EVALUATION OF ANTIDEPRESSANT ACTIVITY OF THE METHANOL ROOT BARK EXTRACT OF *ACACIA SEYAL* DEL. (FABACEAE) IN MICE

BY

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DECLARATION

I declare that the work in this dissertation titled: **EVALUATION OF ANTIDEPRESSANT ACTIVITY OF THE METHNOL ROOT BARK EXTRACT OF *ACACIA SEYAL* DEL.(FABACEAE) IN MICE** was performed by me in the Department of Pharmacology and Therapeutics, under the joint supervision of Dr M.G.Magaji and Prof. N.M.Danjuma. The information derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this dissertation has been previously presented for another Degree or Diploma at this or another University.

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CERTIFICATION

This dissertation **EVALUATION OF ANTIDEPRESSANT ACTIVITY OF THE METHNOL ROOT BARK EXTRACT OF *ACACIA SEYAL* DEL. (FABACEAE) IN MICE** by Gambo Amanda YELMIS meets the regulations governing the award of the degree of Master of Science (Pharmacology) of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty God and to the loving memory of my late mum Mrs DinatuYelmis

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ABSTRACT

Acacia seyal is a small to medium-sized tree, growing up to 17 m tall and 60 cm in diameter and is native to Sudan, Nigeria and several other African countries. It is reported to be used in Zaria, North western Nigeria for the management of depression. The present study investigated the antidepressant like activity of the methanol root bark extract of *Acacia seyal*and its possible mechanisms of action.The preliminary phytochemical screening of the methanol root bark extract of *Acacia seyal*was carried out. The acute toxicity study (LD_{50}) was carried out using oral OECD 425 guidelines. The Irwins test was carried out to assess the effect of the methanol root bark extract of *Acacia seyal* on some autonomic, neurological and behavioural parameters. The antidepressant activity of the methanol root bark extract of *Acacia seyal*(250, 500 and 1000 mg/kg) was evaluated using the Tail suspension test (TST) and forced swim test (FST) followed by the test for motor coordination and stimulant activity using beam walking assay (BWA) and open field test (OFT) respectively.Furthermore, the possible mechanism of action of the extract was determined using the following antagonists; cyproheptadine, metergoline, prazosin, yohimbine, sulpiride atropine, L- arginine and L-NNA. Subsequently the extract was further subjected to chronic unpredictable mild stress (CUMS) following CUMS the effect of the extract was assessed on sucrose preference, OFT and TST. The phytochemical screening of the methanol root bark extract of *Acacia seyal* revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, triterpenes, flavonoids and saponins. The median lethal dose was estimated to be greater than 5000 mg/kg in mice which shows the extract is relatively safe. The extract at all doses tested did not significantly produce neurological, autonomic and behavioural changes in mice. The extract at all doses tested did not significantly alter the number of foot slips nor the number of line crossing in OFT and BWA

respectively.The extract at all tested doses significantly (p<0.05) decreased duration of immobility in the TST and FST in mice. The antidepressant like activity observed in theextract at the highest dose tested (500 mg/kg)in the TST was blocked byintraperitonealpretreatment with sulpiride(50 mg/kg), metergolnie (1 mg/kg), cyproheptadine (4mg/kg), prazosin(1 mg/kg), yohimbine (1 mg/kg), atropine (1 mg/kg,), L-arginine, L-NNA (50 mg/kg) and Naloxone (2 mg/kg). The extract at 500 mg/kg significantly (p<0.05) alleviated the CUMS decreased duration of immobility. The extract at all doses testeddid not significantly alter sucrose consumption in mice. All doses testedsignificantly (p<0.05) decreased the plasma levels ofcortisol and increased the concentrations of brain derived neurotrophic factor (BDNF) following CUMS in mice. These findings suggest that the methanol extract root bark of *Acacia seyal*is relatively safe and possesses antidepressant like activity which may possibly be mediated via monoaminergic, opioidergic, cholinergic, nitric oxide pathway, neurotrophic and neuroendocrine systems.

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ABBREVIATIONS, DEFINITIONS AND ACRONYMS

5-HT 5-Hydroxytryptamine (Serotonin)

ACTH Adrenocorticotropic Hormone

APOE Apolipoprotein E

AS Methanol Root Bark Extract of *Acacia seyal*

BDNF Brain Derived Neurotrophic Factor

cGMP Cyclic Guanosine Mono Phosphate

CNS Central Nervous System

CRF Corticotrophin Releasing Factor

CUMS Chronic Unpredictable Mild Stress

DA Dopamine

DRD4 Dopamine Receptor Gene

e.g: Examples

e.t.c: etcetera

FST Forced Swim Test

GH Growth Hormone

GND3 Guanine Nucleotide-Binding Protein

g Grams

HPA axis Hypothalamic-Pituitary-Adrenal Axis

IL Interleukins

*ip*Intraperitoneal

L-NNA N-Omega-Nitro-L-Arginine

MAO Monoamine Oxidase

MDD Major Depressive Disorder

mg/kg Milligrams per Kilogram

ml/kgMilliliters per Kilogram

MTHFR Methylene Tetrahydrofolate Reductase

NE Norepinephrine

NO Nitric Oxide

NOS Nitric Oxide Synthase

OECD Organization for Economic Co-operation and Development

OFT Open Field Test

p.o Per Oral

pg/ml Picogram per Milliliter

SABC Streptadivin and Biotinylated Horseradish Peroxidase

SLC6A3 Dopamine Transporter

SLC6A4 Serotonin Transporter

SSRI Selective Serotonin Reuptake Inhibitor

T_3 Tri-iodothyronine

T_4 Tetra-iodothyronine

TCA Tricyclic Antidepressants

TRH Thyrotrophin Releasing Hormone

TSH Thyroid Stimulating Hormone

TST Tail Suspension Test

µg/ml Microgram per Milliliter

WHO World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Depression is a common mental disorder that presents with low mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration (WHO, 2006), and often comes with symptoms of anxiety. These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide and almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day. For every person who commits suicide, 20 or more may attempt to end their life (WHO, 2012).

About 322 million people are living with depression worldwide and this has increased by 18.4% between 2005 and 2015 (WHO, 2017). Prevalence rates vary by age, peaking in adulthood (7.5% among older females and 5.5% among males) out of which 25% of patients develop chronic depression (Ferrari *et al*., 2013a). Depression also occurs in children and adolescent below the ageof 15 years, but incidence is low (Ford *et al*., 2003).

As described in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM- V), the hallmark of major depressive disorder (MDD) is the occurrence of depressed mood (dysphoria) and loss of interest in activities that used to be pleasurable

in the past (anhedonia) for a duration of at least two weeks (APA, 2013). These symptoms must also be accompanied by at least four of the following manifestations: changes in appetite or weight, sleep patterns, altered psychomotor activity, feelings of worthlessness or guilt, difficulty concentrating or making decisions and recurrent thoughts of death or suicidal ideation.

The treatment of depressive symptoms involves the use of antidepressants such as the tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). However, these drugs have significant adverse effects following long period of usage. Herbal antidepressants are increasingly being introduced to treat severe depression and are reported to have efficacy rate comparable to the medications with fewer side effects (Woode *et al*., 2010).

1.2 Statement of Research Problem

Depression is a major cause of disability, affective and cognitive impairment (Ford *et al*., 2013). Depression has been estimated to be the single biggest burden of health conditions by 2030 (WHO, 2011). The prevalence of major depression is higher in women (5.5%) than in men (3.2%) (Baxter *et al*., 2014; Albert, 2015). Feelings of helplessness, Suicide and death are complications of depressive illness occurring in up to 15% of patients (Galynker, 2017). In Nigeria, depression is common among people with HIV/AIDS, civil servants and among university students (Chikezie *et al.*, 2013).

Current antidepressants have poor efficacy and high toxicity profile, therefore necessitating the search for novel drugs with better efficacy and safety (Han *et al.*, 2013; Khan *et al.*, 2014).

1.3 Justification of the Study

Depression is a disease that affects men, women and children and if not treated leads to serious complications such as suicide (Weller *et al*., 2006). Conventional antidepressants currently in use have high side effects with low efficacy. Hence, there is growing interest in complementary and alternative medicine among depressed patients with the general belief that "Natural is better" (Sarris and Kavanagh, 2009; Ravindran *et al.,* 2016). Currently, there is widespread use of complementary and alternative medicine in the treatment of physical and psychiatric symptoms and disorders within Western populations (Van der Watt *et al.*, 2008; Fajemiroye *et al.,* 2016). Medicinal plants validated to have therapeutic activity are conserved from extinction (Zheng *et al*., 2010).

Globally, there is increased use of medicinal plants as substitutes for orthodox drugs in the management of diseases (Sarko, 2000; Tiwari and Mehta, 2013).

1.4 Aim of the Study

The aim of this study is to evaluate the antidepressant activity and determine the possible mechanism(s) of action of the methanol root bark extract of *Acacia seyal*

1.5 Specific Objectives

I. To determine the classes of phytochemical constituents present in the methanol root bark extract of *Acacia seyal.*

II. To determine the acute toxicity of the extract.

III. To evaluate the antidepressant activity of the extract.

IV. To establish the possible receptor mechanism(s) of action of the extract.

V. To determine the inherent neurotropic and neuroendocrine mechanism in the antidepressant activity of the extract.

1.6 Research Hypothesis

The methanol root bark extract of *Acacia seyal* possesses antidepressant activity in mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Depression

Depression is a mood disorder characterized by symptoms such as sadness, loss of interest, anhedonia (loss of pleasure), lack of appetite, feelings of guilt, low self-esteem or self-worth, sleep disturbance, feelings of tiredness and poor concentration (WHO, 2012). The individuals in such conditions feel anxious, desperate, irritable, and have poor self-worth (Ingram, 2016). In severe conditions (major depressive disorder), physical changes such as helplessness and hopelessness, inability to concentrate, insomnia, loss of appetite, loss of interest in what they usually find pleasurable, feelings of extreme sadness and guilt, which could be accompanied by thoughts of death. Depression can be recurrent or long-lasting resulting in substantial impairment in ability to function (Marcus *et al.,* 2012).

2.2 Pathophysiology of Depression

Despite the complexity and heterogenecity of the disorder a lot of theories were developed to describe the pathophysiology of depression. They include; biogenic amine hypothesis, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and genetic and Environmental factors (Brigitta, 2002; Nemerroff, 2008). Other possible mechanisms include neurogenesis (Eisch and Petrik, 2012; Gulbins *et al.,* 2015), increased inflammatory cytokines secretion (immunologic factors), elevated levels of

corticotrophin-releasing factor (CRF), and abnormalities of second messenger systems (Connor and Leonard, 1998; Hammen, 2005; Sharpley and Agnew, 2011).

2.2.1 Biogenic amine hypothesis

The brain contains vast numbers of monoaminergic neurons and each play a vital role in brain function. The noradrenergic neurons spread from the brain stem to almost all brain areas where norepinephrine (NE) modulates the function of the prefrontal cortex, the processing of working memory and regulates behaviour and attention (Atzori *et al.,* 2016; Maletic *et al.,* 2017). Serotonin (5-hydroxytryptamine: 5-HT) innervates all brain areas and is the largest cohesive neurotransmitter system in the brain (Delgardo and Morena, 2006), while Dopamine (DA) modulates reward and motivation functions, working memory and attention (Grace, 2016).

Monoaminergic systems are instrumental to many behavioural symptoms, such as low mood, alertness, reduced motivation, fatigue, and psychomotor agitation or retardation (Stahl, 1998; Treadway, 2016). Alterations in cerebral 5-HT levels have been linked to changes in behavioural and somatic functions (including appetite, sleep, sex, pain response, body temperature and circadian rhythm) that are seen in depressed individual (Maes and Meltzer, 1995). Several postmortem studies have demonstrated low 5-HT levels in the brains of depressed patients compared to non-depressed patients further ascertaining the role of monoamines in depression (Stanley *et al.,* 1982; Stockmeier, 2003). Similarly, DA abnormalities have been linked to impaired motivation, concentration and aggression (Seo *et al.,* 2008), while dopamine transmission may improve cognitive outcomes including decision making and motivation (Salamone *et al.*, 2016).

Most fundamental brain functions depend on the presence and actions of various neurotransmitters at the pre- and post-synaptic membranes of the billions of neurons in the brain, and there is evidence to support the role of specific neurotransmitters in the development and clinical manifestations of depression (Nemeroff, 1988; Brigitta, 2002). This hypothesis is also known as the "monoamine hypothesis" and proposes that the reduced availability of these major monoamine neurotransmitters (5HT, NE, and DA) results in decreased neurotransmission and impaired cognitive performance which may lead to depression (Coppen, 1967; Barchas and Altemus, 1999). The functional deficiency of monoamines seen in depression could also be as a result of decreased protein transporter functions and abnormalities in the neurotransmitter receptor function (Brigitta, 2002).

2.2.2 Genetic and environmental factors

Genetic means has been shown to play a major role in the development of depression (Jones and Craddock, 2001; Klein *et al.,* 2004; Mondimore *et al.,* 2006), with chances of inheritance greater in women than in men (Kendler *et al.,* 2001), corroborating the higher incidence and prevalence of most forms of depression in women (Yatham *et al.,* 1997; APA, 2013). Genes have been found to cause the development of depression, (Irie *et al.,* 2008; Hong *et al.,* 2009). Some of these genes include the apolipoprotein E (APOE ε2 and APOE ε4), guanine nucleotide-binding protein (GND3), methylene tetrahydrofolate reductase (MTHFR 677T), dopamine transporter (SLC6A3), the serotonin transporter (SLC6A4) and the dopamine receptor gene (DRD 4) (Kang *et al.,* 2007).

Researches by Sharpley and Agnew (2011) provided basis for the development of depressive symptoms based on the specific associations of genes with depression. For example, two out of four apolipoprotein E isoforms (APOE ε2 and ε4) were reported to possess significant association with depression (Dong *et al.,* 2009; Yuan*et al.,* 2010). Of these, APOE ε4 has been linked to reduced brain anatomical structure implicated in the development of depression (Qiu *et al.,* 2009) with resultant decrease in cognitive function in depressed individuals On the other hand, APOE ε2 is considered to be a protective gene for MDD (Yuan *et al.,* 2010).

Environmental stress and adverse life events play a role on the development of depression, and several researches reported an excess of severely life threatening events before the onset of depression (Brown *et al.,* 1994; Paykel, 2011). Notable life events such as death of a spouse, marital separation, loss of job or redundancy and retirement, unwanted pregnancy, social isolation, rape, childhood abuse, war and major accidents (Brown *et al.,* 1996; Kessler, 1997; Kendler *et al.,* 2005). These stressful life events can limit improvement and increase the probability of depression relapse (Paykel *et al.,* 1996).

It is known that the stress system and depression share many common mediators and circuitries (Gold *et al*., 1988) and that stress has a significant role in precipitating and influencing the clinical course of depression (Gold *et al*., 2015). The absence of stress may confer some protection against the development of depression (Kendler and Halberstadt, 2013), thus, depression could be explained as a possible outcome of

dysregulation of the stress response system (Gold *et al.,* 1988). Evidence from experimental animal studies suggests stress induces structural and functional changes in the brain (Duman, 2009). Following exposure to various stressors, structural changes in the prefrontal cortex, amygdala, hippocampus and nucleus accumbens were reported and shown to contribute to the development of depression (Rajkowska and Miguel-Hidalgo, 2007).

2.2.3 Neuroendocrine

A number of endocrine system abnormalities have been identified as possible contributors to the etiology of depression. These include altered growth hormone (GH) levels, thyroid hormone abnormalities and Hypothalamus-Pituitary-Adrenal (HPA) axis dysfunction (Thase and Howland, 1995; Segerstrom and Miller, 2004).

2.2.3.1 Growth hormone

The role of pituitary hormones such as Growth hormone in the development of depression has been investigated via their direct and indirect influences on the NE (Brigitta, 2002) Growth hormone release (stimulated by catecholaminergic mechanisms) and responses is defective in depressed patients when compared to healthy individuals (Greenberg *et al.,* 2003; Atake *et al.,* 2014). In GH challenge tests using apomorphine and clonidine, patients with recurrent major depression exhibit 'flat' or 'blunted' GH responses compared to healthy subjects (Charney *et al.,* 1984; Hoehe *et al.,* 1986). This suggest an intrinsic abnormality in the GH system to be responsible for the flat GH response seen in patients with recurrent major depression (following the GH challenge tests), thus implicating GH in depression.

2.2.3.2 Thyroid Hormone

The active forms of thyroid hormones, tri-iodothyronine (T_3) and tetra-iodothyronine (T_4), are produced by the thyroid gland following stimulation by Thyroid Stimulating Hormone (TSH) from the pituitary. Secretions of TSH is modulated by the hypothalamic hormone Thyrotropin Releasing Hormone (TRH) (Chiamolera and Wondisford, 2009). The T_3 and T_4 regulate the overall metabolism in the human body (Mullur *et al.,* 2014) and experimental findings suggest that alterations in thyroid function could be a possible link to the development of depression (Duval *et al.,* 1999; Altshuler *et al.,* 2001; Hage and Azar, 2012). This could be responsible for some symptoms of depression such as weight loss, sleep disturbance and psychomotor agitation (Duval *et al.,* 1999; Peng and Li, 2017). Similarly, T_3 administration is an effective adjunctive therapy for many patients with depression (Cooper and Lerer, 2010; Hage and Azar, 2012). The precise mechanism by which thyroid hormone abnormalities contribute to the genesis of depression is yet to be fully elucidated, with a link found between Thyroid hormone precursor and depression (Szpunar and Parry, 2018). However, findings showed that T_3 and T_4 increase cortical 5HT secretion indicating that thyroid hormones act indirectly through the serotoninergic system (Gur *et al.,* 1999).

2.2.3.3 Hypothalamic-pituitary-adrenal axis

The Hypothalamic-Pituitary-Adrenal (HPA) axis also plays significant contributions to the development of depression (Vreeburg *et al.,* 2009; Sher *et al.,* 2013; Martinac *et al*., 2017). Hypothalamic-Pituitary-Adrenal (HPA) axis revealed that depressed individuals to have corticotrophin releasing factor (CRF) and cortisol hyper-secretion, dysfunctional glucocorticoid feedback mechanisms, inadequate HPA axis suppression

in response to exogenous glucocorticoid administration and impaired corticosteroid receptor signalling (Rubin *et al.,* 1989; Holsboer, 2000; Juruena, 2014).

Some depressive signs and symptoms such as excessive personal guilt and hopelessness, decreased appetite, weight loss, decreased sexual behaviour, disrupted sleep, altered psychomotor activity, and overactive response to psychological stressors were shown to be associated with HPA axis dysfunction (Gillespie and Nemeroff, 2005; Lopez-Duran *et al*., 2009). Atypical presentation is in Cushing's disease or syndrome (a clinical condition in which there is hyper-secretion of cortisol) where individuals come down with depression as a symptom (Brigitta, 2002).

Corticotrophin Releasing Factor plays a regulatory role in HPA axis activity (Herman and Cullinan, 1997), increasing adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, which in turn stimulates the release of cortisol from the adrenal gland. In addition, CRF is also found in extra-hypothalamic brain regions where it functions as neurotransmitter coordinating the behavioural, autonomic, endocrine and immune responses to stress (Smith and Vale, 2006; Bonfiglio *et al.*, 2011). It is these functions that may underlie CRF's role in the development of depression (Gillespie and Nemeroff, 2005).

2.2.4 Role of inflammatory cytokines in depression

In current literature, a number of immunologic or inflammatory cytokines have been implicated in the pathogenesis of depression (Strawbridge *et al.*, 2017). These include

interleukins IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10 and interferon gamma, CRP, TNFα, and the chemokine monocyte chemoattractant protein-1 (Strawbridge *et al.,* 2017). The idea that cytokines could have a possible role in the development of depression follows various studies linking chronic stress (a major factor in depression pathology) with altered immune function (Dhabhar, 2000; McEwan, 2000). A number of possible mechanisms have been proposed as direct or indirect links between depression and cytokines (Raison *et al.,* 2006). In addition, some studies which demonstrated an association between inflammatory cytokines and depression were confounded by factors such as personality, gender or body mass index (Miller *et al.,* 2009). Moreover, inflammatory pathways may not be solely responsible for depression as anti-inflammatory drugs (e.g., Cyclooxygenase inhibitors) are ineffective in resolving the symptoms (Dunn, 2008).

Other pathways utilized by peripheral inflammatory cytokines to produce depression include crossing the blood brain barrier to induce neuroinflammation (McCusker and Kelley, 2013). However, identifying markers of inflammation that have an entirely cerebral origin is indicative of a more central role for neuroinflammation in depression pathophysiology (Setiawan *et al.,* 2015). As the case with sickness, the presence of neuroinflammation in specific brain regions may contribute to behavioural responses which overlap with the symptoms of MDD.

2.2.5 Neurogenesis

Neurogenesis in adult individuals involves the generation of entirely new neurons and neuronal connections in the dentate gyrus of the hippocampus and the sub-ventricular

area of the lateral ventricles (Curtis *et al.,* 2012; Miller *et al.,* 2013). In the case of depression, reduction in adult neurogenesis capacity was proposed to cause depression (Malberg and Duman, 2003; Henn and Vollmayr, 2004). However, a number of animal studies and experimental manipulations which reduced neurogenesis capacity in the brains of experimental animals have demonstrated depression-like behaviours (Sahay and Hen, 2007; David *et al.,* 2009). Neurogenesis is essential for restoration of hippocampal structure and function, which may itself be accompanied by improved depression symptomatology (Hanson *et al.,* 2011). Many anti-depressant therapies (e.g. Monoamine oxidase inhibitors, Tricyclic antidepressants, and Mood stabilizers) have been shown to facilitate neurogenesis and as such improved depression therapy (Mnie-Filali *et al.,* 2007; Bjornebekk *et al.,* 2010; Gulbins *et al*., 2015).

Research has shown that antidepressant therapy significantly decreased ceramide levels (a fat molecule in the brain which blocks cell growth), thus enhancing neurogenesis (Gulbins *et al.,* 2015). However, it should be noted that conflicting results in neurogenesis research and depression pathophysiology exists, for instance, the ablation of neurogenesis in experimental animals does not always produce depressive symptoms (Jayatissa *et al.,* 2010), and a number of antidepressant effects are neurogenesis-independent (David *et al.,* 2009). Nevertheless, researchers in the field of depression agree that neurogenesis remains a significant factor in the understanding of the pathophysiology and treatment of depression (Sahay and Hen, 2007; Eisch and Petrik, 2012).

2.3 Etiology of Depression

2.3.1 Environmental factors

Depression is commonly perceived as a reaction to negative environmental circumstances such as acute negative life events, chronically stressful life circumstances, and exposure to adversity in childhood (Afifi *et al.,* 2006).

2.3.1.1 Acute life events

A major risk factor for depression is the experience of undesirable and negative life events. There is ample evidence that most major depressive episodes are triggered by stressful life events (Kessler, 1997; Hammen, 2005). Stressors were 2.5 times more likely in depressed patients compared with normal individuals with eighty percent of depressed cases preceded by major negative life events. Most assessment methods survey the occurrence of stressors within the past 1 to 6 months in relation to depression, (Kendler *et al.,* 2005). Immigrants and refugees experience lengthy or permanent separation from immediate and extended family, with loss of home, property, cultural ties, and customs affecting these communities. The impact of these experiences on the psychological functioning of individuals in these communities is profound with rates of depression reportedly high among these groups (Aguilar-Gaxiola *et al*., 2008). From biological and sociological perspectives, women are likely to be more concerned about others' reactions to them, as well as reactive to the needs of others (Cyranowski *et al*., 2000). Immigrant women may experience significant losses of social support and a sense of isolation on moving to a different country, and this loss may be manifested in a grieving process. The isolation may be related to unfulfilled relationships, or it may result from separation from or loss of family. Thus, women may

be especially likely to be depressed in response to stressful social loss experiences and even to the negative experiences of those in their social networks (Shatell *et al*., 2008).

Gender differences in depression may be accounted for in part by women's greater exposure to interpersonal life events, as well as their greater likelihood, compared with men, of reacting to such events with depression. Results of studies of adults have been mixed with regard to whether or not women experience more overall recent stressors (Spangler *et al.,* 1996 and Kendler *et al.,* 2001), Several studies have found that adolescent females have higher levels of exposure to recent stressors than do males (Ge *et al*., 1994; Shih *et al.,* 2006; Kulesza *et al.,* 2014). Moreover, several studies have shown that at comparable levels of acute stressors, women had higher levels of depressive symptoms than did men (Maciejewski.,*et al*2001; Shih*et al.,* 2006). In general, however, the risk factors for depression in men are likely to be very similar to those of women, involving complex interactions among environmental and neurobiological factors at different developmental stages (Kendler e*t al.,* 2006). Acute stress may precipitate depression in vulnerable individuals, it is more in individuals with depression and those with history of depression experience than those without depression (Hammen, 2006).

2.3.1.2 Chronic stress

Another source of depression although not as commonly studied is exposure to enduring, long-term stressful circumstances. Many studies of stress-depression associations have not adequately distinguished between the effects of ongoing and acute stressors (Caspi *et al.,* 2003), and failure to do so makes it difficult to fully explicate the mechanisms by which stressors have their effects on depression.

Racial and other forms of discrimination are stressors, and, depending on the type of discrimination, such as racial, gender, age, or even social class, they can be either chronic or acute stressors (Banks and Kohn-Wood, 2007) and can increase risk of depression as such. Discrimination, however, can also impact beliefs, self-concept, and coping in ways that increase risk for mood disorders, including depression (Gee *et al*., 2007).

2.3.1.3 Exposure to Early Adversity

There exists a link between childhood exposure to adversity and the development of depression in adolescence or adulthood. One research strategy studies associations between a single specific experience, such as sexual abuse or physical or emotional maltreatment, and depression. There is ample evidence from mostly retrospective community and clinical studies of a significant association between childhood sexual or physical abuse and adult depression particularly among women (MacMillan *et al*., 2001) and similar results from prospective studies (Brown *etal.,* 1994). Some studies suggest that abuse experiences are especially predictive of chronic or recurrent depression (Bifulco *et al*., 2002). However, several studies suggest that physical and sexual abuse is related to diverse adult psychological disorders, not specifically to depression. A large study of psychiatric outpatients found that childhood emotional abuse was most specifically related to depression compared with sexual or physical abuse (Gibb *et al.,* 2003; Alloy*et al.,* 2006).

The mechanisms by which specific childhood stressors, such as physical or sexual abuse, have their effects on later depression are not known directly. However, such experiences are highly likely to occur in the context of parental lack of care, plus

exposure to high levels of chronic and episodic stressors. Such environments contribute to dysfunctional cognitions and coping skills that increase vulnerability to depression. Neurobiological mechanisms may also be implicated, with the speculation that severe stress early in life alters the brain's neuroregulatory processes, which promote susceptibility to depression (Heim and Nemeroff, 2001). Exposure to adverse conditions in childhood may sensitize the youth to stress, so that it may take minimal exposure to later stressful life events to precipitate depression in them compared with those without childhood adversity (Hammen *et al.*, 2000; Harkness *et al.*, 2006).

2.4 Epidemiology

Currently, 29% of the world population suffer from depression, considered to be one of the 10 leading causes of death (Grundmann *et al*., 2010; Singer *et al*., 2011; Cassani *et al.*, 2015) According to the World Health Organization, depression will be the second leading disease in the developed countries in 2020 (Abbasi *et al*., 2013).

Depression is one of the leading causes of disability worldwide. In the United States, the 1-year prevalence of major depressive disorder is 2.7% to 10.3% which is approximately 16 million people (Kessler *et al*., 1996; Brody *et al.*, 2018) and the lifetime cumulative incidence is 16.2% to 17.1%. In 2010, depressive disorders accounted for about 3% of all disability-adjusted life years worldwide (Whiteford *et al.*,2013; Ferari *et al.*, 2013b). Depression is a major contributor to the global burden of disease and affects people in all communities across the world (Murugan *et al.*, 2011).

The lifetime prevalence for major depression is reported to be as high as 14-17% and the one-year prevalence is 4-8%. The lifetime prevalence rates of MDD among women are 10-25%, and for men 5-12% (Garriock, 2006; WHO, 2012; Lim *et al.,* 2018). Almost 10% of the total burden of disease in sub-Saharan Africa is attributed to neuropsychiatric disorders (Tomlinson *et al.,* 2009).

2.5 Treatment of Depression

Antidepressant drugs remain one of the main forms of effective treatment for the amelioration of depressive symptoms (Layous *et al*., 2011). These drugs help in the reduction in symptoms of depressive disorders by altering chemical imbalances of neurotransmitters in the brain. The change in mood and behaviour is due to chemical imbalance. Neurotransmitters are the communication link between neurons in the brain. Neurotransmitters are located in vesicles found in nerve cells. The neurotransmitters such as serotonin, dopamine and noradrenaline or norepinephrine are released by the axonic end of one nerve and received by the other; the phenomenon called as reuptake. The antidepressants inhibit reuptake of neurotransmitters through selective receptors or channels thereby increasing the concentration of specific neurotransmitter around the nerves in the brain (Heninger *et al.,* 1996).

2.5.1 Monoamine oxidase inhibitors

Monoamine oxidase (MAO) inhibitors such as iproniazide, phenelzine, isocarboxazid, tranylcypromine, selegiline and other irreversible inhibitors of the catabolic enzymes, result in an increase in monoamine levels in many regions of the central nervous system (McIntyre *et al.,* 2015). Monoamine oxidase inhibitors indirectly recruit a variety of

receptors postsynaptic to monoaminergic neurons and exert a broad monoaminergic action. It has been shown that the irreversible inactivation of MAO enhances the activity of mesolimbic dopaminergic pathways (Taylor *et al.,* 2013) and down regulates postsynaptic b-adrenergic receptors and inhibitory a_2-adrenergic autoreceptors (Szegedi *et al*., 2009). It also desensitizes 5-HT_1A as well as postsynaptic populations of 5-HT_2C receptors (Reynolds *et al.,* 2011).

Antidepressant effect of MAO inhibitors is clinically established (Thase and Howland, 1995) to be similar to and other antidepressants such as selective serotonin reuptake inhibitors (SSRIs) (Papakostas and Fava, 2007). However, they do differ with respect to their side effect profiles. These drugs are less prescribed because of the many unwanted effects related to their potent activity on a broad number of cholinergic, adrenergic, and histaminergic receptors.

2.5.2 Tricyclic antidepressants

Tricyclic antidepressants (TCA) have long been established as effective agents in the treatment of depressive disorders. Tricyclic agents such as amitriptyline and imipramine combine the blockade of both 5-HT and noradrenaline (NA) reuptake sites in circumscribed limbic regions (Mojtabai, 2014). Desipramine is the more noradrenergic agent, imipramine inhibits the reuptake of both norepinephrine and serotonin, and clomipramine is the most serotonin selective member of this class. As a reduced dopaminergic tone in the frontal cortex can contribute to depressive states (Willner *et al.*, 2005), Trycyclic antidepressants may also strengthen frontocortical dopaminergic transmission through the regulation of NA transporters (Millan *et al.,* 2000). For some other TCAs, such as amitriptyline and clomipramine, a significant affinity for 5-HT_2C and 5-HT_2A receptors subtypes has been reported (Tatsumi *et al*., 1997; Sanchez and

Hyttel, 1999), which may contribute to their antidepressant actions (Reynolds *et al.*,2006). Trycyclic antidepressants are competitive antagonists at the muscarinic, histaminergic, α_1 and α_2 adrenergic receptors, which result in their characteristic side-effect profile.

Amitriptyline, imipramine, and doxepin has the highest anticholinergic activity, whereas nortriptyline and desipramine are less anticholinergic (Sadock and Sadock, 2003; Richelson, 2003). Anticholinergic side effects include dry mouth, constipation, urinary retention, blurred vision, confusion, and delirium. The discontinuation syndrome is mostly related to cholinergic rebound.

Sedation is the most common side effect of TCA and is a result of anticholinergic and antihistaminergic effects (Wilson and Argyropoulos, 2005). The blockade of a_1-adrenergic receptors perturbs cardiovascular function (Glassman, 1998). TCAs may slow down cardiac conduction, causing intraventricular conduction delay, atrioventricular block, flattened T waves, depressed ST segments, and prolonged QT intervals (Roose *et al.*, 1989). All TCAs can cause tachycardia, which is one of the most common reasons for stopping them. Nortriptyline is the least likely to cause orthostatic hypotension. Weight gain and sexual side effects are also common with TCAs (Masand and Gupta, 2002; Rush *et al.*, 2011).

2.5.3 Norepinephrine reuptake inhibitors

The morpholinone derivative reboxetine is the first selective norepinephrine reuptake inhibitor (NRI). Reboxetine is effective in patients with moderate to severe depression

(Montgomery, 1997). Norepinephrine reuptake inhibitors express their actions through ascending adrenergic pathways, and indirectly recruit postsynaptic α-adrenoceptor and β-adrenoceptor by elevating synaptic levels of NA (Stone *et al*., 2003). The recruitment of corticolimbic populations of α_1-adrenergic subtypes as well as frontocortical β_2 and β_1-adrenergic receptors contribute to their favourable influence upon mood (Millan *et al.,* 2000; Nalepa *et al*., 2002). It has also been hypothesized that the NA-induced activation of β_1-adrenergic receptors in the hippocampus participates in mechanisms of synaptic plasticity engaged by NRIs (Zhang *et al.,* 2003).

Dopaminergic receptors in the frontal cortex may also be involved in the antidepressant actions of reboxetine and other NRI actions. Indeed, although associated with low affinities for DA transporters (Millan *et al*., 2000), reboxetine, desipramine, and other NRIs elicit robust elevations in DA levels in the frontal cortex (Yamamoto and Novotney, 1998; Millan *et al.,* 2001).

Reboxetine also acts as a noncompetitive blocker of the α_4 β_2 and $\alpha_3\beta_4$ subtypes of nicotinic receptor (Miller *et al*., 2002), but the precise relationship between this action and its antidepressant activity is unknown. Although clinically effective in both the short- and long-term treatment of depression, no typical 'serotonergic' side effects are reported with NRIs (Page *et al*., 2003). Discontinuation rates, because of intolerance, appear more frequently when using SSRIs (Papakostas *et al*., 2007).

2.5.4 Selective serotonin reuptake inhibitors

Fluoxetine was introduced in 1988 and was followed by sertraline, paroxetine, fluvoxamine, citalopram and its S-enantiomer, escitalopram. The SSRIs vary somewhat in their pharmacologic profile. Although SSRIs have more or less selective effect on serotonin reuptake (citalopram/escitalopram are the most selective). SSRIs cannot be ascribed to one region of 5-HT transporter mediated increase extracellular levels of 5-HT, leading to the engagement of one defined subtype of 5-HT receptor. In fact, SSRIs engage a number of postsynaptic (and presynaptic) 5-HT receptors throughout the CNS, and recruit multiple classes of 5-HT receptor (including 5-HT_1A, 5-HT_1B and 5-HT_2A or 5-HT_2C). For example, fluoxetine and certain other SSRIs are weak antagonists at 5-HT_2C and 5-HT_2A receptors (Palvimaki *et al.,* 1996). Fluoxetine also possesses modest antagonist properties at 5-HT_3 receptors (Eisensamer *et al*., 2003).

Direct actions of SSRIs at other different sites have been reported. Thus, citalopram and escitalopram have little inhibitory effect on norepinephrine and dopamine reuptake and a low affinity for a1 adrenergic receptors, muscarinic cholinergic receptors, and histamine H_1 receptors (Stahl and Grady, 2003; McIntyre, 2013).

Dopaminergic mechanisms have been implicated in the antidepressant actions of SSRIs. SSRI-induced increases in DA and NA levels in the frontal cortex and other cerebral structures may be partially because of the interplay between facilitatory 5-HT and dopaminergic and adrenergic projections. For example, frontocortical 5-HT_1A receptors can enhance extracellular levels of DA, either through local circuits or through the activation of a glutamate input to the neighbouring ventrotegmental area (Bose and Gandhi, 2008).

SSRIs have proved to be popular first-line treatments for depression, but they do not clearly reveal significant clinical advantages over TCAs in terms of efficacy and speed of onset of action (Hirschfeld, 1999; Adell *et al.*, 2005).

SSRIs are generally well tolerated but are not devoid of adverse effects. Sexual dysfunction is the most common side effect of all SSRIs. Delayed ejaculation, anorgasmia, and decreased libido can occur in up to 60% of patients (Rosenberg *et al.*, 2007), and the effects continue as long as the drug is taken.

Anorexia is most common with fluoxetine and occurs early in the treatment (Sporn *et al*., 2000; Masand and Gupta, 2002). CNS side effects include anxiety, insomnia, sedation, nightmares (Masand and Gupta, 2002; Richelson, 2003), and extrapyramidal symptoms. Sleep disturbances, either insomnia or somnolence, have been reported in approximately 25% of patients taking SSRIs.

2.5.5 Norepinephrine and serotonin reuptake inhibitors

Norepinephrine and serotonin reuptake inhibitors (SNRIs) such as venlafaxine, milnacipran, and duloxetine were developed to have dual action on 5HT and NA. Venlafaxine inhibits serotonin (at low dose) and norepinephrine reuptake (at high dose) and is a weak inhibitor of dopamine reuptake (McIntyre *et al.*, 2017). Duloxetine inhibits 5HT and NA reuptake with equal potency both *in vivo* and *in vitro*. The drug has a high affinity for human cloned 5HTand NA transporters in vitro and more potently blocks these transporters than venlafaxine (Bymaster *et al.*, 2003). Microdialysis studies showed that SNRIs elicit marked elevations in extracellular levels of 5-HT, NA, and DA in corticolimbic pathways (Gartlehner *et al.*, 2016).

Experimental and clinical studies indicate that serotonergic mechanisms underlie the effects of low doses of SNRIs, whereas α_1-mediated adrenergic receptors can be recruited with higher doses (Millan *et al*., 2001) to contribute to their antidepressant properties (Redman and Francis, 1998; Stone *et al*., 2003). The most common side effects reported with duloxetine and venlafaxine were nausea, dry mouth, dizziness, insomnia, somnolence (Schatzberg, 2003).

Sexual dysfunction can occur in the course of SNRI treatment, as with SSRIs, but with lower rates (Clayton *et al*., 2002; Goldstein *et al*., 2002). Consistent with their pharmacological profile as a reuptake inhibitor of NA, a mean increase in heart rate can be observed with SNRIs, and, indeed, treatment with duloxetine and venlafaxine can be associated with increased blood pressure. Constipation, fatigue, decreased appetite, and sweating are also reported with duloxetine (Schatzberg, 2003).

2.5.6 Dopamine reuptake inhibition and direct dopamine receptor agonists

Dopamine is well known to be involved in the reward system of the brain and a decline in the activity of both mesocortical and mesolimbic DA release contributes to depressed mood (Willner *et al.*, 2005).

The selective dopamine reuptake inhibitor and clinically effective antidepressant bupropion (Rush *et al*., 1998; Levin *et al*., 2002), which only displays a weak affinity for DA transporters. Bupropion also has an active metabolite, hydroxybupropion, that mediates antidepressant efficacy by blocking reuptake of norepinephrine and dopamine (Tatsumi *et al.*, 1997; Sanchez and Hyttel, 1999) Bupropion bears a resemblance to

NRIs (such as reboxetine) as it elevates both DA and NA (but not 5-HT) levels in frontal cortex (Ascher *et al*., 1995) but it differs from NRIs (and SSRIs) as it elevates DA levels in the nucleus accumbens (Martin *et al*., 1990; Cryan *et al*., 2003). Mesolimbic stimulation of D_3 receptors may also participate in its antidepressant activity (Lambert *et al.,* 2000), perhaps through neuroprotective properties and/or a facilitation of neurogenesis (Coronas *et al*., 2004; Van Kampen and Robertson, 2005). However, dopaminergic mechanisms can be exclusively responsible for its antidepressant action, which remains a matter of debate (Meyer *et al*., 2002). It is noteworthy that the dopamine D_2/D_3 agonist pramipexole exhibits antidepressant properties when given alone (Corrigan *et al*., 2000), or in adjunct to TCAs or SSRIs in patients with treatment-resistant depression (Lattanzi *et al*., 2002; Cassano *et al*., 2004).

Bupropion causes insomnia, headache, tremors, and nausea are common side effects and increased irritability and agitation may also occur. The long-term effects of bupropion still remain a matter of debate, as both sleep-promoting and, more recently, sleep-suppressive actions have been described in depressed patients (Ott *et al*., 2004).

2.6 Medicinal Plants Used in the Management of Depression

2.6.1 *Camellia sinensis* (Green Tea)

Preclinical study demonstrated that polyphenols obtained from *Camellia sinensis* at doses 5, 10, and 20 mg/kg administered orally for 7 days improved depression-like behaviour and decreased serum level of corticosterone. These findings suggest that green tea polyphenols can regulate the HPA axis involved in the pathology of depression (Zhu *et al.*, 2012).

2.6.2 *Lavandula angustifolia* (Lavender)

Lavandula angustifolia is a flowering plant in the family Lamiaceae, native to the Mediterranean. The uses of lavender oil include an antibacterial, antifungal, carminative, sedative, and antidepressant properties (Cavanagh and Wilkinson, 2002). Hritcu *et al.*(2012) reported that chronic lavender oil exposure markedly inhibited depression-like behaviors in rats in forced swimming and elevated plus-maze tests (Hritcu *et al*., 2012).

Effati-Daryani *et al* (2015) studied the effect of lavender cream on depression in pregnancy and demonstrated that the cream can be used for pregnant women to reduce depression. Lavender oil infusion showed several curative benefits on depressed patients, essentially decreasing mean depression score (Nikfarjam *et al*., 2013). Lavender aromatherapy for 4 weeks improved the Edinburgh Postnatal Depression Scale in high risk postpartum women (Conrad and Adams, 2012).

2.6.3 *Hypericum perforatum* (St John's Wort)

Hypericum perforatum is a famous herb that has long been used to treat depression (Butterweck, 2003). A number of clinical studies demonstrated that *Hypericum perforatum* is clinically efficacious for depression (Mannel *et al.,* 2010; Maher *et al.,* 2016).

A long-term follow-up study recruited 426 responders to *Hypericum perforatum* extract (3 × 300 mg/day) to be assessed for remission rates. Results showed a beneficial effect of *Hypericum perforatum* extract for prevention of relapse after recovery from acute depression while long-term maintenance and tolerability (Kasper *et al.,* 2008).

2.7 The Plant *Acacia seyal* Del. Fabaceae

2.7.1 Taxonomy

Kingdom - Plantae

Phylum - Tracheophyta

Class - Magnoliopsida

Order - Fabales

Family - Fabaceae

Genus - *Acacia*

Species - *Acacia seyal*

2.7.2 Vernacular Names

English - Fodder tree, Red Acacia, Shittim wood

French - Epineux

Swahili - Mgunga

Hausa - Dumshee

2.7.3 Description of the plant

Acacia seyal is a small to medium-sized tree that reaches a height of 12-17 m, with umbrella shaped crown. It has bippinnate leaves, dark green, 4-12 pairs of pinnae, 10-12 pairs of leaflets, each 1-2 x 4-12 mm. Flowers are clustered in shining, yellow, globose heads, 1.5 cm diameter, on stems 3 cm long (Hall and McAllan, 1993).

2.7.4 Distribution of plant

Acacia seyal is native to Sudan, Egypt, Eritrea, Ethiopia, Ghana, Iran, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Saudi Arabia, Senegal, Syrian Arab

Republic, Tanzania, Uganda, Yemen, Republic of Zambia, and Zimbabwe. Moreover, it is exotic to Afghanistan, Bangladesh, Bhutan, India, Nepal, Portugal, Sri Lanka and the US (Orwa *et al.,* 2009).

Plate I: The Plant,*Acacia seyal*Del in its Natural Habitat.

2.7.5 Ethnomedicinal uses of *A* *l*

The plant gum is used as aphrodisiac, emollient and astringent for colds, diarrhoea and haemorrhage (Duke, 1983). The bark is used for dysentery, arthritis and leprosy (Orwa*et al*., 2009). Leaves are used for inflammation and stomach disorders (Elgazali *et al*., 1997). Decoction of the root is used in the treatment of depression in Northwestern Nigeria (Shehu *et al*., 2017).

2.7.6 Previous pharmcological studies of *Acacia seyal*

Antibacterial activity: The antimicrobial activity of *Acacia seyal* was evaluated *in vitro*against three bacterial species *Salmonella, Escherichia coli* and *Staphylococcus aureus*. The obtained results showed that *Acacia seyal*has a wide antibacterial activity against most tested bacterial strains (Mariod*et al*., 2014).

Anti inflammatoryactivity: The methanol crude extracts and its active fractions from *Acacia seyal* gum were shown to have antioxidant and anti-inflammatory activities (Elnour *et al.,* 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Animals

Swiss Albino mice of both sexes (18-23 g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were housed in propylene cages and kept under natural day and light cycle. The animals were fed on laboratory animal diet (Vital Feed®) and water *ad libitum*.

Ethical clearance was soughtfrom the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC). All experimental protocols were in accordance with the guideline for the care and use of laboratory animals (NIH, 1996)

3.2 Drugs and Chemicals

The chemicals and drugs used for the studies include; Fluoxetine (Mebidos Laboratories Pvt. Ltd, India), Diazepam (Roche, France), Atropine, Cyproheptadine, Prazosin, Yohimbine and Metergoline (Sigma Aldrich, St. Louis, MO, USA)

3.3 Equipment and other Materials

Animal cages, pestle and mortar, syringes (1ml, 2mls, 5mls and 10mls), filter paper, pair of scissors, measuring cylinders, separating funnel, beakers, test tubes, funnel, test tube holders, Video recording device (JVC, GZ-MG750BUS), stop watch, Open field apparatus, retort stand, water drinkers, weighing balance, Forced swimming cylinder, Tail suspension apparatus, Micro plate reader and incubator

3.4 Plant Collection

The plant sample comprising leaves, stem and root were collected from Malumfashi Local Government Area, Katsina State in June, 2018 and was identified in the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria by a Taxonomist, Namadi Sanusi. A specimen voucher number 900347 previously deposited in the herbarium was used for comparison.

3.4.1 Preparation of extract

The root bark was separated from the root using a knife. The root bark was dried under shade until a constant weight is obtained. The root bark was size-reduced using mortar and pestle. Two thousand grams (2000g) of powdered material was extracted by Cold maceration in 5000 ml of methanol for 72 hours with occasional shaking. The extract was filtered and concentrated at 50°C on a water bath to a constant weight. The extract was kept in airtight containers until needed for use. Aqueous solutions of the extract were freshly prepared for each study using distilled water.

3.5 Phytochemical Screening of *Acacia seyal*

Phytochemical screening was carried out on the methanol root bark extract of the plant *Acacia seyal* using standard methods as follows:

3.5.1 Test for flavonoids

Sodium hydroxide test (Evans, 1996): Few drops of 10% sodium hydroxide was added to 0.5 g of the extract (AS) yellowish colouration indicates the presence of flavonoids.

3.5.2 Test for cardiac glycosides

Keller Killiani's test: A portion of the extract (AS) was dissolved in 1 ml of glacial acetic acid with traces of ferric chloride solution and 1 ml of concentrated sulphuric

acid was added down the side of the test tube to form a lower layer at the bottom. Presence of purple-brown ring at the interphase indicates the presence of deoxy-sugars and a pale green colour in the upper acetic layer indicates the presence of cardiac glycosides.(Evans, 1996).

3.5.3 Test for saponins

Methanol root bark extract (0.5 g) was shaken with 3 mls of water for 30 seconds and allowed to stand for 30 minutes. Formation of honeycomb froth that persists 10-15 minutes indicates the presence of Saponins (Sofowora**,** 1993).

3.5.4 Test for tannins

Lead sub-acetate test: Methanol root bark extract of *A. seyal* (0.5 g) was dissolved in 2 mls of water; 3 drops of lead sub-acetate solution was then added and observed. Formation of black-green coloured precipitate which indicates the presence of tannins.

3.5.5 Test for steroids and triterpenes

Liebermann-Burchard's test: The extract (0.5 g) was dissolved in 5 mls of methanol then filtered. The filtrate was evaporated to dryness on a water bath at 100°C. The residue obtained was shaken with 2 mls chloroform and then filtered into a cleaned and dried test tube. 2 ml of acetic acid anhydride was added to the filtrate and shaken, and then 1ml of concentrated sulphuric acid was also added carefully down the zone of contact of the two liquids. Red colour indicates the presence of triterpenes while blue-green colour indicates steroids (Evans, 1996).

3.5.6 Test for alkaloids

Methanol root bark extract of *A. seyal* (0.5 g) was treated with 5 mls of 1% aqueous HCl and then heated. It was filtered and the filtrate was poured into a test tube **(**Sofowora, 1993).

Dragendoff's reagent: to the test tube, two drops of dragendoff's reagent was added and rose red precipitate indicates presence of alkaloids.

3.5.7 Test for anthraquinones

About 0.5 g of the extract (AS) was placed in placed in a dry test tube and 5 ml of chloroform was added and shaken for at least 5 minutes. This was filtered and equal volume of 10% ammonia solution was added to the filtrate and shaken. The presence of a bright pink colour in the aqueous (upper) layer indicates free anthraquinones (Evans, 1996).

3.6 Toxicity Studies

3.6.1 Acute toxicity studies

Median lethal dose (LD_{50}) was determined using Organization for Economic Co-operation and Development (OECD 425) guidelines in mice. In this method, two groups of three mice each were fasted 3 hours prior to dosing (OECD, 2008). The extract of AS was administered in a single oral dose using a cannula. A start dose of 5000 mg/kg was used for one mouse and observed for 48 hours. The mouse survived, and an additional four mice were dosed and observed individually during the first 30 minutes after dosing, periodically during the first 24 hours, and then daily for 14 days.

3.6.2 Irwin's test

The method described by Irwin (1968) was used. Four groups of five mice each were used. Group I received distilled water (10 ml/kg). Groups II, III and IV received 250, 500 and 1000 mg/kg of the methanol root bark extract of *Acacia seyal* orally, respectively. Mice were observed for 48 hours, with more observation within first 4 hours. Observations include morphological, autonomic, neurological and behavioural changes.

3.6.3 Test for Motor co-ordination deficit (Beam Walking Assay)

The method described by Stanley *et al.* (2005) was used. Five groups of six mice each were used.Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above a bench with wooden support to a goal box. The test beam is made up of wood (8 mm in diameter, 60 cm long) and elevated 30 cm above the bench by a metal support. Group I received distilled water (10 ml/kg), groups II, III and IV mice received 250, 500 and 1000 mg/kg of the methanol root bark extract of *Acacia seyal* orally, respectively. Group V received 10 mg/kg of diazepam.

Mice were individually placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 seconds allowed on the beam. The numbers of foot slips were also recorded.

3.7 Antidepressant Studies

3.7.1 Tail suspension test (TST) in mice

This test was carried out as described by Steru *et al.* (1985). Five groups of eight mice each were used. Group I mice received distilled water (10 ml/kg). Groups II, III and IV received 250, 500 and 1000 mg/kg of the methanol root bark extract of *Acacia seyal* orally, respectively while group V received 20 mg/kg of fluoxetine.

A tape was placed approximately 1cm from the tip of the tail of the mice. One hour later, using adhesive tape on a horizontal bar placed 30 cm above a bench mice were suspended on the tail suspension apparatus for six minutes. The whole procedure was recorded with the aid of a video recorder. The duration of immobility was determined from the video clip with the aid of a stop watch for each mouse. The most effective dose from the TST was adopted for the mechanistic studies.

3.7.2 Forced swim test (FST) in mice

The study was carried out as described by Porsolt *et al.* (1977). Five groups of eight mice each were used. Group I received distilled water (10 ml/kg). Groups II, III and IV mice received 250, 500 and 1000 mg/kg of the methanol root bark extract of *Acacia seyal* orally, respectively, while Group V received 20 mg/kg of fluoxetine. One hour later, mice were forced to swim for 5 minutes in a cylindrical water tank (30 cm in diameter and 50 cm long), filled with water up to 25cm with temperature maintained between24-26°C. The swimming, climbing and immobile behaviour of each mouse within 5 minutes were recorded.

3.7.3 Open field test (OFT) in mice

This test was conducted as described by Rex *et al.* (1996). Five groups of eight mice each were used. Group I mice were given distilled water (10 ml/kg). Groups II, III and IV mice received 250, 500 and 1000 mg/kg of the methanol root bark extract of *Acacia seyal* orally, respectively, while group V received 0.5 mg/kg of diazepam. One hour later, each mouse was exposed to an open field apparatus (72×72×36 cm, length×breadth×height) of which one wall is plexiglass with also a plexiglas floor divided into 16 visible squares (18×18 cm) and a central square.

Each mouse was placed in the central square of the apparatus. The behaviour of the mice in the arena was video recorded within 5 minutes. To determine the number of lines crossed by each mouse.

3.8 Mechanistic Studies

The method described by Zheng *et al.* (2014) was used**.** The most effective dose (500 mg/kg) from the TST was adopted for the mechanistic studies.

3.8.1 Determination of the involvement of the serotonergic system in the antidepressant effect of Methanol root bark extract of *Acacia seyal* in mice

To assess the involvement of the serotonergic system in the antidepressant- like effect of*A. seyal*. Thirty-five mice were divided into 7 groups of 5 mice each. Group I, II and III received distilled water (10 ml/kg), AS (500 mg/kg) (Effective dose) and fluoxetine (20 mg/kg) orally respectively. Group IV and V were pretreated with cyproheptadine (4 mg/kg, *i.p*., a 5-HT_2 receptor antagonist). Fifteen minutes after, each mouse received *A. seyal* (500 mg/ kg, *p.o*.) or fluoxetine (20 mg/kg, *p.o*.) (Ulak *et al.,* 2010). Group VI and VII were pretreated with metergoline (1mg/kg, *i.p*., a non-selective 5-HT_2 receptor antagonist) (Stachowicz *et al.,* 2007). Fifteen minutes after, each mousereceived AS (500 mg/ kg, *p.o*.) and fluoxetine (20mg/kg, *p.o*.). An hour post-treatment, they were subjected to TST.

3.8.2 Determination of the involvement of the noradrenergic system in the antidepressant effect of Methanol root bark extract of *Acacia seyal* in mice

To investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of *A. seya*l, thirty-five mice were divided into 7 groups of 5 mice each. Group I, II and III received distilled water (10 ml/kg), AS (500 mg/kg) and fluoxetine (20 mg/kg) orally respectively. Group IV and V were pretreated with prazosin (1 mg/kg, *i.p*., an α_1-adrenoceptor antagonist). After fifteen minutes, each mouse received AS (500 mg/ kg, *p.o*.) and fluoxetine (20 mg/kg, *p.o*.) and Group VI and VII were pretreated with yohimbine (1 mg/kg, *i.p*., and α_2 - adrenoceptor antagonist) (Gu *et al.,* 2012). After 15 minutes, they received AS (500 mg/kg, *p. o*.) and fluoxetine (20 mg/kg, *p.o*.). Sixty minutes post-treatment, they were subjected to TST.

3.8.3 Determination of the involvement of the dopaminergic system in the antidepressant effect of Methanol root bark extract of *Acacia seyal* in mice

To test the possible involvement of the dopaminergic system in the antidepressant-like effect of *A. seyal,* twenty-*five* mice were divided into five groups of 5 mice each. Group I, II and III were administered distilled water (10 ml/kg), AS (500 mg/kg) and fluoxetine (20 mg/kg) orally respectively. Group IV and V mice were pretreated with sulpiride (50 mg/kg, *i. p*., a dopamine D_2 receptor antagonist) (Gu *et al*., 2012). After 15 minutes, they received AS (500 mg/kg, *p. o*.) and Fluoxetine (20 mg/kg). An hour post-treatment, they were subjected to TST.

3.8.4 Determination of the involvement of the cholinergic system in the antidepressant effect of Methanol root bark extract of *Acacia seyal* in mice

To investigate the involvement of cholinergic system in the antidepressant- like effect of*A. seyal,* twenty-*five* mice were divided into five groups of 5 mice each. Group I, II and III were administered distilled water (10 ml/kg), AS (500 mg/kg) and fluoxetine (20 mg/kg) orally respectively. Group IV and V animals were pretreated with atropine (1 mg/kg, *i. p*.) (Liebenberg *et al.,* 2010). Fifteen minutes post-treatment, the animal received AS (500 mg/kg, p. o.) and fluoxetine (20 mg/kg, *p.o*.). An hour post-treatment, they were subjected to TST.

3.8.5 Determination of the involvement of the opioidergic systemin the antidepressant effect of Methanol root bark extract of *Acacia seyal* in mice

To investigate the involvement of opioidergic system in the antidepressant- like effect of *A seyal,* twentyfive mice were divided into five groups of 5 mice each. Group I, II and III were administered distilled water (10 ml/kg), AS (500 mg/kg) and fluoxetine (20 mg/kg) orally respectively. Group IV and V mice were pretreated with naloxone (2

mg/kg, i. *p*.). Fifteen minutes post-treatment, each mouse received AS (500mg/kg, *p. o.)* and fluoxetine (20 mg/kg, *p.o.*). An hour post-treatment, they were subjected to TST.

3.8.6 Determination of the involvement of the nitric oxide system in the antidepressant effect of Methanol root bark extract of *Acacia seyal* in mice

To investigate the possible involvement of the nitric oxide pathway in the antidepressant-like effect of *A. seyal*, thirty-five mice were divided into 7 groups of 5 mice each. Group I, II and III received distilled water (10 ml/kg), AS (500 mg/kg) and fluoxetine (20 mg/kg) orally respectively. Group IV and V were pretreated with L-arginine (50mg/kg, *i.p*., a nitric oxide substrate) and Group VI and VII were pretreated with L-NNA (50mg/kg *i.p*., a nitric oxide pathway inhibitor). After 15 minutes, they received AS, (500 mg/kg,*p.o*.) and fluoxetine (20 mg/kg, *p.o*.). An hour post-treatment, they were subjected to TST.

3.9 Chronic Unpredictable Mild Stress

The chronic mild stress was induced by chronic variable stress as described by Murua *et al.* (1991). Fifty-six mice were divided into six groups of 9 animals each, matched and assigned to stress and control groups. After three days acclimatization, mice were subjected to different kind of mild stressors for 28 days which varied from day to day to make the stress procedure unpredictable. These stressors were randomly scheduled repeated throughout the 4 weeks experiment. There were a total of nine stressors: (1) Twenty four hour Food deprivation, (2) Twenty four hour Water deprivation, (3) Wet bedding, (4) Two hour immobilization, (5) Cold Swim, (6) Tail pinch, (7) Cage tilt at 45°C (8) Cage reduction, (9) Empty cage.

The same stressor was not applied successively so that the mice will not anticipate the occurring stress. The unstressed groups of animals were housed in one cage with access to food and water without disturbance except for necessary procedure such as weighing and cage cleaning.

On day 27 (60 min after drug administration), animals were subjected to different behavioural (TST and FST) and biochemical tests. For biochemical investigations, mice were sacrificed by decapitation and brains were harvested. Brains samples were then stored in phosphate buffer (0.1 M, pH 7.4) at −80°Ctill further investigations.

3.9.1 Sucrose consumption test

The test was performed as described by Forbes *et al.* (1996). The test was carried out at 0, 2 and 4 weeks of stress. Animals were deprived of food and water for 21 hours after which they were exposed to drinking water and 2% sucrose. Sucrose consumption was measured and recorded.

3.9.2 Tail suspension test

The test was carried out as described by Steru *et al. (*1985). The test was carried out at 0, 2 and 4 weeks of stressors as described previously.

3.9.3 Open field test

This test was conducted according to the method described by Rex *et al.* (1996). The test was carried out at 0, 2 and 4 weeks after stressors as described previously.

3.9.4 Sample Collection

Blood samples were collected in plain bottles which were centrifuged at 1,000 g at 8℃for 30 minutes. Blood plasma and erythrocytes were separated for cortisol competitive ELISA detection method. Brain was homogenized in PBS buffer (0.01M, PH7.4) and centrifuged at 5000xg to obtain the supernatant. Brain homogenates were used for sandwich Enzyme Linked Immunosorbent Assay

3.9.5 Measurement of brain derived neurtropic fator (BDNF) level

Brain derived neurotrophic Factor (BDNF)level was measured using commercially available enzyme linked immunosorbent assay kit (ELISA) (Wuhan Fine Biotech Co., Ltd., Catalogue No. EM0020) according to the manufacturer's instructions. The plates were washed 2 times before adding standard, sample and control (Zero) wells. Thereafter 100 µL standard or sample was added to each well and incubated for 90 minutes at 37°C, after which the plates were aspirated and washed twice. Then 100 µL Biotin labelled antibody working solution was added to each well and incubated for 30 minutes at 37°C. The plates were then aspirated and washed 3 times. Afterwards 100 µL SABC working solution was added into each well and incubated for 30 minutes at 37°C. Plates were then removed aspirated and washed 5 times then 90 µL TMB substrate and incubated lastly for 15 minutes at 37°C. Plates were removed and 50 µL stop solution was added to each well. Absorbance was measured immediately at 450 nm using a micro plate reader (Rayto-RT-2100C). The sensitivity of the assay was <2.0pg/ml of BDNF.

3.9.6 Measurement of serum cortisol level

Serumcortisol level was measured using a commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit (Wuhan Fine Biotech Co. Ltd, Catalogue No. EM1721) according to the manufacturer's protocol. Plates were washed twice before standard, sample and control (Zero) wells were added, then 50 µL sample and standard solutions were added to already precoated antibody plate provided with the kit. After which 50 µL Biotin- labeled antibody was added into each well then incubated for 45 minutes at 37°C. Plates were removed, aspirated and washed 3 times followed by addition of 100 µL SABC working solution into each well then incubated for 30 minutes at 37°C. Plates were further washed and aspirated and washed 5 times followed

by the addition of 90 µL TMB substrate. Then incubated for 15-20 minutes at 37°C. The reaction was stopped by adding 50 µL of stop solution and absorbance was read at 450 nm immediately using a microplate reader (Rayto-RT-2100C). The sensitivity of the assay was <0.234ng/ml of cortisol.

3.10 Data Analysis

Results are presented as Means ±SEM and median scores on tables or figures as appropriate. Data for TST, FST, OFT and BWA were analysed using One Way ANOVA (Analysis of Variance) while weight variation were analysed using Repeated Measure ANOVA. Dunnett post hoc test was used to assess significant differences. Data for Irwins test were analysed using Kruskal Wallis test followed by Dunn post hoc test. Results were considered significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction Yield of Methanol Root Bark Extract of *Acacia seyal*

A dark brown solid residue weighing 103.3 g (5.17 $\%^{w}/_{w}$) was obtained from 2,000 g powdered root bark of *A. seyal*.

4.2 Phytochemical Screening of Constituents Present in the Methanol Root Bark Extract of *Acacia seyal*

Carbohydrates,Flavonoids, alkaloids, steroids, triterpenes, cardiac glycosides,tannins and saponins were found present in the methanol root bark extract of *A. seyal* (Table 4.2)

4.3 The Acute Toxicity Study of Methanol Root Bark Extract of *Acacia seyal*

The LD_{50} was estimated to be >5000 mg/kg orally in mice.

4.4 Effect of Methanol Root Bark Extract of *Acacia seyal* on Irwin's test

The methanol root bark extract of *Acacia seyal* impaired changes on locomotor activity (Table4.2), motor coordination (Table 4.3) and diarrhoea (4.4). No death was observed at all dosestested48 hours after drug administration.

Table 4.1 Phytochemical Constituents of the Methanol Root Bark Extract of *Acacia seyal*

Phytochemical constituents	Inference
Alkaloids	+
Anthraquinones	+
Carbohydrates	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Steroids and triterpenes	-
Tannins	+

Key: + = Present and - = Absent

Table 4.2: Effect of the Methanol Root Bark Extract of *Acacia seyal* on Locomotor Activity- Behavioural Changes

	Behaviour				
Time (h)	Treatment (mg/kg)	Locomotor activity	Grooming	Pain	Vocalisation
0	D/W 10 ml/kg	4	4	4	0
	AS 250 mg/kg	4	4	4	0
	AS 500 mg/kg	4	4	4	0
	AS 1000 mg/kg	4	4	4	0
1	D/W 10 ml/kg	4	4	4	0
	AS 250 mg/kg	3.2	4	4	0
	AS 500 mg/kg	2.8	4	4	0
	AS 1000 mg/kg	2.0*	4	4	0
2	D/W 10 ml/kg	4	4	4	0
	AS 250 mg/kg	3.2	4	4	0
	AS 500 mg/kg	2.6	4	4	0
	AS 1000 mg/kg	2*	4	4	0
3	D/W 10 ml/kg	4	4	4	0
	AS 250 mg/kg	2.8	4	4	0
	AS 500 mg/kg	2.6	4	4	0
	AS 1000 mg/kg	2.2*	4	4	0
4	D/W 10 ml/kg	4	4	4	0
	AS 250 mg/kg	2.6	4	4	0

AS 500 mg/kg	2.0*	4	4	0
AS 1000 mg/kg	1.8**	4	4	0

Values are expressed as median scores * $p \leq 0.05$ significantly different from distilled water (Kruskall Wallis test followed by Dunn post hoc test) n=5, AS=Methanol root bark extract of *Acacia seyal*, DW= 10 ml/kg of Distilled water

Table 4.3: Effect of the Methanol Root Bark Extract of *Acacia seyal* on Motor Coordination- Neurological Changes

	Coordination- Neurological							
Time (h)	Treatment (mg/kg)	Grip strength	Body tone	Motor coordinaion	Staggering gait	Tremor	Convulsion	Twitches
0	D/W 10 ml/kg	4	4	4	4	0	0	0
	AS 250	4	4	4	4	0	0	0
	AS 500	4	4	3	4	0	0	0
	AS 1000	4	4	2.5*	4	0	0	0
1	D/W 10 ml/kg	4	4	4	4	0	0	0
	AS 250	4	4	4	4	0	0	0
	AS 500	4	4	3.5	4	0	0	0
	AS 1000	4	4	3.0*	4	0	0	0
2	D/W 10 ml/kg	4	4	4	4	0	0	0
	AS 250	4	4	4	4	0	0	0
	AS 500	4	4	3.8	4	0	0	0
	AS 1000	4	4	3.2*	4	0	0	0

3	D/W 10 ml/kg	4	4	4	4	0	0	0
	AS 250	4	4	4	4	0	0	0
	AS 500	4	4	4	4	0	0	0
	AS 1000	4	4	4	4	0	0	0
4	D/W 10 ml/kg	4	4	4	4	0	0	0
	AS 250	4	4	4	4	0	0	0
	AS 500	4	4	4	4	0	0	0
	AS 1000	4	4	4	4	0	0	0

Values are expressed as median scores $^{*}p \leq 0.05$ significantly different from distilled water (Kruskall Wallis test followed by Dunn post hoc test) n=5, AS=Methanol root bark extract of *Acacia seyal*, DW= 10 ml/kg of Distilled water

Table 4.4: Effect of Methanol Root Bark Extract of *Acacia seyal* on Diarrhoea-Autonomic Changes

Autonomic Changes and Death				
Time (h)	Treatment (mg/kg)	Diarrhoea (Secretion excitation)	Skin Colour (General)	Death
0	D/W 10 ml/kg		4	0
	AS 250		4	0
	AS 500		4	0
	AS 1000		4	0
1	D/W 10 ml/kg		4	0
	AS 250	+	4	0
	AS 500	+	4	0
	AS 1000	+	4	0
2	D/W 10 ml/kg		4	0

	AS 250	+	4	0
	AS 500	+	4	0
	AS 1000	+	4	0
3	D/W 10 ml/kg		4	0
	AS 250 mg/kg		4	0
	AS 500 mg/kg		4	0
	AS 1000 mg/kg		4	0
4	D/W 10 ml/kg		4	0
	AS 250 mg/kg		4	0
	AS 500 mg/kg		4	0
	AS 1000 mg/kg		4	0

Values are expressed as median score $^{*}p \leq 0.05$ significantly different from distilled water (Kruskall Wallis test followed by Dunn post hoc test) n=5, AS=Methanol root bark extract of *Acacia seyal*, DW= 10 ml/kg of Distilled water

4.5 Effect of Methanol Root Bark Extract of *Acacia seyal* on Motor Coordination Deficit

The methanol root bark extract of *Acacia seyal* did not impair motor coordination deficit at all tested oral doses. The standard drug, diazepam, significantly impaired motor coordination ($p<0.001$) at the dose of 10 mg/kg orally in comparison with the control (distilled water 10 ml/kg) (Figure 4.1).

4.6 Effect of Methanol Root Bark Extract *Acacia seyal*on Tail Suspension Test

The methanol root bark extract of *Acacia seyal* (250, 500 and 1000 mg/kg)produced a significant ($p<0.05$) but not dose-dependentdecrease in the duration of immobility.

Similarly, Fluoxetine (20 mg/kg) significantly (p<0.001) decreasedthe duration of immobility. (Figure 4.2).

4.7 Effect of Methanol Root Bark Extract of *Acacia seyal* on the Open Field Test

In the open field test, the distilled water treated animals produced a mean activities marked by the number of line crossing (48.83 ± 10.16). The methanol root bark extract of *Acacia seyal* (250, 500, or 1000 mg/kg) did not significantly affect the number of line crosses. However, diazepam significantly (p<0.01) increased the number of line crosses activity (Figure 4.3).

4.8 Effect of Methanol Root Bark Extract *Acacia seyal* on Forced Swimming Test

The methanol root bark extract of *Acacia seyal* (250, 500 and 1000 mg/kg)decreased the duration of immobility in the treated mice. Significant (p< 0.05) response was obtained at all the tested doses as compared to the distilled water (10 ml/kg) group. Similarly, the standard drug, Fluoxetine (20 mg/kg), also significantly (p<0.001) decreased the duration of immobility (Figure 4.4)

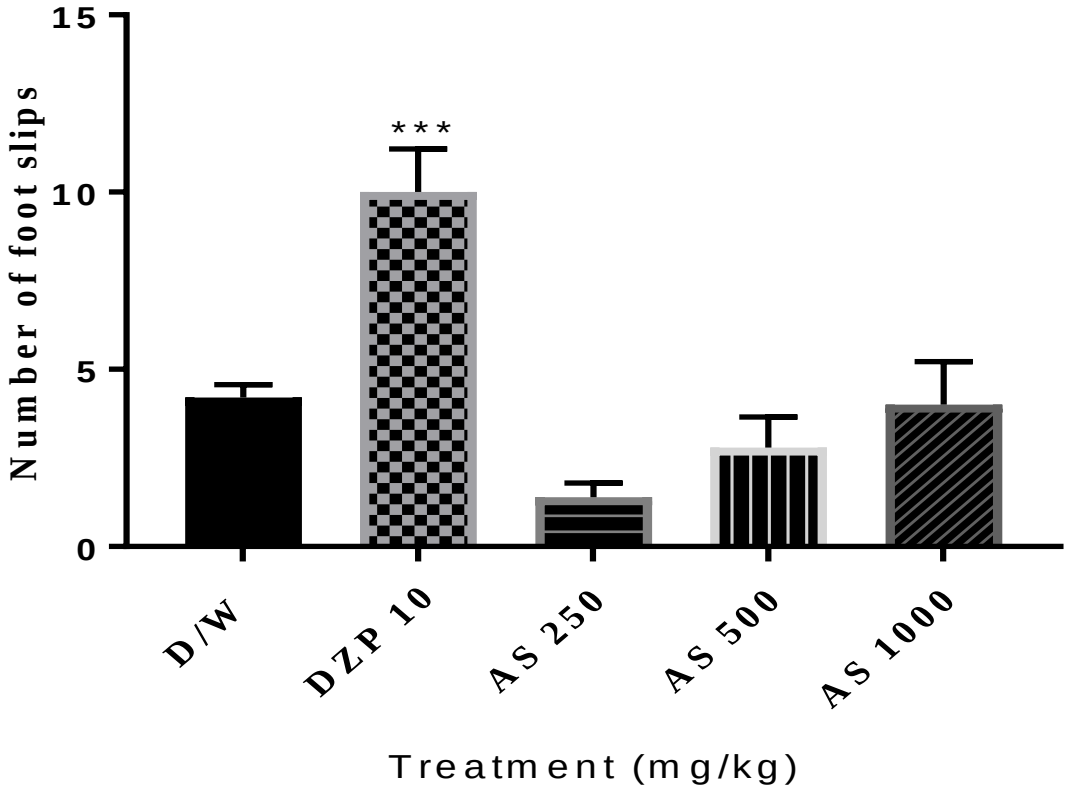

Figure 4.1: Effect of Methanol Root Bark Extract of *Acacia seyal* on Number of Foot Slips in Mice Beam Walking Assay

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.05$ $^{***}p \leq 0.001$significantly different from distilled water treated group(One way ANOVA followed by Dunnet*post hoc* test) n=6, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water, DZP= Diazepam

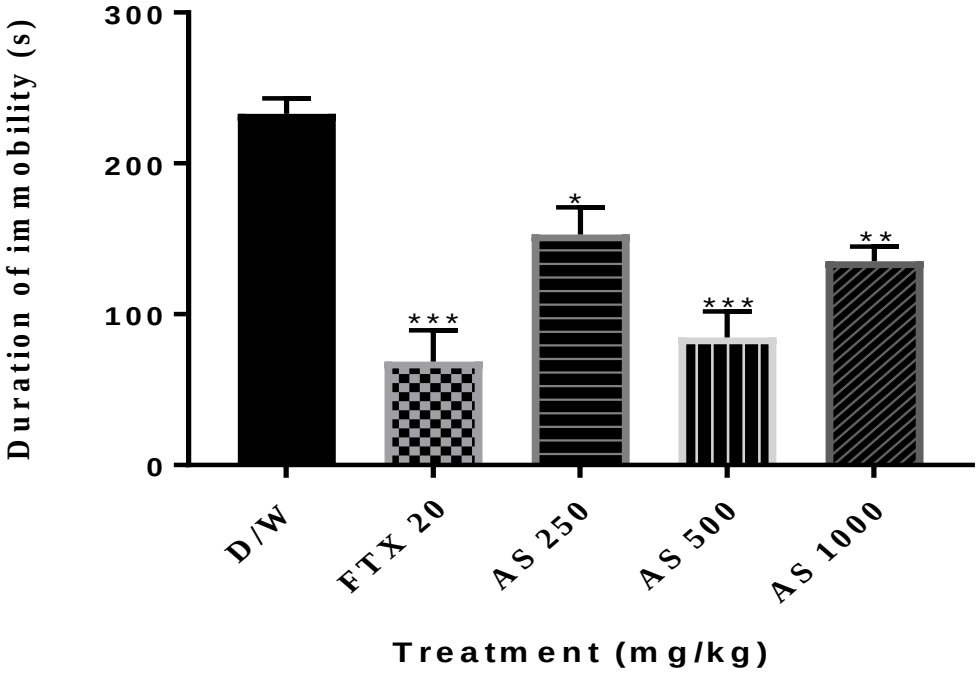

Figure 4.2: **Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test**

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$significantly different from distilled water treated group(One way ANOVA followed by Dunnet*post hoc* test) n=8, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine

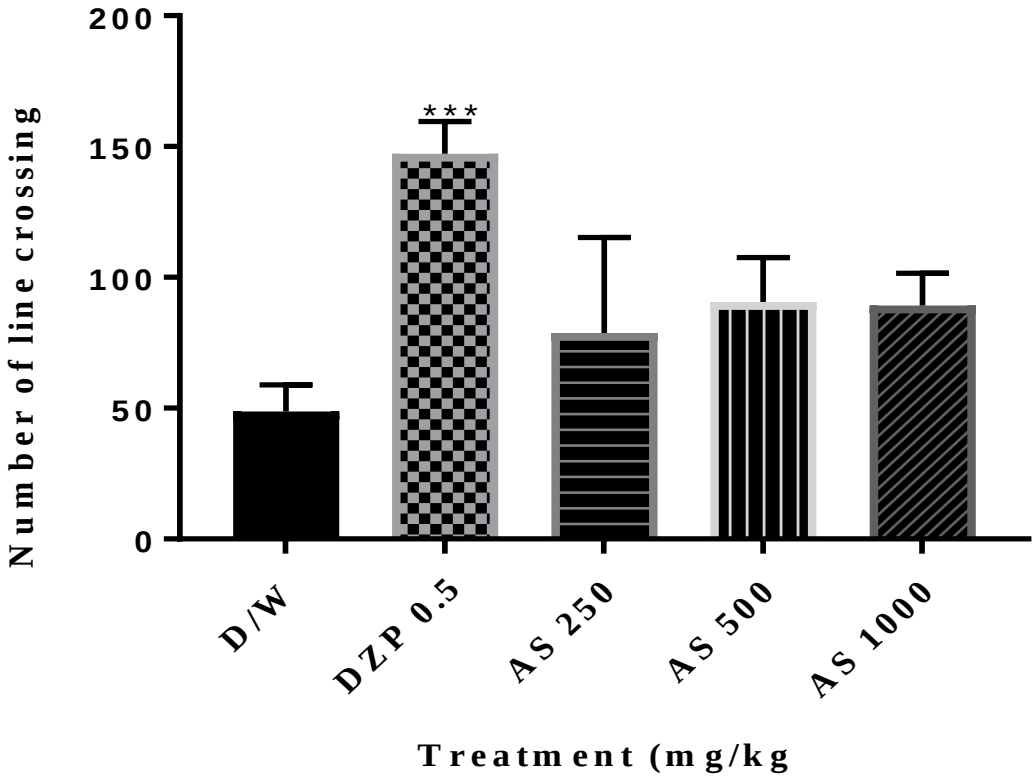

Figure 4.3: **Effect of Methanol Root Bark Extract of*Acacia seyal*on Line Crosses Activity in Mice in the Open Field Test**

Values are expressed as Mean ± S.E.M;$^{***}p \leq 0.001$significantly different from distilled water treated group(One way ANOVA followed by Dunnet*post hoc* test) n=6, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,DZP= Diazepam

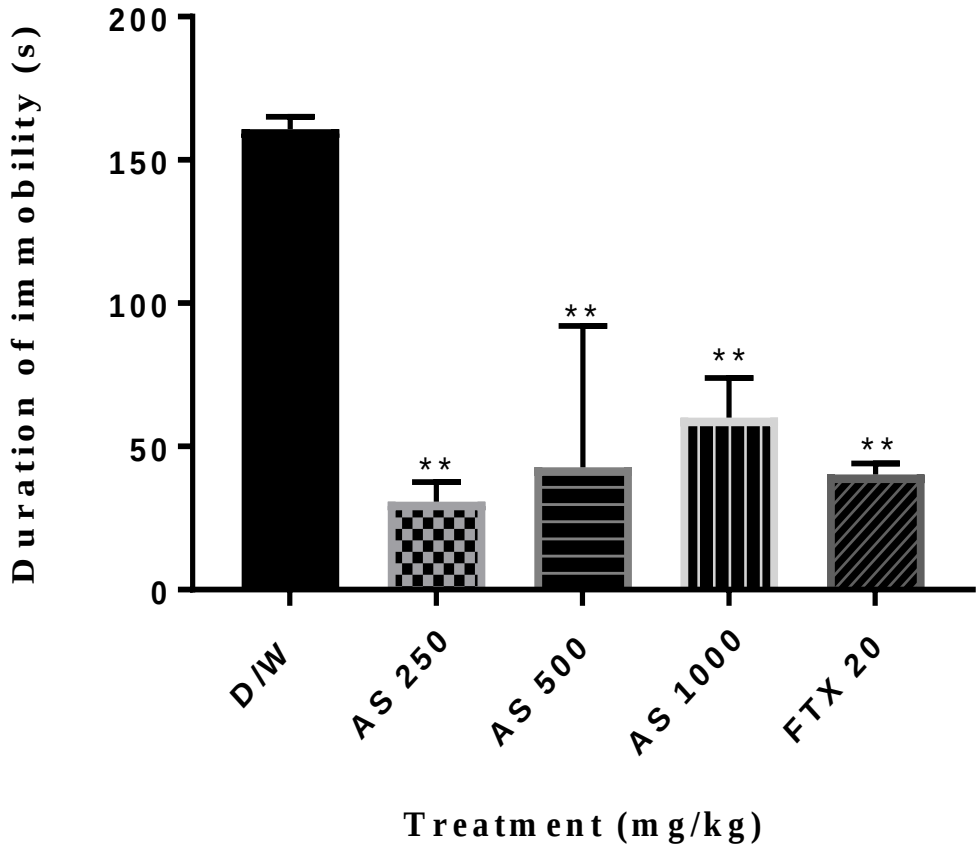

Figure 4.4: Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of mice in Forced Swim Test

Values are expressed as Mean ± S.E.M; $^{**}p \leq 0.01$ significantly different from distilled water treated group(One way ANOVA followed by Dunnet*post hoc* test) n=8, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine

4.9 Mechanistic Studies

4.9.1 Involvement of the dopaminergic system in the antidepressant effect of the Methanol root bark extract of *Acacia seyal* in mice

Pretreatment of mice with sulpiride (50mg/kg, *i.p.*)significantly ($p \leq 0.01$) reversed the anti- immobility effect elicited by the methanol root extract of *Acacia seyal* (500 mg/kg). The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed (Figure 4.5).

4.9.2 Determination of the Involvement of the serotonergic system in the antidepressant effect of the Methanol root bark extract of *Acacia seyal* in mice

Pretreatment of mice with metergoline (1mg/kg, *i.p.*) significantly ($p \leq 0.01$) reversed the reduction in the duration of immobility time elicited by methanol root bark extract of *Acacia seyal* extract (500mg/kg, *p.o.*) The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed. (Figure 4.6). Pretreatment of mice with cyproheptadine (4 mg/kg, *i.p.*) significantly ($p<0.01$) reversed the reduction in immobility time elicited by the methanol root bark extract of *Acacia seyal* (500mg/kg, *p.o.*). The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed (Figure 4.7).

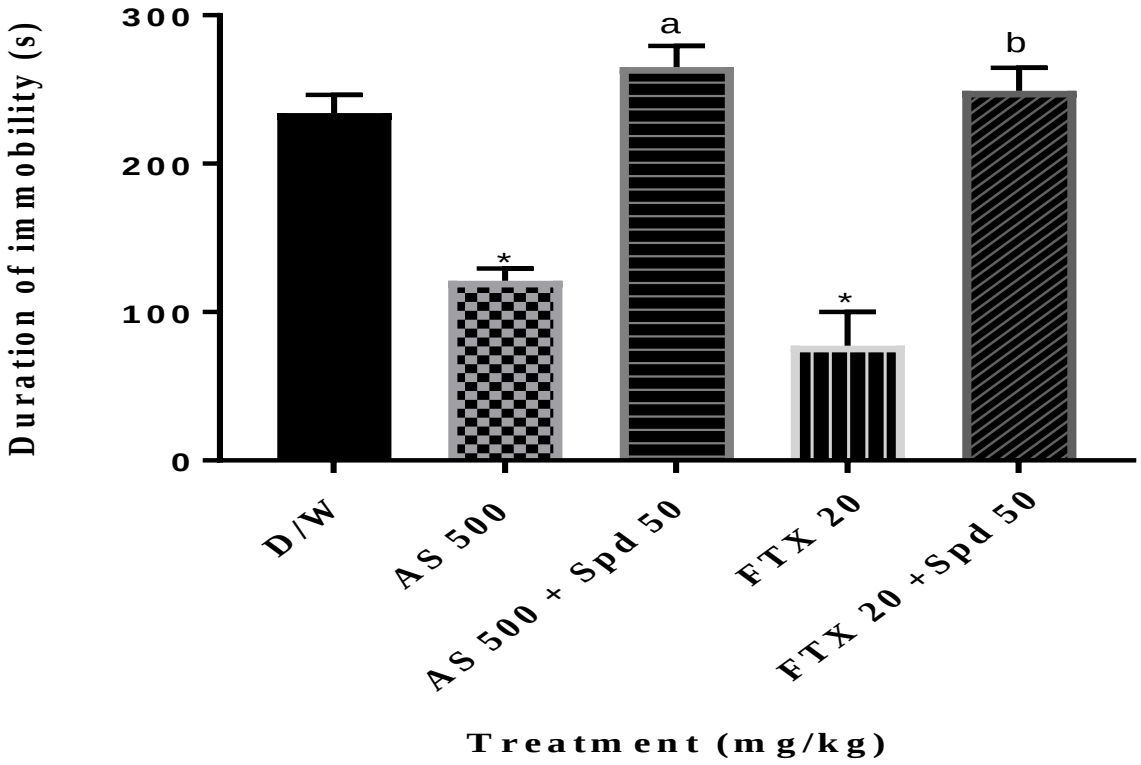

Figure 4.5: Effect of Sulpiride on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test.

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine,Spd=Sulpiride

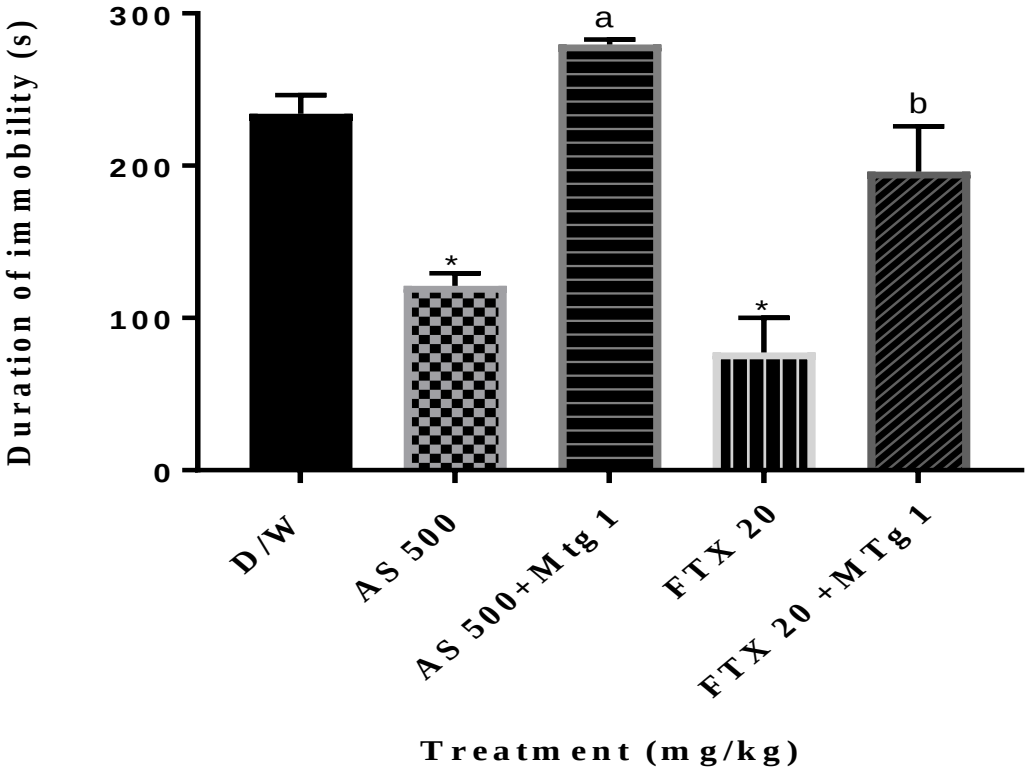

Figure 4.6: **Effect of Metergoline on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test**

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, Mtg=Metergoline

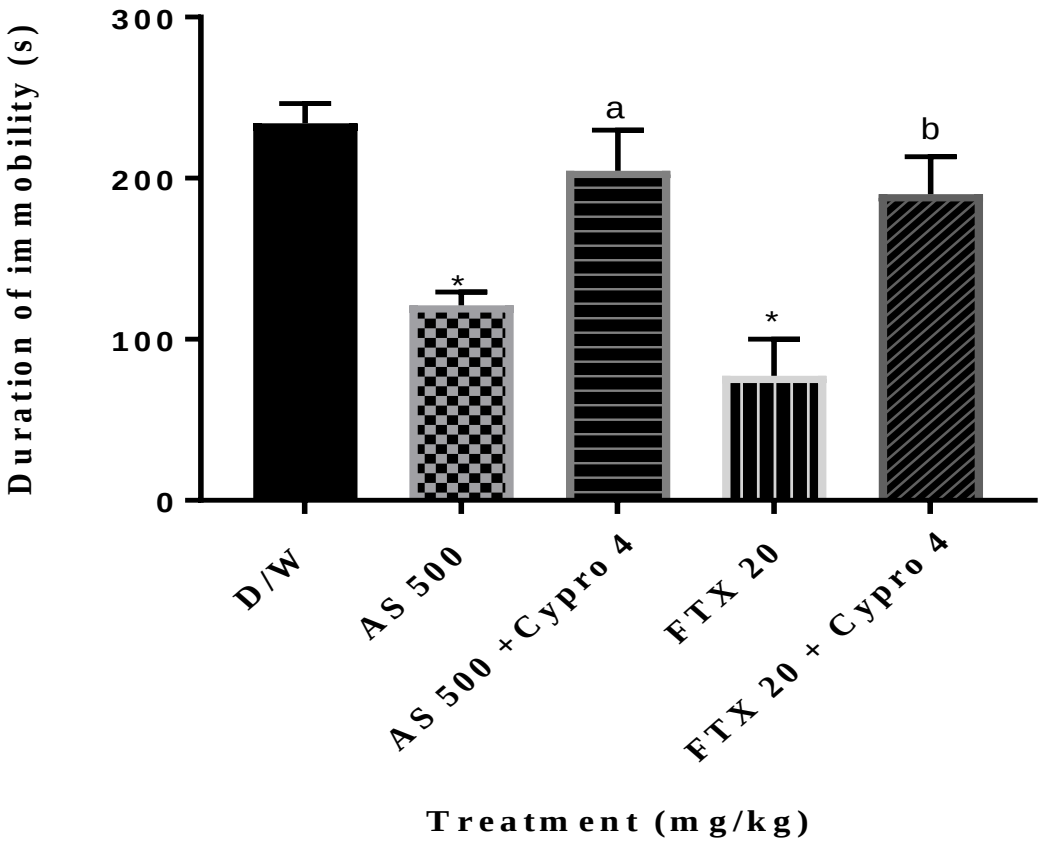

Figure 4.7: Effect of Cyproheptadine on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine,Cypro= Cyproheptadine

4.9.3 Involvement of the cholinergic system in the antidepressant effect of the Methanol root bark extract of *Acacia seyal* in mice

Pretreatment of mice with atropine (1 mg/kg, *i.p.*) significantly ($p<0.01$) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500mg/kg, *p.o.*). The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed (Figure 4.8).

4.9.4 Involvement of the opioidergic system in the antidepressant effect of the Methanol root bark extract of *Acacia seyal* in mice

Pretreatment of mice with naloxone (2 mg/kg, *i.p.*) significantly ($p<0.01$) reversed the reduction in immobility time elicited by the extract (500mg/kg, *p.o.*) The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed (Figure 4.9).

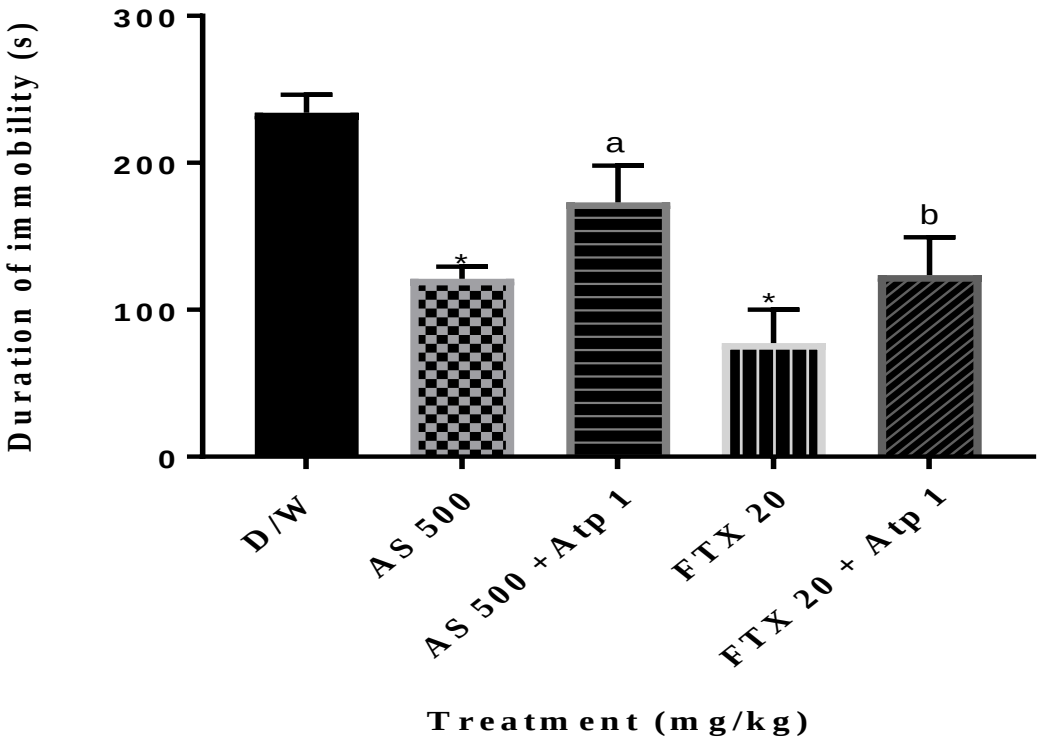

Figure 4.8: Effect of Atropine on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, Atp=Atropine

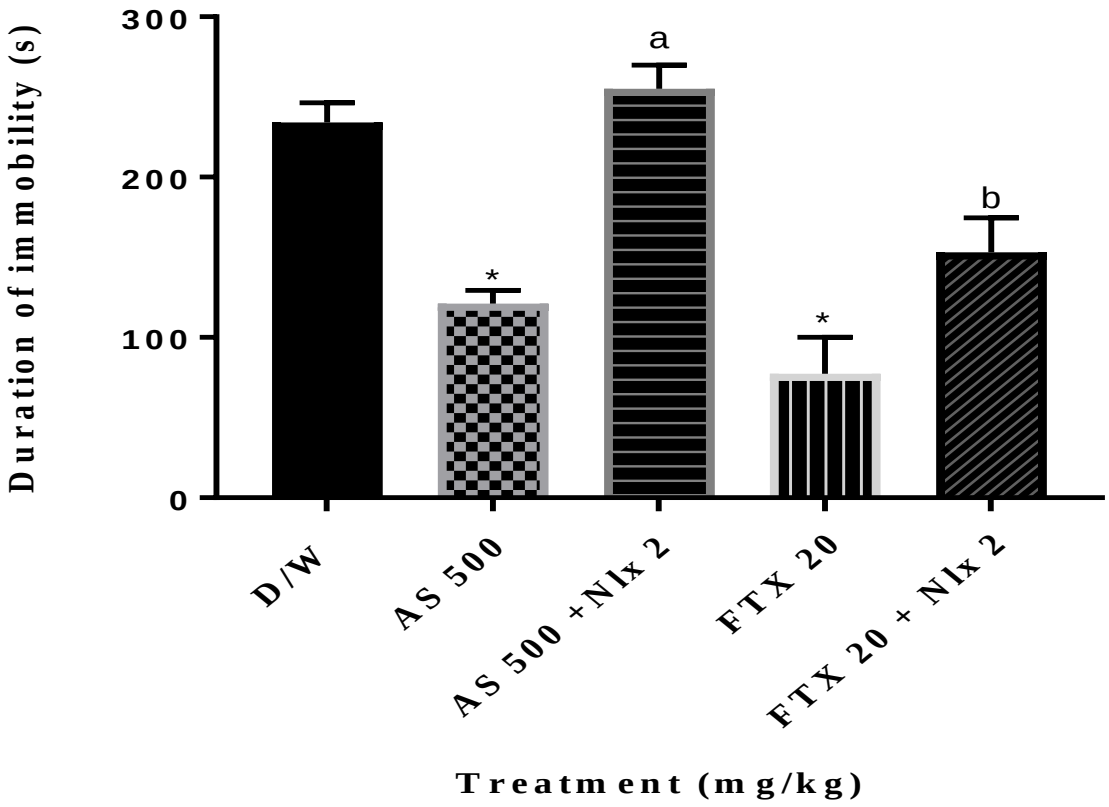

Figure 4.9: Effect of Naloxone on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, Nlx=Naloxone

4.9.5 Involvement of the nitric oxide system in the antidepressant effect of the Methanol root bark extract of *Acacia seyal* in mice

Pretreatment of mice with L- Arginine (50 mg/kg, *i.p*.) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500 mg/kg) orally (Figure 4.10). Pretreatment of mice with L-NNA (50 mg/kg *i.p*.) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500mg/kg) orally. The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed (Figure 4.11).

4.9.6 Involvement of the noradrenergic system in the antidepressant effect of the Methanol root bark extract of *Acacia seyal* in mice

Pretreatment of mice with yohimbine (1 mg/kg, *i.p*.) reversed the reduction in duration of immobility time elicited by methanol root bark extract of *Acacia seyal* (500 mg/kg) orally (Figure 4.12). Similarly, pretreatment of mice with prazosin (1 mg/kg, *i.p*.) reversed the reduction in duration immobility elicited by the methanol root bark extract of *Acacia seyal* (500 mg/kg) orally. The duration of immobility observed after the administration of Fluoxetine (20 mg/kg) was also reversed(Figure 4.13).

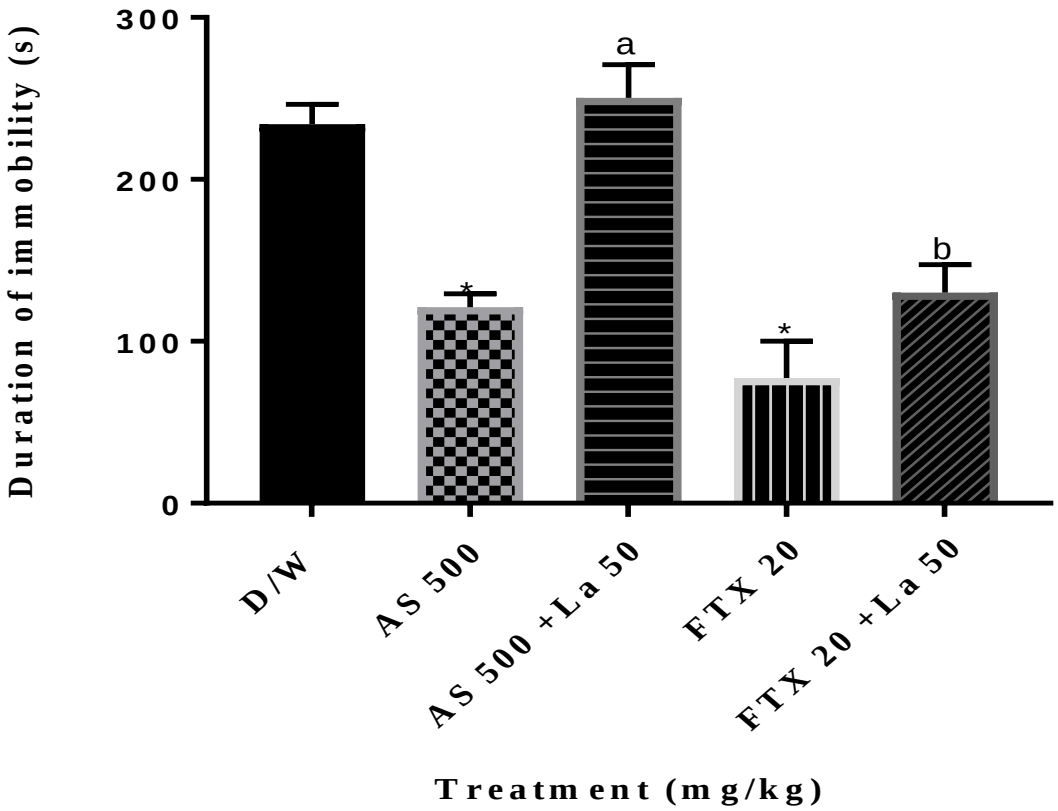

Figure 4.10: Effect of L-Arginine on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, La= L-Arginine

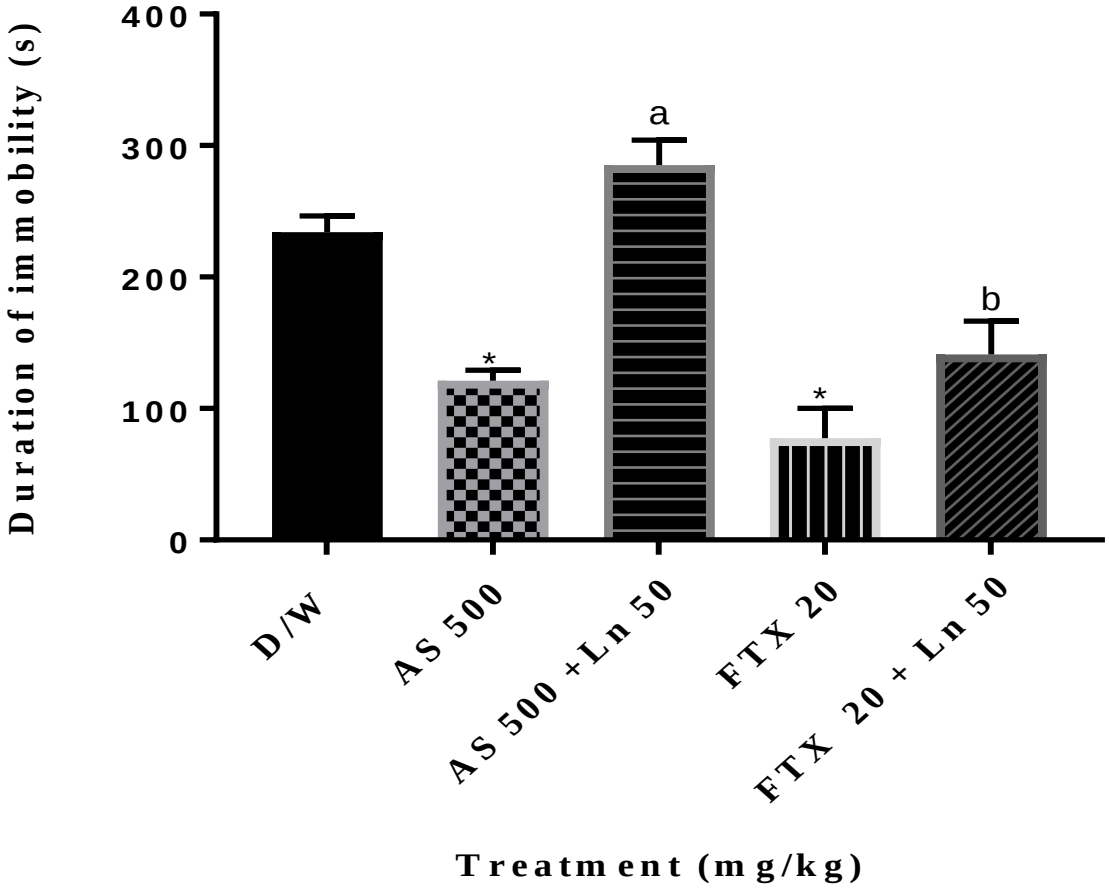

Figure 4.11: Effect of L-NNA on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, Ln=N-Arginine

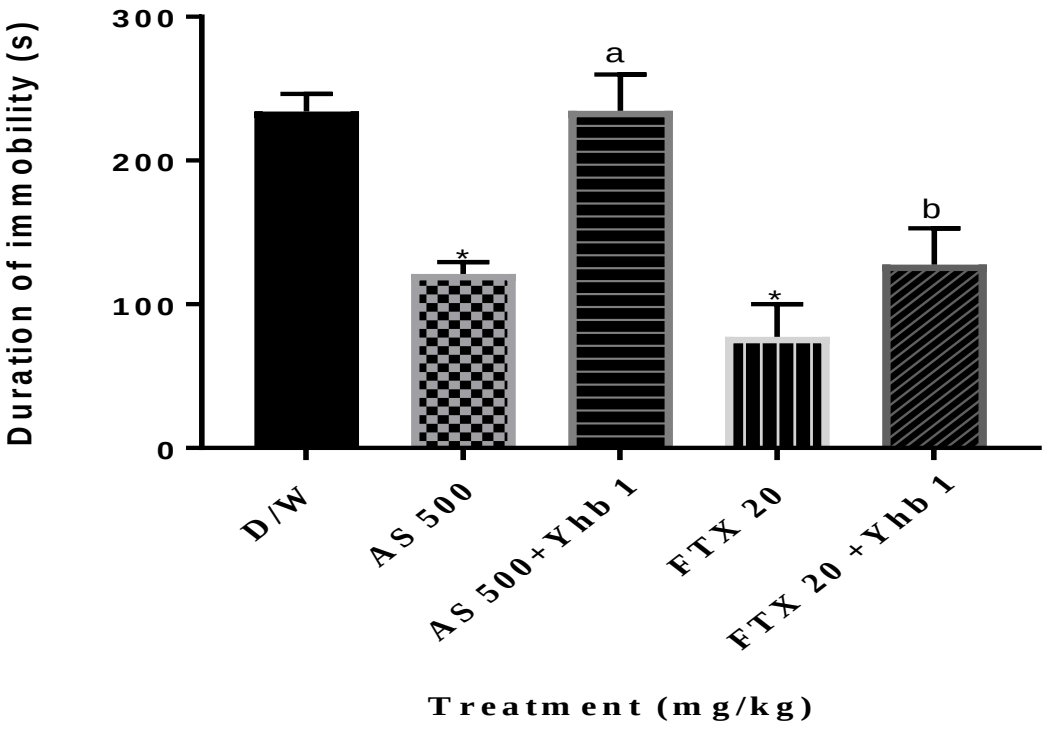

Figure 4.12: Effect of Yohimbine on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, Yhb=Yohimbine

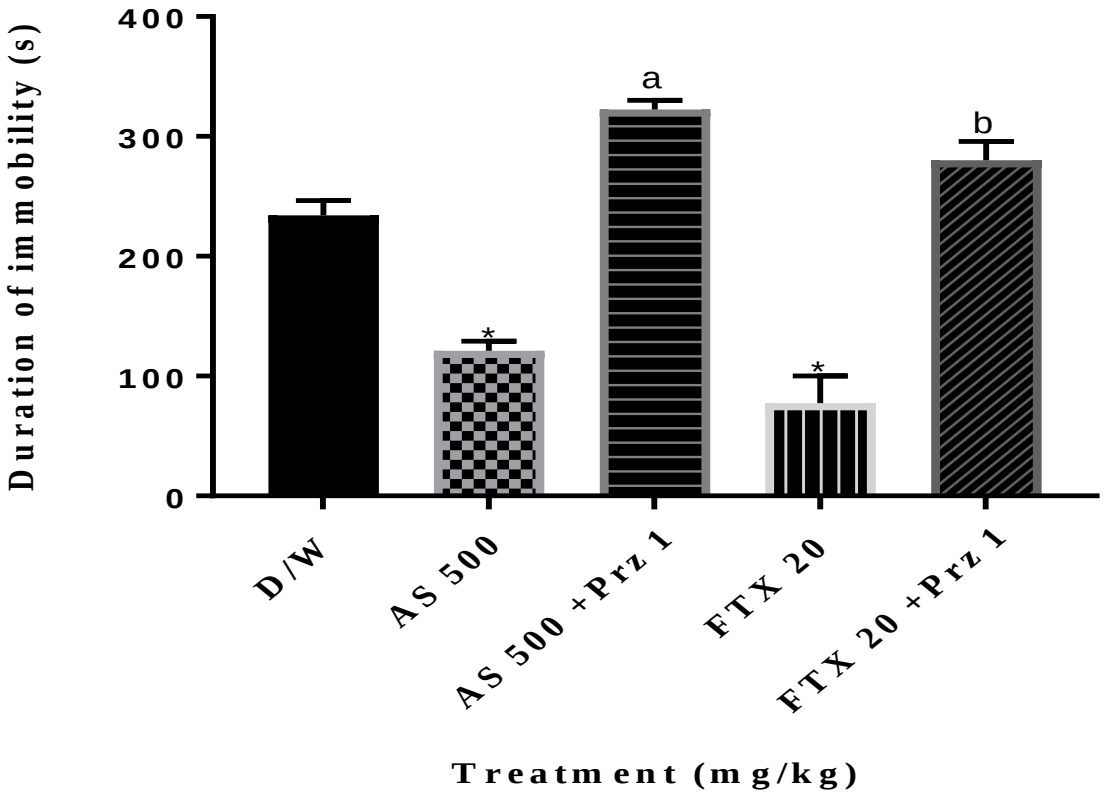

Figure 4.13: Effect of Prazosin on the Activity of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Values are expressed as Mean ± S.E.M; $^{*}p \leq 0.001$ significantly different from distilled water treated group; a & b$p \leq 0.01$= significant differencecompared to AS or FTX treated groupsrespectively(One way ANOVA followed by Dunnet*post hoc* test) n=5, AS = Methanol extract of *Acacia seyal* DW=10 ml/kg of Distilled water,FTX= Fluoxetine, Prz=Prazosin

4.10 Chronic Unpredictable Mild Stress

4.10.1 Effect of Chronic Unpredictable Mild Stress on Body weight

Changes in body weight in the stressed group were statistically significant as compared to the unstressed group for the duration of 4 weeks. There was slight improvement in body weight in all groups in the last two weeks of treatment but was not statistically significant (Figure 4.14).

4.10.2 Effect of Methanol Root Bark Extract of *Acacia seyal* on Sucrose Consumption in Mild Stress

There was no significant difference in sucrose consumption across all groups before stress. However, after two weeks the stressed mice showed significant decrease in sucrose consumption when compared to unstressed mice. After two weeks of treatment, AS (250, 500 and 1000 mg/kg) and FTX (20 mg/kg) did not significantly increase sucrose consumption when compared with distilled water/CMS group (Figure 4.15).

4.10.3 Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration Immobility of Mice Subjected to Tail Suspension Test Followed by Chronic Unpredictable Mild Stress

In the TST, there was no significant difference in immobility time among all groups before stress. However, stressed mice showed significant increase in immobility time when compared to unstressed mice. After two weeks of treatment, AS at the dose (500 mg/kg) and FTX (20 mg/kg) showed significant ($p<0.05$) decrease in duration of immobility when compared with the stressed group (Figure 4.16).

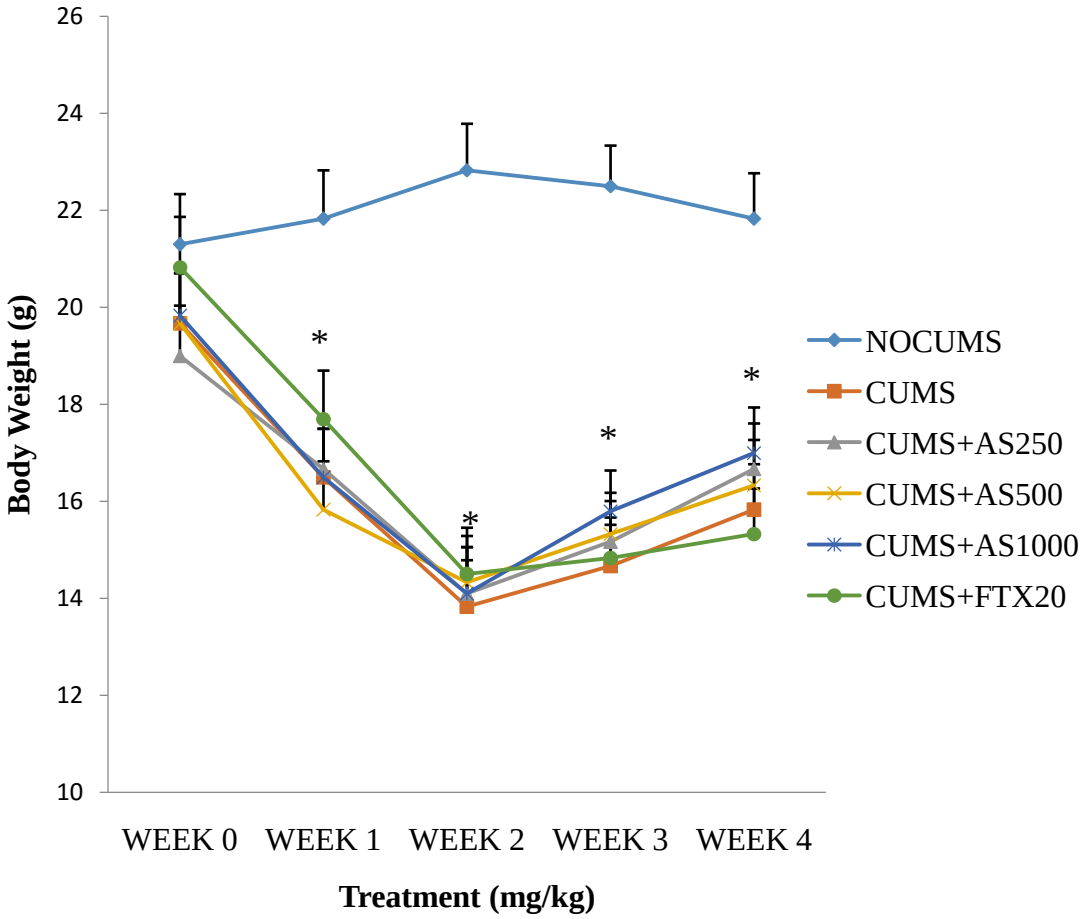

Figure 4.14: Weekly Body Weight Changes of Mice Following Chronic Unpredictable Mild Stress

Results are expressed as Mean ±S.E.M $^{*}p<0.01$= significant difference as compared to the NOCUMS group using Repeated Measure ANOVA followed by Dunnet post hoc test n=9 CUMS= Chronic unpredictable mild stress + distilled water , NOCUMS= No chronic unpredictable mild stress, AS=Methanol root bark extract of *Acacia seyal* , FTX= Fluoxetine

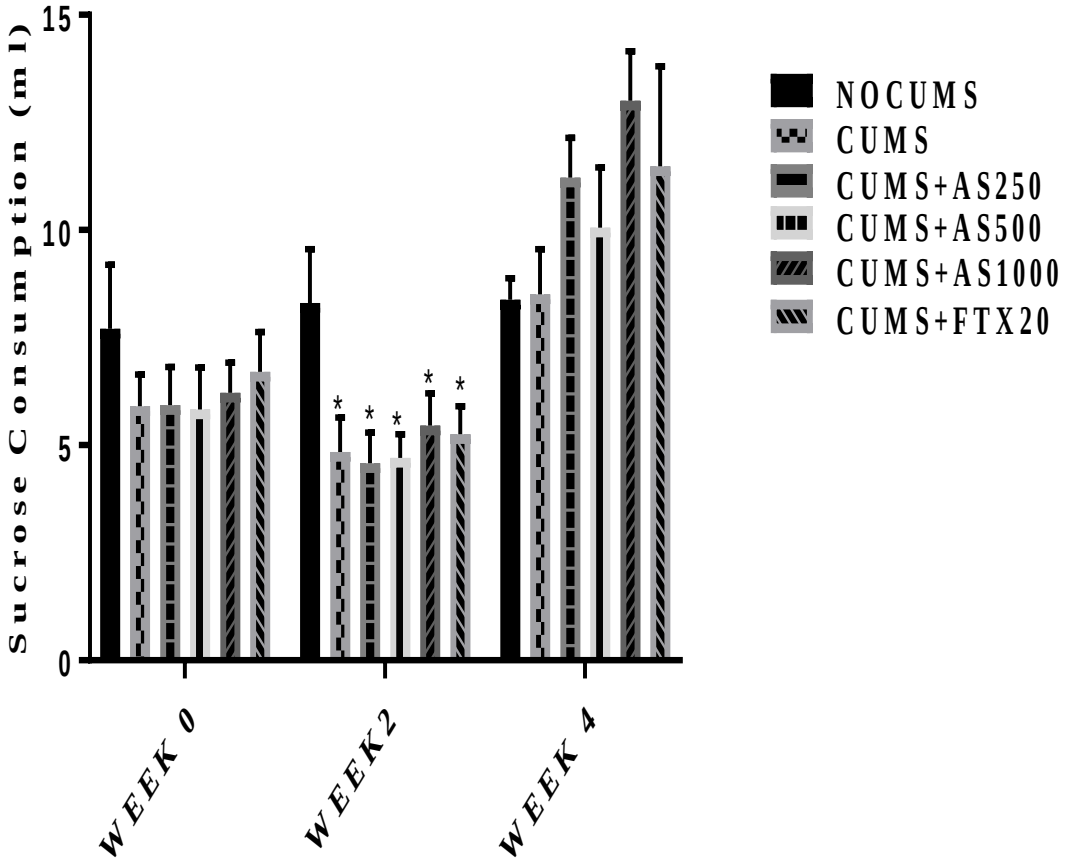

Figure 4.15: Effect of Methanol Root Bark Extract of *Acacia seyal* on Sucrose Consumption Following Chronic Unpredictable Mild Stress

Results are expressed as Mean ±S.E.M $^{*}p<0.01$= significant difference as compared to the NOCUMS group using One Way ANOVA followed by Bonferroni post hoc test n=9 CUMS= Chronic unpredictable mild stress + distilled water , NOCUMS= No chronic unpredictable mild stress, AS= Methanol root bark extract of *Acacia seyal* , FTX= Fluoxetine

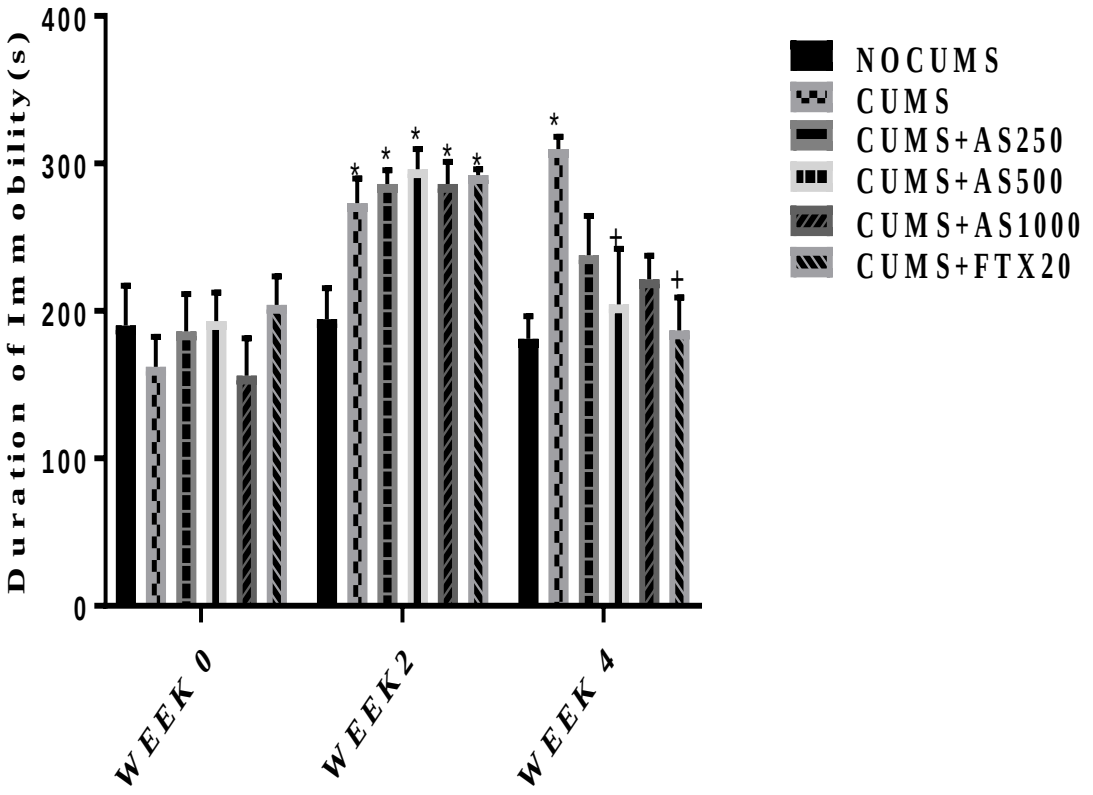

Figure 4.16: Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility Mice Following Chronic Unpredictable Mild Stress

Results are expressed as Mean ±S.E.M $^{*}p<0.01$= significant difference as compared to the NOCUMS group. $^{+}p<0.05$= significant difference as compared to the CUMS group Mice were administered using One-way ANOVA followed by Bonferroni post hoc test CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AS= Methanol root bark extract of *Acacia seyal* , FTX= Fluoxetine

4.10.4 Effect of Methanol Root Bark Extract of *Acacia seyal* on Locomotor Activity of Mice Subjected Open Field Test Followed by Chronic Unpredictable Mild Stress

In the OFT, there was no significant difference in the number of lines crossed among all groups before stress. However, stressed mice showed significant decrease in the number of lines crossed when compared to unstressed mice. After two weeks of treatment, AS (250 mg/kg)and FTX (20mg/kg) showed significant (p<0.05) increase in the number of lines crossed when compared with the stressed group (Figure 4.17).

4.10.5 Effect of Methanol Root Bark Extract of *Acacia seyal* on Cortisol Levels Following Chronic Unpredictable Mild Stress in Mice

The results showed that the stressed group significantly (p<0.05) increase serum cortisol levels when compared with the unstressed group. Administration of AS (250, 500 and 1000 mg/kg) and FTX (20 mg/kg) significantly (p<0.05) decreased cortisol levels as compared to the stressed group (Figure 4.18).

4.10.6 Effect of Methanol Root Bark Extract of *Acacia seyal* on BDNF Levels in Mice

There was a significant (p<0.05) decrease in BDNF levels of mice when compared to the unstressed group following two weeks' exposure to stress. Administration of AS (250, 500 and 1000 mg/kg) and FTX (20mg/kg) significantly (p<0.05) increased the expression of BDNF as compared to the stressed/distilled water group (Figure 4.19).

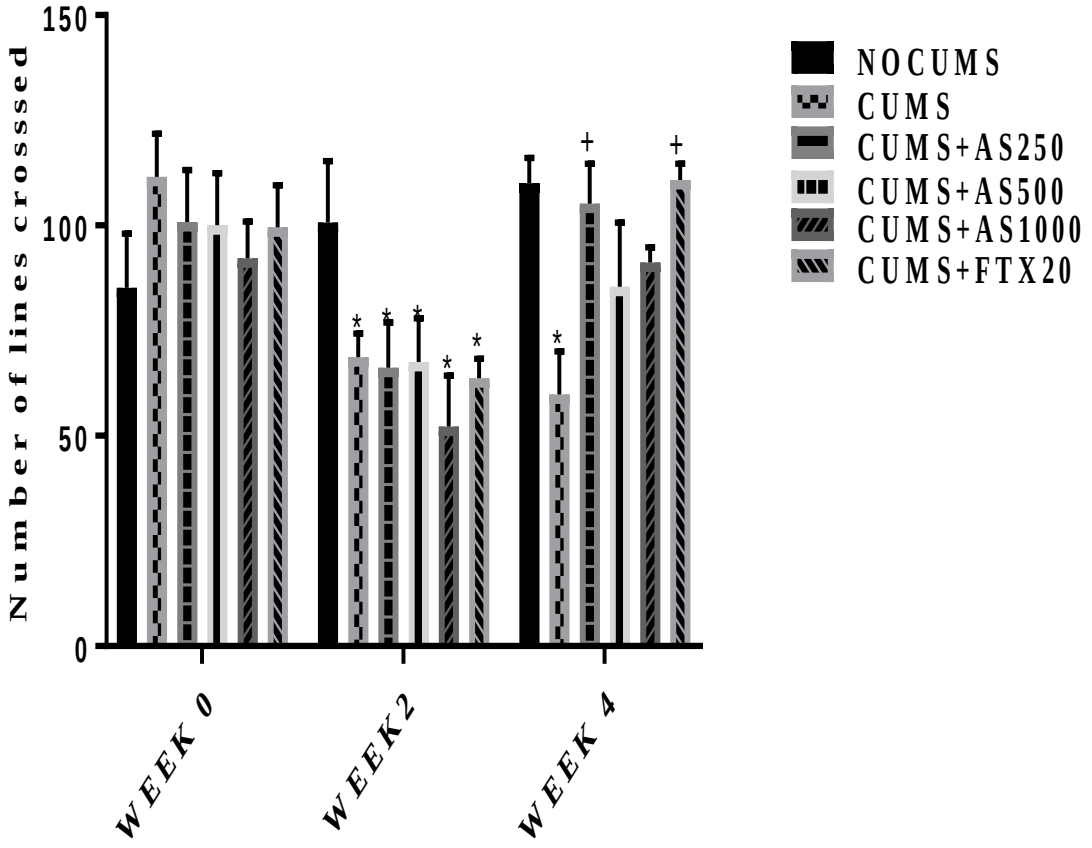

Figure 4.17: Effect of Methanol Root Bark Extract of *Acacia seyal* on Locomotor Activity Following Chronic Unpredictable Mild Stress

Results are expressed as Mean ±S.E.M $^{*}p<0.01$,= significant difference as compared to the NOCUMS group. $^{+}p<0.05$= significant difference as compared to the CUMS group Mice were administered using One-way ANOVA followed by Bonferroni post hoc test CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AS= Methanol root bark extract of *Acacia seyal* , FTX= Fluoxetine

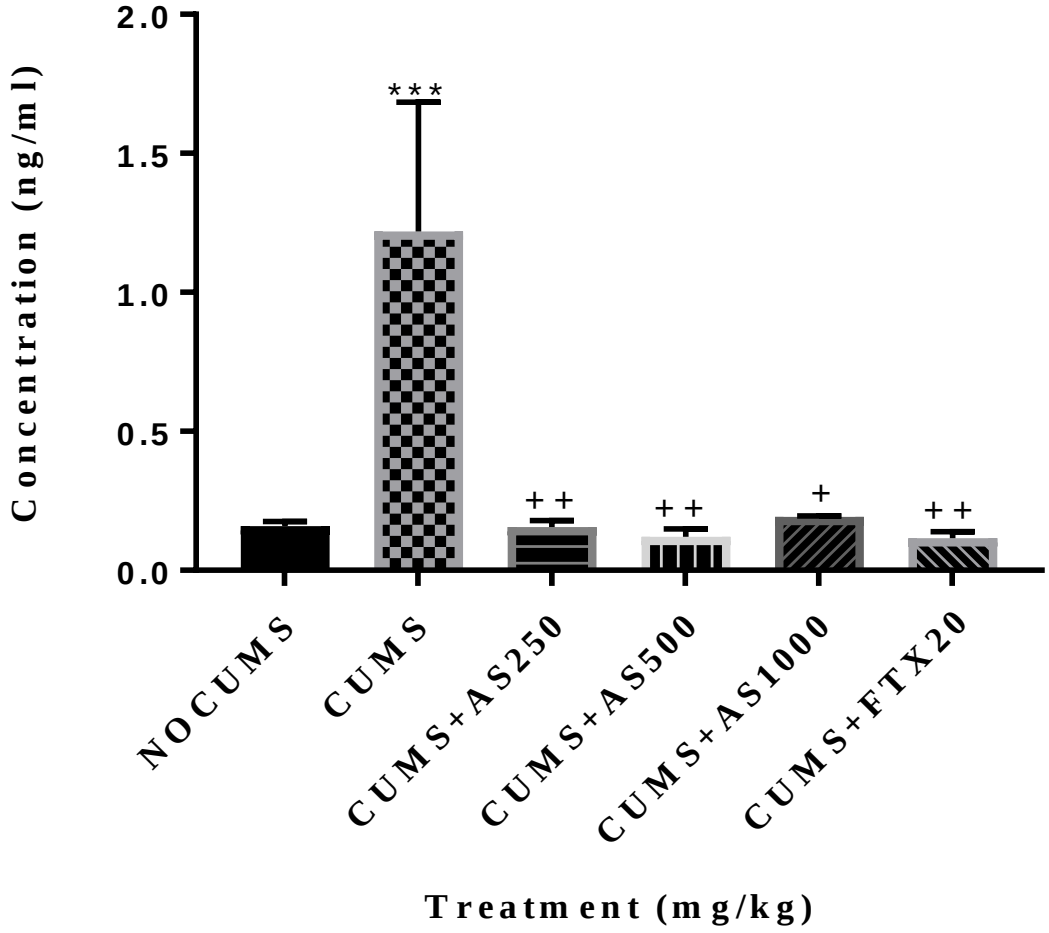

Figure 4.18: Effect of Methanol Root Bark Extract of *Acacia seyal* on Serum Cortisol Levels Following Chronic Unpredictable Mild Stress in Mice

Results are expressed as Mean ±S.E.M $^{***}p<0.001$= significant difference as compared to the NOCUMS group. $^{+}p<0.05$, $^{++}p<0.01$= significant difference as compared to the CUMS group Mice were administered using One-way ANOVA followed by Bonferroni post hoc test CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AS= Methanol root bark extract of *Acacia seyal*, FTX= Fluoxetine

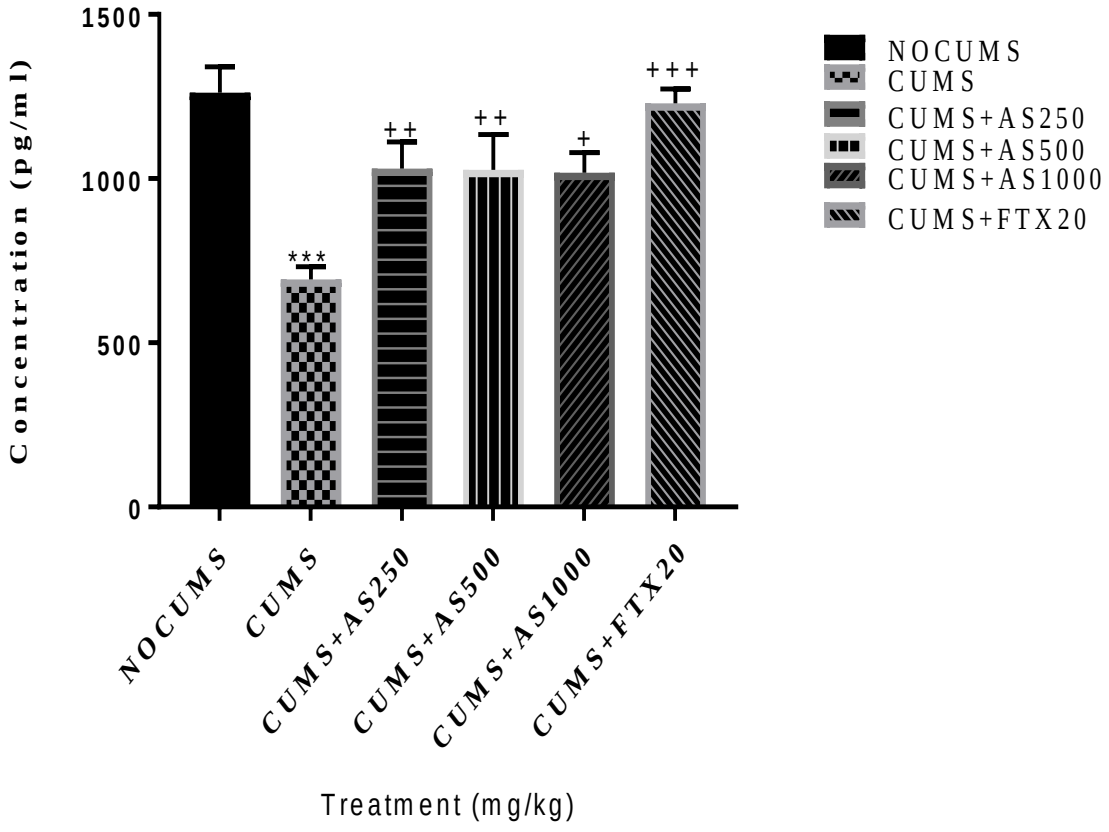

Figure 4.18: Effect of Methanol Root Bark Extract of *Acacia seyal* on Brain Derived Neurotrophic Factor levels in the brain of Mice Following Chronic Unpredictable Mild Stress in Mice

Results were presented as Mean±S.E.M. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test $^{***}p<0.001$ compared to the unstressed group, $^{+}p<0.05$, $^{+++}p<0.001$as compared to the stressed/distilled water group. n=5-9

CHAPTER FIVE

5.0 DISCUSSION

*Acacia seyal*which has been reported to be used in the management of depression in traditional medicine.This study wasaimed at evaluating the antidepressant activity of the methanol root bark extract of *A. seyal* using the tail suspension test, Force swimming test, open field test and chronic unpredictable mild stress.

The median lethal dose of AS was greater than 5000 mg/kg and did not induce mortality or any signs of toxicity implying it is relatively safe. This agrees with the findings of Saleem *et al*. (2016) in which similar result was reported. The Irwin test evaluates the qualitative effects of test substances on the behaviour and autonomic as well as the physiological function of a test animal which provides clues for the classification of the active compounds to proceed for further testing (Irwin, 1968). No death was recorded after administration of the extract. However, an increased ambulation and defecation were noticed suggesting the extract can slightly alter both autonomic and physiologic functions.

The mouse beam walking assay was used to evaluate the effect of AS on motor coordination a very sensitive model in predicting clinical sedation in humans caused by novel drugs (Stanley *et al*., 2005). In the study there was no significant increase in the number of foot slips, an index of motor coordination and balance, thus suggesting that the depressant effect of the extract might be centrally mediated and not due to peripheral muscular blockade (Perez *et al*., 1998).

The antidepressant activity of AS was evaluated using tail suspension test.It is a test approved for the assessment of antidepressant-like effects of medications (Crowley *et al*., 2005; Lad *et al*., 2007). The extract of AS produced a significant decrease in the duration of immobility in the TST. Antidepressants that inhibit serotonin and/or NA reuptake decrease duration of immobility and increase swinging behaviour of mice in the TST (Berrocoso *et al.,* 2013). The findings in this study were similar to some studies that showed that antidepressants decreased the duration of immobility in TST (Jin *et al.,* 2013; Afsar *et al.,* 2017).

The FST differentiates between antidepressants that act via serotonergic and or noradrenergic mechanisms, as noradrenergic compounds selectively increase climbing behaviour (Detke *et al.,* 1995) and drugs with dual effects increased both swimming and climbing (Rénéricand Lucki, 1998). In this study, AS caused a reduction in the immobility time and increase in the swimming behaviour suggesting it has a serotonergic mechanism of action.

The open field test was used to further evaluate exploratory behaviour and general locomotor activity in rodents (Sousa *et al*., 2005; Stanford, 2007). Psychostimulants, convulsants, and anticholinergics are able to increase locomotor activity in the OFT and give a false positive result in the TST and FST (Idayu *et al.,* 2011). Since AS did not produce a significant increase in locomotion, the reduction of immobility time elicited cannot be attributed to any psychostimulant effect.

It is widely accepted that improving brain monoaminergic functions is effective in treating depression and the serotonergic, noradrenergic or dopaminergic systems are targets for development of antidepressants (Lambert *et al.,* 2000; Esposito, 2006; Marazziti *et al*., 2014). In this study, the antidepressant-like effect elicited by AS was reversed by pretreatment of animals with sulpiride (a dopamine D_2 receptor antagonist). This indicates that D_2 receptors might play a role in antidepressant-like effect elicited by AS.

A number of clinical studies indicate that the noradrenergic system is strongly implicated in the pathophysiology of depression (Frazer, 2000; Nutt, 2006). The $\alpha_1$and α_2 -adrenoceptors have been shown to underlie some of the antidepressant-like responses of drugs in behavioural models of depression (Danysz *et al.,* 1986; Masuda *et al.,* 2001).The results obtained from this study showed that pretreatment of mice with prazosin (α_1 adrenoceptor antagonist) and yohimbine (α_2 adrenoceptor antagonist) reversed the antidepressant-like effect of *Acacia seyal* in TST.

The serotonergic system has long been implicated in the pathogenesis of depression (Heninger *et al*., 1996). Some of the most compelling evidence involves the alleviation of depression caused by SSRIs, which increase the availability of serotonin at the synapse (Malagie *et al.,* 2002). Most conventional antidepressants directly affect serotonin turnover in the brain (Kreiss and Lucki, 1995), inhibit serotonin reuptake and also interact with 5-HT $_1$A and 5-HT $_2$ receptors (Cyran *et al.,* 2005). Results obtained from the pretreatment of mice with metergoline (a non-selective 5-HT_2 receptor antagonist) and cypoheptadine (a 5-HT_2 receptor antagonist) reversed the

antidepressant-like effect of ASon TST, suggesting inherent activity of the serotonergic system in the antidepressant effect of AS.

Cholinergic dysfunctions may account for the development of cognitive symptoms associated with depression, especially when the disease is long-lasting and treatment resistant. Moreover, changes in hippocampal neurogenesis may be in part mediated by the cholinergic system and may also relate to the cognitive disturbances diagnosed in depression (Dagyte *et al.,* 2011). Results obtained from the pretreatment of mice with atropine (muscarinic cholinergic receptor antagonist) reversed the antidepressant-like effect of AS on TST.

There is substantial evidence implicating the opioid system in depression (Hegadoren *et al*., 2009), suggesting that compounds that enhance opioid neurotransmission may exert genuine antidepressant effects (Jutkiewicz, 2006; Berrocoso and Mico, 2009). Results obtained from the pretreatment of mice with naloxone (opioid receptor antagonist) reversed the antidepressant-like effect of *Acacia seyal* on TST.

It has become generally accepted that NO plays a significant neuromodulatory role in the nervous system and the pharmacological manipulation of the NO–cGMP signaling pathway may constitute a novel therapeutic approach for the management of depression (Da Silva *et al*., 2000). Pretreatment of mice with L- Arginine a nitric oxide substrate) and L-NNA (a nitric oxide synthase enzyme inhibitor) reversed the antidepressant-like effect of ASon TST.

Findings in this study provide evidence of antidepressant effects produced by AS, which were prevented by 5-HT_2, D_2, α_1 and α_2-adrenoceptor and opiodergic, muscarinic cholinergic receptor antagonists and nitric oxide enzyme inhibitor and substrate.

Chronic stress is an important factor in depression, and changes in various body systems that occur in depression are similar to those observed in response to stress (Muscat *et al*., 1992). Chronic Unpredictable Mild Stress (CUMS), the most promising and valuable rodent model of depression is widely used to investigate and screen for antidepressants. In addition, as proposed by Willner and his colleagues, CUMS appears more suitable for studying the mechanisms of antidepressant drugs (Wilner *et al*., 1992). In the CUMS procedure, animals are exposed to different kinds of mild stress every day, which mimics chronic stressful life events and results in anhedonia and decrease in locomotor activity, the core symptoms of human depression. As a result, these symptoms induced by CUMS procedure can be restored by therapeutically effective drugs for the treatment of depression (Wilner *et al.*, 1992; Muscat *et al*., 1992).

In this study, the effect of AS on CUMS-induced depression was determined, where it reversed the decrease in locomotor activity. Additionally, the mechanism of action of AS was also evaluated regarding the involvement of the neurotrophic, and neuroendocrine systems.

The model was considered successful following application of stressors for two consecutive weeks, after which decreased locomotor behaviour, increased immobility time, weight loss, and decreased sucrose consumptions were observed. Afterwards, an increase in the level of an important neuroendocrine hormone cortisol, on the HPA axis

pathway and the resultant depletion of BDNF, an important growth factor in hippocampal neurogenesis, were all associated with this model. Two weeks aftertreatment with AS, behavioural deficits were reversed. Mice subjected to CUMS showed decreased sucrose consumption, which was an indication of anhedonia (Almeida *et al.,* 2015), and is one of the key symptoms of depressive disorders. It has been postulated that depressed individuals show poor responses to rewards. The depressed mice after treatment with either fluoxetine or AS did not significantly consume more sucrose than the CUMS mice.

In TST, the unstressed mice had shorter durations of immobility compared to the CUMS mice, indicating less behavioural despair. This was so because depressed individuals do not struggle to overcome stressful events in daily life but, instead, show behavioural despair (Evans *et al.,* 2012). In this study, these behavioural symptoms were simulated by CUMS and reversed by treatment with fluoxetine and AS.

The stressed and depressed mice displayed a decrease in locomotor activity in OFT when compared with the unstressed mice, indicating a loss of interest in external stimuli. However, treatment with AS reversed the impaired locomotor activity mice to normal. This result supported the fact that the antidepressant action of AS is specific at the doses tested and is not associated with any stimulating locomotor activity.

A loss in body weight was observed in the CUMS mice, while the unstressed group gained weight all through the experiment. However, there was no statistical difference between AS, fluoxetine, and CUMS mice during 2 weeks' exposure to stressors. Afterwards there was no significant increase in body weight following 2 weeks' treatment with AS and fluoxetine.

The occurrence of depressive symptoms is closely related to the decrease of monoamine neurotransmitters, which are involved in HPA axis hyperactivity (Zhao *et al.,* 2018). Long-term exposure to chronic stress can induce HPA axis hyperactivity, which has been found to cause a reduction in monoamine neurotransmitters in the central and peripheral nervous systems as well as an increase in serum levels of cortisol (Khajehnasiri *et al.,* 2013). The resultant disruption in the HPA axis is a manifestation of depressive behaviour. Antidepressants relieve HPA axis hyperactivity, improve the levels of monoamine neurotransmitter, and also decrease the levels of excess cortisol in circulation. In this study, AS significantly reversed hyperactivity in the HPA axis by reducing serum cortisol levels. Therefore, the antidepressant effect of AS on CUMS mice may involve the inhibition of the HPA axis and upregulation of the monoamine levels. This further support the findings that AS could exert its antidepressant effect by normalizing HPA axis hyperactivity through cortisol levels regulation.

The neurotrophic hypothesis has been found to be worthy of attention in recent years in addition to the monoamine hypothesis (Liu *et al.,* 2018). Brain derived neurotrophic factor (BDNF) is essential for neuronal plasticity and survival in the adult brain (Mizui *et al.,* 2016). Alterations of brain BDNF levels in the pathophysiology of depression and the effects of antidepressants depend on brain areas (Yu and Chen, 2011). An increase of the hippocampal BDNF following chronic antidepressant treatment is associated with neurogenesis (Lyon *et al.,* 2011), while the antidepressant action in the prefrontal cortex relates to synaptic plasticity (Sairanen *et al.,* 2007).

In this study, AS and fluoxetine increased BDNF levels in the brain. The findings of this study suggest that AS possesses neuroprotective effect that may be beneficial against depression. The results obtained in this study are similar to those of others researches in

which BDNF concentrations were reduced in the brain of mice exposed to CUMS and reversed by administration of antidepressants (Dong *et al.*, 2014; Boonlert *et al.*, 2017).

Medicinal plants contain a wide range of metabolites for the management of symptoms associated with various mental disorders (Rahimi and Abdollahi, 2012; Farzaei *et al*., 2013; Farahani *et al*., 2015). Phytoconstituents such as alkaloids, flavonoids and saponins have been reported to possess antidepressant properties (Nesterova *et al.*, 2011; Xiong *et al*., 2011; Fajemiroye *et al*., 2014). Flavonoids have been widely studied for their antidepressant effect. Naringenin is one of the flavonoids in grapefruit (Felgines *et al*., 2000), represented its antidepressant effect by elevation of 5-HT, NE, and BDNF levels as well as glucocorticoidreceptors (Yi *et al.*, 2010, 2012, 2014).

The serotonergic, noradrenergic, and dopaminergiceffects of berberine, an isoquinoline alkaloid of barberry, have been demonstrated in several studies (Peng *et al.*, 2007; Bahramsoltani *et al*., 2015; Perviz *et al.*, 2016). Berberine acts via the l-arginine-NO-cGMP pathway as well as δ opioid receptors. Nitric Oxide synthase (NOS) converts l-arginine to NO, which results in elevation of intracellular cGMP in neuronal cells. Previous studies demonstrated the antidepressant activity of NOS inhibitors and the role of NO in depression pathophysiology (Kulkarni and Dhir, 2007; Freitas *et al.*, 2013). Glycyrrhizin, a triterpene saponin from derived from the underground parts of *Glycyrrhiza* plant known as Chinese licorice (Seki *et al*., 2008) was found to possess antidepressant effect by the involvement of α_1 adrenoceptor and DA D_2receptor (Dhingra and Sharma, 2005).

The phytochemical screening showed that AS contained phytoconstituents such as alkaloids, flavonoids and saponins which may be responsible for the observed antidepressant like effects of AS seen in this study.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Phytochemical screening of methanol root bark extract of *A. seyal* revealed the presence of secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids/triterpenes and carbohydrates.

Oral acute toxicity study of AS showed that it was relatively safe. Pharmacological studies provided some scientific rationale for the use of *Acacia seyal* in the management of depression.

Mice were subjected to chronic unpredictable mild stress (CUMS)-induced depression. The methanol root bark extract of *Acacia seyal* showed no significant effect on weight and anhedonia. However it reversed the decrease in the duration of immobility and the number of lines crossed induced by CUMS-induced depression following two weeks of treatment.

The study established the possible mechanism of action of *Acacia seyal*, which include the involvement of monoaminergic, opioidergic, cholinergic, nitric oxide, neurotrophic and neuroendocrine systems.

6.2 Conclusion

The methanol root bark extract of *Acacia seyal* possesses antidepressant activity providing scientific rationale for the traditional use of the plant in the management of

depression. The antidepressant activity of the extract may possibly be mediated via monoaminergic, opioidergic, cholinergic, nitric oxide pathway, neurotrophic and neuroendocrine systems.

6.3 Recommendations

1. There is need to carry out bioassay guided fractionation to isolate and test the bioactive constituents responsible for the antidepressant activity of *Acacia seyal*
2. A chronic toxicity study needs to be carried out to ascertain the safety of *Acacia seyal*
3. There is a need to evaluate the possible involvement of the neuro-inflammatory pathway in the antidepressant activity of the plant.

6.4 Contributions to Knowledge

1. The study provided some scientific rationale for the folkoric use of *Acacia seyal* in the management of depression.
2. The study showed *Acacia seyal* is a promising plant with antidepressant action on Chronic unpredictable mild stress induced depression.
3. The study established the possible mechanisms of action of methanol root bark extract of *Acacia seyal* which involve neurotransmitter (dopaminergic, serotonergic, adrenergic, opioidergic and muscarinic), neurotrophic (brain derived neurotrophic factor) nitric oxide and neuroendocrine (cortisol) systems.

References

Abbasi, S., Bekhradi, R., Asgharpanah, J., and Abbasi, M.F. (2013) Antidepressant effect of aqueous and hydroalcoholic extracts of *Lavandula officinalis* in forced swim test and tail suspension test in male mice. *Journal of Arak University of Medical Sciences*, *16*: 65-75

Adell, A., Castro, E., Celada, P., Bortolozzi, A., Pazos, A., and Artigas, F. (2005). Strategies for producing faster acting antidepressants. *Drug Discovery Today*, *10*(8): 578-585.

Afifi, T.O., Cox, B.J., and Enns, M.W. (2006). Mental health profiles among married, never-married, and separated/divorced mothers in a nationally representative sample. *Social Psychiatry and Psychiatric Epidemiology*, *41*: 122–129.

Afsar, T., Razak, S., Khan, M.R., and Almajwal, A. (2017). Anti-depressant and anxiolytic potential of *Acacia hydaspica* R. Parker aerial parts extract: modulation of brain antioxidant enzyme status. *BioMed Central Complementary and Alternative Medicine*, *17*(1): 228.

Aguilar-Gaxiola, S., Elliott, K., Deeb-Sossa, N., King, R.T., Magaña, C.G., Miller, E., Sala, M., Sribney, W.M., and Breslau, J. (2008). *Engaging the Underserved: Personal Accounts of Communities on Mental Health Needs for Prevention and Early Intervention Strategies.*Monograph No. 1, Center for Reducing Health Disparities. Sacramento: University of California, Davis. Pp10-15

Albert, P.R. (2015). Why is depression more prevalent in women?*Journal of Psychiatry and Neuroscience*, *40*(4): 219.

Alloy, L.B., Abramson, L.Y., Smith, J.M., Gibb, B.E., and Neeren, A.M. (2006). Role of parenting and maltreatment histories in unipolar and bipolar mood disorders: Mediation by cognitive vulnerability to depression. *Clinical Child and Family Psychology Review*, *9*: 23–64.

Almeida, J., Duarte, J.O., Oliveira, L.A., and Crestani, C.C. (2015). Effects of nitric oxide synthesis inhibitor or fluoxetine treatment on depression-like state and cardiovascular changes induced by chronic variable stress in rats. *Stress*, *18*(4): 462-474.

Altshuler, L.L., Bauer, M., Frye, M.A., Gitlin, M.J., Mintz, J., Szuba, M.P., and Whybrow, P.C. (2001). Does thyroid supplementation accelerate tricyclic antidepressant response? A review and meta-analysis of the literature. *American*

Journal of Psychiatry, *158*(10): 1617-1622.

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders-5*. Washington, DC: American Psychiatric Pub.

Atake, K., Yoshimura, R., Hori, H., Katsuki, A., Ikenouchi-Sugita, A., Umene-Nakano, W., and Nakamura, J. (2014). Duloxetine, a selective noradrenaline reuptake inhibitor, increased plasma levels of 3-methoxy-4-hydroxyphenylglycol but not homovanillic acid in patients with major depressive disorder. *Clinical Psychopharmacology and Neuroscience*, *12*(1): 37.

Atzori, M., Cuevas-Olguin, R., Esquivel-Rendon, E., Garcia-Oscos, F., Salgado-Delgado, R.C., Saderi, N., and Salgado, H. (2016). Locus ceruleus norepinephrine release: a central regulator of CNS spatio-temporal activation?*Frontiers in Synaptic Neuroscience*, 8: 25.

Bahramsoltani, R., Farzaei, M.H., Farahani, M.S., and Rahimi, R. (2015). Phytochemical constituents as future antidepressants: a comprehensive review. *Reviews in the Neurosciences*, *26*(6): 699-719.

Banks, K.H., and Kohn-Wood, L.P. (2007). The influence of racial identity profiles on the relationship between racial discrimination and depressive symptoms. *Journal of Black Psychology*, *33*: 331–354.

Barchas, J. D., and Altemus, M. (1999). Monoamine hypotheses of mood disorders. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Lippincott-Raven, Philadelphia, Available from: ⟨ http://www. ncbi. nlm. nih. gov/books/NBK28257.

Baxter, A.J., Vos, T., Scott, K.M., Ferrari, A.J., and Whiteford, H.A. (2014). The global burden of anxiety disorders in 2010. *Psychological Medicine*, *44*(11): 2363-2374.

Berrocoso, E., and Mico, J.A. (2009). Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. *International Journal of Neuropsychopharmacology*, *12*(8): 1033-1044.

Berrocoso, E., Ikeda, K., Sora, I., Uhl, G.R., Sánchez-Blázquez, P., and Mico, J.A. (2013). Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. *International Journal of Neuropsychopharmacology*, *16*(1): 151-162.

Bifulco, A., Moran, P., Baines, R., Bunn, A., and Stanford, K. (2002. a). Exploring psychological abuse in childhood: II. Association with other abuse and adult clinical depression. *Bulletin of the Menninger Clinic*, *66*: 241–258.

Bjørnebekk, A., Mathé, A.A., and Brené, S. (2010). The antidepressant effects of running

and escitalopram are associated with levels of hippocampal NPY and Y1 receptor but not cell proliferation in a rat model of depression. *Hippocampus,* 20(7): 820-828.

Bonfiglio, J. J., Inda, C., Refojo, D., Holsboer, F., Arzt, E., and Silberstein, S. (2011). The corticotropin-releasing hormone network and the hypothalamic-pituitary-adrenal axis: molecular and cellular mechanisms involved. *Neuroendocrinology,* 94(1): 12-20.

Boonlert, W., Benya-aphikul, H., Umka Welbat, J., and Rodsiri, R. (2017). Ginseng extract G115 attenuates ethanol-induced depression in mice by increasing brain BDNF levels. *Nutrients*, *9*(9): 931.

Bose, A., Li, D., and Gandhi, C. (2008). Escitalopram in the acute treatment of depressed patients aged 60 years or older. *The American Journal of Geriatric Psychiatry*, *16*(1): 14-20.

Brigitta B. (2002). Pathophysiology of depression and mechanisms of treatment. *Dialogues in Clinical Neuroscience.*;*4(*7), 20.

Brody, D.J., Pratt, L.A., and Hughes, J.P. (2018). Prevalence of Depression Among Adults Aged 20 and Over: United States, 2013-2016. *National Centre for Health Statistics data brief,* (303): 1-8.

Brown, C., Schulberg, H.C., Madonia, M.J., Shear, M.K., and Houck, P.R. (1996). Treatment outcomes for primary care patients with major depression and lifetime anxiety disorders. *The American Journal of Psychiatry*, 153(10): 1293.

Brown, G.W., Harris, T.O., and Hepworth, C. (1994). Life events and endogenous depression: a puzzle reexamined. *Archives of General Psychiatry,* 51(7): 525-534.

Butterweck, V. (2003). Mechanism of action of St John's wort in depression. *Central Nervous System Drugs, 17*(8), 539-562.

Bymaster, F.P., Beedle, E. E., Findlay, J., Gallagher, P.T., Krushinski, J.H., Mitchell, S., and Wong, D. T. (2003). Duloxetine (Cymbalta™), a dual inhibitor of serotonin and norepinephrine reuptake. *Bioorganic and Medicinal Chemistry Letters*, *13*(24): 4477-4480.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., and Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science, 301*, 386–389.

Cassani, J., Ferreyra-Cruz, O.A., Dorantes-Barrón, A.M., Villaseñor, R.M.V., Arrieta-Baez, D., and Estrada-Reyes, R. (2015). Antidepressant-like and toxicological

effects of a standardized aqueous extract of *Chrysactinia mexicana* A. Gray (Asteraceae) in mice. *Journal of Ethnopharmacology, 171*: 295-306.

Cassano, P., Lattanzi, L., Soldani, F., Navari, S., Battistini, G., Gemignani, A., and Cassano, G.B. (2004). Pramipexole in treatment- resistant depression: an extended follow- up. *Depression and Anxiety*, *20*(3): 131-138.

Cavanagh, H. M. A., and Wilkinson, J. M. (2002). Biological activities of lavender essential oil. *Phytotherapy Research*, 16(4): 301-308.

Charney, D.S., Heninger, G.R., and Sternberg, D.E. (1984). The effect of mianserin on alpha-2 adrenergic receptor function in depressed patients. *The British Journal of Psychiatry,* 144(4):407-416.

Chikezie, U.E., Otakpor, A.N., Kuteyi, O.B., and James, B.O. (2013). Depression among people living with human immunodeficiency virus infection/acquired immunodeficiency syndrome in Benin City, Nigeria: A comparative study. *Nigerian Journal of Clinical Practice, 16*(2): 238-242.

Clayton, A.H., Pradko, J.F., Croft, H.A., Montano, C.B., Leadbetter, R.A., Bolden-Watson, C., ... and Metz, A. (2002). Prevalence of sexual dysfunction among newer antidepressants. *The Journal of Clinical Psychiatry,10*:62–70

Connor, T.J., and Leonard, B.E. (1998): Depression, Stress and Immunological Activation: The Role of Cytokines in Depressive Disorders. *Life Science*. *62*(7): 583-606.

Conrad, P., and Adams, C. (2012). The effects of clinical aromatherapy for anxiety and depression in the high risk postpartum woman–a pilot study. *Complementary Therapies in Clinical Practice,18*(3): 164-168.

Cooper, R., and Lerer, B. (2010). The use of thyroid hormones in the treatment of depression. *Harefuah,149*(8): 529-34.

Coppen, A. (1967). The biochemistry of affective disorders. *The British Journal of Psychiatry*, *113*(504): 1237-1264.

Coronas, V., Bantubungi, K., Fombonne, J., Krantic, S., Schiffmann, S.N., and Roger, M. (2004). Dopamine D3 receptor stimulation promotes the proliferation of cells derived from the post-natal subventricular zone. *Journal of Neurochemistry, 91*(6), 1292-1301.

Corrigan, M.H., Denahan, A.Q., Wright, C.E., Ragual, R.J., and Evans, D.L. (2000). Comparison of pramipexole, fluoxetine, and placebo in patients with major depression. *Depression and Anxiety, 11*(2), 58-65.

Crowley, J. Blendy, J. and Lucki, I. (2005): Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology* (Berl).*183*: 257-64.

Cryan J.F., Page M.E. and Lucki, I. (2005). Differential behavioural effects of the antidepressant reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology182*, 335–344

Cryan, J.F., Bruijnzeel, A.W., Skjei, K.L., and Markou, A. (2003). Bupropion enhances brain reward function and reverses the affective and somatic aspects of nicotine withdrawal in the rat. *Psychopharmacology*, *168*(3), 347-358.

Curtis, M.A., Low, V.F., and Faull, R..L. (2012). Neurogenesis and progenitor cells in the adult human brain: a comparison between hippocampal and subventricular progenitor proliferation. *Developmental Neurobiology,72*(7): 990-1005.

Cyranowski, J.M., Frank, E., Young, E., and Shear, M.K. (2000). Adolescent onset of the gender difference in lifetime rates of major depression: A theoretical model. *Archives of General Psychiatry, 57*, 21–27.

Dagytė, G., Crescente, I., Postema, F., Seguin, L., Gabriel, C., Mocaër, E., and Koolhaas, J. M. (2011). Agomelatine reverses the decrease in hippocampal cell survival induced by chronic mild stress. *Behavioural Brain Research*, *218*(1), 121-128.

Danysz, W., Kostowski, W., Kozak, W., and Hauptmann, M. (1986). On the role of noradrenergic neurotransmission in the action of desipramine and amitriptyline in animal models of depression. *Polish Journal of Pharmacology and Pharmacy*, *38*(3), 285-298.

Da Silva, G.D. L., Matteussi, A.S., Dos Santos, A.R.S., Calixto, J. B., and Rodrigues, A. L. S. (2000). Evidence for dual effects of nitric oxide in the forced swimming test and in the tail suspension test in mice. *Neuroreport*, *11*(17): 3699-3702.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., and Artymyshyn, R. P. (2009). Behavioral effects of fluoxetine in an animal model of anxiety/depression are mediated by both neurogenesis-dependent and independent mechanisms. *Neuron*, 62(4): 479.

Delgardo P, and Morena F. (2006): Neurochemistry of mood disorders. In: Stein DK, Kupfer DJ, Schatzberg AF, editors. The textbook of mood disorders. Washington DC: American Psychiatric Publishing Inc. p. 101-116.

Detke, M.J., Rickels, M., and Lucki, I. (1995). Active behaviours in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology*, *121*(1): 66-72.

Dhabhar, F.S. (2000). Acute stress enhances while chronic stress suppresses skin mmunity: the role of stress hormones and leukocyte trafficking. *Annals of the New York Academy of Sciences,917*(1): 876-893.

Dhingra, D., and Sharma, A. (2005). Evaluation of antidepressant-like activity of glycyrrhizin in mice. *Indian Journal of Pharmacology*, *37*(6), 390.

Dong, C., Wong, M.L., and Licinio, J. (2009). Sequence variations of ABCB1, SLC6A2, SLC6A3, SLC6A4, CREB1, CRHR1 and NTRK2: association with major depression and antidepressant response in Mexican-Americans. *Molecular Psychiatry*, *14*(12): 1105-1118.

Dong, H., Gao, Z., Rong, H., Jin, M., and Zhang, X. (2014). β-asarone reverses chronic unpredictable mild stress-induced depression-like behavior and promotes hippocampal neurogenesis in rats. *Molecules*, *19*(5): 5634-5649.

Duke, J.A. (1983). Acacia seyal Del. *Handbook of energy crops. unpublished. Web page of Purdue University, Centre for new Crops and Plants Products. Available online at (www. hort. purdue. edu/newcrop/duke_energy/Acacia_seyal. html, Accessed 2006 May, 10)*.

Duman, R.S. (2009). Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. *Dialogues in Clinical Neuroscience*, *11*(3), 239.

Dunn, A.J. (2008). The Cytokines and depression hypothesis: an evaluation. *Neuroimmune Biology, 6*: 485-506.

Duval, F., Mokrani, M.C., Bailey, P., Correa, H., Diep, T.S., Crocq, M.A., and Macher, J. P. (1999). Thyroid axis activity and serotonin function in major depressive episode. *Psychoneuroendocrinology, 24*(7): 695-712.

Effati-Daryani, F., Mohammad-Alizadeh-Charandabi, S., Mirghafourv and, M., Taghizadeh, M.,and Mohammadi, A. (2015). Effect of lavender cream with or without foot-bath on anxiety, stress and depression in pregnancy: a randomized placebo-controlled trial. *Journal of Caring Sciences,4*(1): 63.

Eisch, A. J.,and Petrik, D. (2012). Depression and hippocampal neurogenesis: a road to remission?.*Science*, *338*(6103): 72-75.

Eisensamer, B., Rammes, G., Gimpl, G., Shapa, M., Ferrari, U., Hapfelmeier, G., ... and Holsboer, F. (2003). Antidepressants are functional antagonists at the serotonin type 3 (5-HT 3) receptor. *Molecular Psychiatry*, *8*(12), 994.

Elgazali G.E.B, Tohami MS, Elegami A.A.B, Abdalla W.S and Galal M.A. (1997). Medicinal plants of the Sudan, Medicinal plants of northern Kordofan.Part IV.Omdurman Islamic University printing and publishing house, Sudan, 119.

Elnour, A. A. M., Mirghani, M. E. S., Kabbashi, N. A., Md Alam, Z., and Musa, K. H. (2018). Study of Antioxidant and Anti-Inflammatory Crude Methanol Extract and Fractions of Acacia seyal Gum. *American Journal of Pharmacology and Pharmacother*apeutics *5*(1), 3.

Esposito, E. (2006). Serotonin-dopamine interaction as a focus of novel antidepressant drugs. *Current Drug Targets*, *7*(2): 177-185.

Evans, J., Sun, Y., McGregor, A., and Connor, B. (2012). Allopregnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress. *Neuropharmacology*, *63*(8): 1315-1326.

Evans, W.C. (1996). Trease and Evans Pharmacognosy London Villiere Tindal U.K.., Pp 57–77

Fajemiroye, J.O., da Silva, D.M., de Oliveira, D.R., and Costa, E.A. (2016). Treatment of anxiety and depression: medicinal plants in retrospect. *Fundamental and Clinical Pharmacology*, *30*(3), 198-215

Fajemiroye, J.O., Galdino, P.M., Florentino, I.F., Da Rocha, F.F., Ghedini, P.C., Polepally, P.R., Zjawiony, J.K., and Costa E.A. (2014). Plurality of anxiety and depression alteration mechanism by oleanolic acid. *Journal of Psychopharmacology*. 28: 923–934.

Farahani, M.S., Bahramsoltani, R., Farzaei, M.H., Abdollahi, M., and Rahimi, R. (2015). Plant-derived natural medicines for the management of depression: an overview of mechanisms of action. *Reviews in the Neurosciences*, *26*(3), 305-321.

Farzaei, M.H., Abbasabadi, Z. Ardekani, M.R., Rahimi, R., and Farzaei, F. (2013). Parsley, a review of ethnopharmacology, phytochemistry and biological activities. *Journal of Traditional Chinese Medicine 33*, 815–826.

Felgines, C., Texier, O., Morand, C., Manach, C., Scalbert, A., Régerat, F., and Rémésy, C. (2000). Bioavailability of the flavanone naringenin and its glycosides in rats. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *279*(6), G1148-G1154.

Ferrari, A.J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J., and Whiteford, H. A. (2013a). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *Public Library of Science Medicine*, 10(11), e1001547.

Ferrari, A.J., Somerville, A. J., Baxter, A. J., Norman, R., Patten, S. B., Vos, T., and Whiteford, H. A. (2013b). Global variation in the prevalence and incidence of major depressive disorder: a systematic review of the epidemiological literature. *Psychological Medicine*, 43(3): 471-481.

Forbes, N.F., Stewart, C. A., Matthews, K., and Reid, I. C. (1996). Chronic mild stress and sucrose consumption: validity as a model of depression. *Physiology and Behavior*, *60*(6), 1481-1484.

Ford, A.H., Flicker, L., Singh, U., Hirani, V., and Almeida, O.P. (2013). Homocysteine, depression and cognitive function in older adults. *Journal of Affective Disorders*,

151(2): 646-651.

Ford, T., Goodman, R., and Meltzer, H. (2003). The British child and adolescent mental health survey 1999: the prevalence of DSM-IV disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*(10): 1203-1211.

Frazer, A. (2000). Norepinephrine involvement in antidepressant action. *The Journal of Clinical Psychiatry, 61*(l10), 25-30.

Freitas, A.E., Moretti, M., Budni, J., Balen, G.O., Fernandes, S.C., Veronezi, P.O., and Rodrigues, A.L.S. (2013). NMDA Receptors and the L-Arginine–Nitric Oxide–Cyclic Guanosine Monophosphate Pathway Are Implicated in the Antidepressant-Like Action of the Ethanolic Extract from *Tabebuia avellanedae* in Mice. *Journal of Medicinal Food*, *16*(11), 1030-1038.

Galynker, I. (2017). The suicidal crisis: clinical guide to the assessment of imminent suicide risk. Oxford University Press., Pp 1-9

Garriock, H.A. (2006). Genetics of Major Depressive Disorder in Treatment Resistance and Tryptophan Depletion. University of Arizona USA. Pp 31-42

Gartlehner, G., Gaynes, B.N., Amick, H.R., Asher, G. N., Morgan, L.C., Coker-Schwimmer, E., and Bann, C. (2016). Comparative benefits and harms of antidepressant, psychological, complementary, and exercise treatments for major depression: an evidence report for a clinical practice guideline from the American College of Physicians. *Annals of Internal Medicine*, *164*(5), 331-341.

Ge, X., Lorenz, F.O., Conger, R.D., Elder, G.H., and Simons, R.L. (1994). Trajectories of stressful life events and depressive symptoms during adolescence. *Developmental Psychology*, 30, 467–483.

Gee, G.C., Spencer, M., Chen, J., Yip, T., and Takeuchi, D.T. (2007). The association between self-reported racial discrimination and 12-month DSM-IV mental disorders among Asian Americans nationwide. *Social Science and Medicine*, *64*, 1984–1996.

Gibb, B.E., Butler, A.C., and Beck, J.S. (2003). Childhood abuse, depression, and anxiety in adult psychiatric outpatients. *Depression and Anxiety*, *17*, 226–228.

Gillespie, C.F., and Nemeroff, C.B. (2005). Hypercortisolemia and Depression. *Psychosomatic Medicine,67*: S26-S28.

Glassman, A. H. (1998). Cardiovascular effects of antidepressant drugs: updated. *The Journal of Clinical Psychiatry*, *59*, 13-18.

Gold, P.W., Goodwin, F.K., and Chrousos, G.P. (1988). Clinical and biochemical manifestations of depression. *New England Journal of Medicine, 319*(7): 413-420.

Gold, P.W., Machado-Vieira, R., and Pavlatou, M.G. (2015). Clinical and biochemical manifestations of depression: relation to the neurobiology of stress. *Neural Plasticity*, 2015: 581-976.

Goldstein, D. J., Mallinckrodt, C., Lu, Y., and Demitrack, M.A. (2002). Duloxetine in the treatment of major depressive disorder: a double-blind clinical trial. *The Journal of Clinical Psychiatry*, *63*(3), 225-231.

Grace, A.A. (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nature Reviews Neuroscience*, *17*(8): 524-532.

Greenberg, P.E., Kessler, R.C., Birnbaum, H.G., Leong, S.A., Lowe, S.W., Berglund, P.A., and Corey-Lisle, P. K. (2003). The economic burden of depression in the United States: how did it change between 1990 and 2000? *Journal of Clinical Psychiatry*, *64*(12): 1465-1475.

Grundmann, O.L. V.Y., Lv, Y., Kelber, O., and Butterweck, V. (2010). Mechanism of St. John's wort extract (STW3-VI) during chronic restraint stress is mediated by the interrelationship of the immune, oxidative defense, and neuroendocrine system. *Neuropharmacology*, *58*(4-5): 767-773.

Gu, L., Liu, Y.J., Wang, Y.B., and Yi, L. T. (2012)). Role for monoaminergic systems in the antidepressant-like effect of ethanol extracts from *Hemerocallis citrina*. *Journal of Ethnopharmacology*, *139*(3), 780-787

Gulbins, E., Walter, S., Becker, K. A., Halmer, R., Liu, Y., Reichel, M., and Kornhuber, J. (2015). A central role for the acid sphingomyelinase/ceramide system in neurogenesis and major depression. *Journal of Neurochemistry*, *134*(2): 183-192.

Gur, E., Lerer, B., and Newman, M.E. (1999). Chronic clomipramine and triiodothyronine increase serotonin levels in rat frontal cortex in vivo: relationship to serotonin autoreceptor activity. *Journal of Pharmacology and Experimental Therapeutics*, *288*(1): 81-87.

Hage, M. P., and Azar, S. T. (2012). The link between thyroid function and depression. *Journal of Thyroid Research*, 2012. X. Article ID 590648, 8 pages, 2012. doi:10.1155/2012/590648

Hall, J. B., and McAllan, A. (1993). Acacia seyal: a Monograph. School of Agricultural and Forest Sciences, University of Wales, Bangor. No. 4

Hammen C. (2005): Stress and depression. *Annual Reviews of Clinical Psychology*. 1: 293–319.

Hammen, C. (2006). Stress generation in depression: Reflections on origins, research, and future directions. *Journal of Clinical Psychology*, *62*, 1065–1082.

Hammen, C., Henry, R., and Daley, S.E. (2000). Depression and sensitization to stressors among young women as a function of childhood adversity. *Journal of Consulting and Clinical Psychology*, *68*, 782–787.

Han, P., Han, T., Peng, W., and Wang, X. R. (2013). Antidepressant-like effects of

essential oil and asarone, a major essential oil component from the rhizome of *Acorus tatarinowii*. *Pharmaceutical Biology, 51(5):* 589-594.

Hanson, N.D., Owens, M.J., and Nemeroff, C.B. (2011). Depression, antidepressants, and neurogenesis: a critical reappraisal. *Neuropsychopharmacology,* 36(13): 2589.

Harkness, K.L., Bruce, A.E., and Lumley, M.N. (2006). The role of childhood abuse and neglect in the sensitization to stressful life events in adolescent depression. *Journal of Abnormal Psychology, 115*, 730–741.

Hegadoren, K.M., O'Donnell, T., Lanius, R., Coupland, N.J., and Lacaze-Masmonteil, N. (2009). The role of β-endorphin in the pathophysiology of major depression. *Neuropeptides*, *43*(5), 341-353.

Heim, C., and Nemeroff, C.B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: Preclinical and clinical studies. *Biological Psychiatry, 49*, 1023–1039.

Heninger, G.R., Delgado, P.L., and Charney, D.S. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry, 29*(01): 2-11.

Henn, F.A., and Vollmayr, B. (2004). Neurogenesis and depression: etiology or epiphenomenon?*Biological Psychiatry*, 56(3): 146-150.

Herman, J.P., and Cullinan, W.E. (1997). Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis. *Trends in Neurosciences*, *20*(2): 78-84.

Hirschfeld, R. M. (1999). Efficacy of SSRIs and newer antidepressants in severe depression: comparison with TCAs. *Journal of Clinical Psychiatry*, *60*(5), 326-335.

Hoehe, M., Valido, G., and Matussek, N. (1986). Growth hormone response to clonidine in endogenous depressive patients: evidence for a trait marker in depression. Biological Psychiatry. *Development in Psychiatry, 7*: 788-790.

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology,23*(5): 477-501.

Holsboer, F., and Barden, N. (1996). Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocrine Reviews*, *17*(2): 187-205.

Hong, E.D., Taylor, W.D., McQuoid, D.R., Potter, G.G., Payne, M.E., Ashley-Koch, A., and Steffens, D. C. (2009). Influence of the MTHFR C677T polymorphism on magnetic resonance imaging hyperintensity volume and cognition in geriatric depression. *The American Journal of Geriatric Psychiatry, 17*(10): 847-855.

Hritcu, L., Cioanca, O., and Hancianu, M. (2012). Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats. *Phytomedicine*, 19(6): 529-534.

Idayu, N.F., Hidayat, M.T., Moklas, M.A.M., Sharida, F., Raudzah, A.N., Shamima, A.R., and Apryani, E. (2011). Antidepressant-like effect of mitragynine isolated from *Mitragyna speciosa* Korth in mice model of depression. *Phytomedicine*, *18*(5): 402-407.

Ingram R. (2016): Depression, in: H.S. Friedman, (Ed.), Encyclopedia of Mental Health, (Oxford: Academic Press), 26–33.

Irie, F., Masaki, K.H., Petrovitch, H., Abbott, R.D., Ross, G.W., Taaffe, D.R., and White, L.R. (2008). Apolipoprotein E ε4 allele genotype and the effect of depressive symptoms on the risk of dementia in men: the Honolulu-Asia Aging Study. *Archives of General Psychiatry,65*(8): 906-912.

Irwin, S. (1968). Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia, 13*(3): 222-257.

Jayatissa, M.N., Henningsen, K., Nikolajsen, G., West, M.J., and Wiborg, O. (2010). A reduced number of hippocampal granule cells does not associate with an anhedonia-like phenotype in a rat chronic mild stress model of depression. *Stress, 13*(2): 95-105.

Jin, L., Wu, F., Li, X., Li, H., Du, C., Jiang, Q., and Xu, Y. (2013). Anti-depressant Effects of Aqueous Extract from *Acanthopanax senticosus* in Mice. *Phytotherapy Research, 27*(12): 1829-1833.

Jones, I., and Craddock, N. (2001). Candidate gene studies of bipolar disorder. *Annals of Medicine, 33*(4): 248-256.

Juruena, M.F. (2014). Early-life stress and HPA axis trigger recurrent adulthood depression. *Epilepsy and Behaviour, 38*: 148-159.

Jutkiewicz, E.M. (2006). The antidepressant-like effects of delta-opioid receptor agonists. *Molecular Interventions, 6*(3), 162-169.

Kang, R.H., Hahn, S.W., Choi, M.J., and Lee, M.S. (2007). Relationship between G-protein beta-3 subunit C825T polymorphism and mirtazapine responses in Korean patients with major depression. *Neuropsychobiology, 56*(1): 1-5.

Kasper, S., Volz, H. P., Möller, H.J., Dienel, A., and Kieser, M. (2008). Continuation and long-term maintenance treatment with Hypericum extract WS® 5570 after

recovery from an acute episode of moderate depression—A double-blind, randomized, placebo controlled long-term trial. *European Neuropsychopharmacology*, *18*(11), 803-813.

Kendler, K.S., and Halberstadt, L.J. (2013). The road not taken: life experiences in monozygotic twin pairs discordant for major depression. *Molecular Psychiatry*, *18*(9): 975.

Kendler, K.S., and Gardner, C.O. (2001). Monozygotic twins discordant for major depression: a preliminary exploration of the role of environmental experiences in the aetiology and course of illness. *Psychological Medicine,31*(3): 411-423.

Kendler, K.S., Gardner, C.O., Neale, M.C., and Prescott, C.A. (2001). Genetic risk factors for major depression in men and women: similar or different heritabilities and same or partly distinct genes?*Psychological Medicine*, *31*(4): 605-616.

Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A., and Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Archives of General Psychiatry*, *62*(5): 529-535.

Kendler, K.S., Gardner, C.O., and Prescott, C.A. (2006). Toward a comprehensive developmental model for major depression in men. *American Journal of Psychiatry, 163*, 115–124.

Kessler, R.C. (1997). The effects of stressful life events on depression. *Annual Review of Psychology*, *48*(1): 191-214.

Kessler, R.C., Nelson, C.B., McGonagle, K.A., Liu, J., Swartz, M., and Blazer, D.G. (1996). Comorbidity of DSM-III-R major depressive disorder in the general population: Results from the U.S. National Comorbidity Survey. *British Journal of Psychiatry*, *168*(1), 17–30.

Khajehnasiri, F., Mortazavi, S.B., Allameh, A., Akhondzadeh, S., and Hashemi, H. (2013). Total antioxidant capacity and malondialdehyde in depressive rotational shift workers. *Journal of Environmental and Public Health*, *2013*: 1-5.

Khan, A., Sambunaris, A., Edwards, J., Ruth, A., and Robinson, D. S. (2014). Vilazodone in the treatment of major depressive disorder: efficacy across symptoms and severity of depression. *International Clinical Psychopharmacology, 29*(2): 86.

Klein, D.N., Shankman, S.A., Lewinsohn, P.M., Rohde, P., and Seeley, J.R. (2004). Family study of chronic depression in a community sample of young adults. *American Journal of Psychiatry*, *161*(4): 646-653.

Kreiss, D. S., and Lucki, I. (1995). Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. *Journal of Pharmacology and Experimental Therapeutics*, *274*(2), 866-876.

Kulesza, M., Raguram, R., and Rao, D. (2014). Perceived mental health related stigma, gender, and depressive symptom severity in a psychiatric facility in South India. *Asian Journal of Psychiatry, 9*: 73-77.

Kulkarni, S.K., and Dhir, A. (2007). Possible involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant activity of berberine chloride. *European Journal of Pharmacology*, *569*(1-2), 77-83.

Lad, H.V., Liu, L., Payá-Cano, J.L., Fernandes, C., and Schalkwyk, L.C. (2007). Quantitative traits for the tail suspension test: automation, optimization, and BXD RI mapping. *Mammalian Genome*, *18*(6-7): 482-491

Lambert, G., Johansson, M., Ågren, H., and Friberg, P. (2000). Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: evidence in support of the catecholamine hypothesis of mood disorders. *Archives of General Psychiatry*, *57*(8): 787-793.

Lattanzi, L., Dell'Osso, L., Cassano, P., Pini, S., Rucci, P., Houck, P.R., ... and Cassano, G. B. (2002). Pramipexole in treatment- resistant depression: a 16- week naturalistic study. *Bipolar Disorders*, *4*(5), 307-314.

Layous, K., Chancellor, J., Lyubomirsky, S., Wang, L., and Doraiswamy, P.M. (2011). Delivering happiness: Translating positive psychology intervention research for treating major and minor depressive disorders. *The Journal of Alternative and Complementary Medicine*, *17*(8): 675-683.

Levin, F.R., Evans, S.M., McDowell, D.M., Brooks, D.J., and Nunes, E. (2002). Bupropion treatment for cocaine abuse and adult attention-deficit/hyperactivity disorder. *Journal of Addictive Diseases*, *21*(2), 1-16.

Liebenberg, N., Wegener, G., Harvey, B.H., and Brink, C.B. (2010). Investigating the role of protein kinase-G in the antidepressant-like response of sildenafil in combination with muscarinic acetylcholine receptor antagonism. *Behavioural Brain Research*, *209*(1), 137-141.

Lim, G.Y., Tam, W.W., Lu, Y., Ho, C.S., Zhang, M.W., and Ho, R.C. (2018). Prevalence of Depression in the Community from 30 Countries between 1994 and 2014. *Scientific Reports*, *8*(1): 2861

Liu, H., Zhang, C., Ji, Y., and Yang, L. (2018). Biological and psychological perspectives of resilience: Is it possible to improve stress resistance? *Frontiers in Human Neuroscience*, *12*, 326

Lopez-Duran, N.L., Kovacs, M., and George, C.J. (2009). Hypothalamic–pituitary–adrenal axis dysregulation in depressed children and adolescents: A meta-analysis. *Psychoneuroendocrinology*, *34*(9): 1272-1283.

Lyon, D.E., McCain, N. L., Pickler, R. H., Munro, C., and Elswick, Jr, R. K. (2011).Advancing the biobehavioral research of fatigue with genetics and genomics. *Journal of Nursing Scholarship*, *43*(3), 274-281.

Maciejewski, P.K., Prigerson, H.G., and Mazure, C.M. (2001). Sex differences in event-related risk for major depression. *Psychological Medicine, 31*, 593–604.

MacMillan, H.L., Fleming, J.E., Streiner, D.L., Lin, E., Boyle, M.H., Jamieson, E., Duku, E.K., Walsh, C.A., Wong, M.Y.-Y., Beardslee, W.R. (2001). Childhood abuse and lifetime psychopathology in a community sample. *American Journal of Psychiatry*, *158*: 1878–1883.

Maes M, and Meltzer H. (1995): The serotonin hypothesis of major depression. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: The fourth generation of progress. New York: Raven. pp. 933- 944.

Maes, M., Kubera, M., Obuchowiczwa, E., Goehler, L., and Brzeszcz, J. (2011). Depression's multiple comorbidities explained by (neuro) inflammatory and oxidative and nitrosative stress pathways. *Neuroendocrinology Letters*, *32*(1): 7-24.

Maher, A.R., Hempel, S., Apaydin, E., Shanman, R.M., Booth, M., Miles, J.N., and Sorbero, M.E. (2016). St. John's Wort for major depressive disorder: a systematic review. *Rand Health Quarterly*, *5*(4): 5-12

Malagié, I., David, D.J., Jolliet, P., Hen, R., Bourin, M., and Gardier, A.M. (2002).Improved efficacy of fluoxetine in increasing hippocampal 5-hydroxytryptamine outflow in 5-HT1B receptor knock-out mice. *European Journal of Pharmacology*, *443*(1-3): 99-104.

Malberg, J.E., and Duman, R.S. (2003). Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology*, *28*(9): 1562.

Maletic, V., Eramo, A., Gwin, K., Offord, S.J., and Duffy, R.A. (2017). The role of norepinephrine and its α-adrenergic receptors in the pathophysiology and treatment of major depressive disorder and schizophrenia: a systematic review. *Frontiers in Psychiatry*, *8*: 42.

Mannel, M., Kuhn, U., Schmidt, U., Ploch, M., and Murck, H. (2010). St. John's wort extract LI160 for the treatment of depression with atypical features–a double-blind, randomized, and placebo-controlled trial. *Journal of Psychiatric Research*, *44*(12): 760-767.

Marazziti, D., Rutigliano, G., Baroni, S., Landi, P., and Dell'Osso, L. (2014).Metabolic syndrome and major depression.*Central Nervous System Spectrums*, *19*(4): 293-304.

Marcus M, Yasamy M.T, van Ommeren M, Chisholm D, Saxena S. (2012): Depression: A Global Public Health Concern. Occoquan, VA, USA: World Federation for Mental Health.

Mariod, A.A., Fadle, N., and Hasan, A.A. (2014). Antimicrobial screening of wood extracts of Combretum hartmannianum, *Acacia seyal* and *Terminalia brownie*. *European Journal of Molecular Biology and Biochemistry, 1*(2): 77-80.

Martin, P., Massol, J., Colin, J.N., Lacomblez, L., and Puech, A.J. (1990).Antidepressant profile of bupropion and three metabolites in mice. *Pharmacopsychiatry*, *23*(04), 187-194.

Martinac, M., Babić, D., Bevanda, M., Vasilj, I., Bevanda Glibo, D., Karlović, D., and Jakovljević, M. (2017).Activity of the hypothalamic-pituitary-adrenal axis and inflammatory mediators in major depressive disorder with or without metabolic syndrome.*Psychiatria Danubina*, *29*(1): 39-50.

Masand, P.S., and Gupta, S. (2002). Long-term side effects of newer-generation antidepressants: SSRIS, venlafaxine, nefazodone, bupropion, and mirtazapine. *Annals of Clinical Psychiatry*, *14*(3), 175-182.

Masuda, Y., Ohnuma, S., and Sugiyama, T. (2001). in Mouse Forced Swimming. *Methods and Findings in Experimental Clinical Pharmacology*, *23*(1), 19-21.

McCusker, R.H., and Kelley, K.W. (2013). Immune–neural connections: how the immune system's response to infectious agents influences behavior. *Journal of Experimental Biology,216*(1), 84-98.

McEwen, B.S. (2000). The neurobiology of stress: from serendipity to clinical relevance. *Brain Research, 886*(1-2): 172-189.

McIntyre, R.S. (2013). Using measurement strategies to identify and monitor residual symptoms. *Journal of Clinical Psychiatry, 74*(2), 14-18.

McIntyre, R.S., Lee, Y., and Mansur, R.B. (2015). Treating to target in major depressive disorder: response to remission to functional recovery. *Central Nervous System Spectrums, 20*(1), 17-31.

McIntyre, R. S., Suppes, T., Tandon, R., and Ostacher, M. (2017).Florida best practice psychotherapeutic medication guidelines for adults with major depressive disorder. *The Journal of Clinical Psychiatry, 78*(6), 703-713.

Merali, Z.D.L., Hrdina, P., Palkovits, M., Faludi, G., Poulter, M.O., and Anisman H. (2004). Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA (A) receptor subunits in frontal cortical brain region. *Journal of Neuroscience*, 24: 1478-1485.

Meyer, J.H., Goulding, V.S., Wilson, A.A., Hussey, D., Christensen, B.K., and Houle, S. (2002). Bupropion occupancy of the dopamine transporter is low during clinical treatment. *Psychopharmacology*, *163*(1), 102-105.

Millan, M.J., Gobert, A., Lejeune, F., Newman-Tancredi, A., Rivet, J.M., Auclair, A., and Peglion, J.L. (2001). S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *Journal of Pharmacology and Experimental Therapeutics*, *298*(2), 565-580.

Millan, M.J., Gobert, A., Rivet, J.M., Adhumeau- Auclair, A., Cussac, D., Newman- Tancredi, A., ... and Lejeune, F. (2000). Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of α2-adrenergic and serotonin2C receptors: a comparison with citalopram. *European Journal of Neuroscience*, *12*(3), 1079-1095.

Miller, D.K., Wong, E. H., Chesnut, M.D., and Dwoskin, L.P. (2002). Reboxetine: functional inhibition of monoamine transporters and nicotinic acetylcholine receptors. *Journal of Pharmacology and Experimental Therapeutics*, *302*(2), 687-695.

Miller, A.H., Maletic, V., and Raison, C.L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological Psychiatry*, *65*(9): 732-741.

Miller, J.A., Nathanson, J., Franjic, D., Shim, S., Dalley, R.A., Shapouri, S., and Lee, C.K. (2013). Conserved molecular signatures of neurogenesis in the hippocampal subgranular zone of rodents and primates.*Development*, *140*(22): 4633-4644.

Mizui, T., Ishikawa, Y., Kumanogoh, H., and Kojima, M. (2016). Neurobiological actions by three distinct subtypes of brain-derived neurotrophic factor: Multi-ligand model of growth factor signaling. *Pharmacological Research*, *105*, 93-98.

Mnie-Filali, O., Faure, C., El Mansari, M., Lambás-Señas, L., Bérod, A., Zimmer, L., and Haddjeri, N. (2007). R-citalopram prevents the neuronal adaptive changes induced by escitalopram. *NeuroReport*, *18*(15): 1553-1556.

Mojtabai, R. (2014). Diagnosing depression in older adults in primary care. *New England Journal of Medicine*, *370*(13), 1180-1182.

Mondimore, F.M., Zandi, P.P., MacKinnon, D.F., McInnis, M.G., Miller, E.B., Crowe, R.P., ...and Murphy-Ebenez, K.P. (2006). Familial aggregation of illness chronicity in recurrent, early-onset major depression pedigrees.*American Journal of Psychiatry*, *163*(9): 1554-1560.

Montgomery, S.A. (1997). Reboxetine: additional benefits to the depressed patient. *Journal of Psychopharmacology (Oxford, England)*, *11*(*4*), 9-15.

Mullur, R., Liu, Y.Y., and Brent, G.A. (2014).Thyroid hormone regulation of metabolism.*Physiological Reviews, 94*(2): 355-382.

Murua, V.S., Gomez, R.A., Andrea, M.E., and Molina, V.A. (1991). Shuttle-box deficits induced by chronic variable stress: reversal by imipramine administration. *Pharmacology Biochemistry and Behaviour*, *38*(1), 125-130.

Murugan, S., Umadevi, P., Jennifer Suganthi, S., and Subakanmani, S. (2011). Evaluation of antidepressant like activity of *Cucurbita pepo* seed extracts in rats. International *Journal of Current Pharmaceutical Research, 3*(1): 108-113.

Muscat, R., Papp, M., and Willner, P. (1992). Reversal of stress induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. *Psychopharmacology, 109*, 433–438.

N. I. H. (1996). Revised Guide for the Care and Use of Laboratory Animals. *NIH GUIDE*, *25*.

Nalepa, I., Kreiner, G., Kowalska, M., Sanak, M., Zelek-Molik, A., and Vetulani, J. (2002). Repeated imipramine and electroconvulsive shock increase α1A-adrenoceptor mRNA level in rat prefrontal cortex. *European Journal of Pharmacology*, *444*(3), 151-159.

Nemeroff C.B. (1988): The role of corticotropin- releasing factor in the pathogenesis of majordepression. *Pharmacopsychiatry, 21*(2):76- 82.

Nemeroff, C.B. (2008). Recent findings in the pathophysiology of depression.*Focus,6*(1): 3-14.

Nesterova, Y.V., Povetieva, T. N., Suslov, N. I., Semenov, A. A., and Pushkarskiy, S. V. (2011). Antidepressant activity of diterpene alkaloids of *Aconitum baicalense* Turcz. *Bulletin of Experimental Biology and Medicine*, *151*(4): 425-428

Nikfarjam, M., Parvin, N., Assarzadegan, N., and Asghari, S. (2013). The effects of *Lavandula angustifolia* mill infusion on depression in patients using citalopram: a comparison study. *Iranian Red Crescent Medical Journal*, *15*(8): 734.

Nutt, D.J. (2006). The role of dopamine and norepinephrine in depression and antidepressant treatment.*Journal of Clinical Psychiatry,67*(6), 3–8.

OCDE OECD. (2008) Acute oral toxicity: Up and down procedure. OECD.*Guideline for the Testing of Chemicals*, 425: 1-2

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., and Anthony, S. (2009). Agroforestree Database: a tree reference and selection guide version 4.0. *World Agroforestry Centre, Kenya*, *15*.

Ott, G.E., Rao, U., Lin, K.M., Gertsik, L., and Poland, R.E. (2004). Effect of treatment with bupropion on EEG sleep: relationship to antidepressant response. *International Journal of Neuropsychopharmacology*, *7*(3), 275-281.

Page, M.E., Brown, K., and Lucki, I. (2003). Simultaneous analyses of the neurochemical and behavioral effects of the norepinephrine reuptake inhibitor reboxetine in a rat model of antidepressant action. *Psychopharmacology*, *165*(2), 194-201.

Pälvimäki, E. P., Majasuo, H., Laakso, A., Kuoppamäki, M., Syvälahti, E., Roth, B. L., and Hietala, J. (1996).Interactions of selective serotonin reuptake inhibitors with the serotonin 5-HT 2C receptor. *Psychopharmacology*, *126*(3), 234-240.

Papakostas, G.I., and Fava, M. (2007). A meta-analysis of clinical trials comparing the serotonin (5HT)-2 receptor antagonists trazodone and nefazodone with selective serotonin reuptake inhibitors for the treatment of major depressive disorder. *European Psychiatry*, *22*(7), 444-447.

Paykel, E.S., Cooper, Z., Ramana, R., and Hayhurst, H. (1996). Life events, social support and marital relationships in the outcome of severe depression. *Psychological Medicine, 26*(1), 121-133.

Paykel, E.S.(2011): The evolution of life events research in psychiatry. *Journal of Affective Disorders, 62,* 141- 9

Peng, R., and Li, Y. (2017). Low serum thyroid-stimulating hormone levels are associated with lipid profile in depressive patients with long symptom duration. *Journal of Affective Disorders*, *217*, 99-104.

Peng, W.H., Lo, K.L., Lee, Y.H., Hung, T.H., and Lin, Y.C. (2007). Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice. *Life Sciences*, *81*(11), 933-938.

Perez, G.R.M., Perez,L.J.A., Garcia, D.L.M.,and Sossa, M.H. (1998): Neuropharmacological activity of *Solanum nigrum* fruit. *Journal of Ethnopharmacology*, *62*: 43-48.

Perviz, S., Khan, H., and Pervaiz, A. (2016).Plant alkaloids as an emerging therapeutic alternative for the treatment of depression. *Frontiers in Pharmacology*, *7*, 28.

Porsolt, R.D., Le Pichon, M., Jalfre, M. (1977): Depression: A new animal model sensitive to antidepressant treatments. *Nature, 266*(5604): 730–732.

Qiu, A., Taylor, W.D., Zhao, Z., MacFall, J.R., Miller, M. I., Key, C. R., and Krishnan, K. R. R. (2009). APOE related hippocampal shape alteration in geriatric depression. *Neuroimage*, *44*(3): 620-626.

Rahimi, R., and Abdollahi, M. (2012).An update on the ability of St. John's wort to affect the metabolism of other drugs. *Expert Opinion on Drug Metabolism and Toxicology*, *8*(6), 691-708.

Raison, C.L., Capuron, L., and Miller, A.H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology*, *27*(1): 24-31.

Rajkowska, G., and Miguel-Hidalgo, J.J. (2007).Gliogenesis and glial pathology in depression.*Central Nervous System and Neurological Disorders-Drug Targets*,*6*(3): 219-233.

Ravindran, A.V., Balneaves, L.G., Faulkner, G., Ortiz, A., McIntosh, D., Morehouse, R.L., and MacQueen, G.M. (2016). Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: section 5. Complementary and alternative medicine treatments. *The Canadian Journal of Psychiatry*, *61*(9): 576-587.

Redman, J.R., and Francis, A.J. (1998). Entrainment of rat circadian rhythms by the melatonin agonist S-20098 requires intact suprachiasmatic nuclei but not the pineal. *Journal of Biological Rhythms*, *13*(1): 39-51.

Rénéric, J.P., and Lucki, I. (1998). Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. *Psychopharmacology*, *136*(2): 190-197.

Rex, A., Stephens, D.N., and Fink, H. (1996).“Anxiolytic” action of diazepam and abecarnil in a modified open field test.*Pharmacology Biochemistry and Behavior*, *53*(4): 1005-1011.

Reynolds III, C.F., Dew, M.A., Pollock, B.G., Mulsant, B. H., Frank, E., Miller, M. D., ... and Schlernitzauer, M. A. (2006). Maintenance treatment of major depression in old age. *New England Journal of Medicine*, *354*(11): 1130-1138.

Reynolds, C.F., Butters, M. A., Lopez, O., Pollock, B.G., Dew, M. A., Mulsant, B. H., ... and Houck, P. R. (2011). Maintenance treatment of depression in old age: a randomized, double-blind, placebo-controlled evaluation of the efficacy and safety of donepezil combined with antidepressant pharmacotherapy. *Archives of General Psychiatry*, *68*(1): 51-60.

Richelson, E. (2003). Interactions of antidepressants with neurotransmitter transporters and receptors and their clinical relevance. *The Journal of Clinical Psychiatry*, *64*:5-12.

Roose, S.P., Glassman, A.H., and Dalack, G.W. (1989).Depression, heart disease, and tricyclic antidepressants. *The Journal of Clinical Psychiatry*, *50*: 12-6.

Rosenberg, C., Lauritzen, L., Brix, J., Jørgensen, J.B., Kofod, P., and Bayer, L.B. (2007). Citalopram versus amitriptyline in elderly depressed patients with or

without mild cognitive dysfunction: a danish multicentre trial in general practice. *Psychopharmacology Bulletin*, *40*(1): 63-73.

Rubin, R.T., Poland, R.E., Lesser, I.M., and Martin, D.J. (1989).Neuroendocrine aspects of primary endogenous depression. V. Serum prolactin measures in patients and matched control subjects. *Biological Psychiatry*, *25*(1): 4-21.

Rush, C.R., Kollins, S.H., and Pazzaglia, P.J. (1998).Discriminative-stimulus and participant-rated effects of methylphenidate, bupropion, and triazolam in d-amphetamine-trained humans. *Experimental and Clinical Psychopharmacology*, *6*(1): 32.

Rush, A.J., Trivedi, M.H., Stewart, J.W., Nierenberg, A.A., Fava, M., Kurian, B.T., and Cook, I. A. (2011).Combining medications to enhance depression outcomes (CO-MED): acute and long-term outcomes of a single-blind randomized study. *American Journal of Psychiatry*, *168*(7): 689-701.

Sadock, B.J., and Sadock, V.A. (2003).Synopsis of psychiatry behavioural sciences/clinical psychiatry. 9th ed. Philadelphia, PA: Lippincott Williams and Wilkins.

Sahay, A., and Hen, R. (2007).Adult hippocampal neurogenesis in depression.*Nature Neuroscience*, *10*(9): 1110.

Sairanen, M., O'leary, O.F., Knuuttila, J.E., and Castren, E. (2007). Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. *Neuroscience*, *144*(1): 368-374.

Salamone, J.D., Correa, M., Yohn, S., Cruz, L.L., San Miguel, N., and Alatorre, L. (2016). The pharmacology of effort-related choice behavior: dopamine, depression, and individual differences. *Behavioural Processes*, *127*: 3-17.

Saleem, U., Ahmad, B., Ahmad, M., Erum, A., Hussain, K., and Irfan Bukhari, N. (2016). Is folklore use of Euphorbia helioscopia devoid of toxic effects? *Drug and Chemical Toxicology*, *39*(2): 233-237.

Sánchez, C., and Hyttel, J. (1999).Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cellular and Molecular Neurobiology*, *19*(4): 467-489.

Sarko, J. (2000). Antidepressants, old and new: a review of their adverse effects and toxicity in overdose. *Emergency Medicine Clinics of North America*, *18*(4), 637-654.

Sarris, J., and Kavanagh, D.J. (2009). Kava and St. John's Wort: current evidence for use in mood and anxiety disorders. *The Journal of Alternative and Complementary Medicine*, *15*(8): 827-836.

Schatzberg, A.F. (2003). Efficacy and tolerability of duloxetine, a novel dual reuptake inhibitor, in the treatment of major depressive disorder. *The Journal of Clinical Psychiatry*, *64*: 30-37.

Segerstrom, S.C., and Miller, G.E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychological Bulletin*, *130*(4): 601.

Seki, H., Ohyama, K., Sawai, S., Mizutani, M., Ohnishi, T., Sudo, H., andMuranaka, T. (2008). Licorice β-amyrin 11-oxidase, a cytochrome P450 with a key role in the biosynthesis of the triterpene sweetener glycyrrhizin. *Proceedings of the National Academy of Sciences*, *105*(37), 14204-14209.

Seo, D., Patrick, C.J.,and Kennealy, P.J. (2008). Role of serotonin and dopamine system interactions in the neurobiology of impulsive aggression and its comorbidity with other clinical disorders.*Aggression and Violent Behaviour*, *13*(5): 383-395.

Setiawan, E., Wilson, A.A., Mizrahi, R., Rusjan, P.M., Miler, L., Rajkowska, G., and Meyer, J.H. (2015).Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes.*JAMA Psychiatry*, *72*(3): 268-275.

Sharpley, C.F., and Agnew, L.L. (2011). Cytokines and depression: findings, issues, and treatment implications. *Reviews in the Neurosciences*, 22(3): 295-302.

Shattell, M.M., Smith, K.M., Quinlan-Colwell, A., and Villalba, J.A. (2008). Factors contributing to depression in Latinas of Mexican origin residing in the United States: Implications for nurses. *Journal of the American Psychiatric Nurses Association*, *14*, 193–204.

Shehu, A., Magaji, M.G., Yau, J., and Ahmed, A. (2017).Ethno-botanical survey of medicinal plants used for the management of depression by Hausa tribes of Kaduna State, Nigeria. *Journal of Medicinal Plants Research*, *11*(36), 562-567.

Sher, L., Oquendo, M.A., Burke, A.K., Cooper, T.B., and Mann, J.J. (2013).Combined dexamethasone suppression–corticotrophin-releasing hormone stimulation test in medication-free major depression and healthy volunteers.*Journal of Affective Disorders,151*(3): 1108-1112.

Shih, J.H., Eberhard, N.K., Hammen, C., and Brennan, P.A. (2006). Differential exposure and reactivity to interpersonal stress predict sex differences in adolescent depression. *Journal of Clinical Child and Adolescent Psychology*, *35*, 103–115.

Singer, A., Schmidt, M., Hauke, W., and Stade, K. (2011). Phytomedicine: Duration of response after treatment of mild to moderate depression with Hypericum extract STW 3-VI, citalopram and placebo: A reanalysis of data from a controlled clinical trial. *Alternative Medicine Review*, *16*(2): 200-201.

Smith, S.M., and Vale, W.W. (2006).The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress.*Dialogues in Clinical Neuroscience*, *8*(4): 383.

Sofowora, A. (1993). Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, pp. 134-156.

Sousa, F.C.F., Monteiro, A.P., Melo, C.T.V., Oliveira, G.R., Vasconcelos, S.M.M., Fonteles. M.M.F., Gutierrez, S.J.C., Barbosa-Filho, J.M. and Viana, G.S.B. (2005).Antianxiety Effects of Riparin I from *Anibariparia* (Nees) Mez (Lauraceae) in Mice.*Phytotherapy Research, 19*: 1005–1008

Spangler, D.L., Simons, A.D., Monroe, S.M., and Thase, M.E. (1996). Gender differences in cognitive diathesis-stress domain match: Implications for differential pathways to depression. *Journal of Abnormal Psychology*, *105*, 653–657.

Sporn, J., Ghaemi, S.N., Sambur, M.R., Rankin, M.A., Recht, J., Sachs, G.S., and Fava, M. (2000). Pramipexole augmentation in the treatment of unipolar and bipolar depression: a retrospective chart review. *Annals of Clinical Psychiatry*, *12*(3), 137-140.

Stachowicz, K., Chojnacka-Wójcik, E., Kłak, K., and Pilc, A. (2007). Anxiolytic-like effect of group III mGlu receptor antagonist is serotonin-dependent. *Neuropharmacology*, *52*(2), 306-312.

Stahl, S.M. (1998): Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action'. *Journal of Clinical Psychiatry*.*59*(4): 5- 14.

Stahl, S. M. (1998). Mechanism of action of serotonin selective reuptake inhibitors: serotonin receptors and pathways mediate therapeutic effects and side effects. *Journal of Affective Disorders*, *51*(3): 215-235.

Stahl, S. M., and Grady, M. M. (2003).Differences in mechanism of action between current and future antidepressants. *The Journal of Clinical Psychiatry*, *64*, 13-17.

Stanford, S.C. (2007). The Open Field test: Reinventing the wheel. *Journal of Psychopharmacology*,*21*(2): 134-4

Stanley, J.L., Lincoln, R.J., Brown, T.A., McDonald, L.M., Dawson, G.R., and Reynolds, D.S. (2005). The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. *Journal of Psychopharmacology*, *19*(3): 221-227.

Stanley, M., Virgilio, J., and Gershon, S. (1982): Tritiated imipramine binding sites are decreased in the frontal cortex of suicides. *Science*,*216*: 1337- 1339.

Steru, L., Chermat, R., Thierry, B., and Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology*, *85*(3): 367-370.

Stockmeier, C.A. (2003). Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *Journal of Psychiatric Research,37*(5): 357-373.

Stone, Eric A., Yan Lin, Helen Rosengarten, H. Kenneth Kramer, and David Quartermain. (2003): "Emerging evidence for a central epinephrine-innervated α1-adrenergic system that regulates behavioral activation and is impaired in depression." *Neuropsychopharmacology 28*(8), 1387.

Strawbridge, R., Young, A.H., and Cleare, A.J. (2017). Biomarkers for depression: recent insights, current challenges and future prospects. *Neuropsychiatric Disease and Treatment*, *13*, 1245.

Szegedi, A., Jansen, W.T., van Willigenburg, A.P., van der Meulen, E., Stassen, H.H., and Thase, M.E. (2009). Early improvement in the first 2 weeks as a predictor of treatment outcome in patients with major depressive disorder: a meta-analysis including 6562 patients. *The Journal of Clinical Psychiatry,70*: 344- 53.

Szpunar, M.J., and Parry, B.L. (2018).A systematic review of cortisol, thyroid-stimulating hormone, and prolactin in peripartum women with major depression.*Archives of Women's Mental Health,21*(2): 149-161.

Tatsumi, M., Groshan, K., Blakely, R.D., and Richelson, E. (1997).Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *European Journal of Pharmacology*, *340*(2-3), 249-258.

Taylor, W.D., Aizenstein, H.J., and Alexopoulos, G.S. (2013). The vascular depression hypothesis: mechanisms linking vascular disease with depression. *Molecular Psychiatry*, *18*(9), 963

Thase, M.E., and Howland, R.H. (1995). Biological processes in depression: an updated review and integration.: An updated review and integration. In Handbook of depression Edited by: Beckham EE, Leber WR New York: Guilford, pp. 213- 79.

Tiwari, A.K., and Mehta, R. (2013). Medicinal plants used by traditional healers in Jashpur district of Chhattisgarh, India by Anupam Kumar Tiwari and Rajendra Mehta. *Life Sciences Leaflets*, *35*, 31-50.

Tomlinson, M., Grimsrud, A.T., Stein, D.J., Williams, D.R., and Myer, L. (2009). The epidemiology of major depression in South Africa: results from the South African stress and health study. *South African Medical Journal*, *99*(5).

Tondo, L., Isacsson, G., and Baldessarini, R.J. (2003). Suicidal behaviour in bipolar disorder: risk and prevention. *Central Nervous System drugs.17*: 491-511.

Treadway, M.T. (2016): The Neurobiology of Motivational Deficits in Depression- - An Update on Candidate Pathomechanisms. *Current Topics in Behavioural Neuroscience, 27*:337- 55.

Ulak, G., Mutlu, O., Tanyeri, P., Komsuoglu, F.I., Akar, F.Y., and Erden, B.F. (2010). Involvement of serotonin receptor subtypes in the antidepressant-like effect of TRIM in the rat forced swimming test. *Pharmacology Biochemistry and Behaviour, 95*(3): 308-314.

Van der Watt, G., Laugharne, J., and Janca, A. (2008).Complementary and alternative medicine in the treatment of anxiety and depression. *Current Opinion in Psychiatry, 21*(1): 37-42.

Van Kampen, J.M., and Robertson, H.A. (2005).A possible role for dopamine D3 receptor stimulation in the induction of neurogenesis in the adult rat substantia nigra. *Neuroscience, 136*(2): 381-386.

Vreeburg, S.A., Hoogendijk, W.J., van Pelt, J., DeRijk, R.H., Verhagen, J.C., van Dyck, R., and Penninx, B.W. (2009). Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. *Archives of General Psychiatry, 66*(6): 617-626.

Weller, E. B., Kloos, A., Kang, J., and Weller, R.A. (2006). Depression in children and adolescents: does gender make a difference? *Current Psychiatry Reports, 8*(2): 108-114.

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., ... and Burstein, R. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *The Lancet, 382*(9904): 1575-1586.

Willner, P., Hale, A.S., and Argyropoulos, S. (2005). Dopaminergic mechanism of antidepressant action in depressed patients. *Journal of Affective Disorders, 86*(1): 37-45.

Willner, P., Muscat, R., and Papp, M. (1992). Chronic mild stress- induced anhedonia: a realistic animal model of depression. *Neuroscience and Biobehavioral Reviews, 16*(4): 525-534.

Wilson, S., and Argyropoulos, S. (2005). Antidepressants and sleep. *Drugs, 65*(7): 927-947.

Woode, E., Boakye-Gyasi, E., Amidu, N., Ansah, C., and Duwiejua, M. (2010). Anxiolytic and antidepressant effects of a leaf extract of *Palisota hirsuta* K. Schum.(Commelinaceae) in mice. *International Journal of Pharmacology, 6*(1): 1-17.

World Health Organization (2006) Mental Health: Depression. Available at: http://www.who.int/mental_health/management/ depression/definition/en/(accessed 28 August 2006).

World Health Organization (2011).Global burden of mental disorders and the need for a comprehensive, coordinated response from health and social sectors at the country level.⟨http://apps.who.int/gb/ebwha/pdf_files/EB130/B130_9-en⟩.

World Health Organization. (2012). Public health action for the prevention of suicide: a framework. Available at: https://apps.who.int/iris/bitstream/handle/10665/75166/9789241503570_eng.pdf

World Health Organization. (2017). Depression and other common mental disorders: global health estimates, 1-24.Available at:https://www.who.int/mental_health/management/depression/prevalence_global_health_estimates/en/

Xiong, Z., Jiang, B., Wu, P.F., Tian, J., Shi, L.L., Gu, J., and Chen, J.G. (2011). Antidepressant effects of a plant-derived flavonoid baicalein involving extracellular signal-regulated kinases cascade. *Biological and Pharmaceutical Bulletin*, *34*(2): 253-259.

Yamamoto, B.K., and Novotney, S. (1998). Regulation of extracellular dopamine by the norepinephrine transporter. *Journal of Neurochemistry*, *71*(1): 274-280.

Yatham, L.N., Srisurapanont, M., Zis, A.P., and Kusumakar, V. (1997).Comparative studies of the biological distinction between unipolar and bipolar depressions.*Life Sciences*, *61*(15): 1445-1455.

Yi, L.T., Li, C.F., Zhan, X., Cui, C.C., Xiao, F., Zhou, L.P., and Xie, Y. (2010).Involvement of monoaminergic system in the antidepressant-like effect of the flavonoid naringenin in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(7): 1223-1228.

Yi, L.T., Li, J., Li, H.C., Su, D.X., Quan, X.B., He, X.C., and Wang, X.H. (2012). Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *39*(1): 175-181.

Yi, L.T., Liu, B.B., Li, J., Luo, L., Liu, Q., Geng, D., and Wu, D. (2014). BDNF signaling is necessary for the antidepressant-like effect of naringenin. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*,*48*: 135-141.

Yu, H., and Chen, Z.Y. (2011).The role of BDNF in depression on the basis of its location in the neural circuitry. *Acta Pharmacologica Sinica*, *32*(1): 3.

Yuan, Y., Zhang, Z., Bai, F., You, J., Yu, H., Shi, Y., and Liu, W. (2010). Genetic variation in apolipoprotein E alters regional gray matter volumes in remitted late-onset depression. *Journal of Affective Disorders*, *121*(3): 273-277.

Zhang, H.T., Huang, Y., and O'Donnell, J.M. (2003).Antagonism of the antidepressant-like effects of clenbuterol by central administration of β-adrenergic antagonists in rats. *Psychopharmacology*, *170*(1): 102-107.

Zhao, J., Niu, C., Wang, J., Yang, H., Du, Y., Wei, L., and Li, C. (2018). The depressive-like behaviors of chronic unpredictable mild stress-treated mice, ameliorated by Tibetan medicine Zuotai: involvement in the hypothalamic–pituitary–adrenal (HPA) axis pathway. *Neuropsychiatric Disease and Treatment*, *14*: 129.

Zheng, M., Li, Y., Shi, D., Liu, C., and Zhao, J. (2014). Antidepressant-like effects of flavonoids extracted from *Apocynum venetum* leaves in mice: the involvement of monoaminergic system in mice. *African Journal of Pharmacy and Pharmacology*, *8*(29): 765-774.

Zheng, S., Yu, M., Lu, X., Huo, T., Ge, L., Yang, J., and Li, F. (2010).Urinary metabonomic study on biochemical changes in chronic unpredictable mild stress model of depression. *Clinica Chimica Acta*, *411*(3-4): 204-209.

Zhu, W. L., Shi, H. S., Wei, Y. M., Wang, S. J., Sun, C. Y., Ding, Z. B., and Lu, L. (2012). Green tea polyphenols produce antidepressant-like effects in adult mice. *Pharmacological Research*, *65*(1): 74-80.

APPENDICES

Appendix I: Effect of Methanol Extract of *Acacia seyal s* on Motor Coordination in Mice

Treatment (mg/kg)	Number of foot slips
Distilled water 10 ml/kg	4.20±0.37
AS 250	1.40±0.40
AS 500	2.80±0.86
AS 1000	4.00±0.68
Diazepam 10	10.00±1.22*

Values are expressed as Mean ± S.E.M; *$p \leq 0.05$ compared to distilled water (One way ANOVA followed by Dunnett*post hoc* test) n=6, AS = Methanol extract of *Acacia seyal*

Appendix II: Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Tail Suspension Test

Treatment (mg/kg)	Duration of immobility(s)
Distilled water 10 ml/kg	232.87±10.29
AS 250	152.83±18.02*
AS 500	84.7±17.29***
AS 1000	135±9.85**
Fluoxetine 20	68.67±20.58***

Values are expressed as Mean ± S.E.M; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared to distilled water (One way ANOVA followed by Dunnett*post hoc* test) n=6, AS = Methanol extract of *Acacia seyal*

Appendix III: Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration of Immobility of Mice in Forced Swim Test

Treatment (mg/kg)	Duration of immobility(s)
Distilled water 10 ml/kg	160.67±4.37
AS 250	30.83±6.78**
AS 500	42.67±49.42***
AS 1000	60±13.92**
Fluoxetine 20	40.17±3.83***

Values are expressed as Mean ± S.E.M; ** = $p< 0.01$, *** = $p<0.001$ compared to distilled water (One way ANOVA followed by Dunnett*post hoc* test) n=6, AS = Methanol extract of *Acacia seyal*

Appendix IV: Effect of Methanol Root Bark Extract of *Acacia seyal* on Line Crosses Activity in Mice in the Open Field Test

Treatment (mg/kg)	Number of line crossing
Distilled water 10 ml/kg	48.83±10.16
AS 250	73.83±36.47

AS 500	90.50±17.10
AS 1000	89.17±1.43
Diazepam 0.5	147.17±12.49***

Values are expressed as Mean ± S.E.M; *** = $p<0.001$ compared to distilled water (One way ANOVA followed by Dunnett*post hoc* test) n=6, AS = Methanol extract of *Acacia seyal*

Appendix V: Effect of Methanol Root Bark Extract of *Acacia seyal* on Serum Cortisol Levels Following Chronic Unpredictable Mild Stress in Mice

Treatment (mg/kg)	Concentration(ng/ml)
NOCUMS	0.159±0.017
CUMS	$1.222\pm0.464^{***}$
CUMS + AS 250	$0.155\pm0.025^{++}$
CUMS + AS 500	$0.121\pm0.029^{++}$
CUMS + AS 1000	$0.192\pm0.004^{+}$
CUMS + Fluoxetine 20	$0.116\pm0.023^{++}$

Results are presented as Mean ±S.E.M. Data was analysed using One-Way ANOVA followed by Dunnett post hoc test $^{***}p<0.001$ as compared to the unstressed group, $^{+}p<0.05$,$^{++}p<0.01$, $^{+++}p<0.001$ as compared to stressed/ CMS group

Appendix VI: Effect of Methanol Root Bark Extract of *Acacia seyal* on Brain Derived Neutrophic Factor Levels in the brain of Mice Following Chronic Unpredictable Mild Stress

Treatment (mg/kg)	Concentration(pg/ml)
NOCUMS	1262±78.12
CUMS	$692.6\pm39.21^{***}$
CUMS + AS 250	$1031\pm80.69^{++}$
CUMS + AS 500	$1026\pm107.57^{++}$
CUMS + AS 1000	$1017.8\pm61.05^{+}$
CUMS + Fluoxetine 20	$1229.4\pm44.15^{+++}$

Results are presented as Mean ±S.E.M. Data was analysed using One-Way ANOVA followed by Dunnett post hoc test $^{***}p<0.001$ as compared to the unstressed group, $^{+}p<0.05$,$^{++}p<0.01$, $^{+++}p<0.001$ as compared to stressed/ CUMS group