fluoxetine has only a minor effect on noradrenergic reuptake. Fluoxetine has an activating impact due to its serotonin reuptake blocking activity, and the initial antidepressant effect appears within 2 to 4 weeks due to its lengthy half-life (2 to 4 days). (Robertson and Dodd, 2019).

1.2.7.2. Pharmacokinetics

Fluoxetine was efficiently absorbed and reached maximal plasma concentrations after 6 to 8 hours following oral administration of single doses to healthy fasting participants. During multiple-dose administration, plasma concentrations rise. After 2 to 4 weeks of administration, steady-state plasma concentrations are achieved, and

they stay proportionate to the dose even after long-term treatment. In healthy subjects, repeated dosages resulted in lower plasma clearance and a longer half-life than a single dose. The elimination half-life of the parent medication, fluoxetine, is considerable, averaging 1-3 days after acute treatment and 4-6 days following chronic administration.

About 94 percent of fluoxetine in the blood bound to proteins. Fluoxetine subject to metabolism; CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 convert fluoxetine to norfluoxetine. CYP2D6, CYP2C9, and CYP3A4 appear to be the primary contributing enzymes for phase I metabolism, despite the fact that all of the enzymes mentioned contribute to N-demethylation of fluoxetine. To aid excretion, both fluoxetine and norfluoxetine are glucuronidated. Because both the parent medication and its active metabolite block the CYP2D6 isozymes, individuals who are taking fluoxetine are at risk for drug interactions.

Over the course of 5 weeks, 60 percent of a single dose of radioactive fluoxetine was retrieved in the urine of healthy participants, with only 2.5 percent of the medication remaining unchanged. Fluoxetine has a half-life of approximately 4 days. (Lancaster and Gonzalez, 1989;Liu *et al.*, 2002;Of *et al.*, 2017)

1.2.7.3. Adverse effects

Adults more frequently report these common side effects after fluoxetine trial; insomnia, nausea, diarrhea, anorexia, dry mouth, headache, drowsiness, anxiety, nervousness, yawning, decreased libido, decreased arousal (seen as decreased lubrication in women and decreased erectile function in men). bruising, bleeding (rarely), hyperhidrosis, induction of mania, Increased or decreased weight, decreased orgasm (anorgasmia and delayed ejaculation) reported frequently. weakness of the muscles, tremors, and multiple inflammations of the pharynx. There is also rare activation of suicidal ideation and behavior, especially in children or teenagers. (Pearl and Mullen, 2018;Bahar *et al.*, 2018)

1.2.8. *Cordia myxa*

Boraginaceae (Borage) is a plant family with around 2740 species divided into 148 genera. One of the most well-known members of this family is the genus Cordia. Cordia is a tree or shrub genus that is sometimes classified as a subscandent of the borage family. Around 300 species have been discovered around the world. (Samy *et al.*, 2017)

Picture 1.1. Cordia Myxa Tree, Leaves, and Fruits.

C. myxa, sometimes known as "Bumber" is a species of the genus Cordia that is well-known for its effectiveness in treating lung and urinary infections (Picture 1.1). It is also used as a hepatoprotective and has anthelmintic, diuretic, demulcent, antidiarrheal, antigastritic, and antiworm effects. Several Cordia species formulations have been used in traditional medicine to treat osteoarticular disorders. C. francisci, C. martinicensis, C. myxa, C. serratifolia, and C. ulmifolia have been examined in rats for their analgesic, anti-inflammatory, and antiarthritic properties. (Afzal *et al.*, 2009)
Because it contains demulcent effects, it was used to suppress coughs and treat respiratory infections and sore throats. The leaves were used to treat wounds and ulcers in Mali and Côte d'Ivoire. To cure trypanosomiasis, a macerate of the leaves was taken. In cases of fractured bones, powdered bark is rubbed to the skin before a

plaster is applied to aid healing. Bark powder was applied to the skin to heal skin ailments. Colic was treated with a mixture of bark juice and coconut oil. (Al-Snafi, 2016)

Cordia Myxa decoction mixture was recommended for relieving Influenza in Unani medicine and other COVID-19 like epidemics. (El-Massry *et al.*, 2021)

1.2.8.1. Classification of Cordia Myxa (Al-Ati, 2011)

Kingdom: Plantae , Subkingdom
Tracheobionta (vascular plants)
Superdivision: Spermatophyta (seed plants)
Division: Magnoliophyta (flowering plants)
Class: Magnoliopsida (dicotyledons)
Subclass: Asteridea
Order: Lamiales
Family: Boraginaceae (borage family)
Genus: Cordia L. (cordial)
Species: Cordia myxa L. (Assyrian plum)

1.2.8.2. Constituents

Cordia myxa includes about 6.21 percent water, 12.75 percent glucose, 9.38 percent fructose, 29.09 percent sucrose, and 5.86 percent starch (which is surprisingly low for a fruit), 248 mg per g phytic acid, and 1.39 units per 1 g trypsin inhibitor, according to compositional study. It also has 25.7 grams per 100 grams of total dietary fiber (which is rather high compared to other fruits) and 4.5 percent pectin. The fruit also has 6.7 percent ash, 1.62 milligrams per gram of sodium, 7.83 milligrams per gram of potassium, 0.46 milligrams per gram of calcium, 0.51 milligrams per gram of iron, and 0.35 milligrams per gram of zinc. (Aberoumand and Deokule, 2010) (Aberoumand and Deokule, 2009)

Fruits with improved sensory quality, higher drying ratios, and higher amounts of total soluble solids, ascorbic acid, protein, and carbs were obtained when harvested at 45 days and blanched for 3 minutes with 0.3 percent potassium metabisulphite (KMS). **(**Fageria M., 2003**)**

The chemical components such as phenolic, alkaloid, and terpenoids determine the efficacy of this plant for use as traditional medicine. Flavonoids have been reported to have anti-inflammatory, antibacterial, antiviral, anti-allergic, anticancer, neurodegenerative, and vasodilatory properties. (Artanti N *et al*,. 2006)

Through its antioxidant activity, free radical scavenging, and cation divalent chelators, the flavonoid is also known to decrease lipid-peroxidase, platelet aggregation, capillary blood vessel permeability, and enzyme cyclooxigenase and lipoxygenase activity. Furthermore, various hydrolase enzymes, such as hyaluronidase, alkali phosphatase, arylsulphatase, cAMP-fosfodiesterase, lipase, kinase, and -glucosidase, have been observed to be inhibited by it. (Geethangili and Ding, 2018)

1.3. Oxidants and Antioxidant system

1.3.1. Role of NADH and NADPH Systems

The NADH system is responsible for catabolism and energy capture, whereas the NADPH system is responsible for enzymatically regulated oxidative processes such as NADPH oxidases, nitric oxide synthases (NOS), cytochrome P-450-dependent hydroxylations, and so on. Because of the strong cytosolic NADH binding sites, cytosolic NADH has a significantly higher positive redox potential than mitochondrial NADH, with the set point of free NADH buffered to 1 M (i.e., pNADH = 6). The enzyme energy-linked nicotinamide nucleotide transhydrogenase (NNT) links NADH and NADPH in the mitochondria, using the proton motive force to transfer electrons from NADH to NADP+, therefore supporting NADPH-related antioxidant defense. Because this process is reversible, NADPH is used to sustain NADH and ATP synthesis under pathological metabolic load, weakening antioxidant defense. Oxidative stress is caused by reversed mode through NNT (Figure 1.4). (Helmut Sies, Carsten Berndt, 2017)

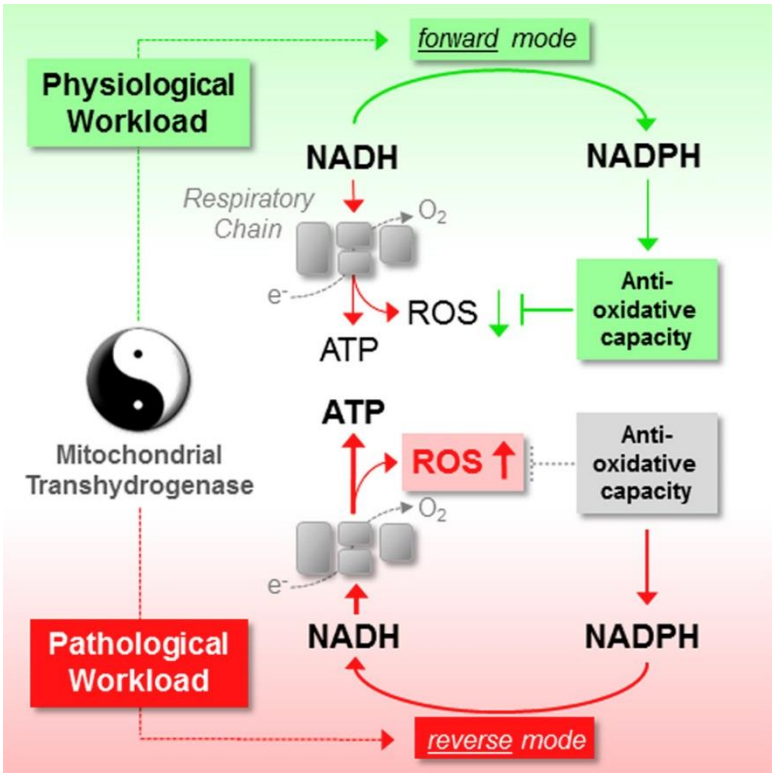
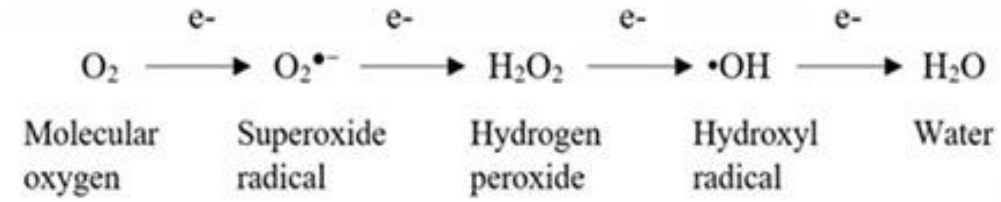


Figure 1.4. Reversed mode and forward mode of NADH and NADPH during workload. (Bertero and Maack, 2018)

1.3.2. Oxidative Stress

Reactive Oxygen Species ROS is created in numerous cellular compartments inside a cell, including the cytosol, peroxisomes, and endoplasmic reticulum; however, the mitochondria generate and compartmentalize over 90% of the ROS. Mitochondria create reactive oxygen species (mtROS) as a consequence of ATP synthesis via oxidative phosphorylation (oxphos). The electron transport chain complexes I and III can indeed produce superoxide anion, which is quickly dismutated to H2O2 by mitochondrial SOD2. As a result, ROS was previously thought to be generated inadvertently in cells as a detrimental by-product of aerobic metabolism. However, ROS have been extensively studied as mediators of both physiological and pathological signals during the last decade. The Fenton reaction produces hydroxyl radical (•OH), the most potent and damaging oxidant, as a result of mitochondrial damage and oxidative stress. mtROS has been linked to a variety of neurological diseases. (Tarafdar and Pula, 2018)



1.3.3. The link between oxidative stress and depression development

The pathophysiology of depression may be influenced by oxidative stress. Inflammation, autoimmune tissue damage, and persistent psychological stress all contribute to oxidative stress, which is a significant cause of severe depression (Bajpai *et al.*, 2014). Oxidative stress has become a prominent cause of depressive illness in the last decade. The etiology and progression of MDD have been linked to increased ROS and amplified expression of genes regulated by OS (Mellon *et al.*, 2016).

Because it is a significant consumer of oxygen and contains oxidizable lipids, the brain is more vulnerable to harm from elevated ROS than any other organ in the body. Deregulation of brain processes and anomalies in neuronal signaling result from an imbalance between ROS and antioxidative defenses. Peroxidation of phospholipids in the brain is caused by reactive oxygen species (ROS). Increased lipid peroxidation in the brain is a key event in the development of MDD. The measurement of malondialdehyde (MDA), which is product of lipid peroxidation, in the brain offers information on OS's role in depression. Antioxidant enzyme activity, including as catalase (CAT) and superoxide dismutase (SOD), as well as reduced glutathione (GSH), are also used to determine the role of oxidative stress in depression. (Bhatt *et al* ,. 2020)

1.3.4. Markers of Oxidative Stress in Depression Disorder

Long-term psychological stress is a risk factor for severe depression, since it raises MDA levels, causes oxidative damage, and causes depressive symptoms. In depressive disorders, the levels of several oxidative stress indicators (SOD, MDA, and nitrite) are shown to be changed. Various biomarkers, including neurodegenerative biomarkers, cytokines, oxidative stress indicators, and tryptophan catabolites, have been identified in individuals with depression, and these findings are also confirmed by animal models of depression (Maes *et al.*, 2009).

As a result, the oxidant and antioxidant valance system require therapy to maintain its equilibrium. Treatments like as fluoxetine and serotonin reuptake inhibitors, which aim to stabilize oxidative stress in a valance state, have had limited success. (Bajpai *et al.*, 2014)

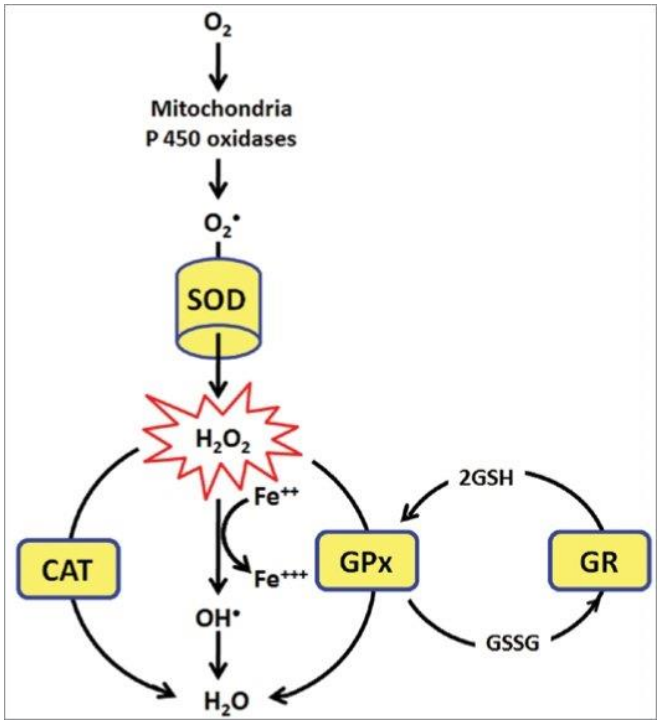


Figure 1.5. Overview on the action of antioxidant enzyme system. (Pandey and Rizvi, 2010)

1.3.4.1 Superoxide dismutase (SOD)

Superoxide dismutases (SODs) play a critical role in the body's antioxidant defense against oxidative stress. The enzyme is an effective treatment for illnesses caused by reactive oxygen species. SODs are the first line of defense against damage caused by reactive oxygen species (ROS). Superoxide dismutase catalyzes the conversion of superoxide to oxygen and, paradoxically, to hydrogen peroxide, which is destroyed by catalase under healthy circumstances. As organism exposed to high levels of superoxides for long periods, SOD consumed and therefore its concentration decreased. While in depressed patients increased and also reduced activity of SOD (Vaváková *et al.*, 2015)

1.3.4.2 Catalase (CAT)

Catalase is a crucial enzyme that feeds on hydrogen peroxide, a nonradical ROS. This enzyme is in charge of neutralizing hydrogen peroxide by breakdown, ensuring that the molecule remains at an optimal level in the cell, which is also important for

cellular signaling activities. In a two-step process, catalase breaks down two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water. The first stage of the chemical process includes the reduction of one hydrogen peroxide molecule to create a spectroscopically unique intermediate product I, which is a covalent oxyferryl species (FeIVO) with a porphyrin -cation radical. Compound I is reduced by redox reactions by a two-electron transfer from an electron donor (the second molecule of hydrogen peroxide) to generate the free enzyme, oxygen, and water in the second step reaction. (Nandi *et al.*, 2019)

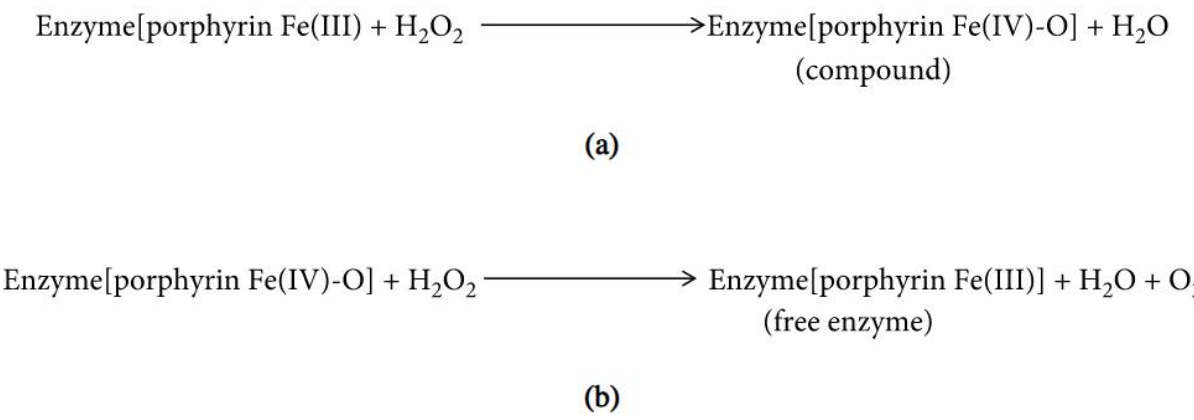


Figure 1.6. Steps in catalase reaction: (a) first step; (b) second step. (Nandi *et al.*, 2019)

1.4. Immune response and depression

In the last decade, several studies have shown that inflammation plays a key role in the etiology of major depressive illness. The macrophage theory of depression proposes that active macrophages generate pro-inflammatory cytokines, greater amounts of the proinflammatory cytokines interleukin (IL)-1, 6 and tumor necrosis factor (TNF) have been found in individuals with severe depression. IL-1 and TNF

may increase glucocorticoid production, alter neurotransmission, and produce stress and anxiety-like behavior in mouse models of depression. (Song *et al*., 2009)

Cytokines are chemical inflammatory mediators produced by lymphoid cells (mainly white blood cells) in response to pathogenic antigens that are foreign or invading. They act as regulators for all other immune cells engaged in the inflammatory process in the body (such as lymphocytes, monocytes, neutrophils, basophils, eosinophils, and natural killer cells). Cytokines are commonly grouped into either pro-inflammatory (IL-1, IL-6, and TNF) or anti-inflammatory (IL-4, IL-8, IL-10, and IL-13) categories. (Jesulola *et al* ,. 2018)

Early studies of the immune system's connection to psychological reactions were made in the context of cytokine-induced illness behavior and immunotherapies like interferon-alpha (IFN-α) in the treatment of hepatitis C. Because of elevated circulation amounts of proinflammatory cytokines, cytokine-induced illness behavior is a condition marked by reduced activity, depression, and energy loss. It's been studied as a model for the immune system's function in behavioral alterations in both animals and humans. Interferon-alpha IFN-α treatments, which trigger an inflammatory antiviral response, are utilized to treat hepatitis C in the clinic.17% of patients treated with IFN-α developed psychiatric side effects, but also noted that the symptoms improved with the cessation of treatment. Patients who developed depression after receiving IFN-α therapy had a substantially greater chance of developing recurring depressive episodes, according to research, suggesting that these mood changes are not a transitory event but are more applicable to typical recurrent depressive episodes. (Giuliani, 2019)

An increase in proinflammatory cytokines, such as TNF-α and IL-6, Higher levels of IL-13, IL-18, IL-12, IL-1RA, and sTNF receptor 2, have been seen in patients suffering from depression. (Köhler *et al.*, 2017) (Dowlati *et al.*, 2010).

The evidence that IFN-α therapy causes depressive symptoms was a piece of solid evidence for a causal connection between inflammatory activation and depression. Furthermore, several researchers have found that greater IL-6 levels indicate the development of clinical depression in the future. (Lee and Giuliani, 2019)

In addition Pro-inflammatory cytokines (such as IL-1β and TNF- α) act on multiple receptors in the brain to cause hyperthermia, nausea, loss of appetite, sleep disturbances, anhedonia, easy-fatigability, and loss of interest in social and physical environments as part of the normal immunologic response to infections. (Micalos *et al* ,. 2018)

The core model of the HPA axis also includes cytokines. Increased pro-inflammatory cytokines cause depressive symptoms by inhibiting brain remodeling and monoamine function and decreasing 5HT synthesis. Pro-inflammatory cytokines enhance glucocorticoid receptor resistance, which prevents CRF down-regulation and allows for cortisol hypersecretion. As a result, immunologic variables indirectly influence depression via the HPA axis. (Jesulola *et al.*, 2018)

Recent research has shown that antidepressant medications, namely SSRIs such as paroxetine, citalopram, and sertraline, inhibit the production of pro-inflammatory cytokines, resulting in an immunosuppressive impact (Gobin et al., 2014; Shenoy et al., 2013).

1.4.1. Inflammation and changes in the brain

The fact that several clinical conditions that cause significant neuroinflammation in the brain (e.g., systemic lupus erythematosus, traumatic brain injury, and multiple sclerosis) are associated with high prevalence rates for major depression supports the link between neuroinflammation and major depression.

Some researchers believe that depressed symptoms are caused by peripheral inflammatory cytokines that penetrate the blood-brain barrier and cause neuroinflammation. Finding indicators of inflammation with a solely cerebral origin, on the other hand, suggests that neuroinflammation plays a more central role in depression pathogenesis. The role of inflammation in depression and tiredness has led researchers to look at the impact of peripheral inflammation on the CNS. (Jesulola et al., 2018)

Depression has been linked to inflammatory alterations in the brain parenchyma. Increased TNFa levels in the hippocampus and striatum have been linked to anxious and depressive behavior, with striatal alterations happening before the development of clinical symptoms. (Peruga *et al.*, 2011)(Haji *et al.*, 2012)

Inflammation induces cellular and structural changes in the CNS, as well as abnormalities in the blood brain barrier BBB. Inflammation reduces neurogenesis in the hippocampus, increases glutamate release from microglia, and affects long-term potentiation LTP, according to in vitro and in vivo animal models. IFNa and endotoxin treatments cause fast alterations in white matter structure, brain global connectivity, and functional activity, all of which are associated with sadness and tiredness, according to human MRI studies. (Lee and Giuliani, 2019)

1.4.2. Interleukin 6

Interleukin 6 (IL-6) helps to host defense by stimulating acute phase responses, hematopoiesis, and immunological reactions. It is generated quickly and transiently in response to infections and tissue damage. IL-6 has a detrimental influence on chronic inflammation and autoimmunity, despite the fact that its production is tightly controlled by transcriptional and posttranscriptional processes. (Tanaka, Narazaki and Kishimoto, 2014)

Increased peripheral or central cytokine interleukin-6 (IL-6) levels have a significant role in stress reactivity and depressive illness, especially physical diseases comorbid

with depression, according to evidence from animal and human research. Increased IL-6 release has been linked to MDD prognosis and treatment response, and it has the potential to impact a wide spectrum of depressive symptoms. Increased IL-6 activity has the potential to produce depression by activating the hypothalamic-pituitary-adrenal axis or influencing neurotransmitter metabolism. Modulation of IL-6 activity by IL-6-related drugs, current antidepressants, herb medicine, pre-/probiotics, or non-pharmacological treatments might be very beneficial for MDD patients with inflammatory characteristics (Figure 1.7). (Ting, Yang, and Tsai, 2020)

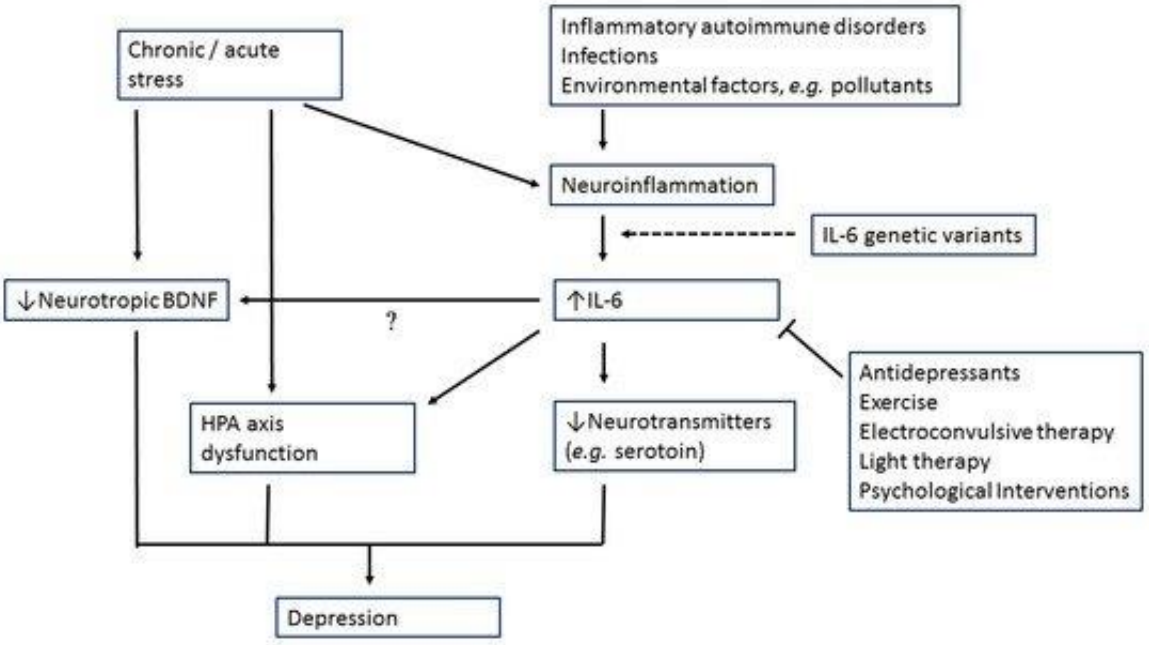


Figure 1.7. possible causes and outcomes of alteration of interleukin-6 level during depression development. (Ting, Yang and Tsai, 2020)

1.5. Animal Model of Depression

An animal model of human behavior reflects a set of emotional and/or cognitive processes that are translated from animals to humans. Behavioral tests used in rodent drug dependence research, such as self-administration and conditioned place

preference focus on measuring the motivation to perform a selected action (Belovicova *et al.*, 2017).

Symptoms of depression such as Anhedonia and depressed mood, apathy, sleep disturbances, weight/appetite changes, psychomotor changes, and other comorbid conditions, such as anxiety and social isolation, can all be easily assessed in animals, whereas other symptoms of depression in humans, such as sadness, guilt, or suicidal thoughts, cannot be simulated in rodents or other animal models. (Cathomas *et al.*, 2015)

Learned helplessness, forced swim test, and tail suspension test, which use relatively short-term exposure to inescapable or uncontrolled stress and may consistently identify antidepressant medication response, are examples of paradigms that use acute or sub-chronic stress exposure. Chronic mild stress models, recurrent open-space models, early-life stress models, and social conflict models are among the longer-term models that may more effectively replicate the mechanisms that contribute to depression. Chronic models are thought to be more practical in inducing a depressive-like state and to have a greater potential connection to actual situations. (Adongo, 2015)

1.5.1. Chronic Unpredictable Mild Stress protocol

In a series of studies published in the early 1980s by Katz and colleagues, rats were progressively subjected to a variety of severe stressors, resulting in the chronic mild stress (CUS) paradigm. To test the effects of stress, the majority of these trials employed changes in open-field behavior, which were shown to be reversed largely by extended therapy with antidepressant medications but not by non-antidepressant drugs. (Willner, 2017) .

Many neuropsychiatric diseases, such as anxiety, depression, and dementia, can be triggered by stressful life experiences, and many of them are associated with immunological dysfunction. Furthermore, immunological dysfunction caused by

persistent stress is thought to be a contributing component in the negative consequences of stress on health. While the acute stress response, in contrast to chronic stress, is a positive occurrence since it is an alarm response that prepares the body for a potential attack. The release of stress mediators such as glucocorticoids and adrenaline characterizes this response, which allows for the stabilization of bodily function through adaptation to the stressor. However, if this reaction persists over time, the system may become unable to cope with the stressor, resulting in chronic stress-related disease. (Monteiro *et al.*, 2015)

1.5.2. Tests for animal depression

1.5.2.1 Forced Swimming Test (FST)

This behavioural test is designed to assess a rodent's sense of hopelessness. FST also known as the Porsolt test, involves forcing rats (or mice) to swim in a tall water-filled cylinder from which they cannot escape. The water is deep enough that the creatures cannot rest their limbs or tails on the bottom. When originally placed in this device, the animal swims frantically to keep its head above water. Attempts to leave the cylinder quickly end as it recognizes the inescapability of its situation. Following that, the animal assumes an immobile position, which has been regarded as a theoretical counterpart of pessimism in terms of escape. The FST has also been shown to produce neurochemical alterations in animals that are consistent with a depressogenic phenotype. It can, for example, temporarily lower serotonin and norepinephrine levels in both cortical and limbic regions, as well as decrease CRH sensitivity. (Pałucha-Poniewiera *et al.*, 2020)

The FST is a highly frequent paradigm in animal studies for a variety of reasons. Firstly, it takes into account the animals' susceptibility to stress, which has been linked to the rise in severe depression. Depression is also associated with an inability to cope

with stress. Second, pharmaceutical antidepressant therapy resulted in a reduction in immobility in the FST. (Yankelevitch-Yahav *et al.*, 2015)

In fact, Animals exposed to depression models adopt this immobility posture earlier and for longer than control groups. Another noteworthy feature is that traditional antidepressant medication treatment can reduce immobility time in healthy control rats, making this test a useful tool for evaluating antidepressant effects of novel medicines without putting the animals through depressed behavior induction methods. (Valvassori *et al.*, 2017)

1.5.2.2. Tail Suspension Test (TST)

The tail-suspension test is a mouse behavioral test that may be used to screen for prospective antidepressant medications as well as examine other treatments that may alter depression-related behaviors. Mice's tails are taped together in such a way that they cannot escape or hang on to adjacent objects. The tail-suspension test is a useful technique for high-throughput screening of potential antidepressant drugs in drug development. TST involves suspending mice above the ground by their tails. A suspension bar or shelf ledge, as well as tape, are all that is required at the most basic level. In the TST, mice are put in an inescapable yet mildly stressful environment, similar to the forced swim test. Immobility is defined as a lack of escape-related activity. The TST, like the forced swim test, is best known for assessing antidepressant efficacy, but it's also used to assess the impacts of environmental, neurobiological, and genetic changes. In contrast to the forced swim test, the TST does not include submersion in water, therefore there is no risk of hypothermia. (Can *et al.*, 2012)

1.5.2.3. Sucrose Preference Test (SPT)

Anhedonia is a basic symptom of depression in humans, characterized by the inability to derive pleasure from rewarding or pleasant activities. We present a methodology for measuring anhedonia in rats using a sucrose preference test (SPT)

based on a two-bottle choice paradigm. Anhedonia is defined as a decrease in the sucrose preference ratio in experimental animals compared to control rats. For the assessment of chronic stress-related anhedonia, our improved technique is extremely sensitive, reliable, and flexible. For decades, diet-intake studies have used a two-bottle choice test to determine preferred nutrient intake (taste preference). Taste preference is determined by the ratio of a particular solution's intake to the overall intake of the solution and water. When given a two-bottle free-choice regimen with access to both sucrose solution and normal water, rats were shown to naturally ingest sweet food and drink sweet solution preference. However, When rats were subjected to stress-based depression models, they refused to consume sweetened water in favor of plain water, but antidepressant treatment restored their preference for sucrose. (Liu *et al.*, 2018)

1.5.2.4. Open Field Test (OFT)

The open field test (OFT) is a popular measure of exploratory behavior and general activity in mice and rats that may be used to assess both the quality and amount of activity. The open field (OF) is essentially an enclosure with surrounding walls that restrict escape. It can be square, rectangular, or circular in shape. The most basic and typical result of interest is movement, although this may be impacted by a variety of factors, including motor output, exploratory drive, fear-related behavior, and relative time in the circadian cycle, to name a few. Many measurments can be recorded, including distance traveled, time spent moving, grooming, time spent in central area, rearing, and change in activity over time. (Dulawa *et al.*, 2004)

Calvin S. Hall created the open-field test (OFT) in 1932 to assess animal emotionality. He utilized an open field with a diameter of 77 feet (about 22 meters) and a food pellet in the center. He saw how animals moved in circular patterns as they approached food. When food was withdrawn, however, animals' desire to investigate a new habitat declined.

Despite the fact that OFT was created by behavioral psychologists, it is now widely utilized in a variety of scientific areas, such as neurology and psychopharmacology.

Despite the fact that OFT is sometimes referred to as a "standardized test" some variables such as open-field size and shape (square, rectangular, circular), level of illumination, familiarity with the apparatus (single or repeated exposures), duration of testing (1–30 min), time of day of testing, motivation (food and/or water deprived animals), and animal housing conditions can all affect the outcome. (Belovicova *et al.*, 2017)

2.1. Materials

2.1.1. Animals

This study is experimental animal study that study was approved by the committee of publication ethics at the College of Medicine included sixty male albino rats, all of whom were adults. Their weights ranged from 185 to 245 grams. The rats were maintained at the Animal House of the College of Medicine/University of Babylon at a temperature approximately 25°C with a 14-hour light/ten-hour dark cycle and free access to water and food. They were kept in 12 cages, each cage contain five rats. This From September 2, 2021, until January 6, 2022, a research was conducted at the college of medicine.

2.1.2. Instruments and Equipment

Table 2.1 instruments and equipment list, which were utilized in the study with their suppliers.

Table 2.1 List of instruments and equipment

No.	Instrument / Equipment	Company / Country
1	Electronic scale	SDT/ China
2	Elisa (reader, washer, printer)	Haman/ Germany
3	Eppendorf plastic tubes	Gondong/ China
4	Eppendrof tubes	Sigma/ England
5	Filter paper	Citotest/ China

6	Gopro hero 9 video camera	Gopro/ USA
7	High speed cold centrifuge	Hettich /Germany
8	Homogenizer drill	Jiao Jie/ China
9	Hot plate stirrer	Labtech /Korea
10	Incubator	Memmert/ Germany
11	Micropipettes (different volumes)	Eppendorf/ Germany
12	Oven	Labcon/ Germany
13	Refrigerator	Gorengi/ Slovenia
14	Refrigerator	Concord/ lebanon
15	Rotary evaporator	Laborota/ Germany
16	Sensitive balance	Sartorius/ Germany
17	Shaker	Gyro-Rocker/ UK
18	Spectrophotometer	Jenway/ England
19	Surgical set	China

2.1.3. Kits

Table 2.2 list of the kits which were used in the study with their companies & countries

Table 2.2: List of kits used

No.	Kit	Company	Country
1	Rat Interleukin 6 ELISA Kit	Bioassay technology laboratory	China
2	Rat Super Oxidase Dimutase ELISA Kit	Bioassay technology laboratory	China
3	Rat Catalase ELISA Kit	Bioassay technology laboratory	China

2.1.4. Chemicals

The chemical and biological materials used in this work are listed in Table 2.4

Table 2.4 List of Chemical materials with their remarks

No.	Chemicals	Company	Country
1	Ethanol Absolute ≥99.8% Solution	Honeywell	Germany
2	Fluoxetine oral suspension	Intas	India
3	Phosphate buffer saline	Labort	India

2.1.5. Drug

Fluoxetine syrup (FLUNIL), 20mg / 5ml manufactured by INTAS (India) was used in the experiment.

Picture 2.1 Fluoxetine syrup used in the experiment

2.1.6. Plant Preparation

The dried fruit of C. Myxa was purchased from Diyala / Iraq in July 2021. According to document no. 3115 on 18 November 2021, the plant was authorized as C. Myxa with the aid of the University of Al-qasim green/ faculty of agriculture/medicinal plant department. The dried fruits of Cordia Myxa was crushed into powder with a mechanical grinder, then kept at 4 °C.

Powder of fruits was extracted using maceration with ethanol. The powdered fruits were macerated in 70% (v/v) ethanol (5:10 w/v) at room temperature for 72 hr. Then, it was shaken for 4 hr. The mixture was filtered with Buchner funnel and Whatman filter paper No.1. The resulting extract concentrated under pressure using a rotary evaporator in 40°C. The extract was stored at refrigerator. For tests the extract was suspended with normal saline. Solution prepared at concentration 200mg/1ml. Normal saline was taken as control. (Salimimoghadam *et al.*, 2019)

Picture 2.2 Dried fruits of Cordia Myxa

2.2. Methods

2.2.1. Study design

The rats were divided into six groups at random manner, each group with 10 male rats, five rats in one cage.

2.2.2. Chronic Unpredictable Mild Stress (CUS)

The Katz method, with minor modifications, was used to induce chronic stress. This protocol was chosen since it has previously been used to generate anxiety in animals. The animals in stress groups were exposed to the CUS protocol as follows (Amiresmaeili *et al.*, 2018).

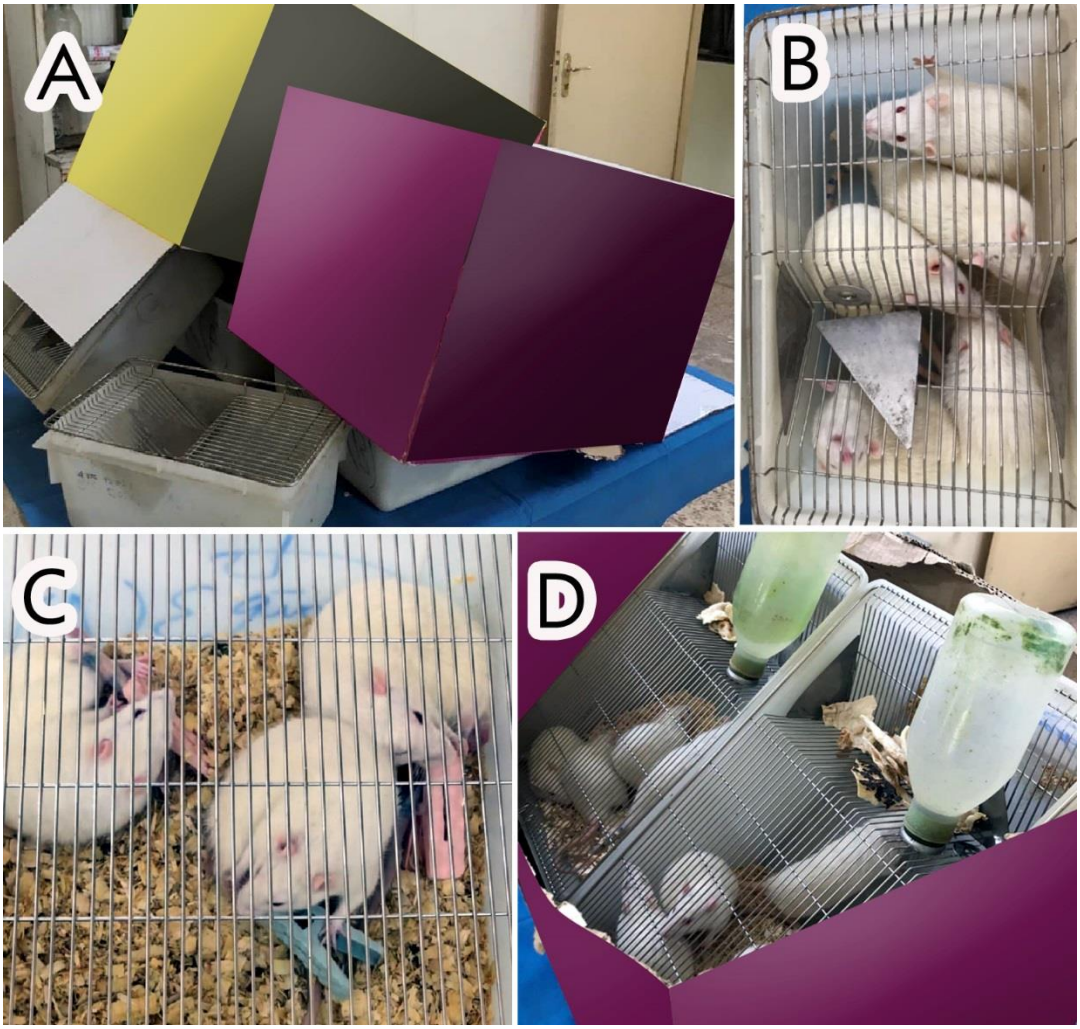
Table 2.5: Chronic unpredictable mild stress protocol used

day	CUS protocol
1	15 min forced swim (20 °C), crowded cage
2	12 h cage tilting (45 °C), 1 h restraint
3	reversal of the light/dark cycle
4	12 h wet bedding, crowded cage
5	24 h food deprivation, 1 h cage rotation
6	12 h cage tilting (45 °C), tail pinch.
7	1 h cage rotation, 1 h cold room isolation
8	reversal of the light/dark cycle, tail pinch
9	24 h water and food deprivation
10	12 h cage tilting (45°C), 15 min forced swim (20 °C)
11	1 h restraint , 24 h water deprivation
12	reversal of the light/dark cycle, 24 h food deprivation
13	12 h cage tilting (45 °C)
14	24 h water deprivation, 1 h restraint

15	12 h wet bedding, 12 h cage tilting (45 °C)
16	1 h cage rotation, reversal of the light/dark cycle
17	1 h restraint, crowded cage
18	12 h wet bedding, 12 h cage tilting (45 °C)
19	reversal of the light/dark cycle, tail pinch
20	15 min forced swim (20 °C), 1 h cold room isolation
21	1 h cage rotation, crowded cage
22	reversal of the light/dark cycle , tail pinch
23	24 h food and water deprivation
24	12 h cage tilting (45 °C), crowded cage

The CUS protocol consisted of the following stressors: (A) Wet bedding: 300 mL of water was poured on and mixed with 1 L of sawdust bedding. (B) Cage tilting: the cage was tilted up to 45 degrees with food and water located at the higher top. (C) Crowded cage : 10 rats per cage (D) Restraint : five rats per small cage render them on immobile position. Animals were exposed to every stressor 3 to 4 times throughout the protocol. (Sequeira-Cordero *et al.*, 2019)

CUS influences animal behaviour. The sucrose preference test (SPT), forced swimming test (FST), and open field test (OFT) were used to investigate depressive-like behavior in the rat CUS model. The animals were evaluated with the FST, OFT, and SPT before starting the CUS procedure to identify their depressed condition (baseline, day 0). Depressive-like behavior was assessed by SPT, OFT, and FST during the CUS exposure period (day 10). The anti-depressive effects of various medications were assessed by SPT, OFT, and FST at the end of the treatments (25 days).



Picture 2.3 Chronic Unpredictable stress CUS A) Reversal of the light/ dark cycle B) Restraint C) Tail pinch D) Cage Tilting

2.2.3. Box of the Forced Swim Test

A cylindrical glass box (30cm*30cm*70cm) was made by researcher and used in this experiment as designed by (Cryan, Valentino and Lucki, 2005).

Individual rats were forced to swim in a cylindrical glass container (70 cm height, 30 cm diameter) filled to a depth of 0.3 meter with water (25°C) (Picture 2.4). Individually, the laboratory animals were permitted to swim for 5 minutes. An observer who was blind to the animal groups recorded and evaluated the test sessions. At 0, 10, and 25 days, the duration of immobility during the first 5 minutes of the

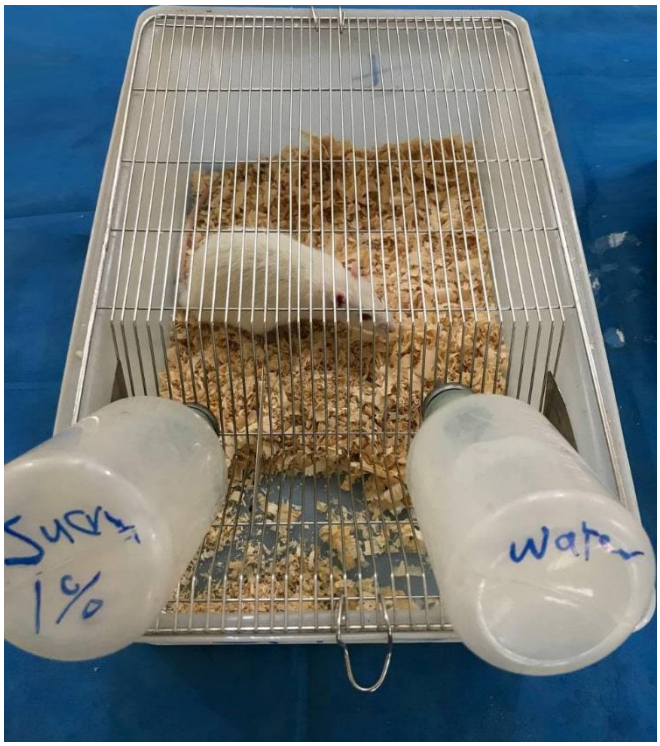
swimming exercise was recorded. After each rat testing, the water in the container was replaced. A depressed phenotype is defined as spending more time stationary or immobile and less time actively swimming or struggling in response to a threat or challenge.

Picture 2.4 Forced swim test session

2.2.4. Sucrose Preference Test (SPT)

SPT assesses anhedonia in rats as one of the fundamental symptoms of depression. Rats were initially trained to drink a 1 percent (w/v) sucrose solution for 72 hours without water access. Then 1% sucrose in one bottle was replaced with tap water for the next 24 h. Following training, rats were subjected to a one-hour test session after 24 hours of food and water restriction. Rats were taken from their home cages and placed separately into test cages before to the test. Each rat was given two bottles containing either tap water or a 1 percent sucrose solution on either the left or right side of the test room. After refilling the bottles, and the rats were free to drink water and sucrose solution for 1 hour (Picture 2.5). Sucrose preference (%) was determined

as sucrose intake (g) divided by total liquid consumption (g) multiplied by 100 during the 1-h test. The test was done on day 0 (for baseline data), 10, and 25. (Sahin Ozkartal *et al.*, 2018)



Picture 2.5 Sucrose preference test

2.2.5. Open-Field Test

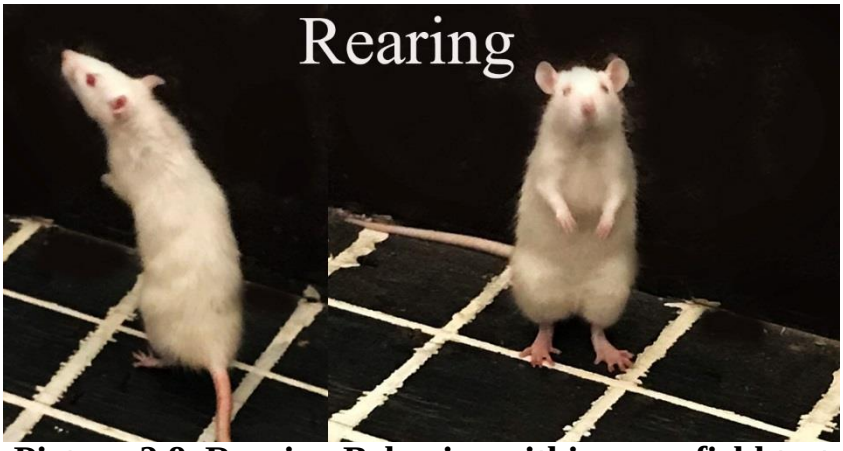
According to (Martinez-Gonzalez *et al.*, 2004), this wooden box (100 x 100cm) was built by a researcher and consists of a square floor divided into 100 equal squares by thin white lines. To prevent escape, the equipment consists of an arena surrounded by high walls. The number of line crossings, rearings, and time spent moving are utilized to evaluate the rat's activity throughout the test period. Crossings and rearings behaviors are used to assess hyperactivity in the open-field apparatus. The total number of square crossings throughout the test time is referred to as crossings, and it is used to determine the animals' locomotor activity. The total number of upright postures taken by the rat with the intention of investigating during the test time is

referred to as rearings. Stereotypy refers to animals' repeated actions, which are more common in animals with brain abnormalities. Grooming and sniffing habits might be used to assess it. Grooming refers to the total time spent grooming during the test period, whereas sniffing refers to the total time spent sniffing during the test period. In order to quantify anxiety-like behavior, the quantity of time spent in the middle of the arena was assessed. This test is based on rats exploring an open field on their own. When rats are anxious, they will naturally seek shelter towards the margins of an open field's perimeter. As a result, less time spent in the center of the arena reflects higher anxiety-like behavior (Valvassori, Varela and Quevedo, 2017).

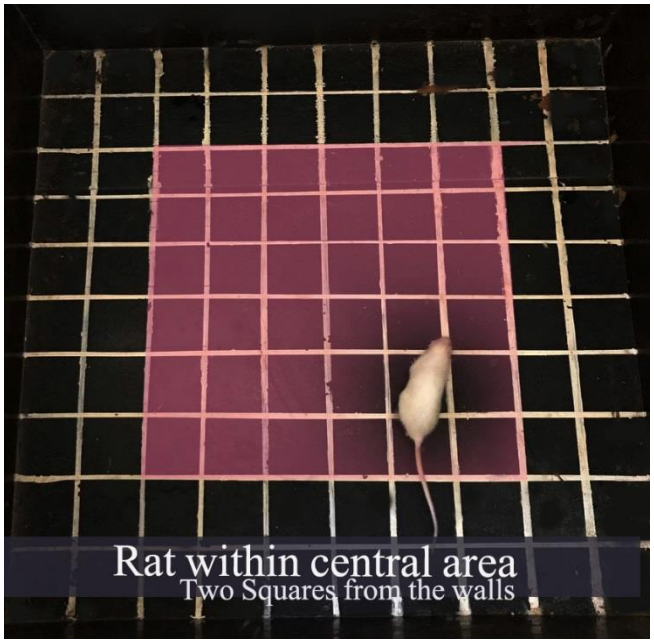
Picture 2.6 Open field test

To minimize worry, the light was kept to a bare minimum. In order to hide animal evidence, the equipment were cleaned with a 95% ethanol solution in between experiments. Over a ten-minute period, animal behavior was recorded.

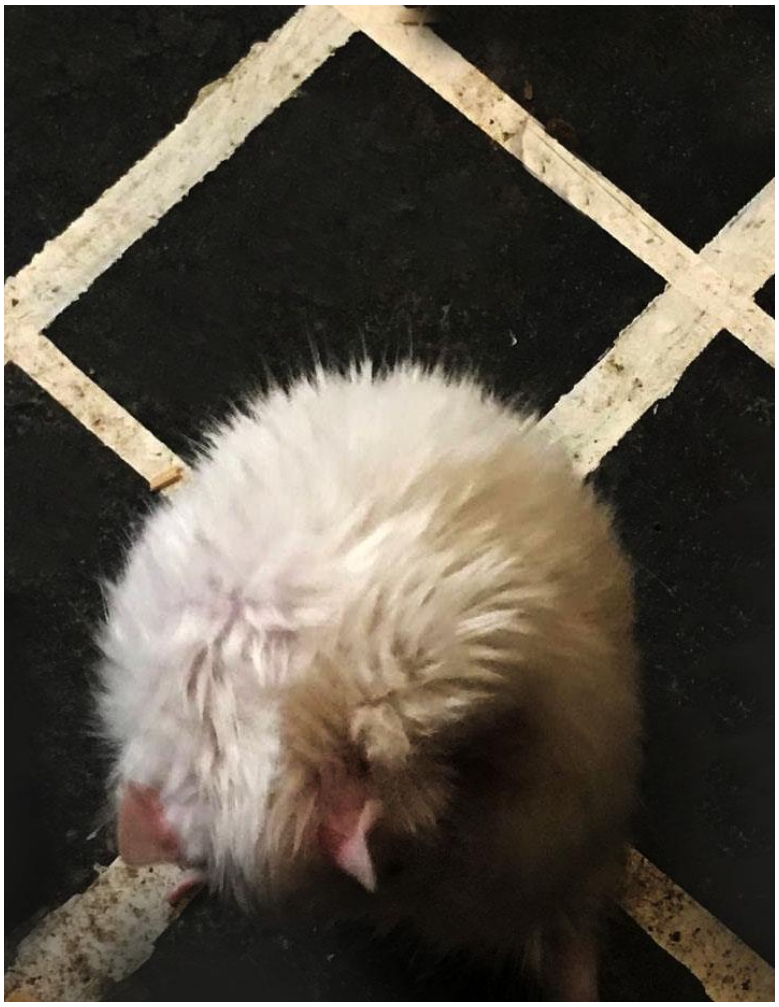
Picture 2.7. Line-crossing within open field test



Picture 2.8. Rearing Behavior within open field test



Picture 2.9. Central Area within open field test



Picture 2.10. Grooming behavior within open field test

2.2.6. Procedure

1. On days 0, 10, and 25, each animal underwent behavioral test including SPT, OFT, and FST.

2. The animals in group 1 (control) did not receive any medication and were not stressed.

3. For 24 days, each animal in groups 2, 3, 4, 5, and 6 was exposed to chronic unpredictable mild stress (CUS).

4. Each animal in group 2 was given 0.2 mL of normal saline by oral gavage for 14 days starting from the tenth day of CUS without any treatment.

5. For 14 days, each animal in group 3 received fluoxetine treatment 10mg/kg by oral gavaging. (López and colleagues, 2019)

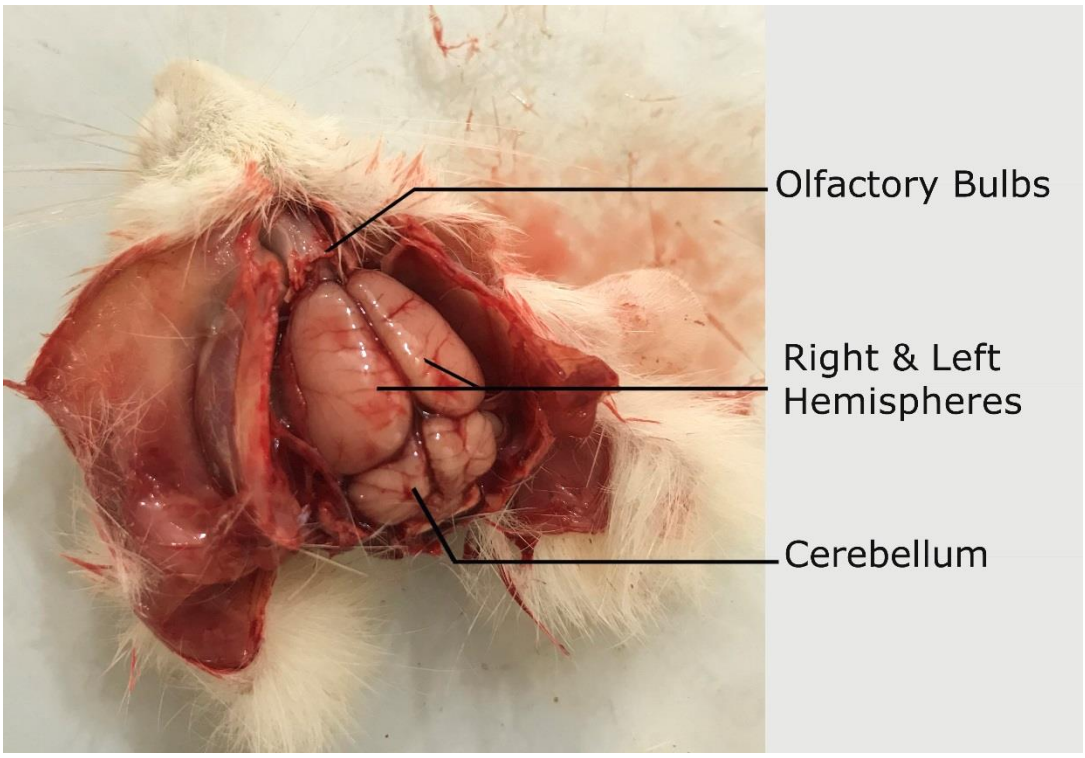
6. For 14 days, each animal in groups 4, 5, and 6 was given a daily dose of C.myxa fruit extract of 125 mg/kg, 250 mg/kg, and 500 mg/kg, respectively by oral gavage. (Shahriari and Moghadamnia, 2021)

2.2.7. Ethical Approval

This study was approved by the committee of publication ethics at the College of Medicine, University of Babylon, Iraq.

2.2.8. Decapitation of Rat Brain and Tissue Samples Preparation

On the 25th day, the animals sacrificed by decapitation 24 h after the last treatment. The brains were removed after dissection of a skull from foramen magnum posteriorly (Picture 2.11). Olfactory bulbs and cerebellum were cut and the brain was removed gently from the skull. The mid and forebrain were dissected out, washed with phosphate buffer saline solution, and weighted. and kept in an sterilized eppendorf tube and was frozen on dry ice and then deep freeze at -20 C^0



Picture 2.11: Skull dissection of rat

2.2.8.1. Preparation of Phosphate Buffer Saline (PBS) solution

According to the manufacturer directions ; Dissolve 10 tablets in 1 liter of purified water and autoclave at 115^0C for 10 mints. Keep it cool, between 15 and 30 degrees Celsius, and out of direct sunlight.

2.2.8.2. Steps in the preparation of brain samples

1. Brain homogenization: any remaining blood was washed away with a precooling PBS solution (pH=7.4).
2. After weighing the brain, it was homogenized in PBS (pH=7.4) using an ice-cold homogenizer.
3. Freeze at -20C or thaw at 2-8C.

4. After thawing, the homogenates were centrifuged for 20 minutes at 2000-3000 RPM.

2.3. Biochemical Assessments using The enzyme-linked immunosorbent assay (ELISA) kits

In our research, we used three different Elisa kits (Sandwich kits) for the accurate quantitative detection of Rat Interleukin 6 (also known as IL-6), Superoxide dismutase (also known SOD), and Catalase (also known CAT), in tissue homogenates of the brain.

2.3.1. Rat Interleukin 6 ELISA Kit

The principle of this kit was that Rat IL-6 antibody has been pre-coated on the plate. IL-6 from the sample is introduced to the wells, where it binds to antibodies. The biotinylated Rat IL-6 Antibody is then added to the sample and binds to IL-6. The biotinylated IL-6 antibody is then bound by Streptavidin-HRP. During the washing phase after incubation, all unbound Streptavidin-HRP is rinsed away. Following that, the substrate solution is added, and the color develops in accordance to the amount of Rat IL-6 present. The process is stopped by adding an acidic stop solution and measuring the absorbance at 450 nm.

2.3.1.1. Reagent Preparation of IL-6 ELISA Kit

Before using, we are bringing all reagents to room temperature. We first prepare standard solution, To make a 24ng/L standard stock solution, we combined 120µl of the standard (48ng/L) with 120µl of standard diluent. Before preparing dilutions, we let the standard sit for 15 minutes with moderate agitation. Then Prepared duplicate standard points by diluting the standard stock solution (24ng/L) 1:2 with standard

diluent to get solutions of 12ng/L, 6ng/L, 3ng/L, and 1.5ng/L. The zero standard is standard diluent (0 ng/L).

Then we prepare wash buffer, 20ml concentrated Wash Buffer solution was diluted to make 500 mL of diluted Wash Buffer. Dilution performed 25 times in distilled water. when crystals have been developed in the concentrate, we gently stir until they have all dissolved.

2.3.1.2. Assay Procedure of of IL-6 ELISA Kit

As directed, we prepare all reagents, standard solutions, and samples. Before using, bring all reagents to room temperature. At room temperature, the assay is carried out. Add 50 µl of standard to the standard well, 40 µl of sample to the sample wells, 10 µl of anti-IL-6 antibody to the sample wells, and 50 µl of streptavidin-HRP to the sample wells and standard wells. Following that mix well. Incubate for 60 minutes at 37°C after sealing the plate. Remove the sealer and use a wash buffer to wash the plate five times. For each wash, soak wells in at least 0.35 mL wash buffer for 30 seconds to 1 minute. Aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer for automatic washing. Using paper towels or another absorbent material, blot the plate. Add 50 µl substrate solution A to each well and then 50 µl substrate solution B to each well. Incubate for 10 minutes at 37°C after sealing the plate. After that 50 µl of Stop Solution added into each well, the blue hue will become yellow almost instantly. Within 10 minutes after applying the stop solution, determine the optical density (OD value) of each well using a microplate reader set to 450 nm.

2.3.2. Rat Superoxide dismutase (SOD) ELISA Kit

The principle of this kit was that Rat SOD antibody has been pre-coated on the plate. SOD from the sample is introduced to the wells, where it binds to antibodies. The biotinylated Rat SOD Antibody is then added to the sample and binds to SOD. The biotinylated SOD antibody is then bound by Streptavidin-HRP. During the

washing phase after incubation, all unbound Streptavidin-HRP is rinsed away. Following that, the substrate solution is added, and the color develops in accordance to the amount of Rat SOD present. The process is stopped by adding an acidic stop solution and measuring the absorbance at 450 nm.

2.3.2.1. Reagent Preparation of (SOD) ELISA Kit

Before using, we were bringing all reagents to room temperature. We first prepare standard solution, to make a 24ng/L standard stock solution, we combined 120µl of the standard (48ng/L) with 120µl of standard diluent. Before preparing dilutions, we let the standard sit for 15 minutes with moderate agitation. Then Prepared duplicate standard points by diluting the standard stock solution (24ng/L) 1:2 with standard diluent to get solutions of 12ng/L, 6ng/L, 3ng/L, and 1.5ng/L. The zero standard is standard diluent (0 ng/L).

Then we prepare wash buffer, 20ml concentrated Wash Buffer solution was diluted to make 500 mL of diluted Wash Buffer. Dilution performed 25 times in distilled water. when crystals have been developed in the concentrate, we gently stir until they have all dissolved.

2.3.2.2. Assay Procedure of of (SOD) ELISA Kit

As directed, we prepare all reagents, standard solutions, and samples. Before using, bring all reagents to room temperature. At room temperature, the assay is carried out. Add 50 µl of standard to the standard well, 40 µl of sample to the sample wells, 10 µl of anti-SOD antibody to the sample wells, and 50 µl of streptavidin-HRP to the sample wells and standard wells. following that mix well. Incubate for 60 minutes at 37°C after sealing the plate. Remove the sealer and use a wash buffer to wash the plate five times. For each wash, soak wells in at least 0.35 mL wash buffer for 30 seconds to 1 minute. Aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer for automatic washing. Using paper towels or another absorbent material,

blot the plate. Add 50 µl substrate solution A to each well and then 50 µl substrate solution B to each well. Incubate for 10 minutes at 37°C after sealing the plate. After that 50 µl of Stop Solution added into each well, the blue hue will become yellow almost instantly. Within 10 minutes after applying the stop solution, determine the optical density (OD value) of each well using a microplate reader set to 450 nm.

2.3.1. Rat Catalase (CAT) ELISA Kit

The principle of this kit was that Rat CAT antibody has been pre-coated on the plate. CAT from the sample is introduced to the wells, where it binds to antibodies. The biotinylated Rat CAT Antibody is then added to the sample and binds to CAT. The biotinylated CAT antibody is then bound by Streptavidin-HRP. During the washing phase after incubation, all unbound Streptavidin-HRP is rinsed away. Following that, the substrate solution is added, and the color develops in accordance to the amount of Rat CAT present. The process is stopped by adding an acidic stop solution and measuring the absorbance at 450 nm.

2.3.1.1. Reagent Preparation of CAT ELISA Kit

Before using, we are bringing all reagents to room temperature. We first prepare standard solution, To make a 24ng/L standard stock solution, we combined 120µl of the standard (48ng/L) with 120µl of standard diluent. Before preparing dilutions, we let the standard sit for 15 minutes with moderate agitation. Then Prepared duplicate standard points by diluting the standard stock solution (24ng/L) 1:2 with standard diluent to get solutions of 12ng/L, 6ng/L, 3ng/L, and 1.5ng/L. The zero standard is standard diluent (0 ng/L).

Then we prepare wash buffer, 20ml concentrated Wash Buffer solution was diluted to make 500 mL of diluted Wash Buffer. Dilution performed 25 times in distilled water. when crystals have been developed in the concentrate, we gently stir until they have all dissolved.

2.3.1.2. Assay Procedure of of CAT ELISA Kit

As directed, we prepare all reagents, standard solutions, and samples. Before using, bring all reagents to room temperature. At room temperature, the assay is carried out. Add 50 µl of standard to the standard well, 40 µl of sample to the sample wells, 10 µl of anti-CAT antibody to the sample wells, and 50 µl of streptavidin-HRP to the sample wells and standard wells. following that Combine well and serve. Incubate for 60 minutes at 37°C after sealing the plate. Remove the sealer and use a wash buffer to wash the plate five times. For each wash, soak wells in at least 0.35 mL wash buffer for 30 seconds to 1 minute. Aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer for automatic washing. Using paper towels or another absorbent material, blot the plate.

After that 50 µl of Stop Solution added into each well, the blue hue will become yellow almost instantly. Within 10 minutes after applying the stop solution, determine the optical density (OD value) of each well using a microplate reader set to 450 nm.

2.4. Analysis of Statistics

The results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out by using one way analysis of variance ANOVA, and the post hoc test (LSD and Bonferoni). Differences were considered statistically significant if the probability p value is lower than 0.05. Statistical analysis was carried out by using the 23th edition of Statistical Package of Social Sciences (SPSS v23) statistics for Windows® 10.

3.1. Behavioral tests results within the group

3.1.1. Forced swimming test (FST) within the group

There were no significant differences in immobility time on the days 10 and 25 as compared to day 0 in group 1 (control group, untreated and unexposed to CUS), whereas in group 2 (untreated and exposed to CUS), the mean immobility time on day 25 significantly increased (p value <0.05) as compared to days 0 and 10 (Table 3.1 and Figure 3.1).

Furthermore, the means of the immobility time on day 10 significantly increases (P-value <0.05) when compared to day 0 in groups 3 (treated with 10mg/kg fluoxetine for 14 days), 4 (treated with 125mg/kg C.Myxa extract for 14 days), 5 (treated with 250mg/kg C.Myxa extract for 14 days), and 6 (treated with 500mg/kg C.Myxa extract for 14 days) (Table 3.1 and Figure 3.1). On day 25, the mean of immobility time in groups 3, 4, 5, and 6 dropped significantly (P-value <0.05) as compared to day 10. (Table 3.1 and Figure 3.1).

Table 3.1 Comparison in immobility time (in seconds) ± SEM between groups on days 0,10,25

FST	Group 1 (sec)	Group 2 (sec)	Group 3 (sec)	Group 4 (sec)	Group 5 (sec)	Group 6 (sec)
Day 0	47.90±1.52	47.50±1.43	46.40±4.48	48.40±3.23	44.40±1.65	42.50±2.65
Day 10	48.60±1.82	64.80± 4.07*	66.00± 4.17*	67.70± 4.86*	63.50± 3.14*	64.00± 4.98*
Day 25	47.70±1.26	67.00± 5.55**	38.90± 2.19***	49.20 ± 2.25 ***	38.10 ± 1.87 ***	48.90 ± 1.13 ***

*** = significantly increased (*P value* <0.05) as compared with day 0.**
**** = significantly increased (*P value* <0.05) as compared with day 0.**
***** = significantly decreased (*P value* <0.05) as compared with day 10.**
Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C. Myxa extract for 14 days), group 5 (treated with 250mg/kg C. Myxa extract for 14 days), group 6 (treated with 500mg/kg C. Myxa extract for 14 days), no.of rats=10 for each group.

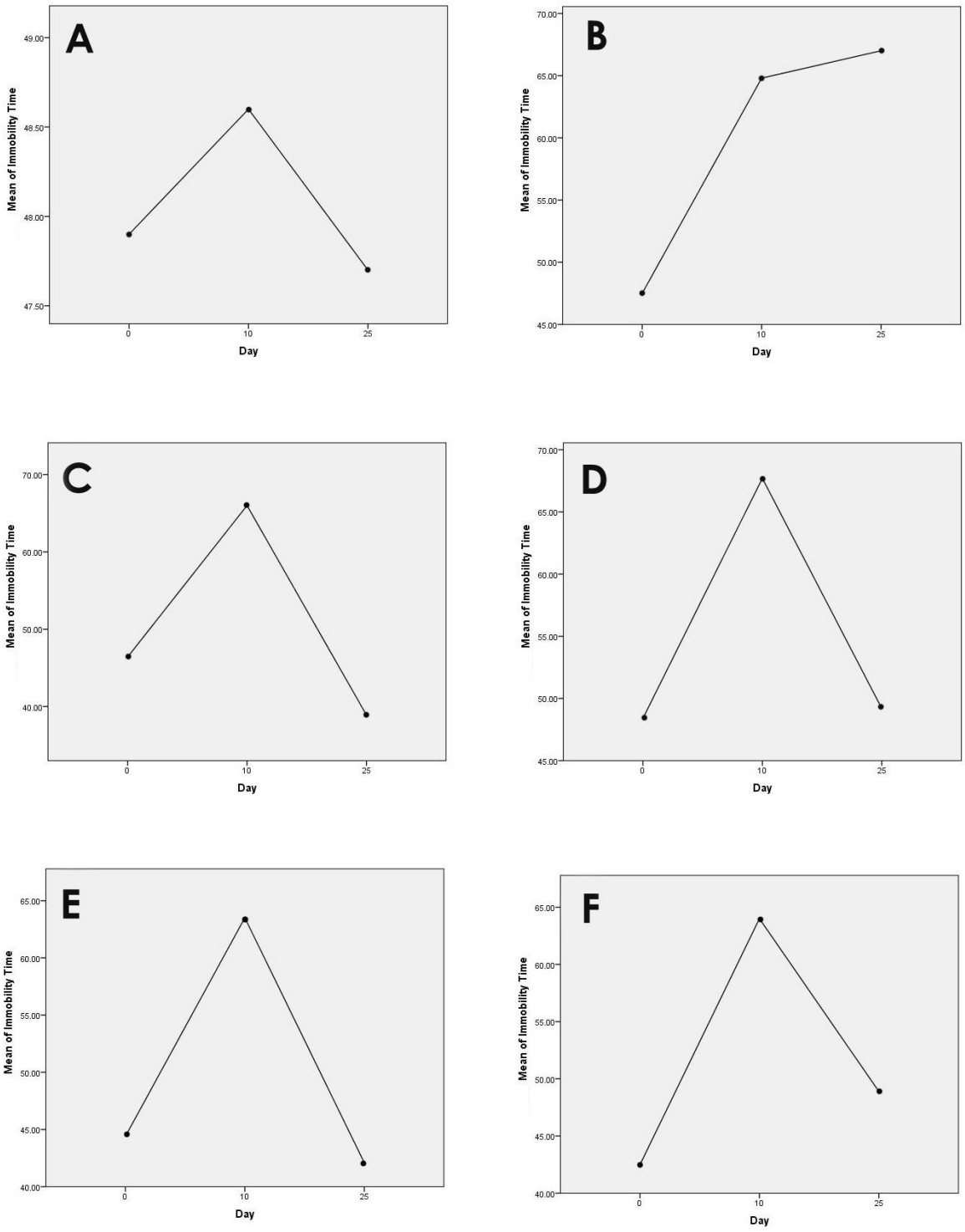


Figure 3.1: the means of the immobility times on days 0, 10, and 25 for all groups.

A: group 1 (control group, untreated and unexposed to CUS), B: group 2 (untreated and exposed to CUS), C: group 3 (treated with 10 mg/kg fluoxetine for 14 days), D: group 4 (treated with 125 mg/kg *C. Myxa* extract for 14 days), E: group 5 (treated with 250 mg/kg *C. Myxa* extract for 14 days), F: group 6 (treated with 500 mg/kg *C. Myxa* extract for 14 days). no. of rats =10 for each group.

3.1.2. Sucrose preference tests within the group

The mean sucrose preference index (SPI) on day 25 in group 1 increased substantially (P-value < 0.05) when compared to days 10 and 0, but the mean SPI on day 25 in group 2 declined significantly (P-value <0.05) when compared to days 0 and 10. (Table 3.2 and Figure 3.2)

On day 10, the means of SPI in groups 3, 4, 5 and 6 substantially reduced (P-value < 0.05) when compared to day 0, but the means of SPI on day 25 in groups 3,4,5 and 6 considerably rose (P-value < 0.05) when compared to day 10. (Table 3.2 and Figure 3.2)

Table 3.2 Comparison in the means of sucrose preference index ± SEM between groups on days 0, 10, 25.

SPT	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 0	79.20±1.34	79.10±1.87	76.20±2.40	77.50±1.57	80.90±1.68	80.70±1.61
Day10	78.60±1.80	51.40± 3.19*	57.60± 1.79*	57.70± 2.22*	56.30± 2.78*	55.80± 2.64*
Day25	82.00± 1.90 ***	53.40± 1.70*	74.00± 1.69 **	74.30± 1.55 **	78.10± 2.36**	80.20± 1.23**

* = significantly decreased (P <0.05) as compared with day 0,
** = significantly increased (P <0.05) as compared with day 10,
*** = significantly increased (P <0.05) as compared with day 0.
Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

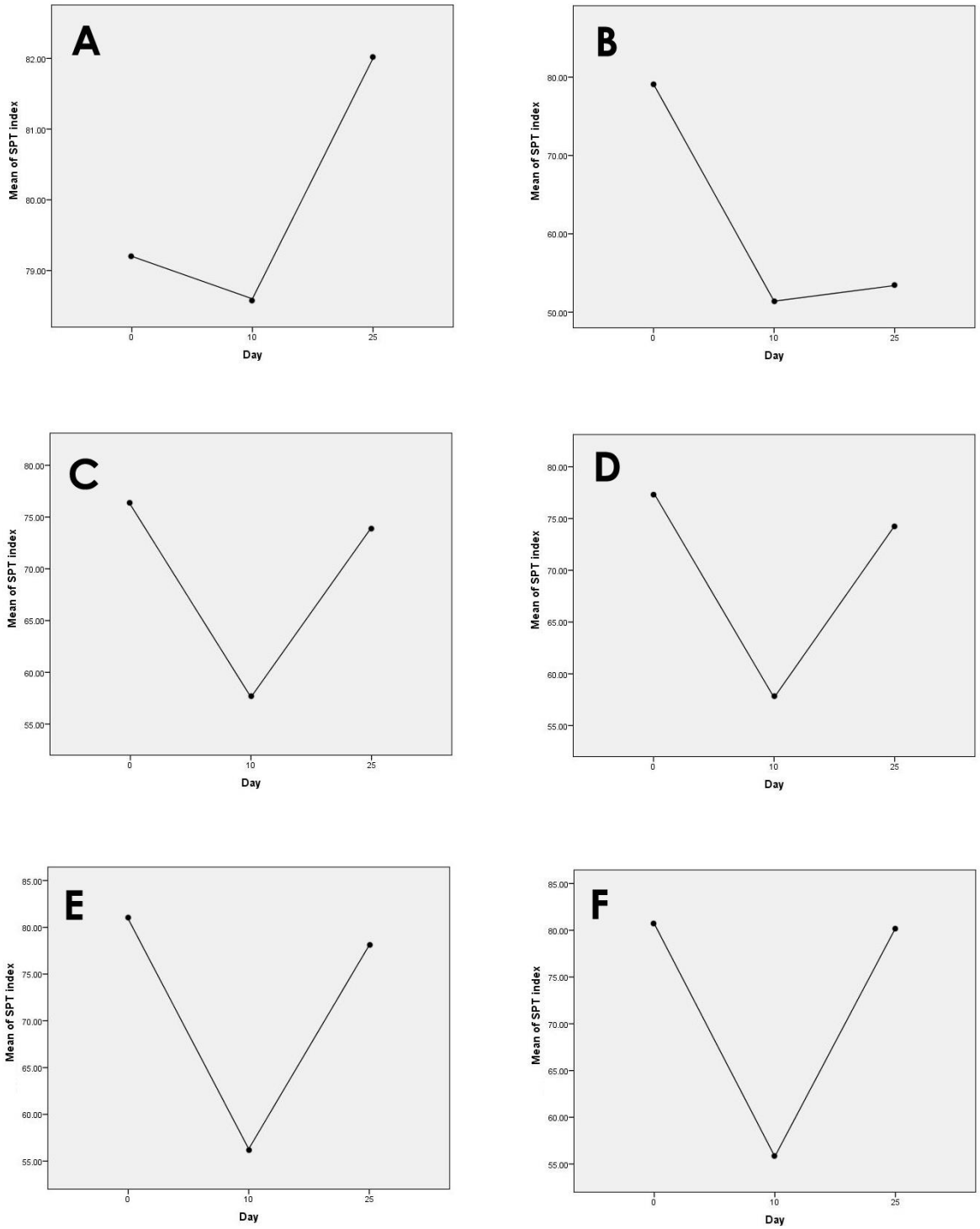


Figure 3.2: the means of SPT indexes on days 0, 10, and 25 for all groups.

A: group 1 (control group, untreated and unexposed to CUS), B: group 2 (untreated and exposed to CUS), C: group 3 (treated with 10 mg/kg fluoxetine for 14 days), D: group 4 (treated with 125 mg/kg *C. Myxa* extract for 14 days), E: group 5 (treated with 250 mg/kg *C. Myxa* extract for 14 days), F: group 6 (treated with 500 mg/kg *C. Myxa* extract for 14 days). no. of rats =10 for each group.

3.1.3. Open Field Test (OFT) results

3.1.3.1. Frequency of Rearing behavior within the group

There were no significant differences in means of rearings on days 10 and 25 as compared to day 0 in group 1 (control group, untreated and unexposed to CUS), whereas in group 2 (untreated and exposed to CUS), the means of rearing frequency on day 25 significantly increased (*P-value* <0.05)as compared to day 10 (Table 3.3 and Figure 3.3).

In groups 2, 3, 4, 5 and 6, the means of rearing frequency on day10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of rearing frequency on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day 10. (Table 3.3 and Figure 3.3)

Table 3.3 Comparison in the means of Rearings frequency ± SEM between groups on days 0, 10, 25.

Rearings	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 0	50.20±5.41	59.00±2.48	53.50±4.21	59.60±5.75	50.20±4.18	53.30±4.67
Day10	48.20±2.69	9.80± 1.43*	10.00± 2.21*	10.30± 1.81*	11.20± 1.63*	9.30± 1.48*
Day25	48.00±2.43	8.90±1.06*	45.70± 5.11 **	35.70± 3.82 **	39.10± 3.47 **	37.60± 4.90 **

* = significantly decreased (P <0.05) as compared with day 0,
** = significantly increased (P <0.05) as compared with day 10,
Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

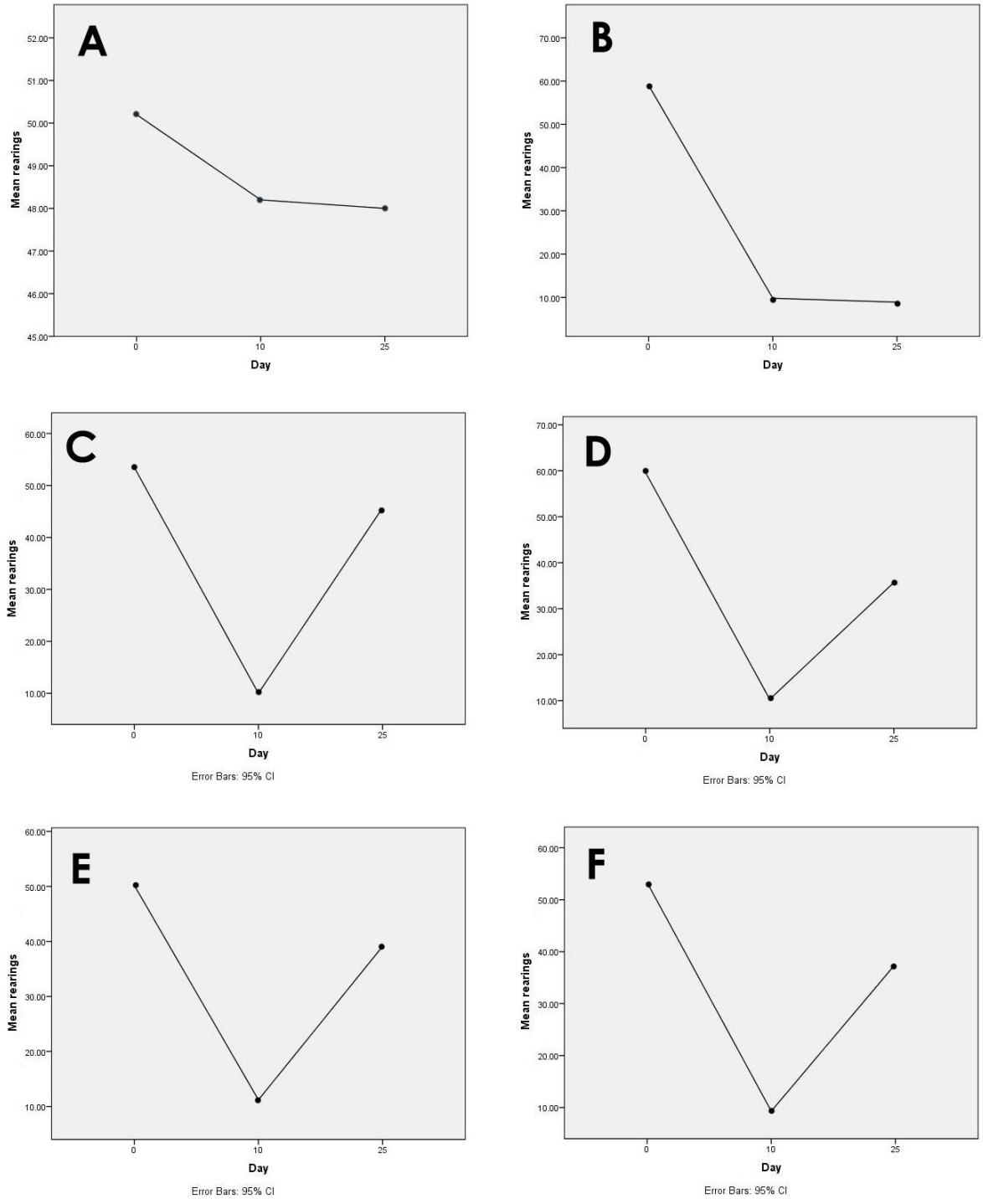


Figure 3.3: the means of Rearings frequencies on days 0, 10, and 25 for all groups.

A: group 1 (control group, untreated and unexposed to CUS), B: group 2 (untreated and exposed to CUS), C: group 3 (treated with 10 mg/kg fluoxetine for 14 days), D: group 4 (treated with 125 mg/kg *C. Myxa* extract for 14 days), E: group 5 (treated with 250 mg/kg *C. Myxa* extract for 14 days), F: group 6 (treated with 500 mg/kg *C. Myxa* extract for 14 days). no. of rats =10 for each group.

3.1.3.2. Frequency of line crossing within the group

There were no significant differences in means of line crossings on days 10 and 25 as compared to day 0 in group 1 (control group, untreated and unexposed to CUS), whereas in group 2 (untreated and exposed to CUS), the mean of line crossing frequency on day 25 significantly increased (*P-value* <0.05) as compared to 10 (Table 3.1 and Figure 3.1). (Table 3.4 and Figure 3.4)

In groups 2, 3, 4, 5 and 6, the means of line crossing frequency on day10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of line crossing frequency on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day 10. (Table 3.4 and Figure 3.4)

Table 3.4 Comparison in the means of Line-crossing frequency ± SEM between groups on days 0, 10, 25.

Line-Crossings	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 0	314.60± 21.58	353.00± 8.78	312.50± 16.17	311.10± 16.55	320.30± 12.29	301.40± 18.61
Day10	316.60± 8.58	121.90± 14.83*	121.50± 12.41*	104.00± 11.31*	128.60± 11.88*	122.80± 11.69*
Day25	307.70± 14.09	116.50± 14.78*	278.40± 20.55**	253.10± 13.02**	246.10± 10.69**	218.60± 8.08**

* = significantly decreased (P <0.05) as compared with day 0,
** = significantly increased (P <0.05) as compared with day 10,

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

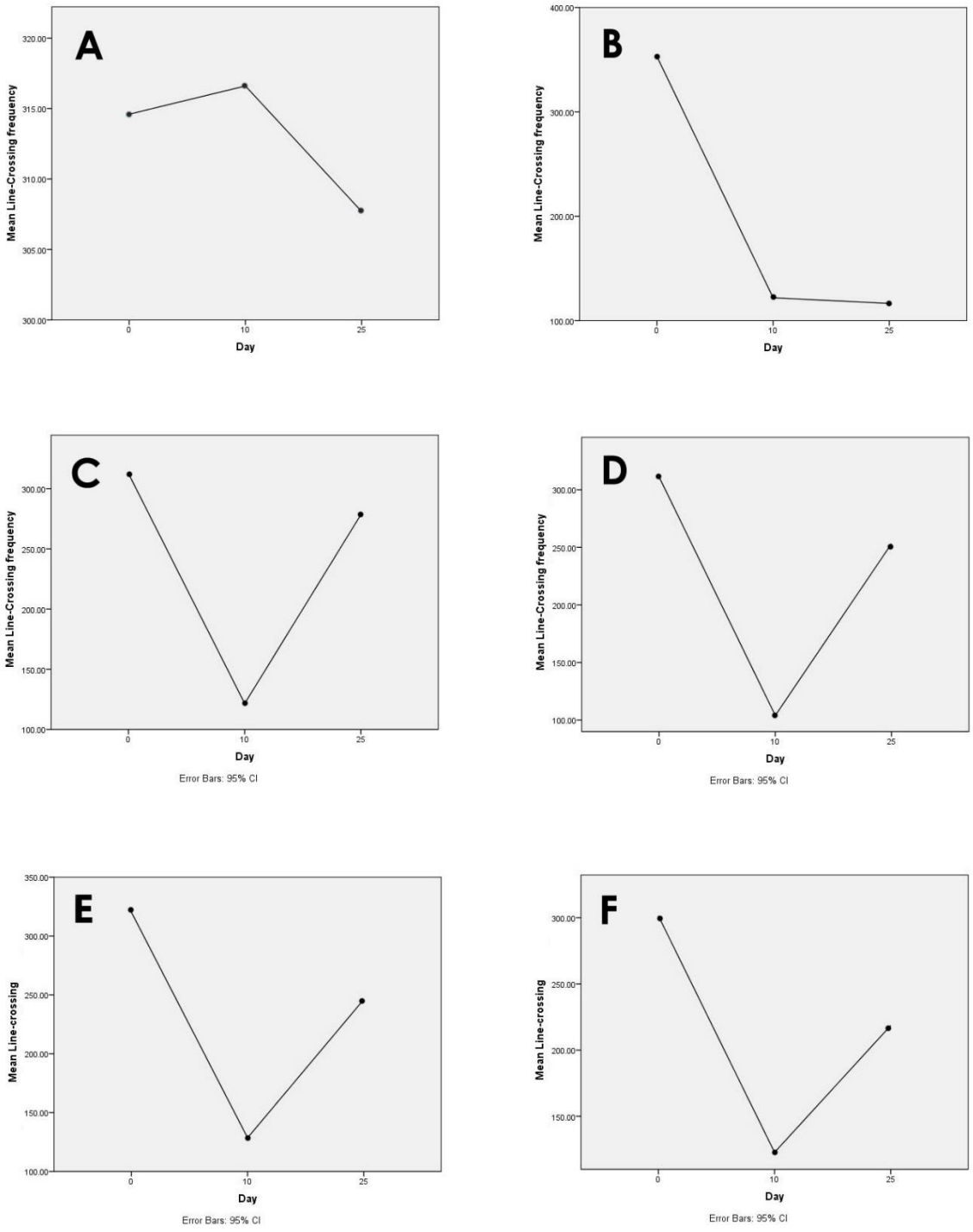


Figure 3.4: the means of Line-crossing frequencies on days 0, 10, and 25 for all groups.

A: group 1 (control group, untreated and unexposed to CUS), B: group 2 (untreated and exposed to CUS), C: group 3 (treated with 10 mg/kg fluoxetine for 14 days), D: group 4 (treated with 125 mg/kg *C. Myxa* extract for 14 days), E: group 5 (treated with 250 mg/kg *C. Myxa* extract for 14 days), F: group 6 (treated with 500 mg/kg *C. Myxa* extract for 14 days). no. of rats =10 for each group.

3.1.3.3. frequency of groomings within the group

There were no significant differences in number of groomings on days 10 and 25 as compared to day 0 in group 1 (control group, untreated and unexposed to CUS), whereas in group 2 (untreated and exposed to CUS), the mean of grooming frequency on day 25 significantly increased (*P-value* <0.05) as compared to 10 (Table 3.5 and Figure 3.5).

In groups 2, 3, 4, 5 and 6, the means of grooming frequency on day10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of groomingfrequency on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day 10. (Table 3.5 and Figure 3.5)

Table 3.5 Comparison in the means of Grooming frequency ± SEM between groups on days 0, 10, 25.

Groomings	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 0	7.80±0.90	6.60±0.94	6.80±0.44	5.50±0.40	8.10±0.99	5.60±0.70
Day10	7.30±0.63	0.60± 0.22*	0.90± 0.17*	1.10± 0.31*	1.30± 0.36*	1.30± 0.30*
Day25	7.70±0.78	1.10± 0.31*	4.80± 0.35**	4.70± 0.59 **	5.50± 0.68**	5.30± 0.55**

* = significantly decreased (P <0.05) as compared with day 0,
** = significantly increased (P <0.05) as compared with day 10,

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

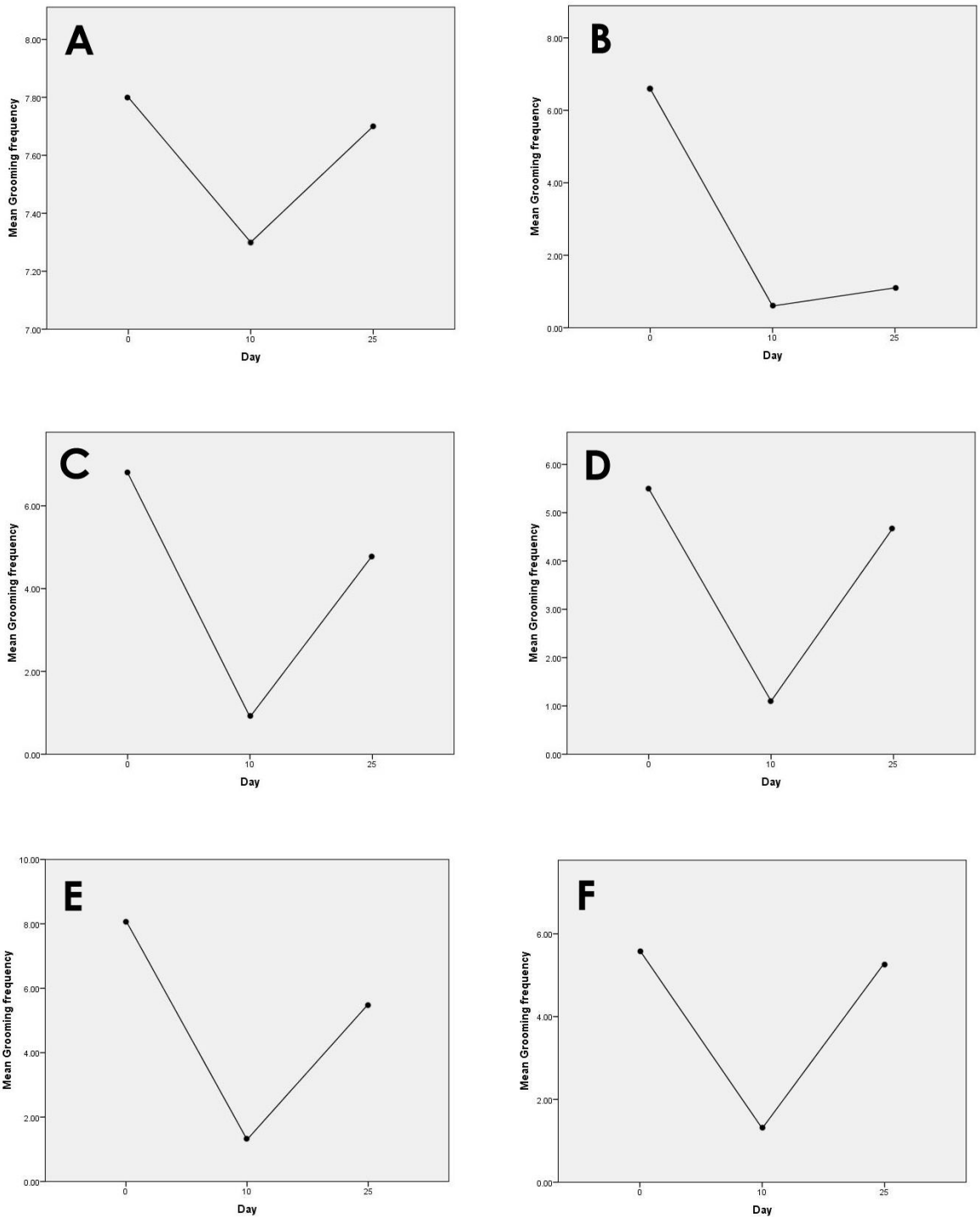


Figure 3.5: the means of Groomings frequencies on days 0, 10, and 25 for all groups.

A: group 1 (control group, untreated and unexposed to CUS), B: group 2 (untreated and exposed to CUS), C: group 3 (treated with 10 mg/kg fluoxetine for 14 days), D: group 4 (treated with 125 mg/kg *C. Myxa* extract for 14 days), E: group 5 (treated with 250 mg/kg *C. Myxa* extract for 14 days), F: group 6 (treated with 500 mg/kg *C. Myxa* extract for 14 days). no. of rats =10 for each group.

3.2. Forced swimming test, Sucrose preference test, and Open field test on Day 25

3.2.1. Forced swimming test on day 25

The mean of the immobility time of group 2 significantly increased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of the immobility time significantly decreased (*P-value* <0.05) as compared with group 2. (Table 3.6 and Figure 3.6).

3.2.2. Sucrose preference tests on day 25

In group 2, the mean of SPI significantly decreased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of SPI significantly increased (*P-value* <0.05) as compared with group 2 (Table 3.6 and Figure 3.7).

3.2.3. Rearing frequency in open field test on day 25

In group 2, the mean of rearing frequency significantly decreased (*P-value* <0.05) as compared with group1, while in groups 3,4, 5, and 6 the means of rearing frequency significantly increased (*P-value* <0.05) as compared with group 2. In groups 4 and 6 the mean of Rearing frequency significantly decrease (*P-value* <0.05) as compared with group 1 (Table 3.6 and Figure 3.8).

3.2.4. Line-crossing frequency in open field test on day 25

In group 2, the mean of Line-crossing frequency significantly decreased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of Line-crossing frequency significantly increased (*P-value* <0.05) as compared with group 2 (Table 3.6 and Figure 3.9) and in groups 4,5, and 6 the mean of Line-crossing frequency significantly decrease (*P-value* <0.05) as compared with group 1 (Table 3.6 and Figure 3.9). In group 5 , the mean of line crossing frequency significantly decreased (*P-value* <0.05) as compared with group 3 (Table 3.6 and Figure 3.9).

3.2.5. Grooming frequency in open field test on day 25

In group 2, the mean of Grooming frequency significantly decreased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of Grooming frequency significantly increased (*P-value* <0.05) as compared with group 2 (Table 3.6 and Figure 3.10).

Table 3.6: comparison between the means of immobility time (FST)±SEM, the means of SPI±SEM , means of rearing frequency (OFT)±SEM, means of line-crossing frequency (OFT)±SEM, and means of grooming frequency (OFT)±SEM on day 25 for all groups.

Test	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
FST (immobility Time in seconds)	47.70± 1.26	67.00± 5.55 **	38.90± 2.19*	49.20± 2.25*	38.10± 1.87*	48.90± 1.13*
SPI (Sucrose preference index)	82.00± 1.90	53.40± 1.70****	74.00± 1.69 ***	74.30± 1.55 ***	78.10± 2.36 ***	80.20± 1.23 ***
OFT (Rearing frequency)	48.00± 2.43	8.90± 1.06****	45.70± 5.11 ***	35.70± 3.82 ***	39.10± 3.47 ***	37.60± 4.90 ***
OFT (Line-Crossing frequency)	307.70± 14.09	116.50± 14.78****	278.40± 20.55 ***	253.10± 13.02 ***	246.10± 10.69 ***	218.60± 8.08 ***
OFT (Grooming Frequency)	7.70± 0.78	1.10± 0.31****	4.80± 0.35 ***	4.70± 0.59 ***	5.50± 0.68 ***	5.30± 0.55 ***

*= significantly decrease (*P value* <0.05) as compared with group 2,
** = significantly increase (*P value* <0.05) as compared with group 1,
*** = significantly increase (*P value* <0.05) as compared with group 2,
**** = significantly decrease (*P value* <0.05) as compared with group 1.

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

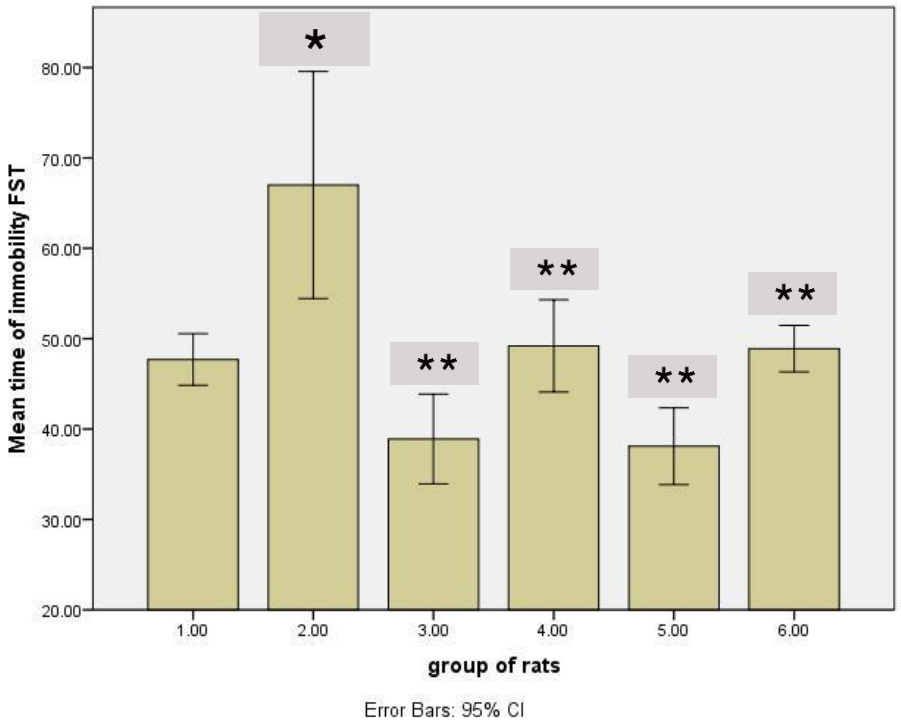


Figure 3.6: Means ± SEM of the immobility time of forced swimming test on day 25 for all groups.

* = significantly increase (*P value* <0.05) as compared with group 1,
**= significantly decrease (*P value* <0.05) as compared with group 2.

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

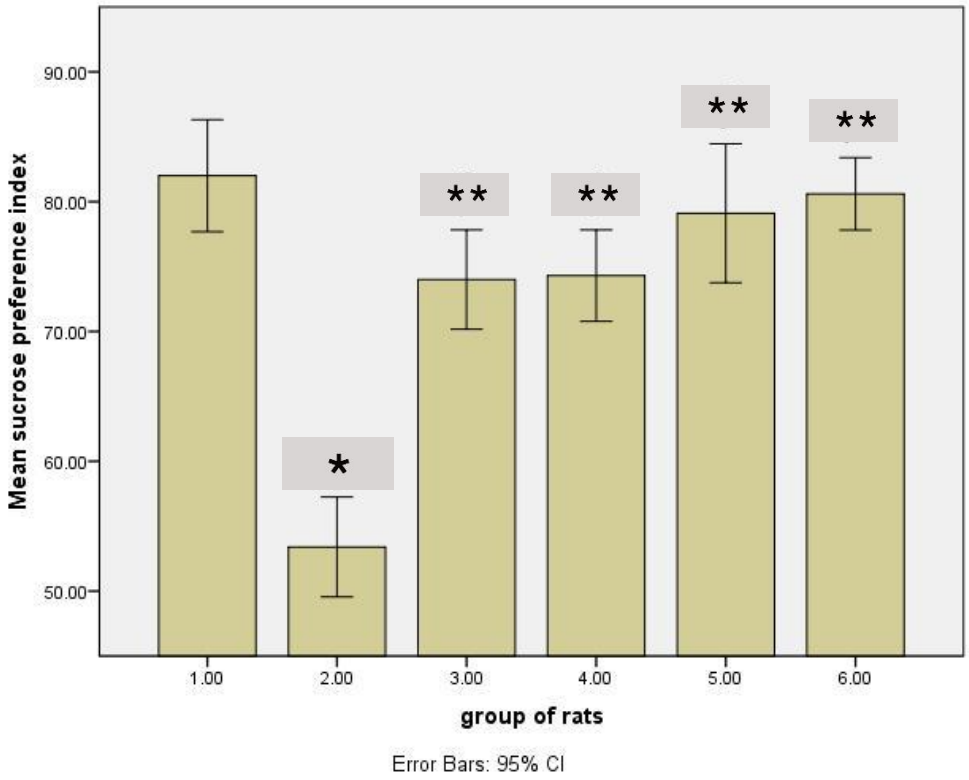


Figure 3.7: Means ± SEM of the SPT index of sucrose preference test on day 25 for all groups.

*= significantly decrease (*P value* <0.05) as compared with group 1

** = significantly increase (*P value* <0.05) as compared with group 2.

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

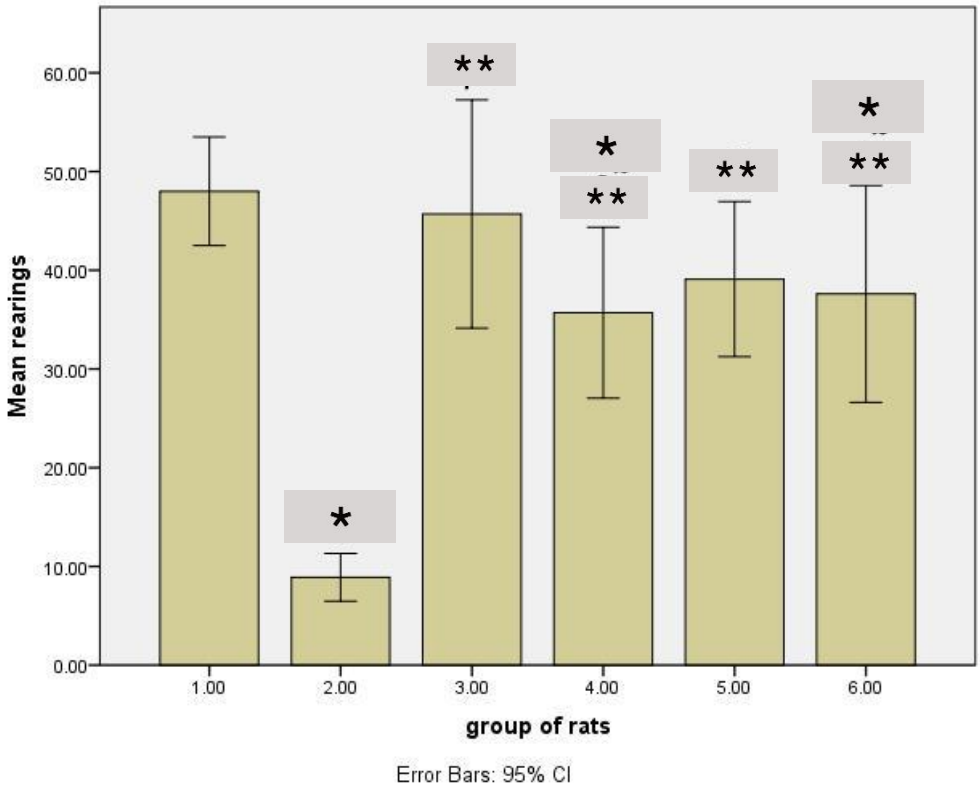


Figure 3.8: Means ± SEM of the rearing frequencies in open field test on day 25 for all groups.

* = significantly decrease (*P value* <0.05) as compared with group 1

** = significantly increase (*P value* <0.05) as compared with group 2

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

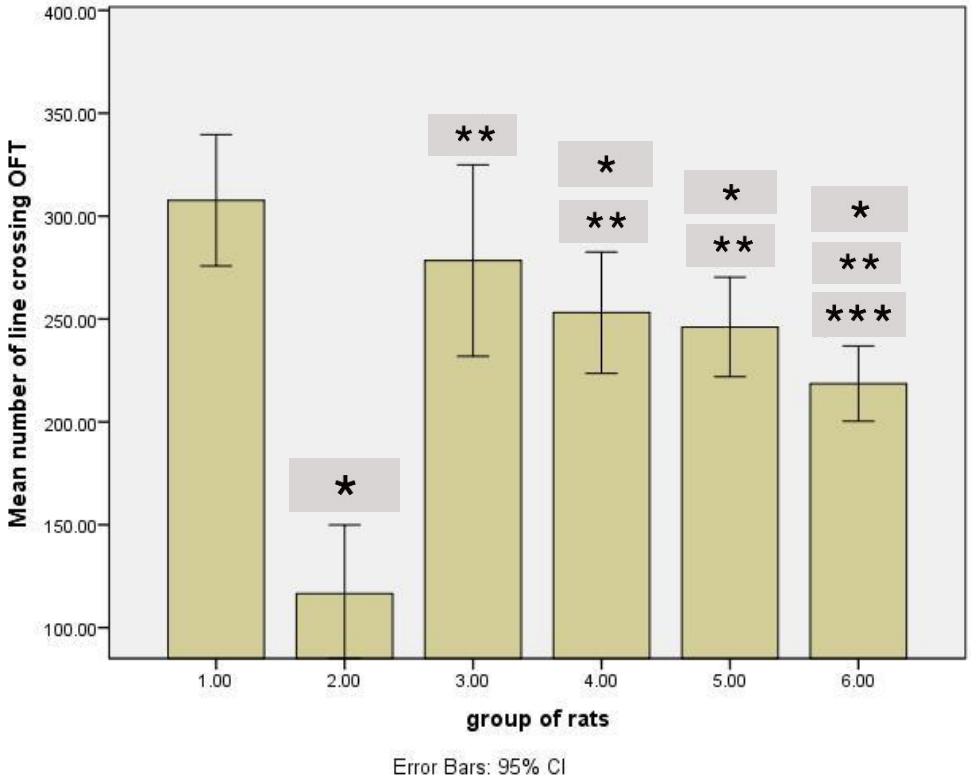


Figure 3.9: Means ± SEM of line-crossings frequencies in open field test on day 25 for all groups.

* = significantly decrease (*P value* <0.05) as compared with group 1
** = significantly increase (*P value* <0.05) as compared with group 2,
*** = significantly decrease (*P value* <0.05) as compared with group 3.

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

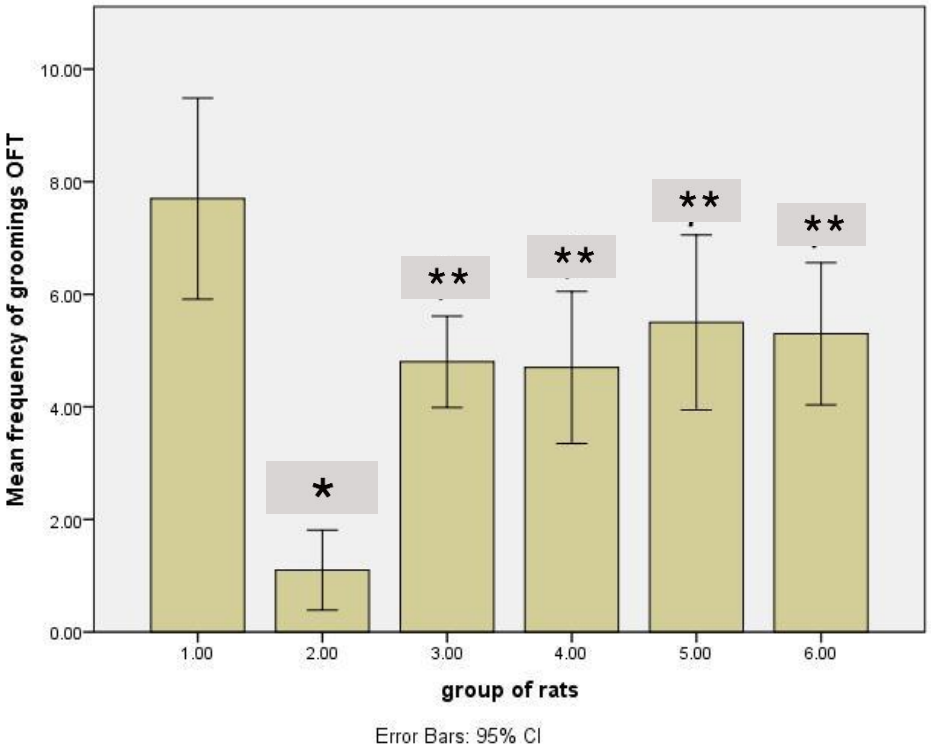


Figure 3.10: Means ± SEM of the Groomings frequencies in open field test on day 25 for all groups.

** = significantly increase (*P value* <0.05) as compared with group 2.
* = significantly decrease (*P value* <0.05) as compared with group 1

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

3.3. Biochemical tests results after day 25

3.3.1. Interleukin-6 (IL-6)

The means of the IL-6 concentrations in brain tissue of group 2 significantly increased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of the IL-6 concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group 2. (Table 3.7 and Figure 3.11).

3.3.2. Superoxide dismutase enzyme concentration (SOD)

In group 2, the means of the SOD concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group1, while in groups 4, 5, and 6 the means of the SOD concentrations in brain tissue significantly increased (*P-value* <0.05) as compared with group 2. In group 3 there are no significant increases in the means of the SOD concentrations in brain tissue as compared with group 2 that (*P-value* > 0.05) (Table 3.7 and Figure 3.12).

3.3.3. Catalase enzyme concentration (CAT)

In group 2, the means of the CAT concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group1, while in groups 4, 5, and 6 the means of the CAT concentrations in brain tissue significantly increased (*P-value* <0.05) as compared with group 2. In group 3 there are no significant increases in the means of the CAT concentrations in brain tissue as compared with group 2 that (*P-value* > 0.05) (Table 3.7 and Figure 3.13).

Table 3.7: Elisa results of IL-6, SOD and CAT concentrations in brain tissue

Concentrations	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
IL-6 (ng/L)	8.8257±0.42629	15.5160±0.71749 **	9.3387±0.62002*	10.1393±0.42055*	9.3183±0.57823*	10.7692±0.55333*
SOD (ng/ml)	5.0756±0.31169	3.7913±0.54339****	4.4937±0.51124	5.3509±0.24535 ***	5.8615±0.35779 ***	5.7067±0.33619 ***
CAT (ng/ml)	34.7461±1.90833	27.2349±1.72104****	33.8669±2.01764	36.8036±2.25583 ***	40.3442±2.66987 ***	37.2042±1.83820 ***

*= significantly decrease (*P value* <0.05) as compared with group 2.
** = significantly increase (*P value* <0.05) as compared with group 1,
*** = significantly increase (*P value* <0.05) as compared with group 2,
**** = significantly decrease (*P value* <0.05) as compared with group 1,

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

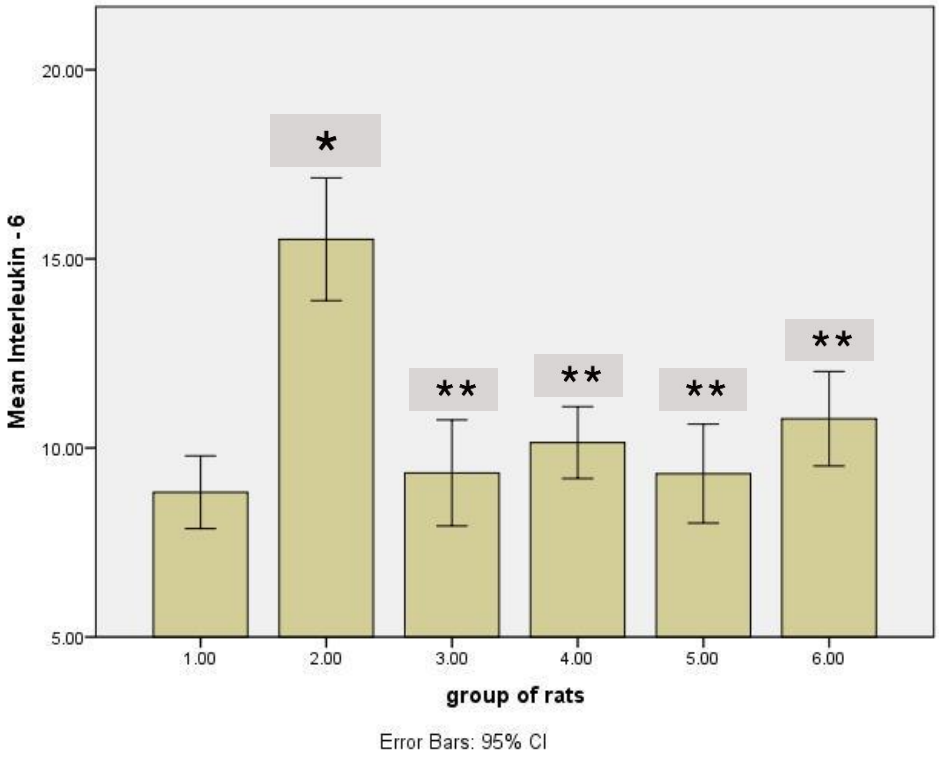


Figure 3.11: Means ± SEM of the Interleukin-6 concentrations (ng/L) in brain tissue for all groups.

** = significantly decrease (*P value* <0.05) as compared with group 2
* = significantly increase (*P value* <0.05) as compared with group 1

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

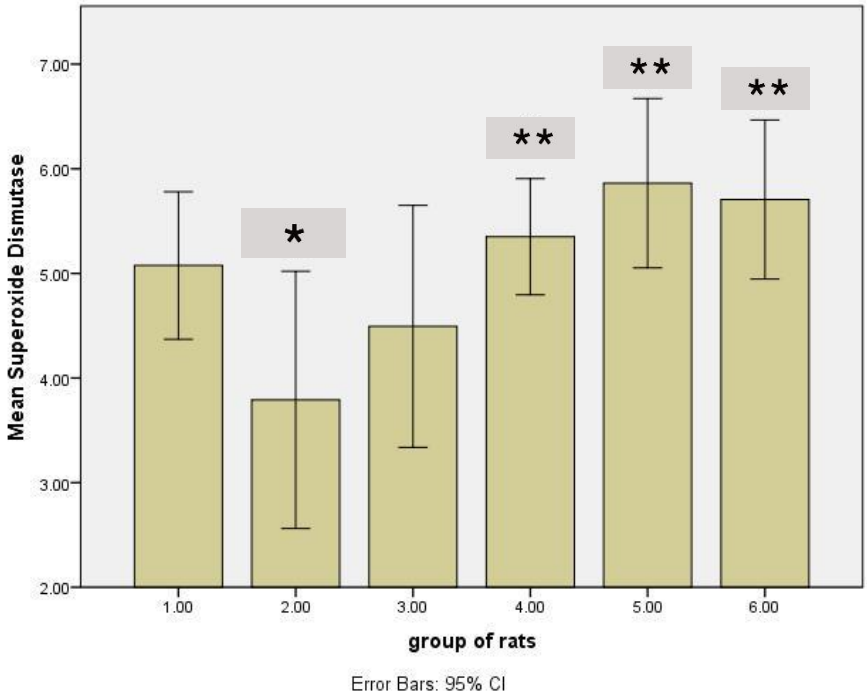


Figure 3.12: Means ± SEM of the Superoxide dismutase enzyme concentrations (ng/ml) in brain tissue for all groups.

* = significantly decrease (*P value* <0.05) as compared with group 1

** = significantly increase (*P value* <0.05) as compared with group 2

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

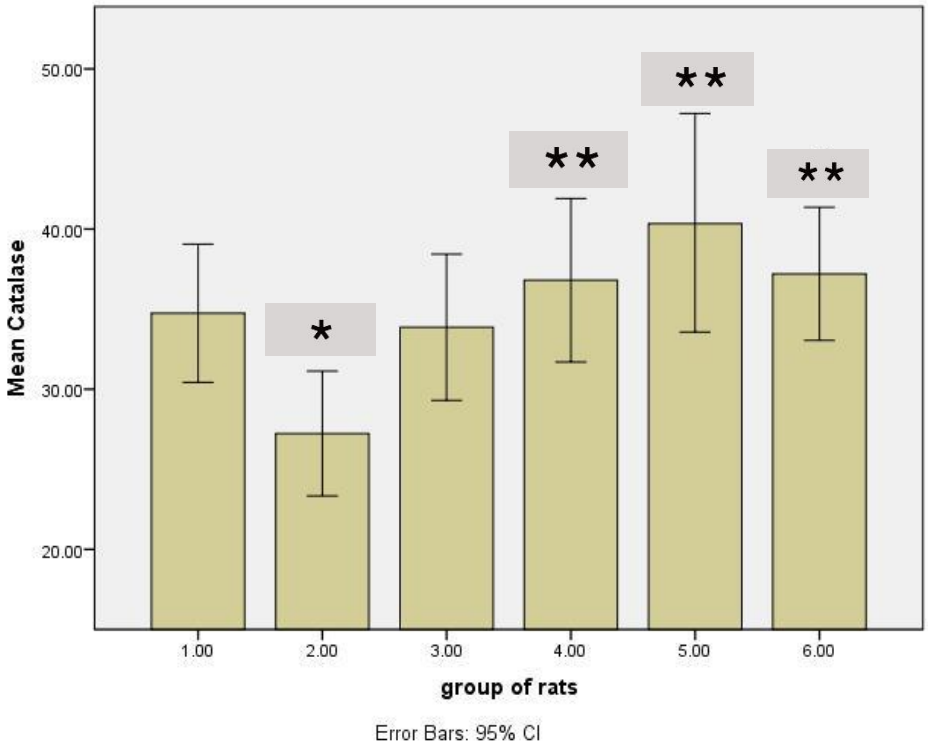


Figure 3.13: Means ± SEM of the Catalase enzyme concentrations (ng/ml) in brain tissue for all groups.

* = significantly decrease (*P value* <0.05) as compared with group 1

** = significantly increase (*P value* <0.05) as compared with group 2

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

4.1. Chronic Unpredictable/Mild Stress (CUS) Model:

Animal models created with maternal separation and chronic unpredictable stress-exposure, for example, are commonly used models that simulate stress experienced by humans during childhood and maturity. (Zhang *et al.*, 2017)

Several studies have found that CUS can cause long-term behavioral changes that are similar to clinical depressive symptoms. As a matter of fact, this study looked into the anti-depressant effect of Cordia Myxa fruit extract against CUS-induced depression using behavioral tests like the forced swim test FST, open field test OFT, and sucrose preference test SPT, as well as the effect on inflammatory mediators like interleukin-6 and antioxidant enzyme systems like superoxide dismutase SOD and catalase CAT in the brain tissues of rats. This is the first study that we are aware of that correlates the effects of C. Myxa extract on the chronic stress-induced male rat.

The immobility duration, sucrose preference index, and rates of rearings, groomings and line crossings in OFT were not significantly different between animals from all groups on day 0 (baseline) in this study. When compared to baseline (day 0), there was a substantial increase in immobility time during FST, a significant decline in sucrose preference during SPT, and rates of rearings, groomings and line-crossings in OFT for all groups of rats exposed to CUS after 10 days of unpredictable stress. This indicates that these animals have evolved a depression model. These results agree with previous studies had found that rats acquired a behavioral model of depression after being exposed to unpredictable stress procedures utilizing various stressors, as seen by increased immobility periods in behavioral despair tests, notably the tail suspension test TST and forced swimming test FST. Furthermore, this model causes a reduction in sucrose consumption as well as a decrease in self-care and motivating behavior (Isingrini et al., 2010).

In a forced swimming test, CUS has been shown to significantly increase immobility time, which indicates "behavioral despair" and hence may reflect

"recurrent thoughts of death," a common hallmark of severe depression in humans (Wang, An and Zhang, 2008).

The reduction in sucrose consumption is thought to represent anhedonia in animals, which is one of the two fundamental symptoms needed to diagnose a severe depressive episode in humans. (Zhang *et al.*, 2017)(Sequeira-Cordero *et al.*, 2019)(Moretti *et al.*, 2012)

The Open field test OFT was used as behavioral index of locomotor activity, exploratory activity and anxiety-like behavior. This test introduced in our research to strength interpretation of forced swim test findings. Our results agree with Hazra findings that after exposure to 10 days of CUS, the number of rearing and line crossing in OFT significantly decreased (Hazra *et al.*, 2017). The decreased activity in open field arena contrast with group 1 (rats not exposed to CUS) that show increased rearing, line crossing and grooming, which is driving by the interests of rats to explore a new environment. However, rats show reduced rearing, line crossing and grooming in an unfamiliar open field after CUS , which might imply a "refractory loss of interest", which is another fundamental characteristic of human severe depression (Wang, An and Zhang, 2008).

It is worth noting that the results of the open field test can be also affected by several factors which are mentioned in previous studies including age, and circadian rhythm. In addition, adaptation, room temperature, humidity, lighting, noise, and even odor can affect assessment outcomes. (Tatem *et al.*, 2014)

Several pathways have been postulated for CUS to produce depressive-like behavior. The first is BDNF, which affects neural plasticity, suppresses cell death cascades, and promotes cell survival proteins necessary for the proliferation and maintenance of central nervous system neurons, suggesting that it might have a role in the development and treatment of depression. It was discovered in experimental

studies that a decrease in BDNF expression in the hippocampus and frontal cortex among animals exposed to chronic stress can be reversed by antidepressant treatment showed that conditional BDNF knockout mice displayed an increase in depression-like behavior, as measured by the forced swim test and sucrose preference tests, demonstrating that BDNF deficit can be reversed by antidepressant treatment. These findings support the function of BDNF in depression and suggest that upregulating BDNF expression may help antidepressants work more effectively. (Banerjee *et al.*, 2014)

The function of astrocytes in the genesis of depression may provide some clues for the mechanism investigation. The quantity and volume reduction of hippocampal astrocytes following exposure to a range of environmental stresses supports the theory that glial atrophy is an etiological component in depression. In addition to their housekeeping tasks, astrocytes, which are the primary producers of numerous neurotrophic factors, have been discovered to be important regulators of postnatal neurogenesis, neuronal development, maintenance, and plasticity. Reduced neurotrophic factor availability can lead to increased cellular susceptibility or possibly cell death. By decreasing the synthesis of neurotrophic factors, glial atrophy can cause functional deficits in neuronal activity, increased neuronal susceptibility, or even neuronal death, and a reduction in postnatal neurogenesis. Stress can decrease the expression of neurotrophic factors generated by glia cells, as well as the number and size of astrocytic cells in the hippocampus, which can be avoided by long-term antidepressant therapy. (Q. Liu *et al.*, 2011)

A meta-analysis of neuroimaging data from people with depression shows that cortical GABA levels (anterior cingulate and/or occipital cortex) are consistently lower, and pharmacotherapeutic treatment outcomes in people with treatment-resistant depression show that ketamine can quickly relieve depressive symptoms. These disparate lines of evidence point to GABA's role in depression pathogenesis and glutamate's role in its therapy. (Perrine *et al.*, 2014)

CUS may have caused monoaminergic stimulation of stress-related glutamate neurotransmission, resulting in excitotoxic damage in the apical dendritic field of cortical pyramidal cells and the observed cognitive impairment. (Bondi *et al.*, 2008)

Histone acetylation, which has been related to depression, is another mechanism (histones are composed of mostly positive charged amino acid residues such as lysine and arginine that provide structural support to a chromosome). Histone acetylation, particularly targeting the N-terminal lysine residues in histone 3 (H3) and histone 4 (H4), has raised concerns in depression because it affects the transcription of certain genes, including brain-derived neurotrophic factor (BDNF) and serotonin transporter. The rate-limiting enzymes for the production of monoaminergic transmitters are tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) (such as norepinephrine, dopamine, and serotonin). Histone acetylation and regulation of these enzymes' production, TH, and TPH, in the hippocampus, are linked in the CUS-induced depressed model of rats, suggesting that they may play a role in the genesis of depression. (Liu *et al.*, 2014)

Finally, clinical and laboratory studies show that chronic stress causes oxidative stress, which causes an imbalance in the body's free radical generation and antioxidative system. Reactive oxygen species (ROS)-based oxidative stress may damage proteins, lipids, and DNA, as well as cause chromatin remodeling by interfering with histone acetylation and deacetylation. (Rahman, Marwick and Kirkham, 2004)

Furthermore, in group 1, glucose intake was substantially higher on day 25 compared to day 0 and day 10. This might be due to glucose's rewarding impact. When feeding was limited with planned sucrose availability, the rat dopamine transporter was up-regulated in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) of the brain. Dopamine regulates dopaminergic activation of the reward region of the brain by interacting with several strong neurotransmitters such

as serotonin, enkephalins, and GABA. Additionally, by activating the β3 adrenoceptors, dopamine can decrease uptake of glucose in rat white adipocytes that lack dopaminergic receptors. (Klietz *et al.*, 2019)

When compared to group 1, group 2 (untreated and exposed to CUS) had depressive-like behavior on day 25, as evidenced by a substantial increase in immobility time during FST, a significant reduction in the sucrose preference index during SPT, and lower rates of rearing and line-crossing. These findings matched those of a prior research (Q. Liu *et al.*, 2011). In comparison to group 2, there was a substantial decrease in immobility durations during FST and a significant rise in sucrose preference index during SPT, and increased frequencies of rearing, line-crossing in OFT in groups 3,4,5, and 6. The antidepressant properties of fluoxetine and C. Myxa fruit extracts were shown by these findings.

Moreover, this study has revealed that after the treatment of rats with either fluoxetine or three concentrations of Cordia Myxa fruit extract; antidepressant effect has been shown. That, the immobility time was lowered, sucrose preference index were significantly increased, and increased rearing, line crossing and grooming in open field on day 25 as compared with day 10 for group 3 rats (treated with 10 mg/kg fluoxetine for 14 days). This findings has been demonstrated in the previous study (Shen *et al.*, 2019). Shen et al. have reported that fluoxetine increased serotonin levels in the brain, where insufficient levels of monoamine neurotransmitters, particularly dopamine (DA), 5-HT, and NE, are related to the neurobiological mechanism of depression.

The antidepressant action of fluoxetine has been linked to a number of mechanisms. In people suffering from depression, activation of the serotonergic system, for example, can enhance the release of DA. Serotonergic neurons connect to the mesolimbic dopamine system and control dopamine transmission via several serotonin receptor subtypes. Serotonergic activity has been found to impact the NAc

(a prominent candidate for reward and pleasure processing). (Kranz, Kasper and Lanzenberger, 2010)

The neurotrophic theory of depression proposes that low levels of the neurotrophin BDNF may contribute to the development of mood disorders, and fluoxetine has been found to raise BDNF levels in limbic areas. Fluoxetine, in particular, has been demonstrated to enhance BDNF mRNA levels in the meso-corticolimbic pathway specifically. The ventral tegmental area, prefrontal cortex, and shell region of the nucleus accumbens in the hippocampus have all been found to have considerably elevated BDNF levels. Fluoxetine therapy increases BDNF levels not only in limbic but also in the dopaminergic regions, which may help improve the function of this system in depressed people. (Molteni *et al.*, 2006)

Depression has lately been linked to the activation of the inflammatory response system, as research has shown an increase in the production of proinflammatory cytokines such as interleukin-1b (IL-1b), IL-6, and interferon-c (IFN-c). Increased production of proinflammatory cytokines might have a role in depression's genesis. Indeed, in animal and human studies, IL-1, IL-6, and IFN-c were found to cause behavioral changes and symptoms comparable to those seen in depression. An inability to experience pleasure, anorexia, weight loss, retreat from social settings, psychomotor slowness, a lack of energy, impatience, and sleep problems are only a few of the symptoms. Fluoxetine has been shown to have anti-inflammatory and pain-relieving properties in experimental inflammation models. The mechanism by which this medication decreases inflammation, however, is still unknown yet. (Caiaffo *et al.*, 2016)

There is limited information on fluoxetine's effect on antioxidant enzymes. While some research suggests that this antidepressant improves antioxidant capacity in the brain, others claim that it affects the liver antioxidant system in rats (Caiaffo *et al.*,

2016). Pro-inflammatory cytokines and prostaglandin E2, which are implicated in increasing ROS, have been found to be inhibited by fluoxetine. It's been proposed that protein kinase A is involved in its inhibitory actions. Fluoxetine, according to a research by Desai et al., is beneficial in reducing the negative effects of oxidative stress on peripheral defense cells. Because Fluoxetine therapy reduces stress-induced oxidative damage, this research suggests that an improvement in cellular oxidative state might be a key mechanism underpinning Fluoxetine's protective pharmacological benefits in the treatment of depressive disorders. (Desai, Kori and Aladakatti, 2017)

After 10 days of CUS exposure, there was a substantial increase in immobility time and a significant drop in the sucrose preference index in groups 4,5 and 6 (treated with C. Myxa extract 125 mg/kg, 250 mg/kg, and 500 mg/kg, respectively) as compared to day 0 (baseline). This illustrates a depressed state and a depression model.

On day 25, there was a substantial decrease in immobility time, a significant increase in the sucrose preference index and activity in open field arena as compared to day 10 after treatment with various doses of C. Myxa for 14 days from CUS. C. Myxa was given repeatedly, and the effectiveness was equivalent to that of the clinically active antidepressant fluoxetine. C. Myxa's capacity to reverse CUS-induced anhedonia-like behavior, reduce immobility time, and increase exploratory and locomotor activity in open field test lends credence to the hypothesis that it possesses antidepressant properties.

4.2. Biochemical Tests

Stress is a pathogenic component that promotes neuroinflammation and neuronal degeneration (Knutson, 2014). At the end of the experiment, each animal was decapitated and the brain was removed from the skull and homogenized to evaluate

the antioxidant enzyme system concentrations superoxide dismutase (SOD) and catalase (CAT) and the inflammatory response system (interleukin 6).

4.2.1. Interleukin 6 (IL-6)

According to previous studies, several inflammatory and oxidative cytokines have been implicated in the mechanisms underpinning the occurrence of depression. Several lines of evidence point to this conclusion that interleukin-1 and interleukin-6 (IL-1 and IL-6) may play a role in the development of major depression as well as in antidepressant treatment therapeutic processes. (Zong *et al.*, 2018)

In group 2 (exposed to CUS, and receive normal saline after 10 days from beginning of CUS for 14 days), There was significant increases in interleukin 6 (IL-6) concentrations in brain tissues as compared with group 1 (control ; untreated and unexposed to CUS). This finding supports previous study that revealed IL-6 production was substantially greater in MDD patients than in healthy controls, and that monocytic proinflammatory cytokines have a role in MDD (Kim *et al.*, 2007). So, This finding reinforces the Cytokine Hypothesis. External (psychological) and internal (organic inflammatory illnesses or disorders) stresses can both trigger the inflammatory process, that proinflammatory cytokines increased and play role in development of depression. according to the cytokine theory of depression (Song *et al.*, 2009).

Chopra *et al*, also reported the similar results that immunological deregulation in MDD is linked to the activation of monocytic proinflammatory cytokines (ILs and TNF), as well as the suppression of both Th1 and Th2 cytokines in addition TGF-beta1 may have a role in the control of monocytic cytokines, as well as Th1 and Th2 cytokines (Chopra, Kumar and Kuhad, 2011). Our results meet previous Raison *et al.* findings that Increased plasma and CSF concentrations of a number of cytokines

and their receptors, including IL-1, IL-2, IL-6, and TNF-a, are linked with immunological activation in major depression. (Raison *et al.*, 2006)

The effects of cytokines on behavior are thought to be linked to their impacts on neurotransmitter and neuropeptide function, synaptic plasticity, and neuroendocrine functions. The effects of cytokines on neuroendocrine function in depression might be linked to their effects on the glucocorticoid receptor (GR) and signaling pathways that contribute to glucocorticoid resistance. Glucocorticoid resistance may be an adaptive mechanism that allows inflammatory healing to proceed despite glucocorticoid levels caused by stress. (Pace, Hu and Miller, 2007)

Increases in IL-6 concentrations affect the metabolism of neurotransmitters such as serotonin and dopamine and thus influences their synthesis, release, and reuptake. Cytokines activate enzyme Indolamine 2,3 dioxygenase which causes conversion of tryptophan to kynurenin and thus favoring glutamate synthesis instead of conversion of tryptophan to serotonin. Cytokine also modulate serotonin signaling by elevate expression of monoamine transporters that increases the reuptake of serotonin. (Roohi, Jaafari and Hashemian, 2021)

Indeed, in animal and human studies, IL-1, IL-6, and IFN-c were found to cause behavioral changes and symptoms similar to those seen in depression, including an inability to feel pleasure, anorexia, weight loss, withdrawal from social situations, psychomotor retardation, irritability, and sleep disorders. (Makkonen *et al.*, 2011)

In group 3 (exposed to CUS and received fluoxetine treatment for 14 days), there was significant decrease in interleukin-6 (IL-6) concentrations as compared with group 2 (untreated and exposed to CUS). According to this finding, fluoxetine have anti-inflammatory properties and this confirm with previous researches that shown that fluoxetine can lower the production of prostaglandin E2 and proinflammatory cytokines, as well as lessen the symptoms of major depression caused by IL-1 (Song *et al.*, 2009). Our result also supports Liu et al. findings that demonstrate anti-

inflammatory activity of fluoxetine that reduces the production of IL-6, TNF-a, and nitric oxide in microglia (D. Liu *et al.*, 2011)

Although the molecular mechanism by which fluoxetine exerts anti-inflammatory activity is unclear, some authors indicate that fluoxetine operates by lowering gene expression as seen by lower transcription levels of mRNA of IL-6 and TNF-a . Additionally, fluoxetine may block the phosphorylation of mitogen activated protein kinase, a key signaling route for proinflammatory cytokines, as well as the activation of nuclear factor kappaB (NF-kB), a key inflammatory signaling molecule. (Caiaffo *et al.*, 2016)

Fluoxetine can act by inhibiting NF-kB, can reverse the polarization of modified macrophages, suggesting that fluoxetine could be a useful drug for reprogramming macrophages to favor the host in inflammatory situations. (Ghosh *et al.*, 2015)

In group 4, 5, 6 (exposed to CUS and treated with three diffirent concentration of C. Myxa extract 125mg/kg, 250mg/kg, 500mg/kg respectively), there was significant decrease of interleukin-6 (IL-6) concentrations as compared with group 2 (received normal saline 14 days and exposed to CUS), So the plant extract have anti-inflammatory action. Polyphenols, alkaloids and flavonoids in plants are known to exert active anti-inflammatory effects in the plant extract (Olukunle *et al.*, 2011).

According to Afzal *et al.* study, the high polyphenolic content of C. myxa fruit may be responsible for its preventive effect against liver injury in rats that prevent inflammation (Afzal *et al.*, 2009). An investigations on the anti-inflammatory effects of C. myxa fruit in an experimental colitis model and found that C. Myxa preparation inhibits the oxidative stress factors that lead to colitis progression, resulting in improvement in total antioxidant status, and return to normal levels. Furthermore, in terms of bioavailability, C. Myxa fruit is a great provider of numerous nutrients (Al-Awadi *et al.*, 2001).

Arachidonic-acid-derived eicosanoids modulate the production of pro-inflammatory and immunoregulatory cytokines. Phenolic compounds were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways. This inhibition reduces the release of arachidonic acid. The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, thus diminishing the formation of these inflammatory metabolites. (Nijveldt *et al.*, 2001)

The Cordia myxa fruit's anti-inflammatory properties can be also linked in part to its antioxidant properties and the restoration of trace element levels in inflamed tissues. (Ranjbar *et al.*, 2013)

4.2.2. Oxidative stress and estimation of Superoxide Dismutase (SOD) & Catalase (CAT) concentrations in Brain tissues

The brain has a number of antioxidant systems that help to counteract the negative effects of ROS. However, due to the activation of phagocytic cells, the loss of antioxidant efficacy and changes in the pro-inflammatory cytokine system in depression leads to an increase in free radical formation and development of oxidative stress according to previous reports. (Pizzino *et al.*, 2017)

One of the primary causes of neurodegeneration and the progression of depression and its symptoms is oxidative stress. Chronic stress causes the activation of various proapoptotic proteins, the production of excessive ROS, and necrosis, resulting in neurodegeneration, which plays an important part in the pathophysiology of depression. The brain homogenate's antioxidant capacity is a sign of oxidative stress. (Rai *et al.*, 2019)

The effect of the CUS paradigm on oxidative stress-related parameters in the rat brain, such as antioxidant enzyme system SOD and CAT was investigated in our study.

In group 2 (exposed to CUS and received normal saline for 14 days), there was significant decrease in superoxide dismutase (SOD), catalase (CAT) levels as compared with group 1 (control; untreated and unexposed to CUS) that means development of oxidative stress status and elevated levels of free radicals which are superoxide radicals and hydrogen peroxide radicals due to consumption of enzymes.

Lower antioxidant levels define CUS-induced depression in rats and also reported in previous study that CUS has been shown to elicit depressive and anxiety-like symptoms and lower SOD and GPX levels (Hu *et al.*, 2016) . Overproduction of ROS results from the activation of immune cells by pro-inflammatory cytokines, which leads to an increase in lipid peroxidation levels. Overproduction of ROS interferes with the structure and ratio of polyunsaturated fatty acids (PUFA), causing a loss of fluidity in the biological membrane. As a result, the biological membranes trigger cytokine production as a result of these changes. Increased lipid peroxidation, on the other hand, activates phospholipase A2, which alters receptor activities in cell membranes, activates immune cells, and causes T cells to secrete ILs, potentially increasing lipid peroxidation. (Chopra, Kumar and Kuhad, 2011)

Previous study shows that superoxide generation was enhanced in all brain areas studied in a CUS rat model, which mimics some of the symptoms of a severe depressive episode in humans. (Lucca *et al.*, 2009)

Increased monoamine metabolism could be a factor in increased ROS production in depression. Sanacora *et al.*, noted that depression is associated with increased glutamatergic transmission. In the presence of high glutamate levels, can cause excitotoxicity by allowing pathologically high levels of calcium to enter the cell,

which can stimulate the production of reactive oxygen species (ROS). (Sanacora, Treccani and Popoli, 2012)

Excessive oxidative stress lowers catalase levels, resulting in a buildup of harmful hydrogen peroxide and reactive oxygen species (ROS) in the body. Reduced catalase (CAT) concentrations means that significant amount of H_2O_2 accessible to react with transition metals and release the radical hydroxyl (the most dangerous radical), resulting in increased lipid peroxidation and, as a result, neuronal damage. (Moretti *et al.*, 2012)

ROS can be antibacterial, killing antigen-bearing cells by damaging lipid membranes and protein structures. However, oxidative damage is not confined to microbial targets, and severe damage to host tissue can occur. ROS produced by active phagocytes can damage DNA bases and cause strand breaks in surrounding cells, leading some to believe that hydroxyl radicals and peroxynitrite produced during inflammation are the main contributors to DNA oxidation. (Russo, 2010)

Our results doesn't meet with Gazal *et al.*, findings that The levels of the antioxidant enzymes SOD and CAT was not affected in mice subjected to CUS. (Gazal *et al.*, 2014)

In our study, fluoxetine improved the behavioral deficits caused by CUS but had no protective benefits against oxidative stress that antioxidant enzymes concentration show no significant increases between group 3 (exposed to CUS and receive fluoxetine treatment for 14 days) and group 2 (exposed to CUS and receive normal saline treatment for 14 days). This finding support Zhao *et al.*, results Because fluoxetine primarily exerts its antidepressant effect by selectively blocking serotonin reuptake (Zhao *et al.*, 2008).

In groups 4, 5, 6 (exposed to CUS and treated with 125mg/kg, 250mg/kg, 500mg/kg of C. Myxa extract respectively), there was significant increase of SOD, CAT concentrations as compared with group 2 (untreated and exposed to CUS) that

related to antioxidant properties of the plant constituents. According to these findings C. Myxa extract have a different mechanism of antidepressant-like activity than fluoxetine.

Increased reactive oxygen generation during depression may result in depletion of endogenous scavenging chemicals as mentioned before. Our results demonstrate that the plant have antioxidant action as mentioned by previous studies. Aljeboury report that flavonoids, one of the most important constituents of Cordia Myxa fruits, may have an additive effect on endogenous scavenging compounds. Endogenous antioxidants may benefit from flavonoids as well. When flavonoids are combined with radical, the flavonoid hydroxyl groups render the radicals inactive.(Aljeboury, 2021)

Antioxidant activities of Cordia Myxa extract may be ascribed to high concentration of polyphenolics including flavonoids. The active flavonoids of cordia myxa extract according to HPLC analysis performed by El-Massry *et al.*, study are catechin, quercetin, and gallic acid. The presence of chrysin and apigenin supported the medicinal value of the cordia myxa extract as they are potent antioxidants and can protect the cells against oxidative damage. In addition chrysin and apigenin possess anxiolytic effect on the benzodiazepine receptor with no sedative effect or muscle relaxation. (El-Massry *et al.*, 2021)

Flavonoids may have an additive effect to the endogenous scavenging compounds and interfere with ≥3 different free radical–producing systems, but they can also increase the function of the endogenous antioxidants. First way is the direct scavenging of free radicals that Flavonoids oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. The second way interfering with inducible nitric-oxide synthase activity, the much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage by production of both nitric oxide and

superoxide anions. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. The third way was inhibition of xanthine oxidase pathway especially by quercetin and therefore prevent production of free radicals by favoring xanthine dehydrogenase reaction. The fourth way was Quercetin is known for its iron-chelating properties therefore inhibit the casual factor for development of free radicals and lipid peroxidation. (Nijveldt *et al.*, 2001)

The antioxidant efficacy of catechins is exerted through either direct mechanisms of scavenging ROS, chelating metal ions or indirect mechanisms by inducing antioxidant enzymes SOD, CAT, and GPx, inhibiting pro-oxidant enzymes such as Xanthine Oxidase, and production of antioxidant enzymes with suppression of stress related signaling pathways. (Bernatoniene and Kopustinskiene, 2018)

As a result, this study found that C. Myxa fruit extract reversed the effects of chronic stress on antioxidant enzymes and interleukin 6 that increases SOD and CAT concentrations in rats brain tissues and decreases the proinflammatory cytokines IL-6. This could be linked to the plant's antioxidant, anti-inflammatory, and ability to scavenge free radicals produced during oxidative stress. Despite the small size of the groups in this study, the findings support the cytokine hypothesis (Depression Macrophage Theory) that plays a role in MDD pathogenesis. Increased antioxidant enzyme levels, decreased IL-6 levels, and their relationship with treatment outcome in depressed rats suggest a link between inflammation, depression, and treatment.

The current study did not look at monoamine levels or changes in protein expression in the brain; however, these features may be investigated in future studies. Future research may look at more fundamental processes in addition to the antioxidant axis.

5.1. Conclusions

1. This study reveals that prolonged unpredictable stress causes depressive-like behavior in rats, as well as greater levels of interleukin-6 (IL-6) in the brain and lower levels of brain antioxidant enzymes including catalase (CAT) and superoxide dismutase (SOD).

2. Stress-induced high levels of interleukin-6 (IL-6) and low levels of catalase (CAT) and superoxide dismutase (SOD) are reversed by *Cordia myxa* treatments in treated groups, in addition to its antidepressant-like action as shown by behavioral tests.

3. In addition, this study shows that catalase (CAT) and superoxide dismutase (SOD) measured were not significantly increased during antidepressant treatment (fluoxetine) in animals with major depressive disorder.

4. Increased formation of cytokines and development of oxidative stress plays an important role in the pathogenesis of depression.

5.2. Recommendations

1. Further studies are needed to evaluate the possible antidepressant effects of other parts of *Cordia myxa* such as leaves.

2. More studies are needed to determine the effects of other types of cytokines in the pathogenesis of depression and the effect of *C. Myxa* extract on them such as IL-1b.

3. *Cordia myxa'*s antidepressant properties might be tested in human clinical studies.

4. More studies needed for extraction of flavonoids from *Cordia myxa* fruits such as quercetins and catechins and evaluate antidepressant activities of them

5. Further studies on combination *Cordia myxa* with fluoxetine in order to lower fluoxetine dosage and side effects.

6.1. References

Abdel-aleem, E. R., Seddik, F. E. F. and Samy, M. N. (2017) 'Botanical studies of the leaf of Cordia myxa L .', 6(6), pp. 2086–2091.

Aberoumand, A. and Deokule, S. S. (2009) 'Determination of elements profile of some wild edible plants', *Food Analytical Methods*, 2(2), pp. 116–119. doi:10.1007/s12161-008-9038-z.

Aberoumand, A. and Deokule, S. S. (2010) 'Assessment of the nutritional value of plant-based diets in relation to human carbohydrates: A preliminary study', *Advance Journal of Food Science and Technology*, 2(1), pp. 1–5.

Adongo, D. W. (2015) 'Anticonvulsant and Antidepressant Effects of an Ethanolic Extract of the Leaves of Pseudospondias Microcarpa'. doi: 10.1155/2015/397943.

Afzal, A., Obuekwe, C., Khan, A. R. and Barakat, H. (2009) 'Influence of Cordia myxa on chemically induced oxidative stress', *Nutrition & Food Science*, 39(1), pp. 6–15. doi: 10.1108/00346650910930761.

Al-Ati, T. (2011) *Assyrian plum (Cordia myxa L.)*, *Postharvest Biology and Technology of Tropical and Subtropical Fruits*. Woodhead Publishing Limited. doi: 10.1533/9780857092762.116.

Al-Snafi, A. E. (2016) *The Pharmacological and therapeutic importance of Cordia myxa-A review Medicinal plants with antimicrobial effects View project Plants with antioxidant effects View project The Pharmacological and therapeutic importance of Cordia myxa-A review*. Available at: www.iosrphr.org.

Arnér, E. S. J. and Holmgren, A. (2000) 'Physiological functions of thioredoxin and thioredoxin reductase', *European Journal of Biochemistry*, 267(20), pp. 6102–6109. doi: 10.1046/j.1432-1327.2000.01701.x.

Artanti N, Maarifa Y, and H. M. (2006) 'Isolation and Identification of Active Antioxidant Compound in Dendrophthoe Pentandra.Pdf', *Journal of Applied Sciences*, pp. 1659–1663.

Amiresmaeili, A., Rooholl ahi, S., Mostafavi, A. and Askari, N. (2018) 'Effects of oregano essential oil on brain TLR4 and TLR2 gene expression and depressive-like behavior in a rat model', *Research in Pharmaceutical Sciences*, 13(2), pp. 130–141. doi: 10.4103/1735-5362.223795.

Al-Awadi, F. M., Srikumar, T. S., Anim, J. T. and Khan, I. (2001) 'Antiinflammatory effects of Cordia myxa fruit on experimentally induced colitis in rats', *Nutrition*,17(5), pp. 391–396. doi: 10.1016/S0899-9007(01)00517-2.

Aljeboury, G. H. (2021) 'Anti-Inflammatory Effect of Cordia Myxa Extract on Bacteria that Infected Wounds in Rats as a Model for Human', 25(4), pp. 16040–16045.

Bahar, M. A., Kamp, J., Borgsteede, S. D., Hak, E. and Wilffert, B. (2018) 'The Impact of CYP2D6 mediated Drug-Drug Interaction : A Systematic Review on a Combination of Metoprolol and Paroxetine / Fluoxetine'. doi: 10.1111/bcp.13741.

Bajpai, A., Verma, A. K., Srivastava, M. and Srivastava, R. (2014) 'Oxidative stress and major depression', *Journal of Clinical and Diagnostic Research*, 8(12), pp. CC04–CC07. doi: 10.7860/JCDR/2014/10258.5292.

Belardi, P. and Lucertini, G. (2003) 'Cerebral vasoreactivity does not predict cerebral ischaemia during carotid endarterectomy', *Journal of Cardiovascular Surgery*, 44(6), pp. 731–735. doi: 10.1016/j.neuroscience.2015.05.053.Pathogenesis.

Belovicova, K., Bogi, E., Csatlosova, K. and Dubovicky, M. (2017) 'Animal tests for anxiety-like and depression-like behavior in rats', *Interdisciplinary Toxicology*, 10(1), pp. 40–43. doi: 10.1515/intox-2017-0006.

Benazzi, F. (2006) 'Various forms of depression', *Dialogues in Clinical Neuroscience*, 8(2), pp. 151–161. doi: 10.31887/dcns.2006.8.2/fbenazzi.

Benhar, M. (2020) 'Oxidants, antioxidants and thiol Redox switches in the control of regulated cell death pathways', *Antioxidants*, 9(4). doi: 10.3390/antiox9040309.

Benhar, M., Shytaj, I. L., Stamler, J. S. and Savarino, A. (2016) 'Dual targeting of the thioredoxin and glutathione systems in cancer and HIV', *Journal of Clinical Investigation*, 126(5), pp. 1630–1639. doi: 10.1172/JCI85339.

Bertero, E. and Maack, C. (2018) 'Calcium signaling and reactive oxygen species in Mitochondria', *Circulation Research*, 122(10), pp. 1460–1478. doi: 10.1161/CIRCRESAHA.118.310082.

Bernatoniene, J. and Kopustinskiene, D. M. (2018) 'The Role of Catechins in Cellular Responses to Oxidative Stress', *Molecules*, 23(4), pp. 1–11. doi: 10.3390/molecules23040965.

Bhatt, S., Nagappa, A. N. and Patil, C. R. (2020) 'Role of oxidative stress in depression', *Drug Discovery Today*. Elsevier Ltd, 25(7), pp. 1270–1276. doi: 10.1016/j.drudis.2020.05.001.

Banerjee, R., Hazra, S., Ghosh, A. K. and Mondal, A. C. (2014) 'Chronic administration of bacopa monniera increases BDNF protein and mRNA expressions: A study in chronic unpredictable stress induced animal model of depression', *Psychiatry Investigation*, 11(3), pp. 297–306. doi: 10.4306/pi.2014.11.3.297.

Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A. and Morilak, D. A. (2008) 'Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment', *Neuropsychopharmacology*, 33(2), pp. 320–331. doi: 10.1038/sj.npp.1301410.

Cryan, J. F., Valentino, R. J. and Lucki, I. (2005) 'Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test', *Neuroscience and Biobehavioral Reviews*, 29(4–5), pp. 547–569. doi: 10.1016/j.neubiorev.2005.03.008.

Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S. and Gould, T. D. (2012) 'The Tail Suspension Test', (January), pp. 3–7. doi: 10.3791/3769.

Cathomas, F., Hartmann, M. N., Seifritz, E., Pryce, C. R. and Kaiser, S. (2015) 'The translational study of apathy—An ecological approach', *Frontiers in Behavioral Neuroscience*, 9(September), pp. 1–7. doi: 10.3389/fnbeh.2015.00241.

Cortese-Krott, M. M., Koning, A., Kuhnle, G. G. C., Nagy, P., Bianco, C. L., Pasch, A., Wink, D. A., Fukuto, J. M., Jackson, A. A., Van Goor, H., Olson, K. R. and Feelisch, M. (2017) 'The Reactive Species Interactome: Evolutionary Emergence, Biological Significance, and Opportunities for Redox Metabolomics and Personalized Medicine', *Antioxidants and Redox Signaling*, 27(10), pp. 684–712. doi: 10.1089/ars.2017.7083.

Caiaffo, V., Oliveira, B. D. R., de Sá, F. B. and Evêncio Neto, J. (2016) 'Anti-inflammatory, antiapoptotic, and antioxidant activity of fluoxetine', *Pharmacology Research and Perspectives*, 4(3), pp. 1–9. doi: 10.1002/prp2.231.

Chopra, K., Kumar, B. and Kuhad, A. (2011) 'Pathobiological targets of depression', *Expert Opinion on Therapeutic Targets*, 15(4), pp. 379–400. doi: 10.1517/14728222.2011.553603.

Dhenain, T. and Francine, C. (2019) 'Biochimie Serotonin and orthodontic tooth movement', 161, pp. 73–79. doi: 10.1016/j.biochi.2019.04.002.

De Zwart, P. L., Jeronimus, B. F. and De Jonge, P. (2019) 'Empirical evidence for definitions of episode, remission, recovery, relapse and recurrence in depression: A systematic review', *Epidemiology and Psychiatric Sciences*, 28(5), pp. 544–562. doi: 10.1017/S2045796018000227.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K. and Lanctôt, K. L. (2010) 'A Meta-Analysis of Cytokines in Major Depression', *Biological Psychiatry*. Elsevier Inc., 67(5), pp. 446–457. doi: 10.1016/j.biopsych.2009.09.033.

Dulawa, S. C., Holick, K. A., Gundersen, B. and Hen, R. (2004) 'Effects of chronic fluoxetine in animal models of anxiety and depression', *Neuropsychopharmacology*, 29(7), pp. 1321–1330. doi: 10.1038/sj.npp.1300433.

Desai, S. D., Kori, R. S. and Aladakatti, R. H. (2017) 'Effect of anti-stress activity of buspirone on restrained stress induced male albino rats', *Journal of*

Pharmaceutical Sciences and Research, 9(11), pp. 2068–2071. doi: 10.5530/jyp.2017.

Eren, I., Naziroğlu, M., Demirdaş, A., Çelik, Ö., Uğuz, A. C., Altunbaşak, A., Özmen, I. and Uz, E. (2007) 'Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat', *Neurochemical Research*, 32(3), pp. 497–505. doi: 10.1007/s11064-006-9258-9.

Fageria M., P. K. and D. R. (2003) '"Effect of fruit harvesting stages and processing treatments on the quality of sun dried fruits of lehsua (Cordia myxa Roxb.)"', *Journal of Applied Horticulture*, 5 (1), pp. 32 – 38.

Fekadu, N., Shibeshi, W. and Engidawork, E. (2017) 'Major Depressive Disorder: Pathophysiology and Clinical Management.', *Journal of Depression and Anxiety*, 06(01), pp. 1–7. doi: 10.4172/2167-1044.1000255.

Fu, C. M. and Parahoo, K. (2009) 'Causes of depression: Perceptions among people recovering from depression', *Journal of Advanced Nursing*, 65(1), pp. 101–109. doi: 10.1111/j.1365-2648.2008.04845.x.

Gannon, J. M., Brar, J., Rai, A. and Chengappa, K. N. R. (2019) 'Effects of a standardized extract of withania somnifera (ashwagandha) on depression and anxiety symptoms in persons with schizophrenia participating in a randomized, placebo-controlled clinical trial', *Annals of Clinical Psychiatry*, 31(2), pp. 123–129.

Geethangili, M. and Ding, S. T. (2018) 'A review of the phytochemistry and pharmacology of Phyllanthus urinaria L', *Frontiers in Pharmacology*, 9(OCT), pp. 1–20. doi: 10.3389/fphar.2018.01109.

Go, Y. M., Chandler, J. D. and Jones, D. P. (2015) 'The cysteine proteome', *Free Radical Biology and Medicine*. Elsevier, 84, pp. 227–245. doi: 10.1016/j.freeradbiomed.2015.03.022.

Guilliams, T. G. and Edwards, L. (2010) 'Chronic stress and the HPA axis: Clinical assessment and therapeutic considerations', *The Standard*, 9(2), pp. 1–12.

Gazal, M., Ortmann, C. F., Martins, F. A., Streck, E. L., Quevedo, J., de Campos, A. M., Stefanello, F. M., Kaster, M. P., Ghisleni, G., Reginatto, F. H. and Lencina, C. L. (2014) 'Antidepressant-like effects of aqueous extract from Cecropia pachystachya leaves in a mouse model of chronic unpredictable stress', *Brain Research Bulletin*. Elsevier Inc., 108, pp. 10–17. doi: 10.1016/j.brainresbull.2014.07.007.

Ghosh, S., Mukherjee, S., Choudhury, S., Gupta, P., Adhikary, A., Baral, R. and Chattopadhyay, S. (2015) 'Reactive oxygen species in the tumor niche triggers altered activation of macrophages and immunosuppression: Role of fluoxetine', *Cellular Signalling*. Elsevier B.V., 27(7), pp. 1398–1412. doi: 10.1016/j.cellsig.2015.03.013.

Haji, N., Mandolesi, G., Gentile, A., Sacchetti, L., Fresegna, D., Rossi, S., Musella, A., Sepman, H., Motta, C., Studer, V., De Chiara, V., Bernardi, G., Strata, P. and Centonze, D. (2012) 'TNF-α-mediated anxiety in a mouse model of multiple sclerosis', *Experimental Neurology*. Elsevier Inc., 237(2), pp. 296–303. doi: 10.1016/j.expneurol.2012.07.010.

Hasler, G. (2010) 'Pathophysiology of depression: Do we have any solid evidence of interest to clinicians?', *World Psychiatry*. Blackwell Publishing Ltd, pp. 155–161. doi: 10.1002/j.2051-5545.2010.tb00298.x.

Hazra, S., Kumar, S., Saha, G. K. and Mondal, A. C. (2017) 'Reversion of BDNF, Akt and CREB in hippocampus of chronic unpredictable stress induced rats: Effects of phytochemical, Bacopa Monnieri', *Psychiatry Investigation*, 14(1), pp. 74–80. doi: 10.4306/pi.2017.14.1.74.

Hu, M., Zou, W., Wang, C. Y., Chen, X., Tan, H. Y., Zeng, H. Y., Zhang, P., Gu, H. F. and Tang, X. Q. (2016) 'Hydrogen Sulfide Protects against Chronic Unpredictable Mild Stress-Induced Oxidative Stress in Hippocampus by Upregulation of BDNF-TrkB Pathway', *Oxidative Medicine and Cellular Longevity*, 2016. doi: 10.1155/2016/2153745.

Isingrini, E., Camus, V., Le Guisquet, A. M., Pingaud, M., Devers, S., & Belzung, C. (2010). Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. *PloS one*, *5*(4), e10404. https://doi.org/10.1371/journal.pone.0010404

Jaddoa, E. (2018) 'Analysis of rat brain lipids and metabolites after antidepressant drug treatment Analysis of rat brain lipids and metabolites after antidepressant drug treatment', pp. 155–161 (April) 2018.

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, Z., Abera, S. F., Abil, O. Z., Abraha, H. N., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., Accrombessi, M. M. K., *et al.* (2018)

'Global, regional, and national incidence, prevalence, and years lived with disability for 354 Diseases and Injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017', *The Lancet*, 392(10159), pp. 1789–1858. doi: 10.1016/S0140-6736(18)32279-7.

Janssen-Heininger, Y. M. W., Mossman, B. T., Heintz, N. H., Forman, H. J., Kalyanaraman, B., Finkel, T., Stamler, J. S., Rhee, S. G. and van der Vliet, A. (2008) 'Redox-based regulation of signal transduction: Principles, pitfalls, and promises', *Free Radical Biology and Medicine*, 45(1), pp. 1–17. doi: 10.1016/j.freeradbiomed.2008.03.011.

Jesulola, E., Micalos, P. and Baguley, I. J. (2018) 'Understanding the pathophysiology of depression: From monoamines to the neurogenesis hypothesis model - are we there yet?', *Behavioural Brain Research*. Elsevier B.V., 341, pp. 79–90. doi: 10.1016/j.bbr.2017.12.025.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G. M. and Schatzberg, A. F. (2017) 'HPA axis in major depression: Cortisol, clinical symptomatology and genetic variation predict cognition', *Molecular Psychiatry*. Nature Publishing Group, 22(4), pp. 527–536. doi: 10.1038/mp.2016.120.

Kessler, R. C. and Bromet, E. J. (2013) 'The epidemiology of depression across cultures', *Annual Review of Public Health*, 34, pp. 119–138. doi: 10.1146/annurev-publhealth-031912-114409.

Köhler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes, B. S., Stubbs, B., Solmi, M., Veronese, N., Herrmann, N., Raison, C. L., Miller, B. J., Lanctôt, K. L. and Carvalho, A. F. (2017) 'Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies', *Acta*

Psychiatrica Scandinavica, 135(5), pp. 373–387. doi: 10.1111/acps.12698.

Kim, Y. K., Na, K. S., Shin, K. H., Jung, H. Y., Choi, S. H. and Kim, J. B. (2007) 'Cytokine imbalance in the pathophysiology of major depressive disorder', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31(5), pp. 1044–1053. doi: 10.1016/j.pnpbp.2007.03.004.

Klietz, M.-L., Kaiser, H. W., Machens, H.-G. and Aitzetmüller, M. M. (2019) 'Ac c ep te d Corresponding Author : an us cr ip t Ac c ep te d us cr t', pp. 1–31.

Knutson, J. S. (2014) 'Genetic changes NIH Public Access', *Bone*, 23(1), pp. 1–7. Availableat:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf.

Kranz, G. S., Kasper, S. and Lanzenberger, R. (2010) 'Reward and the serotonergic system', *Neuroscience*. Elsevier Inc., 166(4), pp. 1023–1035. doi: 10.1016/j.neuroscience.2010.01.036.

Lancaster, S. G. and Gonzalez, J. P. (1989) 'Lofepramine: A Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy in Depressive Illness', *Drugs*, 37(2), pp. 123–140.doi: 10.2165/00003495-198937020-00003.

Lee, C. H. and Giuliani, F. (2019) 'The Role of Inflammation in Depression and Fatigue', *Frontiers in immunology*, 10(July), p. 1696. doi: 10.3389/fimmu.2019.01696.

Li, Xiao, Li, J., Li, Xianrui, Wang, Jisheng, Dai, H. and Wang, Jingshang (2019) 'Effectiveness and safety of fluoxetine for premature ejaculation', 0(January), pp. 1–5.

Liu, L., Liu, C., Wang, Y., Wang, P., Li, Y. and Li, B. (2015) 'Herbal Medicine for Anxiety, Depression and Insomnia', *Current Neuropharmacology*, 13(4), pp. 481–493. doi: 10.2174/1570159x1304150831122734.

Liu, M. Y., Yin, C. Y., Zhu, L. J., Zhu, X. H., Xu, C., Luo, C. X., Chen, H., Zhu, D. Y. and Zhou, Q. G. (2018) 'Sucrose preference test for measurement of stress-induced anhedonia in mice', *Nature Protocols*. Springer US, 13(7), pp. 1686–1698. doi: 10.1038/s41596-018-0011-z.

Liu, Z. Q., Zhu, B., Tan, Y. F., Tan, Z. R., Wang, L. S., Huang, S. L., Shu, Y. and Zhou, H. H. (2002) 'O-dealkylation of fluoxetine in relation to CYP2C19 gene dose and involvement of CYP3A4 in human liver microsomes', *Journal of Pharmacology and Experimental Therapeutics*, 300(1), pp. 105–111. doi: 10.1124/jpet.300.1.105.

Liu, D., Qiu, H. M., Fei, H. Z., Hu, X. Y., Xia, H. J., Wang, L. J., Qin, L. J., Jiang, X. H. and Zhou, Q. X. (2014) 'Histone acetylation and expression of mono-aminergic transmitters synthetases involved in CUS-induced depressive rats', *Experimental Biology and Medicine*, 239(3), pp. 330–336. doi: 10.1177/1535370213513987.

Liu, D., Wang, Z., Liu, S., Wang, F., Zhao, S. and Hao, A. (2011) 'Anti-inflammatory effects of fluoxetine in lipopolysaccharide(LPS)- stimulated microglial cells', *Neuropharmacology*. Elsevier Ltd, 61(4), pp. 592–599. doi: 10.1016/j.neuropharm.2011.04.033.

Liu, Q., Li, B., Zhu, H. Y., Wang, Y. Q., Yu, J. and Wu, G. C. (2011) 'Glia atrophy in the hippocampus of chronic unpredictable stress-induced depression model rats is reversed by electroacupuncture treatment', *Journal of Affective Disorders*. Elsevier B.V., 128(3), pp. 309–313. doi: 10.1016/j.jad.2010.07.007.

Lucca, G., Comim, C. M., Valvassori, S. S., Réus, G. Z., Vuolo, F., Petronilho, F., Gavioli, E. C., Dal-Pizzol, F. and Quevedo, J. (2009) 'Increased oxidative stress in submitochondrial particles into the brain of rats submitted to the chronic mild stress paradigm', *Journal of Psychiatric Research*. Elsevier Ltd, 43(9), pp. 864–869. doi: 10.1016/j.jpsychires.2008.11.002.

López, C., Gómez-Roso, M., García-Pedraza, J. Á., Martín, M. L., Morán, A. and GarcíaDomingo, M. (2019) 'Fluoxetine oral treatment discloses 5-HT 1D receptor as vagoinhibitor of the cardiac cholinergic neurotransmission in rat', Canadian Journal of Physiology and Pharmacology, 97(2), pp. 90–98. doi: 10.1139/cjpp2018-0390.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., Kubera, M., Bob, P., Lerer, B. and Maj, M. (2009) 'The inflammatory & neurodegenerative (I&ND) hypothesis of depression: Leads for future research and new drug developments in depression', *Metabolic Brain Disease*, 24(1), pp. 27–53. doi: 10.1007/s11011-008-9118-1.

Mao, Q. Q., Ip, S. P., Ko, K. M., Tsai, S. H., Xian, Y. F. and Che, C. T. (2009) 'Effects of peony glycosides on mice exposed to chronic unpredictable stress: Further evidence for antidepressant-like activity', *Journal of Ethnopharmacology*, 124(2), pp. 316–320. doi: 10.1016/j.jep.2009.04.019.

Mao, Q. Q., Ip, S. P., Xian, Y. F., Hu, Z. and Che, C. T. (2012) 'Anti-depressant-like effect of peony: A mini-review', *Pharmaceutical Biology*, 50(1), pp. 72–77. doi: 10.3109/13880209.2011.602696.

Maurer, D. M., Raymond, T. J. and Davis, B. N. (2018) 'Depression: Screening and diagnosis', *American Family Physician*, 98(8), pp. 508–515.

Martinez-Gonzalez, D., Obermeyer, W., Fahy, J.L., Riboh, M., Kalin, N.H. and Benca, R.M., 2004. REM sleep deprivation induces changes in coping responses that are not reversed by amphetamine. *Sleep*, *27*(4), pp.609-617.

Mellon, S. H., Wolkowitz, O. M., Schonemann, M. D., Epel, E. S., Rosser, R., Burke, H. B., Mahan, L., Reus, V. I., Stamatiou, D., Liew, C. C. and Cole, S. W. (2016) 'Alterations in leukocyte transcriptional control pathway activity associated with major depressive disorder and antidepressant treatment', *Translational psychiatry*. Nature Publishing Group, 6(March), p. e821. doi: 10.1038/tp.2016.79.

Ménard, C., Hodes, G. E. and Russo, S. J. (2016) 'Pathogenesis of depression: Insights from human and rodent studies', *Neuroscience*. IBRO, 321(May), pp. 138–162. doi: 10.1016/j.neuroscience.2015.05.053.

Monteiro, S., Roque, S., de Sá-Calçada, D., Sousa, N., Correia-Neves, M. and Cerqueira, J. J. (2015) 'An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice', *Frontiers in Psychiatry*, 6(FEB), pp. 1–12. doi: 10.3389/fpsyt.2015.00006.

Muñoz, R. F., Cuijpers, P., Smit, F., Barrera, A. Z. and Leykin, Y. (2010) 'Prevention of major depression', *Annual Review of Clinical Psychology*, 6, pp. 181–212. doi: 10.1146/annurev-clinpsy-033109-132040.

Makkonen, I., Kokki, H., Kuikka, J., Turpeinen, U. and Riikonen, R. (2011) 'Effects of fluoxetine treatment on striatal dopamine transporter binding and cerebrospinal fluid insulin-like growth factor-1 in children with autism', *Neuropediatrics*, 42(5), pp. 207–209. doi: 10.1055/s-0031-1291242.

Molteni, R., Calabrese, F., Bedogni, F., Tongiorgi, E., Fumagalli, F., Racagni, G. and Riva, M. A. (2006) 'Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions', *International Journal of Neuropsychopharmacology*, 9(3), pp. 307–317. doi: 10.1017/S1461145705005766.

Moretti, M., Colla, A., De Oliveira Balen, G., Dos Santos, D. B., Budni, J., De Freitas, A. E., Farina, M. and Severo Rodrigues, A. L. (2012) 'Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress', *Journal of Psychiatric Research*. Elsevier Ltd, 46(3), pp. 331–340. doi: 10.1016/j.jpsychires.2011.11.009.

Nandi, A., Yan, L. J., Jana, C. K. and Das, N. (2019) 'Role of Catalase in Oxidative Stress- And Age-Associated Degenerative Diseases', *Oxidative Medicine and Cellular Longevity*, 2019. doi: 10.1155/2019/9613090.

Neumeister A, Nugent AC, W. T. et al (2004) 'Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls', *Arch Gen Psychiatry*.

Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E. C., Boelens, P. G., Van Norren, K. and Van Leeuwen, P. A. M. (2001) 'Flavonoids: A review of probable mechanisms of action and potential applications', *American Journal of Clinical Nutrition*, 74(4), pp. 418–425. doi: 10.1093/ajcn/74.4.418.

Olukunle, J. O., Adenubi, O. T., Oladele, G. M., Sogebi, E. A. and Oguntoke, P. C. (2011) 'Studies on the anti-inflammatory and analgesic properties of Jatropha curcas leaf extract', *Acta Veterinaria Brno*, 80(3), pp. 259–262. doi: 10.2754/avb201180030259.

Ondicova, K., Tillinger, A., Pecenak, J. and Mravec, B. (2019) 'The vagus nerve role in antidepressants action: Efferent vagal pathways participate in peripheral anti-inflammatory effect of fluoxetine', *Neurochemistry International*, 125(December 2018), pp. 47–56. doi: 10.1016/j.neuint.2019.02.003.

Pałucha-Poniewiera, A., Podkowa, K., Rafało-Ulińska, A., Brański, P. and Burnat, G. (2020) 'The influence of the duration of chronic unpredictable mild stress on the behavioural responses of C57BL/6J mice', *Behavioural Pharmacology*, 31(6), pp. 574–582. doi: 10.1097/FBP.0000000000000564.

Pandey, K. B. and Rizvi, S. I. (2010) 'Markers of oxidative stress in erythrocytes and plasma during aging in humans', *Oxidative Medicine and Cellular Longevity*, 3(1), pp. 2–12. doi: 10.4161/oxim.3.1.10476.

Pearl, P. and Mullen, S. (2018) 'Major depressive disorder in children and adolescents', 8(6), pp. 275–283. doi: 10.9740/mhc.2018.11.275.

Peruga, I., Hartwig, S., Thöne, J., Hovemann, B., Gold, R., Juckel, G. and Linker, R. A. (2011) 'Inflammation modulates anxiety in an animal model of multiple sclerosis', *Behavioural Brain Research*. Elsevier B.V., 220(1), pp. 20–29. doi: 10.1016/j.bbr.2011.01.018.

Pace, T. W. W., Hu, F. and Miller, A. H. (2007) 'Cytokine-effects on glucocorticoid receptor function: Relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression', *Brain, Behavior, and Immunity*, 21(1), pp. 9–19. doi: 10.1016/j.bbi.2006.08.009.

Perrine, S. A., Ghoddoussi, F., Michaels, M. S., Sheikh, I. S., McKelvey, G. and Galloway, M. P. (2014) 'Ketamine reverses stress-induced depression-like behavior and increased GABA levels in the anterior cingulate: An 11.7T 1H-MRS study in rats', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. Elsevier B.V., 51, pp. 9–15. doi: 10.1016/j.pnpbp.2013.11.003.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. and Bitto, A. (2017) 'Oxidative Stress: Harms and Benefits for Human Health', *Oxidative Medicine and Cellular Longevity*. Hindawi, 2017. doi: 10.1155/2017/8416763.

Ramic, E., Prasko, S., Gavran, L. and Spahic, E. (2020) 'Assessment of the Antidepressant Side Effects Occurrence in Patients Treated in Primary Care', *Materia Socio Medica*, 32(2), p. 131. doi: 10.5455/msm.2020.32.131-134.

Ravichandran, H., Janakiraman, B., Sundaram, S., Fisseha, B. and Yitayeh, A. (2017) 'Active Release Technique in Hamstrings Strain : Rehabilitation and Return to Play – A Case Study', pp. 71–74. doi: 10.4103/amhs.amhs.

Robertson, O. D. and Dodd, S. (2019) 'Putative neuroprotective pharmacotherapies to target the staged progression of mental illness', (December 2018), pp. 1–18. doi: 10.1111/eip.12775.

Rahman, I., Marwick, J. and Kirkham, P. (2004) 'Redox modulation of chromatin remodeling: Impact on histone acetylation and deacetylation, NF-κB and pro-inflammatory gene expression', *Biochemical Pharmacology*, 68(6), pp. 1255–1267. doi: 10.1016/j.bcp.2004.05.042.

Raison, C. L., Capuron, L. and Miller, A. H. (2006) 'Cytokines sing the blues: Inflammation and the pathogenesis of depression', *Trends in Immunology*, 27(1), pp. 24–31. doi: 10.1016/j.it.2005.11.006.

Ranjbar, M., Varzi, H. N., Sabbagh, A., Bolooki, A. and Sazmand, A. (2013) 'Study on analgesic and anti-inflammatory properties of Cordia myxa fruit Hydro-alcoholic extract', *Pakistan Journal of Biological Sciences*, 16(24), pp. 2066–2069. doi: 10.3923/pjbs.2013.2066.2069.

Roohi, E., Jaafari, N. and Hashemian, F. (2021) 'On inflammatory hypothesis of depression: what is the role of IL-6 in the middle of the chaos?', *Journal of Neuroinflammation*. Journal of Neuroinflammation, 18(1), pp. 1–15. doi: 10.1186/s12974-021-02100-7.

Russo, A. J. (2010) 'Increased Serum Cu/Zn SOD in Individuals with Clinical Depression Normalizes after Zinc and Anti-oxidant Therapy', *Nutrition and Metabolic Insights*, 3, p. NMI.S5044. doi: 10.4137/nmi.s5044.

Sanacora, G., Treccani, G. and Popoli, M. (2012) 'Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders', *Neuropharmacology*. Elsevier Ltd, 62(1), pp. 63–77. doi: 10.1016/j.neuropharm.2011.07.036.

Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J. and Brenes, J. C. (2019) 'Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats', *Scientific Reports*. Springer US, 9(1), pp. 1–21. doi: 10.1038/s41598-019-53624-1.

Shen, M., Yang, Y., Wu, Y., Zhang, B., Wu, H., Wang, L., Tang, H. and Chen, J. (2019) 'L-theanine ameliorate depressive-like behavior in a chronic unpredictable mild stress rat model via modulating the monoamine levels in limbic–cortical–striatal–pallidal–thalamic-circuit related brain regions', *Phytotherapy Research*, 33(2), pp. 412–421. doi: 10.1002/ptr.6237.

Song, C., Halbreich, U., Han, C., Leonard, B. E. and Luo, H. (2009) 'Imbalance between Pro- and Anti-inflammatory cytokines, and between Th1 and Th2 cytokines in depressed patients: The effect of electroacupuncture or fluoxetine treatment', *Pharmacopsychiatry*, 42(5), pp. 182–188. doi: 10.1055/s-0029-1202263.

Sapozhnikov, I. (2019) 'Lost Connections: Uncovering the Real Causes of Depression-and the Unexpected Solutions', *The Permanente journal*, 23, pp. 1–2. doi: 10.7812/TPP/18-231.

Sarris, J., Panossian, A., Schweitzer, I., Stough, C. and Scholey, A. (2011) 'Herbal medicine for depression, anxiety and insomnia: A review of psychopharmacology and clinical evidence', *European Neuropsychopharmacology*. Elsevier B.V., 21(12), pp. 841–860. doi: 10.1016/j.euroneuro.2011.04.002.

Shen, M., Yang, Y., Wu, Y., Zhang, B., Wu, H., Wang, L., Tang, H. and Chen, J. (2019) 'L-theanine ameliorate depressive-like behavior in a chronic unpredictable mild stress rat model via modulating the monoamine levels in limbic–cortical–striatal–pallidal–thalamic-circuit related brain regions', *Phytotherapy Research*, 33(2), pp. 412–421. doi: 10.1002/ptr.6237.

Shin, I. J., Son, S. U., Park, H., Kim, Y., Park, S. H., Swanberg, K., Shin, J. Y., Ha, S. K., Cho, Y., Bang, S. Y., Lew, J. H., Cho, S. H. and Maeng, S. (2014) 'Preclinical evidence of rapid-onset antidepressant-like effect in radix polygalae extract', *PLoS ONE*, 9(2). doi: 10.1371/journal.pone.0088617.

Swartz C, S. E. (2007) *Psychotic Depression*, *Cambridge University Press*. 32nd Avenue, New York, USA.

Sl, P., Pollok, J., Aj, E., Al, G., Knowles, S., Jm, A., Sl, P., Pollok, J., Aj, E., Al, G., Knowles, S. and Jm, A. (2019) 'inflammatory bowel disease (Review)'. doi: 10.1002/14651858.CD012680.pub2.www.cochranelibrary.com.

Song, C., Halbreich, U., Han, C., Leonard, B. E. and Luo, H. (2009) 'Imbalance between Pro- and Anti-inflammatory cytokines, and between Th1 and Th2 cytokines in depressed patients: The effect of electroacupuncture or fluoxetine treatment', *Pharmacopsychiatry*, 42(5), pp. 182–188. doi: 10.1055/s-0029-1202263.

Sahin Ozkartal, C., Aricioglu, F., Tuzun, E. and Kucukali, C. İ. (2018) 'Chronic mild stress-induced anhedonia in rats is coupled with the upregulation of inflammasome sensors: a possible involvement of NLRP1', *Psychiatry and Clinical Psychopharmacology*, 28(3), pp. 236–244. doi: 10.1080/24750573.2018.1426694.

Salimimoghadam, S., Ashrafi, A., Kianidehkordi, F. and Najafzadehvarzi, H. (2019) 'Hypoglycemic, Antitussive and Analgesic Effects of Nanoparticles of Cordia myxa Fruits Extract', *International Journal of Pharmaceutical Investigation*, 9(4), pp. 205–209. doi: 10.5530/ijpi.2019.4.38.

Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J. and Brenes, J. C. (2019) 'Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats', *Scientific Reports*. Springer US, 9(1), pp. 1–21. doi: 10.1038/s41598-019-53624-1.

Shahriari, M. and Moghadamnia, D. (2021) 'Protective effect of cordia myxa extract on changes in body weight, serum proteins, albumin and liver histology of adult male rats induced by cadmium chloride toxicity', *Iranian Journal of Toxicology*, 13(2), pp. 43–49. doi: 10.32598/IJT.13.2.575.3.

Samy, M. N Abdel-aleem, E. R., Seddik, F. E. F.. (2017) 'Botanical studies of the leaf of Cordia myxa L .', 6(6), pp. 2086–2091.

Valvassori, S. S., Varela, R. B. and Quevedo, J. (2017) *Animal Models of Mood Disorders: Focus on Bipolar Disorder and Depression*. Second Edi, *Animal Models for the Study of Human Disease: Second Edition*. Second Edi. Elsevier Inc. doi: 10.1016/B978-0-12-809468-6.00038-3.

Tanaka, T., Narazaki, M. and Kishimoto, T. (2014) 'Il-6 in inflammation, Immunity, And disease', *Cold Spring Harbor Perspectives in Biology*, 6(10). doi: 10.1101/cshperspect.a016295.

Tarafdar, A. and Pula, G. (2018) 'The role of NADPH oxidases and oxidative stress in neurodegenerative disorders', *International Journal of Molecular Sciences*, 19(12). doi: 10.3390/ijms19123824.

Ting, E. Y. C., Yang, A. C. and Tsai, S. J. (2020) 'Role of interleukin-6 in depressive disorder', *International Journal of Molecular Sciences*, 21(6), pp. 1–22. doi: 10.3390/ijms21062194.

Tolentino, J. C. and Schmidt, S. L. (2018) 'DSM-5 criteria and depression severity: Implications for clinical practice', *Frontiers in Psychiatry*, 9(OCT), pp. 1–9. doi: 10.3389/fpsyt.2018.00450.

Trevor, A. J. (2015) *Pharmacology Examination & Board Review Eleventh Edition*.

Tatem, K. S., Quinn, J. L., Phadke, A., Yu, Q., Gordish-Dressman, H. and Nagaraju, K. (2014) 'Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases', *Journal of Visualized Experiments*, (91), pp. 1–7. doi: 10.3791/51785.

Van Ruitenbeek, P., Vermeeren, A., Smulders, F. T. Y., Sambeth, A. and Riedel, W. J. (2009) 'Histamine H 1 receptor blockade predominantly impairs sensory processes in human sensorimotor performance', *British Journal of Pharmacology*, 157(1), pp. 76–85. doi: 10.1111/j.1476-5381.2008.00103.x.

Van Bronswijk, S., Moopen, N., Beijers, L., Ruhe, H. G. and Peeters, F. (2019) 'Effectiveness of psychotherapy for treatment-resistant depression: A meta-analysis and meta-regression', *Psychological Medicine*, 49(3), pp. 366–379. doi: 10.1017/S003329171800199X.

Valvassori, S. S., Varela, R. B. and Quevedo, J. (2017) *Animal Models of Mood Disorders: Focus on Bipolar Disorder and Depression*. Second Edi, *Animal Models for the Study of Human Disease: Second Edition*. Second Edi. Elsevier Inc. doi: 10.1016/B978-0-12-809468-6.00038-3.

Vaváková, M., ɯuračková, Z. and Trebatická, J. (2015) 'Markers of Oxidative Stress and Neuroprogression in Depression Disorder', *Oxidative Medicine and Cellular Longevity*, 2015. doi: 10.1155/2015/898393.

El-Massry, K. F., Farouk, A., Mahmoud, K. F., El-Ghorab, A. H., M, S. S., Musa, A., Mostafa, E. M., Ghoneim, M. M., Naguib, I. A. and Abdelgawad, M. A. (2021) 'Chemical characteristics and targeted encapsulated Cordia myxa fruits extracts nanoparticles for antioxidant and cytotoxicity potentials', *Saudi Journal of Biological Sciences*. The Author(s), 28(9), pp. 5349–5358. doi: 10.1016/j.sjbs.2021.05.064.

Wang, J., Wu, X., Lai, W., Long, E., Zhang, X., Li, W., Zhu, Y., Chen, C., Zhong, X.,

Liu, Z., Wang, D. and Lin, H. (2017) 'Prevalence of depression and depressive symptoms among outpatients: A systematic review and meta-analysis', *BMJ Open*, 7(8), pp. 1–14. doi: 10.1136/bmjopen-2017-017173.

Willner, P. (2017) 'The chronic mild stress (CMS) model of depression: History, evaluation and usage', *Neurobiology of Stress*. Elsevier Inc, 6, pp. 78–93. doi: 10.1016/j.ynstr.2016.08.002.

Wu, J., Du, J., Xu, C., Le, J., Xu, Y., Liu, B. and Dong, J. (2011) 'Icariin attenuates social defeat-induced down-regulation of glucocorticoid receptor in mice', *Pharmacology Biochemistry and Behavior*. Elsevier Inc., 98(2), pp. 273–278. doi: 10.1016/j.pbb.2011.01.008.

Wang, D., An, S. C. and Zhang, X. (2008) 'Prevention of chronic stress-induced depression-like behavior by inducible nitric oxide inhibitor', *Neuroscience Letters*, 433(1), pp. 59–64. doi: 10.1016/j.neulet.2007.12.041.

Yankelevitch-Yahav, R., Franko, M., Huly, A. and Doron, R. (2015) 'The forced swim test as a model of depressive-like behavior', *Journal of Visualized Experiments*, 2015(97), pp. 1–7. doi: 10.3791/52587.

Yi, L. T., Li, J., Geng, D., Liu, B. Bin, Fu, Y., Tu, J. Q., Liu, Y. and Weng, L. J. (2013) 'Essential oil of Perilla frutescens-induced change in hippocampal expression of brain-derived neurotrophic factor in chronic unpredictable mild stress in mice', *Journal of Ethnopharmacology*. Elsevier, 147(1), pp. 245–253. doi: 10.1016/j.jep.2013.03.015.

Zunszain, P. A., Anacker, C., Cattaneo, A. and Carvalho, L. A. (2012) 'Europe PMC Funders Group Glucocorticoids , cytokines and brain abnormalities in

depression', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 35(3), pp. 722–729. doi: 10.1016/j.pnpbp.2010.04.011.Glucocorticoids.

Zhang, Y., Wang, Y., Lei, H., Wang, L., Xue, L., Wang, X. and Zhu, X. (2017) 'Optimized animal model to mimic the reality of stress-induced depression in the clinic', *BMC Psychiatry*. BMC Psychiatry, 17(1), pp. 1–9. doi: 10.1186/s12888-017-1335-x.

Zhao, Z., Wang, W., Guo, H. and Zhou, D. (2008) 'Antidepressant-like effect of liquiritin from Glycyrrhiza uralensis in chronic variable stress induced depression model rats', *Behavioural Brain Research*, 194(1), pp. 108–113. doi: 10.1016/j.bbr.2008.06.030.

Zong, J., Liao, X., Ren, B. and Wang, Z. (2018) 'The antidepressant effects of rosiglitazone on rats with depression induced by neuropathic pain', *Life Sciences*. Elsevier, 203(October 2017), pp. 315–322. doi: 10.1016/j.lfs.2018.04.057.

Chapter One

Introduction and Literature Review

Chapter Two

Materials and Methods

Chapter Three

Results

Chapter Four

Discussion

Chapter Five

Conclusions and Recommendations

Chapter Six

References

الخلاصة

الاكتئاب هو أحد أكثر الأمراض العقلية شيوعًا حيث يتميز بنسب عاليه من العزله الاجتماعيه والانتحار. وهو رابع سبب رئيسي للإعاقة حسب منظمة الصحة العالمية WHO. تعتبر الآثار الجانبية الكثيره ، وزيادة السمية ، وتأخر بدء تأثير العلاج المضاد للاكتئاب كلها من الاهتمامات الرئيسية في علاجات الاكتئاب حاليا. اكتسبت الأدوية العشبية اهتمامًا كبيرًا مؤخرًا في علاج الاكتئاب نظرًا لسلامتها وفعاليتها وكلفتها الواطئه، لذلك هدفت هذه الدراسة إلى تقييم آثار مستخلص ثمار البمبر على نموذج الإجهاد الخفيف المزمن في ذكور الجرذان (CUS) وعلى إنزيمات مضادات الأكسدة في انسجة الدماغ والسيتوكينات المسببة للالتهابات في ذكور الجرذان بسبب المعرفة المقدمة من الدراسات السابقة حول نظرية السايتوكين (macrophage hypothesis) في التسبب في الاكتئاب و حسب المميزات المضادة للأكسدة و الالتهاب لمكونات ثمار البمبر.

حيث تم تقسيم ستين من ذكور الجرذان إلى ست مجموعات. لم تتعرض المجموعة 1 (مجموعة التحكم) لـ CUS ولم تتلق أي علاج بينما تعرضت المجموعة 2 لـ CUS لمدة 24 يومًا مع العلاج بمحلول normal saline لمدة 14 يومًا ، وتعرضت المجموعة 3 لـ CUS لمدة 24 يومًا وتلقيت 10 مجم / كجم من عقار الفلوكستين يوميًا في اليوم 10 لمدة 14 يومًا ، وتعرضت المجموعة 4 و5 و 6 إلى CUS لمدة 24 يومًا وتلقوا مستخلص ثمار البمبر (125 و250 و 500 مجم / كجم على التوالي) في اليوم العاشر ولمدة 14 يومًا. تم تقييم التأثير المضاد للاكتئاب للفلوكستين ومستخلص ثمار البمبر باستخدام اختبار السباحة القسري واختبار المجال المفتوح واختبار تفضيل السكروز. في نهاية التجارب ، تم التضحية بالحيوانات عن طريق قطع الرأس ، وتم تحديد مستويات إنزيم مضادات الأكسدة الكاتلاز (CAT) وسوبروكسيد ديسموتاز (SOD) بالإضافة إلى الانترلوكين - 6 (IL-6) بواسطة اختبارات الاليزا (Elisa) في أنسجة دماغ الجرذان.

تبين في المجموعات 3 (تمت معالجتها بـ 10 مجم / كجم فلوكستين لمدة 14 يومًا) ، و 4 (تمت معالجتها بـ 125 مجم / كجم من مستخلص ثمار البمبر لمدة 14 يومًا) ، و 5 (تمت معالجتها بـ 250 مجم / كجم من مستخلص البمبر لمدة 14 يومًا) ، و 6 (تمت معالجته بـ 500 مجم / كجم من مستخلص البمبر لمدة 14 يومًا) ، تزداد متوسطات وقت عدم الحركة في اليوم العاشر بشكل ملحوظ (*P-value* <0.05) مقارنة باليوم 0. بينما في المجموعات 3 و 4 و 5 و 6 انخفض متوسط زمن عدم الحركه في اليوم 25 بشكل ملحوظ (*P-value* <0.05) مقارنة باليوم 10. زاد متوسط زمن عدم الحركه للمجموعة 2 بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 1 ، بينما في المجموعات 3 و 4 و 5 و 6 ، انخفض متوسط زمن عدم الحركة بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 2 في اليوم 25.

علاوة على ذلك ، في المجموعات 3 و 4 و 5 و 6 ، انخفض متوسط مؤشر تفضيل السكروز (SPI) في اليوم 10 بشكل ملحوظ (*P-value* <0.05) مقارنة باليوم 0 ، بينما زاد متوسط SPI في اليوم 25 للمجموعات 3 و4 و5 و 6 بشكل ملحوظ (*P-value* <0.05) مقارنة باليوم 10. بينما في المجموعة 2 ، انخفض متوسط SPI بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 1 ، بينما في اليوم 25 في المجموعات 3 و 4 و 5 و 6 ، زاد متوسط SPI بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 2.

أظهر اختبار المجال المفتوح (OFT) أنه في المجموعات 2 و 3 و 4 و 5 و 6 ، انخفضت بشكل ملحوظ متوسطات الوضعيات المستقيمه وترددات عبور الخط والعنايه في اليوم 10 (*P-value* <0.05) مقارنة باليوم 0 ، بينما زادت الوضعيات المستقيمه ، وتكرار عبور الخط ، والعنايه في اليوم 25 للمجموعات 3 ، 4 ، 5 و 6 بشكل ملحوظ (*P-value* <0.05) مقارنة باليوم 10. في المجموعة 2 ، انخفض متوسط ترددات الوضعيات

المستقيمه وترددات عبور الخط والعنايه بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 1 ، بينما في المجموعات 3 و 4 و 5 و 6 ازداد النشاط (*P-value* <0.05) مقارنة بالمجموعة 2 في اليوم 25 .

فيما يتعلق بمستويات إنترلوكين 6 ، فإن متوسطات تركيزات IL-6 في أنسجة المخ للمجموعة 2 زادت بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 1 ، بينما في المجموعات 3 و 4 و 5 و 6 تراكيز IL-6 في أنسجة المخ انخفضت بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 2.

تظهر مستويات الإنزيمات المضادات للأكسدة أنه في المجموعة 2 ، انخفضت متوسطات تركيز SOD و CAT في أنسجة المخ بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 1 ، بينما في المجموعات 4 و 5 و 6 ، زاد متوسط تركيز SOD و CAT في أنسجة المخ بشكل ملحوظ (*P-value* <0.05) مقارنة بالمجموعة 2. في المجموعة 3 كانت زيادة SOD، CAT في أنسجة المخ مقارنة بالمجموعة 2 غير ملحوظه حيث (P> 0.05) .

في الختام و من خلال النتائج المتوفره يمكن القول ان مستخلص ثمار البمبر له نشاطًا شبيهًا بمضادات الاكتئاب كما تبين في الاختبارات السلوكيه و الاختبارات الكيميائيه الحياتيه حيث قام بعكس الزياده الحاصله في مستويات الانترلوكين 6 في انسجة دماغ الجرذان بسبب الاجهاد المزمن و قام ايضا برفع مستويات الانزيمات المضاده للاكسده التي قل تركيزها بسبب الاجهاد.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل - كلية الطب

تأثير مستخلص ثمار البمبر *Cordia myxa* في علاج الاكتئاب المستحث في ذكور الجرذان

رسالة

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كجزء من متطلبات نيل درجة الماجستير في الأدوية / الأدوية و السموم

من قبل

ياسر جاسب حبيب علاوي

بكلوريوس صيدلة

(2017-2016)

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