CHAPTER ONE

1.0. INTRODUCTION

Depression is a chronic recurrent neuropsychiatric disease that affects millions of individuals worldwide and impacts patients' social function and quality of life (Kessler *et al*., 2003) or overall productivity (Schechter *et al*., 2005). Depression is an affective disorder characterized by change in mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, psychomotor retardation and melancholia. It is a major health condition with life time prevalence in the range of 10-15% (Lépine and Briley, 2011). The course of the disease is recurrent and most patients who recover from major depressive episodes still become depressed afterwards (Lavori *et al.,* 1994). Moreover, repeated episodes of depression have been shown to cause atrophy of the hippocampus thereby increasing the risk of dementia and also contributing to treatment failures (Kessing, 2012; Gorwood *et al*, 2014). The prevalence of clinically significant depressive syndromes in those people over 60 years of age (i.e. 'late-life depression') ranges from 9% to 18% and incidence rates of 19.3 per 1000 person years have been reported (Luijendijk *et al*., 2008; Mulsant and Ganguli, 1999). While a wide range of pharmacological treatments for major depression are moderately effective, as many as 50 % of patients across all age groups will not achieve remission with their first treatment (Roose and Schatzberg, 2005). Ultimately, 20–30% will not achieve full recovery despite access to multiple interventions (Whyte *et al*., 2004).

In contrast to the epidemiological magnitude of the disease, the progress of pharmacological therapy for depression is still limited (Nestler *et al.,* 2002). All available antidepressants act via monoamine neurotransmitter systems, but only 50-70% of the patients exhibit acceptable responses to treatment (Morilak and Frazer, 2004). Antidepressant drugs in use today are based on the strategy of blocking the reuptake or degradation of monoamine neurotransmitters (Morilak and Frazer, 2004). Despite existing antidepressants, there are drawbacks (slow therapeutic onset, low remission rates, and treatment resistant patients). (Nestler *et al*., 2002; Sonawalla and Fava, 2001). Improvement of therapeutic options (especially designing treatments for patients that do not respond to currently available drugs) depends on the identification of underlying pathological processes in depression and potential mechanisms for their reversal.

There has been a growing interest in the use of natural products on mental disorders. Studies have shown that herbs have been used as alternative therapy for depression (Kessler et al., 2005). Jasmonates are involved in regulation of plant growth, development and protection against infections and mechanical injury (Farmer and Ryan, 1990). Jasmine flower has been used in aromatherapy for depression, nervousness, tension, alertness and memory improvement (Kuroda *et al.,* 2005). Jasmonates have been used in the production of jasmine tea, which has been found to be a powerful antioxidant (Zhang and Zhu, 1997), calm nerves (Kuroda *et al*., 2005) and reduce fat and cholesterol absorption (Fong and Chan, 1999).

Methyl Jasmonate (MJ) is a plant stress hormone that was first isolated from the essential oil of *Jasmonium grandiflorum* (Demole *et al.,* 1962). Methyl jasmonate is a bioactive compound obtained from Jasmine, a perennial climbing plant that is well known for its sweet and highly scented flowers. Methyl jasmonate is a volatile compound and possess the ability to diffuse through membranes. It is an airborne signal molecule potentially responsible for mediating intra- and interplant communications, modulating plant defense responses (Avanci *et al*., 2010) and regulating plant reproductive processes. This compound is secreted by plants in response to external stress and its level rises rapidly when plants suffer injury and pathogenic invasions (Fingrut *et al.,* 2005). It is one of the most well studied members of the jasmonate family and has gained international recognition as a potential agent for the treatment of cancer (Cesari *et al.,* 2014). Previous investigations have also shown that methyl jasmonate has anti-parasitic, anti-malarial (Gold *et al*., 2003), anti-inflammatory (Dang *et al*., 2008), antinociceptive (Umukoro and Olugbemide, 2011) and anti-aggressive (Umukoro *et al*., 2012) activities. Moreover, methyl jasmonate has been reported to demonstrate memory enhancing effects (Eduviere et al., 2015) and also decreased the brain levels of biomarkers of neuroinflammation that are implicated in cognitive dysfunction (Umukoro and Eduviere, 2017). In addition, preliminary studies have also revealed that methyl jasmonate exhibited anti-depressant activity in mice subjected to tail suspension and forced swim test, but the mechanism of action remains unknown. Thus, the present study was designed to investigate in details the antidepressant-like activity of methyl jasmonate and the mechanisms underlying its action employing the mice model.

1.1 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to evaluate the mechanisms via which methyl jasmonate mediates its antidepressant-like activity in mice with the following specific objectives:

- To assess the effect of methyl jasmonate on immobility time in mice subjected to forced swim and tail suspension tests.
- To evaluate the role of monoaminergic system in the antidepressant-like effect of methyl jasmonate using tail suspension paradigm in mice.
- To determine the effect of methyl jasmonate on the levels of biomarkers of oxidative stress and pro-inflammatory cytokine (tumor necrosis factor alpha) in the brains of mice pre-treated with lipopolysaccharide or exposed to chronic unpredictable mild stress.
- To evaluate the effect of methyl jasmonate on serum corticosterone and nitric oxide levels in the brains of mice subjected to lipopolysaccharide treatment or chronic unpredictable mild stress.
- To evaluate the effect of methyl jasmonate on the cyto-architectural alterations and population of viable neurons in the hippocampal and cortical regions of the brains of mice treated with lipopolysaccharide or exposed to chronic unpredictable mild stress.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Depression

Major depressive disorder or depression is an heritable neuropsychiatric syndrome characterized by relatively subtle cellular and molecular alterations distributed across a circuit of neural substrates (Krishnan and Nestler 2008). Major depressive disorder is a leading cause of disability worldwide, with a lifetime population prevalence as high as 20% (Kessler *et al*., 2005). Depressive disorders cause morbidity across the entire age spectrum (Kessler *et al*. 2005): they can be difficult to diagnose and treat in the paediatric and adolescent period (Prager 2009), complicate the course of patients with chronic illness (Evans *et al*. 2005), and increase overall medical burden in the elderly (Lyness *et al*. 2006). Depression is a heterogeneous group of illnesses that vary in symptomology and most likely in etiology. Depression, officially termed major depressive disorder ranks among the most prevalent diseases worldwide. According to the estimations of the World Health Organization, depression will be the second leading cause of disability in 2020 (Murray and Lopez, 1997). Major depressive disorder is characterized by at least five of the following symptoms: depressed or irritable mood, decreased interest or loss of pleasure, weight gain or loss, insomnia or hypersomnia, psychomotor retardation or agitation, fatigue or loss of energy, feelings of worthlessness or inappropriate guilt, diminished ability to think or concentrate, recurrent thoughts of death and suicide (Richards *et al.*, 2014). The symptoms must be evident almost daily for at least 2 weeks (Berton and Nestler, 2006) Additionally, symptoms of anxiety are also often seen in depressed individuals (Berton and Nestler, 2006). Major depressive disorder arises not because of a single traumatic event (or even multiple insults), but is the result of many factors that work together over time to produce changes in brain functions that ultimately give rise to the disease state (Anisman and Merali, 2003).

Exposure to stress is a main environmental risk factor associated with the occurrence of depression (Kessler, 1997). Previous studies have indicated that stress exposure may interact with genetic risk factors to increase susceptibility to depression (Caspi *et al*., 2003; Kaufman *et*

al., 2006). For these reasons, many animal models have attempted to reproduce some core components of major depressive disorder through exposure of laboratory animals to stress (Chakravarty *et al*., 2013). There are some risk factors of depression which include job loss, alienation from family and isolation from friends, which are known to be potent stressors (Oken *et al*., 2015). Addictive substances become more attractive, because they make the horrible emotional stress associated with depression seem a little better for a short period of time. Even if major depression is accurately diagnosed and with good treatment compliance, the best remission rates with standard antidepressants are known to be about 30–40% (Rapaport *et al.* 2003; Trivedi *et al*. 2006) which suggests that various mechanisms may be involved in the aetiology of depression. It is worthy to note that antidepressant drugs act by increasing synaptic levels of norepinephrine, serotonin, and also dopamine (Nutt, 2006). However, there are evidences implicating neuroinflammation as a major factor in the pathology of the disease. Indeed, studies have shown increased levels of inflammatory cytokines and antidepressants were reported to suppress pro-inflammatory cytokine production (e.g. interleukin-1b (IL-1β), IL-6, tumor necrotic factor-alpha (TNFα) and stress hormone increase (Lanquillon *et al*., 2000) but may also stimulate anti-inflammatory cytokine release (e.g. IL-10) (Castanon *et al.*, 2004; Kubera *et al*., 2001). These findings indicate that neuroimmune and neuroendocrine system perturbations likely play an important role in the etiology of depression (Konsman *et al*., 2002).

2.2 Biology of depression

Scientific studies have found that numerous brain areas show altered activity in patients suffering from depression and the brain is the "command centre" of the human body. It controls the basic functions of our bodies, our movements, and our thoughts and emotions (McEwen, 2003). The brain is flexible, able to grow and assume new shapes termed neuroplasticity. Specific receptors help neurons sense the environment and turn the genes which cause production of neurotransmitters and their receptors on or off (Nemade *et al.*, 2007). Neurons can adjust their responsiveness, literally growing new synapses and strengthening existing synapses, depending on the sorts of stimulation they receive (Nemade *et al.*, 2007). New neurons are constantly generated in neuronal pathways within certain areas of the brain that are involved in memory. In

addition, research suggests that antidepressant medications seem to increase the growth of new neurons in these key brain areas. In contrast, chronic stress seems to decrease cell growth in these areas (Nemade *et al.*, 2007).

Ongoing research continues to work towards finding definite causes for depression, developing diagnostic tests, and better treatments based on these key brain systems (Nemade *et al.*, 2007).

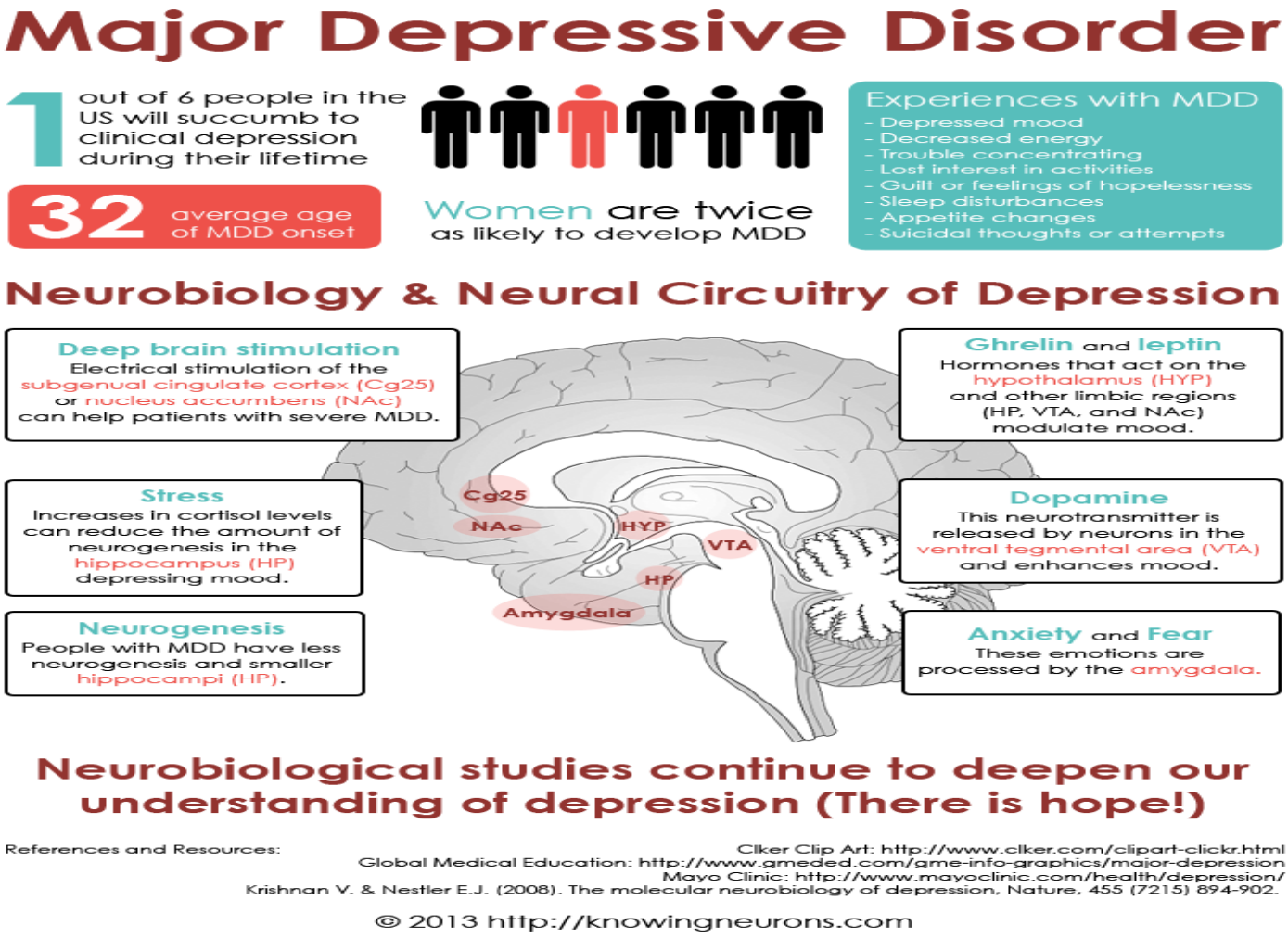


Figure 1: The molecular neurobiology of depression (Krishnan and Nestler, 2008)

2.3 Brain regions associated with depression

There is increasing evidence that stress and the resulting depression may involve structural changes in the brain. Brain imaging studies have shown that brain areas involved in mood, memory and decision making may change in size and function in response to depressive episodes (McEwen, 2003). Three brain structures the hippocampus, amygdala and prefrontal cortex help the brain determine what is stressful and how to respond (McEwen, 2003).

2.3.1 The Raphe nuclei

The sole source of serotonin in the brain is the raphe nuclei, a group of small nerve cell nuclei in the upper brain stem, located directly at the mid-line of the brain. There is some evidence for neuropathological abnormalities in the raphe nuclei in depression (Arango *et al.*, 2001). Despite their small size, they reach very widely through their projections, and are involved in a very diverse set of functions.

2.3.2 The Subgenual cingulate

This region is extremely rich in serotonin transporters and is considered a governor for a vast network involving areas like hypothalamus and brain stem, which influences changes in appetite and sleep; the amygdala and insula, which affect the mood and anxiety; the hippocampus, which plays an important role in memory formation; and some parts of the frontal cortex responsible for self-esteem (Carlson, 2013; Insel, 2010). Thus, disturbances in this area may contribute to depression. Deep brain stimulations of this area have been successful in curing depression in patients that could not be cured by anti-depressants (Mayberg *et al*., 2005). One meta analysis found this area to be overactive in youths with major depressive disorder compared to control (Miller *et al*., 2015).

2.3.3 The Ventricles

Multiple studies have found evidence of ventricular enlargement in people who have depression, particularly enlargement of the third ventricle (Hendrie and Pickles, 2010; Sheline, 2003). These

observations were interpreted as indicating loss of neural tissue in brain regions adjacent to the enlarged ventricle, leading to suggestions that cytokines and related mediators of neurodegeneration may play a role in giving rise to the disease (Miller *et al.*, 2009; Raison *et al.*, 2006).

2.3.4 The Prefrontal cortex

The prefrontal cortex (PFC), a key structure in emotional regulation, decision-making and memory, may also shrink with depression (McEwen, 2003). One of the regions of the prefrontal cortex that is most affected both by depression and by the manic phase of manic depression is the ventromedial cortex. This region is strongly modulated by the neurotransmitters involved in depression. Studies of people with a family form of depression or manic depression have shown that the ventromedial cortex was up to 40% smaller in people who were clinically depressed. One review reported hypoactivity in the prefrontal cortex of those with depression compared to controls (Wessa and Lois, 2016). One study on antidepressant treatment found an increase in PFC activity in response to administration of antidepressants (Outhred *et al.*, 2013). Another study suggested that the areas of the prefrontal cortex are part of a network of regions including dorsal and pregenual cingulate, bilateral middle frontal gyrus, insula and superior temporal gyrus that appear to be hypoactive in depressed patients. However the authors cautioned that the lack of consistency and small samples are limitations of this study (Fitzgerald *et al.*, 2010).

2.3.5 The Amygdala

The amygdala, a structure involved in emotional processing appears to be hyperactive in those with major depressive disorder (Miller *et al.,* 2015). The amygdala, where emotional memories are stored, becomes more active in depressive illness and post-traumatic stress disorder. Repeated stress may enlarge the amygdala (McEwen, 2003). A hyperactive amygdala, along with abnormal activity in other brain regions, leads to disrupted patterns of sleep and physical activity. It can also cause abnormal secretion of hormones and other chemicals that affect many systems of the body (McEwen, 2003).

2.3.6 The Hippocampus

Atrophy of the hippocampus has been observed during depression, consistent with animal models of stress and neurogenesis (Cole *et al*., 2011; Videbech and Ravnkilde, 2004). The endocrine and nervous systems are linked by the hypothalamus (a centrally located 'switching station' within the brain). The hypothalamus is an exceptionally complex brain region, which controls many different body functions such as blood pressure, appetite, immune responses, body temperature, maternal behavior, and body rhythms pertaining to circadian and seasonal rhythms. Both brain wave activity and hormone production are coupled to the circadian rhythm, and when the circadian rhythm is disturbed, mood disturbances can also result (Nemade *et al.*, 2007).

Many mental disorders, including depression, may cause the hippocampus to shrink or weaken. In the dentate gyrus, part of the hippocampal formation, new neurons (brain cells) are produced throughout adult life. Repeated stress slows the production of new neurons in the dentate gyrus and may also cause neurons in the hippocampus to shrink (McEwen, 2003)

2.4 Types of depression

2.4.1 Major depression

Major depressive disorder (MDD), also known as depression is characterized by a combination of symptoms that last for at least two weeks in a row, including sad, and/or irritable mood, that interfere with the ability to work, sleep, eat, and enjoy activities that were once pleasurable (National Institute of Mental Health, 2011). Its impact on functioning and well-being has been compared to that of other chronic medical conditions such as diabetes (Hays *et al*., 1995). People may also occasionally have false beliefs or see or hear things that others cannot (National Institute of Mental Health, 2016). About 7% of adults with major depression die by suicide and about 60% who die by suicide had depression or another mood disorder (Lynch and Duval, 2010). Major depressive disorder is usually caused by a combination of genetic, environmental, and psychological factors (National Institute of Mental Health, 2016). Risk factors include a family history of the condition, major life changes, chronic health problems, and substance abuse (National Institute of Mental Health, 2016).

2.4.2 Dysthymia

Dysthymia, now known as persistent depressive disorder (PDD), is a mood disorder consisting of the same cognitive and physical problems as depression, with less severe but longer-lasting symptoms (Gilbert *et al*., 2011). The concept was coined by Robert Spitzer as a replacement for the term "depressive personality" in the late 1970s (Brody, 1995). Dysthymia has a number of typical characteristics: low energy and drive, low self-esteem, and a low capacity for pleasure in everyday life (Niculescu and Aiskal, 2001). Mild degrees of dysthymia may result in people withdrawing from stress and avoiding opportunities for failure. In more severe cases of dysthymia, people may even withdraw from daily activities (Niculescu and Aiskal, 2001). Additionally, dysthymia often occurs at the same time as other psychological disorders, which adds a level of complexity in determining the presence of dysthymia, particularly because there is often an overlap in the symptoms of disorders (Sansone and Sansone, 2009). It is vital to look for signs of major depression, panic disorder, generalised anxiety disorder, alcohol and substance misuse and personality disorder (Baldwin and Thomas, 1995). Sometimes, people with dysthymia also experience episodes of major depression. This combination of the two types of depression is referred to as double-depression (Klein *et al*., 2006).

2.4.3 Bipolar disorder (manic depression)

Bipolar disorder, previously known as manic depression, is a mental disorder that causes periods of depression and periods of elevated mood (Anderson *et al*., 2012). The elevated mood is significant and is known as mania or hypomania, depending on its severity, or whether symptoms of psychosis are present (Anderson *et al.,* 2012). During mania, an individual behaves or feels abnormally energetic, happy, or irritable. (Anderson *et al.,* 2012). Bipolar disorders are often chronic and recurring (Debjit *et al*., 2012). When in the manic cycle, any or all of the depressive symptoms may be experienced. Mania often affects thinking, judgment, and social behavior in ways that cause serious problems and embarrassment. For example, indiscriminate or otherwise unsafe sexual practices or unwise business or financial decisions may be made when an individual is in a manic phase (Debjit *et al*., 2012). There are some mental health issues such as anxiety disorders and substance use disorder that are

commonly associated (Anderson *et al.*, 2012). The causes are not clearly understood, but both environmental and genetic factors play a role (Anderson *et al.*, 2012). Many genes of small effect contribute to risk (Goodwin, 2012). Environmental factors include a history of childhood abuse, and long-term stress (Anderson *et al*., 2012).

2.4.4 Postpartum depression (PPD)

Postpartum depression (PPD), also called postnatal depression, is a type of mood disorder or condition that describes a range of physical and emotional changes that many mothers can have which is usually associated with childbirth and can affect both sexes (Paulson, 2010). Post Partum Depression can happen a few days or even months after childbirth and this condition can negatively affect the person's child (Pearlstein *et al*., 2009). A woman can have feelings of sadness, despair, anxiety and irritability. It often keeps a woman from doing the things she needs to do every day (Debjit *et al.*, 2012). When a woman's ability to function is affected, this is a sure sign that she needs to see her healthcare provider right away. If a woman does not get treatment for PPD, symptoms can get worse and last for as long as one year (Debjit *et al*., 2012).

2.5 Theories of depression

Several theories concerning the cause of depression have been suggested over the years, of which the most prominent and widely researched is the monoamine hypothesis.

2.5.1. Monoamine theory

In its original formulation, the theory proposed that “some, if not all, depressions are associated with an absolute or relative deficiency of catecholamines or monoamines, particularly noradrenaline, at functionally important receptor sites in the brain (Bunney and Davis, 1965; Prange, 1964). This deficiency model of depression probably had its roots in two types of clinical data. The first of these consisted of observations of mood states of patients suffering from diseases involving the deficiency of a particular neurotransmitter, vitamin, or hormone,

such as in patients with hypothyroidism. The second consisted of observations of depressive reactions caused by pharmacological therapies which were thought to deplete particular substances, such as the depletion of norepinephrine stores by the antihypertensive agent, reserpine (Golden and Potter, 1986).

Monoamines are neurotransmitters and neuromodulators that include serotonin, dopamine, norepinephrine, and epinephrine (Neil, 2005). The brain dopaminergic system is crucially involved in reward behavior and/or motivation, especially the mesolimbic projections to the nucleus accumbens (Nac) and prefrontal cortex. A reduced plasma concentration of Homovanilic acid (HVA, a dopamine metabolite), is found in depressed patients (Altshuler *et al*., 2001). Serotonin and norepinephrine dysfunctions are the leading candidates, although problems with acetylcholine and dopamine function are thought by a number of researchers to be of critical importance as well. More recent studies suggest that there are indeed a subset of depressed people who have low levels of norepinephrine (Altshuler *et al*., 2001). The main assumption of this hypothesis is that clinical depression is due to impairment of central monoaminergic function, a deficiency in the neurotransmission mediated by serotonin (5-HT), norepinephrine (NA) and dopamine (DA). Monoamine concentrations may be altered as a result of disrupted synthesis, storage or release, or the concentrations may be normal but the postsynaptic receptors and/or sub-cellular messenger activity may be impaired (Hindmarch, 2001). Depressed mood as well as problems concentrating may be linked to deficient functioning within the monoamine projections to frontal cortex, and emotional symptoms (Stephen, 2004).

The treatment of depression is supposed to increase the availability of the amines in the brain. Different mechanisms such as blocking the reuptake of the monoamines in the synapse, inhibiting the intraneuronal metabolism of the monoamine or blocking the presynaptic inhibitory auto or heteroreceptors (Kiss, 2008) may increase the availability of brain monoamines. Monoamines affect a wide range of functions central in depression like sleep, vigilance, appetite, motivation, motor activity and reward, and their imbalance may produce symptoms like aggression, euphoria and impulsiveness. Loss of interest or pleasure in activities that are normally pleasurable is one of the core symptoms of depression (Victor, 2007). "Norepinephrine

may be related to alertness and energy as well as anxiety, attention, and interest in life; serotonin to anxiety, obsessions, and compulsions; while dopamine to attention, motivation, pleasure, and reward, as well as interest in life" (Victor, 2007). Many antidepressant drugs increase synaptic levels of the serotonin, but they may also enhance the levels of two other neurotransmitters, norepinephrine and dopamine. Serotonin may help to regulate other neurotransmitter systems, and decreased serotonin activity may "permit" these systems to act in unusual and erratic ways. Facets of depression may be emergent properties of this dysregulation (Mandell and Knapp, 1979).

An offshoot of the monoamine hypothesis suggests that monoamine oxidase A (MAO-A), an enzyme which metabolizes monoamines, may be overly active in depressed people. This would, in turn, cause the lowered levels of monoamines. This hypothesis received support from a study, which found significantly elevated activity of MAO-A in the brain of some depressed people (Meyer *et al*., 2006).

2.5.2. ENDOCRINE THEORY

The endocrine system is made up of small glands within the body, which secrete hormones and release them into the blood. The hormones that are released into the body by the glands regulate processes such as reaction to stress and sexual development (Kim, 2016). Hormonal imbalances also may play a role in depression. A possible mechanism is a default in the hypothalamic-pituitary-adrenal (HPA) axis—the system that manages the body's response to stress (Oken *et al*., 2015). Chronic activation of the HPA axis may contribute to depression. Indeed, depressed patients often exhibit higher blood levels of stress hormones than those who are not depressed (Nemeroff and Vale, 2005). One theory suggests that abuse or neglect early in life may contribute to permanent changes in the brain that result in depression, for example, a persistent overproduction and increased activity of corticotrophic hormone releasing factor (CRF) and hyperactivity of the HPA axis (Sanchez *et al*., 2001).

The main hormone of the adrenal glands, cortisol, is higher in depressed individuals (Oken *et al*., 2015). Decreased levels of estrogen can alter the activity of neurotransmitters such as serotonin and norepinephrine, which can then lead to depression (Nemade *et al*., 2007). The ovaries, which

produce estrogen, are thought to be one of the main reasons why women run a higher risk of developing depression than men. Testosterone, a hormone produced by the testes in males, may also be linked to depression. A decrease in testosterone after the age of 50 is well documented, and may possibly be one of the reasons that men of this age become more prone to depression (Nemade *et al*., 2007). There is a well documented relationship between the brain and the endocrine system. Neurotransmitters have profound effects on the release of stress hormones and likewise, stress hormones have significant effects on neurotransmitters and mood. The elevated level of stress hormones usually found in depressed patients is strong support for the importance of the brain-endocrine system relationship in depression (Deussing and Wurst, 2005).

There is a large body of literature which showed that corticosteroids can influence neurotransmitter tone and, vice versa, that corticosteroid secretion is regulated by the neurotransmitters implicated in depression. It is widely believed that high levels of corticotropin releasing hormone (CRH) are rather prodepressive (Holsboer and Ising, 2008) and it was shown that CRHR1 antagonists have an effect similar to paroxetine and other antidepressants (Holsboer and Ising, 2008). Studies confirmed a major role of corticosteroid receptors and of the CRH system in the pathogenesis of affective disorders including depression (Deussing and Wurst, 2005). Much attention has been focused on brain areas showing high levels of corticosteroid receptor expression, namely the hippocampus, and the prefrontal cortex. These two brain areas, which are reciprocally connected, exert inhibitory neural control over the hypothalamo–pituitary–adrenal (HPA) axis, and thus restrain excess corticosteroid secretion (Plotsky *et al*., 1998).

During the past decade, several research groups formulated a hypothesis relating aberrant stress hormone dysregulation to causality of depression and submitted that antidepressants may act through normalisation of these HPA changes (Holsboer and Barden, 1996). This hypothesis was derived from the following clinical observations in depressive patients:

- the number of ACTH and cortisol secretory pulses is increased which is also reflected in elevated urinary cortisol production rates (Rubin *et al*. 1987);
- levels of CRH in the CSF are elevated, the number of CRH secreting neurons in limbic brain regions is increased (Raadsheer *et al.* 1994); and

- the number of CRH binding sites in the frontal cortex is reduced secondary to increased CRH concentration (Nemeroff *et al*. 1988).

Considering the findings that depressed patients are frequently hypercortisolemic and that the degree of hippocampal atrophy in aged humans correlates with the degree of plasma cortisol increase over time and the current basal cortisol levels (Lupien *et al*. 1998), it has been proposed that the neuroendocrine changes in depression may account for the changes in hippocampal size seen in this disease. The hypothalamus is also responsible for releasing stress hormones. Many studies show that depressed individuals have increased levels of stress hormones. In addition to the hypothalamus, other endocrine organs such as the thyroid, adrenal glands, the ovaries and testes have been linked to depression. As mentioned previously, depression is frequently associated with low levels of thyroid hormone (or hypothyroidism), and mood elevation is often associated with high levels of thyroid hormone (or hyperthyroidism). Treating hypothyroidism by supplementing or replacing thyroid hormone may sometimes alleviate depression (Nemade *et al.,* 2007).

2.5.3 CYTOKINE THEORY

Various reviews have found that inflammation may play a role in depression (Krishnadas and Cavanagh, 2012; Patel, 2013). Various sources of inflammation in depressive illness have been hypothesized and include trauma, sleep problems, diet, smoking and obesity (Leonard and Maes, 2012). Cytokines, by manipulating neurotransmitters, are involved in the generation of sickness behavior, which shares some overlap with the symptoms of depression. One meta analysis of cytokines in depressed patients found increased IL-6 and TNF-α levels relative to controls (Dantzer *et al*., 2016). Meta analysis on cytokine levels in depressed patients have demonstrated increased levels of IL-1, IL-6 and C-reactive protein in depressed patients (Howren *et al*., 2009; Maes, 2011). One review found normalization of cytokine levels after successful treatment of depression (Kohler *et al.,* 2014).

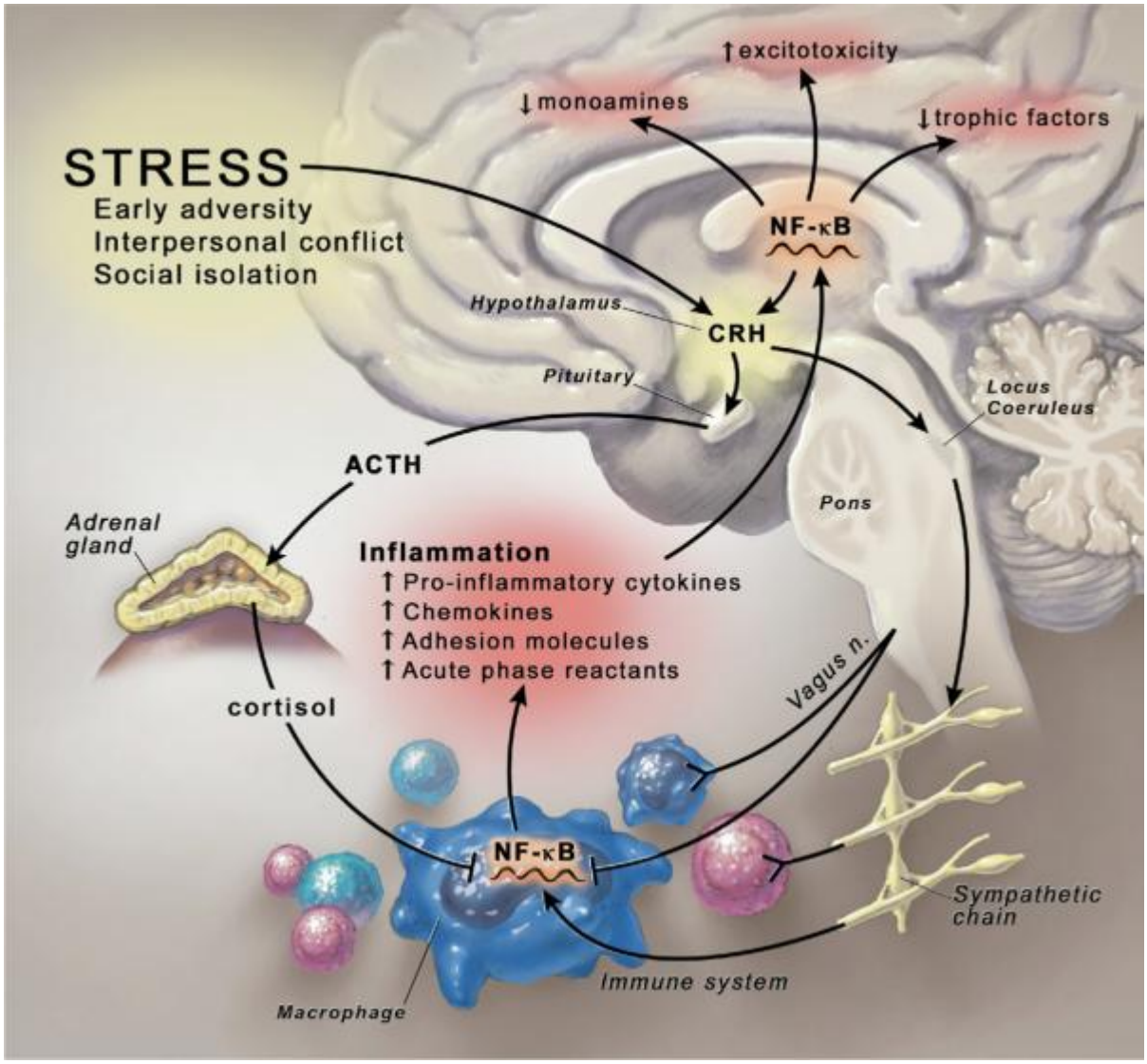


Figure 2: Stress-induced activation of the inflammatory response.

Psychosocial stressors activate central nervous system stress circuitry, including CRH and ultimately sympathetic nervous system outflow pathways via the locus coeruleus. Acting through alpha and beta adrenergic receptors, catecholamines released from sympathetic nerve endings can increase NF-κB DNA binding in relevant immune cell types, including macrophages, resulting in the release of inflammatory mediators that promote inflammation. Proinflammatory cytokines, in turn, can access the brain, induce inflammatory signaling pathways including NF-κB, and ultimately contribute to altered monoamine metabolism, increased excitotoxicity, and decreased production of relevant trophic factors. Cytokine-induced activation of CRH and the hypothalamic-pituitary-adrenal axis, in turn, leads to the release of cortisol, which along with efferent parasympathetic nervous system pathways (e.g., the vagus nerve) serve to inhibit NF-κB activation and decrease the inflammatory response (Fig. 2). In the context of chronic stress and the influence of cytokines on glucocorticoid receptor function, activation of inflammatory pathways may become less sensitive to the inhibitory effects of cortisol, and the relative balance between the proinflammatory and anti-inflammatory actions of the sympathetic and parasympathetic nervous systems, respectively, may play an increasingly important role in the neural regulation of inflammation (Miller *et al*., 2009).

A meta analysis found the use of anti-inflammatory drugs such as non steroidal anti inflammatory drugs (NSAIDs) and investigational cytokine inhibitors reduced depressive symptoms (Black *et al.*, 2015). Cytokines secreted by activated immune cells can alter the moods, behaviors and thoughts characteristic of depression and schizophrenia. Cytokines can also cause the neurotransmitter, hormone and other physiological abnormalities found with depression (Mizel, 1989). Physical stressors always activate the immune system and increase cytokine secretion. The immune system and cytokines always inform the brain and endocrine system about serious physical stressors (Mizel, 1989). There are certain cytokines that have been shown to induce depression-like behavior in rodents and primates (Dunn *et al*. 2005; Felger *et al*. 2007), and several models of chronic stress produce significant changes in immune function (Miller *et al*. 2009). One such example is IL-1β (interleukin-1β): increases in IL-1β signaling in the hippocampus play a role in mediating the anhedonic and antineurogenic effects of chronic stress through the actions of the transcription factor NFκB (nuclear factor-κB) (Koo and Duman, 2008; Koo *et al*., 2010).

The immune system, via the secretion of cytokines, has a powerful ability to affect brain function (Fig. 2). The brain, via the secretion of various chemicals and direct nerve connections with the immune system, has a potent ability to affect immune system function. Therefore, the linkage between the immune system and the brain is a two-way communication channel (Mizel, 1989). Depression causes psychological, social and economic stressors (i.e. mental stressors) to increase. The arrow number 6 (in the figure below), going from depression to mental stressors, points out the phenomena of depression helping to induce more depression (Mizel, 1989).

A unique feature of this paradigm is that the immune system has a documented two-way communications link with the endocrine system. This is very important, because endocrine activation is a common finding in depression. Another feature is that physical stressors (infection, trauma, cancer, organ dysfunction, autoimmune disease and physical diseases of any sort) activate the immune system, causing increased cytokine secretion (Fig. 3). The cytokines are carried to the brain, where they help produce the symptoms and signs of depression (Mizel, 1989).

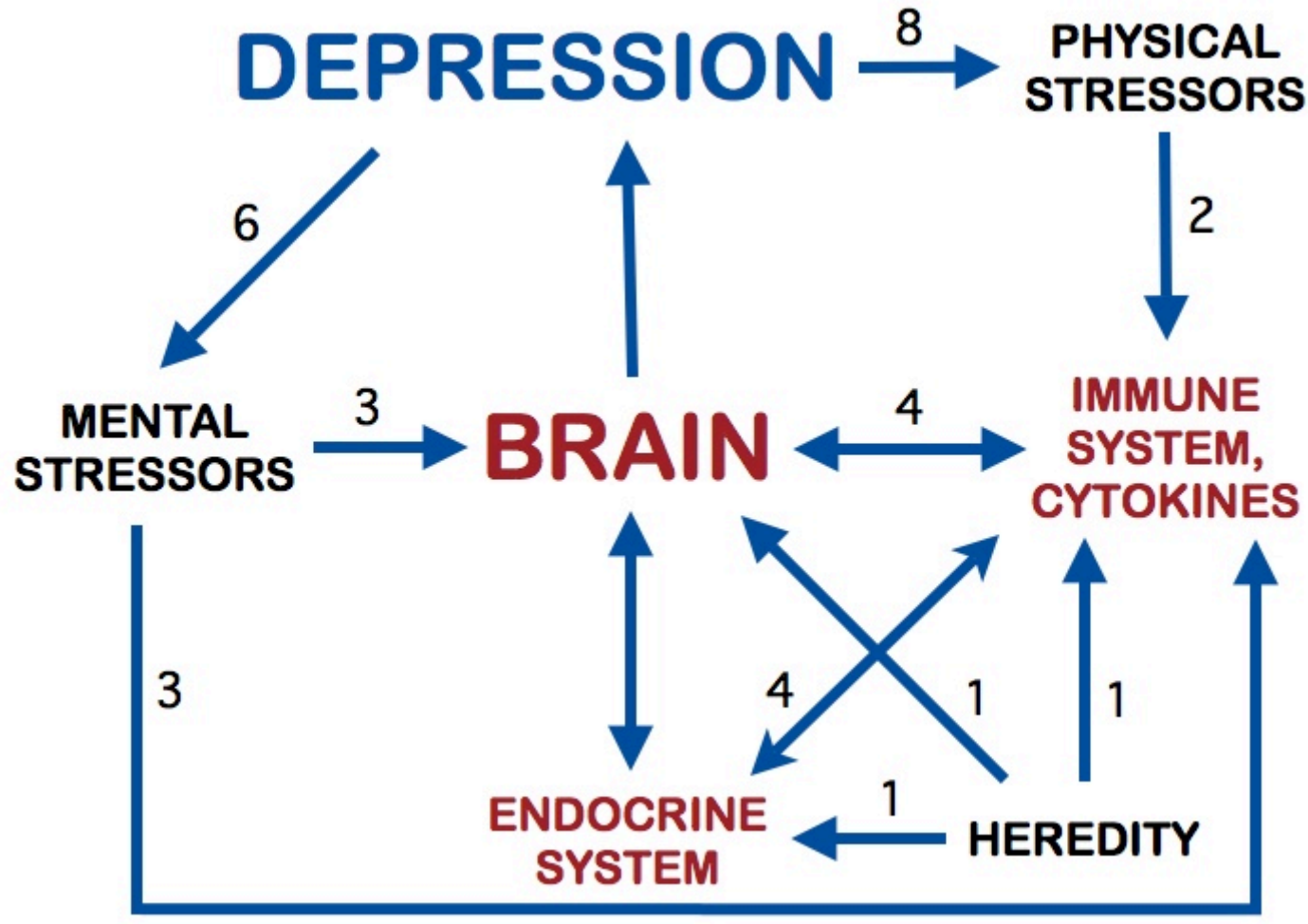


Figure 3: The Immune-Cytokine Paradigm of Depression

2.5.4. Neurotrophic hypotheses

This hypothesis proposes that depression results from decreased neurotrophic factors, leading to neuronal atrophy, decreased hippocampal neurogenesis and loss of glia, and that antidepressant treatment blocks or reverses this neurotrophic factor deficit, and thereby reverses the atrophy and cell loss (Duman and Monteggia, 2006; Duman *et al*., 1997). Different types of acute or chronic physical and psychsocial stressors decrease neurogenesis, while chronic antidepressant treatments, including serotonin-selective reuptake inhibitors (SSRIs) and norepinephrine-selective reuptake inhibitors (NSRIs), increase neurogenesis (Duman and Monteggia, 2006; Sahay and Ren, 2007). Brain imaging studies demonstrate a reduction in the volume of limbic brain regions implicated in depression, notably the hippocampus and prefrontal cortex (PFC) (Drevets *et al*., 2008; Macqueen *et al*., 2008). Post-mortem studies reported a reduction in the size of neurons and loss of glia (Drevets *et al*., 2008; Miguel-Hidalgo and Rajkowska, 2002), and preclinical studies show that exposure to repeated stress causes atrophy of neurons in the hippocampus and PFC, as well as loss of glia (Duman and Monteggia, 2006; Krishnan and Nestler, 2008). These studies provide strong evidences that atrophy and loss of neurons and glia are contributing factors to depression- and stress-related disorders.

The role of neurotrophic factors in cell atrophy and loss is supported by evidence that stress or depression decreases the expression of certain factors in limbic brain regions. One of the most highly studied factors is brain-derived neurotrophic factor (BDNF), one of the most prevalent neurotrophic factors in adult brain (Mirescu and Gould, 2006). Brain derived neurotrophic factor (BDNF) is a member of the nerve growth factor family (Murakami *et al*., 2005). It is essential for growth, maintenance, cellular differentiation and survival of neurons in the central nervous system. Postmortem analyses have revealed lower levels of BDNF in patients with major depression (Castren *et al.,* 2007), while BDNF infusion into the brain has been found to produce antidepressant like action (Siuciak *et al*., 1997). Exposure to different types of physical or social stress decreases the levels of BDNF in the hippocampus and prefrontal cortex (Duman and Monteggia, 2006; Krishnan and Nestler, 2008; Castren and Rantamaki, 2010). There is also evidence that antidepressants increase hippocampal BDNF levels in humans. It is possible that the down regulation of BDNF may contribute to the atrophy of cornu ammonis 3 (CA3) neurons and reduced neurogenesis of granule cells in the hippocampus, although elevated levels of

adrenal glucocorticoids could also account for these effects (Duman, 2002; Yu et al., 2008). Neuronal atrophy is demonstrated by a decrease in the number and length of branch points of the apical dendrites of CA3 neurons (Duman, 2002; Gold *et al*., 1988). Repeated stress is reported to cause atrophy of CA3 pyramidal neurons in the hippocampus, including a decrease in the number and length of apical dendrites. In addition, exposure to acute stress decreases the proliferation of cells in the dentate gyrus of the hippocampus (Eugene *et al.,* 2005). Earlier studies reported that the numbers of cells in prefrontal cortex are decreased in patients with depression. Previous studies have also reported a decrease in neuronal size and the number of neurons and glia in the prefrontal and rostral orbitofrontal cortex (Duman, 2002; Sendtner, 2001). These findings suggest that atrophy and survival of neurons may also contribute to certain symptoms of depression, such as depressed mood and working memory that can be attributed to prefrontal cortex (Lu, 2007).

In addition to BDNF, other neurotrophic/growth factors have been implicated in depression, including vascular endothelial growth factor (VEGF), fibroblast growth factor 2 and insulin-like growth factor 1 (IGF-1). Some of these factors have been best known for their effects on peripheral tissues, but they are also expressed in neurons and glia and influence brain function (Duman and Monteggia, 2006; Akil *et al*., 2008; Fournier and Duman, 2011).

2.6 Causes of depression/ risk factors

Many researchers believe depression is caused by chemical changes in the brain. This may be due to a problem with the genes, or triggered by certain stressful events. More likely, is combination of both (Fava and Kendler, 2000). The genetic risk in depression is about 40–50% (Levinson, 2006), but there are also several environmental risk factors for major depressive disorder (MDD). These include gender, stressful life events, adverse childhood experiences and certain personality traits (Fava and Kendler, 2000). An association of psychological stress and oxidative stress has been described repeatedly, both in animal models and humans. Psychological stress can induce a constellation of physiological responses (including nervous, endocrine and immune systems) which otherwise could be harmful under some conditions (Hall *et al*., 2012).

Among those responses, hyperactivity of HPA axis is one of the commonest neurobiological changes which are manifested in depressive patients (Holsboer, 2000). Furthermore, depression is associated with cardio-vascular problems. Interestingly, oxidative stress is a risk factor linking both disorders on biochemical levels.

Heredity profoundly influences the endocrine and brain function and both of these systems play key roles in depression. It is estimated that heredity accounts for about 30 to 50% of the incidence of depression (Sullivan *et al.*, 2000). Anything that will affect the brain or the endocrine system will affect the incidence and severity of depression (Oken *et al.*, 2015). There are various causes of depression which can be classified into environmental, biological or genetic causes.

2.6.1 Genetic causes

There is strong evidence that genetic factors play a significant role in a person's predisposition towards developing depression, especially melancholic depression, psychotic depression and bipolar disorder. No single gene is likely to be responsible, but rather a combination of genes (Levinson, 2006). Epidemiologic studies show that roughly 40%–50% of the risk for depression is genetic (Fava and Kendler 2000) which makes depression a highly heritable disorder. Some types of depression run in families, indicating that a biological vulnerability to depression can be inherited. This seems to be the case, especially with bipolar disorder. Families in which members of each generation develop bipolar disorder have been studied (Anderson, 2000). The investigators found that those with the illness have a somewhat different genetic makeup than those without bipolar disorder (Anderson, 2000). However, not everybody with the genetic makeup that causes vulnerability to bipolar disorder will develop the illness. Major depression also seems to occur in generation after generation in some families, although not as strongly as in bipolar I or II. Indeed, major depression can also occur in people who have no family history of depression (Anderson, 2000).

2.6.2 Environmental causes

Environmental causes of depression are concerned with factors that are outside of ourselves, not directly related to brain function, inherited traits from parents, medical illnesses, or anything else that may take place within us (Bilsker and Paterson, 2012). Instead, environmental events are those things that happen in the course of our everyday lives. These may include situations such as prolonged stress at home or work, coping with the loss of a loved one, or traumatic events (Bilsker and Paterson, 2012). It has long been understood that experiences we have in our lives can affect our state of mind and how we react to these environmental events may influence the development of various psychiatric diseases (Gilman *et al*., 2007). Stress can also occur as the result of a more positive event such as getting married, moving to a new city, or starting a new job. It is not uncommon for either positive or negative events to become a crisis that precedes the development of clinical depression (Ingram *et al*., 1987). It is not certain why stress may lead to depression in this way. However, researchers have theorized an explanation called the "kindling effect," or "kindling-sensitization hypothesis." This theory surmises that initial depressive episodes spark changes in the brain's chemistry and limbic system that make it more prone to developing future episodes of depression (Post, 1992). Since early episodes of depression make a person more sensitive to developing depression, even small stressors can lead to later depressive episodes (Post, 1992).

People who become clinically depressed have generally experienced more severe difficulties in childhood than those who do not become depressed. These difficulties may include sexual or physical abuse, a turbulent upbringing, separation from a parent, or mental illness in a parent. Some researchers believe that a problematic childhood may trigger an early-onset of depression (Klein *et al.,* 2009). Experiencing great difficulties as children, these individuals may be more likely to have low self-esteem, feel powerless, and become dependent on others to make them feel good about themselves. These kinds of traits may increase a person's susceptibility to depression (Alloy *et al*., 2006a).

2.6.3 Biological causes

The biologic factors that might have some effect on depression include hormones and brain chemicals (Nemeroff, 1998). Much of our research and knowledge, however, has focused on four of these neurochemical systems: norepinephrine, serotonin, dopamine, and acetylcholine (Palmer *et al.*, 2003). Research has found that there are some hormonal changes that occur in depression, which might result in an over- or under-production of some hormones, which may account for some of the symptoms of depression (Palmer *et al*., 2003). It is also believed that during depression, there is reduced activity of one or more certain neurotransmitter systems, and these disturb certain areas of the brain that regulate functions such as sleep, appetite, sexual drive and perhaps mood (Palmer *et al*., 2003). The reduced level of these neurotransmitters results in reduced communication between nerve cells and accounts for typical symptoms of depression.

2.6.4 Medications

Certain medications used for a variety of medical conditions are more likely than others to cause depression as a side effect. Specifically, some medications that are used to treat high blood pressure, cancer, seizures, extreme pain, and to achieve contraception can result in depression. Even some psychiatric medications like some sleep aids and medications to treat alcoholism and anxiety can contribute to the development of depression (Guaiana *et al*., 2007).

2.7 Depression and the hypothalamic-pituitary-adrenal (HPA) axis

The hypothalamic-pituitary-adrenal axis (HPA) is a chain of endocrine structures that are activated during the body's response to stressors of various sorts. Early life stress has been hypothesized as a potential cause of HPA dysfunction (Juruena, 2014; Heim *et al*., 2008). The HPA axis involves three structures; the hypothalamus, which release corticotropin releasing hormone (CRH) that stimulates the pituitary gland to release adreno-corticotropic hormone (ACTH), which stimulates the adrenal glands to release cortisol. Increased basal cortisol levels have been observed in patients with depression (Belvederi *et al*., 2014). The main regulatory centre of the axis is the hypothalamic paraventricular nucleus (PVN). Under basal conditions, corticotropin-releasing hormone (CRH) produced within the medial parvicellular division of this nucleus is the dominant regulator of the

axis (Pariante and Lightman, 2008), mediating the endocrine response to stress. In situations of chronic stress, many parvicellular neurones co-express vasopressin, which plays an important role in sustaining HPA axis activation through a synergistic action with CRH (Dinan and Scott, 2005). CRH stimulate the release of adrenocorticotropic hormone (ACTH), causing increased synthesis and release of cortisol, the main stress hormone of the axis, from the adrenal glands (Aguilera and Rabadan-Diehl, 2000).

The CRH test has also been administered following pretreatment with dexamethasone (DEX), which results in augmented, rather than reduced, ACTH and cortisol responses in depressed patients compared to normal controls (Heuser *et al*., 1994). HPA axis monitoring by DEX/CRH before and after active medication shows that cortisol and ACTH response normalizes with effective antidepressant therapy (Himmerich *et al*., 2007). Overall, the DEX/CRH test is probably the most reliable neuroendocrine test for assessing HPA axis dysregulation in depression (Ising *et al*., 2005). It has a reasonable predictive value for the risk of depressive relapse.

Abnormalities in the function of the HPA axis have been described in people who experience psychiatric disorders (Nemeroff, 1996). Studies conducted in both animals and humans suggest that stress experienced during the early phases of development can induce persistent changes in the ability of the HPA axis to respond to stress in adulthood, increasing the susceptibility to depression (Glover and O'Connor, 2002). Findings derived from multiple lines of research have provided evidence that during depression, dysfunction of limbic structures, including the hypothalamus and hippocampus, results in hypersecretion of corticotropin-releasing factor (CRF), dehydroepiandrosterone, and vasopressin, which in turn determines HPA activation. A flaw in this system caused by factors such as excessive stress, high glucocorticoid levels, social isolation, and depressive symptoms results in difficulty adapting to stress and can predispose the individual to depression by impairing hippocampal serotonergic neurotransmission (Holsboer, 2000; Joca *et al*., 2003; Juruena *et al*., 2004). Another group of states is characterized by hypoactivation of the stress system, rather than sustained activation, in which chronically reduced secretion of CRF may result in pathological hypoarousal and enhanced HPA negative feedback. Patients with atypical depression, seasonal depression, and chronic fatigue syndrome fall into this category (Kellner and Yehuda, 1999; Juruena *et al.*, 2004; Juruena and Cleare, 2007).

Table 1 Clinical conditions associated with hyperactivation or hypoactivation of the HPA axis (Juruena et al., 2004).

Increased HPA axis activity	**Decreased HPA axis activity**	**Disrupted HPA axis activity**
Severe chronic disease	Atypical depression	Cushing syndrome
Melancholic depression	Seasonal depression	Glucocorticoid deficiency
Anorexia nervosa	Chronic fatigue syndrome	Glucocorticoid resistance
Obsessive-compulsive disorder	Fibromyalgia	
Panic disorder	Hypothyroidism	
	Adrenal suppression	
Malnutrition	Post glucocorticoid therapy	
Diabetes mellitus	Posttraumatic stress disorder	
Hyperthyroidism	Nicotine withdrawal	
Central obesity	Postpartum	
Childhood maltreatment	Menopause	

One of the mechanisms thought to be involved in HPA axis hyperactivity in depression is impaired feedback inhibition of the HPA axis by circulating glucocorticoids (Pariante and Miller, 2001). This impaired feedback inhibition has been demonstrated in depressed patients in various studies, many conducted in the 1970s and 1980s (Juruena *et al.,* 2009; Juruena *et al*., 2010; Ribeiro *et al.,* 1993). Many recent studies support the hypothesis that stressful events correlate with an increased vulnerability for depression in a way that stressful situations often precede the onset of illness and are also associated with the severity of depression (Holsboer, 2001; Nemeroff, 1988). Such stressors can lead to a transient hyperactivation of the hypothalamic-pituitary-adrenocortical (HPA) axis, resulting in increased glucocorticoid secretion. In this regard, depression is often associated with a dysregulation of the HPA axis under chronic stress conditions (Holsboer and Barden, 1996).

2.8 Oxidative stress and depression

It has been suggested that oxidative stress plays a major role in the etiology of depression. Furthermore, it has been shown that antidepressants influence oxidative stress during a depressive episode. It has been shown that oxidative stress contributes to neuronal and glial cell loss in the central nervous system (CNS) (Goetz *et al*., 1994; Grunblatt *et al*., 2004; Koutsillieri *et al*., 2002). Increased markers of oxidative stress relative to controls have been found in patients with major depressive disorder (MDD).

Immunological processes have been discussed in the pathophysiology of depressive disorder (DD), since a mild systemic inflammation seems to take place in patients with depressive symptoms (Raison *et al*., 2006). Furthermore, reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite are produced by phagocytes as part of the cytotoxic host response (Raison *et al*., 2006). Increased oxidation of lipid molecules in depression might be due to activation of the immune response followed by induction of pro-inflammatory mediators like tumor necrosis factor and interleukins (Miller *et al*., 2002; Penninx *et al*., 2003; Lesperance *et al*., 2004).

Consistently, volumetric reductions in the prefrontal cortex (PFC) and the hippocampus have been reported from structural brain imaging studies of patients with depression (Campbell *et al*., 2004). One of the most plausible causes for these neuronal alterations is elevated oxidative stress due to increased production of free radicals (Michel *et al*., 2007; Michel *et al*., 2010). Oxidative stress is a result of either increased production of ROS or decreased antioxidant defence. ROS are free radicals or reactive anions/molecules containing oxygen atoms such as superoxide, the hydroxyl-radical, and peroxynitrite (Fridovich, 1986; Halliwell and Gutteridge, 1989). Antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GPX) metabolise ROS into less toxic molecules. SOD catalyses the reaction of superoxide to the less toxic H_2O_2 (Winterbourn, 1993). SOD is one of the most important antioxidant enzymes and interacts with other neuroprotective substances (Gsell *et al.*, 1995).

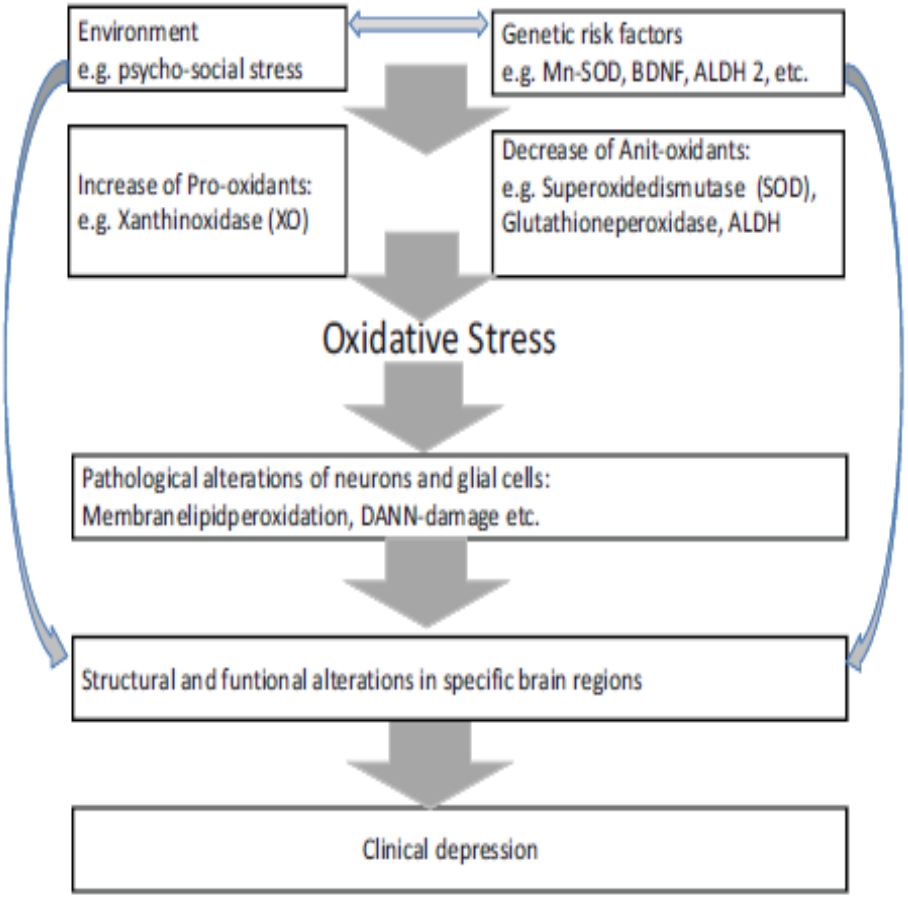


Figure 4: Oxidative stress aetiology of depression: environmental and genetic factors leading to increased induction of oxidative stress related membrane-lipidperoxidation and DNA damage. These are responsible for structural and functional neuronal and glial alterations in certain brain regions known to play a role in the development of depression. These lead to some of the clinical presentations of depression (Michel *et al*., 2011).

Depressive disorder has been linked to increased serum levels of malondialdehyde (MDA), a breakdown product of oxidized apolipoprotein B-containing lipoproteins, and thus a marker of the rate of peroxide breakdown (Bilici *et al.,* 2001; Sarandol *et al.,* 2006). In patients with depression, elevated levels of MDA adversely affect the efficiency of visual-spatial and auditory-verbal working memory, short-term declarative memory and delayed recall declarative memory (Talarowska *et al*., 2012). The results of altered antioxidant enzyme levels in the blood of patients with MD have been somewhat conflicting, some showed increased levels of SOD, GSH-Px and GR (Bilici *et al*., 2001; Sarandol *et al*., 2007), others showed decreased SOD (Herken *et al*., 2007). Patients with depression seem to have lower albumin levels, which means decreased antioxidant activity (Van Hunsel *et al*., 1996). One report of unmedicated patients (for at least 2 months) with depression found a decreased total antioxidant potential and uric acid levels in plasma as well as increased total plasma peroxide and oxidative stress index levels compared to healthy control subjects (Yanik *et al*., 2004). A study in medication-free patients with depression compared to healthy controls showed a lower total antioxidant potential, uric acid concentration, increased lipid peroxidation markers and total oxidative stress index in plasma of these patients (Yanik *et al*., 2004). Maes *et al*., (2010) found increased plasma peroxides and serum oxidized low density lipoptrotein antibodies in patients with depression. These findings emphazise the role of oxidative stress pathways in depression as well as in coronary heart syndrome, which might partly explain the high comorbidities of these disease entities (Maes *et al*., 2010).

Increasing evidence suggests an anti-oxidative role of antidepressants via complex interactions with growth factors etc. Antidepressants seem to help regaining the so-called “oxidative balance” (Michel, 2010; Herken *et al*., 2007). In the serum of patients with depression, initial lower concentrations of SOD are normalized through antidepressant treatment (Herken *et al*., 2007). This is accompanied by inverse alterations of the activity of SOD (Atmaca *et al*., 2004).

2.9 Anhedonia and depression

The most widely accepted approach to assess reward-seeking behaviour is via the sucrose consumption and preference tests. Decreased intake of palatable solutions, such as sucrose is

regarded as a behavioural measure of anhedonic deficit/depressive-like state (Willner, 2005). Anhedonia, a hallmark of depression is defined as the inability to experience pleasure from activities formally found enjoyable (American Psychiatric association, 2000). Dopamine neuronal functioning is essential in sustaining a wide variety of pleasurable and rewarding experiences (Wise and Bozarth, 1985; Wise, 2002). Anhedonia is specified as a core symptom of MDD and is characterized by a loss of interest in, or ability to derive pleasure from, rewarding stimuli (or activities) (American Psychiatric Association, 2000). Anhedonia may not be present in all individuals diagnosed with major depressive disorder, yet its occurrence in a significant number of depressed patients makes it a feature worth investigating in animal models (Borowski *et al*., 1998). Reduced consumption of palatable substances has been determined in a number of animal procedures involving manipulations that are hypothesized to induce depression. These include the chronic mild stress model (Konkle *et al*., 2003), models of social stress (Von Frijtag *et al*., 2002), a model of prenatal drug (i.e. cocaine, nicotine) exposure (Sobrian *et al*., 2003). It is well established that humans exhibit decreased appetite during illness and disease, including depression (Plata-Salaman, 1996). In rodents, infection and inflammation are similarly accompanied by reduced food intake, and endotoxins and cytokines have specifically been shown to reduce food intake (Dunn and Swiergiel, 2001).

In animals, exposure to endotoxins (e.g. lipopolysaccharide; LPS) or cytokines induces a 'sickness behavior' syndrome that is characterized by anhedonia, increased sleep, and decreases in food intake, body weight, locomotor activity, social interaction, sexual behavior and grooming (Konsman *et al*., 2002). Long-term biological changes following repeated endotoxin exposure include significant increases in serum IL-1 (Zuckerman *et al*., 1991). Acute exposure to endotoxins, IL-1β, IL-6 or TNF-α, each disrupts consumption of palatable substances (Anisman *et al*., 2002). Repeated cytokine exposure produced persistent alterations in HPA axis functioning (Linthorst *et al*., 1994).

2.10 Consequences of Depression

Patients with depression have very high rates of physical illnesses. Furthermore, depression appears to increase the severity of physical illnesses. For example, patients that are vulnerable or prone to heart attack who develop depression have higher death rates and more complications than those without depression. The same is true for kidney disease and rheumatoid arthritis. Thus, depression is thought to have very serious health consequences.

2.11 ANIMAL MODELS OF DEPRESSION

Animal models of depression are research tools used to investigate depression and action of antidepressants as a simulation to investigate the symptomatology and pathophysiology of depressive illness or used to screen novel antidepressants.

2.11.1 LEARNED HELPLESSNESS

One of the well validated animal models is learned helplessness, in which the depressive-like state in animals is induced by uncontrollable and unpredictable electrical foot-shock stress (Seligman and Maier, 1967; O'Neil and Moore, 2003). The rationale is that exposure to uncontrollable and stressful life events makes people to feel like losing control, and sometimes leads to a depressive like behavior ultimately. The model is based on the observation that animals also develop deficits in escape, cognitive and rewarded behaviors when they have been subjected to repeated unavoidable and uncontrollable shocks (Drugan *et al*., 1997; Grahn *et al.*, 2000). Following one or more sessions of inescapable shock, rats have been shown to develop persistent changes including weight loss, alterations in sleep patterns and HPA axis activity and loss of spine synapses in hippocampal regions (Cryan and Mombereau, 2004; Haddjeri *et al*. 1998; Nestler *et al*. 2002).

Learned helplessness was first observed in the early 1960s by Richard L. Solomon, a graduate student of Dr. Mowrer, who observed that moderately-extended experience with uncontrollable traumatic events resulted in later unexpected behavioral changes (Seligman, 1972). Thereafter, Overmier and Seligman found that exposure to an uncontrollable traumatic event for a total of 3-

5 min distributed over the course of a couple of hours resulted in dramatic deficits in behavioral coping, associative learning, and emotional expression, and they called these phenomena "learned helplessness". Animals with learned helplessness show several changes that are reminiscent of depression, such as altered rapid eye movement sleep (Adrien *et al*., 1991), reduced body weight (Dess *et al*., 1988), diminished sexual behavior (Dess *et al.,* 1988), and elevated levels of corticotrophin-releasing factor (CRF) and corticosterone (Greenberg *et al*., 1989). Currently, studies have indicated that repeated dosing with antidepressants (Sherman *et al.,* 1982) or electroconvulsive seizure therapy (ECS) (McKinney *et al*., 1986) reduces the latency to escape and decreases the number of animals that show learned helplessness.

One advantage of learned helplessness as a model is that its symptoms are parallel to those of major depression, and most of them can be reversed by multiple acute (subchronic) treatment with AD (Takamori *et al.,* 2001). These excellent face and predictive validities make learned helplessness an interesting model to explore the pathophysiology of depression (Volmayr and Henn, 2003). However, the major drawback of this model is that most of the depression-like symptoms do not persist long enough following cessation of the uncontrollable shock (Cryan *et al.,* 2002).

2.11.2. Chronic mild stress (CMS)

The first CMS paradigm was introduced by Katz and colleagues, which was further developed by Willner (Katz *et al*., 1981a; Willner *et al*., 1987). It provides the basis for most of the currently used paradigms. Repeated presentation of the same stressor usually leads to adaptation which can, however, be excluded by presenting a variety of stressors in an unpredictable sequence. Thus, the chronic stress procedure was developed. Initial protocols included 3 weeks of exposure to electric shocks, immersion in cold water, immobilization, reversal of the light/dark cycle and a variety of other stressors (Katz *et al*., 1981b). These series of stressors could cause an increase in plasma cortisol level and a reduction in sucrose preference (Katz, 1982), which suggests that chronic stress may cause anhedonia. The revised procedure involves relatively continuous exposure of rats (Willner *et al*., 1987) or mice (Monleon *et al.,* 1995) to a

variety of mild stressors, such as periods of food and water deprivation, small temperature reductions, changes of cage mates, and other similar individually innocuous, but unpredictable, manipulations. Chronic Mild Stress causes the appearance of many other symptoms of depression, such as decreases in sexual, aggressive, and investigative behaviors, and a decrease in locomotor activity. The reduction in sucrose preference, as well as other symptoms induced by CMS, can be gradually reversed by chronic, but not acute treatment with a wide variety of antidepressants including TCAs, SSRIs, SNRIs, MAOIs, atypical antidepressants such as mianserin, buspirone and amisulpride, and electroconvulsive schock (ECS) (Wilner, 2005).

The advantages of this model are its good predictive validity (behavioral changes are reversed by chronic treatment with a wide variety of antidepressants), face validity (almost all demonstrable symptoms of depression have been reproduced), and construct validity (CMS causes a generalized decrease in responsiveness to rewards comparable to anhedonia, the core symptom of depression) (Willner, 1997). However, the CMS model has 2 major drawbacks. One is the practical difficulty in carrying out CMS experiments, which are labor intensive, demanding of space, and of long duration. The other is that the procedure can be difficult to be established in a new laboratory setting, and data can be hardly replicated across laboratories (Willner, 1997; Willner, 2005).

2.11.3. Social defeat stress

Social defeat stress paradigm is the most frequently used model in rodents (Yan *et al*., 2010; Berton *et al*., 2006). Firstly, experimental male animals are introduced into the territory of aggressive conspecific males. The intruders are rapidly investigated, attacked and defeated by the residents. To ensure the desired outcome of the social conflict, residents usually have a higher body weight and are familiarized with fighting. They usually belong to a strain with a relatively higher level of aggression (Buwalda *et al*., 2005). After a few minutes of physical interaction, residents and intruders are usually separated by a plastic divider with holes, which allows visual, olfactory and auditory contacts for the remainder of the 24-h period. The experimental rodents are exposed to a different resident aggressor each day for several days (Yan *et al*., 2010;

Krishnan *et al.,* 2007; Tsankova *et al.,* 2006). Previous studies have proved that behavioral and pharmacological tools in treating human depression are also beneficial for reducing the behavioral, physiological, neuroendocrinal and neurobiological changes following defeat.

Antidepressants such as clomipramine (Fuchs *et al*., 1996), imipramine and fluoxetine (Yan *et al.,* 2010; Krishnan *et al*., 2007; Tsankova *et al.,* 2006), as well as social interaction (Von *et al.,* 2000) can prevent many of the consequences of social stress. In addition, this model gives another validity that only chronic but not acute antidepressant administration can reverse the social aversion (Cryan and Slattery, 2007). However, this model has 2 major disadvantages. One is that a short period paradigm results more likely in the phenotype of anxiety (Kaleuff *et al.,* 2006). The other is that only male rodents can be used for this model, since female rats or mice do not fight each other in a resident–intruder confrontation (Bjorkqvist, 2001).

2.12 Behavioral tests on antidepressant (AD) activity

2.12.1 Forced swim test (FST)

The forced swim test (FST), also known as behavioral despair test or the Porsolt test, was developed in 1977 by Porsolt and colleagues in the rat and subsequently in the mouse (Porsolt *et al.,* 1977). Although it works in subacute condition (30 min after drug injection), it does remain highly reliable in predicting the therapeutic potential of the tested compounds (Petit-Demouliere *et al*., 2005). The forced swimming test (FST) is the most widely employed screening test for antidepressants in rodents (Cryan *et al*., 2002; Porsolt *et al.,* 1978). The test is based on the observation that animals develop an immobile posture in an inescapable cylinder filled with water. When rodents are placed in a cylinder of water without an opportunity to escape, they typically display an immobile posture that is said to reflect a state of 'behavioural despair' (Harkin *et al.,* 2002). After AD administration, the animals will actively perform escape-directed behaviors with longer duration than animals with vehicle treatment. In the FST, subacute AD (including SSRIs) administration decreases immobility, with a corresponding increase in

climbing or swimming behavior (Lucki, 1997) without altering locomotor activity in an open field test (Nestler *et al*., 2002; Cryan *et al*., 2005).

Numerous agents that act independently of monoamine signaling have also been shown to reduce immobility time, such as recombinant ghrelin (Lutter *et al.* 2008), ketamine (Maeng *et al*. 2008), and estradiol (Dhir and Kulkarni 2008). The advantages of FST are that it is low-costing and is a fast and reliable tool to test potential antidepressant activities with a strong predictive validity. Besides, it is readily automated, allowing rapid screening of large numbers of compounds (Petit-Demouliere, 2005). The FST is still one of the most used testing methods for screening antidepressants.

2.12.2 Tail suspension test (TST)

The TST, which was first introduced in 1985 to measure the potential effectiveness of antidepressants (Steru *et al*., 1985), shares a common theoretical basis and behavioral measure with the FST. In this procedure, tails of rodents (mainly mice, although rats are also used) are suspended using adhesive tape to a horizontal bar for 6 min, and the time of immobility is recorded. Typically, the suspended rodents are immediately engaged in several agitation or escape-like behaviors, followed temporally by developing an immobile posture (Cryan *et al*., 2005).

Like the FST, the advantages of this test are that it can detect a broad spectrum of antidepressants irrespective of their underlying mechanisms (Cryan *et al*., 2005), it's inexpensive, and it is methodologically unsophisticated and easily amenable to automation. Thus, the use of TST has been substantially increased in recent years to assess AD-relevant behaviors. Although TST and FST share a common theoretical basis, there are many differences between them, therefore they could complement each other in some situations. For example, TST avoids problems of hypothermia or motor dysfunction that could interfere with the performance in swimming test, while FST could overcome the tail-climbing problem in TST (Dalvi and Lucki, 1999; Steru et al., 1985). Similar to FST, one of the shortcomings of TST is that AD takes effect quickly, which is in contrast with chronic treatment with the same drugs in patients (Cryan *et al*., 2005).

2.12.3 Sucrose preference test

This is a reward-based test. Rodents are born with an interest in sweet foods or solutions. Reduced preference for sweet solution in sucrose preference test represents anhedonia, while this reduction can be reversed by treatment with chronic antidepressants (Christina *et al.,* 2000). Anhedonia, the loss of pleasure or interest in normally rewarding stimuli, represents an extremely feasible symptom to study, both in humans and in preclinical research, which coupled with being a core symptom of depression makes it an attractive proposition. Anhedonia has been used as a behavioural endpoint for a number of the existing animal models of depression, such as chronic mild stress and maternal separation, with sucrose preference being measured (Slattery *et al.,* 2007). The sucrose preference test can measure the affective state and motivation of rodents; however, further validation is needed for working as a model of depression.

2.12.4 Spontaneous motor activity

This is an experiment developed by Calvin S. Hall used to assay genera locomotor activity levels and anxiety in rodents (Denenberg, 1969; Stanford, 2007). Animals such as rats and mice display a natural aversion to brightly lit areas. Decreased anxiety leads to increased exploratory behaviour. Increased anxiety will result in less locomotor motion and preference for the edges of the field (Ennaceur, 2013). Rodents tend to avoid brightly illuminated areas, and this avoidance is interpreted as a symptom of anxiety (Holmes, 2001). The open field is an arena with walls to prevent escape. Open field is a bright enclosure and during the test rodents are placed in this arena thus forced to interact with a novel and bright environment (Holmes, 2001). Commonly, the field is marked with a grid and square crossings. In the modern open field apparatus, infrared beams or video cameras with associated software (Samson *et al*., 2015) can be used to automate the assessment process. Behavioral patterns measured in the open field test include: Line crossing, center square entries, center square duration, rearing, stretch attend postures, defecation and urination (Brown *et al*., 1999).

2.12.5 Elevated plus maze test

This is also an anxiety-based test. For the elevated plus maze test, the rodents are placed at the intersection of the four arms of the maze (two open, two closed), facing an open arm. The number of entries and time spent in each arm is recorded and valid results are obtained in a single 5-minute testing session. An increase in the open-arm time is an index of anti-anxiety behavior of rodents (Holmes, 2001).

Open field test and elevated plus maze test can work as an antidepressant screen by measuring anxiety-related behavior as an accompanying endophenotype of depression.

2.13 Management of depression

The management of depression involves a number of different pharmacological, behavioral, and medical device-based therapies (Lanier, 2003).

2.13.1 Psychotherapy

With more chronic forms of depression, the most effective treatment is often considered to be a combination of medication and psychotherapy (Thase, 1999). The most studied form of psychotherapy for depression is cognitive behavioral therapy (CBT), thought to work by teaching clients to learn a set of cognitive and behavioral skills, which they can employ on their own (Roth, 2006). A systematic review of data comparing low-intensity CBT (such as guided self-help by means of written materials and limited professional support, and website-based interventions) with usual care found that patients who initially had more severe depression benefited from low-intensity interventions at least as much as less-depressed patients (Bower *et al.*, 2013). Behavior therapy, a component of CBT, is sometimes referred to as behavioral activation (Hopko *et al.*, 2004). Studies exist showing behavioral activation to be superior to CBT (Dimidjian *et al.*, 2006). In addition, behavioral activation appears to take less time and lead to longer lasting change (Spates *et al.*, 2006).

Interpersonal psychotherapy focuses on the social and interpersonal triggers that may cause depression. Here, the therapy takes a structured course with a set number of weekly sessions

(often 12) as in the case of CBT; however, the focus is on relationships with others (Weissman *et al.*, 2000). This therapy can be used to help a person develop or improve interpersonal skills in order to allow him or her to communicate more effectively and reduce stress (Weissman *et al.*, 2000). Psychoanalysis, a school of thought founded by Sigmund Freud that emphasizes the resolution of unconscious mental conflicts (Dworetzky, 1997), is used by its practitioners to treat clients presenting with major depression (Doidge *et al*., 2002).

2.13.2 Pharmaceutical drug treatment

To find the most effective pharmaceutical drug treatment, the dosages of medications must often be adjusted, different combinations of antidepressants tried, or antidepressants changed. Response rates to the first agent administered may be as low as 50% (Depression guideline panel, 1999).

(a) Medications

Selective serotonin reuptake inhibitors (SSRIs), such as sertraline (Zoloft, Lustral), escitalopram (Lexapro, Cipralex), fluoxetine (Prozac), paroxetine (Seroxat), and citalopram, are the primary medications considered, due to their relatively mild side effects and broad effect on the symptoms of depression and anxiety, as well as reduced risk in overdose, compared to their older tricyclic alternatives (Susan, 2017). Another popular option is to switch to the atypical antidepressant bupropion or to add bupropion to the existing therapy (Zisok *et al*., 2006); this strategy is possibly more effective (Rush *et al.*, 2006; Trivedi *et al*., 2006). Fluoxetine is the only antidepressant recommended for people under the age of 18, though, if a child or adolescent patient is intolerant to fluoxetine, another SSRI may be considered (Nelson and Devanand, 2011). Tricyclic antidepressants have more side effects than SSRIs (but less sexual dysfunctions) and are usually reserved for the treatment of inpatients, for whom the tricyclic antidepressant amitriptyline, in particular, appears to be more effective (Anderson, 2000; Krishnan, 2007). A different class of antidepressants, the monoamine oxidase inhibitors, have historically been plagued by questionable efficacy and life-threatening adverse effects. They are still used only rarely, although newer agents of this class, with a better side effect profile, have been developed (Valenstein *et al.*, 2006).

(b) Augmentation

Physicians often add a medication with a different mode of action to bolster the effect of an antidepressant in cases of treatment resistance (Bauer and Dopfmer, 1999). Lithium has been used to augment antidepressant therapy in those who have failed to respond to antidepressants alone (Guzetta *et al.*, 2007). Furthermore, lithium dramatically decreases the suicide risk in recurrent depression (Bender, 2008). Addition of atypical antipsychotics when the patient has not responded to an antidepressant is also known to increase the effectiveness of antidepressant drugs, albeit at the cost of more frequent and potentially serious side effects (Nierenberg *et al.*, 2006). Stephen M. Stahl, renowned academician in psychopharmacology, has stated resorting to a dynamic psychostimulant, in particular, d-amphetamine as the "classical augmentation strategy" for treatment-refractory depression (Kraus and Burch, 1992).

2.13.3 Medical devices

A variety of medical devices are in use or under consideration for treatment of depression including devices which offer electroconvulsive therapy, vagus nerve stimulation, repetitive transcranial magnetic stimulation, and cranial electrotherapy stimulation. Factors considered were whether drugs had been effective, how many different drugs had been tried, and what tolerance for suicides should be in field trials (Beloucif, 2013).

(a) Electroconvulsive therapy (ECT)

Electroconvulsive therapy is a standard psychiatric treatment in which seizures are electrically induced in patients to provide relief from psychiatric illnesses (Dierckx *et al.*, 2012). A usual course of ECT involves multiple administrations, typically given two or three times per week until the patient is no longer suffering symptoms. ECT is administered under anesthetic with a muscle relaxant (Emily, 2013). Electroconvulsive therapy can differ in its application in three ways: electrode placement, frequency of treatments, and the electrical waveform of the stimulus. ECT appears to work in the short term via an anticonvulsant effect mostly in the frontal lobes, and longer term via neurotrophic effects primarily in the medial temporal lobe (Lakhan and

Callaway, 2010). Electroconvulsive therapy is usually the last line of intervention for major depressive disorder and is used with informed consent (Jelovac *et al.,* 2013).

(b) Deep brain stimulation (DBS)

Deep brain stimulation involves brain surgery for the implantation of electrodes in a specific region of the brain and then constant pulses of stimulation to maintain its effects (Eliza, 2017). Results suggest that disrupting pathological activity in limbic-cortical circuits using electrical stimulation of the subgenual cingulate white matter can effectively reverse symptoms in treatment-resistant depression (Mayberg *et al.,* 2005). Functional neuroimaging studies have had a critical role in characterizing these limbic-cortical pathways (Brody *et al*., 2001; Mayberg, 2003). Studies have demonstrated consistent involvement of the subgenual cingulate in both acute sadness and antidepressant treatments effects, suggesting a critical role for this region in modulating negative mood states (Mayberg *et al.,* 1999; Seminowicz *et al.,* 2004). A March 2010 systematic review found that "about half the patients did show dramatic improvement" and that adverse events were "generally trivial" given the younger psychiatric patient population than with movements disorders (Michael, 2012).

(c) Vagus nerve stimulation (VNS)

Vagus nerve stimulation (VNS) is a more invasive procedure than electroconvulsive therapy, but it has been shown to be well tolerated. During the procedure a stimulating electrode is surgically attached to the vagus nerve; this allows for continuous stimulation after implantation. Like electroconvulsive therapy, it is usually only used in severe cases of treatment-resistant depression that have been non-responsive to medication (Rogers and Anderson, 2009). The support for this method comes mainly from open-label trials, which indicate that several months may be required to see a benefit (Marangell *et al.,* 2007). The only large double-blind trial conducted lasted only 10 weeks and yielded inconclusive results; VNS failed to show superiority over a sham treatment on the primary efficacy outcome, but the results were more favorable for one of the secondary outcomes. The authors concluded "This study did not yield definitive evidence of short-term efficacy for adjunctive VNS in treatment-resistant depression (Rush *et al*., 2005).

2.14 PROPERTIES OF METHYL JASMONATE

Jasmonic acid (JA), methyl jasmonate (MJ), 12-oxophytodienoic acid (12-OPDA), JA conjugated to some amino acids such as leucine (JA-leucine) and isoleucine (JA-isoleucine), among other jasmonates, are widespread in the plant kingdom. These compounds are involved in crucial processes related to plant development and survival, including direct and indirect defense responses, secondary metabolism, reproductive process, senescence, fruit development, and tritrophic interactions (Seo *et al.,* 2001; Wasternack, 2007).

Methyl jasmonate was first identified as a component of the essential oil of several plant species, while JA was first obtained from a fungal culture filtrate. Jasmonate accumulates in response to plant wounding (Creelman *et al*., 1992). Methyl jasmonate (MJ) is one of the phytochemicals belonging to the jasmonate family that was first isolated from the essential oil of *Jasminum grandiflorum* (Demole *et al.*, 1962; Cohen and Flescher, 2009). Due to its volatile property, MJ initially aroused considerable commercial interest from the perfume industry, stimulating studies focused on its structure and synthesis. During the 1980's the first reports of physiological effects attributed to JA and MJ appeared, describing activities related to senescence in *Artemisia absinthium* (Ueda and Kato, 1980), and growth inhibition in *Vicia faba* (Dathe *et al.*, 1981). Due to its volatile nature and ability to diffuse through membranes, MJ has been considered an important candidate for an airborne signal molecule mediating intra- and interplant communications, modulating plant defense responses (Demole *et al*., 1962; Creelman and Mullet, 1995; Seo *et al*., 2001; Wasternack, 2007).

2.14.1 BIOSYNTHESIS AND ACTION OF JASMONATES

The biosynthesis of jasmonates begins with linolenic acid (LA). This fatty acid is converted to 13-hydroperoxylinolenic acid by lipoxygenase. 13-hydroperoxylinolenic acid is a substrate for allene oxide synthase [also known as hydroperoxide dehydratase or hydroperoxide dehydrase (Simpson and Gardner, 1995) and allene oxide cyclase resulting in the formation of 12-oxo-phytodienoic acid (12-oxo-PDA). Following reduction and three steps of beta oxidation, (-)-7-

iso-JA is formed. Jasmonic acid can be catabolized to form MJ and numerous conjugates and catabolites that may have biological activity (Hamberg and Gardner, 1992).

The jasmonic acid (JA), MJ and their octadecanoid precursors (e.g., 12-OPDA) are lipidic molecules with a great capacity to regulate diverse physiological processes. They are produced from α-linolenic acid present in chloroplast membranes. Jasmonates play a crucial role in plant intracellular signaling and defense in response to stress and pathogenic invasions (Cohen and Flescher, 2009).

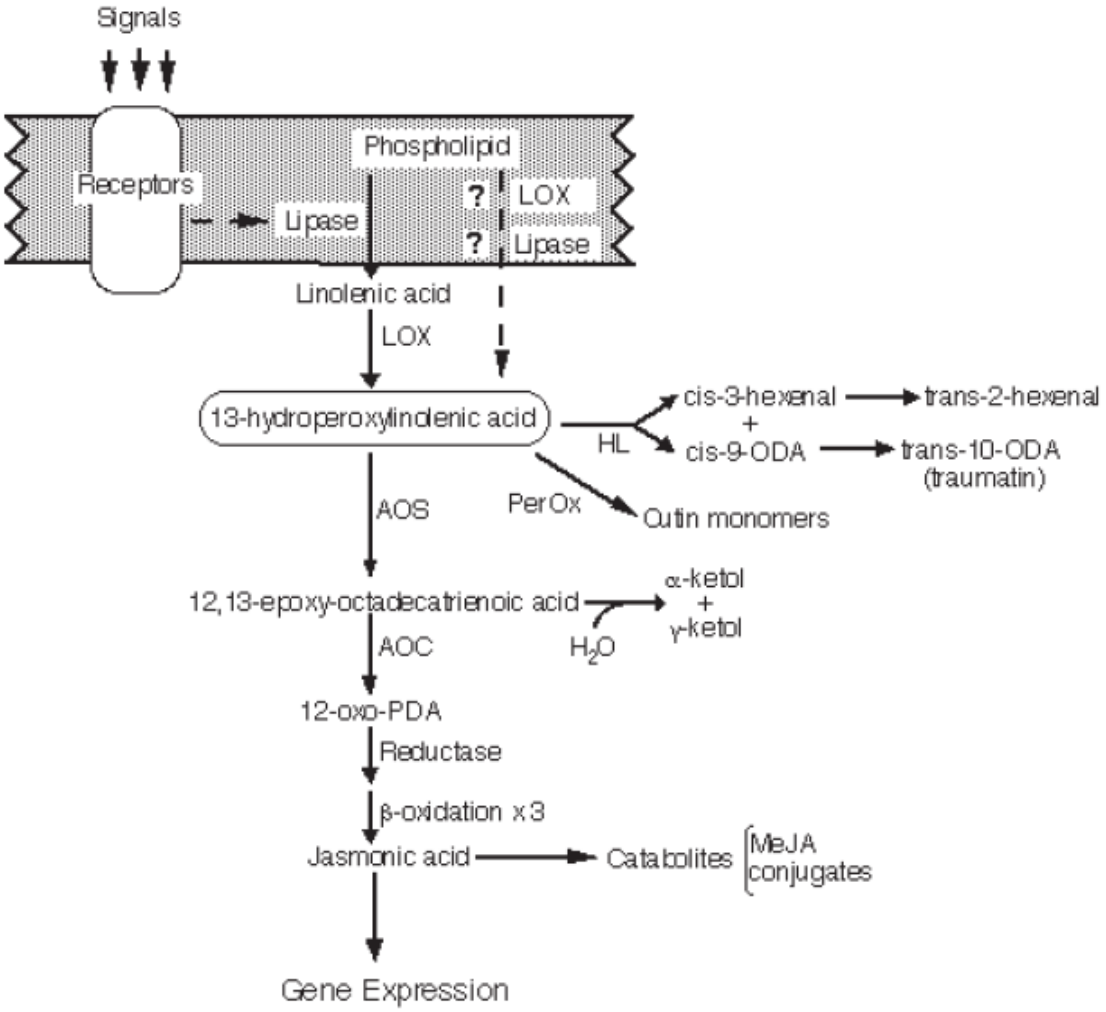


Figure 5: Biosynthetic pathway of jasmonic acid (Simpson and Gardner, 1995)

2.14.2 Biological properties of Methyl jasmonate (MJ)

Methyl jasmonate has also been found in a variety of plant species especially fruits, which forms essential components of human diet (Cesari *et al*., 2014). Detailed toxicological studies have also shown that MJ, irrespective of the route of exposure, was not toxic to humans and has been approved as a food additive by the World Health Organization (Cesari *et al*., 2014; Belsito *et al*., 2012; Environmental Protection Agency, 2013). Methyl jasmonate has been reported to have high lipid solubility, a property that has been ascribed to its ability to cross various biological membranes including the blood brain barrier (Cesari *et al*., 2014).

Small molecular weight chemicals from plants (phytochemicals) often accomplish multitargeted anticancer activities including cell cycle arrest, inhibition of cell growth, proliferation, and metastasis and promote apoptosis and cell death (Mou *et al.*, 2011). Methyl jasmonate is one of the most well studied members of the jasmonate family and has gained international recognition as a potential agent for the treatment of cancer (Cesari *et al*., 2014). Methyl Jasmonate is a prominent multi drug resistant reversal agent and deserves significant value in cancer patients (Wang *et al*., 2013). Previous investigations showed that MJ has anti cancer activities (Fingrut and Flescher, 2002). MJ has been shown to have powerful anticancer activity through multiple mechanisms (Cohen and Flescher, 2009; Elia and Flescher, 2013; Raviv *et al*., 2013). Methyl jasmonate has been shown to be selectively toxic to human cancer cells without affecting normal cells (Cohen and Flescher, 2009; Rotem *et al*., 2005). Anticancer activity of MJ is mainly exhibited through inhibition of lipo oxygenase 5 (LOX 5) which is generated in arachidonic pathways an important mediator in cancerous cells (Flescher and Cohen, 2009). Some investigators demonstrated anticancer activity against human bladder cell line and proposed that the anticancer activity is due to activation and induction of caspase 3 and caspase 9 and decreases expression of cancer cell regulators (Wang *et al*., 2014). Cooperative properties of MJ with other chemotherapeutic agents clearly indicate that MJ in a dose dependent manner helps to induce death of several types of carcinoma cells (Heyfets and Flescher, 2007).

It has also been overwhelmingly proposed that MJ is a novel class of anticancer drugs that act directly and selectively against tumor cells both *in vitro* and *in vivo*, without affecting normal

cells such as lymphocytes (Rotem *et al*., 2005; Cesari *et al*., 2014; Flescher, 2007; Goldin *et al.*, 2008). The susceptibility of cancer cells and mitochondria to MJ was shown to be dependent on the evaluated expression of hexokinase (Goldin *et al*., 2008), a key hallmark of many types of cancer cells. The complete lack of toxicity to normal cells and the rapidity by which MJ causes damage to cancer cells turn MJ into a promising anticancer agent that can be used alone or in combination with other agents (Shivshankar and Shanmugarajan, 2016**)**. This property may encourage the development of MJ as a therapeutic agent for cancer and perhaps for the treatment of other ailments (Rotem *et al*., 2005; Cohen and Flescher, 2009).

Previous investigations have also shown that MJ has antinociceptive, antiaggressive, and antidepressant properties (Umukoro *et al.,* 2012; Umukoro and Olugbemide, 2011). It has demonstrated inhibitory activity against acetic acid-induced nociception in mice. The ability of MJ to suppress acetic acid-induced nociceptive behaviours suggests a peripheral analgesic effect similar to that of the NSAIDs (Umukoro and Olugbemide, 2011). Methyl jasmonate and its analogues have been shown to inhibit the formation of inflammatory mediators, suggesting its role in conditions with inflammation as the underlying factor (Dang *et al.,* 2008). It is our opinion that the efficacy of MJ in these painful states may be related to the inhibition of the release of inflammatory mediators, especially prostaglandins, which sensitize nociceptors to cause pain (Umukoro and Olugbemide, 2011). This suggestion is in agreement with literature, as MJ and its analogues inhibited the formation of pro-inflammatory mediators, which are also known to cause pain (Dang *et al*., 2008). MJ may be used as a potential application in the treatment of inflammatory pain (Umukoro and Olugbemide, 2011).

Previous investigations also showed that MJ reduced offensive aggression in both intruder- and isolation-evoked aggression paradigms after peripheral administration in mice. the ability of MJ to suppress these violent acts in the resident–intruder paradigm indicates antiaggressive activity (Umukoro *et al*., 2012). MJ did not produce cataleptic effect, which may encourage its development as antiaggressive agent (Umukoro *et al.,* 2012). Methyl jasmonate might be acting like 5-HT_{1B} agonists in offensive aggression which is based on the findings that MJ like 5-HT_{1B} agonists did not affect latency to attack but reduced the overall amount of aggression (Umukoro *et al*., 2012). Although, several neurochemicals are involved in the pathogenesis of aggression,

preclinical and clinical studies have confirmed that the propensity to exhibit violent behavior is more closely connected with the reduced brain level of 5-HT (Sietse *et al.*, 2005;; Gowin *et al.*, 2010). Evidences in mice show that the antiaggressive activity of MJ might be mediated in part, through the stimulation of 5-HT_{1B} receptors and could be relevant in the treatment of reactive aggression (Umukoro *et al.*, 2012).

Investigations have revealed that MJ demonstrated adaptogenic-like property during acute stress responses in the forced swim endurance test in mice, especially in delaying the onset of exhaustion, which suggests an action that resembles adaptogens (Aluko *et al*., 2015). It has also been reported that MJ exhibited anti-stress activity, as indicated by the increase in duration of anoxic stress tolerance time in mice (Aluko *et al.*, 2015). The anti-anoxic effect of MJ might be related to increased cerebral resistance and efficient utilization of oxygen during the acute hypoxic stress response in mice (Aluko *et al.*, 2015). Literature also suggests that MJ may be acting like yohimbine and other adaptogens, in boosting energy and resilience in the face of acute stress. Due to the adaptogenic-like property of MJ, it may be useful in mitigating the deleterious effect of stress (Aluko *et al*., 2015).

Previous studies revealed that MJ improved memory performance in mice in the object recognition test. Earlier investigations also showed that methyl jasmonate attenuated memory deficits induced by lipopolysaccharide (LPS) via mechanisms related to antioxidation, neuroprotection and inhibition of cholinesterase enzymes (Eduviere *et al*., 2016). In earlier studies, the ability of MJ to improve memory performance in a similar manner to donepezil in LPS-treated mice suggests an action related to enhancement of central cholinergic neurotransmission through inhibition of AChE activity (Eduviere e*t al*., 2016). Thus, MJ may be a potential agent for the treatment of cognitive dysfunction caused by impairment of central cholinergic transmission (Eduviere *et al*., 2016). Methyl jasmonate might also improve memory performance via suppression of oxidative stress as evidenced by decreased brain levels of malondialdehyde (MDA) and increased antioxidant defense systems of the brain cells (Eduviere *et al*., 2016). Furthermore, it has also been shown to reduce concentrations of nitric oxide in the

brain of mice and decrease LPS-induced cytoarchitectural alterations of the neuronal cells in the prefrontal cortex and hippocampal regions of the brain (Eduviere *et al*., 2016).

Literature search have also shown that methyl jasmonate has anti-parasitic, anti-malarial (Gold *et al*., 2003) and anti-inflammatory (Dang *et al*., 2008) activities. It shows cytotoxic effect against two human parasites, *Schistosoma mansoni* and *Plasmodium falciparum* with *P. falciparum* being the more susceptible. Jasmonates did not cause any damage to control human erythrocytes at maximum concentration that was used in the studies (Gold *et al*., 2003). The anti-inflammatory activity of MJ is associated with inhibitory effect on production of proinflammatory mediators like NO, IL and TNF and also exhibits potent anti-inflammatory potential similar to prostaglandin A1 and A2 (Dang *et al*, 2008).

Umukoro *et al* (2011) reported that methyl jasmonate significantly reduced immobility period in the forced swim and tail suspension tests in mice, suggesting an anti-depressant effect. The immobility displayed in rodent subject to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair, which claims to reproduce condition similar to human depression (Willner, 1984).

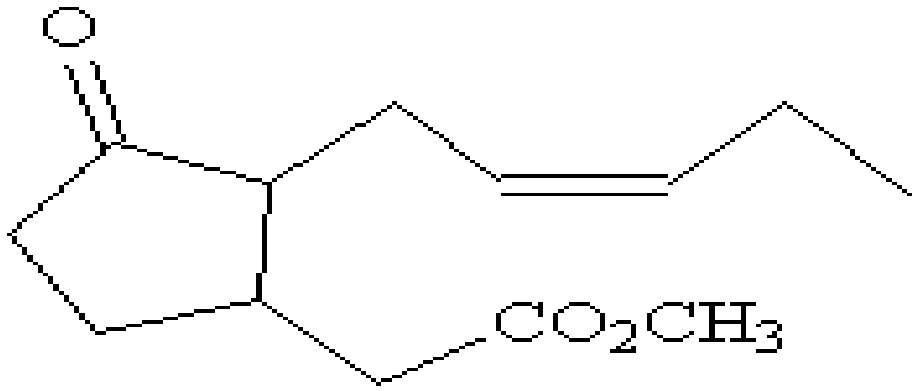


Figure 6: Chemical structure of methyl jasmonate

The acute toxicity test as reported by Umukoro and Olugbemide (2011) showed that MJ was well tolerated by the animals, as no death was observed at a dose range of 100-500 mg/kg in mice. However in literature, no study has been carried out in details to evaluate the antidepressant effect of methyl jasmonate and its probable mechanism of action in rodents. The present study was designed to investigate the antidepressant activities of methyl jasmonate in details and its probable mechanisms of action in mice.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental animals

Male Swiss mice (22-25g) used in the study were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature with free access to commercial food pellets and water *ad libitum.* The animals were allowed to acclimatize for one week before commencement of experiments. The experimental procedures were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/2016/023).

3.2 Equipment and Apparatus

Centrifuge (ATKE, China), water bath (Equitron, India), spectrophotometer (Spectrumlab, China), pH meter (EDT instruments), weighing balance (Ohaus), test tubes, eppendorf tubes, tube racks and dissecting kits and boards were all used in this study.

3.3 Drugs, Chemicals and Preparations

Methyl jasmonate (Sigma, Germany), Yohimbine (Sigma, Germany) and Imipramine (pfizer, USA) were used. Lipopolysaccharide—LPS (Sigma, Germany), trichloroacetic acid—TCA (Burgoyne Burbidges & Co., Mumbai, India), thiobarbituric acid—TBA (Sigma, Germany), 5,5′-dithio-bis(2-nitrobenzoicacid)–DTNB (Sigma Aldrich, Germany) and Tris (hydroxymethyl)-amino-methane (Tris-buffer) (Hopkin & Williams Company, USA) were also used in the study. 0.1mL of methyl jasmonate (MJ) was dissolved in 1mL of ethanol to get the stock solution and this solution was further diluted with distilled water to give the various doses of methyl jasmonate used. The final concentration of ethanol in the solution used for the study did not exceed 1%. The other drugs were dissolved in distilled water immediately before use.

3.4 Doses selection

The doses of methyl jasmonate used in the study were selected based on the results obtained from preliminary investigations. The preliminary studies involved the use of 100, 50, and 25mg/kg of methyl jasmonate and it was observed that the effects of the doses were less with higher doses. Methyl jasmonate 100mg/kg had the least effect with the 25mg/kg dose showing the greater effect so I went further to test lower doses and stepped down gradually to 5mg/kg methyl jasmonate.

3.5 Drug Treatment

This study was divided into three namely; acute, lipopolysaccharide (chronic study) and the chronic unpredictable mild stress (chronic study) phases. Drugs were all administered via intraperitoneal (i.p) route. In the acute studies, the animals were divided into five treatment groups of n = 6 each. Mice in group 1 which served as control were treated with vehicle (1% ethanol, 10 mL/kg), group 2 received imipramine (10 mg/kg), which served as the reference drug while groups 3 to 5 received MJ (5, 10, and 20 mg/kg) 30 minutes prior to the start of each experiment.

In the lipopolysaccharide (LPS) studies, the animals were divided into 6 groups of 6 animals each. Mice in group 1 received vehicle (1% ethanol, 10mL/kg) without LPS, group 2 received imipramine (10 mg/kg), groups 3-5 received MJ (5, 10, 20 mg/kg) while group 6 received vehicle. LPS (0.83 mg/kg) was administered to groups 2-6 thirty minutes after drug treatment on the seventh day.

In the chronic unpredictable mild stress (CUMS) studies, the animals were also divided into 6 groups of 6 animals each. Mice in group I, which served as non-stress control were not subjected to CUMS and were given vehicle (10 mL/kg), group 2 served as stress control but also received vehicle (10 mL/kg), group 3 received imipramine (10 mg/kg), which served as the reference drug while groups 4 to 6 received methyl jasmonate (5, 10, and 20 mg/kg).

3.6 Experimental procedures

3.6.1 Acute Studies

3.6.1.1 Effect of methyl jasmonate on forced swim test (FST)

This test was carried out according to the method previously described (Porsolt *et al.*, 1977). The animals were divided into various treatment groups and administered with the various drugs as described earlier. Then, 30 min later, mice were forced to swim individually in Plexiglas cylinder (20 cm height, diameter 10 cm) containing water to a depth of 10 cm at room temperature and observed for a period of 6 min. A mouse was judged immobile if it floats in the water in an upright position and made only slight movements to prevent sinking. The total duration of immobility was recorded during the last 4 min of the 6 min test.

3.6.1.2 Effect of methyl jasmonate on tail suspension test (TST)

This test was carried out according to the method described earlier (Rodrigues, 2002). The animals were treated with the various drugs as described earlier and thirty minutes after treatment each mouse was suspended by the tail on a retord stand, placed 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. After the initial 2 min period of vigorous motor activity, the mice became still and the immobility time (s) was recorded with the aid of a stopwatch for a period of 4 min. A mouse was considered to be immobile when it hung passively and completely motionless.

3.6.1.3 Effect of methyl jasmonate on locomotor activity (SMA)

In order to rule out any unspecific locomotor effect of methyl jasmonate, mice were administered with the same regimen as in the FST or TST. The effect of MJ on locomotor activity in mice was assessed utilizing an automated activity cage (Ugo Basile, Italy). Mice were individually placed in the activity cage. The animals were then allowed to habituate to the apparatus for 2 min and

the spontaneous motor activity was recorded as activity counts automatically over a 5 min period (Rodrigues *et al.*, 1996). The apparatus was cleaned with a solution of 70% alcohol between each tests in order to hide animal clues and to prevent each mouse from being influenced by the odors present in the urine and feces of the previous mouse.

3.6.2 Evaluation of the involvement of monoaminergic system in the antidepressant activity of methyl jasmonate

3.6.2.1 Involvement of noradrenergic and dopaminergic systems

In order to investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of MJ in the TST, the animals were pretreated with prazosin (62.5 µg/kg, i.p., an α_1-adrenoceptor antagonist) or yohimbine (1 mg/ kg, i.p., an α_2-adrenoceptor antagonist) 15 min before administration of MJ (20 mg/kg, i.p). Thirty minutes afterward, the animals were tested in the TST and the period of immobility was measured as earlier described. Moreover, in order to investigate the possible involvement of dopaminergic pathway in the antidepressant-like effect of MJ, mice were pretreated with sulpiride (50 mg/kg, i.p., a D_2 dopamine receptor antagonist) or haloperidol (0.2 mg/kg, i.p.; dopamine receptor antagonist) and after 15 min, they received MJ (20 mg/kg, i.p) before being tested in the TST 30 min later (Cardoso *et al*., 2009).

3.6.2.2 Involvement of serotonergic system

In order to investigate the possible contribution of the serotonergic system to the antidepressant-like activity of MJ in the TST, the animals were pretreated with metergoline (4 mg/kg, ip., a 5-HT_2 receptor antagonist) 15 min prior to administration of the most effective dose of MJ (20 mg/kg, i.p.) and were tested in the TST 30 min later (Gigliucci *et al*., 2010). The possible role of the serotonergic system in the antidepressant-like effect of MJ in the TST was also evaluated in a different group of animals (n =6) pretreated with pCPA (100 mg/kg, ip., an inhibitor of serotonin biosynthesis) once daily for 4 consecutive days (Sherman *et al*., 1982). Then, 24 h after the last

dose of pCPA, the animals were given MJ (20 mg/kg, i.p.) and were tested in the TST 30 min later.

3.6.3 EVALUATION OF THE ROLE OF NITRIC OXIDE (NO) PATHWAY IN THE ANTIDEPRESSANT-LIKE EFFECT OF METHYL JASMONATE

3.6.3.1 Influence of L-arginine pre-treatment on methyl jasmonate-induced antidepressant-like effect

Mice (n = 6) were pretreated either with L-arginine, a precursor of NO (250 mg/kg, i.p), a dose that had no effect on the TST (Heiberg *et al*., 2002) alone or with MJ (2.5 mg/kg, i.p). L-arginine was given 30 min before adminsitration of MJ (2.5 mg/kg, i.p). The TST was carried out 30 min after treatment as earlier described.

3.6.3.2 Influence of L-NAME, L-NNA and methylene blue on MJ-induced antidepressant effect in the TST

Nitric oxide synthase inhibitor L-NAME (50 mg/kg, i.p.) (Harkin *et al*., 2003), L-NNA (3 mg/kg, i.p.) and methylene blue (3.75 mg/kg, i.p., an inhibitor of NOS) were further used to investigate the influence of nitrinergic pathway on the antidepressant-like effect of MJ (Eroglu and Caglayan, 1997). Mice (n=6) were pretreated with MJ (2.5 mg/kg) 15 min before administration of L-NAME, L-NNA or methylene blue. Also, another set of mice (n = 6) were given NAME, L-NNA or methylene blue alone. Thirty minutes after each treatment, TST was carried out as described earlier.

3.6.4 Chronic Studies

3.6.4.1 Effect of MJ on Lipopolysaccharide-induced depressive-like behaviour (LPS study)

Mice were randomly divided into 6 treatment groups (n = 6). Mice in group 1 were given vehicle (1% ethanol; 10mL/kg), groups 2-4 received MJ (5, 10 and 20 mg/kg) whereas group 5 were pretreated with imipramine (10 mg/kg) daily for 7 days as earlier described. On day 7, treatments were carried out 30 min prior to administration of LPS (0.83 mg/kg, i.p). Twenty four hours after LPS administration, the animals were divided into two sets for various behavioral studies. The first set of animals was used for SMA and TST while the second set was used for sucrose preference test. The animals were sacrificed for biochemical studies immediately after behavioural studies.

3.6.4.1.1 Locomotor activity

The effect of MJ on locomotor activity in LPS-exposed mice was assessed utilizing an automated activity cage (Ugo Basile, Italy). Accordingly, 24 h after the last stress session in the LPS protocol, stressed and unstressed mice were individually placed in the activity cage. The animals were then allowed to habituate to the apparatus for 2 min and thereafter, the frequency of movements, used as an index of spontaneous motor activity was recorded automatically over a 5 min period.

3.6.4.1.2 Tail suspension test

This test was carried out according to the procedure described by Cryan *et al.* (2005). The animals were treated with the various drugs and 30 min after treatment each mouse were suspended individually on a retord stand, placed 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility were recorded during the last 4 min of the 6 min test. An animal was considered to be immobile when it did not show any movement of the body and hangs passively.

3.6.4.1.3 Sucrose Preference Test:

Sucrose preference test was carried out 24h after LPS administration. Briefly, 72 hours before the test, mice were trained to adapt to 1% sucrose solution (w/v): two bottles of 1% sucrose solution were placed in each cage, and 24 h later 1% sucrose in one bottle will be replaced with tap water for 24 h. After adaptation, mice were deprived of water and food for 12 h, followed by the sucrose preference test, in which mice were allowed free access to two bottles containing 100 mL of 1% sucrose and 100 mL of tap water. Afterwards, the volumes of consumed sucrose solution and water were recorded, and sucrose preference was calculated using the following formula:

$$\frac{\text{Sucrose consumption}}{\text{Sucrose consumption + water consumption}} \times 100$$

3.6.4.1.4 Ex vivo assays (Biochemical studies)

Immediately after behavioral assessment, the animals were anaesthetized via deep ether anaesthesia and the whole brains were harvested and blood samples were collected. A portion of brain tissues were homogenized and the supernatant were assayed for malondialdehyde (MDA), nitrite, reduced glutathione and superoxide dismutase activities using spectrophotometric methods, while histological studies was carried out on the other portion of the brain samples. The blood samples and some set of the brain samples were also kept and used for ELISA studies to assay for corticosteroids and TNF alpha.

(i) Determination of glutathione (GSH) concentration

Aliquots of brain homogenates of individual mouse in the respective treatment groups were taken and GSH concentration was determined using the method of Moron *et al* (1979). Equal volume (0.4 mL) of brain supernatant and 20% trichloroacetic acid (TCA) (0.4 mL) was mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4^{o}C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm against blank reagent

using a spectrophotometer. The concentrations of GSH in the brain tissues are expressed as micromoles per gram tissue (µmol/g tissue).

(ii) Effect on lipid peroxidation

The MDA level was estimated according to the method of Adam-Vizi and Seregi (1982). An aliquot of 0.4 mL of the sample was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30% TCA was added. Then, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^{o}C. This was then cooled in ice and centrifuged at 3000 rpm for 15 minutes. The clear supernatant was collected and absorbance was measured against a reference blank of distilled water at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1} and values were expressed as µmoles of MDA per gram tissue.

(iii)Effect on superoxide dismutase (SOD) activity

The level of SOD activity was determined by the method of Misra and Fridovich (1972). An aliquot of 0.1 mL of the brain sample was added to 2.5 mL of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3 mL of adrenaline and 0.2 mL of water. The increase in absorbance at 480 nm was monitored and recorded at 0 sec and every 60 seconds for 180 seconds (Misra and Fridovich, 1972).

(iv)Estimation of Brain Nitrite Concentration

Brain nitrite concentration was estimated using Griess reagent, which serves as an indicator of nitric oxide production. One hundred microliter of Greiss reagent (1:1 solution of 1 % sulfanilamide in 5 % phosphoric acid and 0.1 % of N-1-naphthyl ethylenediamine dihydrochloride) was added to 100 µL of the supernatant and absorbance was measured at 540 nm (Green *et al*., 1982). The brain nitrite concentration was estimated from a standard curve obtained from sodium nitrite (0–100 uM).

(v) Estimation of serum corticosterone level

After behavioral testing, 1 mL of blood sample was obtained through cardiac puncture from all the animal groups under ether anesthesia for the determination of serum corticosterone level. The serum corticosterone (ng/mL) level was estimated using ELISA kit (Oxford Biomedical Research, USA) according to the manufacturer's instructions. Briefly, blood sample was centrifuged at 3000 rpm for 15 min and serum was collected for estimation of corticosterone levels. Samples, standards, controls and Cortisol-HRP conjugate were added to a micro-plate coated with mAb to cortisol and incubated at room temperature for 1 hr. The bound cortisol-HRP was measured using tetramethylbenzidine (TMB) substrate. The TMB (150 µL) substrate was added to each well and incubated at room temperature for 30 min and the reading was taken at 650 nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional plate reader equipped with Soft max Pro v 5.4 (SMP 5.4), and a 5-parameter sigmoid minus curve fit determined unknown concentrations.

(vi)Estimation of tumor necrosis factor-alpha

The brain tumor necrosis factor-alpha (TNFα) concentration was estimated using ELISA kit (Assaypro, USA) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. Samples, standards, controls and Streptavidin-peroxidase conjugate were added to a micro-plate coated with Biotinylated mouse TNF-α antibody and incubated at room temperature for 2 h. The Chromogen substrate (50 µL) was added to each well and incubated at room temperature for 20 min before the addition of Stop solution (50 µL) and the reading was taken at 450nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional plate reader.

(vii) Assessment of histological alterations in mice brains

The LPS-treated mice were perfused and their brains fixed with 10 % phosphate buffered formaldehyde. The fixed brain tissues were processed to obtain paraffin wax embedded tissue blocks, which was sectioned in the sagittal plane using a microtome (Leica, Germany). The sections were stained with Hematoxylin and Eosin to demonstrate general histology of the cortex and hippocampal region of mice brains using the method of Eltony and Elgayar (2014). Thereafter, images were acquired using an the eyepiece of an Olympus CH (Japan) binocular research microscope.

3.6.4.2 Chronic Unpredictable Mild Stress (CUMS) induced depression

The CUMS paradigm was used to induce depression-like behavior in mice as previously described (Yalcin *et al*., 2005) with minor modifications. Thirty mice were randomly allotted into six treatment groups (n = 6). Mice in group 1 received vehicle (1% ethanol; 10 mL/kg, i.p.), groups 2-4 received MJ (5, 10, 20 mg/kg, i.p.) while the fifth group had imipramine (10 mg/kg, i.p) 30 min before exposure to different stressors in the CUMS paradigm (Table 2). This procedure was repeated daily for 2 weeks and 24 h after the last stress session, SMA, TST and SPF were assessed. Mice in the last group, which served as non-stress control, also received vehicle (1% ethanol; 10 mL/kg, i.p.) but were not subjected to CUMS. Then, the animals were sacrificed, brains were extracted and blood samples were collected and kept for further studies.

3.6.4.2.1 Effect of methyl jasmonate on locomotor activity

The effect of MJ on locomotor activity in CUMS-mice was assessed utilizing an automated activity cage (Ugo Basile, Italy). Accordingly, 24 h after the last stress session in the UCMS protocol, stressed and unstressed mice were individually placed in the activity cage. The animals were then allowed to habituate to the apparatus for 2 min and thereafter, the frequency of movements, used as an index of spontaneous motor activity was recorded automatically over a 5 min period (Rodrigues *et al*. 1996).

3.6.4.2.2 Tail suspension test (TST)

The TST was used to evaluate the effect of MJ on CUMS-induced depressive-like behavior in mice. The TST was carried out according to the method previously described (Steru *et al.*, 1985). Briefly, 24 h after the last stress session, both stressed and unstressed mice in the respective treatment groups were individually suspended by the tail to a cord of about 50 cm in length stretched between two metal retort at a height of 70 cm. After the initial 2 min period of vigorous motor activity, the mice became still and the immobility time (s) was recorded with the aid of a stopwatch for a period of 4 min. A mouse was considered to be immobile when it hung passively and completely motionless.

3.6.4.2.3 Sucrose preference test (SPT)

Anhedonia is one of the major symptoms of depression in human and the SPT had served as a useful animal model for evaluating the effects of compounds with anti-depressive-like activity. The SPT was used to assess the effect of MJ on CUMS-induced depressive-like behavior in mice using the modified method of Schweizer *et al*. (2009). Briefly, the test consists of training and test sessions. In the training session, mice were housed individually in a cage trained to consume 1% (w/v) sucrose solution from two identically sized bottles (100 mL) 72 h prior to the commencement of CUMS. Then, 24 h later, one of the bottles containing 1% (w/v) sucrose solution was replaced with a bottle containing distilled water for the next 24 h. Thereafter, mice were subjected to 12 h food and water deprivation and a 15 min baseline SPT was performed, in which mice had free access to two equally sided bottles (100 mL), one with 1% (w/v) sucrose solution and the other with distilled water. In the test phase, 24 h after the last stress session, both stressed and unstressed mice in the respective treatment groups were subjected individually to SPT as described above and the frequency of visits to the two bottles (one with 1% (w/v) sucrose solution and the other with distilled water) were recorded. Sucrose preference (%) was calculated as: (frequency of visit to bottle containing sucrose solution) ÷ (frequency of visit to bottle containing sucrose solution + frequency of visit to bottle containing distilled water) × 100.

3.6.4.2.4 Biochemical assays

After behavioral testing for depressive-like behaviors, the animals were decapitated under ether anesthesia and their brains were immediately removed and kept in the refrigerator with ice block for 30 min. Thereafter, each brain was weighed and homogenized with 10% w/v phosphate buffer (0.1 M, pH 7.4) and centrifuged for 10,000 rpm at 4 °C for 15 min. Each supernatant was separated into various portions for ELISA and other biochemical assays.

(i) Determination of brain level of malondialdehyde

The brain level of malondialdehyde (MDA), an index of lipid peroxidation, was estimated according to the method of Okhawa *et al*. (1979). An aliquot of 0.4 mL of the supernatant was mixed with 1.6 mL of Tris–KCl buffer to which 0.5 mL of 30% TCA was added. Then, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1} and values were expressed as µmoles of MDA per gram tissue (µmol/g tissue).

(ii) Determination of reduced glutathione concentration

The brain concentration of reduced glutathione (GSH) was determined using the method of Moron *et al.* (1979). Equal volume (0.4 mL) of brain homogenate and 20% TCA (0.4 mL) was mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4 °C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0). The absorbance was then read at 412 nm against blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues were expressed as micro moles per gram tissue (µmol/g tissue).

(iii)Estimation of superoxide dismutase activity

The activity of superoxide dismutase (SOD) in mice brains was determined by the method of Misra and Fridovich, (1972). Briefly, 0.1 mL of brain supernatant was added to 2.6 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.6 mL buffer, 0.3 mL of adrenaline and 0.1 mL of distilled water. Then, the increase in absorbance at 480 nm was monitored at 60 s intervals for 3 min. Superoxide dismutase (SOD) activity was expressed as units of adrenaline consumed per mg protein (units/mg protein).

(iv)Estimation of serum corticosterone level

After behavioral testing, 1 mL of blood sample was obtained through cardiac puncture from both stressed mice and unstressed counterparts under ether anesthesia for the determination of serum corticosterone level. The serum corticosterone (ng/mL) level was estimated using ELISA kit (Oxford Biomedical Research, USA) according to the manufacturer's instructions. Briefly, blood sample was centrifuged at 3000 rpm for 15 min and serum was collected for estimation of corticosterone levels. Samples, standards, controls and Cortisol-HRP conjugate were added to a micro-plate coated with mAb to cortisol and incubated at room temperature for 1 hr. The bound cortisol-HRP was measured using tetramethylbenzidine (TMB) substrate. The TMB (150 µL) substrate was added to each well and incubated at room temperature for 30 min and the reading was taken at 650 nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional plate reader equipped with Soft max Pro v 5.4 (SMP 5.4), and a 5-parameter sigmoid minus curve fit determined unknown concentrations.

(v) Estimation of tumor necrosis factor-alpha

The brain tumor necrosis factor-alpha (TNFα) concentration was estimated using ELISA kit (Assaypro, USA) according to the manufacturer's instructions. All reagents, standard solutions

and samples were brought to room temperature before use. Samples, standards, controls and Streptavidin-peroxidase conjugate were added to a micro-plate coated with Biotinylated mouse TNF-α antibody and incubated at room temperature for 2 h. The Chromogen substrate (50 µL) was added to each well and incubated at room temperature for 20 min before the addition of Stop solution (50 µL) and the reading was taken at 450nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional plate reader.

(vi)Estimation of Brain Nitrite Concentration

Brain nitrite concentration was estimated using Griess reagent, which serves as an indicator of nitric oxide production. One hundred microliter of Greiss reagent (1:1 solution of 1 % sulfanilamide in 5 % phosphoric acid and 0.1 % of N-1-naphthyl ethylenediamine dihydrochloride) was added to 100 µL of the supernatant and absorbance was measured at 540 nm (Green *et al.,* 1982). The brain nitrite concentration was estimated from a standard curve obtained from sodium nitrite (0–100 uM).

(vii) Assessment of histological alterations in mice brains

Mice were perfused and their brains fixed with 10 % phosphate buffered formaldehyde. The fixed brain tissues were processed to obtain paraffin wax embedded tissue blocks, which was sectioned in the sagittal plane using a microtome (Leica, Germany). The sections were stained with Hematoxylin and Eosin to demonstrate general histology of the cortex and hippocampal region of mice brains using the method of Eltony and Elgayar (2014). Thereafter, images were acquired using an the eyepiece of an Olympus CH (Japan) binocular research microscope.

Table 2: Schedule of stressors in chronic unpredictable mild stress (CUMS) induced depression paradigm in mice

Days	Stressors	Duration
1	Damp sawdust	2 hours
2	Cage tilt	24 hours
3	Water deprivation	12 hours
4	Background noise	30 minutes
5	Food deprivation	12 hours
6	Illumination over night	12 hours
7	Tail pinch	1 minute
8	Forced swim	5 minutes
9	Sawdust free cage+200mL water	120 minutes
10	Damp sawdust	2 hours
11	Cage tilt	24 hours
12	Forced swim	5 minutes
13	Sawdust free cage	2 hours
14	Tail pinch	1 minute

3.7 Statistical analysis

The data obtained were all expressed as mean ± S.E.M (standard error of mean) and analyzed with Graph Pad Prism software version 4.00. Statistical analysis of data was done using One-way or two-way ANOVA, followed by Newman-Keuls *post-hoc* test. P-values less than 0.05 ($p < 0.05$) were considered statistically significant.

CHAPTER FOUR

4.0. RESULTS

4.1 Effect of Methyl Jasmonate on the duration of immobility in mice

The effects of MJ on the period of immobility in mice subjected to FST and TST are shown in Figures 7a and 7b. It showed that intraperitoneal injection of MJ (10 and 20 mg/kg; i.p) significantly reduced the immobility time in the FST and TST when compared with the control groups. However, the reference drug, imipramine (10 mg/kg; i.p) could only decrease the period of immobility in the TST (Figures 7a and 7b).

4.2 Involvement of the noradrenergic system on the antidepressant effect of Methyl Jasmonate

Figures 8a and 8b show the effect of pretreatment of mice with prazosin (62.5 μg/kg; i.p) or yohimbine (1 mg/kg, i.p) on MJ (20 mg/kg, i.p)-induced decrease in immobility time in the TST. The results presented in Figure 8a showed that pretreatment of mice with prazosin (62.5 μg/kg, i.p) reversed the antidepressant-like effect of MJ (20 mg/kg, i.p.) in the TST. Also, similar effect was observed in mice pretreated with yohimbine (1 mg/kg, i.p) in the TST (Figure 8b).

4.3 Involvement of the dopaminergic pathway on the antidepressant effect of Methyl Jasmonate

The effect of pretreatment of mice with sulpiride (50 mg/kg; i.p) or with haloperidol (0.2 mg/kg, i.p) on MJ (20 mg/kg, i.p)-induced antidepressant-like activity in the TST is shown in Figures 9a and 9b. As shown in figure 9a and 9b, pretreatment of mice with sulpiride (50 mg/kg, i.p) or haloperidol (0.2 mg/kg, i.p) significantly attenuated the anti-immobility effect of MJ (20 mg/kg, i.p.) in the TST in mice.

7a.

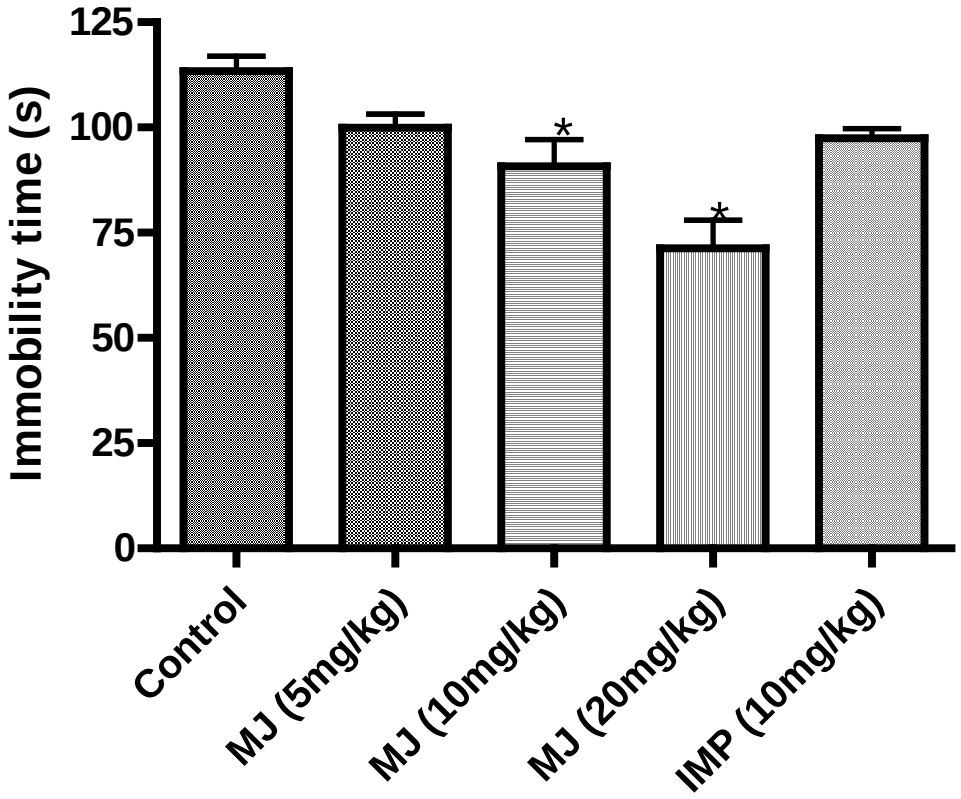


Figure 7a: Methyl jasmonate significantly decreased duration of immobility in the forced swim test paradigm in mice. Vertical bars represent mean ± S.E.M for 6 animals per group. $^{*}P < 0.05$ compared to Vehicle (ANOVA followed by Newman Keuls test).

Control mice received 10 mL/kg 1% ethanol, i.p; MJ- Methyl Jasmonate; IMP- Imipramine

7b.

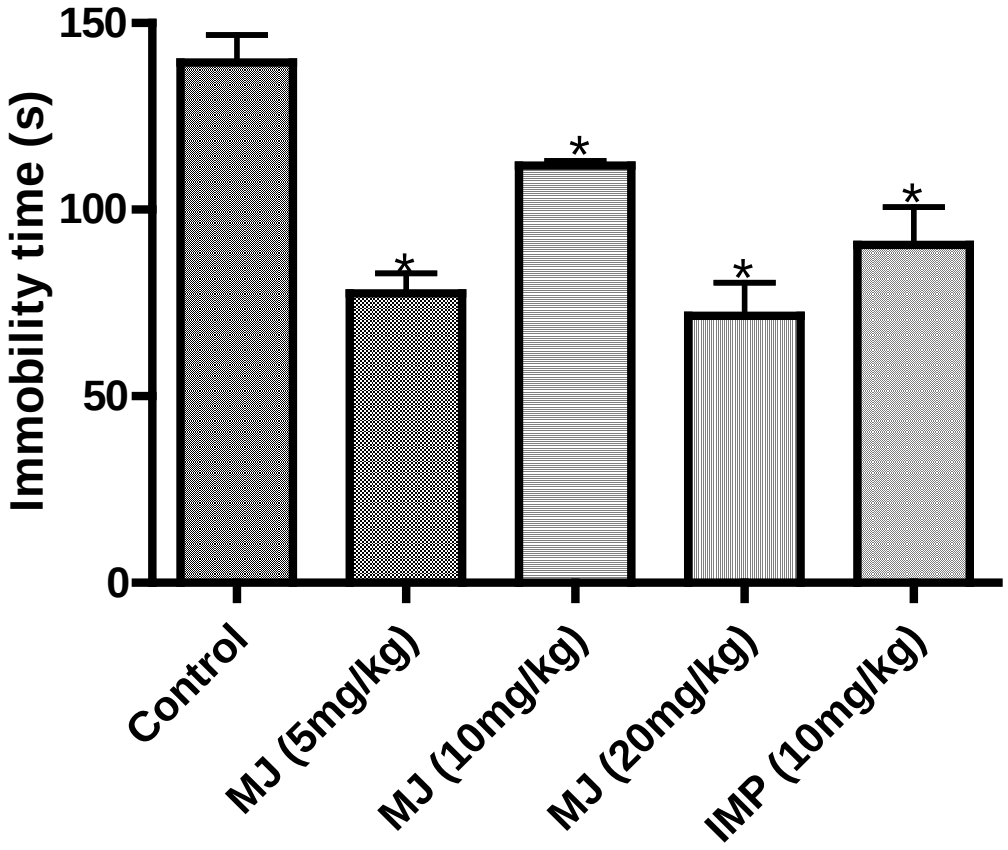


Figure 7b: Methyl jasmonate significantly decreased duration of immobility in the tail suspension test in mice. Vertical bars represent mean ± S.E.M for 6 animals per group. $^{*}P < 0.05$ compared to vehicle (ANOVA followed by Newman Keuls test).

Control mice received 10 mL/kg 1% ethanol, i.p; MJ- Methyl Jasmonate; IMP- Imipramine.

8a.

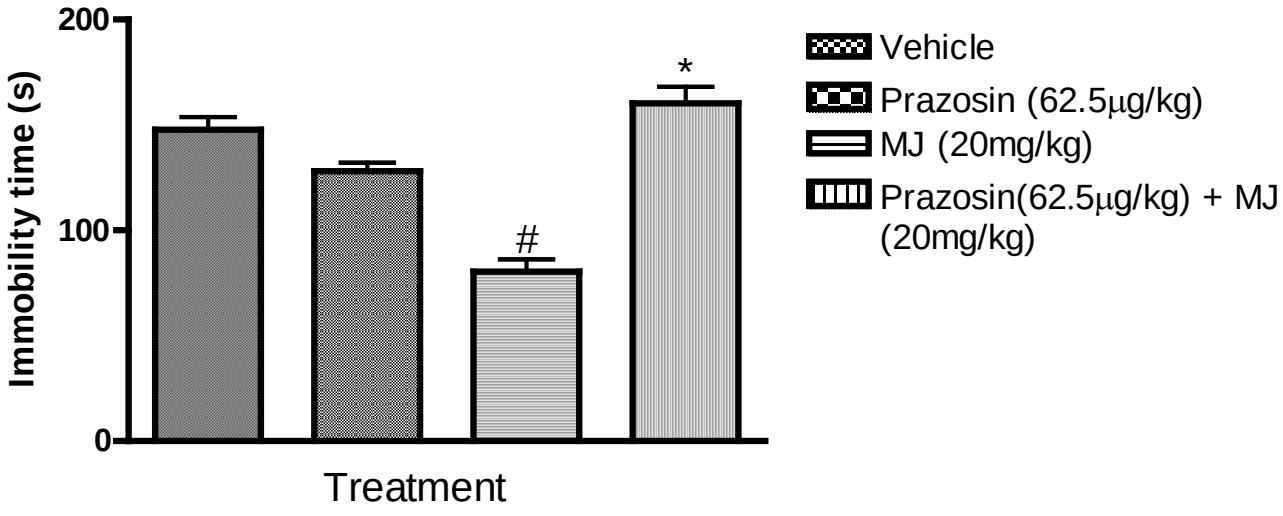


Figure 8a: Effect of pretreatment of mice with prazosin on methyl jasmonate induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #p < 0.05 when compared with vehicle and *p < 0.05 when compared to MJ (ANOVA followed by Newman Keuls test).

MJ- Methyl Jasmonate; Vehicle (10mL/kg 1% ethanol, i.p.), TST- Tail suspension test

8b.

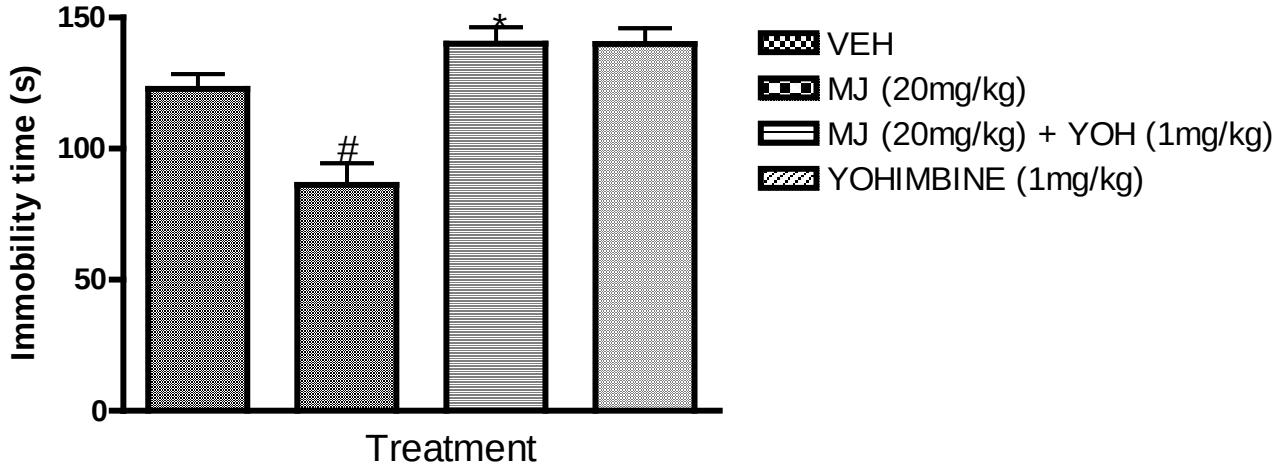


Figure 8b: Effect of pretreatment of mice with prazosin (a) and yohimbine (b) on methyl jasmonate induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to MJ (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol, i.p.); YOH- Yohimbine; TST- Tail suspension test

9a.

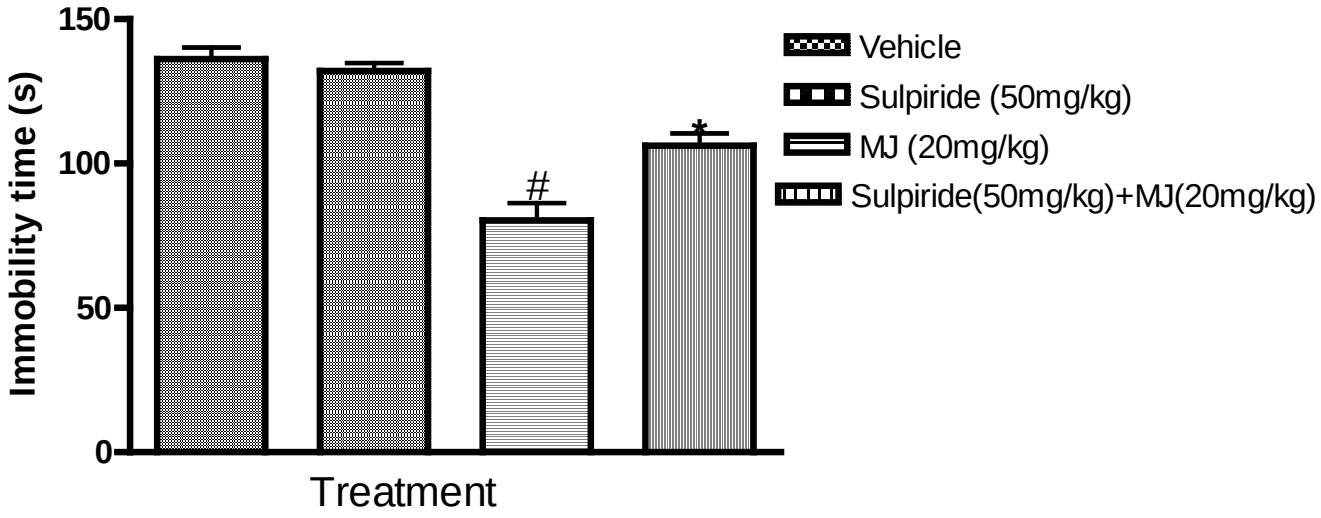


Figure 9a: Effect of pretreatment of mice with sulpiride on methyl jasmonate induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #p < 0.05 when compared with vehicle and *p < 0.05 when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test

9b.

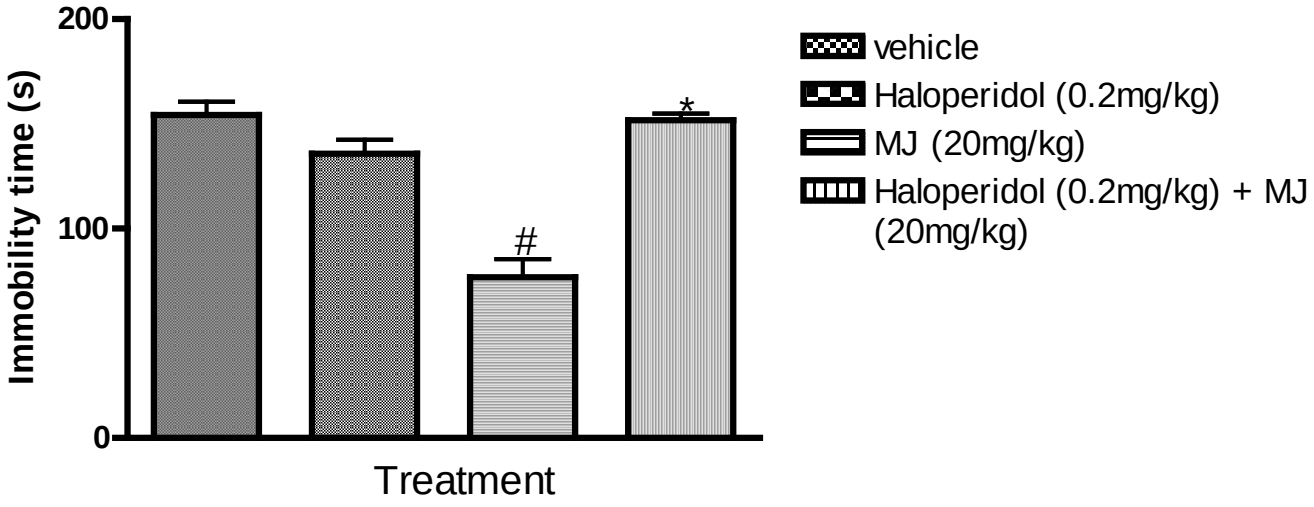


Figure 9b: Effect of pretreatment of mice with haloperidol on methyl jasmonate induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test

4.4 Involvement of serotonergic pathway on the antidepressant effect of Methyl asmonsate in mice

The effect of pretreatment of mice with p-chlorophenylalanine (pCPA) (100 mg/kg; i.p) or metergoline (4 mg/kg, i.p) on MJ (20 mg/kg, i.p)-induced reduction in immobility time in the TST is shown in Figures 10a and b. Pretreatment of mice with pCPA (100 mg/kg; i.p) once daily for 4 consecutive days or metergoline (4 mg/kg, i.p) for 15 min significantly attenuated the anti-immobility effect of MJ (20 mg/kg; i.p) in the TST.

10a.

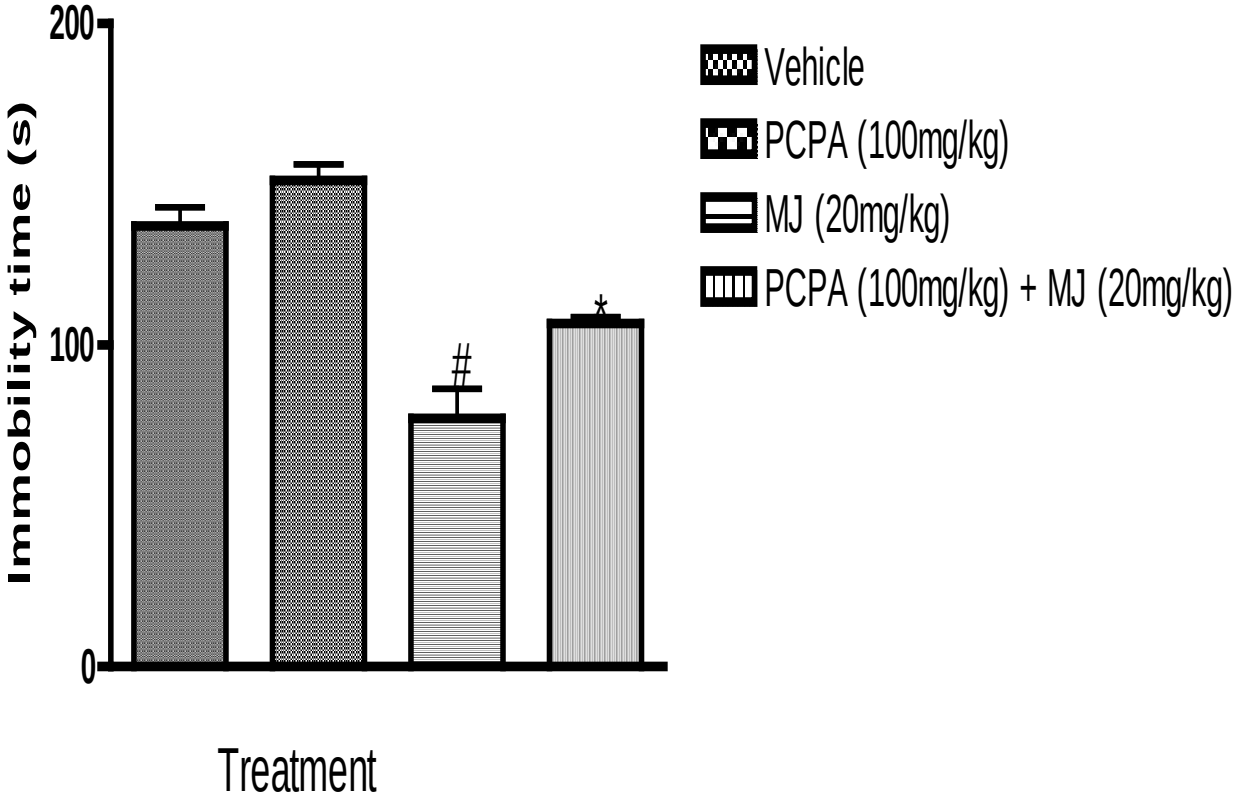


Figure 10a: Effect of pretreatment of mice with PCPA on methyl jasmonate-induced reduction in immobility time in the TST is shown. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test;

PCPA- p-Chlorophenyl alanine

10b.

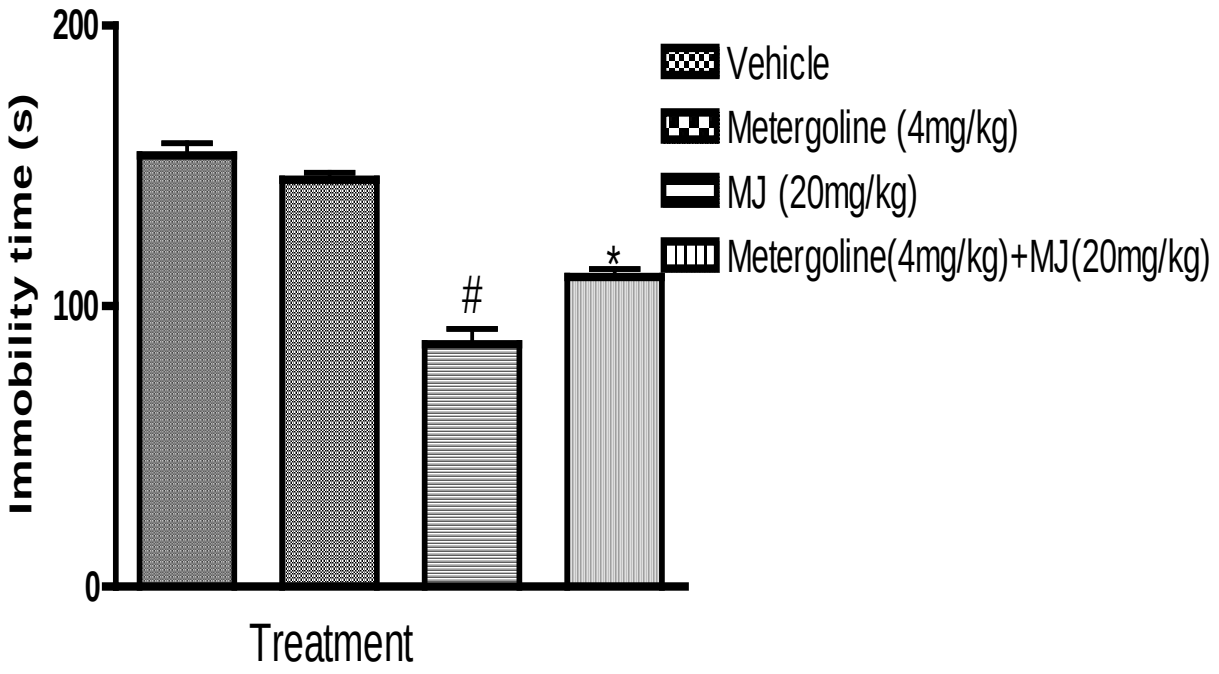


Figure 10b: Effect of pretreatment of mice with metergoline on methyl jasmonate-induced reduction in immobility time in the TST is shown. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test

4.5 Involvement of the nitric oxide pathway in the antidepressant effect of methyl jasmonate

The influence of the pretreatment of mice with L-arginine (750 mg/kg, i.p) and methylene blue (3.75 mg/kg) is shown in Figures 11a and 11b. Pretreatment with L-arginine did not significantly alter the anti-immobility effect of methyl jasmonate (20 mg/kg, i.p) in the TST. However, methylene blue significantly enhanced the anti-immobility effect of methyl jasmonate (2.5 mg/kg, i.p) in the TST in mice.

As shown in Figures 12a and 12b, pretreatment with L-NNA did not significantly alter the anti-immobility effect of methyl jasmonate (2.5 mg/kg, i.p) treatment in the TST while pretreatment with L-NAME significantly increased the immobility time.

11a.

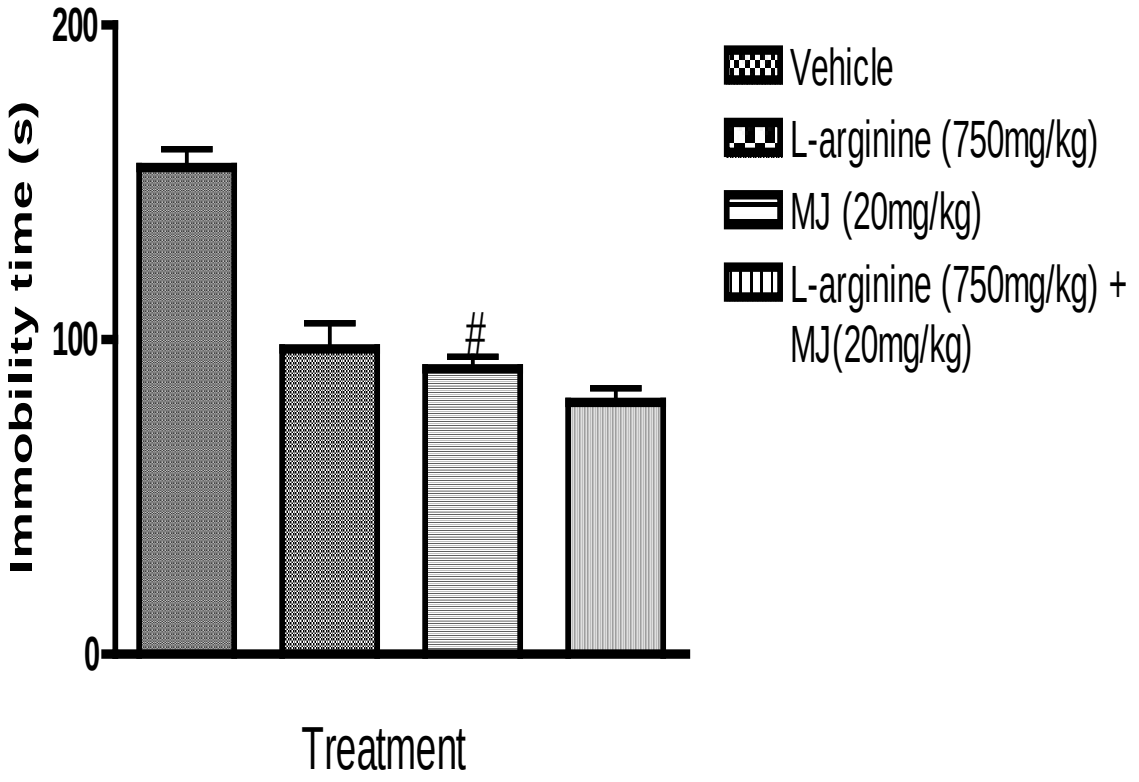


Figure 11a: Effect of pretreatment of mice with L-arginine on methyl jasmonate-induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #p < 0.05 when compared with vehicle and *p < 0.05 when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test

11b.

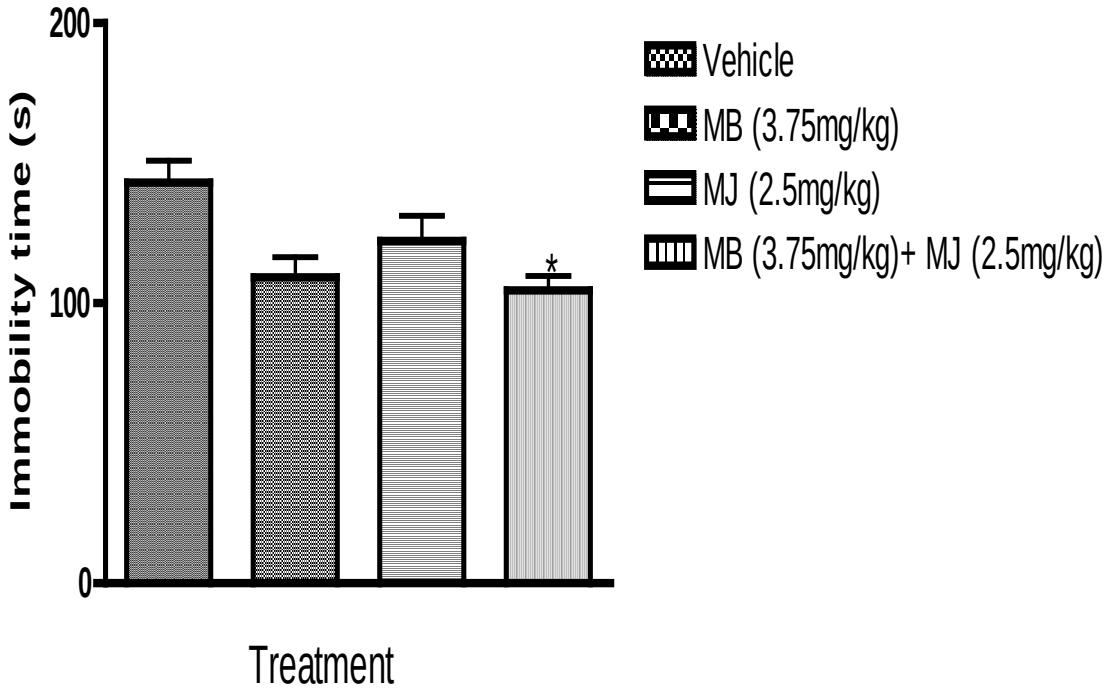


Figure 11b: Effect of pretreatment of mice with methylene blue on methyl jasmonate-induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test; MB- Methylene blue

12a.

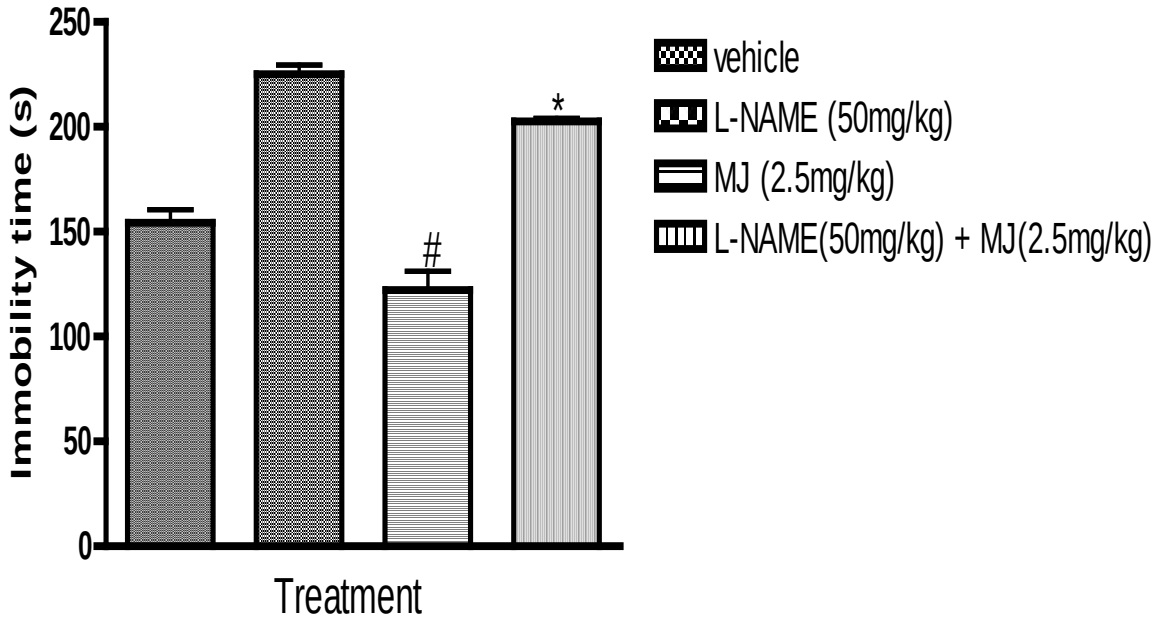


Figure 12a: Effect of pretreatment of mice with L-NAME on methyl jasmonate-induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test

b.

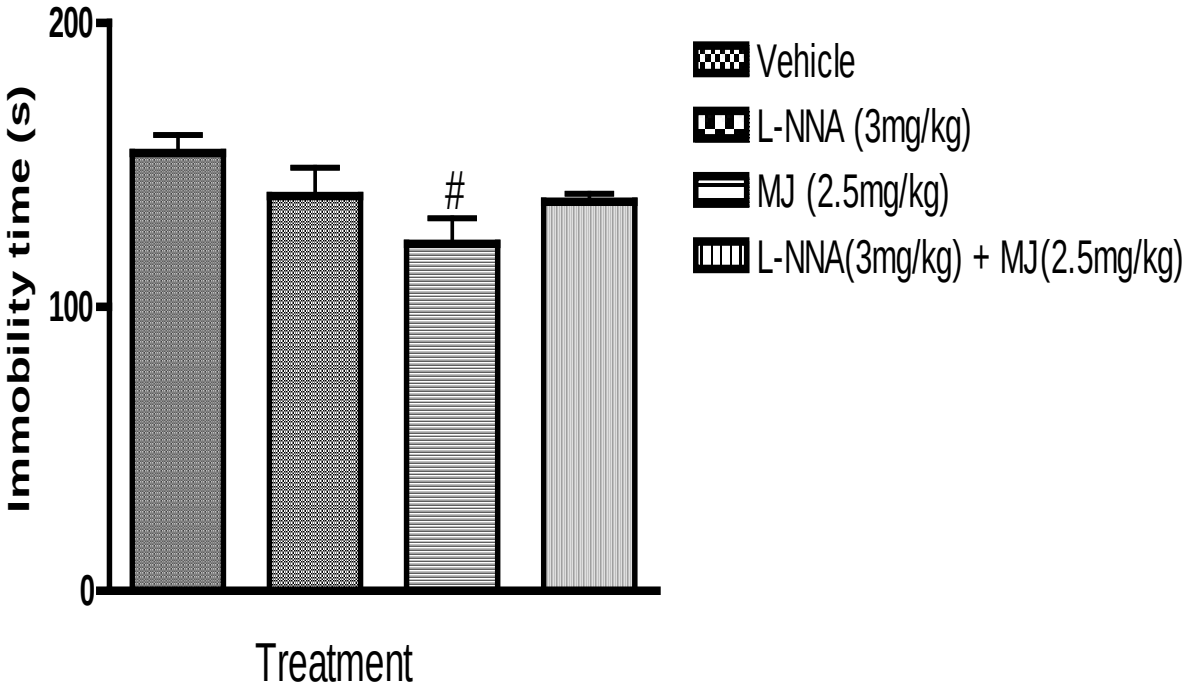


Figure 12b: Effect of pretreatment of mice with L-NNA on methyl jasmonate-induced reduction in immobility time in the TST. Vertical bars represent mean ± S.E.M for 6 animals per group. #p < 0.05 when compared with vehicle and *p < 0.05 when compared to MJ (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test

4.6 Effect of methyl jasmonate on the spontaneous motor activity (SMA)

As shown in Figure 13, the effect of methyl jasmonate on SMA, as expressed by the activity count revealed that it did not significantly alter the values when compared with vehicle suggesting absence of CNS stimulant or depressant effect. The reference drug showed similar effect.

4.7 Effect of Methyl Jasmonate on the duration of immobility in Lipopolysacharride-induced depressive-like behavior

Intraperitoneal injection of LPS (0.83 mg/kg) significantly increased the immobility time in the TST and FST when compared with vehicle which suggests induction of depressive-like behavior in mice. However, MJ (5, 10 and 20 mg/kg) produced significant ($p < 0.05$) decrease in the period of immobility in both TST and FST when compared with LPS (Figures 14a and 14b). As shown in Figures 14a and 14b, the reference drug, IMP (10 mg/kg) also reduced the duration of immobility in a significant manner.

4.8 Effect on spontaneous motor activity (SMA) in mice exposed to Lipopolysacharride

Intraperitoneal injection of LPS showed that there was no significant alteration in the activity count that was recorded over a 5 min period (Figure 15). Also, as shown in Figure 15, LPS did not change SMA in MJ-treated mice.

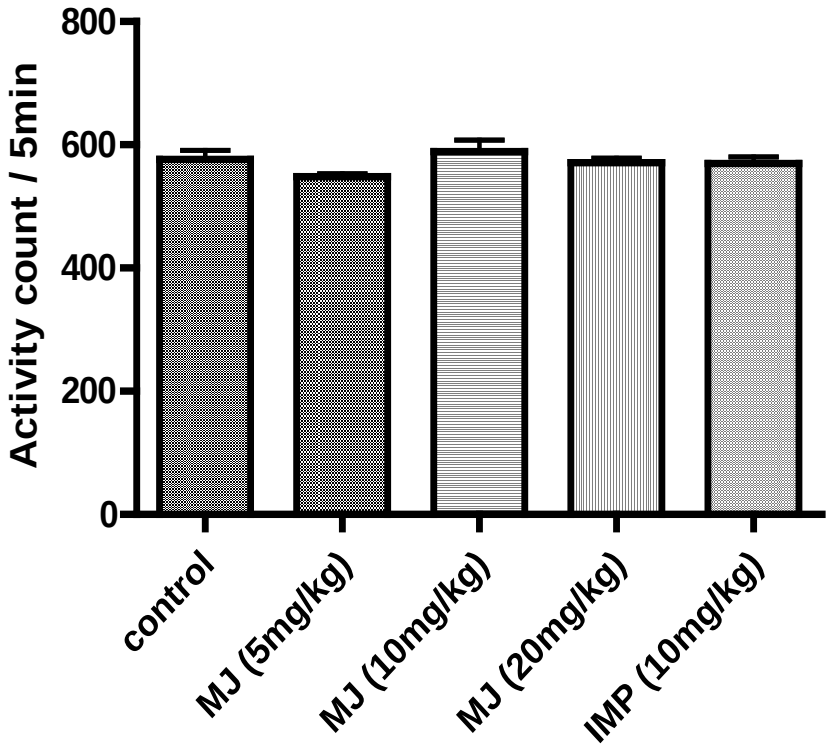


Figure 13: Effect of administration of Methyl jasmonate (MJ) and imipramine (IMP) on activity count of mice in the activity cage. Vertical bars represent mean ± S.E.M for 6 animals per group. (ANOVA followed by Newman Keuls test).

Control mice received 10 mL/kg 1% ethanol, i.p.; MJ- Methyl jasmonate

14a.

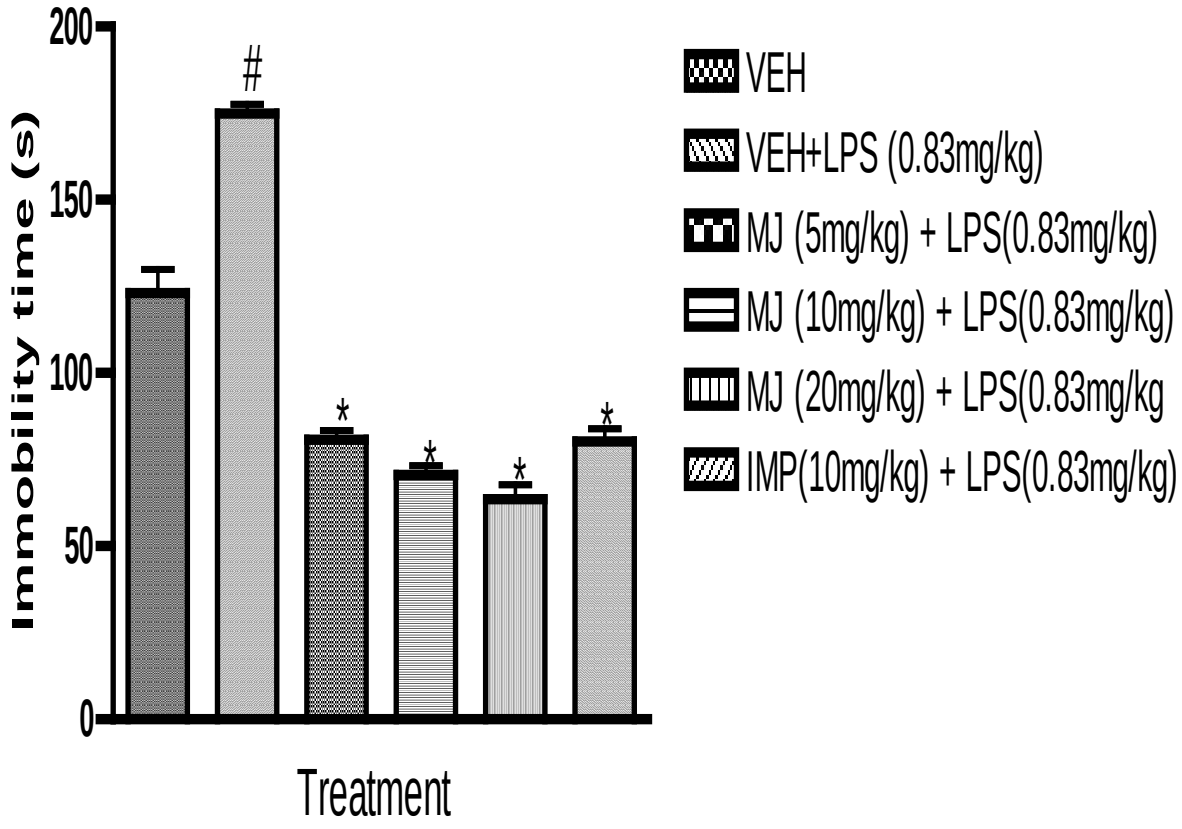


Figure 14a: Effect methyl jasmonate (MJ) on lipopolysaccharide (LPS)-induced depressive-like symptoms in the TST in mice. Vertical bars represent the mean ± S.E.M for 6 animals per group. #$p < 0.05$ compared to vehicle (VEH) group; *$p < 0.05$ compared to LPS (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; TST- Tail suspension test, LPS- Lipopolysacharride, IMP- Impramine

14b.

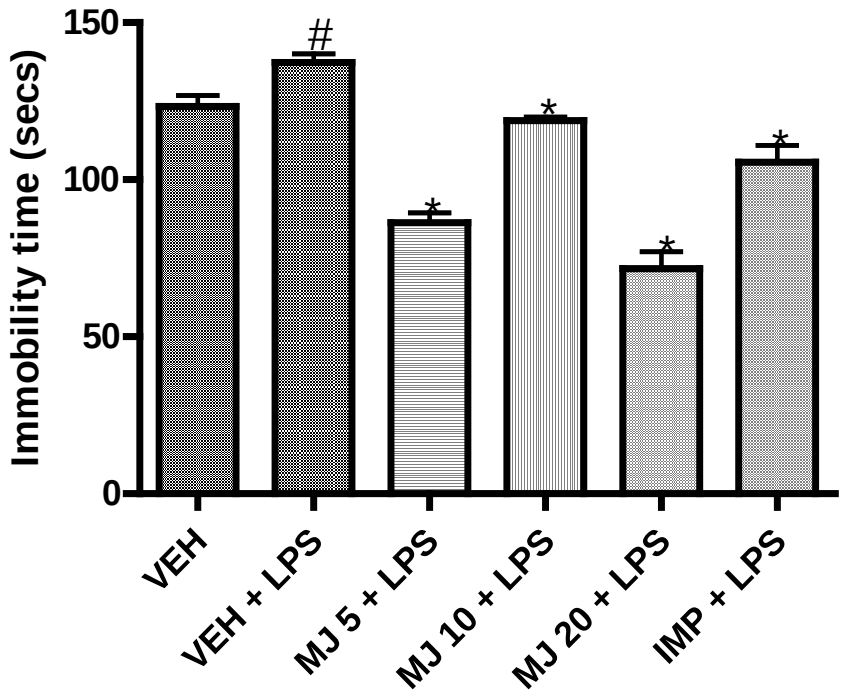


Figure 14b: Effect of methyl jasmonate (MJ) on lipopolysaccharide (LPS)-induced depressive-like symptoms in the FST in mice. Vertical bars represent the mean ± S.E.M for 6 animals per group. #$p < 0.05$ compared to vehicle (VEH) group; *$p < 0.05$ compared to LPS (ANOVA followed by Newman Keuls test).

Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; FST- Forced swim test, LPS- Lipopolysacharride, IMP- Impramine

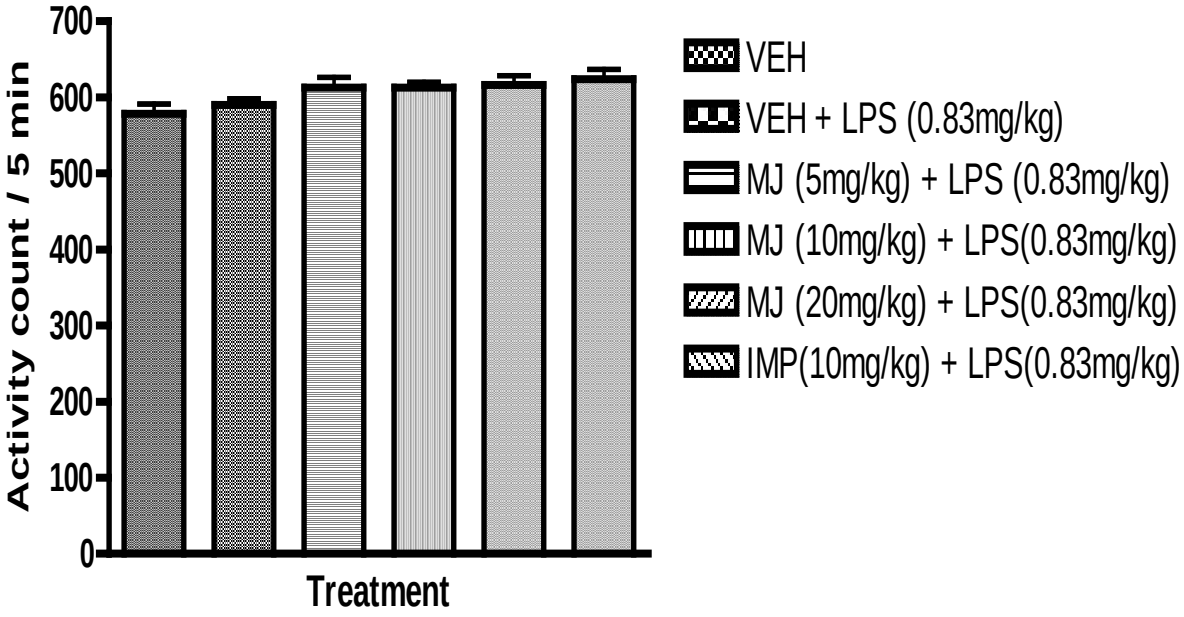


Figure 15: Effect of administration of Methyl jasmonate (MJ) and imipramine (IMP) on activity count of mice exposed to LPS administration in the activity cage. Vertical bars represent mean ± S.E.M for 6 animals per group. (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; LPS- Lipopolysacharride, IMP- Impramine

4.9 Effect of Methyl Jasmonate on LPS- induced anhedonia in mice

The effect of LPS-induced anhedonia as measured by the preference for sucrose intake in mice is presented in Figure 16. It is observed that LPS (0.83 mg/kg) reduced the preference for sucrose intake ($p< 0.05$) in comparison with the control group, however, MJ (5, 10 and 20 mg/kg, i.p) or imipramine (10 mg/kg, i.p) produced a significant increase for the intake of sucrose.

4.10 Effect of Methyl Jasmonate on LPS-induced oxidative stress parameters in mice brains

The effects of MJ on LPS-induced increases in oxidative stress parameters in mice brains are shown in Table 3. LPS produced a significant ($p < 0.05$) elevation of MDA level and decreased antioxidant defense system (GSH and SOD) in brains of mice. However, MJ (5, 10, 20 mg/kg, i.p) reduced MDA and elevated GSH concentrations in the brains of mice treated with LPS ($p < 0.05$). Similar effects were also produced in the group pretreated with imipramine (10 mg/kg, i.p). Increased brain content of SOD in LPS-treated mice was not significantly affected by MJ or imipramine ($P>0.05$).

4.11 Effect of Methyl jasmonate on nitric oxide concentration in the brains of LPS-treated mice

The effect of methyl jasmonate on nitric oxide concentration in mice subjected to LPS (0.83 mg/kg, i.p) is shown in Figure 17. Decreased brain levels of nitric oxide concentration induced by LPS was significantly reversed by methyl jasmonate (5, 10, 20 mg/kg, i.p) in mice.

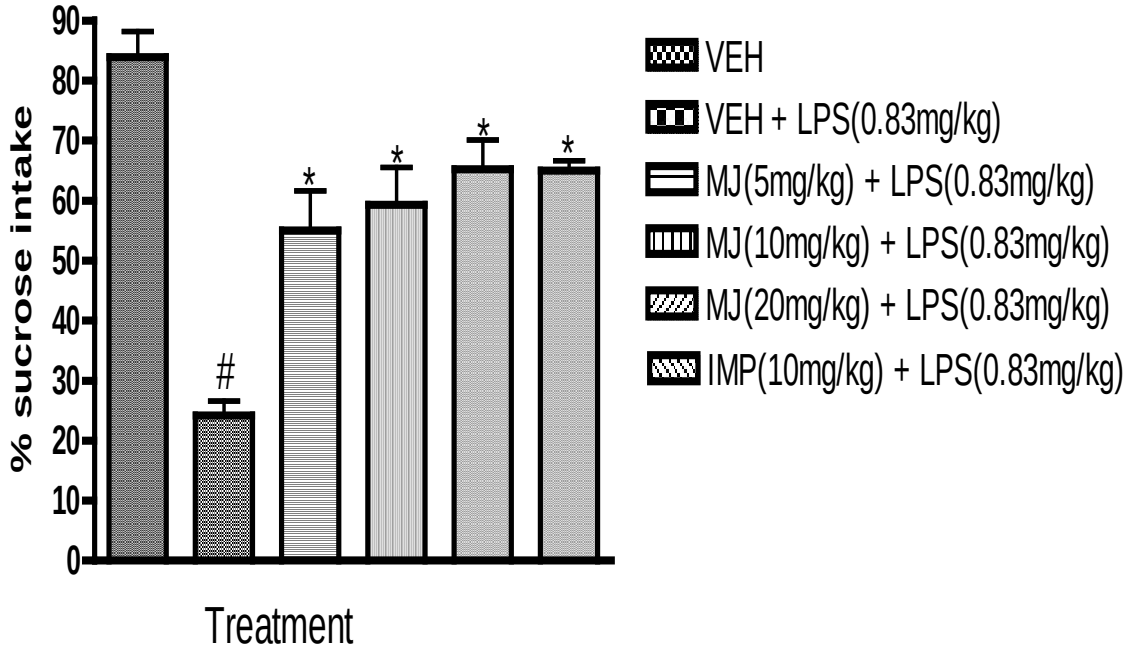


Figure 16: Methyl jasmonate increased percentage sucrose intake in mice exposed to LPS. Vertical bars represent mean ± S.E.M for 6 animals per group. $^{\#}P < 0.05$ compared to vehicle (VEH), $^{\#}P < 0.05$ compared to vehicle + LPS group (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; LPS- Lipopolysacharride, IMP- Impramine

Table 3: Effect of Methyl jasmonate on antioxidant parameters in the mice brains in the Lipopolysacharride (LPS) model

Treatment	GSH concentration (µmol/g tissue)	MDA levels (µmol/g tissue)	SOD levels (units/mg protein)
Vehicle	5.61 ± 0.11	85.70 ±1.08	4.14 ± 0.32
Vehicle + LPS 0.8mg/kg	4.52 ± 0.07#	99.88 ± 1.80#	1.29 ± 0.36#
MJ 5mg/kg + LPS	6.24 ± 0.37*	30.89 ± 2.47*	2.59 ± 0.42
MJ 10mg/kg + LPS	9.34 ± 0.29*	68.15 ± 3.95*	2.02 ± 0.23
MJ 20mg/kg + LPS	10.42 ± 0.30*	40.02 ± 1.38*	2.23 ± 0.44
IMP 10mg/kg + LPS	5.38 ± 0.20*	90.75 ± 0.50*	3.15 ± 0.27*

Values represent mean ± S.E.M for 6 animals per group. $^{\#}P < 0.05$ compared to vehicle, $^{*}P < 0.05$ compared to vehicle + LPS group (ANOVA followed by Newman Keuls test).

GSH- Glutathione; MDA- Malondialdehyde; SOD- Superoxide dismutase; Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; LPS- Lipopolysacharride, IMP- Impramine

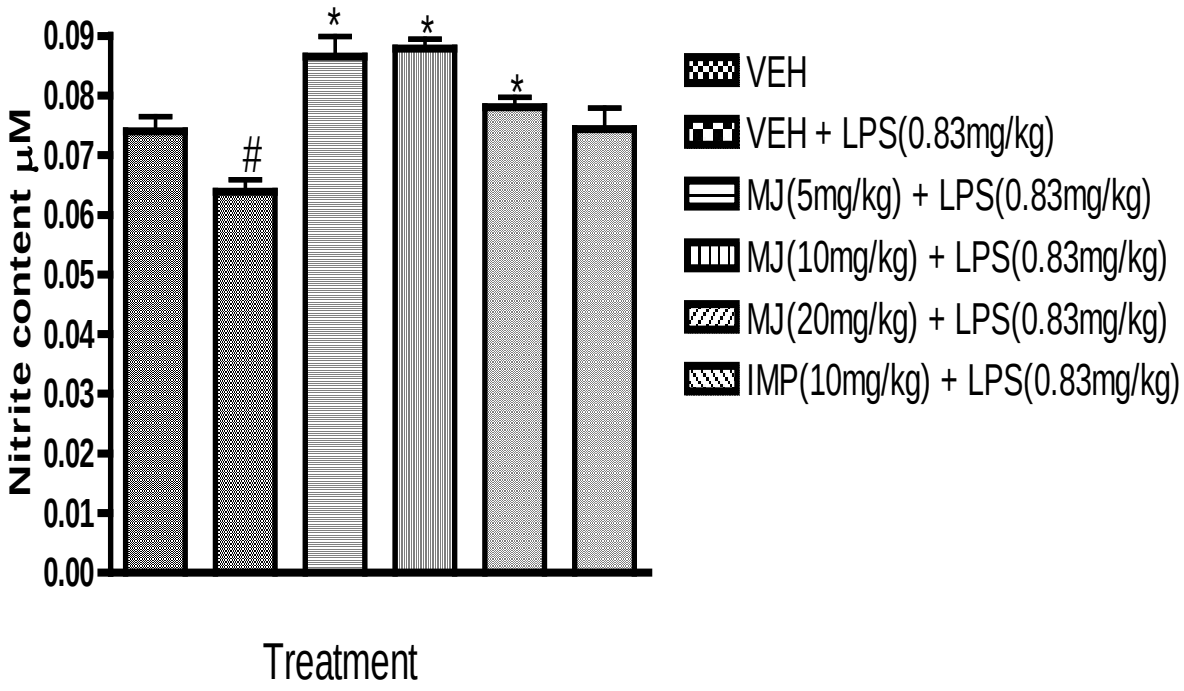


Figure 17: Effect of methyl jasmonate (MJ) on nitric oxide concentrations in the mice brains. Vertical bars represent mean ± S.E.M for 6 animals per group. $^{*}P < 0.05$ compared to vehicle (VEH) + LPS group (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; LPS- Lipopolysacharride, IMP- Impramine

4.12 Effect of MJ on lipopolysacharride-induced elevated corticosterone level

Figure 18 shows that intraperitoneal injection of LPS produced significant increases in the levels of corticosterone in comparison with vehicle. However, MJ (5-20 mg/kg) reduced the increases in serum corticosterone levels in LPS-treated mice in a significant manner.

4.13 Effect of MJ on lipopolysacarride-induced elevated TNF alpha levels

Intraperitoneal injection of LPS (0.83 mg/kg) produced significant increases in the levels ofTNF alpha in comparison with vehicle; however, MJ (20 mg/kg, i.p) reduced the increases in the levels of TNF alpha in LPS-treated mice (Figure 19).

4.14 Effect of MJ on immobility time in TST in mice subjected to CUMS

Figure 20 showed the effect of MJ administration on immobility time in the TST. Mice subjected to 14 days chronic unpredictable mild stress experienced a significantly prolonged immobility time (s) in the TST as compared to non-stress control. However, there was a significant ($p < 0.05$) reduction in immobility time (s) in MJ (5-20 mg/kg, i.p.)-treated groups when compared with stressed- control (Figure 20).

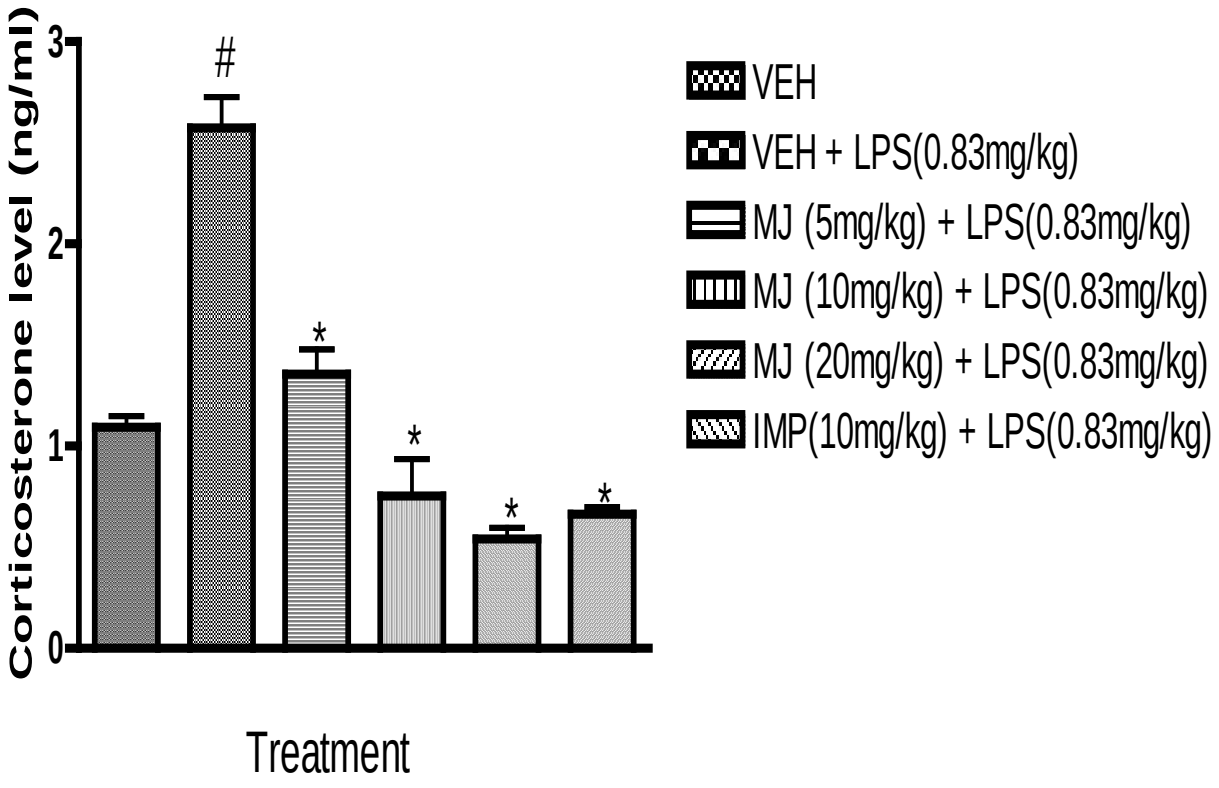


Figure 18: Effect of methyl jasmonate (MJ) on corticosterone levels in mice treated with lipopolysaccharides (LPS). Vertical bars represent mean ± S.E.M for 6 animals per group. $^{\#}P < 0.05$ compared to vehicle, $^{*}P < 0.05$ compared to vehicle + LPS group (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; LPS- Lipopolysacharride, IMP- Impramine

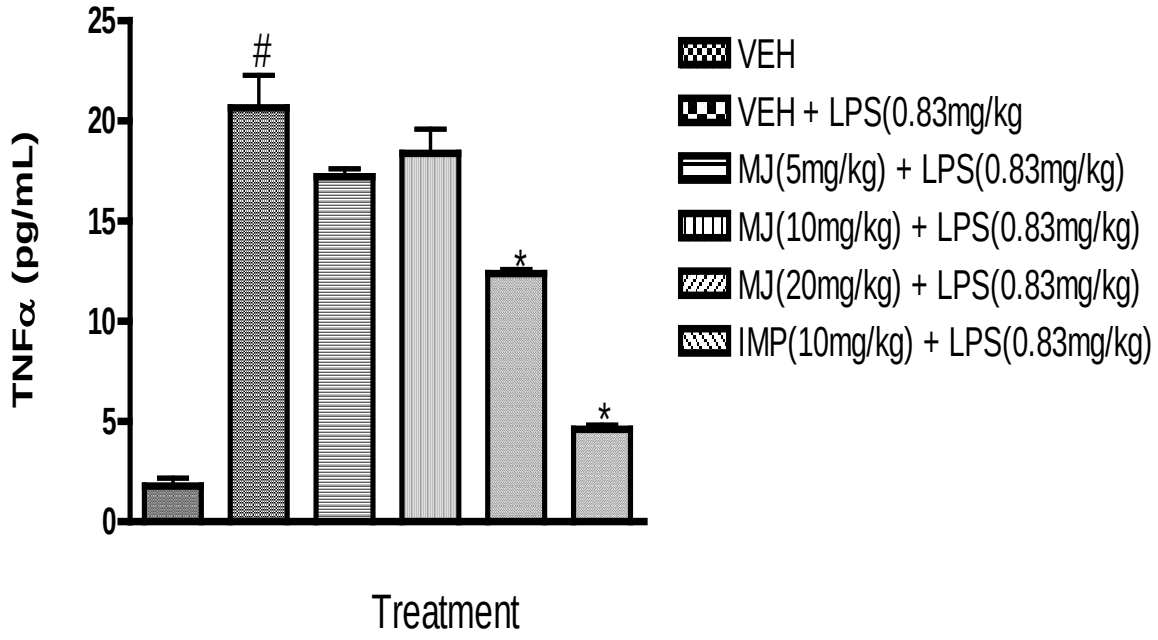


Figure 19: Effect of methyl jasmonate (MJ) on TNF alpha levels in mice treated with lipopolysaccharides (LPS). Vertical bars represent mean ± S.E.M for 6 animals per group. $^{\#}P < 0.05$ compared to vehicle, $^{*}P < 0.05$ compared to vehicle + LPS group (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; LPS- Lipopolysacharride, IMP- Impramine

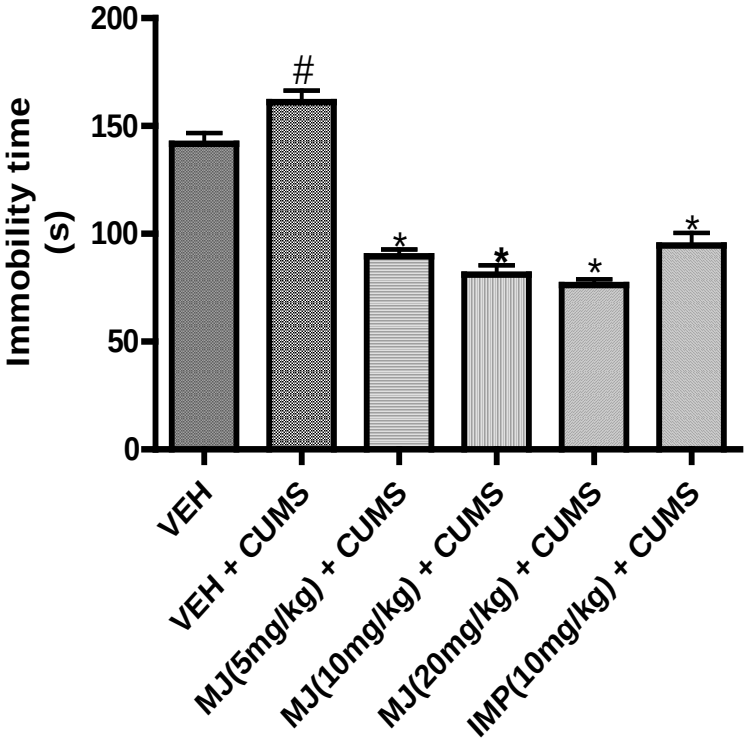


Figure 20: Effect of methyl jasmonate (MJ) on immobility time (s) in TST after exposure of mice to chronic unpredictable mild stress (CUMS). Each column represents mean ± S.E.M for 6 animals per group. $^{\#}p < 0.05$ compared with vehicle-treated unstressed control (ANOVA followed byNewman–Keuls test). $^{*}p < 0.05$ compared with vehicle (VEH)-treated stressed group (ANOVA followed by Newman–Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine; TST- Tail suspension test

4.15 Effect of Methyl Jasmonate on locomotor activity in mice exposed to Chronic Unpredictable Mild Stress (CUMS)

The effect of MJ on SMA of mice exposed to CUMS as measured by the activity counts in the activity cage is presented in Figure 21. There was a significant difference in the SMA in mice exposed to CUMS for 14 days as compared with non-stress control. However, MJ (5-20 mg/kg, i.p.) or imipramine (10 mg/kg, i.p) significantly reversed the motor deficits in mice subjected to CUMS (Figure 21).

4.16 Effect of Methyl Jasmonate on sucrose preference in mice subjected to Chronic Unpredictable Mild Stress (CUMS)

The effect of MJ on anhedonia as measured by sucrose preference (%) in CUMS-induced depressive-like behavior in mice is shown in Figure 22. There was a significant decrease in sucrose preference in stressed mice after 14 days of exposure to CUMS relative to non-stress counterparts. However, the results showed that MJ (5, 10, 20 mg/kg, i.p.) significantly ($p < 0.05$) restored the impaired sucrose preference caused by CUMS in mice (Figure 22).

4.17 Effect of methyl jasmonate on CUMS-induced oxidative stress in mice brains

Table 4 showed the effect of MJ on the brain levels of MDA, GSH and SOD levels in CUMS-exposed mice. It showed that CUMS caused an increase in the brain levels of MDA and also significantly caused decreases in the brain concentrations of GSH and SOD when compared to non-stress controls respectively. Methyl jasmonate significantly ($p < 0.05$) reduced brain levels of MDA and increased GSH and SOD concentrations in CUMS-exposed mice. Similar effects were also produced in the group pretreated with imipramine (10 mg/kg, i.p).

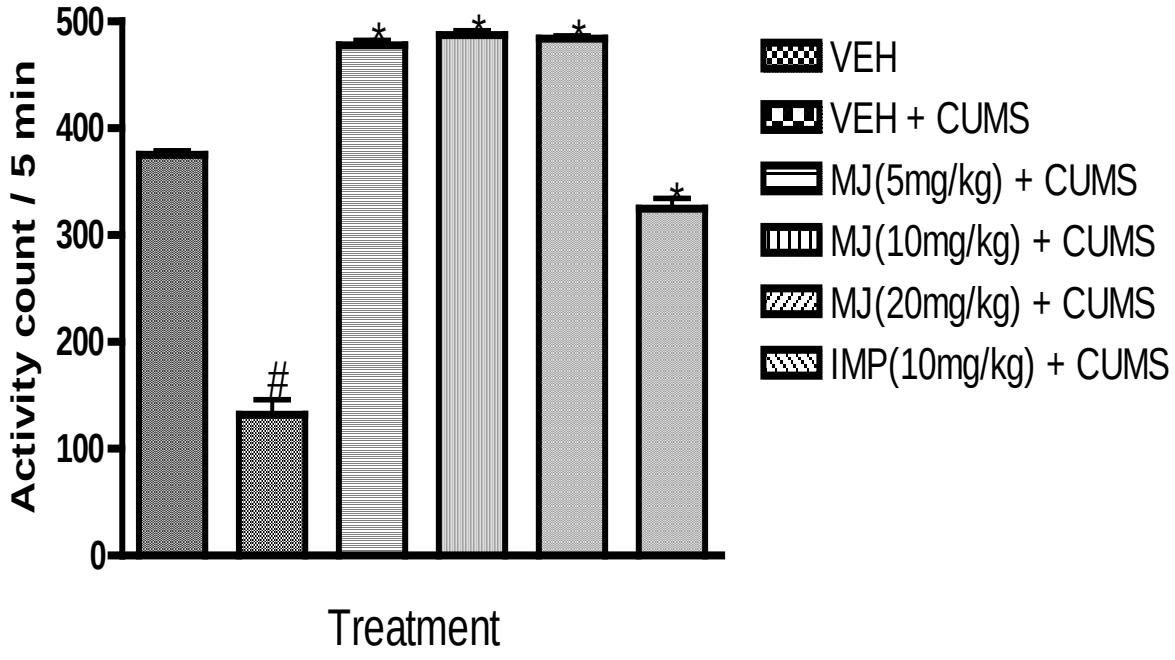


Figure 21: Effect of methyl jasmonate (MJ) on spontaneous motor activity in mice exposed to chronic unpredictable mild stress (CUMS). Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle and *$p < 0.05$ when compared to vehicle (VEH) + CUMS (ANOVA followed by Newman Keuls test)

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine.

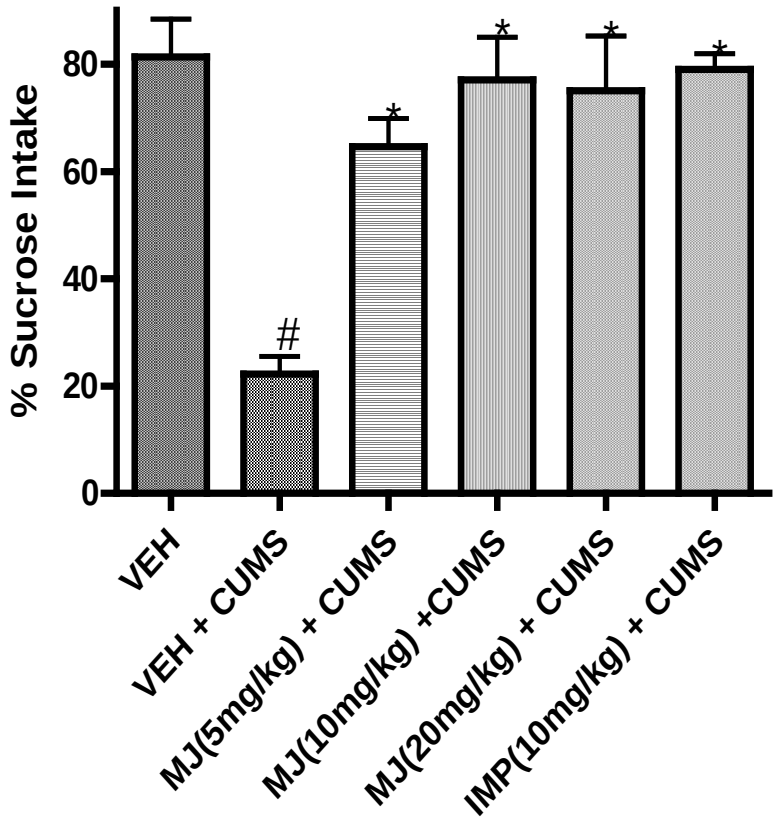


Figure 22: The effect of methyl jasmonate (MJ) (5, 10, 20 mg/kg) on % sucrose intake in mice exposed to chronic unpredictable mild stress (CUMS). Values represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle (VEH) and *$p < 0.05$ when compared to vehicle + CUMS group (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine

Table 4: Effect of methyl jasmonate on chronic unpredictable mild stress-induced oxidative stress

Treatment	GSH concentration (µmol/g tissue)	MDA levels (µmol/g tissue)	SOD levels (units/mg protein)
Vehicle	12.28 ± 0.35	11.93 ± 0.75	4.70 ± 0.21
Vehicle + CUMS	6.47 ± 0.64#	40.14 ± 1.07#	0.90 ± 0.03#
MJ 5mg/kg + CUMS	13.27 ± 0.47*	37.62 ± 1.72	1.77 ± 0.22*
MJ 10mg/kg + CUMS	16.41 ± 0.62*	26.80 ± 0.86*	4.38 ± 0.36*
MJ 20mg/kg + CUMS	18.93 ± 0.38*	21.27 ± 1.17*	4.79 ± 0.13*
IMP 10mg/kg + CUMS	11.74 ± 0.48*	28.61 ± 1.12*	4.65 ± 0.35*

Values represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle (VEH) and *$p < 0.05$ when compared to vehicle + CUMS (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine; GSH- Glutathione; MDA- Malondialdehyde; SOD- Superoxide dismutase.

4.18 Effect of methyl jasmonate on concentration of nitric oxide in brains of mice subjected to chronic unpredictable mild stress (CUMS)

As shown in Figure 23, the effect of methyl jasmonate on the concentration of nitric oxide in mice subjected to CUMS is shown. Elevated brain levels of nitric oxide concentration induced by CUMS were significantly reversed by methyl jasmonate (5 mg/kg, i.p) in mice. Also, similar effects were observed in the group that were given imipramine (10 mg/kg, i.p) (Figure 23).

4.19 Effect of methyl jasmonate on serum corticosterone level in chronic unpredictable mild stress (CUMS)-exposed mice

The effect of MJ on serum corticosterone level in mice exposed to CUMS is shown in Figure 24. It showed that there was a significant increase in the concentration of serum corticosterone in chronic stressed group when compared with non-stress control. However, the increase in the concentration of serum corticosterone produced by CUMS was attenuated by MJ and imipramine in a significant manner (Figure 24).

4. 20 Effect of Methyl jasmonate on tumor necrosis factor-alpha (TNFα)

The effect of MJ on brain levels of TNF-α in mice subjected to CUMS is presented in Figure 25. It showed that there was a significant increase in the concentrations of TNF-α in the brain of mice exposed to CUMS relative to non-stress control. However, MJ (5-20 mg/kg, i.p.) pretreatment significantly reduced the increased levels of TNF-α induced by CUMS in mice brains (Figure 25).

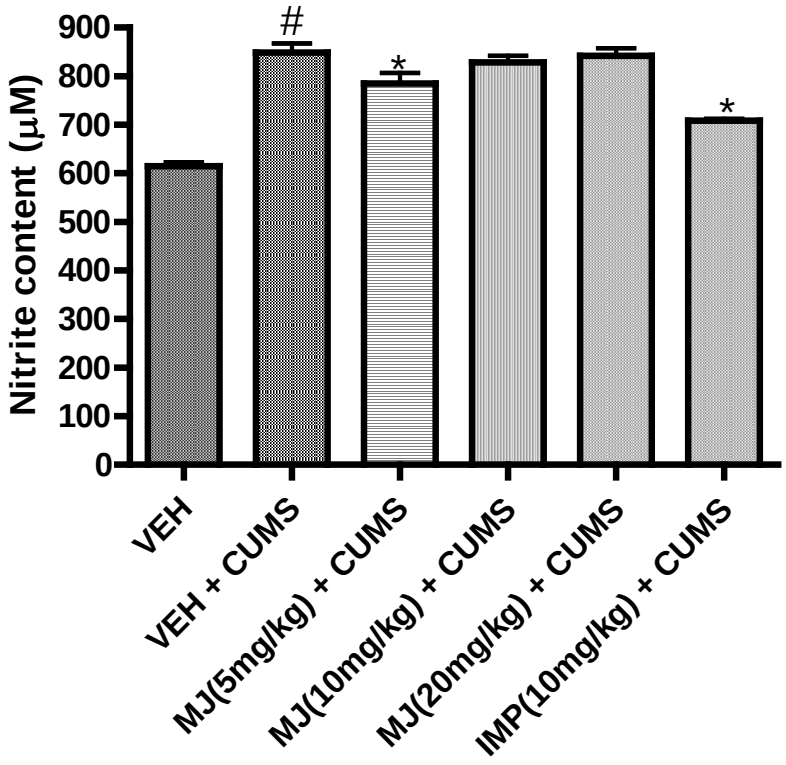


Figure 23: Effect of methyl jasmonate (MJ) on nitrite concentration in chronic unpredictable mild stress (CUMS)-exposed mice. Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle (VEH) and *$p < 0.05$ when compared to vehicle + CUMS (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine

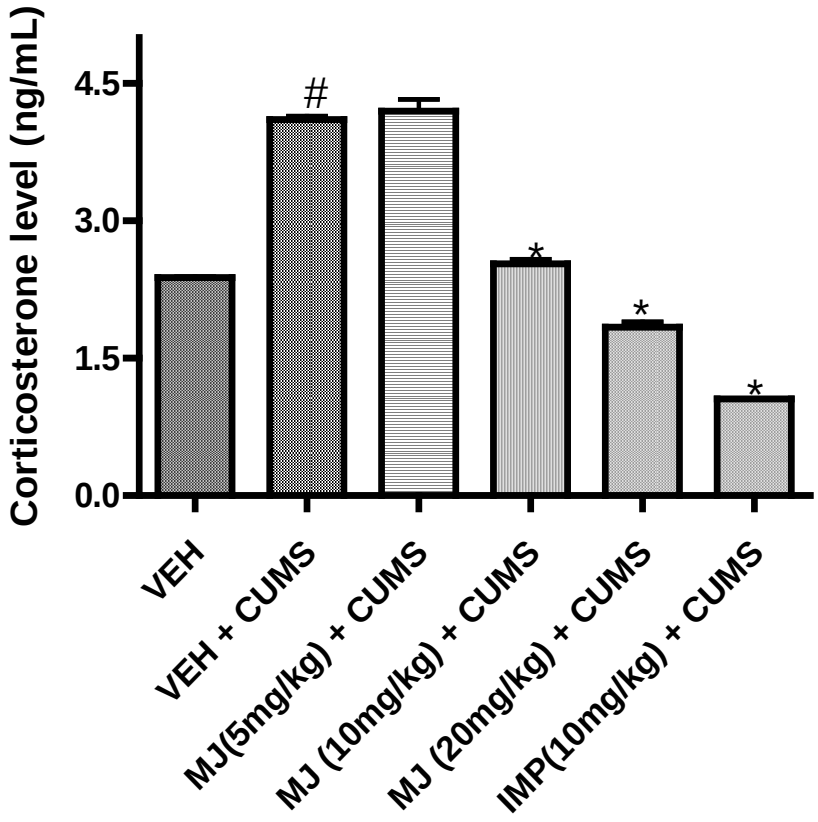


Figure 24: Effect of methyl jasmonate (MJ) on corticosterone level in mice subjected to chronic unpredictable mild stress (CUMS). Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle (VEH) and *$p < 0.05$ when compared to vehicle + CUMS (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine

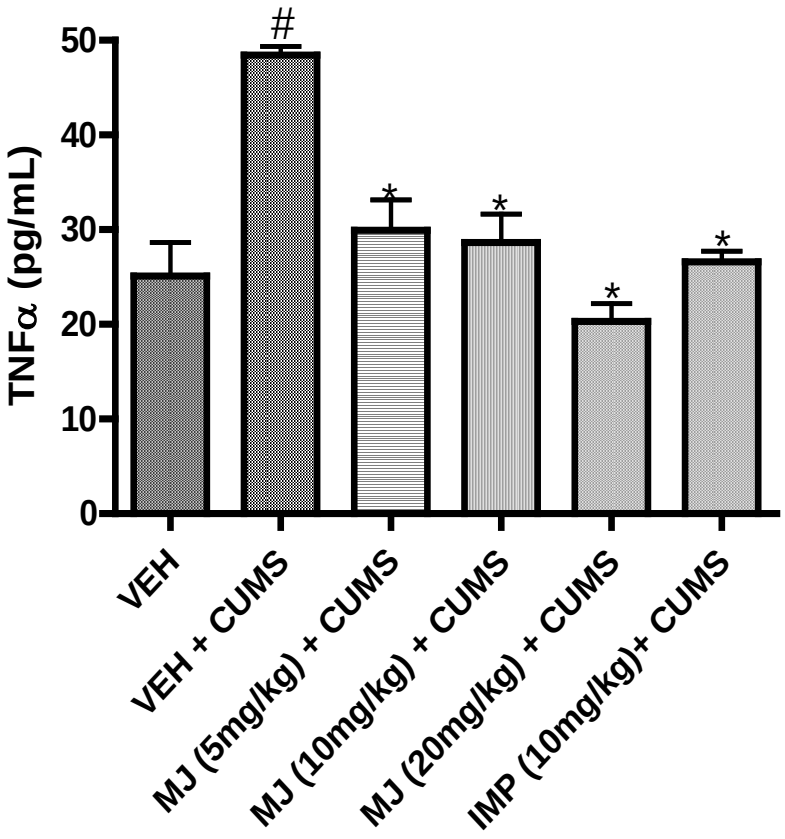


Figure 25: Effect of methyl jasmonate (MJ) on TNF alpha level in mice subjected to chronic unpredictable mild stress (CUMS). Vertical bars represent mean ± S.E.M for 6 animals per group. #$p < 0.05$ when compared with vehicle (VEH) and *$p < 0.05$ when compared to vehicle + CUMS group (ANOVA followed by Newman Keuls test).

VEH- Vehicle (10mL/kg 1% ethanol solution, i.p.); MJ- Methyl Jasmonate; IMP- Impramine

4.21 Effects of Methyl Jasmonate on histological alterations of the hippocampus and cortex of mice brains subjected to LPS treatment

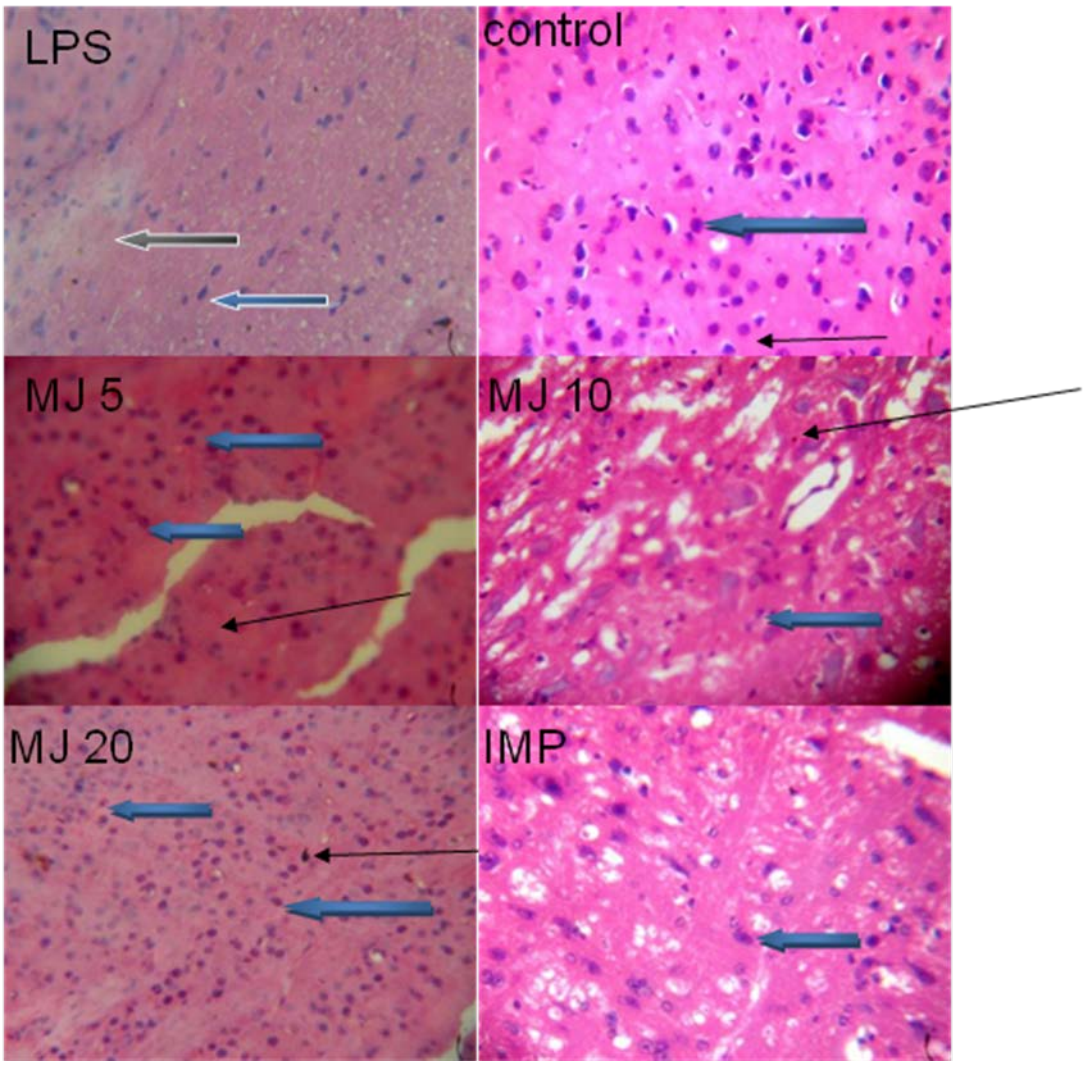
Plates 1a and 1b show the effect of methyl jasmonate on the neuronal cells in mice brain after treatment with LPS. The neuronal cells in the LPS treated group without methyl jasmonate were observed to have degenerated and were shrunken when compared with the vehicle group without LPS treatment. The neuronal cells seem to have improved in the methyl jasmonate (5, 10 and 20 mg/kg, i.p) pretreated groups when compared with the vehicle + LPS treated group. Similar results were observed in the imipramine-treated group.

4.22 Effects of Methyl jasmonate on histological alterations of the hippocampus and cerebral cortex of mice brains subjected to chronic unpredictable mild stress (CUMS)

Plates 2a and 2b show the effect of methyl jasmonate on the neuronal cells in mice brain after exposure to CUMS. The neuronal cells in the CUMS treated group without methyl jasmonate were observed to have degenerated when compared with the vehicle group not subjected to CUMS, while the neuronal cells seem to have improved in the methyl jasmonate (5, 10 and 20 mg/kg, i.p) pretreated groups when compared with the vehicle + CUMS treated group. Similar improvements were observed in the imipramine-treated group.

Plate 1a: Photomicrograph of the representative sections of the hippocampal region of mice brains treated with LPS. LPS slide shows sparse neurons which appear shrunk and pathological lesion were observed. Control slide shows normal area of hippocampal formation (white arrow) and neuronal cells (blue arrow) and no pathological lesion seen. LPS slide showed neurons exhibiting distorted cytoarchitecture and degeneration. MJ 5 mg/kg shows some normal neuronal cells and some degenerating cells. MJ 10 mg/kg shows normal neuronal layers while MJ 20 mg/kg shows cellular layers and neurons which are relatively normal with no pathological lesion seen. Imipramine also showed relatively normal neurons. Magnification x400

MJ- Methyl jasmonate, IMP-Imipramine; LPS- Lipopolysacharride



Plates 1b: Photomicrograph of the representative sections of the cerebral cortex of mice brains treated with LPS. LPS slide shows poor architecture and the cerebral cortex show extensive areas of necrosis (black arrow), some of the neuronal cells seen appear ovoid and shrunk (blue arrow). In the MJ (5 and 10 mg/kg, i.p)-treated groups, there are various normal cells seen. MJ 20 mg/kg shows cellular layers and neurons which are relatively normal when compared with control and no pathological lesion seen. Imipramine also showed similar features as control. Magnification x400

MJ-methyl jasmonate, IMP- imipramine; LPS- Lipopolysacharride

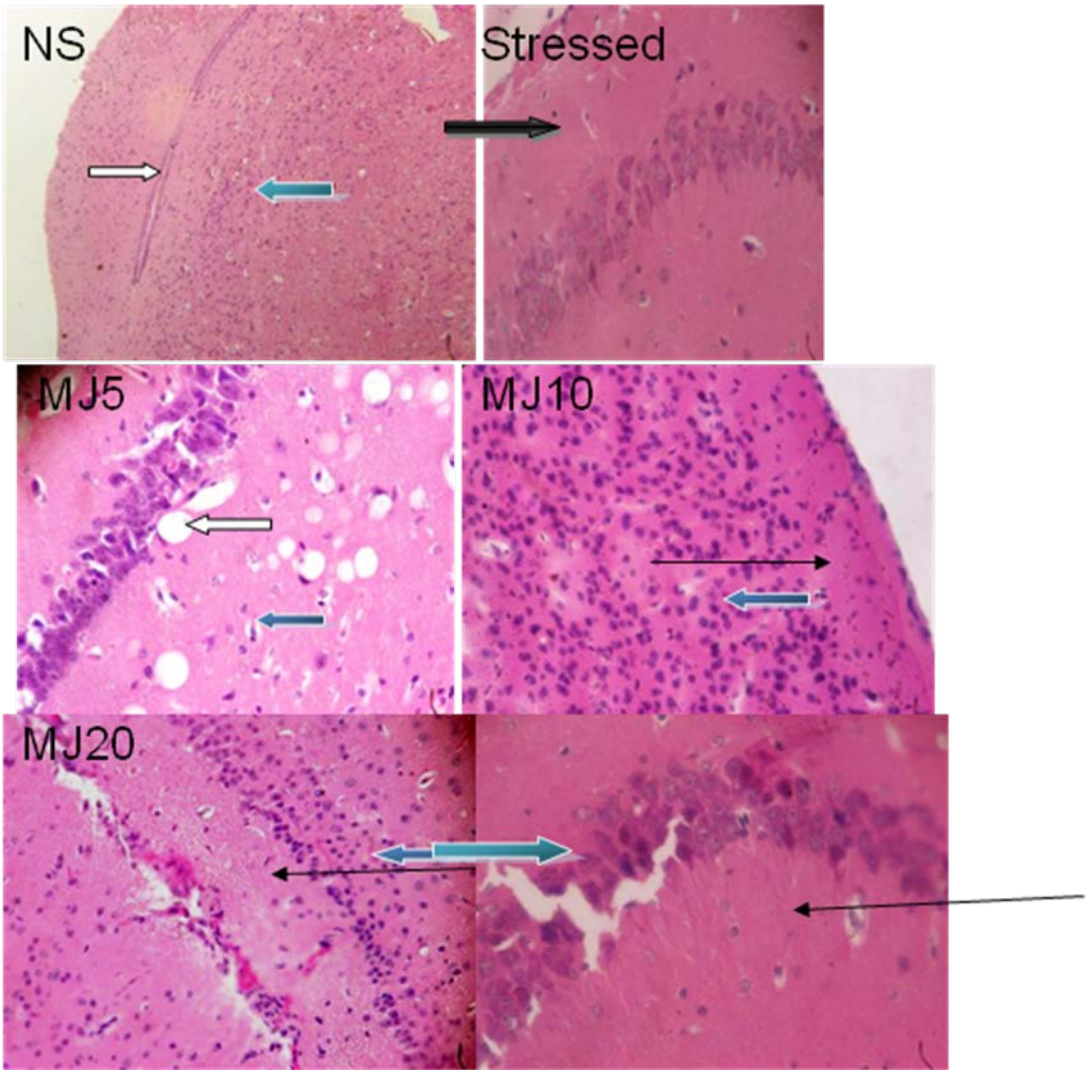


Plate 2a: Photomicrograph of a representative section of hippocampus stained by haematoxylin and eosin (H & E). NS shows normal partial area of hippocampal formation noted and normal neuronal cells (blue arrow). In the stressed slide, there are scarcely distributed neurons and they are mostly shrunk neuronal cells with pathological lesion seen. MJ 5 mg/kg shows some normal neuronal cells and some degenerating cells. MJ 10 mg/kg shows normal neuronal layers while MJ 20 mg/kg shows cellular layers and neurons which are relatively normal with no pathological lesion seen. Imipramine also showed relatively normal neurons comparable to control. Magnification x400

NS-non stressed, MJ-methyl jasmonate, IMP-imipramine

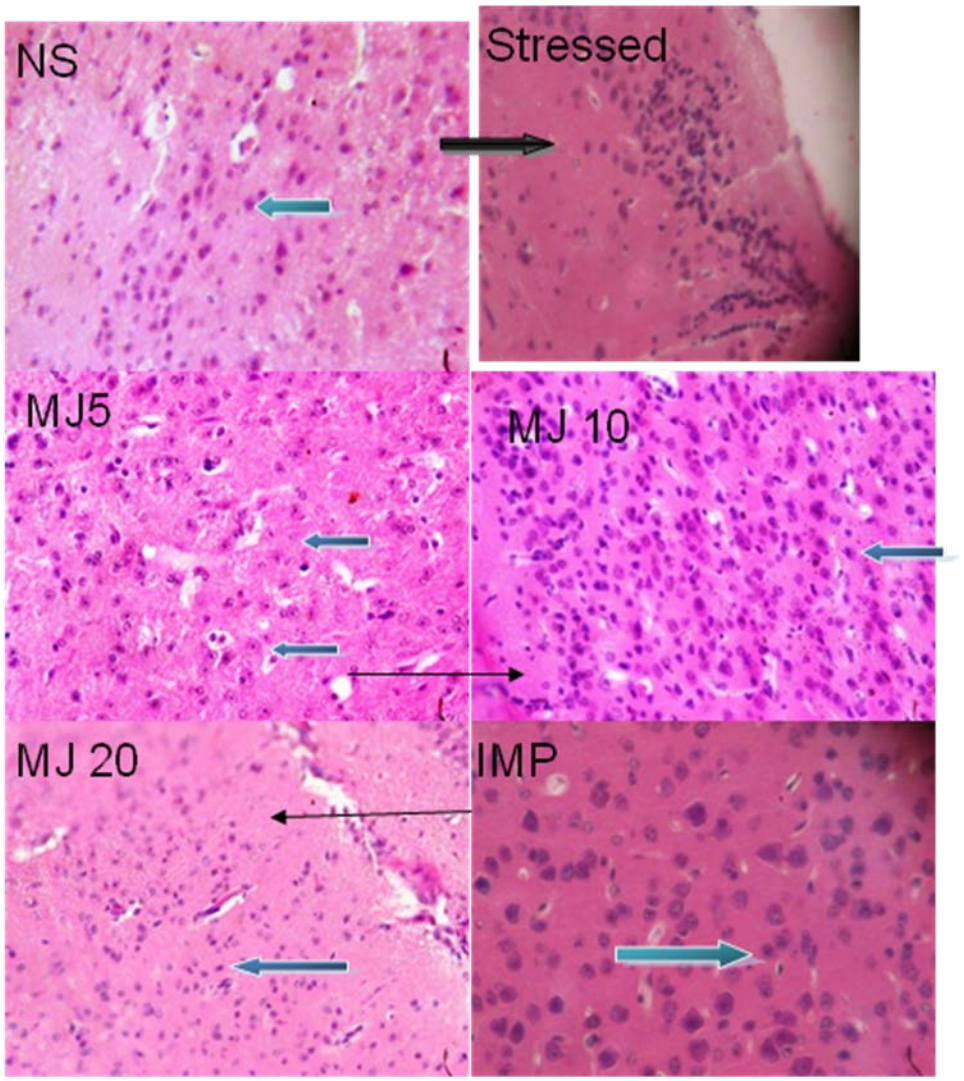


Plate 2b: Photomicrograph of a representative section of the cerebral cortex stained by haematoxylin and eosin (H & E). In the NS slide, the brain stroma appears clean with no pathological lesion seen. The stressed slide shows areas of necrosis and sparse neurons (black arrow). MJ 5 mg/kg shows some normal neuronal cells and some degenerating cells. MJ 10 mg/kg shows normal neuronal layers while MJ 20 mg/kg shows cellular layers and neurons which are relatively normal with no pathological lesion seen. Imipramine also showed relatively normal neurons comparable to control. Magnification x400

NS-non stressed, MJ-methyl jasmonate, IMP-imipramine

CHAPTER FIVE

5.0 DISCUSSION

The results of this study showed that MJ produced a significant decrease in the period of immobility in the forced swim and tail suspension tests in mice, which suggest antidepressant-like activity. The forced swim test (FST) and tail suspension test (TST) are well recognized validated experimental models for the detection of compounds with antidepressant property in rodents (Porsolt *et al.*, 1978, Steru *et al.*, 1985). Both tests are based on the observation that when a mouse or rat is placed in an inescapable situation, that is, cylinder filled with water, or suspended by their tail, after initial escape-directed behaviour, the animals quickly adopt an immobile posture (Cryan *et al*., 2005; Petit-Demouliere *et al.,* 2005). This switch in behaviour is hypothesised to reflect either a failure to persist with escape-directed behaviour or a passive behaviour to cease active forms of coping with the stressful stimuli (Cryan and Slattery, 2007; Geyer and Markou, 1995; Slattery and Cryan, 2006). The indicative parameter in these tests is the duration of immobility. Thus, the state of immobility is akin to a state of despair or hopelessness that often characterizes depressive illnesses and antidepressant drugs are known to decrease the period of immobility in rodents. The FST, a widely accepted behavioural model predictive of antidepressant activity is sensitive to all major classes of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors and monoamine oxidase inhibitors (Cryan *et al*., 2002; Porsolt *et al*., 1977). These tests have proven to be highly selective for antidepressants, with all clinically effective drugs prolonging the time that animals spend in active behaviours. Immobility is quantified during brief test periods and classical antidepressants such as the monoamine oxidase inhibitors, tricyclics, and atypical antidepressants all decrease the duration of immobility in rats and mice (Borsini and Meli, 1988). Acute antidepressant treatment given prior to the test reduces immobility time in the tail suspension test and it is considered to have good predictive validity (Cryan *et al.,* 2005). However, antidepressant drugs have been found to cause little or no significant effect on the spontaneous motor activity (SMA) in rodents unlike psychomotor stimulants such as amphetamine and cocaine (Sherman *et al*., 1982; Kang *et al*., 2010). The finding that the anti-immobility effect of MJ was not accompanied by increase in SMA shows that it has antidepressant-like activity in mice. The results for methyl

jasmonate on activity count or spontaneous motor activity showed no significant difference across the groups which suggests that it neither decreases nor increase locomotion. Thus, methyl jasmonate has no sedating nor excitatory effects. The assessment of spontaneous locomotor activity is imperative as part of the routine procedures for detecting prospective antidepressant drugs (Porsolt *et al.*, 1978).

Monoamine depletion in synaptic clefts in the brain has been suggested to be an important paradigm of depressogenic pathogenesis. Thus, the mechanism of action of antidepressant drugs has been investigated on this basis (Elhwuegi, 2004; Millan, 2004). Since, monoaminergic system has been recognized as the neurochemical pathway implicated in the pathophysiology and treatment of depression (Elhwuegi 2004; Millan, 2004), I decided firstly to investigate the involvement of the noradrenergic, dopaminergic and serotonergic systems in its anti-immobility effect in the TST. The TST is widely used for screening new antidepressant drugs and for elucidation of their mechanism (s) of action (Cryan *et al.,* 2005). The TST is also known to be highly sensitive to the major classes of the clinical antidepressants, including the selective serotonin reuptake inhibitors (SSRIs), the tricyclic antidepressants (TCAs), and the monoamine oxidase inhibitors (MAOIs) (Cryan *et al*., 2005; Kwon *et al*., 2010). Thus, the more sensitive method than the FST was used to investigate the likely mechanism(s) of action involved in the antidepressant-like effect of MJ. To this end, the effects of some of the pharmacological agents that affect monoaminergic systems on the anti-immobility activity of MJ were investigated in this study using the TST.

Studies implicating serotonin and noradrenaline in the pathogenesis of depression are well reported in literature both in preclinical and clinical investigations (Blier, 2003; Anguelova *et al*., 2003). The role of noradrenaline in the pathophysiology of depression has also been extensively studied (Brunello *et al*., 2003; Delgado and Murano, 2000). Depression seems to be associated with a hypofunction of the noradrenergic system (Brunello *et al*., 2003; Wang *et al*., 1999). The tricyclic antidepressants like imipramine or desipramine with marked noradrenaline selectivity have enjoyed long patronage for the treatment of depressive illnesses but are known to have high affinity for cholinergic and histaminergic receptors, resulting in a wide range of adverse effects

(Nutt, 2006; Diaz *et al*., 2012). However, in this study, we use prazosin (an α_1-adrenoceptor antagonist) and yohimbine (an α_2-adrenoceptor antagonist) to evaluate the involvement of noradrenergic pathway in the antidepressant-like effect of MJ. Previous studies have shown that these adrenergic receptors are involved in some of the antidepressant-like effects of drugs in behavioral models of depression (Kaster *et al*., 2007; Masuda *et al*., 2001). Moreover, the blockade of α_1-adrenergic receptors have been shown to produced depressive-like behaviours and also desensitized α_1-adrenoceptors (Stone *et al.,* 2003) whereas, antidepressant therapy enhanced the density and functional activity of these receptors in the frontal cortex and hippocampus (Stone *et al*., 2003). However, upregulation of α_2-adrenoceptor autoreceptors has been reported in patients with depression while downregulation was observed with antidepressant treatments (Flugge *et al*., 2003; Ordway *et al*., 2003). In the present study, we observed that pre-treatment with prazosin or yohimbine significantly inhibited the antidepressant-like effect of MJ in a manner similar to previous results with other antidepressant agents (Cardoso *et al*., 2009; Giggliucci *et al.,* 2010), which suggests its possible interaction with both α_1 and α_2-adrenergic receptors. These adrenoceptors have been shown to underlie some of the antidepressant-like responses of drugs in behavioural models of depression (Danysz *et al.,* 1986; Kaster *et al.,* 2007b; Masuda *et al*., 2001). The decrease in adrenergic activity due to antagonism of central α_1-adrenergic receptors may perhaps accounts for the reduced anti-immobility effect of MJ caused by prazosin in this study. Meanwhile, antagonism of α_2-adrenoceptors has been reported to produce a complex, variable and opposing effects depending on the receptor subtypes involved (Cottingham and Wang, 2012). However, the role of these receptor subtypes in yohimbine-induced attenuation of antidepressant effect of MJ and other substances reported in literature required further investigations.

The role of dopamine (DA) deficiency in the pathophysiology of depression is supported by studies demonstrating reduced levels of DA and its metabolite homovanillic acid (Papakostas, 2006) as well as increased dopamine D_1/D_2 receptor binding (Shah *et al.,* 1997) in depressed patients when compared to normal individuals. The dopaminergic pathway is also involved in the regulation of mood and behaviours and plays a role in the pathophysiology of depression (Dailly *et al.,* 2004). Biochemical evidence obtained from clinical studies have also shown that the

plasma levels of dopamine metabolites (homovanillic acid and 3,4-dihydroxyphenylacetic acid) were significantly lower in the depressed patients indicating a diminished dopamine turnover in depressive illnesses (Mitani *et al*., 2006). Moreover, it has been reported that the potentiation of dopaminergic neurotransmission contribute to the therapeutic effect of antidepressant treatments (D'Aquila *et al*., 2000; Papakostas, 2006). Indeed, the anti-immobility effects of the tricyclic antidepressant imipramine were reported to be reduced by antisense dopamine D_2 receptor (Papakostas, 2006), which further implicates dopaminergic system as an important target for antidepressant action (Dziedzicka-Wasylewska, 2000; Willner, 1997). Moreover, clinical studies have also reported that dopamine D_2 receptor agonists are efficacious for treating patients with depressive illnesses (Waehrens, 1981). As shown in our results, sulpiride (a D_2 receptor antagonist) and haloperidol prevented the anti-immobility effect caused by MJ in the TST. These findings suggest that dopaminergic pathway might also play a role in the antidepressant-like activity demonstrated by MJ in mice. This indicates that methyl jasmonate may also exert its effect by interacting with D_2 antagonists. There is considerable amount of pharmacological evidence regarding the efficacy of antidepressants with dopaminergic effects in the treatment of depression (Papakostas, 2006).

The serotonergic system plays a major role in the action of antidepressants (Millan, 2004). A large number of experimental and clinical studies show that the serotonin (5-HT) system is strongly associated to the neural regulation of mood and evidences indicate abnormalities in 5-HT neurotransmission in the pathophysiology of depression (Elhwuegi, 2004). Drugs affecting 5-HT neurotransmission, such as those inhibiting 5-HT reuptake at nerve terminals or inhibiting monoamines metabolism (MAO inhibitors) are effective in treating depression (Elhwuegi, 2004). Several reports have suggested an involvement of the $5\text{-}HT_{1A}$ receptors in the mechanism of action of several classes of antidepressant drugs, including tricyclics, SSRIs (selective serotonin reuptake inhibitors) and MAOi (monoamine oxidase inhibitors) and in the pathophysiology of depression (Hirvonen *et al*., 2008). The involvement of the serotonergic system in the antidepressant-like effect of MJ was studied using p-Chloropenyl alanine (PCPA) and metergoline in TST paradigm. The pCPA is an inhibitor of tryptophan hydroxylase and its administration, for four consecutive days, depletes the endogenous stores of 5-HT (Rodrigues *et*

al., 2002; Gigliucci *et al*., 2010). Previous studies have shown that pCPA specifically block the antidepressant action of serotonin reuptake inhibitors like fluoxetine (Rodrigues *et al*., 2002; Redrobe *et al*., 1998). The findings that pCPA attenuated the immobility effect of MJ in the TST suggest that its antidepressant property may be mediated via enhancement of serotonergic neurotransmission. Moreover, the role for 5-HT_2 receptors in the pathophysiology and treatment of depressive illnesses has been recognized over the years (Redrobe *et al*., 1998; Zomkowsk *et al*., 2004). Several preclinical studies have established that the pretreatment of animals with 5-HT_2 receptor antagonists like ketanserin reversed antidepressant-like effect of some compounds (Redrobe *et al*., 1998; Diaz *et al*., 2012). Conversely, the preferential 5-HT_{2A} receptor agonist was reported to enhance the antidepressant-like effect of some compounds (Deakin, 1988; Khisti and Chopde, 2000). Thus, the reduction in anti-mobility effect of MJ caused by pretreatment with metergoline, a 5-HT_{2A} receptor antagonist suggests the participation of 5-HT_2 receptors in its antidepressant-like effect in the TST.

The L-arginine–nitric oxide pathway has been implicated in the pathogenesis of depression (Mantovani *et al*., 2003). Nitric oxide (NO) plays a significant role in the central nervous system and pharmacological manipulation of the NO pathway may constitute a novel therapeutic approach for the treatment of depression (Harkin *et al*., 2003, 2004). To date a number of studies have demonstrated that inhibition of nitric oxide synthase (NOS) produces anxiolytic and antidepressant-like behavioural effects in a variety of animal paradigms (Spiacci *et al*., 2008). In this study, I showed that the reduction of immobility time elicited by methyl jasmonate in the TST was not reversed by pre-treatment with L-arginine (nitric oxide synthase (NOS) substrate) thus, L-arginine did not prevent the antidepressant effect of MJ which suggests that methyl jasmonate probably did not work via the L-arginine NO pathway. Several studies have demonstrated that NOS inhibitors, depending on their concentration, exert antidepressant-like effects (Harkin *et al*., 2004). In addition to clinical studies in bipolar disorder, methylene blue has been recognised as a potential antidepressant and anxiolytic in animal models, (Eroglu and Caglayan, 1997) possibly by increasing both serotonin and dopamine levels in the hippocampus through various mechanisms (Wegener *et al*., 2000). There are certain effects of methylene blue which include non-specific inhibition of nitric oxide synthase. Previous study has shown that methylene blue potentiated the antidepressant-like effect of venlafaxine in the FST (Dhir and

Kulkarni, 2007). The results of this study showed that methylene blue potentiated the antidepressant-like effect of MJ in the TST. However, L-NAME and L-NNA did not potentiate the antidepressant activity of methyl jasmonate.

The validity of these models in the discovery of antidepressant drugs is based on the observations that rodents exposed to TST and FST experienced behavioural despairs characterized by increased period of immobility (Cryan *et al.,* 2005; Liu *et al.,* 2016). Clinically useful antidepressant drugs are known to reduce the duration of immobility in these tests (Cryan and Slattery, 2007; Kang *et al.,* 2010; Liu *et al*., 2016).

The effect of MJ on lipopolysacharride (LPS)-induced depressive-like behaviour was further investigated based on the preference for sucrose consumption (as may be noticed in anhedonia), duration of immobility and SMA in rodents (Willner *et al*., 1992). In this study, LPS was found to increase the duration of immobility but did not significantly alter SMA. The ability of MJ to reduce the duration of immobility in mice subjected to LPS treatment, therefore suggesting the possession of an antidepressant-like activity in mice. These findings also corroborated our previous reports that MJ demonstrated anti-depressant-like effect in naïve mice subjected to TST and FST (Umukoro *et al.,* 2011). Moreover, the effects of antidepressant drugs in these models have been reported to be specific since they do not increase spontaneous motor activity of the animals (Cryan and Slattery, 2007; Kang *et al.,* 2010; Liu *et al*., 2016). In this study, the anti-immobility effect of MJ was not associated with central nervous system stimulation; as it did not produce any significant changes in the SMA of the animals. This is in agreement with previous reports (O' Connor *et al.,* 2009; Ohgi *et al.,* 2013) which states that locomotor activity is decreased in mice up to 6 hours after LPS administration normalizing 24 hours after LPS administration.

Anhedonia has been described as a loss of interest in pleasurable activities and the results of this study further confirm that LPS can impair preference for sucrose intake suggesting precipitation of depressive-like behaviour. Indeed, preclinical studies have shown that rodents exposed to LPS display characteristic behaviours consistent with a loss of responsiveness to reward, such as

sucrose consumption, which typify major depression (Moreau, 1997). Earlier studies confirm previous reports that LPS can induce depressive-like behavior, such as anhedonia, anorexia, and body weight loss (Kentner *et al*., 2010; Pitychoutis *et al.,* 2009; Singal *et al.,* 2006; Yirmiya, 1996). However, antidepressant drugs are known to reverse LPS-induced impaired preference for sucrose consumption in rodents (Matthews *et al.,* 1995). Methyl jasmonate was seen to increase percentage of sucrose intake in the sucrose preference test when compared with the control group to which lipopolysaccharide (LPS) was administered. The results also showed that LPS decreased the percentage of sucrose intake when compared with the control group. Thus, the finding that MJ produced increase in sucrose consumption in LPS-mice further indicates antidepressant-like activity. Acute activation of the peripheral innate immune system in laboratory animals, through the administration of the cytokine inducer lipopolysaccharide (LPS), induces depressive-like behavior, as measured by increased immobility in the forced-swim test (FST) and tail suspension test (TST), decreased consumption of a sweetened solution and a suppression of sexual behavior, which can be attenuated by chronic antidepressant administration (Yirmiya, 1996; Frenois *et al*., 2007). The sweet taste of sucrose is strongly rewarding for animals, including rodents and primates (Bachmanov *et al*., 1997; Berridge, 2000; Levine *et al.,* 2003). Anhedonia has been used as a behavioural endpoint for a number of the existing animal models of depression, such as chronic mild stress and maternal separation, with sucrose preference being measured (Slattery *et al*., 2007).

Although altered brain levels of monoamines have been accepted for many years as the major pathological hallmark of major depression, current evidences have implicated increased levels of oxidative stress and neuroinflammation as the major factors for the genesis of the disease (Miller *et al*., 2009; Catena-Dell'Osso *et al*., 2011; Vaváková *et al.,* 2015). The brain cells are known to be more susceptible to the deleterious effect of free radicals and the extent of tissue damage have been reported to be associated with increased level of MDA and decreased antioxidant defense mechanisms of the cells (Vaváková *et al.,* 2015). High levels of MDA, a major biomarker of oxidative stress have been reported in patients with depressive illnesses (Bakunina *et al.,* 2015; Vaváková *et al.,* 2015). Oxidative stress has been implicated in cell death, reduced

neurogenesis, reduced neuronal plasticity and increased autoimmune responses, which in turn trigger and propagate neuroinflammation that further enhanced tissue destruction (Behr *et al.*, 2012; Bakunina *et al*., 2015). Various biomarkers of neuroinflammation including tumor necrotic factor have been reported to be up-regulated in depressive illnesses suggesting that the disease has inflammation as the most important underlying factor (Catena-Dell'Osso *et al.*, 2011; Leonard and Maes, 2012). Accumulating evidence suggests that inflammation may play a role in the pathophysiology of major depressive disorder (MDD) (Miller *et al.*, 2009; Ohgi *et al.*, 2013). The proinflammatory cytokine tumor neurosis factor-α (TNF-α) is increased in serum of depressed patients (Dowlati *et al*., 2010). Thus, increased TNF-α brain level in LPS-treated mice observed in this study may play a role in depressive-like behaviour due to intraperitoneal administration of LPS. In fact, previous studies have established that TNF-α was the first cytokine released in response to peripheral administration of LPS and was also shown to cause activation of microglia and subsequent increase of other brain pro-inflammatory cytokines (Qin *et al.*, 2007). This result also showed that LPS increased the serum levels of corticosterone, which may contribute to its ability to induce oxidative stress and neuroinflammation.

It has been suggested that inhibition of oxidative stress and neuroinflammation may serve an important targets for the development of novel antidepressant drugs (Lee *et al*., 2012). Antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GPX) metabolise ROS into less toxic molecules. SOD catalyses the reaction of superoxide to the less toxic H_2O_2 (Winterbourn, 1993). The results showed that methyl jasmonate was able to restore the decreased antioxidant defense mechanisms induced by LPS. Methyl jasmonate significantly reduced lipid peroxidation as shown by decrease in level of malondialdehyde in LPS-treated mice. Depression is known to be accompanied by increased levels of malondialdehyde (MDA), a major biomarker of oxidative stress associated with lipid peroxidation and often indicates the degree of tissue damage (Winterbourn, 1993; Gsell *et al*., 1995). The findings that methyl jasmonate reduced the increased level of MDA in LPS-treated mice suggest that its anti-depressant-like effect may be related to reduction of oxidative stress in the brain of depressive patients. Moreover, MJ significantly increased LPS-induced decrease in the concentration of nitric oxide in mice brains. The possible explanation for the decreased nitrite

level due to LPS may be related to the possible role of endogenous NO in restraining the activation of the hypothalamic-pituitary-adrenal (HPA) axis during periods of increased cytokine and/or neuropeptide secretion, such as during immune stimulation (Uribe *et al.,* 1999; Jankord *et al.,* 2009). Previous study also demonstrated that patients with mood disorders exhibited decreased levels of of nitrite/ nitrate in the cerebrospinal fluid indicating a more general decrease of NO production in this disorder (Gao *et al.,* 2012). In addition, it was previously demonstrated that both excess and shortage of NO may result in depressive-like behaviours (Hu *et al.,* 2012).

The results of this study revealed that MJ reduced the brain levels of TNF-α in LPS-treated mice, which suggest that inhibition of this pro-inflammatory cytokine may contribute to its antidepressant-like effect. It has been reported that depression is accompanied by activation of immune-inflammatory pathways consisting of chronic mild inflammation and activation of cell-mediated immunity, secondary auto-immune reactions and neuroprogression (Maes *et al.,* 1993; Leonard and Maes, 2012; Moylan *et al.,* 2013). Thus, TNF-α has been described as the prime mediator that convey inflammation from periphery to the brain, that ultimately leads to progressive neurodegeneration. TNF-α may also be involved in the perpetuation of neuroinflammation by stimulating the activation of the transcription factor NF-κB, a potent stimulus of the production of several other pro-inflammatory mediators and concomitant impairment of behaviour (Capuron and Miller, 2011) . Thus, the decrease in TNF-α brain level in mice pretreated with MJ before exposure to LPS may play a role in its antidepressive-like effect. Furthermore, the findings that MJ reduced the elevated level of serum corticosterone induced by LPS suggest that suppression of this steroid may contribute to its antidepressant-like activity in mice. It is worthy to note that previous studies have shown that MJ and its congeners exhibited anti-inflammatory activity by decreasing the levels of pro-inflammatory cytokines in LPS-activated murine macrophage cells via inhibition of the NF-B pathway (Lee *et al.,* 2011).

Intraperitoneal injection of LPS has also been shown to trigger cascade of cellular and molecular events that are capable of initiating neurodegeneration and inhibition of adult neurogenesis (Bastos *et al.,* 2008; Bachstetter *et al.,* 2010; Ormerod *et al.,* 2013). In this study, LPS administration led to damaged and reduced neurons in the mice brain and this damage was

reversed by methyl jasmonate as evidenced by increased number of normal neurons. Normal cerebral cortex and hippocampal formation were observed in the methyl jasmonate treated groups and there were no pathological lesion seen in the methyl jasmonate and imipramine treated groups unlike the group administered with LPS. Previous studies have demonstrated that intraperitoneal administration of LPS in rodents induces an early decrease in the number of dentate gyrus proliferating cells (Fujioka and Akema, 2010; Sierra *et al.*, 2010). Thus, the neuroprotective effect of methyl jasmonate as seen in this study may contribute to its anti depressant effect. It is worthy to note that previous studies have shown that MJ demonstrated neuroprotective activity in mice exposed to LPS (Eduviere *et al*., 2016), however, more studies are necessary before concluding the relevance of these findings in the ability of MJ to attenuate depressive-like behaviour induced by LPS in mice.

The effect of MJ on CUMS-induced depressive-like behaviour was also investigated based on the preference for sucrose consumption (typifying anhedonia), duration of immobility in the TST and SMA in rodents (Willner et al., 1992). The direct or indirect involvement of stress has been suggested in the development of human depression (Anisman and Zacharko, 1982; Brown, 1993). The CUMS involves exposure of rodents to a variety of stressors in an unpredictable manner over a period of time, thus, mimicking the ways humans encounter stressors or life-time events on daily basis. Animals exposed to CUMS present with behavioral changes such as decreased sucrose preference, altered locomotion, reduced social interaction, anxiety and increased immobility (Chakravarty *et al*., 2013) that typify endogenous depression. The results of this study revealed that CUMS increased the duration of immobility in the tail suspension test which was reversed by MJ and thus further suggests that MJ has antidepressant-like activity. The reference drug, imipramine, also showed an effect similar to MJ. The immobility that characterized the TST, also referred to as behavioural despair in animals, is believed to resemble loss of energy or stamina seen in human depression. Additionally, many studies have shown that the test is highly sensitive to the major classes of the clinical antidepressants, including the selective serotonin reuptake inhibitors (SSRIs), the tricyclic antidepressants (TCAs), and the monoamine oxidase inhibitors (MAOIs) (Cryan *et al*., 2005). Chronic mild stress involves exposure to unpredictable mild stressors over several weeks, designed to mimic the daily hassles

that reportedly provoke the onset of depression in humans (kindler *et al.,* 1995). In animals, unpredictable stressors have also been shown to induce other behavioral changes such as impaired sexual behavior and altered locomotor or explorative activity (Strekalova *et al*., 2004; Bennabi *et al.,* 2013). The results of this study further confirmed previous investigations that CUMS altered locomotor activity and explorative behavior in rodents (Bennabi *et al*., 2013; Sobin and Sackeim, 1997). Although MJ did not alter SMA in naïve mice, it was found in this study to reverse impaired locomotion induced by CUMS. This finding might contribute to the antidepressant-like property of MJ. Most of the motor deficits associated with psychomotor retardation seen in depressed patients include disturbances in speech, facial expression, fine motor behavior and gross locomotor activity (Bennabi et al., 2013; Sobin and Sackeim, 1997).

In the CUMS model, the major symptom of human depression, anhedonia, is claimed to be reflected in the animals' decreased consumption of palatable solutions (Willner, *et al.,* 1987). D' Aquila et al. (1994) found a decreased sucrose intake together with a decreased sexual behavior in CUMS exposed rats. Sucrose and saccharine intake are both commonly accepted measures of anhedonia, and many investigators have reported that consumption is inhibited by CUMS (Muscat *et al*., 1992; Willner *et al*., 1987). This study showed that there was a significant decrease in percentage sucrose consumption in the CUMS exposed control group. This decrease was reversed by methyl jasmonate which showed a significant increase in percentage sucrose consumption. Imipramine also showed a similar increase in percentage of sucrose intake.

Within the last decade, a growing body of literature not only in humans, but also preclinical findings from animal models indeed support this "oxidative stress hypothesis of depressive disorder" (Michel *et al*., 2007; Michel *et al*., 2010). There is a growing body of literature from both preclinical and clinical studies that support the role of oxidative stress and neuroinflammation in the pathophysiology of depression (Munhoz *et al*., 2008; Vaváková *et al*., 2015). Oxidative stress is a result of either increased production of ROS or decreased antioxidant defence. Oxidative stress has been suggested as an important contributive factor in the pathogenesis of depression (Ng *et al*., 2008). Studies have also shown a close correlation between clinical outcomes of antidepressant treatment and level of oxidative stress in the brains

of depressed patients (Munhoz *et al.,* 2008; Vaváková *et al.,* 2015). Glutathione is an endogenous antioxidant which plays a vital role in the detoxification of xenobiotics and scavenging of free radicals or reactive oxygen species (ROS) in cells (Albano *et al*., 1998). Repeated stress on a daily basis may impair the antioxidant defenses in the body leading to oxidative damage by changing the balance between oxidant and antioxidant factors. In this study, CUMS reduced the levels of the antioxidants GSH, SOD and increased the levels of MDA. The results showed that methyl jasmonate was able to restore the decreased antioxidant defense mechanisms induced by exposure to CUMS. Methyl jasmonate significantly reduced lipid peroxidation as shown by decrease in level of malondialdehyde in CUMS-exposed mice. The findings that methyl jasmonate reduced the increased level of MDA in CUMS-exposed mice suggest that its anti-depressant-like effect may be related to reduction of oxidative stress in the brain of depressive patients. This suggests that methyl jasmonate could also be working via mopping of free radicals and thus improving the anti-oxidative defense system to alleviate depressive symptoms. Depressive disorder has been linked to increased serum levels of malondialdehyde (MDA) (Bilici *et al*., 2001; Sarandol *et al.,* 2006). Different studies have also shown associations between the severity of depressive disorder and oxidative stress-indices (Forlenza and Miller, 2006; Sarandol *et al.*, 2007).

Elevation of the endogenous inhibitor of endothelial NO, synthase asymmetric dimethylarginine (ADMA) (Selley, 2004) was found, as well as diminished NO in certain studies (Selley, 2004). Previous studies demonstrated the role of oxidative and nitrosative stress in the pathophysiology of depression (Maes, 2011 and Maes *et al,* 2011). Elevated levels of reactive oxygen species and nitric oxide (Dhir and Kulkarni, 2011; Suzuki and Colasanti, 2001) were observed in depressed patients. The results of this study showed that CUMS increased the levels of nitric oxide while methyl jasmonate as well as imipramine reversed the increased levels of nitric oxide caused by CUMS exposure. Oxidative and nitrosative mechanisms have been suggested as targets for new antidepressant drugs (Lee *et al.,* 2013).

It is well established in literature that chronic stress activates the pituitary-adrenal axis resulting in the release of steroids from the adrenal glands (Oken *et al*., 2015; de Kloet *et al.,* 2005). Thus,

increased corticosterone level is often used as an important indicator of chronic stress response (Oken *et al*., 2015; de Kloet *et al*., 2005). Previous studies have confirmed that excess cortisol produced during chronic stress response damage vital organs of the body especially the brain via the induction of oxidative stress and neuroinflammation (Umukoro *et al.*, 2015; Oken *et al*., 2015). Glucocorticoid hormones, mainly corticosterone in rats and cortisol in human, participate in the response of the organism to stressors (Habib *et al*., 2001). Previous studies (Song *et al*., 2006; Li *et al.*, 2007) showed that fluoxetine reduced serum corticosterone concentrations in rats exposed to CUMS. These confirmed that reduction in the hypothalamic pituitary adrenal (HPA) axis hyperactivity of depressed subjects was of primary importance for therapeutic effects of antidepressants. In this study, the result revealed that CUMS significantly increased the levels of corticosterone which was reversed by methyl jasmonate significantly. Similar decrease was observed with imipramine. Under stressful conditions, there is an increase in corticotropin releasing hormone secretion and hence adrenocorticotropic hormone is released which acts on the adrenal cortex to stimulate the synthesis and release of cortisol in humans and corticosterone in rodents (Kulkarni and Juvekar, 2008). The results of this study complement the findings of previous investigations that chronic stress is a major trigger of oxidative stress-mediated neuroinflammation, which in turn precipitates depressive-like behaviour in animals (Munhoz *et al.*, 2008; Vaváková *et al* ., 2015). Tumor necrosis factor alpha has been implicated as the major cytokine involved in stress-induced depression (Munhoz *et al*., 2008; Vaváková *et al*., 2015). Also, elevated levels of inflammatory cytokines in the brains of patients with depressive disorders have been reported in literature (Bajpai *et al*., 2004; Munhoz *et al*., 2008; Vaváková *et al.*, 2015). Thus, it has been suggested that oxidative-mediated neuroinflammatory mechanisms could serve as potential targets for the development of new drugs for depression (Munhoz *et al.*, 2008; Vaváková *et al* ., 2015).

The results of this study revealed that methyl jasmonate decreased the levels of TNFα which was increased by chronic unpredictable mild stress. A similar trend was observed in the reference drug, imipramine. Thus, increased TNF-α brain level in CUMS-exposed mice observed in this study may play a role in depressive-like behaviour due to exposure to CUMS.

Reduced hippocampal neurogenesis has also been proposed as a possible underlying mechanism of depression (Jacobs *et al*., 2000). A previous report found that rats exposed to chronic unpredictable stress show some dendritic atrophy of CA3 neurons (13% loss), but not to the same degree as repeated immobilization stress (29% loss) (Vyas *et al*., 2002). It has been shown that oxidative stress contributes to neuronal and glial cell loss in the central nervous system (CNS) (Goetz *et al*., 1994; Grunblatt *et al*., 2004; Koutsillieri *et al.,* 2002). Evidence from post-mortem studies has shown that oxidative stress is responsible for cerebral morphological changes seen in depressive disorder (Michel *et al.,* 2007; Michel, 2010). In this study, CUMS exposure led to damaged and reduced neurons in the mice brain and this damage was reversed by methyl jasmonate as evidenced by increased number of viable neurons. Normal cerebral cortex and hippocampal formation were observed in the methyl jasmonate treated groups and there were no pathological lesion seen in the methyl jasmonate and imipramine treated groups. This result further showed the anti depressive effect of methyl jasmonate as observed in its ability to reverse the loss of neurons observed in CUMS exposed mice. In depressive disorder, glial cells have repeatedly been described as altered, especially in the frontal cortex (Rajkowska, 2000; Harrison, 2002).

CHAPTER 6

6.1 SUMMARY AND CONCLUSION

The results of this study provide evidence which suggest that methyl jasmonate produced antidepressant-like activity in mice, which may be related to activation of serotonergic, noradrenergic and dopaminergic pathways. It also revealed that MJ exhibited antidepressant-like activity in LPS-treated mice and suggests its potential usefulness for the treatment of depression associated with neuropsychiatric disorders. The normalization of deregulated levels of oxidative stress parameters and inhibition of tumor necrotic factor as well suppression of corticosterone may be playing significant roles in its antidepressant-like property observed in this study. The results also suggest that the antidepressant-like activity of MJ is mediated through decreases in levels of corticosterone, oxidative stress and neuroinflammation in mice subjected to chronic unpredictable mild stress (CUMS) which closely mimics depression in humans.

6.2 CONTRIBUTION TO KNOWLEDGE

- The study has provided data which further confirms the antidepressant-like property of methyl jasmonate and its usefulness in treating depression.
- This study provided experimental data which showed that the antidepressant effect of methyl jasmonate is related to inhibition of oxidative stress, corticosterone and the pro-inflammatory cytokine (TNF alpha).
- This study also showed that enhancement of monoaminergic transmission may underlie the antidepressant effect of methyl jasmonate.
- This study provided data that which showed that methyl jasmonate has no sedatory nor stimulatory effect on the central nervous system which gives it an added advantage over existing antidepressants.

6.3 RECOMMENDATION FOR FURTHER STUDIES

There is need for further evaluation of methyl jasmonate such as its effect on brain derived neurotrophic factor (BDNF) and the use of immunohistochemistry techniques to further assess its neuroprotective effect. There is also the need for further toxicity studies to be done chronically on methyl jasmonate and determine if it's totally safe when taken over a long period of time.

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